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A practical guide

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Foreword

At the last international conference on 'Food Oral Processing – Physics, Physiology, and Psychology of Eating' (Beaune, France, 1–5 July 2012), I participated in a workshop on instrumental techniques for eating and sensory studies. Having been hugely impressed by the latest technical developments and the enthusiasm of participants, I came to the conclusion that a book on this topic would be tremendously helpful to both industrial and academic researchers. And here comes a book in this area. I am really grateful to David for this excellent work.

Since I started my research career as a food scientist many years ago, I have devoted a lot of time and effort to trying to understand the controlling mechanisms of food oral processing and sensation. The complications still overwhelm me. I often wonder how lucky and how unusual we are as human beings. We have uniquely developed (or evolved) some very smart sensory systems which make us capable of detecting and responding to all sorts of external stimuli, such as light, sound, warmth/coldness, smell, taste, touch, pain, etc. For pleasure, we tend to voluntarily expose ourselves to stimuli which are perceived to be pleasant and, of course, for self-protection, we always avoid those which are perceived to be not pleasant or possibly harmful. This simple principle applies in particular when we come into contact with food. Our sensation of a food is through a combination of looking, smelling, tasting, touching (both hand and oral), and even listening. We receive pleasure and enjoyment from consuming a food which provides desirable sensory features, but reject one which gives no such pleasing sensory effect. From this perspective, our eating experience and preference for a food is fully psychologically emotional. That's why providing sensory pleasure is always claimed to be one of the most important basic functions

of food. It is this function which uniquely distinguishes food from medicines.

However, the reliable characterisation or quantification of sensory features of a food product is not as easy as it seems. The use of human subjects as taste panellists is a classic method of food sensory assessment, which has been applied by the food industry as long for as we can trace the industry itself. Even though human testing is still commonly used in cases of research and development, it is always not favoured as a tool for the purposes of routine food quality control owing to reasons of high cost, human error, as well as increasingly complicated ethical issues involving human tests. Therefore, there is a great need from the food industry for reliable instrumental methods to measure various sensory properties of food.

To this end, food scientists and technologists have been working tirelessly over the past half century and great progress has been made in terms of both instrumental hardware and software. Despite this progress, how to correlate a physical/instrumental process to a highly complicated psychological/physiological process of human sensation still remains a great challenge. This is fundamental in order for meaningful interpretations of instrumental results in relation to human sensation and perception to be made. In this context, the contents of the first part of this book will provide background theories and some very useful clues. I also tend to believe that the application of instrumental methods for food sensory quality assessment has to overcome two practical challenges. Firstly, there is a wide range of food sensory properties appreciated and referred to by consumers. Some of these sensory properties are directly sensed or detected by our sensory systems, but many of them appear to be not. They are derived perceptions based on a combination of different sensory stimuli. Secondly, food exists in so many different forms and types. Very often the same sensory feature could have very different implications in the context of different foods. Therefore, it is no doubt to me that specific approaches will have to be applied to deal with different sensory features in different food systems. I am particularly glad to see that the second part of this book is organised based on material categories and a wide range of food systems are covered by the individual chapters. Certain sensory properties associated with each food system are discussed in great depth as well as in precise detail.

I find this book extremely useful and I hope readers will enjoy reading this book as much as I have.

*Dr Jianshe Chen
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Preface

The perception of food quality is an enormously complex subject that, in spite of many significant advances made over recent years, we are only beginning to understand. Take the many varied physiological inputs that are generated by the human sense organs when consuming food, couple these with the psychological and neurological factors that modify these sensations in the brain, and it is clear that the human perceptual process is extraordinarily difficult to mimic by any instrumental methods, however sophisticated.

The primary measuring instrument for assessing quality is, of course, the human subject, and the many methods now available for assessing both perceived sensory quality and consumer liking are used to some degree by even the smallest companies. Improved understanding of both physiological and psychological processes has led to the development of a wide range of validated test methods and analytical procedures, both statistical and non-statistical, for extracting meaningful information from the extensive data sets generated. Why, then, is there a continuing focus on developing instrumental quality measurement systems that many sensory and consumer scientists regard as invalid and misleading measures of perceived quality?

Whilst measurement of quality using sensory methods can justifiably be seen as having the highest level of validity, it must be remembered that sensory science is relatively youthful; for example, instrumental measurement of texture developed in the latter half of the 19th century, whereas formalised sensory methods only became available about 100 years later. Consequently it is not surprising that many instrumental methods developed some considerable time ago have become established and continue to be used, even if they were not originally validated against sensory quality

measures. Whilst it is tempting to dismiss such methods on grounds of non-validation, their continuing use suggests that they do have some practical value as quality measures.

Increasing consumer demands for consistent high quality, however, has generated corresponding demands for instrumental quality measures that can be shown to correlate strongly with sensory quality measures. This has been accompanied by the increasing cost and logistical difficulties experienced by many companies in setting up and maintaining trained sensory panels (this topic is expanded upon in Chapter 1). As a result, interest in the development of instrumental methods has continued to grow, and the content of this book is intended to reflect this continuing activity.

The book is structured into three main sections. Following an introductory chapter outlining the sensory test methods that instrumental methods are designed to complement, Part I comprises chapters detailing the underlying principles that are used in the measurement of the key factors that contribute to perceived quality: appearance, flavour, texture (of solid foods) and viscosity (of liquid foods). These chapters focus primarily on current practices used by the industry.

In Part II of the book, the six chapters describe more advanced instrumental methods that are being developed to complement the more established methods. The contributors address colour measurement, flavour measurement and non-destructive texture measurement, and also encompass in-mouth measurement and aspects of food authentication. In addition, advances in the handling of instrumental data relevant to sensory quality are described.

Part III comprises eight chapters that cover the practical application of instrumental measurements for individual food and beverage categories. The categories covered are meat, poultry and fish; baked goods; dry crisp products; dairy products; fruit and vegetables; wine; beer; and juices.

It is increasingly recognised that effective assessment of food quality requires the use of reliable sensory techniques coupled with instrumental measures that have been shown to relate to sensory measures. This book will give direction to quality control personnel who need to ensure that they are using the most appropriate and relevant instrumental test methods that relate to perceived sensory quality.

David Kilcast

1

Measurement of the sensory quality of food: an introduction

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Abstract: This chapter presents an overview of the nature of the sensory data that are generated by different types of quantitative sensory test procedures, the available types of instrumental measurements and principles, and analysis and statistical validation of instrumental data. The focus is primarily on methods that can be applied within quality control functions, but more advanced methods suitable for the investigation of instrumental–sensory relationships are also covered in outline.

Key words: human senses, sensory test procedures, instrumental–sensory correlations, statistical analysis, data validation.

1.1 Introduction: food quality and consumer choice

Consumers fortunate enough to live in prosperous societies have the choice of an enormous and ever-increasing range of foods, and manufacturers find themselves in an intensely competitive situation. In less well-developed societies, hunger will be the constant driving force, and our diet will be determined by availability of any food that satisfies our basic nutritional needs. It is increasingly clear that if we are to understand what drives consumer choice of food, no single factor can be considered in isolation from other factors. For some years, psychology researchers have been developing models to understand consumer behaviour (e.g. Shepherd & Sparks, 1994). Although there are many circumstances under which non-sensory factors such as price, advertising and nutritional image can have strong effects, delivering the sensory characteristics of foods is required by consumers central to continued purchase of foods.

The importance of a holistic approach is also becoming more clear when the components of sensory perception are considered. During the sequences of actions that constitute food consumption we perceive a whole

2 Instrumental assessment of food sensory quality

range of different characteristics relating to the appearance, flavour and texture of the food. Traditionally, it has been common industrial practice to consider these characteristics individually when analysing and designing food sensory quality, and this can be seen in the development of sensory methods that are specific to certain characteristics, for example the Flavour Profile Method and the Texture Profile Method (details of both methods can be found in Lawless & Heymann, 1998). Current sensory measurement systems, however, are increasingly focused on assessing all sensory factors that are likely to be important to perceived quality, and on understanding how these interact at both physiological and psychological levels.

Numerous sensory methods have consequently been developed to assess various aspects of sensory quality for both research and quality control (QC) purposes. The relevance of these sensory quality measurements (made by trained panels) to likely consumer response should ideally also have been established by carrying out appropriate correlation studies. The information secured by such research is vital in maximising product success, but can be out of reach of smaller companies operating on limited budgets, and there is a danger of falling prey to the temptation of extrapolating too far from a limited number of non-validated quality measurements. Similar considerations can also lead to the uncritical use of instrumental measurements that are assumed to be relevant to sensory quality.

The main uses of instrumental measurement are found in QC functions, for reasons given in the following section, and the remainder of this chapter will focus primarily on such uses. This is not to diminish the need for researchers to be aware of the value of instrumental–sensory quality relationships, particularly in the investigation of how factors such as product composition, product structure, processing and storage relate to sensory perception and consumer liking. However, the increasingly large numbers of such investigative techniques that can be used for this purpose are outside the scope of this chapter.

This chapter presents an overview of (i) the nature of the sensory data that are generated by different types of sensory test procedures, (ii) the types of instrumental measurements that are available and (iii) the analysis and statistical validation of instrumental data. The content of this overview is not intended to be exhaustive; more relevant accounts are presented in the subsequent chapters contributed by individual experts.

1.2 The role of instrumental measurement

The development of applied sensory techniques for evaluating the quality of consumer goods has been most extensive in the food and beverages industry, reflecting the intimate contact that users have with the finished product. In contrast, until relatively recently other manufacturers of

consumer goods have relied almost exclusively on using various types of appropriate instrumental measurement methods to ensure that any important perceived sensory characteristics of the product are as intended. The extent of the use of instrumental methods in different industries therefore reflects the difficulties inherent in the availability of validated sensory techniques. Given the wide range of sensory techniques available to the food and beverage sector then, why is there such extensive usage of instrumental measurement methods in quality control functions, and why is there a growing demand for the development of new methods?

Many possible answers to these questions can be proposed, for example:

- Logistical difficulties (both time and cost) in setting up, operating and maintaining sensory panels, especially in small companies.
- Staff downsizing policies, giving rise to difficulties in securing adequate panellist numbers.
- The realisation that in order to maintain even a basic sensory QC system, resources in terms of facilities and panellist training require investment.
- Instability of results from sensory panels over long time periods.
- Possibility of contamination (accidental or malicious) of product by toxic chemicals, especially when investigating consumer complaint returns.
- The manufacturing business produces large numbers of small batches of different products, and key customers demand 100% batch testing.
- An unfounded expectation that there will be a simple and invariant 1:1 correlation between an instrumental parameter with a key sensory characteristic.
- Lack of appreciation of the power and relevance of formal testing procedures, and a failure to recognise that uncontrolled informal sampling procedures are not an adequate substitute.
- A naive faith in data that is generated by modern electronic instrumentation.

Lawless & Heymann (1998) have also pointed out that instrumental measurements should be used for evaluations that are repetitive, fatiguing and dangerous, and when decisions made with the data are not business critical – again, providing that a correlation can be established.

Irrespective of these concerns, consumer enjoyment of foods and beverages will be determined principally by a wide range of responses from the senses, and no instrument (or set of instruments) will be able to mimic these in the foreseeable future. However, the concerns listed above are not trivial, and although all companies must take all possible steps to employ sensory methods in QC, instrumental methods will continue to provide valuable quality input, provided that steps are taken to establish that the measurements relate to relevant sensory characteristics.

1.3 Sensory assessment of quality

1.3.1 The human senses

It is generally accepted that humans have five senses in operation, namely sight, smell, taste, touch and hearing, although warmth, cold, movement and pain may also be considered as senses of importance in a food context. Foods are complex mixtures of chemical compounds, arranged into structural units. The perception of the sensory characteristics of foods results from the stimulation of all our senses to some extent by the physicochemical properties of the foods. The sensory characteristics of food are generally grouped into three modalities – appearance, flavour and texture. These modalities are, however, not independent of one another. For example, colour, which is obviously an important appearance characteristic, can be shown to have an influence on flavour perception; consumers will assign higher scores for flavour intensity to darker foods than to lighter foods. The interaction between appearance and flavour is referred to as ‘visual flavour’. Similarly, textural characteristics such as viscosity can influence the perception of flavour, and some flavour characteristics, e.g. acidity, can affect textural characteristics. One means of defining flavour, texture and appearance is by taking into account the fact that each can be attributed to the stimulation of one or more senses. On this basis the International Standards Organisation (ISO, 1992) has proposed working definitions for flavour, texture and appearance, as given below.

Appearance: sensory characteristics of foods perceived largely by way of the visual sense. Input from other senses, especially smell, may contribute.

Flavour: the combination of taste and odour. Pain, heat, cold, tactile and visual sensations may also contribute.

Texture: sensory characteristics perceived largely by way of the senses of movement and touch. Input from other senses, especially vision and taste, may sometimes contribute.

1.3.2 Sensory test procedures

The main sensory test procedures available to sensory analysts are shown schematically in Fig. 1.1. There is a fundamental distinction between the analytical methods, which use trained panels as an instrument to measure sensory properties, and the hedonic methods, which measure consumer responses to the sensory characteristics. The choice of sensory test depends critically on the nature of the information required, and on how that information will subsequently be used. Analytical sensory testing can be carried out in both quality control and in research and development functions. QC testing has specific requirements that need to be chosen and adapted to the restrictions imposed within most manufacturing environments (Muñoz *et al.*, 1992; Costello, 2002; Rogers, 2010). Consequently, heavy use is made of discrimination tests and simple quantitative tests that do not in general

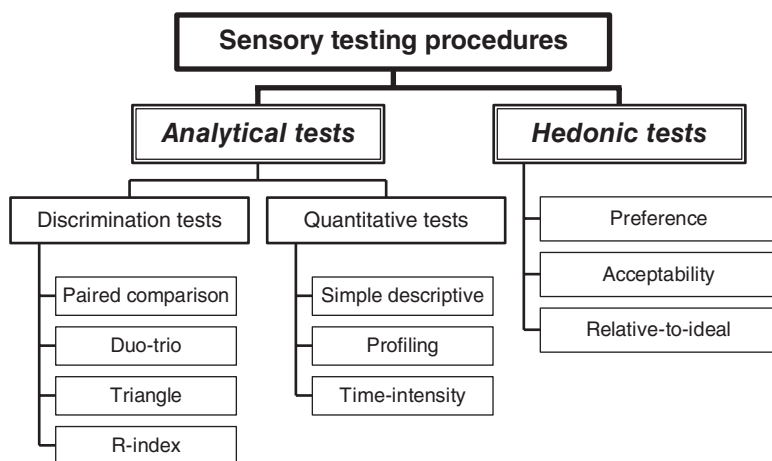


Fig. 1.1 A classification of sensory testing procedures.

provide the quantitative sensory data needed to validate instrumental measurements, and validation programmes commonly need quantitative sensory data that necessitate the use of fully trained R&D sensory panels. Consumer acceptability data have not been used extensively in instrumental data correlations, although developments might yet be seen in this respect. The range of sensory techniques that are of potential value has been summarised by Hugi & Voirol (2001), and the remainder of this section will focus on some of the main quantitative test procedures.

Quantification of sensory data through the recording of perceived intensity of attributes or liking requires some form of scaling procedure. These procedures should be distinguished from quality grading systems, which are used to sort products into classes defined by a combination of sensory characteristics, and which are not open to quantitative numerical analysis. Scaling procedures are mainly used to generate numeric data that can be manipulated and analysed statistically. The most commonly used types of scale are outlined below.

- *Category scales* use a defined number of boxes or categories (often 5, 7 or 9, although other numbers are often used). The scale ends are defined by verbal anchors, and intermediate scale points are often given verbal descriptions.
- *Graphic scales* (line scales) consist of a horizontal or vertical line with a minimum number of verbal anchors, usually at the ends. Other anchors can be used, for example to define a central point, or to denote the position of a reference sample.
- *Unipolar scales* have a zero at one end, and are most commonly used in quantitative profiling, especially for flavour attributes.

6 Instrumental assessment of food sensory quality

- *Bipolar scales* have opposite attributes at either end. Definition of the central point can often give rise to logical difficulties, as can ensuring that the extreme anchors are true opposites.
- *Ratio scales*, in which the intensity of a sensory characteristic is scored as ratio against a reference. Some advantages are claimed against graphic scales, and these are often used to scale extreme characteristics (e.g. chilli burn), but the resulting ratios need to go through a geometric transformation for statistical analysis.
- *Hedonic scales* are used to measure consumer liking or acceptability. Category scales are usually used.
- *Relative to ideal scales* are a type of hedonic scale which measures deviation from a personal ideal point.

The type of scale used, and its construction, depends on a number of factors:

- *Purpose of test.* Both category and graphic scales are commonly used with trained panels. In consumer testing, category scaling methods are usually used.
- *Expertise of panellists.* Trained panels can start with 5- or 7-point category scales, but, as their discrimination ability increases, they can use effectively more scale points or graphic scales. When using inexperienced panellists, scales incorporating a 'neutral point', such as the central point in an odd-numbered category scale, are sometimes avoided in order to minimise the risk of 'fence-sitting'.
- *Number of panellists.* Using small panellist numbers with a low number of category scale points will limit statistical analysis options.
- *Data-handling facilities.* Category scaling responses can be entered relatively quickly onto a spreadsheet, whereas data from line scales must be measured, and this can be a time-consuming procedure. Computerised data acquisition, either directly from a terminal or tablet computer, or indirectly from optical readers, is now commonly used to streamline data handling.

Sensory analysts need to be aware of difficulties that panellists encounter in using scales, and careful training is needed to ensure that scales are unambiguous and can measure the intended response.

Scaling may often be needed in order to quantify a single, well-defined attribute, especially when validating a single instrumentally measured parameter. However, it should be established that there is no ambiguity in the attribute of interest. This is particularly relevant during product development or modification, when the assumption that a process or ingredient modification will change only a single attribute is frequently violated. Such changes are especially common when textural changes are a consequence of process or ingredient modifications. If it is suspected that several attributes might be of interest, then the quantitative profiling procedures described

in the subsequent sections should be considered. In practice, strict application of the methods described below is too resource-hungry and time-consuming for QC operations in all but the largest companies, and the methods undergo considerable simplification in order to comply with local circumstances.

Quantitative descriptive analysis (QDA™)

Variants of the original quantitative descriptive analysis (QDA™) procedures are probably used more than any other quantitative profiling procedure. The QDA™ technique uses typically 6 to 12 panellists, screened for sensory acuity and trained to perform the descriptive task. Three major steps are required: development of a standardised vocabulary, quantification of selected sensory characteristics and statistical analysis of the results.

Development of the vocabulary is a group process for creating a complete list of descriptors for the products under study. Panellists freely describe the flavour, appearance, odour, mouthfeel, texture and aftertaste characteristics of different samples. No hedonic (good or balanced), general (full or typical) or intensity-based (strong or weak) terms are permitted. Terminology should be consistent from product to product and tied to reference materials. The references decrease panellist variability, reduce the amount of time necessary to train sensory panellists, and allow calibration of the panel in the use of intensity scales.

The attributes are collected and compiled into a master list. This individual preliminary evaluation of the samples may be revised during an open discussion to eliminate any redundant or synonymous descriptors. New terms might be added and physical references proposed. The panel leader condenses and formats the information into a proposal for standardised vocabulary. This vocabulary is then modified and improved in several interactive sessions. Multivariate statistical methods (e.g. factor analysis) are sometimes used to reduce the number of descriptors. Finally, definitions for the attributes are agreed.

Once agreement is reached on the vocabulary, further training is performed. The number of training sessions depends on the subject's performance, product and attribute difficulties and the time allowed for QDA™ testing. Panel training increases panellist sensitivity and memory and helps panellists to make valid, reliable judgements independent of personal preferences. Following the establishment of satisfactory panel performance, and removal of ambiguities and misunderstandings, the test samples can be evaluated. This is usually carried out in replicated (commonly three) sessions, using experimental designs that minimise biases.

The Spectrum™ method

The method resembles QDA™ in some respects; for example the panel must be trained to fully define all product sensory attributes, to rate the intensity of each and to include other relevant characterising aspects such

as change over time, difference in the order of appearance of attributes, and integrated total aroma and/or flavour impact. However, the perceived intensities are recorded in relation to absolute or universal scales, which allow the comparison of relative intensities among attributes within a product and among products tested.

Panellists develop their lists of descriptors by first evaluating a broad array of products that define the product category. The process includes using references to determine the best choice of term and to best define that term so that it is understood in the same way by all panellists. Words such as vanilla, chocolate or orange must describe authentic vanilla, chocolate and orange characters for which clear references are supplied. All terms from all panellists are then compiled into a list that is comprehensive yet not overlapping.

The Spectrum™ method is based on an extensive use of reference points. The choice of scaling technique may depend on the available facilities for computer manipulation of data and on the need for sophisticated data analysis. Whatever the scale chosen, it must have at least two, or preferably three or five reference points distributed across the range.

Free choice profiling (FCP)

Free choice profiling (FCP) is a very different concept, which removes the need to generate a compromise consensus vocabulary (Williams & Langron, 1983), and that can also be used in consumer research (Jack & Piggott, 1992). Assessors are allowed to develop their own individual vocabularies to describe sensory perceptions of sample sets and to assign intensity scores to these attributes. As a consequence of removing the need to agree vocabularies, FCP requires little training – only instruction in the use of the chosen scale. Assessors merely have to be objective, capable of using line scales, and able to use their developed vocabulary consistently. Thus, assessors can be still regarded as representing naive consumers. Characteristics being judged can be restricted by the panel leader, but the number of descriptors produced is limited only by the perceptual and descriptive skills of the assessors. A range of sensory characteristics such as appearance, flavour, aroma or texture can be examined. One particular advantage of the technique for shelf-life assessment is that new attributes that develop on storage can readily be incorporated into the profile. Disadvantages include the need to use a complex statistical analysis technique (generalised Procrustes analysis), and the absence of any agreed terminology.

Time-related methods

Time–intensity methods are used to measure intensity of a specific attribute as a function of time in the mouth, and have been used extensively to investigate the temporal behaviour of tastants, such as sweet and bitter molecules, and the release of volatile flavour materials from foods. Such studies are particularly important in the reformulation of foods that results

in structural modifications, and in changes that can occur on storage. These structural modifications are often accompanied by textural changes, and these often result in complex perceptual phenomena that are direct consequences of the changes in texture with time producing different flavour release phenomena. Although the use of time–intensity for flavour measurement is relatively well established, textural changes can also be monitored using the method.

A major limitation of the time–intensity method is that only a single attribute can be tracked with time, or, with some software packages, two attributes. If several important attributes are thought to be time-dependent, separate sessions are needed for each attribute. Difficulties encountered in time–intensity profiling prompted the development of a hybrid technique, progressive profiling (Jack *et al.*, 1994). In this technique, assessors carried out a profile on a set of texture descriptors at each chew stroke over the mastication period. Such a method has a number of potential advantages: several attributes can be assessed in one session; scaling is reduced to a unidimensional process; and the most important aspects of the shape of a time–intensity curve are retained.

Temporal dominance of sensations (TDS) is a more recent time-related descriptive sensory method in which sensations are assessed repeatedly until they end (e.g. Labbe *et al.*, 2009). At any given time point, the attribute that is being selected by the largest number of assessors is called dominant and its intensity is scored.

1.4 Instrumental measurement of quality factors

1.4.1 General principles

It is possible to identify an enormous range of measurement variables that can be used to quantify aspects of product quality and product safety, in addition to those used to quantify factors such as production efficiency and compliance (Kress-Rogers, 2001a). Uncritical choice of the types of instrumental measurements to be used will generate substantial difficulties in data handling and interpretation. Further, as a consequence of the development of sophisticated software used for instrument control and data analysis, a given instrument will frequently generate a large number of numeric parameters. If confusion is to be avoided, and erroneous conclusions are to be minimised, careful consideration must be given both to the choice of instrumental measurement and to the selection of measured parameters.

Steps that should be taken in the use of instrumental measurements are outlined below:

1. Identify the sensory quality factors that the instrumental measurements are intended to mimic.
2. Select appropriate sensory methods for quantifying the sensory quality factors, and any necessary data manipulation and formatting.

3. Select appropriate instrumental test procedures and measured parameters, and any necessary data manipulation and formatting.
4. Correlate the instrumental measurements against sensory measurements using appropriate methods.
5. Validate any sensory–instrumental relationships developed for their value as a predictive tool.

One important distinction between instrumental and sensory assessments is that the instrumental measurements comprise measurement of discrete, well-defined physicochemical properties, whereas sensory perception is rarely discrete, and different stimuli (within or across different sensory modalities) interact at both physiological and psychological levels. As a consequence, whilst an instrumental measurement of flavour components can usually be assumed to be free from the influence of other product characteristics, it has long been established that the sensory perception of flavour can be strongly influenced by product colour (for example see Zampini *et al.*, 2007). One notable and important exception, however, lies in the measurement of flavour, in which case the physicochemical structure of the products can influence the release of flavour components, with profound effects on both perceived flavour and on the measured flavour components (for example, Taylor, 2002; Taylor & Roberts, 2004). The following sections give brief summaries of the main types of measurements that are relevant to the key sensory modalities; more detailed treatments are given in other chapters in this volume.

1.4.2 Appearance measurement

For many food products, the visual senses are the first to be used by purchasers, consumers and trained sensory assessors. If a negative impression is communicated at point of sale, then purchase might not go ahead. More subtle influences can also affect the perception of non-visual sensory attributes through interactive mechanisms. Even if the product is packaged at point of sale and not directly visible, then visual information associated with the packaging system, including product images, product descriptions and ingredient lists will generate an expectation of product quality.

Colour is usually regarded as the most important visual product characteristic (Francis & Clydesdale, 1975), and many instrumental systems have been developed for colour measurement, varying in sophistication from colour reference atlases to highly sensitive electronic instruments (detailed information can be found in, for example, MacDougall, 2001, 2002). The most common ones in practical use that are capable of generating quantitative data are based on the Hunter Lab system, in which colour is measured in terms of three parameters: *L* (lightness), *a* (red/green) and *b* (blue/yellow).

Many foods carry important visual cues other than colour. For example, the glossiness of chocolate, turbidity of beverages and visual composition

of prepared meals can all influence consumer liking, and many systems have been developed for measuring this wider range of visual characteristics (Kress-Rogers & Brimelow, 2001). A more holistic view has also been taken in appearance assessment, through the concept of *total appearance* (Hutchings, 1999). In addition to direct visual information, the importance of indirect visual information about the product should also be considered, commonly available in printed form on packaging as photographic images, product labelling and ingredient information.

1.4.3 Texture measurement

Texture perception is complex, with two major components: a tactile, surface response from skin (somesthesia) and a deep response from muscles and tendons (kinesthesia or proprioception) (Kilcast, 2004). In addition to perception in the mouth, manipulation of products by fingers and hands can generate textural responses, together with visual information (visual texture) and information arising from sounds released when handling and chewing products. As a consequence, many types of instrumental measurements have been devised to cover food categories (for more detailed information on texture measurement methods, see Bourne, 2002; McKenna, 2003; Kilcast, 2004; Rosenthal, 1999).

The majority of these methods measure a wide range of mechanical characteristics of food which, although related to texture, do not give a complete picture of textural characteristics. As an example, the frictional properties of foods that are related to the perception of attributes such as roughness and creaminess have received relatively little attention, and measurement methods have not been developed to the extent of those used for other textural characteristics, but in recent years the importance of understanding such processes has been stressed (de Wijk & Prinz, 2006; Engelen & van der Bilt, 2008). Although the incomplete nature of instrumental texture measurement is widely recognised, the use of texture measurement in QC protocols is extensive, and this in part results from the greater degree of difficulty often encountered in using sensory panels for texture assessment in comparison with other sensory modalities.

The types of texture measurement employed have been categorised as empirical, imitative and fundamental. Empirical methods measure often ill-defined variables that are indicated through practical experience to be related to some aspect of textural quality, and are frequently dedicated to a specific product type. Imitative methods mimic conditions that the product is subjected to during eating. Fundamental methods measure well-defined physical properties of the product which can be independent of the measurement method. Both imitative and fundamental methods can usually be applied to a wide range of food types, and instrument manufacturers supply a wide range of test cells for this purpose. In general, empirical methods generate a single measurement parameter, whereas imitative and

fundamental methods can generate a wide range of measurement parameters, some of which might be correlated. Selection of appropriate parameters is important if valid correlations with sensory data are to be achieved.

1.4.4 Flavour measurement

Flavour is conventionally regarded as a combination of sensations derived from several distinct types of chemical stimuli. Tastes, detected by receptors on the tongue and other oral surfaces, are involatile chemical stimuli that are carried in solution by saliva from the food to the receptors. It is now widely accepted that there are five basic tastes – sweet, salt, bitter, acid, savoury (umami) – although this list is sometimes extended to include other sensations. Odours (aromas) are volatile chemical stimuli detected by receptors located in the olfactory epithelium in the nasal cavity. These are transmitted to the receptors directly through sniffing (orthonasal route), or from the mouth during eating (retronasal route). The odour response is complex, with around 2500 odorous chemicals found in food (Taylor & Roberts, 2004). A third component of flavour, a chemical sense that stimulates the trigeminal nerves, is responsible for sensations such as burning and cooling. Trigeminal sensations can arise from both chemicals in dissolved saliva, for example the tingling sensation from carbonic acid in fizzy drinks, and from volatile chemicals, for example pungent thiocyanates in mustard and horseradish.

Measurement of flavour components is consequently strongly influenced by the widely differing volatilities of flavour-active chemicals. The relatively large number of volatile chemicals contributing to flavour has been reflected in the wide range of instrumental methods that are now commonly used for volatile analysis, in particular gas chromatography/mass spectrometry systems (for example, see Kress-Rogers, 2001a). More recently, considerable publicity has been given to the development of so-called ‘electronic noses’, which are more correctly volatile sensors operating on a pattern recognition basis. Although these are finding numerous uses in other fields, relatively few routine uses have been recorded within the food industry (Röck *et al.*, 2008). Measurement of taste chemicals has relied predominantly on traditional methods of chemical analysis for salty and acidic stimuli, with high-pressure liquid chromatography being used for less volatile chemicals such as sugars. However, ‘electronic tongues’ have now appeared on the market, and whilst considerable research is being carried out, very few practical applications have been reported.

1.4.5 Other measurements

In addition to physicochemical measurements that can be related directly to the sensory quality of products, other measured data can be used to help build a model of likely sensory quality. This can take the form of data such

as solution concentrations of components, pH, process temperature and emulsion droplet size. Depending on the type of data incorporated, care needs to be taken in the use of correlation methods.

1.4.6 Selection of instrumental measurement methods

When developing instrumental–sensory relationships, careful consideration must be taken in both R&D and QC environments in selecting the instrumental methods to be used. Researchers often fall prey to the temptation to list all the instruments that *might* generate data relevant to sensory perception, leading to consequential problems in data analysis and interpretation. On the other hand, QC methods are often those that are inexpensive, rapid and convenient, but which are not necessarily the most appropriate. In either situation, an additional danger is that methods will be selected on the basis of outdated information (or, worse, hearsay) regarding their relevance to perceived sensory characteristics, and correlations assumed rather than being checked and validated.

In analytical investigations of aroma, Reineccius (2006) has stressed the importance of giving careful consideration to the sample, volatiles of interest, analysis time and study objectives in selecting analytical procedures, and has pointed out that analytical objectives such as those listed below will strongly influence the choice of procedures:

- Obtain a complete aroma isolate to accurately identify and quantify all aroma constituents.
- Identify key components responsible for the characteristic aroma.
- Identify any off-notes.
- Monitor aroma changes with time.
- Predict sensory attributes.
- Determine if a food flavouring is adulterated.

The validity of all instrumental–sensory data correlations found in published or internal company literature should always be questioned, especially if product design factors such as ingredient composition, physical structure, processing conditions, storage conditions and packaging have changed substantially since the reported investigations.

1.5 Analysis and validation of instrumental measurements

Section 1.2 listed some of the driving forces underlying the use of instrumental measurement of food quality. In a QC environment, the most pressing requirement is to find instrumental methods that are rapid and inexpensive and which can reduce the dependence of the company on sensory panels (or even replace the use of sensory panels, although fortunately regulations in most developed countries recognise the importance

of sensory quality assessments). One consequence is that all too often instrumental data are used uncritically, and several steps are needed to ensure that any instrumental measurement(s) used are valid. The following sections give a brief account of some of the available methods. More detail on appropriate statistical methods can be found in standard statistical texts.

1.5.1 Data inspection

Most authorities on statistical data analysis stress the need to carry out appropriate visual inspection of numeric data before any statistical or correlation analysis is carried out. This applies not only to instrumental data, but also to sensory data used for correlation studies. The primary purpose of this stage is to check for any anomalies in the data that would compromise the quality of any data associations achieved. This could take the form, for example, of an instrument recalibration during an experiment, an uncorrected temperature change, or a simple transcription error.

Inspection of small data sets in tabular form is feasible, and for many instruments, such as pH meters and empirical texture measuring instruments generating just a single-point measurement, this will give a good indication of data anomalies. Increasingly, however, multi-purpose instruments are used that carry out a continuous recording during a test, for example deformation–force measurements in texture assessment. Instrumental software will then often calculate a summary parameter that experimentation has shown to relate to sensory characteristics. Although convenient to the user, care should always be taken to inspect the form of the data recording to ensure that valid parameters are being measured.

As an example, testing of gels and solid foods for firmness usually involves penetrating the product with a probe of defined geometry, and recording the force continuously during penetration. By convention, firmness is usually measured as the force recorded at a set penetration distance. Relatively minor changes to the gel structure and the probe geometry can result in distinctly different force–deformation curve shapes, as shown in Fig. 1.2. In the case of a simple brittle gel (e.g. gelatin) penetrated by a cylindrical probe, a break in the gel structure occurs at a short penetration distance, giving a discontinuity to the smooth curve. This has two consequences in practice. First, this initial break occurs at the 4 mm penetration distance conventionally used for gel firmness testing, and unwanted variability on this measurement. Secondly, this initial break results in chaotic breakdown patterns at higher penetration distances, and as a consequence high variability in any parameters measured at these penetration distances (Kilcast *et al.*, 1984; Kilcast, 2001). This initial break does not occur when using a hemispherically ended probe, and firmness measurements associated with the simpler breakdown pattern show lower variability.

A further example of the importance of visual observation of product behaviour during instrumental testing can be seen in research on devising

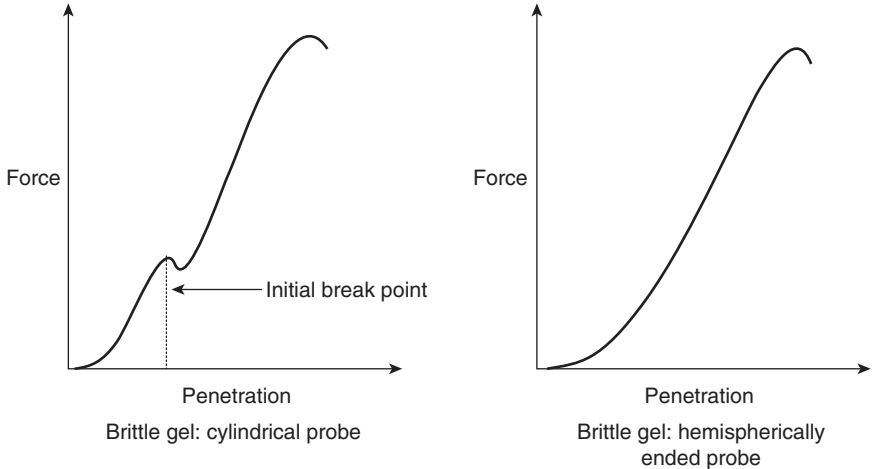


Fig. 1.2 Effect of penetration test probe geometry on gel breakage patterns.

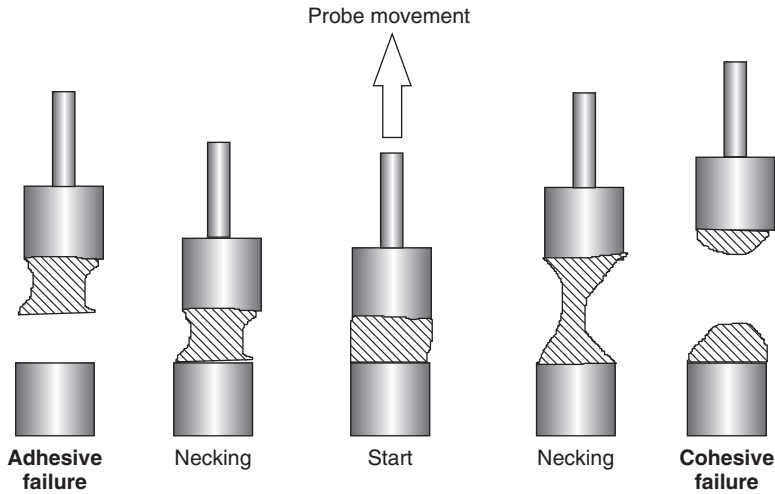


Fig. 1.3 Adhesive failure and cohesive failure in stickiness measurement.

instrumental measurements to measure stickiness in foods (Kilcast & Roberts, 1998; Kilcast, 2001). The perception of oral stickiness during sensory testing relates to the force needed to remove product from the teeth (ISO 5492, 1992). Instrumental testing of stickiness commonly uses a procedure in which the product is placed between two plates, is compressed, and then the force recorded as the plates are separated (Fig. 1.3). Perceived stickiness can then be related to the force when the product separates from the plate. In some situations, however, the product remains stuck to the

plates and undergoes an internal failure, termed cohesive failure. Whilst this is an important characteristic in some contexts, for example in the sticking of unwanted material to equipment surfaces, it is less likely to relate to perceived stickiness. As the behaviour of products during such tests can be influenced by a range of factors, such as product rheology, test conditions and the surface energy of the materials used for testing, observation can help to minimise the risk of misinterpretation.

1.5.2 Correlation analysis

The most common objective in the use of instrumental relationships is to set up an empirical statistical model that relates the intensity of a sensory characteristic to a measured instrumental parameter, or to a set of instrumental parameters. Another relationship that is sometimes considered is to use instrumental data to directly model consumer liking. This requires, however, reliable consumer liking data that are relevant to the intended market, and an understanding of consumer segmentation patterns. A step-wise approach is therefore usually taken, first to relate instrumental data to key sensory attributes, and then to relate the sensory attributes to consumer liking. (Examples of different approaches to modelling consumer liking can be found in MacFie, 2007.)

An important prerequisite to carrying out any statistical analysis of instrumental data is to carry out a visual inspection of the data using scatter plots, usually by plotting one measure on the x -axis against a second measure on the y -axis. The visual form of the resulting plot will often give useful information on the data relationship, and guidance on further data analysis. In addition, the plots will often highlight problems with the data set. Examples of the form of plots that might be seen are shown in Fig. 1.4. Figure 1.4a shows a plot in which it is difficult to discern any structure in the data set, and a significant correlation is unlikely to be seen in such a plot. In Fig. 1.4b, there is sufficient indication of a possible linear relationship that would warrant further investigation. Evidence for a relationship can also be seen in Fig. 1.4c, but in this case the curvilinear form of this plot points to using non-linear modelling. The form of the plot in Fig. 1.4d is found occasionally, and indicates a possible change in the product structure (especially in texture testing) or some environmental factor such as temperature during the test. In this case (sometimes called a broken-stick model) two different linear relationships are evident, with the intersection occurring at the presumed change.

If the scatter plots reveal possible linear relationships, then the next step is usually to calculate Pearson product moment correlation coefficients (r). A perfect positive correlation gives $r = +1$, a perfect negative correlation gives $r = -1$, and no correlation gives $r = 0$. However, it should be noted that these coefficients are relevant to only linear correlations, and strong data relationships can exist in which the correlation coefficient is very low. This is illustrated in Fig. 1.5. The scatter plot shown in Fig. 1.5a will give a

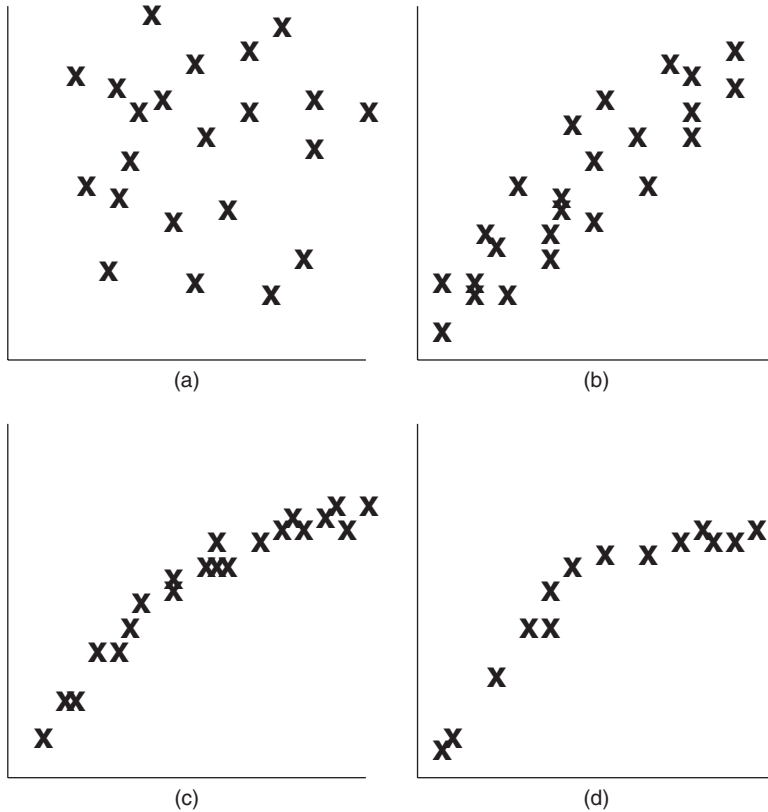


Fig. 1.4 Different forms of scatter plots: (a) no relationship; (b) linear relationship; (c) curvilinear relationship; (d) broken stick model.

correlation coefficient that is close to zero. The plots shown in Fig. 1.5b indicate near-perfect positive and negative correlations, and will give correlation coefficients close to +1 and -1, respectively. The scatter plot shown in Fig. 1.5c shows a strong non-linear correlation, but which will give a very low correlation coefficient. (This inverted-U relationship is commonly encountered in relationships between consumer liking and sensory attributes.) Figure 1.5d shows a situation in which there is a strong linear correlation, but an outlying point reduces the correlation coefficient. This situation often occurs through data transposition errors, and can also indicate a step change in a measurement.

The square of the correlation coefficient (r^2 , or coefficient of determination) gives a measure of the data variance accounted for by the linear correlation. For example, a correlation coefficient of 0.7 indicates that 49% of the data variance is accounted for in the correlation. Statistical software packages commonly available will often associate a significance value to the

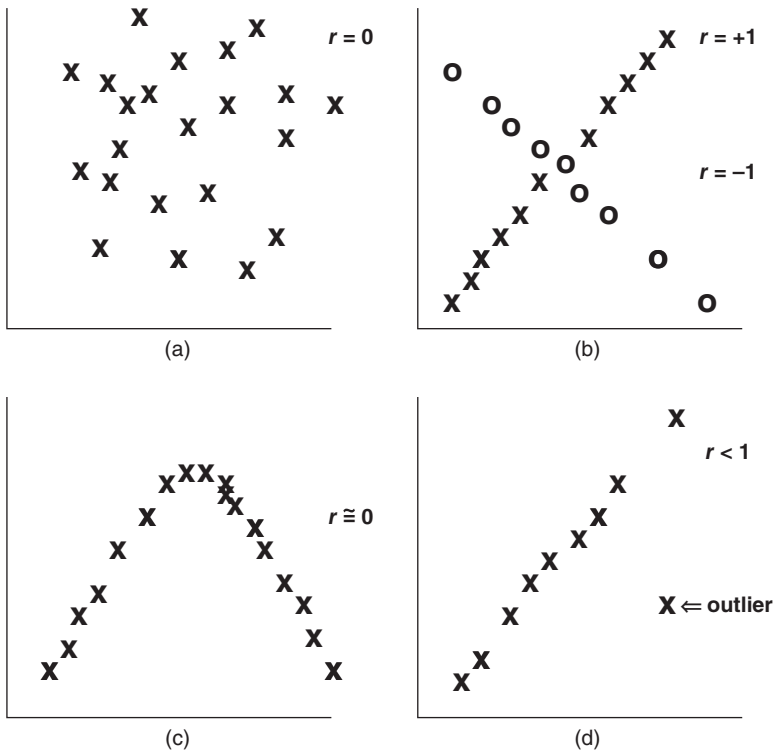


Fig. 1.5 Pearson product moment correlations: (a) no correlation; (b) perfect positive and negative correlations; (c) strong non-linear relationship, low correlation coefficient; (d) strong linear correlation with outlier, low correlation coefficient.

correlation coefficient. Lawless & Heymann (1998) have described the use of the so-called Anscombe data sets in demonstrating the dangers of using correlation coefficients without first examining the form of the data.

1.5.3 Regression analysis

Linear regression is used to fit a linear mathematical function between two variables, and which takes the general form:

$$y = a + bx$$

As with Pearson product moment coefficients, the regressions will be invalid for highly correlated but non-linear data sets, such as that shown in Fig. 1.5c. Statistical software packages can conveniently be used to generate such a regression equation, together with an associated coefficient of determination. Many packages can also be used to generate confidence bands, for example a 95% confidence band means that there is a 95% probability that

the 'real' trend is in that band. Confidence bands should not be used for predictive purposes, however – prediction bands are wider than confidence bands, but are not generated by some software packages. A further limitation of such an equation is that the relationship might only be valid for a limited range of measurements. For example, there is a linear relationship between sweetness intensity and solution concentration for a wide range of bulk sweeteners, but this relationship can become curvilinear at both low and high sweetener concentrations (Portmann & Kilcast, 1996).

Multiple linear regression (MLR) methods are used to find a linear relationship between a variable and a set of variables, for example between a sensory attribute (y) and a set of instrumental variables ($x_1, x_2, x_3, \dots$). The relationship developed takes the form:

$$y = a + b_1x_1 + b_2x_2 + b_3x_3 + \dots$$

Most statistical analysis packages have available a range of MLR methods. For example, all variables can be included in the relationship, or variables can be added (and removed) and the effect on the calculated R^2 observed to find the best correlation. However, problems can arise if a large number of instrumental variables are included. Firstly, as the number of instrumental variables rises, this requires a larger number of sensory observations. Secondly, if there are inter-correlations between the instrumental variables (which is likely if several parameters are measured from a graphical plot) then the regression relationship can become misleading. Thirdly, if inter-correlations exist, then the order in which they are introduced into the equation can influence the relationship (Lawless & Heymann, 1998). Consequently, the temptation to include every measurable instrumental parameter into a predictive relationship must be resisted, and careful consideration given to the choice of parameters.

Many statistical software packages are also able to carry out non-linear multiple regressions, for example using square terms or log terms. Such regressions should only be used if there is a good logical reason for including non-linear terms, for example in investigating a sensory characteristic such as creaminess, in which fat droplet size is thought to be important, and in which case the cube of the mean droplet size (i.e. fat particle size volume) might be included. Again, the temptation to develop complex non-linear relationships that are easily set up with readily available software packages must be resisted, and the simpler relationships investigated first – the visual form of a valid relationship of practical value will very frequently have a clear logical basis.

1.5.4 Multivariate methods

Multivariate statistical methods are used almost routinely by sensory analyst, reflecting the complex multidimensional character of sensory data, and the need for tools to help rationalise and understand sensory

phenomena. Instrumental data can be equally complex, and unsurprisingly many multivariate approaches have been developed to examine the relationship between these data sets. Reineccius (2006) identifies two approaches in the use of multivariate methods. Firstly, the unsupervised approach is taken, in which all the data are entered into a statistical program that searches for relationships, trends or groupings of samples. Secondly supervised methods, in which there is an *a priori* knowledge of the sample groupings. The main unsupervised methods used are principal component analysis (PCA), factor analysis (FA), cluster analysis and multidimensional scaling (MDS). Supervised methods include MRA (discussed above), principal component regression (PCR), canonical correlation analysis (CCA) and partial least squares regression (PLS). The use of some of these methods in the analysis of flavour data has been described by Qannari & Schlich (2006).

PCA is a data reduction technique that replaces a large number of original variables by a smaller number of linear combinations, whilst still explaining a substantial proportion of the original variation in the data. Essentially, PCA projects an n -dimensional space onto a 2-dimensional plot. PCA analyses the correlation structure in the data set and identifies the axis along which the maximum variation occurs. A second principal axis is then identified orthogonal to the first axis, corresponding to the second greatest amount of variation, and so on. The new axes are linear combinations of the original axes, and the coefficients, or loadings, measure the importance of the original variables on each principal component. A useful reduction will often retain 70–80% of the variation in the first three dimensions. PCA is now a routine statistical procedure for analysing sensory profile data, and increasingly used to examine for structure in combined sets of sensory and instrumental data. When analysing such data sets, it is important to carry out the analysis using the correlation matrix to compensate for the different measurement scales used. An example of the use of PCA on combined data sets is shown in Fig. 1.6, redrawn from Kilcast & Clegg (2002).

PCR takes the first few principal components from a PCA carried out on the instrumental data, and then using these in a multiple linear regression against the sensory variables. One pitfall in the use of this method is that those principal components not used in the regression might contain information relevant to the sensory data. This defect is addressed in the use of CCA and PLS. Both these methods analyse the structure of each data set, and then measure the association between these structures – CCA uses the correlation coefficient as a measure, and PLS uses the covariance. PLS is currently one of the most popular methods used to relate two data sets (Qannari & Schlich, 2006).

1.6 Conclusion and future trends

The traditional approach described above to devise instrumental measurement systems that can be used as predictors of sensory quality has been

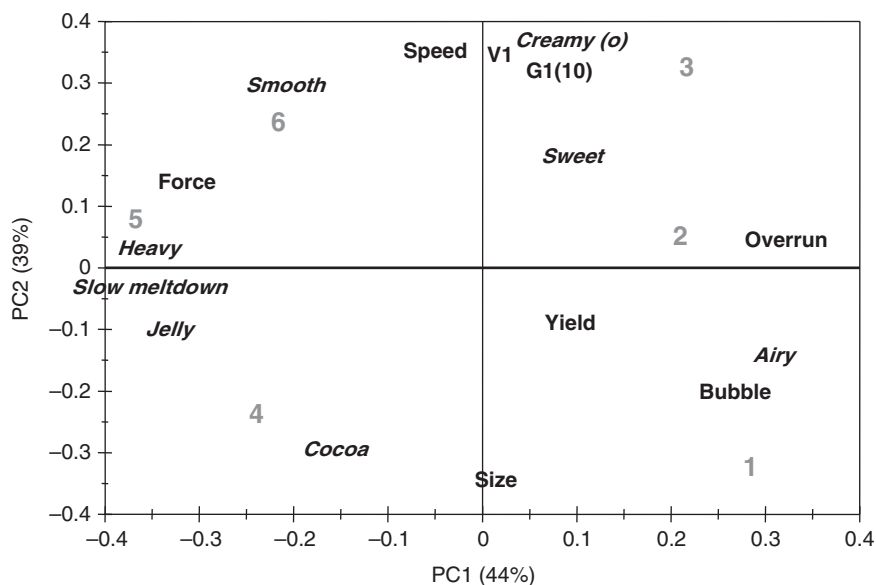


Fig. 1.6 Principal component plot from a data set comprising sensory profile data and measured instrumental parameters. The numbers correspond to the positioning of different products, and the measured parameters associated with the products are superimposed, with the sensory attributes shown in italics.

extended in recent years to developing instrumental measurements that give a more direct measure of sensory response. In addition, different modelling procedures have been investigated.

1.6.1 *In vivo* measurements

One of the most important milestones in texture measurement was the development of the General Foods Texturometer (Friedman *et al.*, 1963), which was designed to mimic as far as possible human chewing actions. Although it has subsequently been replaced by more general purpose instruments, the principle behind mimicking oral action was taken further to measuring electrical activity in chewing muscles during mastication using electromyography (EMG). The technique has been used to measure changes in texture during mastication (e.g. Brown *et al.*, 1998; Kilcast, 2001) and to investigate differences in human chewing behaviour and texture perception (e.g. Brown *et al.*, 1994). Other oral texture measurement methods have been reviewed by Smith (2004). The principle of *in vivo* measurement has also been applied to the measurement of flavour. Systems have been developed for extracting and analysing volatiles released from the food in the mouth (e.g. Taylor & Hort, 2004; Cook *et al.*, 2005; Linforth & Taylor, 2006), and these have been extended to the *in vivo* measurements of tastes (Taylor

& Hort, 2004). Whilst *in vivo* measurements have proved of great value for understanding the physical processes underlying sensory perception of food, they are generally complex and have yet to see extensive use in a QC environment.

Related researches utilising various types of measurement of brain activity during eating (e.g. Rolls, 2005) are generating important information on the understanding of sensory perception mechanisms, but are again unlikely to see practical use for QC purposes.

1.6.2 Non-destructive testing

Many instrumental test methods, and particularly those used for texture measurement, are inherently destructive, and for many purposes, for example in assessing the ripeness of fruits, there is considerable interest in developing non-destructive test methods (Kress-Rogers & Brimelow, 2001; Irudayaraj & Reh, 2008). Examples of non-destructive methods for texture measurement include sound input techniques, particularly for fruit (Duizer, 2004) and near-infrared techniques applied to a wide range of foods (Millar, 2004). Other techniques that have potential applications in the QC laboratory are nuclear magnetic resonance (NMR) and magnetic resonance imaging (MRI) (Thybo *et al.*, 2004).

1.6.3 Electronic noses and tongues

As has been stated previously, a substantial amount of research has been carried out to develop electronic noses for volatile measurement, and electronic tongues for involatile measurement (Kress-Rogers, 2001b; Röck *et al.*, 2008). Extensive publicity given to such systems led to an over-expectation of their potential in the early years of their development, and many companies that invested in these instruments did not find their expectations realised. Some applications have been reported, mainly in the beverage and water areas (e.g. Deisingh *et al.*, 2004; Marti *et al.*, 2005), but routine usage is currently not high. Specific applications that might be expected in the future include screening incoming ingredients such as raw milk for taints, and screening packaging materials for residual volatiles. Continuing development of new systems is likely to increase the range of applications in the industry.

1.6.4 Data analysis

The traditional data analysis methods described above continue to prevail in most of the industry, but other (non-statistical) approaches to relating instrumental and sensory data are under active investigation, and it is highly likely that some of these will be developed into practical methods.

Artificial neural networks (ANN), which are designed to mimic the structure and functionality of the biological nervous system (Kress-Rogers, 2001b), can be trained to relate complex instrumental data sets to sensory quality data. They have been used in conjunction with both electronic nose systems (Yu *et al.*, 2008) and with electronic tongue systems (Chen *et al.*, 2008) in the quality evaluation of green tea. Fuzzy logic analysis has been used to investigate the relationship between instrumental parameters and sensory quality of mango drinks (Jaya, 2003), and ANN and fuzzy logic systems have the potential to be used in combination (Kress-Rogers, 2001b).

A criticism that is often levelled at ANN systems is their lack of transparency, and their use as a black box. An alternative approach that is being made is the use of novel belief rule-based (BRB) models (Wang *et al.*, 2009; Yang *et al.*, 2012) which can be used to support quality analysis and consumer acceptance prediction in rapid retro-design and testing of new food and drink products.

1.6.5 Summary

Considerable efforts will almost certainly continue to be made to identify instrumental methods that can be used to give valid measures of sensory quality. It is equally certain, however, that these efforts will fail to find a single instrument, or combination of instruments, that will be capable of measuring the full range of sensory information that the human senses respond to and interpret as quality. This does not diminish the value of instrumental measurement as a valuable adjunct to sensory assessment, provided that the instrumental measurements can be validated satisfactorily. A key issue is that any useful set of instrumental measurements should be compatible with the working constraints of a busy quality function.

1.7 Sources of further information and advice

General sensory methods available to industry are detailed in standard sensory texts (e.g. Lawless & Heymann, 1998; Meilgaard *et al.*, 2006) and in texts focusing more closely on QC methods (e.g. Muñoz *et al.*, 1992; Costello, 2002; Kilcast, 2010). Instrumental methods tend to be classified according to sensory modality, e.g. appearance in Hutchings (1999) and MacDougall (2002); texture in Bourne (2002) and Kilcast (2004); and flavour in Taylor & Roberts (2004) and Voilley & Etiévant (2006). A broader picture of instrumental methods for food quality measurement can be found in Kress-Rogers & Brimelow (2001). Correlation studies are less well covered in a comprehensive form, and the reader should examine published papers, in conjunction with standard statistical texts.

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Food appearance quality assessment and specification

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Abstract: Total appearance properties form an introduction to the food product in front of us. They determine the extent and quality of the expectations we subconsciously predict for the eating and drinking experience ahead. Such properties can be understood, analysed and measured using instruments as well as the senses. Colour forms an important part of the perceptions of quality and plays a role in the sensory assessment of visual structure, translucency, gloss and surface texture. A full understanding must take account of population differences, halo effects and the effects of lighting. A combination of the new digital approach to instrumental measurement and sensory appearance profile analysis is a powerful tool in the armoury of all concerned with product appearance at all stages along the supply chain.

Key words: colour, appearance, total appearance, expectations, halo effects, sensory, appearance profiling, instrumental measurement, digital imaging.

2.1 Introduction

Colour is of vital importance to the food industry. Although use of the word *colour* is widespread this should be merely shorthand for *appearance*. This is because other aspects of the way a product looks may be vital to its performance as a signal of quality. For example, clarity is vital to the perception of quality of beer and wine, translucency is an important indicator for smoked salmon condition, the distribution of meat and vegetable elements across the surface of a pizza govern its visual impact. Appearance is subject to commercial exploitation. For example, the wax used to coat apples to reduce weight loss and fungal growth in the store is also designed to be glossy. Study of product appearance is an important step in the understanding of the effects of processing and subsequent impact on consumer behaviour. That is, colour is important but it is not the whole story because we have evolved to respond to food appearance as a whole.

This chapter contains definitions for *appearance* of the food material itself and *total appearance* which includes the reactions we all have when viewing the product on the shelf or on the plate. This involves psychological concepts such as halo effects which in turn lead to expectations, population differences, exploitation of the customer and food ethics. The chapter will finish with an account of assessment and measurement and includes application of modern digital imaging techniques to specification and as an aid to the sensory scientist.

2.2 Appearance, total appearance and expectations

A commonly found belief within the food industry is that it is the *colour* of the product that is the major visually perceived factor influencing customer selection from a display. Choice based on appearance is, in fact, governed by a hierarchy of appearance properties of which the colour is just one. This hierarchy, which results in the formation of expectations, is controlled by the order of importance of the constituent elements of appearance. Colour is the paramount attribute influencing the selection of paints, colours and shapes are the paramount attributes influencing selection of clothes, but it is total appearance and expectations arising therefrom that govern food selection.

As a visual phenomenon the appearance of a material consists of visual structure, for example a slice of bacon made up of definable areas of muscles, fat, rind and gristle. Each element of this visual structure has colour, translucency, gloss and surface texture properties. Each of these attributes will include variation or patterning. The presence of or defect in any of these individual properties can lead to rejection of the product. For completion, each of these attributes changes in a characteristic way with time, cooking and processing. However, each of these attributes can be assessed and instrumentally specified.

Total appearance is formed by interaction of appearance properties with the human response, so consists of two parts – the scene, which may be an individual food material or a composite product, and the viewer. For a complex food layout, the scene consists of the elements of the scene (perhaps the meat and carrots on the plate), the design of the elements (the way they are arranged), and the illumination. The human response is governed by our individual sensory characteristics, our upbringing, our psychology, our preferences and our immediate environment, which includes our appetite, needs and health. We respond to total appearance in terms of sensory, emotional, intellectual images and expectations. Appearance properties drive expectations that control our responses along the whole supply chain. The term total appearance was first used with reference to foods and since then the approach has been extended and refined (Hutchings 1999).

Colour and appearance of food as well as that of the food environment both drive and are intimately linked through the information transfer process with expectations (Hutchings 2003). Food expectations are influenced by the event as well as by the viewer's needs and wants at a particular time. Expectations comprise:

- Visually assessed safety – will this food harm me?
- Visual identification – what is it, what flavour is it, what texture is it?
- Visually assessed usefulness – will this food answer my present needs, will it give me energy?
- Visually assessed pleasantness – will I like it, how pleasant will this eating/drinking experience be?
- Visually assessed satisfaction – how satisfied will I be when I have finished eating this meal, will it have been value for money?

Hence, the total appearance of a particular food communicates in a number of ways. First, it tells us about the food itself and second, through the halo effect, it will inform us about the properties of *other* foods we may come across. That is, appearance enables us to recognise a food as well as helping us to recognise its quality and set up our expectations prior to consumption.

Hence the information transfer process, through which we derive images and expectations, is complex but it provides methods for studying and understanding the formation of appearance images and expectations relevant to a wide range of situations. These include the product, the package, the pack in the freezer and shop, as well as even the design of the restaurant and supermarket. In fact, to all appearance images however they arise. Within the information transfer process, designers, developers and manufacturers manipulate material properties to create a scene consisting of scene physics. The scene encourages in the viewer basic perceptions (e.g. colour and gloss) of elements of the scene as well as total appearance images (e.g. this is a ripe orange) and expectations of the scene (e.g. this orange will have a excellent flavour). Appearance profile analysis can be used formally to assess images and expectations of the scene, and we can thus attempt linking scene material properties with total appearance images and consumer expectations and actions.

The scene in view can be described in terms of the physics of the elements of the scene coupled with the way the elements have been assembled, that is, the design. The scene physics and design working together contribute to the stimulus which is converted into appearance images in the brain of the perceiver of the scene. There are two broad types of image, basic perceptions, such as size and colour, and derived perceptions (or *visual expectations*), such as creaminess and value.

A product or scene possesses physics properties that can be summarised as spatial (properties of dimension), spectral (properties dependent on wavelength of light reflected or transmitted), goniophotometric (properties

dependent on angles of illumination and viewing), translucency (properties dependent on internal scattering and absorption) and temporal (properties dependent on movement and time). The product viewed under illumination, which itself can be defined, results in two types of perception. Basic perceptions are of size, shape, surface texture, colour, translucency, gloss and their patterns and uniformities. Derived perceptions, formed through repeated eating experience, comprise visual expectation. The extent to which these expectations are subsequently confirmed or otherwise has a profound effect on acceptance.

When viewing a scene, our images are normally the gestalt expectations. Nevertheless, they are linked with the basic perceptions through the specific properties of the viewer's visual mechanisms. The total appearance model includes consideration of appearance images, what they are, how they arise, how they can be measured and how they can be manipulated. It can be applied to any situation in which the individuals find themselves, but applications described here are confined to foods.

The total appearance approach can also be applied to the food and drink consumption environment. For example, a study of a number of venues revealed that there are key customer sensitive rules. These can be grouped under the general headings of spatial dimension, cleanness, quality and privacy (Hutchings 2003; Hutchings *et al.* 2012).

2.3 Food appearance

In very many ways the study of the colour of foods is completely different from the study of the colour of other mass marketed materials. One of the differences is that foods have a natural variation in appearance properties. Normally, solid foods are non-uniform in colour, they are translucent and vary, often irregularly, in surface texture (i.e. roughness) and gloss. It is the complete package of these properties that leads to identification and preference for a particular food.

Many foods have a visual structure that reveals different elements within the total product. For a piece of meat it is evident as to the number of muscles present, the degree of lean, fat, connective tissue and gristle contents. Manufactured desserts may consist of layers having different colour or translucency or surface texture.

Each element of the visual structure possesses colour, gloss, translucency and surface texture properties characteristic of a particular food material that is vital to identification and judgement of quality. Fish and red meats depend for their perceived quality on the balance between colour and translucency. Cooking and processing changes the absolute values and balance of visually perceived attributes. Ancient Egyptians clarified drinks by filtration and today a reduction in clarity leads to rejection of most

alcoholic drinks. Turbidity in fruit juices, such as apple, can be a positive or a negative attribute depending on the expectations of the consumer.

Gloss, also perceived as a colour contrast, and specific gloss characteristics are associated with different food materials. High-quality chocolate normally has a high gloss and light scattered from the surface is near mirror-like specular reflection. When chocolate blooms there is a redistribution of the fat crystalline properties which contributes to the loss of gloss, the specular reflection changing to diffuse scatter and the surface becomes dull. In the store glossiness of moist surfaces such as fish reinforce perceptions of freshness, and looks 'dead' if there is not sufficient directional light. Glossy surfaces look attractive and are perceived as clean. Hence, along the supply chain apples may be coated with wax designed to reduce gas exchange, weight loss and fungal growth but it is also designed to be glossy. Gloss or glaze is achieved in the kitchen for vegetables and maintained for the dining room by coating with butter. Other products may be coated with a jam or fruit purée, or with an aspic glaze.

Surface texture is also a perceptual characteristic of a number of foods. Meat flesh possesses a visually perceptible fibrous structure, breakfast cereals have differing degrees and types of roughness, and roughness is a characteristic of some varieties of apple. We perceive this roughness as colour contrast set up by product colour variation and shadow.

These individual appearance attributes are not linked to quality in isolation. For example, the visually perceived quality of fish and meat depends on colour, translucency and gloss, that of chocolate depends on colour and gloss, breakfast cereals depends on colour, colour distribution and surface texture, and drinks depend on colour and translucency. That is, all perceived individual attributes of appearance depend on lightness and colour but a study of colour alone will not reveal the story behind the product.

In summary, there are a number of attributes of appearance that affect the look of the product. Effects of processing, cooking and consumer preference should be considered in terms of appearance as a whole because each element of appearance changes characteristically with time and processing and combine to affect our expectations. That is, temporal properties of appearance must be included as a vital element of how we recognise the product and in what we expect of the food in front of us.

2.4 Halo effects

Expectations are fundamental to food marketing and when a food pack, advertisement, dining room or a particular food dish is being designed it is helpful to consider the five types (visual safety, visual identification, visual usefulness, visual pleasantness and visual satisfaction, as described in Section 2.2). An important consequence of expectations is that they generate halo

effects. It is relatively easy to confuse tasters by giving them inappropriately coloured foods, and raspberry flavoured orange coloured drinks, for example, may be identified as tasting of orange. Such effects have profound implications for the sensory testing of foods. Panel in-mouth scores can be influenced by sample appearance, the environment, panel organisation and panel organiser attitude. There are, however, two groups of subjects, field-independent, who attend to taste and smell stimuli even in the presence of an inconsistent visual stimulus, and the field-dependent, who make more mistakes when trying to identify flavours in the absence of visual cues as to their origin. Often, little account is taken of the extreme influence of the brand. The investigative focus may concentrate too much on the product, marketers may over-focus on the concept, or on the potential customers, but the brand can dominate expectations.

Preconditioning also influences taster beliefs. For example, knowledge of the fat content may affect consumer responses to the product. Preferences of young children can be changed by identification of the product with hero figures. Product advertising affects panel members. For example, the British have been subjected to the persistent advertising claims that ‘smaller peas are sweeter’ and that ‘larger peas are tougher’. An unwary approach to the panelling of peas may well result in erroneous findings founded upon awareness of this claim.

First, the total appearance of the food tells us about the food itself. The colour, size, shape, skin translucency and surface texture may tell us that this object is an orange. Through what we have learned about oranges, these properties also may tell us that it is ripe, mature, perhaps juicy, not rotten in the middle and is good enough to eat. Also, the appearance tells us that this orange is healthy, that it is full of those antioxidants and free radical scavengers that play vital roles within our cardiovascular system. These are positive expectations.

There may be negative expectations also. The colour uniformity resulting from the blemish-free surface of this orange tells us it may have been heavily treated with pesticides and herbicides, and therefore that the fruit as well as our hands must be washed before we eat it. Also from the glossiness of the skin we must expect that a coating of wax has been applied after harvesting to prevent loss of moisture, and therefore that we must scrub it before using the zest for cooking. We may indeed conclude that eating this orange will be bad for us.

Second, the total appearance, hence expectations, of the orange itself tells us about the properties of *other* similarly coloured foods. Fresh orange juice may have the same health-giving properties as the orange itself, but similarly marketed orange-coloured products containing added sugar and only 5% orange juice do not.

For many or most natural products, the degree of ripeness automatically triggers the response of preference, for example of bananas – see Fig. 2.1. Hence, a visual clue of ripeness will trigger expectations of sensory attributes

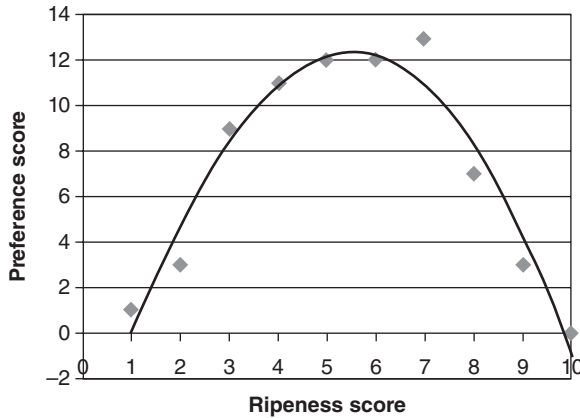


Fig. 2.1 The relationship between perceived ripeness and preference for bananas.

such as flavour degree and in-mouth texture. Removing such ripeness cues may be difficult but in most instances, for example, skin can be removed before the taster sees the sample (Park *et al.* 2005). It is postulated that this curve will move towards green (under-ripe) or towards brown for different populations of the world.

Another consequence of expectations is that attitudes to specific foods can be regional. For example, in the UK there are different preferences for tomato soup colour. Dark, deep red is the preferred colour for those used to tomato purée or tomato powder-based soup, but those raised on tins of Heinz cream of tomato soup prefer an orange red colour. In experiments that looked at orange juice properties more than one population was observed – see later.

Colour properties can combine and may be instrumental in initiating a rejection response, for example, both clarity and colour can be used for rejecting a beer sample – see later. Colour perception is a function of the wavelengths of light reflected from the product, the illumination and the sensitivities of the different retinal cones in the eye. That is, colour perceptions are affected by lighting and no single lighting regime is optimal for all foods. According to food folklore diners eating in the dark can be made physiologically sick by switching on the lights, revealing that they are eating highly inappropriately coloured food. In the store red-biased light is often used in the marketing of red meats. This conceals the brown specks in fresh beef that indicate pigment oxidation and the presence of metmyoglobin. Such meat may be perfectly edible but the customer normally sees this as undesirable. Some regard such a use of store lighting as bordering on fraud, but, is it unethical to display foods to their best possible advantage? That is, expectations can be optimised but also can be commercially exploited, sometimes to what may be seen as an unethical extent.

During optimisation of product flavour or texture, low illumination levels or coloured lighting is often used in the tasting area. However, although the actual colour of the product may be completely lost, conclusions may still be drawn by the panellist about the sample from the light reflected. For example, the extent of baking of bread products can be detected even under low illumination levels as greater baking will make the bread browner, that is, darker. The halo effect is so powerful that variations in appearance should be entirely eliminated while judgements of flavour and texture are required.

2.5 Appearance assessment and measurement

When we view the product on the shelf, although we perceive the appearance quality as a whole it is in fact a combination of individual attributes. Perceptions of these are formed from the presence of light scattering and absorption within the material. These two physical properties lead to individual perceptions of visual structure, colour, translucency, gloss and surface texture. Perceptions of these attributes have their foundation in colour and colour contrast. Colour can be specified visually as well as instrumentally.

2.5.1 Total appearance profiling

As described above there are a number of elements of appearance any of which visual cues may be used singly or in combination by the consumer when judging quality. All can be quantitatively assessed, by trained panel using the total appearance profile approach, and measured, using instrumentation.

Gestalt properties of the product may suggest that this product looks different or that something about it looks wrong. Future success of the product may well hinge on these differences being identified and quantified. This can be achieved using the total appearance profile approach while observing the rules concerning halo effects. The profile comprises both basic and derived (expectation) perceptions properties. For example, a comprehensive examination of three types of lemon dessert revealed significant differences in the basic properties of colour, opacity and gloss. Differences were also found in the derived properties of visually assessed flavour attributes of acidity, sweetness and lemonness, and the visually assessed texture attributes of graininess and bounciness (Hutchings 1999).

Visually assessed basic properties of beer and lager include visual structure (quantity and density of head), colour (amberness, greyness and intensity), translucency (clarity), and the temporal properties of head persistence, quantity of rising bubbles and bubble size (Spooner 1997). Basic properties of apples are skin colour and specific types of colour uniformity, skin gloss

and gloss uniformity, skin translucency and its uniformity, surface texture (in terms of ridges, white dust, white specks and dark specks). Derived perceptions include visually assessed texture in terms of soft to hard (Dailliant-Spinnler *et al.* 1996). Visually assessed derived properties of fresh pork are judged to be taste, leanness, juiciness and tenderness. The visually assessed satisfaction attributes are nutritional value, wholesomeness and freshness (Bredahl *et al.* 1998).

2.5.2 Visual specification

Visual specification of colour can be made using a colour atlas or by magnitude estimation techniques. The most popular colour atlases used in the food industry are the Munsell system and the Natural Colour System (NCS). The Munsell colour order system arranges colours according to their hue (H), value (V) and chroma (C). The NCS orders colours with reference to the six elementary colour sensations: whiteness (V), blackness (S), redness (R), yellowness (Y), greenness (G) and blueness (B).

Advantages to the food industry of using such comparative standards include low cost, portability, normally only a few colour chips will be needed for a product; stability and consistency; although colour chips may become contaminated they are reproduced to precise and accurate tolerances. Disadvantages include the necessity for a standard viewing and lighting system, the necessity to check that the observer has normal colour vision and behaves consistently, the comparatively large steps between adjacent chips in each system, and the difficulty of imagining that a piece of uniformly coloured card or plastic looks like a particular food.

Colours can be quantified visually, observers estimating the lightness, colourfulness and hue of the sample (Luo *et al.* 1991). Hue is scaled according to the quantities of four unique hues: red, yellow, green, blue. Lightness can be scaled against a reference of lightness 100, black being the blackest colour imaginable. A reference colourfulness sample is always presented to observers in the viewing field. For example observers may be told that a given reference sample has a colourfulness value of 40 and be asked to assign a colourfulness number to each of the test samples compared with this. Considerable training and practice are needed to obtain consistency of performance.

2.5.3 Instrumental colour specification

Colour measurement is based on the observation that it is possible to match the colour of an object using a mixture of light stimuli. Colour perception results from a combination of the light illuminating the material, the light reflected from the material and our colour vision characteristics. It is specified accordingly – see Fig. 2.2.

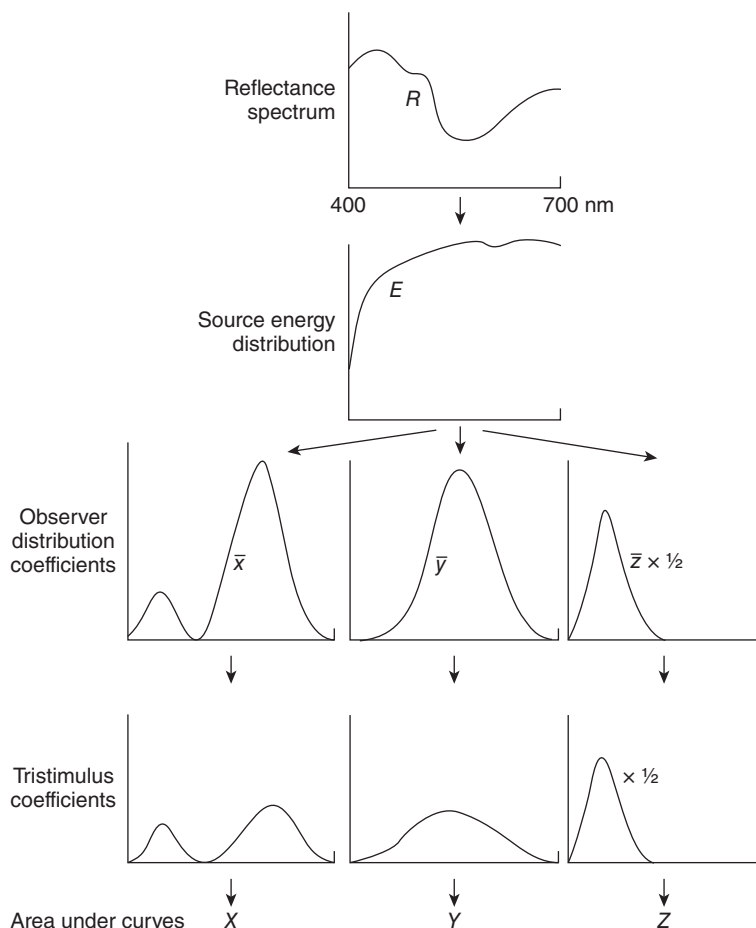


Fig. 2.2 Derivation of tristimulus values X , Y , Z of an object from its reflectance spectrum. At each wavelength the reflectance is multiplied by the relative energy of the light source and the colour-matching functions $\bar{x}$, $\bar{y}$, $\bar{z}$ to yield three curves. The areas under these curves give the tristimulus values X , Y , Z .

At each wavelength the spectral radiance factor (we use reflectance (R) here) is multiplied by the relative energy of the light source (E) and the colour-matching functions $\bar{x}(\lambda)$, $\bar{y}(\lambda)$, $\bar{z}(\lambda)$. The latter are functions of the cone sensitivities of a defined standard viewer using subtended angles of 2° or 10° . Three curves result and the areas under them yield the tristimulus values X , Y , Z . Tristimulus values are defined as the amounts of three matching stimuli in a given trichromatic system required to match the stimulus considered. These are the units from which all systems of colour specification are derived.

Colour is three dimensional but the colour space represented by X , Y and Z is not uniform, differences in colour not being represented by equal distances. A common system of colour specification which is much more uniform is the CIELAB 1976 space in which the axes L^* , a^* and b^* are plotted at right angles to one another (CIE 2004a; Hunt and Pointer 2011). Measurements are always made with reference to white, which itself is defined by the illumination.

The CIELAB 1976 space is a nonlinear cube root transformation of the tristimulus values X , Y and Z and this space is obtained by plotting L^* , a^* and b^* at right angles to each other.

$$L^* = 116(Y/Y_n)^{1/3} - 16 \quad \text{for } Y/Y_n > (24/116)^3$$

$$L^* = 903.3(Y/Y_n) \quad \text{for } Y/Y_n \leq (24/116)^3$$

$$a^* = 500[(X/X_n)^{1/3} - (Y/Y_n)^{1/3}]$$

$$b^* = 200[(Y/Y_n)^{1/3} - (Z/Z_n)^{1/3}]$$

where X_n , Y_n and Z_n are values of X , Y and Z for the appropriate reference white.

The CIELAB 1976 space approximates to Munsell space, which expresses colour in terms of hue, chroma and value (lightness). Note that the following values do not constitute an expression of Munsell space.

$$\begin{aligned} \text{CIELAB 1976 hue angle } h_{ab} & \text{ (derived from the } a^*/b^* \text{ plot)} \\ & = \arctan(b^*/a^*) \end{aligned}$$

$$\text{CIELAB 1976 chroma } C_{ab}^* = (a^{*2} + b^{*2})^{1/2}$$

That is, a colour can be expressed either in terms of (L^*, a^*, b^*) or (L^*, C_{ab}^*, h_{ab}) . The near linear nature of CIELAB 1976 space makes the colour difference calculation more meaningful and the colour difference between two objects (Euclidean distance) can be calculated from

$$\Delta E_{ab}^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2} = [(\Delta L^*)^2 + (\Delta C_{ab}^*)^2 + (\Delta H_{ab}^*)^2]^{1/2}$$

where $\Delta H_{ab}^* = 2(C_1 C_2)^{1/2} (\Delta h/2)$

C_1 and C_2 are the C_{ab}^* values for the sample and batch. Δh is calculated between hue angles of batch minus standard. Commercial paint and textile colour tolerances may be, typically, 1 and 2 ΔE units, respectively. The just noticeable colour difference for foods tends to be larger due to natural colour variation across the sample and the irregular nature of the surface. A further transformation can be made into colour appearance dimensions using the CIECAM02 approach (CIE 2004b).

Colour of an object changes with the viewer's eyesight, with lighting, and with the angles of illumination and viewing. All these are standardised when measurements are made. The above calculations are based on standard illuminations, standard viewer and standard measurement geometry (CIE 2004a) and all are built into the operation of commercial colour measuring instrumentation.

Reflection of light from materials depends on the ratio of absorption to scatter which depends on the pigmentation, refractive index and light scattering properties of the material. The different contributions of absorption and scattering can be determined using the Kubelka–Munk Theory (Kubelka 1948) together with the Saunderson correction (Judd and Wyszecki 1975). For opaque materials colour can be determined directly from the surface reflectance. Non-light scattering transparent materials can be measured directly from the transmitted radiation. For a full understanding of translucent materials, however, use of the Kubelka–Munk approach is essential.

Colour vision is used in the perception of all appearance attributes. Translucency is perceived through colour contrast which indicates that an object partially transmits light. Gloss is perceived as a continuing change in colour contrast with angles of illumination and view. The presence of surface texture is perceived through colour contrast originating from the presence of shadows. This means that sophisticated colour measurement, for example using digital imaging methodology, can be used in the specification of all these properties.

2.5.4 Colour instrumentation

When viewing or measuring the colour of any object by reflection or transmission, three factors must be present for there to be a colour: a light source, the object and a viewing mechanism. If changes occur in any of these elements the colour may change. Hence, any colour measurement system must include a specification of these factors.

There are eight instrument geometries recommended by the CIE (CIE 2004a). Quoting the illumination angle ($^\circ$) first, and the viewing angle second, they are designated as $45^\circ a:0^\circ$, $45^\circ x:0^\circ$, $0^\circ:45^\circ a$ and $45^\circ z:0^\circ$, whereas illumination:viewing angles and a and x are the annular and particular direction of illumination or viewing. For integrating spheres, the geometries are: $de:8^\circ$, $8^\circ:de$, $di:8^\circ$ and $8^\circ:di$, where e and i are the exclusion and inclusion of gloss component.

All measurements are made relative to a white standard, which the CIE nominates to be a perfect diffuser. Such perfection does not exist, but compensation for this can be made during measurement and calculation. A white working standard is frequently used to calibrate each instrument. Some coloured working standards are sometimes used to increase measurement reliability and sensitivity. This technique has been found particularly useful when inter-laboratory colour-quality specification procedures are being established. Instrumental performance may be checked with highly stable reflection or transmission standards. When comparing results made on different occasions, or at different laboratories, or when comparing instrumental measurements and visual assessments, great care must be taken to ensure that all measurement and calculation details correspond.

Colour measurement can be carried out using conventional or digital instrumentation. The former is designed to be used as specified by the CIE with so-called 'perfect sample' that are flat, uniform in colour and perfectly matt and opaque. Instruments were designed for the measurement of paint surfaces and textiles, but have become widespread in the plastics industries also. All these materials although they may not be truly opaque can be regarded as such in the applications to which they are put in everyday life. Hence, the conventional instrument approach is used in direct contact with the sample and eminently suited for highly accurate measurements on those materials being used in highly testing applications such as dye and pigment recipe prediction. Accurate measurements can also be made of transparent materials.

Results from non-perfect samples will be subject to error, and appropriate measurement techniques must be used. Unfortunately most foods are translucent, uneven and partially glossy so extreme care needs to be exercised when measuring them. For example, the relationships between illumination, sample and measuring areas are highly important for successful colour measurement of translucent materials (Hutchings 1999). In digital measurement there is no contact with the sample and the methodology is highly suitable for materials that vary in colour across the surface, such as most foods, and are useful for those having irregular geometries. These include fibrous samples such as meats and three-dimensional samples such as fruits that cannot be presented to instrumentation in the form of a flat surface.

The most accurate instrument used for measuring colour is the spectrophotometer, which measures the ratio of spectral reflected radiance (reflectance) in a given cone under the eight CIE specified geometries as introduced earlier. The spectral radiance factor measured can then be used to calculate X , Y and Z for particular illuminant and observer conditions.

The tristimulus colorimeter is the simplest means for conventional instrumental colour specification. A light source, filters and a set of photodetectors are combined such that together they yield a direct evaluation of X , Y and Z . As outlined above spectrophotometers may also be used for colour specification. The spectral curve produced by a spectrophotometer may also yield useful information regarding possible chemical mechanisms or colour-change kinetics of the system under investigation.

The advent of digital analysis has presented the industry for the first time with a methodology that can be used to examine the appearance of food materials in detail. That is, in terms not only of average colour, achieved by the standard aperture capability of conventional instrumentation, but in terms of a detailed examination of the type of colour variation that occurs across the surfaces of biological materials. Using colour calibrated digital technology measurements of gloss, surface texture (as well as visual structure) can also be attempted on irregular three-dimensional materials, such as fruit, vegetables and prepared products. The technology for specifying

total appearance is based upon the digital camera which can capture images rapidly in digital format. These digital images can be easily processed, duplicated, modified or transmitted via a network.

Unfortunately camera R , G , B sensors do not have the same spectral sensitivities as the CIE standard observer. Hence, in order to measure an object in terms of device independent colour from a digital camera, there is a need to correlate the camera RGB signals and CIE XYZ values. The most common technique for digital camera characterisation consists of presenting the camera with a series of colour patches in a standardised reference chart with known XYZ values and recording the averaged RGB signals for each patch. Polynomial fitting techniques are then applied to interpolate the data over the full range and to generate inverse transformations. An overall accuracy of approximately 0.5 to 2 ΔE_{ab}^* units is obtainable (Hong *et al.* 2001).

2.6 Applications

2.6.1 Measurement

Currently there are two broad applications of colour and appearance specifications – to obtain an understanding of the particular material, perhaps in terms of its chemistry and its response to storage or processing, and to provide an understanding of its sensory properties including consumer behaviour. Using conventional instrumentation, classic studies have been carried out on the chemistry of tuna and wine colour (Little 1969, 1980) and on processing effects on meat and translucent liquids (MacDougall 2002).

More recently advances have become possible with the advent of the more flexible digital approach. This technique is very well suited to all natural as well as manufactured and processed foodstuffs. Measurements are performed on a pixel by pixel basis, hence unwanted effects due to background, sample gloss and shadow can be eliminated. This can be done automatically with appropriate software tools – see later. Examples of such applications concerning a number of products are included here.

Colour measurement can reveal the subtleties associated with the perceived colour of wines (Martin *et al.* 2007). Figure 2.3 illustrates this for four Spanish wines. The figure shows colours in terms of CIECAM02 colour appearance values of the coordinates a and b . Constant Munsell hue loci at a lightness (V) of 0.5 are plotted to illustrate the changes. For example, increasing depth of the more heavily pigmented wines leads to a visually obvious characteristic change of hue. The rim colour originating from a shallow layer of Oloroso has a greenish tinge ($-a$ values) which changes through a yellowish (increase in $+b$) then to a reddish hue (increase in $+a$) as depth is increased.

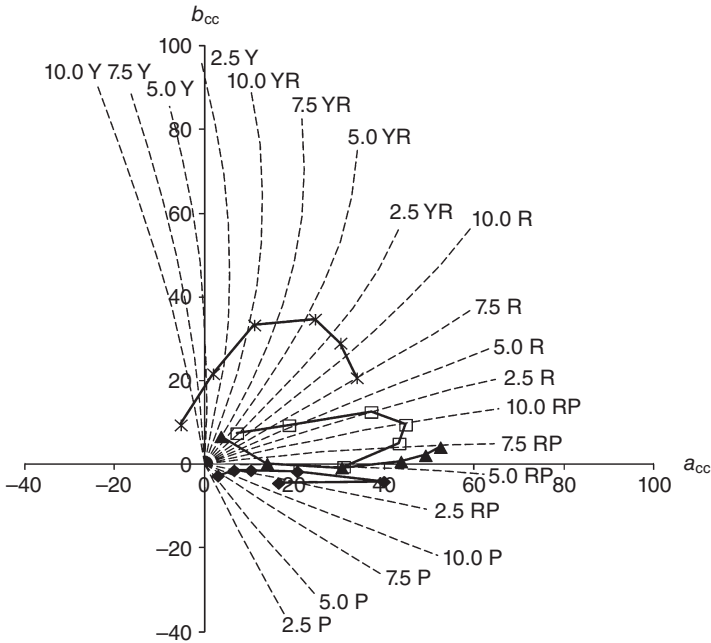


Fig. 2.3 The effect of increasing depth of wine on CIECAM02 bcc and acc values. The four wine types are Oloroso (*), Tawny (□), Rosé (▲) and Table Red (◆).

Colour measurement can be used to indicate acceptability. Judgements were made on a series of beers adjusted for colour using red and yellow dyes and for clarity involving addition of a light scatterer. Plate I (between pages 242 and 243) is a graph demonstrating that beer can be rejected (set in this case at an acceptability of 0.5) because the colour difference from 'standard' is too large or because there is too much light scattering (Jung *et al.* 2009).

Translucency is a key quality factor of fruit juice preference. Figure 2.4 shows the internal transmittance, effectively the light transmission properties, of a number of orange juice samples is plotted against visually perceived strength and visually perceived preference (Ji *et al.* 2005).

Although there was general agreement as to order of visually perceived strength there was disagreement as to preference. The panel response was split between those preferring the strongest colour juice and those preferring a drink of more moderate strength.

One great advantage of digital imaging is that it can be used to examine materials that vary in colour. For example, samples of a breakfast product were baked at 180°C and scanned at 15 minute intervals. All samples exhibited a range of colours from whitish to darkish brown the degree of which

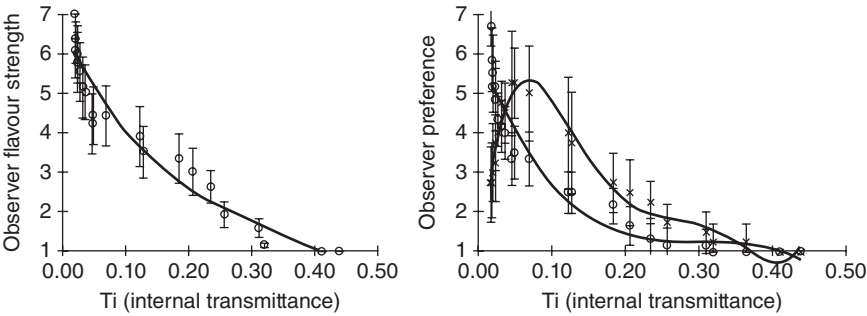


Fig. 2.4 Relationships between visually perceived properties of orange juice and internal transmittance. The higher the transmittance, the greater the dilution, the lower the perceived strength. There are two populations for observer preference, one favouring the most concentrated samples others favouring lower concentrations.

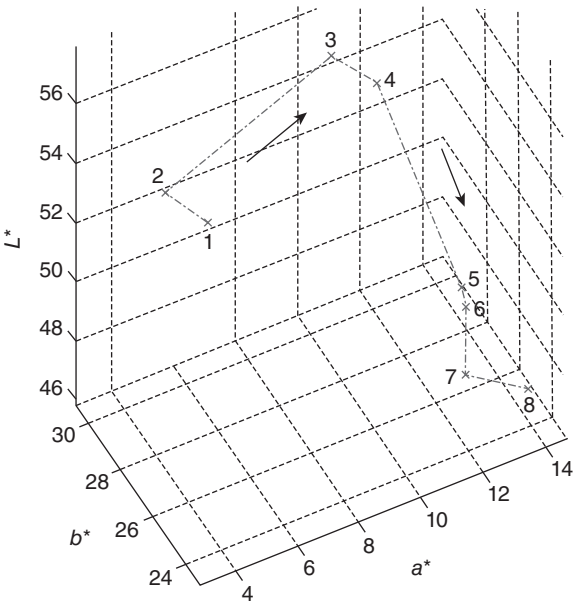


Fig. 2.5 A plot of the median pixel colour values of baked breakfast cereal.

increased with baking time. Median values of L^* , a^* and b^* are plotted in Fig. 2.5. The changes in the coordinate direction indicate the presence of a number of different major pigment development reactions. As cereals are heated carbohydrate/amino acid browning reactions occur during which colour and flavour develop. Caramelisation involving thermal degradation of sugars without amine participation also takes place and lipid oxidation

decomposition products react with proteins. Finally there are complex pigment reactions involving the greying of natural carotenoids present in the outer layers of cereals (Dworschak 1980).

More detailed examination of the data reveals the nature of the variability. For example, although mean values of L^* decreased by 21% over the baking time the values for the lightest parts of structure decreased by only 2%. This demonstrates the relative inertness of the paler parts of the structure, which are possibly caused by poorly processed starch. Also, it was observed that the minimum value of L^* for all biscuits was always zero. These areas correspond to the gaps in the structure that always appear black and hence an increase in caramelisation can be followed as baking progresses by examining the minimum values of L^* . Specific areas of an image can be identified visually, isolated and analysed for change in colour values (Hutchings *et al.* 2002). In this way, it is possible to specify the colour and colour patterning of irregular three-dimensional objects that vary in colour, for example, the surface of a ripening fruit (Ji *et al.* 2012).

Appearance measurements can now be made outside the laboratory. For example, growing and ripening patterns of plants as well as of individual fruits and vegetables can be studied and monitored in the field, store, laboratory and factory. Coupled with studies in the supermarket these techniques make possible coordinated studies along the complete supply chain.

This technology is transferable to the measurement of all appearance attributes. Exploitation of digital imaging can extend to automatic on-line monitoring of food product appearance, to online product assembly as well as for communication and archiving.

2.6.2 Sensory and consumer studies

There are many applications of digital colour measurement science that help the sensory scientist. Examples included here are following colour changes in samples during panelling and construction of visual aids for use during sensory examination.

Colour is an important sensory attribute, often scored during panel sessions but natural pigments are not stable and can change significantly during the actual process of the sensory assessment. Until now the extent of colour change during the session has been more difficult to examine. A sample of green peas was cooked, cooled rapidly and placed as a monolayer on a white plate simulating conditions used for a panel test. The sample was digitally scanned immediately and at short intervals over the period of an hour. Figure 2.6 is a black and white view of a typical a^* vs b^* (on the left) and L^* vs C^* (right) scan result. Included are pixels resulting from the peas, areas of gloss, shadow and plate. Those arising from the plate fall around the a^*/b^* neutral point and contribute to the vertical line of low C^* and high L^* (the area to the left of the superimposed white line). The near horizontal area occurring at high L^* was caused by specular gloss from the

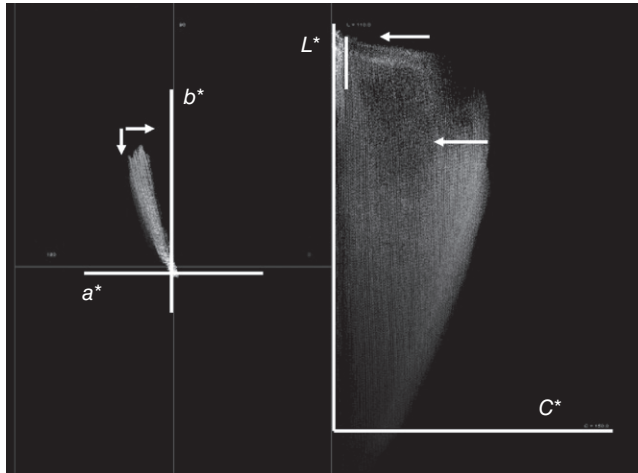


Fig. 2.6 The pixel distribution arising from a colour calibrated digital image of a plate of peas. The a^*/b^* plot is on the left, L^*/C^* on the right. The superimposed white arrows indicate directions of changes occurring with time over a period of 60 minutes – see text.

peas. The remainder of the pixels arise from the peas, shadow and areas of less obvious pea gloss.

Superimposed white arrows indicate directions of changes occurring with time. Pea gloss (high L^*) decreased, as did the maximum C^* values of the peas. In general these changes were accompanied by a change towards a^*+ and towards lower values of b^*+ . This confirmed the lowering of C^* values, indicating the greying of the peas with time. The contribution from the plate (low C^* , high L^*) remained constant with time as expected. Significant colour and gloss changes occur immediately and consistently over the hour, emphasising the extreme need for consistency in timing of the panelling organisation and process (Hutchings *et al.* 2005).

When a colour specification is needed the food sample can be matched to a chip obtained from an established colour order system or by using an ad hoc form of colour reference plaque. Advantages to the food industry of using such comparative standards include: low cost, only those colour chips covering the relevant ranges need to be purchased; stability and consistency, although chips may become easily contaminated, they are portable and commercial colour atlas chips can be reproduced to precise and accurate tolerances. Disadvantages include: the necessity for a standard viewing and lighting system; the necessity to check that the observer has normal colour vision and is consistent at the task of matching colours; the comparatively large steps between adjacent chips in established colour order systems often result in only an approximate match being possible; the difficulty of imagining that a piece of uniformly coloured card or plastic looks like a

particular food; saturated colours tend to be omitted from atlases and matching intense dark colours is difficult, although this is not a problem for most foods.

Many colour atlases have been produced but those most commonly mentioned in the food literature are the Munsell System and the Natural Colour System. Both divide colours first by hue then by two other dimensions – Munsell by chroma and value, and NCS by chromaticity (or nuance) and blackness. A scale for a particular food material, say frozen peas, can be constructed by selecting appropriate chips from an atlas and forming these into a scale. However, existing colour atlases are not easily applied to the problems of food specification. Gaps between adjacent colour chips tend to be large and the colour change routes of natural pigment systems run skew to the orthogonal lay out of existing atlases. This tends to result in a widely spaced and unevenly spaced scale.

It is now possible using digital technology to construct a series of colour plaques which are easy and quick to use by those less experienced in visually specifying colours. The process of producing physical pea colour standards for panel use has been explored. Six samples of frozen peas from different producers were purchased, cooked and imaged. Areas of gloss and shadow were eliminated from each pea image the resulting colours forming an ad hoc colour collection which was then ordered in colour space. This formed a matrix displaying the world of cooked frozen pea colour. It incorporated seven hues (at approximately five degree h^* intervals), each at eight lightnesses (approximately four L^* units apart), and two chroma (10–12 C^* units apart). The volume of this colour space is depicted in Fig. 2.7 as that bounded by the two planes.

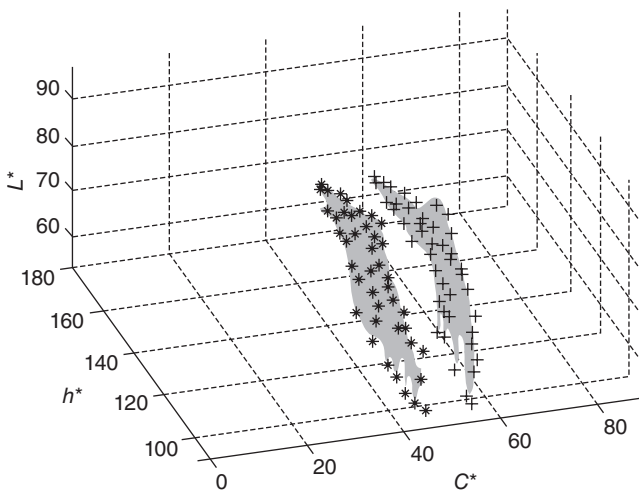


Fig. 2.7 The volume of colour space occupied by the world of individual pea colour is bounded by the two planes.

In trial experiments involving the colour matrix panellists used only a range of colours of low L^* and high h^* , corresponding to darker, greener peas. Although lighter, yellower peas were present in the samples they were not present in sufficient numbers for panellists to use areas of higher L^* and lower values of h^* . Using colours selected by this panel a routine panel tool was devised. This comprised a fan of 12 colours constructed using a fully calibrated printer and imaging system. The fan has been used successfully in panel trials involving the screening of pea varieties. An advantage of this approach is that the resulting colour chart is built using scientifically sound colour space. That is, the chart produced represents the three-dimensional colour volume occupied by the peas and arithmetic interpolations can be made which have a concrete meaning. This enables more realistic comparisons to be made between batches.

Until now studies of the sensory aspects of food appearance have been hampered by the three-dimensional nature of colour itself. This has prohibited the production of physical colour scales that mimic the sequences of colour changes occurring with natural pigments. This is made even more difficult because of the different ways the balance of these pigments change from year to year due to differing soil and weather conditions. The understanding and technology now available will render the current casual approach to the preparation of physical colour scales obsolete. Using the new digital technology physical colour scales can be developed with respect to the colour and pigment science of the particular food system under examination.

An example of the increased flexibility possible with digital technology is the ability to manipulate images so that specific parts can be examined separately. Plate II (between pages 242 and 243) is a screen shot of the process in action. The imaged banana on the left has its background removed (middle), making possible segregation of the area into different colours. The a^* , b^* area occupied is shown on the right and the mean values of the four colour clusters are displayed together below, with their colour values. In this way colour variation across a surface can be analysed. Figure 2.8 shows the mean values of ripening colour across the surface of the fruit.

As bananas ripen there is an increase in freckling. Figure 2.9 shows the increasing percentage of the banana area occupied by freckling is plotted

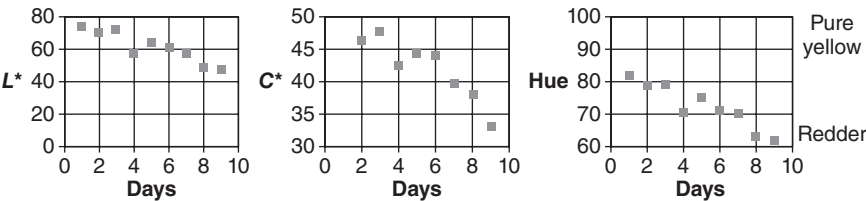


Fig. 2.8 Lightness, colourfulness and hue changes of banana with time.

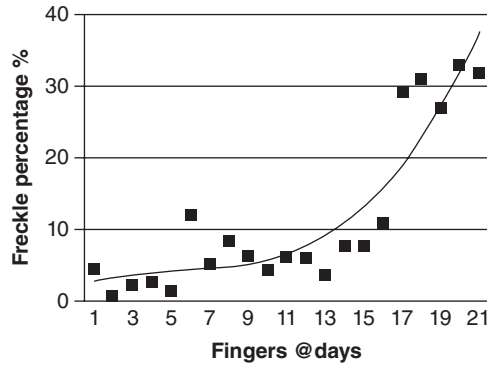


Fig. 2.9 Freckle percentage changes of a ripening banana.

against storage time. Digital analysis can be applied to all aspects of appearance measurement with confidence. Detailed examination can be made of all aspects of appearance-related phenomena throughout the business.

Other applications of calibrated digital technology to sensory and consumer studies include the ability to carry out studies of diet appearance effects on consumers in the home and in the hospital. Also on-screen visual sensory assessments can be made and on-screen panel assessments can be made of products whose virtual colour has been carefully manipulated. For example, Fig. 2.10 illustrates the results from an on-screen assessment experiment involving virtual pictures of orange juice. Pictures of bottles containing orange juice were judged by panel members for the expectations of sourness, sweetness, bitterness, flavour strength and freshness.

The results are plotted on a^* , b^* diagrams, the size of the spot signifying the magnitude of the perception. Sourness and bitterness gave similar results, increased perceptions tending towards greenish, that is towards negative values of a^* . Perceptions of sweetness tended towards high yellowness and highish redness and flavour strength tended towards high yellowness and/or high redness. Apparent increase in freshness was perceived for samples of a^* values close to zero (neither green nor red) and highish yellowness (greater b^*) (Wei *et al.* 2009). In this way it is also possible to use digital technology for crop and product monitoring and can be used to create international links between growing, packaging and producing companies.

Digital technology can be used to aid pack design – see Fig. 2.11. This illustrates a concept map for predicting expectations of fruit juice flavours from the pack colours. The pack illustrated on the left is subjected to digital and visual examination which lead to two models, one for colour harmony the other for freshness. They in turn lead to predictions for liking, quality and freshness (Wei *et al.* 2012).

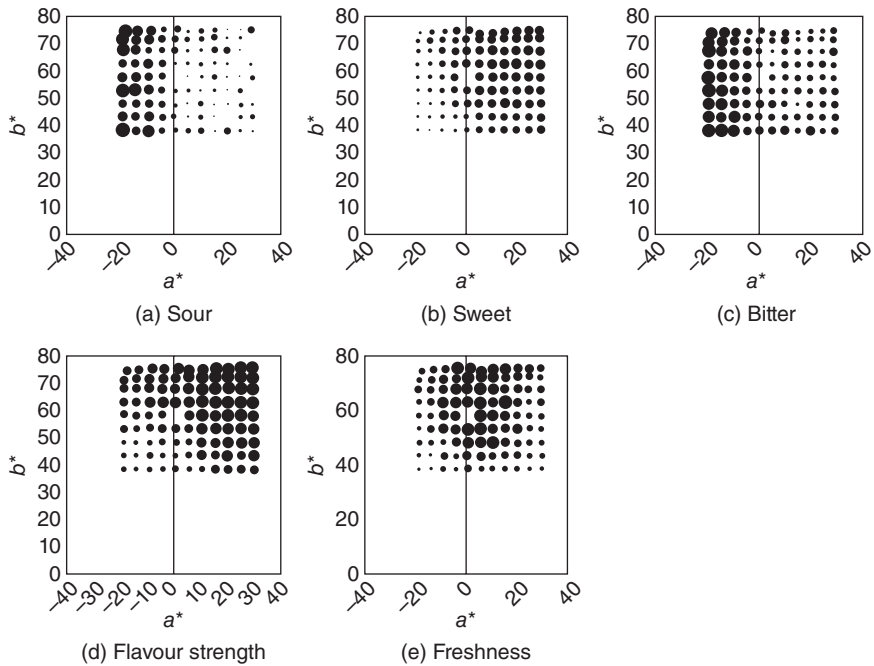


Fig. 2.10 Mean scores of expected flavours for the 98 equal-lightness colours.

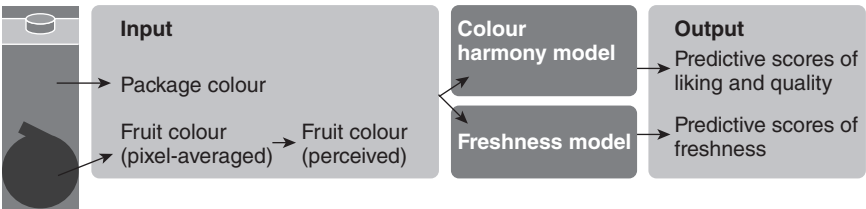


Fig. 2.11 A concept map for predicting expectations of a new juice product.

2.7 Future trends

Conventional colour measurement techniques are well established and potential advancement of the methodology and widening of the application are restricted. Much of the ground work of digital technology has been laid and there is a huge area for development ahead that is unavailable to the conventional scene.

Areas of development will include gaining a detailed understanding of the behaviour of fresh and processed foods along the supply, processing and storage chains. Digital reproduction hardware including cameras, scanners, monitor screens and printers can be calibrated for the production of colour standards for use on and offline. Calibrated systems also enable us to use

on-screen visual assessments and on-screen panel assessments of products and packaging whose virtual colour has been carefully manipulated for development studies. Portable equipment will also allow us to make in-home and in-hospital studies of the effect of diet appearance on consumer consumption and acceptance. Also, it is possible to make development studies on the effect of appearance make-up of products on the plate, on the pack and on the shelf. It is now possible to facilitate remote crop and product monitoring. Calibrated digital systems can be used to assess and standardise product and pack permitting visual link communication and archiving between the growing, producing and packaging companies to occur across the world.

2.8 Sources of further information and advice

Advice on colour and appearance specification and measurement as well as on application of measurement technology to sensory problems can be obtained from the University of Leeds, Colour, Imaging and Design Research Centre, led by Professor M. Ronnier Luo and the food research institute Campden BRI. Currently there are two relevant books in print. These are *Food Color and Appearance* (Hutchings 1999) and *Colour in Food* (ed MacDougall 2002). These contain further details on measurement and sensory techniques and chemistry. Although the latter contains a chapter on digital measurement methodology there has been much progress since 2002. The present chapter contains an overview of the current situation. General sensory expertise can be gained from membership of the Institute of Food Science and Technology Professional Sensory Group where there is considerable expertise on the subjects of flavour and statistics as applied to sensory methodology. Publications dealing with specific applications of food colour and appearance assessment and measurement occasionally appear in a wide number of journals including *Food Quality and Preference*, *Color Research and Application*, *Journal of Food Science*, *Food Science and Technology*, and numerous food and postharvest journals.

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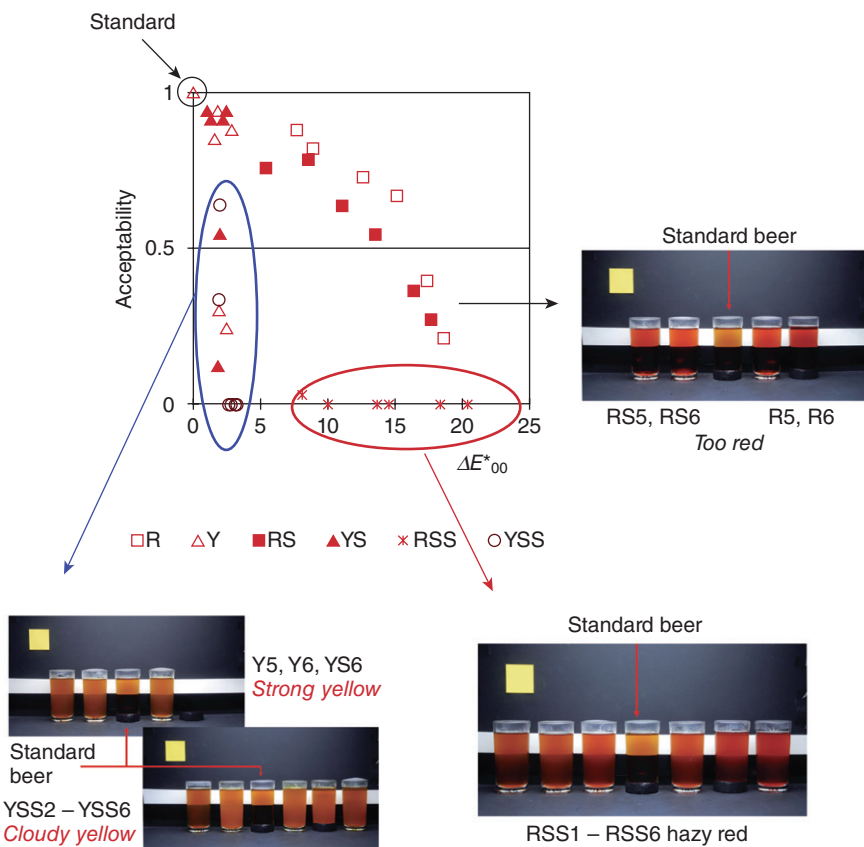


Plate I (Chapter 2) Colour as well as haze cause rejection of beers – see text.

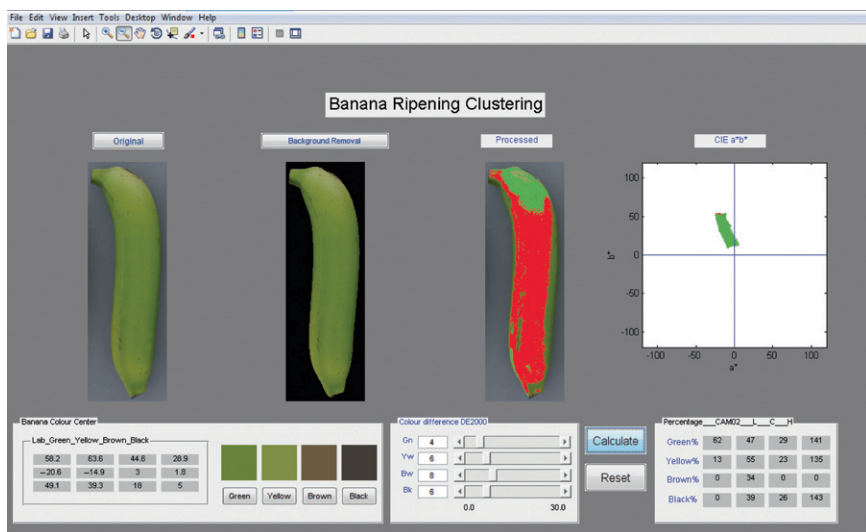


Plate II (Chapter 2) A screenshot of the image processing software for banana colour clustering.

3

Principles of food flavor analysis

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Abstract: This chapter presents a discussion of the most commonly used methods for the analysis of aroma, taste and chemesthetic compounds found in foods. Each group of flavor compounds presents unique challenges and thus has a different focus. The methodology for aroma analysis (i.e. primarily isolation of an aroma fraction) has been well researched and, thus, these methods are discussed in some detail. The analysis of taste compounds has been studied much less and having no standard methods, is discussed using examples from the literature. Since chemesthetic compounds may be either volatile or non-volatile, this discussion is again done by example. In all cases, the discussion focuses on getting an appropriate food flavor isolate to the proper instrument (primarily gas chromatography/mass spectrometry (GC-MS) or high-performance liquid chromatography/mass spectrometry (HPLC-MS)) with no discussion of the instrumental methods used: these instruments are described in detail in many texts.

Key words: flavor analysis, taste analysis, chemesthetic, aroma.

3.1 Introduction: flavor perception

Flavor is a sensory impression which occurs during the eating of a food. While at one time flavor was considered to be primarily due to aroma, it is now generally recognized to be due to numerous sensory inputs. As shown in Fig. 3.1, the final conclusion, i.e. perception, is the result of numerous chemical and physical stimuli. As flavor chemists, we typically narrow our view to focus on our chemical sensing systems, i.e. aroma, taste and somatosenses (chemesthesis).

3.1.1 Olfaction

Aroma compounds must be volatile to be sensed. They are liberated during eating, contributing the chemical stimuli responsible for the odor of foods.

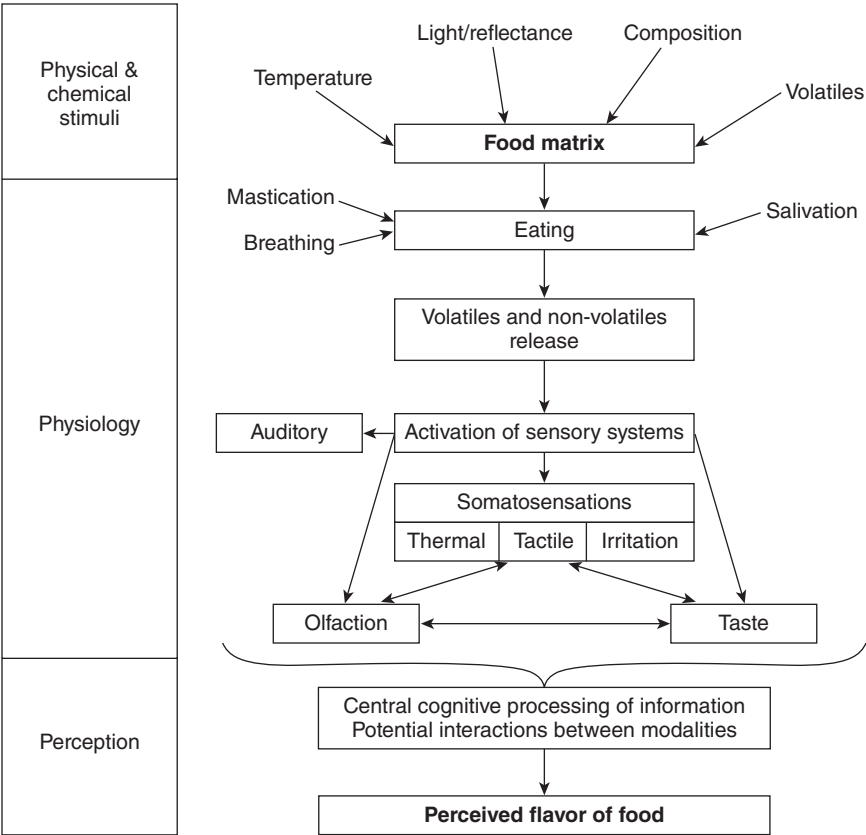


Fig. 3.1 Flow diagram outlining stages and factors involved in flavor perception (adapted from Keast *et al.*, 2004 by Charve, 2010).

These compounds are carried by the inhaled air to the olfactory receptors of the nasal cavity via two pathways: orthonasal and retronasal (Linforth and Taylor, 2006). The orthonasal pathway is the direct route through the nostrils, whereas the retronasal pathway is indirect, and implies travel through the nasopharynx. The binding of volatiles to the receptor cells triggers nerve impulses (olfactory sensations) that are transmitted to the brain, where they are decoded and finally interpreted (olfactory perception; Bell, 1996).

It is generally recognized that foods may contain a large number of volatiles but of these volatiles, only a limited number can be perceived and fewer likely contribute to the characteristic aroma of a food (key odorants). The sensory threshold of an odorant depends on its vapor pressure (influenced by the food matrix and other compounds present) and a human's inherent ability to detect it. A good illustration of the broad range

in sensory thresholds exhibited by odorants is β -pinene and ethyl-2-methylbutanoate with orthonasal odor thresholds in a orange juice matrix of 37.2 mg/L and 8×10^{-5} mg/L, respectively (Plotto *et al.*, 2004, 2008).

3.1.2 Taste

Taste compounds are generally non-volatile compounds and include a broad array of molecules (100–20000 Da) including, for example, sugars, salts, acids, nucleotides, and a diverse group of chemicals that are bitter. These compounds contribute to the five basic tastes: sweet, salty, sour, bitter, and umami (Delwiche, 1996; Laing and Jinks, 1996). Taste compounds interact primarily with taste receptors located on the surface of taste buds of the tongue (Lindemann, 1996), and are transported to the taste buds by saliva. Similar to olfaction, the binding of taste compounds to specific taste receptors leads to taste perception. The transduction of sour and salty tastes involves the opening of ion channels at the surface of the taste receptor cells, whereas bitter, umami and sweet tastes result from the binding of taste compounds to surface receptor sites (e.g., G proteins) and subsequent transduction cascades (Lindemann, 2001). Genes encoding for taste receptors were recently identified (Matsunami *et al.*, 2000; Max *et al.*, 2001). This is of particular interest for the discovery of bitter-masking compounds or design of new sweeteners (e.g., reduced calorie content) since knowing the binding-site structure could help in tailoring taste compounds that activate or inhibit specific taste receptors.

3.1.3 Chemesthesis

Lastly, chemesthetic compounds (irritants) are responsible for oral and nasal chemosensory sensations (chemesthesis) and also contribute to flavor perception. They generally have a molecular weight in the range of volatile compounds (<400 Da). The burn from hot peppers and mustard (capsaicin and allyl isothiocyanate, respectively), the coolness from mint (menthol), and the tingling/stinging of carbonation (carbon dioxide) are examples of chemesthesis (Green, 1996). Mouthfeel sensations, such as astringency, can also be considered part of chemesthetic sensations. These sensations were once called a 'trigeminal' response until being renamed 'chemesthesis'. The more general term was preferred because nerves other than the trigeminal nerve are responsible for this sensation (i.e., glossopharyngeal and vagus nerves for oral chemesthesis; Rentmeister-Bryant and Green 1997). Chemesthetic sensations result from the stimulation of receptors usually associated with pain (nociceptors), thermal perception (thermoreceptors), and touch (mechanoreceptors). These receptors are primarily located in the oral, nasal and ocular mucosae (Rentmeister-Bryant and Green, 1997).

3.2 The analysis of aroma contributors in foods: an introduction

3.2.1 Introduction to aroma analysis

The task of identifying volatile flavor components (aroma compounds) particularly in a food matrix is one of the most formidable tasks faced by an analytical chemist. A primary reason is that laboratory instrumentation is not as sensitive to many odors as is the human olfactory system. It is widely accepted that as few as 8 molecules of a potent odorant can trigger one olfactory neuron and that only 40 molecules may provide an identifiable sensation. Making a few assumptions about air concentration versus absorption on the olfactory membrane, it is postulated that the nose has a theoretical odor detection limit of about 10^{-19} moles, which surpasses even the most sensitive analytical instrumentation. The low concentrations at which these analytes may be present in a food and have sensory significance requires that they be isolated from the food system and concentrated to permit instrumental analysis.

The fact that trace quantities of aroma components are distributed throughout a food matrix greatly complicates the aroma isolation/concentration process. The isolation of exceedingly low concentrations of flavor compounds from food systems containing sugars, complex carbohydrates, lipids, proteins, and water is problematic. Aroma isolation methods based on volatility are complicated by the fact that water is the most abundant volatile in a food. Thus, any procedure that draws a vacuum or involves distillation will also extract/isolate the water from the sample. Isolation methods based on solubility (most aroma compounds are lipophilic), e.g., solvent extraction, will not only extract aroma compounds but lipids. Proteins are great emulsifiers and foam stabilizers, which complicate a simple flavor extraction process using organic solvents. Carbohydrates often add viscosity, foaming or emulsification properties to a product thereby complicating aroma isolation. Food matrices greatly complicate this endeavor.

Aroma isolation and analysis are made difficult also by the fact that flavors comprise a large number of chemical classes. If they were made up from one or just a few classes of compounds, isolation methods could focus on molecular properties characteristic of a given class of compounds. Rather, the chemist must attempt to effectively extract and concentrate alcohols, aldehydes, acids, ketones, amines, carbonyls, heterocyclics, aromatics, gases, non-volatiles (or nearly so), etc.

The absolute number of flavor compounds in a food further complicates flavor analysis. It is a rather simple, natural flavor that has fewer than 200 identified constituents. In fact those with fewer than 200 identified constituents probably have not been adequately researched. It is not uncommon for the browning flavors (e.g., meats) to have nearly 1000 volatile constituents. To date, over 8000 volatile substances have been found in foods.

A final problem complicating the instrumental study of flavor is instability. The food product being examined is a dynamic system, readily undergoing flavor changes while being stored awaiting analysis to begin. The flavor isolation process may initiate chemical reactions (e.g., thermally induced degradation or oxidations) which alter the flavor profile and introduce artifacts. Thus, we have to be very cautious that the volatile components we find in a food product are truly native to that product.

Unfortunately, once we have considered each of the points above and obtained some instrumental profile of the aroma compounds in a food, we are left with the huge question of attempting to determine the importance of each volatile to the perceived flavor. This has been the topic of countless research articles over the past 40+ years. Unfortunately, analytical instrumentation has no sense of taste or smell. Instrument response for the flame ionization detector (most commonly used detector in gas chromatography, GC) is related to the number of carbon-carbon bonds, whereas the human olfactory system varies greatly in response to different odors. For example, 2-methoxy-3-hexyl pyrazine has an odor threshold of 1 part in 10^{12} parts water, while pyrazine has an odor threshold of 175 000 parts/ 10^{12} parts water (Seifert *et al.*, 1970). On pyrazines alone, the human threshold varies by nearly 2×10^8 . It could be that the smallest peak in a gas chromatogram may be more important to flavor than the largest peak. It must also be recognized that the instrument is providing no appreciation for flavor character of each component. It is not apparent, for example, that peak 3 may be buttery while peak 48 may contribute an oxidized flavor note. There is no question that aroma analysis offers a most challenging analytical problem.

3.2.2 Sample selection/preparation

The first step is to select samples of the food most typical of the flavor or off-flavor to be studied. If one is studying an off-flavor problem, the strongest yet characteristically flavored samples need to be selected. Recalling the extreme sensitivity of the human olfactory system demands that the most intense samples be selected in order to improve the probability that our relatively insensitive instruments can detect the volatiles of interest. While this may seem to be an easy task for an off-flavor, often it is not. There may be disagreement amongst individuals as to which samples have the most intense off notes. If one is considering a study of desirable flavor, the task can become very difficult. For example, what if one was asked to obtain an aroma profile of aged Cheddar cheese? Cheddar cheese flavor varies around the world and there is absolutely no consensus as to what is a typical Cheddar cheese. Sample selection becomes arbitrary and is left to the prejudices of the researcher.

Assuming one gets by the first hurdle of sample selection, then sample preparation becomes the next issue. One cannot simply put an apple or a

pie into an instrument and expect a response thus, one must somehow extract the aroma from the food and concentrate it for analysis. This generally requires that the food be crushed, homogenized, blended, gas stripped or extracted in some manner. Most fresh plant and animal tissues contain active enzyme systems which may quickly alter the flavor profile once cellular disruption has occurred (Drawert *et al.*, 1965; Dirinck *et al.*, 1981). Singleton *et al.* (1976) demonstrated how sample handling during aroma isolation may influence the flavor profile of peanuts. Peanuts ground in water following immersion in liquid N₂ contained 62% less pentane and 8% less carbonyls than the control which was ground in water at room temperature. Peanuts ground in liquid N₂ showed an 81% decrease in pentane and an increase in total carbonyls compared to dry grinding at room temperature. Blending time, temperature and pH were all shown to have a pronounced effect upon the flavor profile of peanuts.

The inactivation of enzymes of fresh plant and animal tissue when the isolation procedure exceeds only a few minutes is essential. A common method used to inactivate enzymes is homogenization in methanol (Drawert *et al.*, 1969; Schreier *et al.*, 1976). This does, however, dilute the sample, decrease the polarity of an aqueous food slurry, and may interfere with later isolation methods. Thermal processes may also be employed if the product is a juice and may be rapidly passed through a high-temperature short-time heat exchanger. One must be aware of the artifacts or interferences contributed by the means of enzyme inactivation.

One must be extremely careful of water quality if the sample is mixed with any water (or steam). Organic solvents are seldom sufficiently pure to be used in aroma isolation without additional cleanup (typically distillation). Any polymer-based materials (containers or tubing) are common sources of contamination. Antifoam additives may contribute as many components to an aroma isolate as the food itself. Stopcock or vacuum greases are known sources of contamination. Bottle closures must be Teflon coated rather than rubber to prevent the closure from both absorbing some aroma components and contributing others.

Long isolation procedures may even permit fermentation to occur. Ribereau-Gayon *et al.* (1975) added sodium fluoride to crushed grapes to inhibit microbial growth. In addition to enzyme or microbially induced changes in flavor profile during the isolation procedure, one must also be aware of chemical changes. Long isolation times may permit oxidative changes to occur. Thought must be given to extracting under CO₂ or N₂. Some researchers have chosen to add antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) or ascorbic acid to their sample. High temperatures (greater than 60°C) for extended periods can promote non-enzymatic browning reactions. Reduced temperatures (e.g., with vacuum distillations) should be used whenever possible. Inadequate consideration for artifact formation during sample preparation can lead to erroneous results.

3.3 Aroma isolation

3.3.1 Principles of aroma isolation

Most of the techniques used in aroma isolation take advantage of either solubility (including adsorption) or volatility of the aroma compounds for extracting from a food matrix. Inherently, aroma compounds must be volatile to be sensed and most other food constituents have little or no volatility, thus it is logical that volatility is a common basis for separation from a food matrix. Likewise, aroma compounds tend to have greatest solubility in organic solvents while most major food constituents are water soluble, thus aroma isolates may be prepared by solvent extraction processes. The caveats to the above generalizations are that water is the most abundant volatile in most foods, complicating isolation based on volatility and lipids complicate isolation based on solvent extraction. Thus, one will find combinations of solubility and volatility are often used in preparing aroma isolates.

3.3.2 Methods of aroma isolation

As noted earlier, none of these methods will truly reflect the aroma compounds actually present in a food or their proportions. Thus, one has to have the analytical objective clearly in mind when choosing a method of aroma isolation. The objective and the food matrix will define the method chosen. For example, attempting to find a given off-aroma in a food is very different from wanting to have an overall aroma profile of that food. The first task only requires that the aroma isolation method extract the volatile component with the objectionable character. It is not important if *any other* volatile is present in the isolate other than the one causing the off-note. However, if one wants an overall view of the aroma profile, then one will likely combine several methods of aroma isolation, each isolation technique providing a part of the overall profile. With this said, a brief description of the common methods of aroma isolation will be presented including the primary strengths and weaknesses of each.

Static headspace

Direct analysis of the equilibrium headspace above a food product would appear to be an ideal method for aroma studies. It is very simple, gentle and easily automated. In the simplest form of this method, one puts a food sample into a jar, closes the jar with an inert septum (Teflon lined), allows equilibration (between the food and the sample headspace – 30–60 min), and then draws a few ml of headspace above a food into a gas-tight syringe and makes a direct injection into a GC analyzer.

The primary limitation of this method is its lack of sensitivity (Fig. 3.2). Note that the *Y* axis on all chromatograms have the same range so one might see some peaks on a headspace chromatogram if the sensitivity were increased but they are few. Since direct headspace injections into a GC

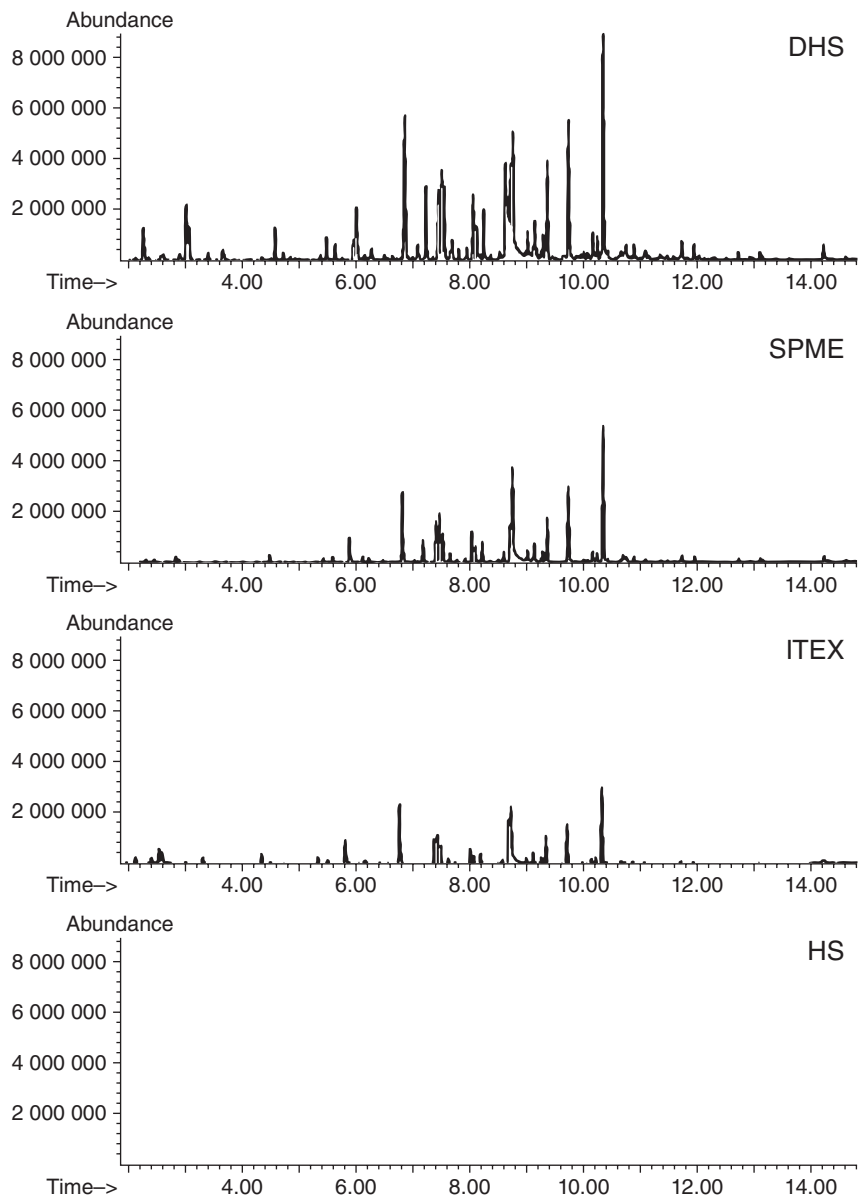


Fig. 3.2 Comparison of headspace (HS) methods for the isolation of coffee volatiles. (DHS = dynamic headspace, SPME = solid phase microextraction, ITEX = in-tube extraction) (Gil *et al.*, 2007).

Table 3.1 Minimum concentrations of a substance in a given volume of air required for GC analysis or identification by mass spectrometry

Air volume ^a	GC (g/L)	MS (g/L)
1 mL	10^{-5} – 10^{-6}	10^{-3} – 10^{-4}
10 mL	10^{-6} – 10^{-7}	10^{-4} – 10^{-5}
100 mL	10^{-7} – 10^{-8}	10^{-5} – 10^{-6}
1 L	10^{-8} – 10^{-9}	10^{-6} – 10^{-7}
10 L	10^{-9} – 10^{-10}	10^{-7} – 10^{-8}
100 L	10^{-10} – 10^{-11}	10^{-8} – 10^{-9}
1 m ³	10^{-11} – 10^{-12}	10^{-9} – 10^{-10}

Source: Schaefer (1981).

^aSample volume put into GC or MS analyzer.

analyzer are generally limited to 10 mL or less, one can see from Table 3.1 that only volatiles present at concentrations exceeding 10^{-7} g/L (headspace) will be detected by GC, and only those at concentrations exceeding 10^{-5} g/L will be adequate for mass spectrometry. Since the concentration of volatiles above a food product generally ranges from about 10^{-4} to 10^{-10} g/L (or less; Weurman, 1974), only the most abundant volatiles will be detected by direct headspace sampling. Trace component analysis will require some method of headspace concentration (e.g. adsorption using solid phase microextraction (SPME) or stir bar, or dynamic sampling using an adsorbent such as Tenax) which permits sampling of large volumes of headspace (100–1000 L) thereby compensating for low headspace concentrations.

Headspace methods have a second disadvantage in that it is difficult to do quantitative studies using them. The analytical data one receives reflect the amount of an aroma constituent in the headspace. The relationship between concentration in the headspace (equilibrium or non-equilibrium) versus the food can be very complex and must be determined experimentally.

Sensitivity of the method may be enhanced to some extent via headspace enrichment. This may be accomplished by preparing a distillate of a food product and analyzing the distillate by headspace methods. Enrichment of the headspace may also be accomplished through the addition of soluble salts to the aqueous food product. The salts tend to drive some of the organic volatiles from solution into the vapor phase. It is relevant that the enhancing effect is not similar for all volatiles (Roberts and Pollien, 2000). Thus, the use of NaCl to enrich headspace volatiles may further quantitatively distort the headspace profile.

The advantages and shortcomings of static headspace sampling dictate its applications (Wampler, 2002). It is often used in quality control situations where only major components need to be measured. Although the components measured may not actually be responsible for the flavor attributes

being monitored, if there is a good correlation between flavor quality and the component(s) measured, the goal has been accomplished. For example, Buttery and Teranishi (1963) used headspace analysis of 2-methyl propanal and 2- and 3-methyl butanal as indicators of nonenzymatic browning in potato granules. Sullivan *et al.* (1974) have used this technique to do additional work on the flavor quality of dehydrated potatoes. It is commonly used to indicate the oxidative quality of edible oils (monitor hexanal formation). Today the method finds some application as being complementary in getting a complete aroma profile for gas chromatography–olfactometry (GC/O) work. That is, a researcher may use multiple aroma isolation techniques, e.g. static headspace, purge and trap and solvent-assisted flavor evaporation (SAFE), to get a more complete look at aroma compounds in a food (Qian, 2000).

Headspace concentration methods

Headspace methods employing some type of gas stripping and concentration are commonly called dynamic headspace (DHS) sampling or purge and trap (P&T) methods. In these methods, a sample is purged with an inert gas, such as nitrogen or helium, which strips aroma constituents from the sample (Fig. 3.3). The volatiles in the purge gas must then be trapped (removed) from the gas stream. The aroma constituents may be trapped via a cryogenic, Tenax (or alternative polymer), charcoal, or other suitable trapping material/method. This method is biased in that it favors the isolation

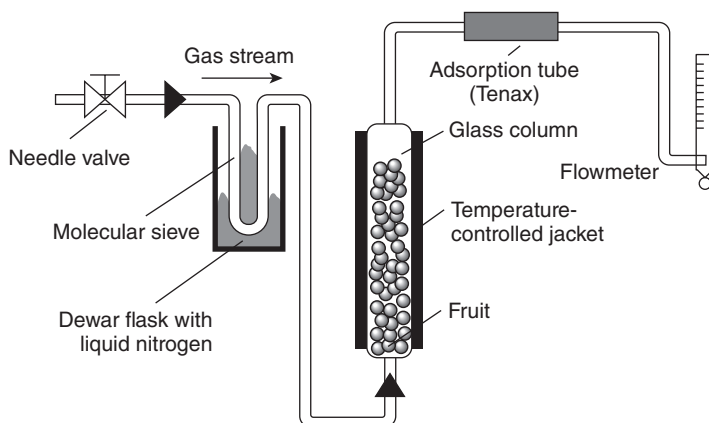


Fig. 3.3 Example of a set up for the isolation of volatiles via dynamic headspace methodology (Tenax trap with ambient pressure operation) (Reprinted with permission from Guntert, M; Krammer, G; Sommer, H; Werkhoff, P. 1998. The importance of vacuum headspace methods for the analysis of fruit flavors, in *Flavor Analysis: Developments in Isolation and Characterization*, CJM Mussinan, M Morello, eds. ACS Books 705: Washington, DC, p. 38–60. Copyright 1998. American Chemical Society.)

of constituents with the highest vapor pressure above the food. The aroma isolate is further biased due to the aroma-trapping technique. A cryogenic trap is the least selective of the traps: it will remove and thus contain virtually any aroma constituent if properly designed and operated. The primary problem with a cryogenic trap is that it will also trap water – the most abundant volatile in nearly all foods. Thus, one obtains an aqueous distillate of the product which must then somehow be treated to remove the water (again more biases enter the profile).

A Tenax trap is very widely used for aroma trapping since it has a low affinity for water and high affinity for non-polar organic compounds. Despite its wide usage, it has a low surface area and, therefore, a low adsorption capacity. Additionally, Tenax has a low affinity for polar compounds (hence it does not retain much water) and a high affinity for nonpolar compounds. An aroma compound such as hydrogen sulfide would be retained very poorly.

Buckholz *et al.* (1980) demonstrated the biases associated with Tenax traps during a study on peanut aroma. They found a ‘breakthrough’ of peanut aroma (through two traps in series) after only 15 min of purging at 40 mL/min. In an evaluation of the sensory properties of the material collected on the Tenax trap, they found a representative peanut aroma had been collected by the trap after 4 h of purging. Shorter or longer purge times did not produce an aroma characteristic of peanuts. In fact, the majority of purging conditions did not yield an aroma isolate characteristic of the sample.

The work of Guntert *et al.* (1998) found that the Tenax trapping method (ambient pressure) did not yield as true of an aroma profile as vacuum distillation (with cryo-trapping). This difference in performance may have been partially due to the operating conditions used for the Tenax system. As Buckholz *et al.* (1980) noted, operating conditions have a strong influence on the composition of the aroma isolate. While this is an ‘old’ method for volatile analysis, it is still very popular. A search on SciFinder using the terms ‘flavor AND Purge and Trap’ resulted in 97 hits in the last 5 years. The reason for its continued popularity is evident from Fig. 3.2, DHS (aka purge and trap) gave the highest recovery of volatile compounds of the methods tested.

The traditional purge and trap (DHS) has been adapted to work with small samples which can readily be automated. This modified method is known as in-tube extraction (ITEX) and various companies provide detailed information on how this method works and comparative data with other traditional methods (e.g. Leap Technologies, Gerstel, and CTC Analytics). The schematic in Fig. 3.4 illustrates this method. Analyte is transferred from the sample to a trapping medium, e.g. Tenax, by pumping the syringe up and down to draw sample headspace through the trap. After the desired number of syringe strokes, the trapping material is thermally desorbed to provide a headspace analysis.

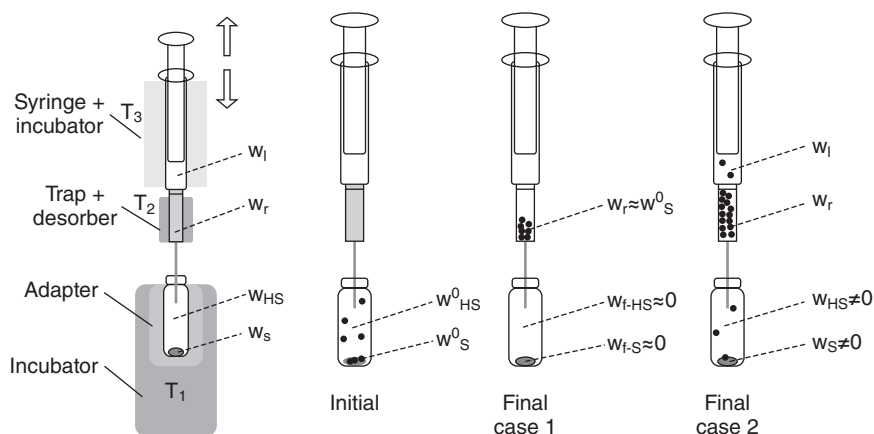


Fig. 3.4 Schematic illustrating how analyte is transferred from sample to trapping material. (T_i = temperatures in different zones of the ITEX device; w represents the mass of an analyte in: the sample (s), the headspace (HS), the trap (r) and in the syringe (I), respectively. Superscript 0 and subscript f indicate initial or final condition. In the final case it is possible to get two scenarios: in case 1 the extraction is completed before reaching the breakthrough of the trap, and in case 2 the breakthrough situation is reached before the extraction is completed). (Zapata *et al.*, 2012).

An appreciation for the relative sensitivity of ITEX isolation to traditional methods (static headspace and dynamic headspace) is shown in Fig. 3.2 (ITEX, SPME, Static Headspace and DHS sampling of a coffee powder). (The choice of a powdered coffee is odd considering that the powder would liberate few volatiles – reconstituted coffee powder would be more reasonable.) As was noted earlier, the normal direct headspace sample provided little data due to poor sensitivity. The DHS method provided substantially more data than any of the other methods. This is not unexpected since the DHS sample was obtained by purging the sample container with 200 or 300 ml of inert gas. As is obvious from this figure, ITEX was not as sensitive as DHS and about equal to SPME (which will be discussed later).

In searching SciFinder for 'ITEX AND flavor', no references came up. In using a combination of ITEX and headspace, nine references were returned. Of the nine, only two were related to flavor: one by Zhong *et al.* (2010) for the determination of chloroanisoles in wine and a paper by Zapata *et al.* (2012) reporting on the use of the method for wine volatiles. The paper by Zapata *et al.* includes substantial information on sensitivity and precision of the method. While ITEX has been around for 4 years, it has not found significant use in the flavor field. Perhaps this is since it performs about the same as the very popular SPME method. Wampler (2002) has provided a good review of DHS which is also relevant to ITEX.

Distillation methods

Distillation can be defined broadly to include high vacuum molecular distillation, steam distillation or simply heating of the food (e.g. a spice or other essential oil-bearing material) and sweeping the 'distilled' aroma constituents into a GC analyzer. High-vacuum distillation may be applied to pure fats or oils (use a thin film and high vacuum), solvent extracts of fat-containing foods or aqueous-based foods (e.g. fruit). Since fats and oils are essentially anhydrous, additional extractions (or sample manipulations) would not be required to remove any co-distilled water. The use of high vacuum distillation for the isolation of volatiles from solvent extracts has been frequently used to provide good quality extracts for aroma extraction dilution assays (AEDA; Engel *et al.*, 1999). This distillation process would likely require an additional solvent extraction step since diethyl ether is commonly used as the extracting solvent and it will extract some water as well.

The most common steam distillation method employs simultaneous distillation/solvent extraction (SDE may be called a Likens-Nickerson method). This is one of the oldest methods for obtaining aroma isolates. Searching SciFinder on the key words 'SDE AND flavor' returned 181 hits (last 5 years). It is interesting that 150 of these hits were from Chinese authors: the technique is very popular there. Chaintreau (2001) has provided a very good review of this method and its evolution. A traditional atmospheric pressure system is shown in Fig. 3.5. Vacuum SDE systems

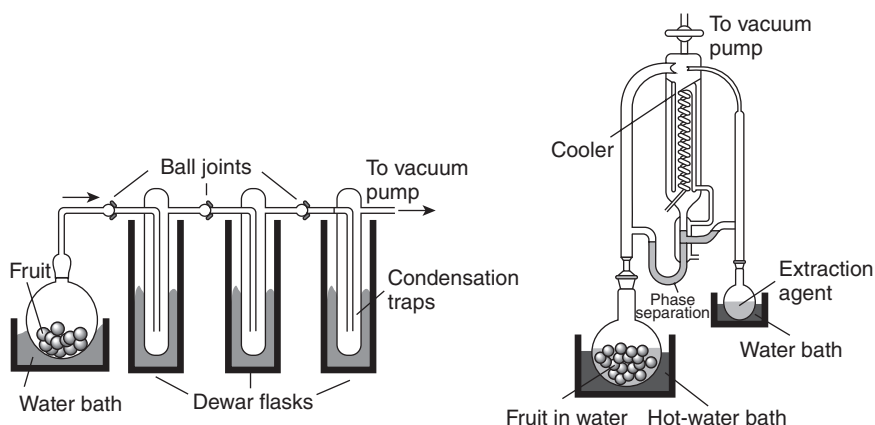


Fig. 3.5 Distillation equipment used to isolate volatiles from foods. Left – high vacuum distillation of volatiles from an aqueous food. Right – simultaneous distillation/extraction apparatus. (Reprinted with permission from Guntert, M; Krammer, G; Sommer, H; Werkhoff, P. 1998. The importance of vacuum headspace methods for the analysis of fruit flavors, in *Flavor Analysis: Developments in Isolation and Characterization*, CJM Mussinan, M Morello, eds. ACS Books 705: Washington, DC, p. 38–60. Copyright 1998. American Chemical Society.)

have been developed but require joints that are air-tight and all parts of the apparatus must be under rigid temperature control.

Over time various modifications in the method have occurred, for example, Ferhat *et al.* (2007) developed a microwave SDE that allowed very rapid extraction and concentration (30 min in total). The method was not as efficient as the standard SDE but was considerably faster. In all of the SDE methods, the aroma profile ultimately obtained is influenced by volatility of the aroma compounds (initial isolation), solubility during solvent extraction of the distillate, and, finally, volatility again during the concentration of the solvent extract. The aroma isolate prepared by SDE contains a good sampling of the volatiles in a food, but their proportions may only poorly represent the true profile in a food. The popularity of this method comes from the fact that medium to low boilers are recovered well and a liquid isolate is obtained. This liquid isolate is quite concentrated which facilitates mass spectrometry (MS) work or repeated injections for further studies.

Distillation, as defined above, also includes direct thermal analysis techniques. These techniques involve the heating of a food sample in an in-line (i.e., in the carrier gas flow of the GC) desorber. Generally, aroma compounds are thermally desorbed from the food and then cryofocused to enhance chromatographic resolution. This technique has been used for a number of years for the analysis of lipids and was later modified to include aqueous samples (Dupuy *et al.*, 1971; Legendre *et al.*, 1979). Aqueous samples were accommodated by including a water trap after the desorption cell. This general approach has been incorporated into the short path thermal desorption apparatus discussed by Hartman *et al.* (1993) and Grimm *et al.* (2002). More recent applications are the analysis of complex flavorings (Ibanez and Sola, 2010) and of tea leaves (Zhu *et al.*, 2008).

A schematic of this apparatus is shown in Fig. 3.6. In the schematic shown, a sample of food is placed in the desorption (sample) tube and quickly heated. The volatiles are distilled into the gas flow which carries them into the cooled injection system where they are cryofocused prior to injection into the analytical column.

The issue of water in the sample often limits sample size even when it is a minor component of the food. Virtually all of these methods/instruments require cryofocusing prior to GC and small amounts of water will tend to freeze the cryotrap blocking the carrier gas flow. Thus, the sample size (and therefore, sensitivity) is often limited by the moisture content of the sample (the method is applied to samples <5% moisture; Rothaupt, 1998).

The primary bias inherent in this approach is again the relative volatility of the aroma constituents. Additional concerns involve the technique used to remove water from the sample (if aqueous-based sample) and the potential for artifact production due to heating of the sample. There is a substantial body of information demonstrating aroma formation (in this case

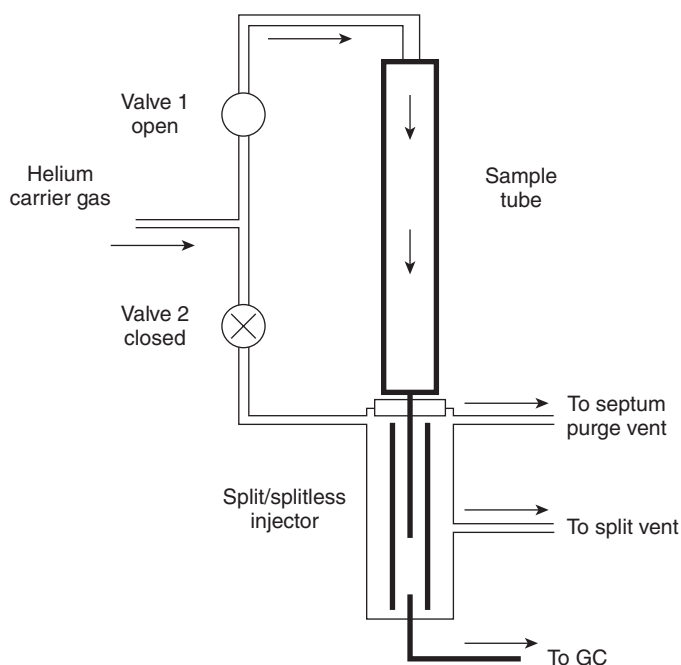


Fig. 3.6 Short path thermal desorption apparatus (Grimm *et al.*, 2002).

artifact formation) due to heating. Some reactions proceed rapidly at temperatures as low as 60 °C. Therefore, the aroma profile can be greatly altered via the addition of artifacts due to heating the sample during isolation.

Solvent extraction

One of the simplest and most efficient approaches for aroma isolation is direct solvent extraction. The major limitation of this method is that it is most useful on foods that do not contain any lipids. If the food contains lipids, the lipids will also be extracted along with the aroma constituents, and they must be separated from each other prior to further analysis. Aroma constituents can be separated from fat-containing solvent extracts via secondary techniques such as molecular distillation, steam distillation, and purge and trap.

A second consideration in solvent extraction is for solvent purity. Solvents must be of the highest quality which often necessitates in-house distillation prior to use. One must be mindful that various qualities of solvents can be purchased and GC grade is highly recommended (not high-pressure liquid chromatography (HPLC) or other quality). Furthermore, a reagent (solvent) blank must always be run to monitor solvent artifacts irrespective of the quality of the solvent.

Solvent extraction can be as simple as putting a food sample into a separatory funnel (e.g. apple juice), adding a solvent (e.g. dichloromethane) and shaking. The solvent is collected from the separatory funnel, dried with an anhydrous salt and then concentrated for GC analysis. Alternatively, the process may be much more costly and complicated involving, for example, a pressure chamber and supercritical CO₂ (Jennings and Filsoof, 1977). Supercritical CO₂ has the advantage of being very low boiling (efficiently separated from extracted volatiles), leaves no 'residue' to interfere with any subsequent sensory analysis, penetrates food matrices and its solvent properties can be altered through temperature and pressure changes or chemical modifiers (e.g. methanol). Negative aspects of this solvent include its high cost due to pressure requirements, small sample sizes (most commercial extractors) and its highly non-polar nature (without modifiers). The use of modifiers such as methanol negates some of the advantages noted earlier. Morello (1994) has provided a good example of its use and thoughtful discussion of the technique.

The biases imposed on the aroma profile by solvent extraction relate to the relative solubility of various aroma constituents in the organic/aqueous phases. Cobb and Bursey (1978) have made a comparison of solvent effect on recovery of a model aroma system from 12% (v/v) ethanol in water (Table 3.2). Recoveries of aroma constituents were low and variable, depending on the solvent chosen and aromatic component being extracted.

While it is obvious that even a simple solvent extraction introduces substantial bias into an aroma profile, combining solvent extraction with another technique (e.g. to separate aroma components from extracts containing lipids) adds more bias. For example, applying a distillation technique to a solvent extract that contains lipids selects for the most volatile components (now from an oil phase). Despite these considerations, this combination (solvent extraction followed by distillation – SAFE method) has been

Table 3.2 Recovery of model compounds from an alcohol-water (12% v/v) system

Compound extracted	Solvent recovery in % ^a			
	Freon II	Dichloromethane	Ether	Isopentane
Ethyl butanoate	66	43	–	16
2-Me-1-propanol	34	55	22	32
3-Me-1-butanol	63	66	50	48
1-Hexanol	85	67	23	38
Benzaldehyde	83	54	18	20
Acetophenone	53	41	34	20
Benzyl formate	75	56	21	25
2-Phenethyl butanoate	46	48	25	17
Me anthranilate	62	59	57	27

Source: Cobb and Bursey (1978).

^aBatch separatory funnel extraction, 757 mL of model system extracted 6 × 50 mL solvent.

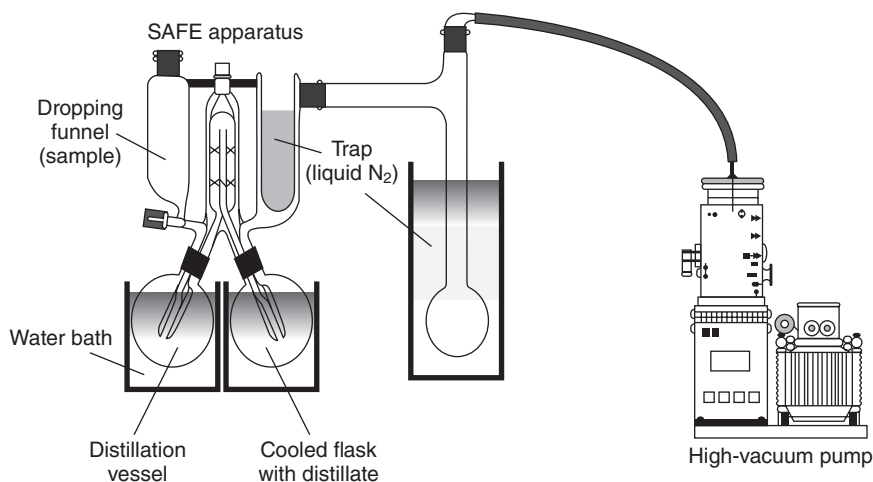


Fig. 3.7 Solvent-assisted flavor evaporation system for the isolation of aroma compounds from solvent extracts (Werkhoff *et al.*, 2002).

widely applied due to its efficiency at isolating a broad range of volatiles. The distillation was originally done using a simple high vacuum system (Fig. 3.6) but this is fairly tedious due to the requirement that the solvent be slowly added to the high vacuum flask. Engel *et al.* (1999) have developed a much more rapid and yet highly efficient SAFE distillation head that is now widely used (Fig. 3.7).

Sorptive extraction

Sorptive extractions in various forms (e.g. solid phase extraction (SPE), SPME, stir bar Sorptive extraction (SBSE) and polydimethylsiloxane (PDMS) foam) are relatively new techniques for the isolation of food aromas. SPE has found application in sample preparation for free (Du *et al.*, 2010), or glycosidically bound (Gomez Garcia-Carpintero *et al.*, 2012) volatiles and taste compounds (Charve, 2010). In SPE extraction, a liquid sample is passed through a SPE cartridge: the non-absorbed components pass through the cartridge and the adsorbed would be subsequently eluted by a solvent wash. Essentially, one gets non-polar substances adsorbed on the SPE packing and polar substances pass through the cartridge.

A very specific application of this method was published for the selective isolation of mercaptans from wine (Mateo-Vivaracho *et al.*, 2009). In this example, an SPE cartridge was prepared which contained *p*-hydroxymercurybenzoate. Red wine was passed through the cartridge and then washed with both polar (water/methanol) and non-polar (pentane or pentane/ether) solvents to remove all volatiles other than the thiols. The remaining thiols were eluted with 1,4-dithioerythritol in an organic solvent. To provide

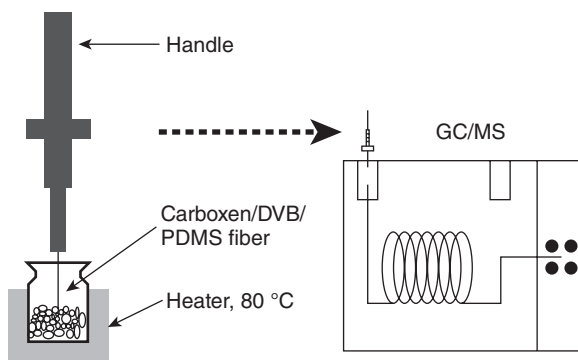


Fig. 3.8 Schematic of a SPME device (Grimm *et al.*, 2002).

a comparative measure of the use of this technique, a search on SciFinder (2007–present) came up with 30 hits on the key words ‘flavor or aroma AND SPE’.

SPME was first used in environmental work (Pawliszyn, 2001). Since then, it has become the most widely used technique for the analysis of volatiles in foods (a search on SciFinder (2007–present) returned 393 hits on the key words ‘flavor or aroma AND SPME’). In this technique, an inert fiber is coated with an adsorbent (several choices). The adsorbent-coated fiber is generally placed in the headspace of a sample (it may be placed in the sample) and allowed to adsorb volatiles. The ‘loaded’ fiber is then thermally desorbed into a GC carrier flow and the released volatiles are analyzed. A schematic of the device is presented in Fig. 3.8. The coated fiber is a modified syringe where the needle is retractable and coated with adsorbent. The retractable feature affords protection to the fiber against physical damage and contamination.

Similar to all of the other methods described thus far, SPME affords a certain view of the volatile composition of the food. When used as an equilibrium technique, the volatile profile one obtains is strongly dependent upon sample composition and careful control of all sampling parameters is required. While Harmon (1997) notes that the method can give excellent results, Coleman (1996) and Marsili (2002a) caution that the fibers have a definite linear range and competition between volatiles for binding sites can introduce errors. Nongonierma *et al.* (2006) have provided a very comprehensive discussion of absorptive methods and should be required reading for anyone planning to use these methods.

The basic SPME method has been modified to include an SBSE version. In this method, a coating of adsorbent phase is placed on an inert stir bar (glass). The coating acts as an extracting solvent as opposed to an adsorbent, thus, phase volume as opposed to surface area is important. The stir bar may be placed in the sample headspace or immersed in the product to be

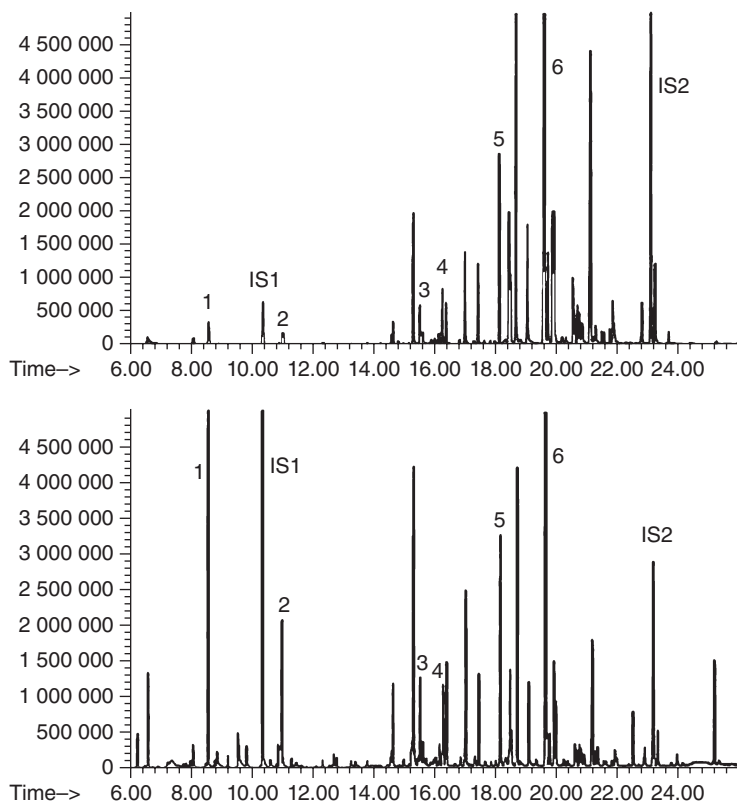


Fig. 3.9 A comparative analysis of the headspace above a lotion by SPME (top) and Stir Bar (bottom) (Natascha and Labor, 2012).

analyzed, allowed to come to equilibrium with the sample being analyzed (30–240 min), rinsed with water, dried and then either thermally desorbed into a GC or solvent extracted. It has been found that food samples containing fat levels below 2–3%, or alcohol levels below 10% can be directly extracted (by immersion) with this technique. The availability of larger extraction phase volumes results in better quantitative data as well as greatly improved sensitivities compared to SPME (Fig. 3.9). The speed and simplicity of this method, and refinements over SPME, make it quite attractive.

The most recent adaptation of this methodology involves using a foamed PDMS as an absorbent (Marsili *et al.*, 2007). One can either use the loaded foam trap as part of a purge and trap device or couple it with a commercial thermal desorption unit (TDU). In the latter case one puts a sample into a microvial (ca. 250 μL), places the loaded microvial into a TDU tube, and then heats it such that the volatile contents of the vial pass through the foam. The foam traps volatiles while allowing solvent to be vented. Further

heating then desorbs the volatiles onto the GC column. Bazemore (2011) has provided a detailed description of this method plus examples.

Concentration for analysis

Some of the methods for aroma isolation discussed above produce dilute solutions of aroma compounds in an organic solvent (e.g. distillations followed by solvent extract, and direct solvent extraction). The solvent needs to be partially evaporated to facilitate GC analysis. Evaporative techniques take advantage of the difference in boiling point between the flavor compounds and solvent. Thus, low boiling point solvents are commonly used in the isolation process to facilitate concentration (e.g., pentane, dichloromethane, diethyl ether and isopentane).

A disadvantage of evaporative techniques is that volatiles of interest may be lost by co-distillation. Unfortunately, the loss of different components will not be the same nor readily predictable. Therefore, quantitative results may be in error even when multiple internal standards are employed. Since the flavor isolate typically contains a small amount of water (from the food product or distillate), care must be taken to remove water prior to concentration. This is typically done via the addition of desiccants (e.g., anhydrous magnesium sulfate or sodium sulfate). Failure to remove the water will result in steam distillation of the flavor components and substantial flavor losses during the concentration step.

Equipment used for evaporative concentration may be very simple or quite sophisticated. The simplest approach is to evaporate the solvent from an Erlenmeyer flask using gentle heating under a stream of nitrogen. Assuming one is using a low boiling solvent and the most volatile flavor constituents are not of interest, this is a very suitable method for concentration. However, if one is interested in the low boiling volatiles, a reflux system should be employed. A very efficient reflux set up is the Kuderna-Danish. A high reflux ratio provides effective solvent removal with minimal loss of volatile flavors. A more efficient system is to use a spinning band fractionating column. Proper care in operation will result in nearly complete recovery of volatiles that differ in boiling point from the solvent by less than 1 °C.

Aroma isolation summary

Since every method preferentially selects those aroma constituents that meet certain physical or chemical criteria (e.g., solubility, or volatility), one must 'make do' and compensate for having a very biased analytical view of the aroma constituents in a food product. This biased view does not mean that it is useless or even of lesser value than a truly accurate picture. We need to choose our methodology wisely so that we measure the aroma components we need to monitor to solve our problem, i.e. they are contained in the aroma 'view' we *consciously* select. Furthermore, one must recognize that the most commonly used approaches in the literature may not be the best or even suitable for a given task. A particular task requires

a unique method. The frequency of a method appearing in the literature is more often linked to the size of the research group than anything else. A given research group may be large and doing similar work and thus, their particular methodology appears frequently. Also, individuals have certain biases – no two researchers will approach the same problem in the same manner. Thus, every aroma isolation task should be approached as a unique analysis.

3.3.3 Analysis of aroma isolates

The analytical method used to analyze an aroma isolate depends on the task at hand. If one wishes to determine the amount of an aroma compound(s) in a food, GC may suffice. If one is looking for odorous compounds in a food (desirable or undesirable), then one will use GC/O. If one wishes to identify the aroma compounds in a food, this would require GC and MS (or GC/O/MS). While other instrumental methods may also be applied (e.g., infrared (IR) or nuclear magnetic resonance (NMR)) the bulk of aroma research is done by these three methods.

Pre-fractionation

Traditionally some prefractionation method was occasionally used prior to GC analysis. As column resolution has improved and rapid scanning MS has evolved (permitting peak deconvolution without resolution), these techniques have been used less. Some of the more common methods to pre-fractionate flavor isolates prior to GC analysis have included acid/base separations, HPLC, silicic acid column chromatography and preparative GC. One can find a discussion of these methods in the literature such as Reineccius (2006).

Gas chromatography

Aroma research has benefited tremendously from the development of GC. In 1963, only 500 aroma compounds had been identified in foods. The development of GC in the mid-1960s and subsequent application to flavor research has resulted in over 8000 compounds being identified to date. Gas chromatography is ideally suited to aroma studies since it has excellent separation powers (up to 200000 plates/column) and extreme sensitivity (picogram detection levels). Resolution and sensitivity are essential for analysis of the complex aroma isolates encountered routinely in flavor work. The primary disadvantage that capillary columns have brought is their low sample capacities. Sample capacity is needed if the analyst wishes to collect a component for further work, e.g., NMR or IR. Capacity is enhanced through the use of thick phase coatings (can work with up to 500 ng/component).

Some of the most difficult flavor studies need to use two-dimensional GC (or comprehensive GC). Two-dimensional GC involves breaking a GC

run into segments and then rechromatographing each trapped segment on a second column (Ramos, 2009). These systems typically permit the collection of a selected part of *several* GC runs which can improve on sensitivity. GC as a technique will not be discussed in this book since there are many excellent offerings on this subject (McNair and Miller, 2009).

GC/O or GC-MS/O

GC/O and GC-MS/O are techniques uniquely applied to aroma studies. In these techniques, the nose is used as a GC detector. The GC system may be set up such that the column effluent is split so that a portion of the effluent goes to a sniffing port and the remainder goes to a GC detector (flame ionization or an MS detector), or the GC run may be made by passing *all* of the GC column effluent to the nose one time, the column is then connected to the instrument detector and a second run made. This latter alternative provides the maximum amount of sample to the nose one time and the flame ionization detector (FID) or MS detector the second time enhancing the performance of each detector. The primary disadvantages of making two separate GC runs is analysis time and the difficulty of determining which GC peak is responsible for what odor. In 'busy' chromatograms, there may be several GC peaks in the vicinity of the retention time of a given odor thereby making it difficult to assign an odor to a specific GC or MS peak.

It is generally desirable to use a GC-MS/O method (as opposed to a GC/O method). Most commonly, the analyst is interested in the identification of components that have odor (may be a desirable odor or an off-odor). Unfortunately, if one does GC/O work and is able to assign a given odor to a given GC peak, it is problematic to determine which GC peak is which MS peak. While one may think it is simple to make this assignment, the MS works under a vacuum and this changes the GC column elution time even when using the same GC column in the GC and MS. A second complication is that an FID detector gives different responses to compounds than an MS detector. This means that retention times may shift *and* the analytical profiles (peak heights) change between a GC run and a GC-MS run. If one can get an odor profile and an MS profile at one time, the identification of odorants is greatly simplified.

GC/O produces what is called an 'aromagram': a listing of the odor character of each peak in a GC run. Since more than one sniffer is used in this analysis (minimize data error due to any specific anosmia), data are often presented in table form (e.g. Table 3.3). These data are extremely valuable since they indicate which GC peaks have given odors. This information is useful in directing where an analyst should focus his/her efforts. For example, if one is studying a particular off-flavor, once the offending odorants are located in the aromagram, efforts can then be directed towards the identification of the compound, or compounds, of interest. There is no need to identify all of the GC peaks, only selected GC peaks. The

Table 3.3 Sensory descriptions (and compound identifications) of GC peaks noted by three different sniffers. Aroma isolate was obtained by dynamic headspace analysis from Parmesan cheese

GC peak number	Compounds	Male subject	Female subject 1	Female subject 2
1	Acetaldehyde	Strong pungent	Strong pungent	Strong pungent
2	Propanal	Pungent	n.d.	n.d.
3	2-Methylpropanal	Strong pungent	Stale bread crust	Strong pungent
4	Butanal	Weak pungent	Alcoholic, fruity	Weak Parmesan
5	3-Methylbutanal	Strong malty	Strong stale	Parmesan-like
6	2-Methylbutanal	Strong cocoa	n.d.	n.d.
7	Diacetyl	Strong buttery	Strong buttery	Diacetyl
8	2-Butenal	Weak malty	n.d.	n.d.
9	2,3-Pentadione	Weak buttery	Warm buttery	n.d.
10	Dimethyl disulfide	Putrid, cabbage	Weak buttery	Weak sulfury
11	Ethyl butyrate	Strong fruity	Sweet, fruity	Strong esters
12	Hexanal	Green, grassy	Weak green	Rubbery
13	Isoamyl alcohol	Fruity, alcoholic	n.d.	n.d.
14	Ethyl pentanoate	Weak fruity	n.d.	n.d.
15	2-Heptanone	Strong fruity	n.d.	Weak fruity
16	Heptanal	Green	n.d.	n.d.
17	Ethyl hexanoate	Strong fruity	Sweet candy	Ripe banana
18	2,6-Dimethylpyrazine	Baked, fried potato	n.d.	n.d.
19	Dimethyl trisulfide	Strong cabbage	Strong putrid	Heavy gassy
20	Methional	Strong baked	Cooked cabbage	Burnt, browned
21	Butyric acid	Cheesy	Cheesy	Dirty socks
22	Phenylacetaldehyde	Strong floral	n.d.	n.d.

Source: Qian and Reineccius (2002).

n.d., not detected.

aromagram may show that the most important area of the chromatogram is where there are *no* GC peaks. This would suggest going ‘back to the drawing-board’ and trying to do a better job in isolation.

GC/O may be criticized as being a subjective method yielding inconsistent results. If one looks at the descriptors presented in Table 3.3, it is clear that while there is substantial agreement, there also is frequent disagreement amongst assessors. These types of results are common despite the use of well-trained subjects (consistent with sensory panel results). One makes an effort to minimize sensory fatigue by limiting the time a subject is asked to do sniffing (may limit to 20 min) and uses only experienced panelists.

Occasionally GC/O data may be misleading due to concentration effects. Odor characteristics of some flavor compounds tend to vary as a function of concentration. For example, skatole (3-methyl indole) has a characteristic fecal odor at high levels but becomes pleasant, sweet, and warm at very low levels. Fortunately, there are not many aroma compounds exhibiting such a large concentration-dependent odor character. Additional errors in the perceived intensity of a GC peak can be due to masking in mixtures (i.e. situations where components are not resolved on the GC column). Finally, in-line condensation of some compounds can result in persistent background odors. The splitter and the transfer lines should be well conditioned and adequately heated to render them odor free. Despite these potential pitfalls, GC/O is an invaluable tool to the flavor chemist and has found frequent application in this field (Leland *et al.*, 2001).

MS

The very high sensitivity inherent to MS (10–100 pg) and compatibility with GC makes a GC-MS combination extremely valuable. Mass spectrometers may be classed as low-resolution (LR) or high-resolution (HR) instruments. The LR instruments provide mass measurements to the closest whole mass unit. Since many combinations of elements may give the same unit mass, LR MS may provide the molecular weight of a compound but does not provide elemental composition. HR instruments provide sufficiently accurate mass measurements to permit determination of elemental composition. The majority of flavor work in the past has utilized LR instruments; however, HR instruments are becoming more common.

Mass spectrometry is generally used in the flavor area to either determine the identity of an unknown or to act as a mass-selective GC detector. As mentioned, MS as an identification tool is unequaled by other instruments. The systems have largely become turn-key systems that require little or no operator expertise. If the operator can do GC, he/she can do MS. Comprehensive MS libraries and efficient searching algorithms make identification simple; however, herein lies a danger. An MS will provide a best match (suggest identity) for any unknown irrespective of the validity of the match. The neophyte often accepts the proposed identifications without question and obtains incorrect identifications. It is essential that any MS identifications be supported by other data, most commonly GC retention data.

Obtaining full mass spectra to generate mass chromatograms has been problematic with older quadrupole MS since they require significant time to scan a typical mass range. Time of flight (TOF), ion trap and newer quadrupole MS instruments can collect spectra much faster (ion trap about 10–15 spectra/s and TOF up to 500 spectra/s) and are more suited to capillary column GC and more recently, fast GC. The TOF instrument uniquely offers the ability to take a large number of spectra across a GC peak and then to sum groups of spectra thereby reducing noise, improving sensitivity

and detection limits. The ability to take many spectra per unit time offers another advantage in facilitating the deconvolution of mixed spectra, i.e. resolving the MS data from one compound from a mixture of compounds that coelute. While the analyst has traditionally been nearly required to resolve one compound from another to obtain an MS identification, newer MS systems with the proper software are able to do identifications and quantification without the need for peak resolution.

3.3.4 Electronic noses (Enose)

The goal of finding an instrument that functions as our olfactory system dates back to 1961 (Moncrieff, 1961). Thus, over 50 years have passed since this initial effort. The term 'electronic nose' was coined by Gardner (1989) who described an instrument consisting of a set of chemical sensors interfaced with pattern recognition software to give sample discrimination based on volatiles. The technique gained widespread visibility in the early to mid-1990s when commercial companies (e.g., AlphaMOS, Aromascan and Neotronics) made instruments available. The technique was hailed as a remarkable breakthrough in instrumentation for studying the flavor of foods. However, over time researchers have determined where this technique has value and where it does not. The following section will provide an overview of the technique. The interested reader is encouraged to read more detailed reviews provided by Bonnefille (2011), Dymerski *et al.* (2011) and Goodner and Rouseff (2011).

The Enose functions by analyzing a sensor array response to a complete aroma, i.e., there is no separation of aroma components. The sensor array response to any given aroma is correlated (pattern recognition software) to sensory panel data. Using neural network software and many training samples, the system determines a sensor response pattern that is representative of a fresh milk versus a spoiled milk, for example, thereby providing sample discrimination. The primary developments in the evolution of this technique have been in the sampling systems, sensor arrays and neural network capabilities. Over time improvements have been made in all aspects of the technique hardware and software and today about 20 companies offer Enose instruments.

The sensors are key components of this system. There are numerous types of sensors, the most common being semiconductor gas sensors (metal oxides), surface acoustical wave devices, conducting polymers and MS-based sensors. In the current instruments, it is common to combine sensor types to gain a wider range in responses.

At first glance the technique appears to be ideal in that there is no need for separation of volatiles. This can result in very rapid analysis. Also, it seems to be based on a process similar to the human olfactory system in that both the electronic nose and human olfactory systems consist of a host of receptors (sensors) and yield a pattern of response to any given aroma.

The brain, in the case of the human, and the computer, in the case of the electronic nose, make judgments based on a pattern recognition process as to the aroma and its quality. Thus, the speed is attractive and theoretical foundation appears to be rational.

The primary weakness of such instrumentation is that one has no clear idea of what the instrument is responding to in making a judgment. One chooses to evaluate some sensory parameter (e.g. staling or rancidity during storage) and then asks the instrument to develop a means of predicting that sensory parameter. In the end, the instrument uses some stimuli/response pattern to make a prediction but, with the exception of MS or fast GC sensor systems, we have no idea *what* the instrument was responding to. For example, in a storage study where one is determining the sensory quality of coffee (e.g. oxidized flavor) and obtaining electronic nose correlations, the instrument could be responding to CO₂ as opposed to any oxidized flavor. As long as CO₂ out-gassing is correlated to lipid oxidation, the relationship is good. If it is not correlated in all situations, then the relationship is likely to be invalid in other systems or studies. The point is that the human brain uses causative input/patterns to make judgments while the electronic nose uses patterns that are not necessarily causative and may be only casually or haphazardly related.

Another potential concern for this type of instrumentation is that some sensors respond to water vapor or CO₂ and these responses may dominate or unduly alter sensor patterns. The sensors also deteriorate with time (or can be 'poisoned') changing response which makes shelf-life studies problematic or frequent calibration necessary. These weaknesses largely relegate the technique to discriminating between samples as opposed to research studies. Despite these words of caution, the technique has substantial potential to be applied in our field. Publications by Marsili (2002b), Bonnefille (2011), and Dymerski *et al.* (2011) illustrate some of these applications.

3.3.5 Specific analyses

Key components in foods

Patton and Josephson (1957) proposed estimating the importance of an aroma compound to the sensory character of a food by calculating the ratio of the concentration of a compound in a food to its sensory threshold in that food. This ratio is known now as the odor activity value (OAV) (also as: odor value, odor unit, flavor unit, or aroma value). They suggested that compounds present above their sensory threshold concentrations in a food are significant contributors to its aroma, whereas those occurring below their threshold are not. Patton and Josephson (1957) proposed this method as a guide 'that may not hold in some instances'.

Since the introduction of the OAV concept, various research groups have developed their own approach to screen for 'significant' odorants in food.

AEDA (Ullrich and Grosch, 1987), CHARM (Acree *et al.*, 1984), OSME (McDaniel *et al.*, 1990), and NIF (nasal impact frequency) or SNIF (surface of nasal impact frequency; Pollien *et al.* 1997; Chaintreau, 2002). All depend upon subjects sniffing compounds eluting from a GC column as single compounds and using resultant data to estimate sensory importance. Mahatantatawee and Rouseff (2011) have provided a very thorough discussion of these methods.

In our opinion, there is no clear choice in methodology to use when screening for key aroma components of a food. All methods are complicated by biases in preparing aroma isolates for analysis, by anosmia amongst panelists, human variability and bias, as well as problems interpreting the contribution of an aroma compound singly and out of the food matrix as opposed to being in a food and in a complex aroma mixture (Frijters, 1978; Mistry *et al.*, 1997). These weaknesses are being acknowledged but there is no alternative, ‘correct’ methodology. If one is to do sensory studies to determine what aroma compounds are truly needed to reproduce the aroma of a food, there must be some preselection of aroma compounds to use in the sensory studies. It is impossible to try all possible combinations of all of the volatiles in a food.

Aroma release during eating

Research on determining the key aroma compounds responsible for aroma has brought us closer to reproducing the aroma of a food. However, it has become clear that there are other factors playing into sensory perception of a flavor for one can put exactly the same flavoring into two different food products and find that they have different aromas. It was postulated that this effect is due to how aroma compounds are released from a food. Thus, analytical methods for analyzing aroma release during eating have been developed.

Taylor *et al.* (2000) developed a method to measure aroma release during eating using an atmospheric pressure ionization (API) MS. Grab and Gfeller (2000) developed an alternative system based on an ion trap MS and Yeretzian *et al.* (2000) used proton transfer reaction mass spectrometry (PTR-MS). Buettner *et al.* (2002) have used other methodologies (not real time MS) including analyzing the amount of an odorant remaining in a food and spit-off during eating (spit off-odor measurement – SOOM) and a breath trapping technique (exhaled odor measurement) to quantify the aroma compounds that have been released from food during eating (Lasekan *et al.*, 2009).

The systems designed to measure aroma compounds in the breath during eating in real time use similar sampling methods: they primarily differ in type of MS used. Taylor *et al.* (2000) introduced breath into the MS ionization source by a venturi effect created by high nitrogen gas flows in the MS ion source. Volatiles in the breath were ionized in the MS ion source by the corona discharge pin and drawn into the MS analyzer. The

MS then monitored individual ions characteristic of the compounds of interest, giving us a measurement of the compounds in the breath that result in perception.

This technique has found very broad usage by numerous groups. As a result we have come to understand a great deal about what controls flavor release from foods as well as some of the linkages between stimuli and perception. Researchers have found that aroma release is influenced by the chemical interactions that occur within a food (thereby altering vapor pressure), the physical properties of the food, and the eating process. Unfortunately, the interactions that occur are difficult to quantify and mathematically model (Roberts and Taylor, 2000): we know they happen but cannot accurately predict them. Also, the latest results suggest that sensory perception is multi-modal – we must not only consider the aroma portion of a food, but the taste, texture, and visual stimuli. Thus, it appears that understanding aroma release from a food is not the final answer in understanding human perception, we must also understand the cognitive and interactive aspects of this sense.

3.4 Taste

3.4.1 Introduction to taste analysis

Historically, flavor chemists (and the flavor industry) have largely ignored taste, considering it largely irrelevant, or not their problem. As time has gone on, a growing body of research has pointed to the importance of taste in defining flavor: taste and olfaction are *both* essential to creating the desired perception. Beyond the idea of taste contributing to creating a desired perception, taste can modify perception in potentially undesirable ways through interactions with volatiles or present objectionable properties in themselves, e.g., bitterness. Thus, the study of taste components has become more main stream. However, since taste has largely been ignored by the research community for the last 40+ years, the developments of tools for its study lag behind those for olfaction.

It is generally considered that taste is due to water-soluble, non-volatile compounds in foods. However, these stimuli can also be volatile (i.e., acetic acid) or have very low water solubility (i.e., quinine). Typically taste compounds are larger, more complex molecules than those that contribute to olfaction, which complicates their study. Being largely water soluble, organic solvent extraction techniques can be limited in application and the utilization of water or more polar solvents (i.e. methanol) is common. Due to their nature (semi- or non-volatile), we lose volatility as a principle for isolation prior to analysis. Furthermore, we cannot use GC (without derivatization) and thus we lose a very powerful separation technique and have to work with LC which at its best is inferior to GC. A final problem is that we lack comprehensive LC/MS libraries for the identification of potential tastants.

With that said, substantial progress has been made in developing methods for taste substances. For example, instead of volatility we can use either membrane or gel permeation as an isolation tool, or we can use adsorption methods separating the non-volatiles based on polarity.

This chapter will not present any discussion of the common tastants, i.e., those compounds traditionally considered responsible for sweet, sour, tartness, and umami. We have well-established methods for these well-known compounds so there is no need for a discussion here. Instead we will focus on more complex measurements of tastants, for example, bitterness or the determination of compounds that influence flavor in some manner.

3.4.2 Sample preparation/non-volatiles isolation

Tastants must be extracted from foods prior to any analytical separations. While different approaches can be taken depending upon the researcher's preferences and experimental goals, we will describe some commonly used techniques/protocols.

Unless the food is a liquid, it must first be extracted with a solvent: the solvent may be pure water, methanol or ethanol, an aqueous:alcohol dilution (i.e. 80:20 solvent:water) or an acidified solvent extract. (The 80:20 dilution comes from the fact that this ratio will extract lower molecular weight food components that one would expect to be taste active but not larger molecular weight food polymers.) As noted, the solvent of choice may be acidified (often 1% formic acid) to enhance the extraction of ionic compounds. The treatment hereafter depends upon the food being analyzed and goals of the study. One may directly analyze the sample extract or use size fraction (gel permeation chromatography (GPC) or membranes), SPE or HPLC for additional prefractionation. Since the next steps are sample and goal dependent, a view of subsequent sample treatment is left to specific examples presented later in this chapter.

3.4.3 Principles of separation and instrumental analysis

HPLC-MS and perhaps GC-MS are used for the separation of non-volatiles. GC-MS is used only when a volatile derivative is made to determine the more polar non-volatiles, e.g., sugars and organic acids. The principles of HPLC separations are readily found in numerous texts or reference books on HPLC and thus, are not discussed here (e.g., Snyder *et al.*, 2010; Ferrer and Thurman, 2009).

3.4.4 Sensory-directed taste research

Taste research has followed a similar pathway to research on volatiles (olfaction), i.e., used sensory evaluation to determine compounds of importance for further study. The steps in this process are: extraction of

non-volatiles from the food matrix, separation by appropriate methods, fraction collection and then tasting to determine compounds of interest.

There are some significant differences in how sensory is used in taste vs. olfactory work due to the non-volatiles being collected from an HPLC effluent in a fraction collector. This potentially allows numerous evaluators to evaluate a fraction or provide the evaluators the time to evaluate fractions more accurately: they generally are not evaluated by a human subject in real time as volatiles are in GC/O methods. Furthermore, the fractions collected are adjusted in strength (may be diluted [if too strong] or concentrated and then diluted [if too dilute]) to reflect the concentration in the real food sample, thus one is tasting the fractions at realistic concentrations. A further advantage is that individual fractions can be combined, adjusted in concentration to that of the original food, and then tasted to determine what amount of a given sensory property is provided by a subgroup of fractions. The ultimate approach is to add fractions to the real, whole food and determine if specific sensory attributes are enhanced by the addition of a compound or group of compounds. In this case, one is able to evaluate the contribution of a compound or group of compounds to sensory in a real food context: volatiles and non-volatiles interactions and the food matrix are all considered.

As one might expect, initially sensory driven taste protocols paralleled those developed for odor research. TDA (taste dilution analysis – parallel is AEDA; Dunkel and Hofmann, 2009), cTDA (comparative taste dilution analysis; Ottinger *et al.*, 2003), and DoT (dose over threshold – parallel is OAV). As time has progressed, we find much more rigorous methods being applied, e.g. recombination studies with omission testing (e.g., Scharbert and Hofmann, 2005).

A creative approach to determine if an isolated components has an enhancing effect on flavor has been developed by Reichelt *et al.* (2010) and is presented in Fig. 3.10. The device makes it possible to blend compounds being eluted from an HPLC into a liquid matrix and then delivered to sensory panel members for evaluation. This could be used to evaluate sweetness enhancers or bitter blockers, for example. In the sweetness enhancer application, the ‘Standard Tastant’ would be a sugar solution or other sweetener with compounds being delivered by the HPLC for blending from some natural extract. One can see numerous applications for the device.

3.4.5 Applications of taste research

As noted earlier, the approaches to sample pretreatment and subsequent analysis are dependent upon the sample type, research goals, and also researcher preferences. Thus, the next section will highlight six publications which we feel will give the reader an overview of methods applied in analyzing taste compounds. As one would expect, each approach has certain

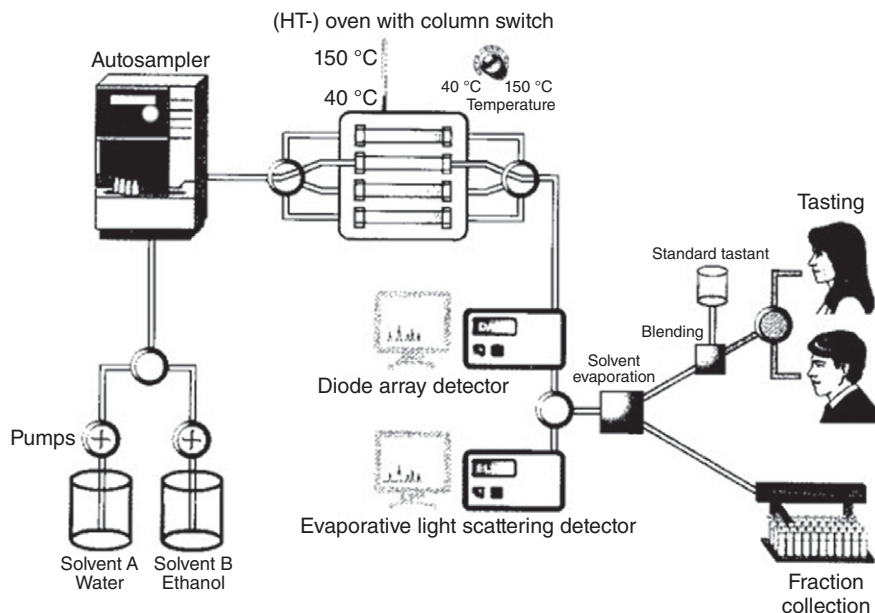


Fig. 3.10 Schematic representation of LC taste device (Fig 1 in Reichelt *et al.*, 2010).

advantages and disadvantages and represents compromises in choice by the researcher. We will start with some work on cheese non-volatiles.

Pionnier *et al.* (2002) were interested in determining how non-volatiles influenced the release of aroma compounds from cheese. They chose to prepare a water extract of cheese (Camembert) and then centrifuge it to remove the fat and larger, insoluble proteins. The soluble portion was subjected to size fractionation initially via tangential microfiltration and then ultra- and nanofiltration. This yielded lower molecular weight fractions which were analyzed for composition, and used in experiments to determine the influence of the non-volatiles on olfactory compounds. This study did not focus on individual non-volatiles so there was no need to do HPLC fractionation.

A later study on cheese (Gouda) had the goal of identifying the molecules responsible for the 'kokumi' taste (Toelstede *et al.*, 2009). This analytical protocol also started with a water extraction followed by centrifugation and then was freeze dried. Since their goal was to determine the taste contribution of specific molecules, they then used GPC (Sephadex G15) for fractionation and tasting.

As is obvious from Fig. 3.11, GPC gave very poor peak resolution, leaving numerous components in each collected fraction. The crude fractionations were tasted and then those fractions which yielded a kokumi taste were further analyzed by LC-MS/MS (MonoChrom MS column) to determine the chemical components in each fraction. The authors then had to quantify

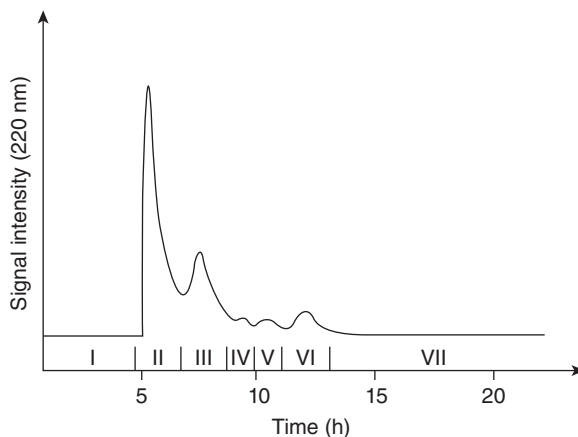


Fig. 3.11 GPC chromatogram of the WSE prepared for a 44-week-old cheese. (Reprinted with permission of Toelstede, S; Dunkel, A; Hofmann, T. 2009. A series of kokumi peptides impart the long-lasting mouthfulness of matured Gouda cheese. *J. Agric Food Chem.* **57**: 1440–1448. Copyright 2009. American Chemical Society.)

the compounds suspected of giving the kokumi taste and evaluate their potential contribution by recombination and omission sensory studies. In this manner, six γ -L-glutamyl dipeptides were proposed as being the primary contributors of the kokumi sensation.

A very different application of taste analysis is exemplified by Brühl *et al.* (2007) who identified the primary bitter compound (cyclolinopeptide E, CLE) formed during the storage of linseed oil. This procedure started with a methanol:water extraction of linseed oil diluted in heptane. The water:methanol extract was taken to dryness and evaluated by sensory methods determining that the bitter compound was extracted in it. (In a later study, Brühl *et al.*, 2008, SPE was used for an initial isolation prior to direct LC-MS analysis.) This dried water:methanol extract was dissolved in solvent and then fractionated on a silica gel column. Fractions were taken to dryness, reconstituted in water:ethanol and tasted again. This further purified the compounds of interest. A final separation was done on preparative reversed phase HPLC with fraction collection, drying each fraction and then tasting fractions after reconstitution in water. Two sequential fractions were found to be very bitter. Larger amounts of these two combined fractions were collected and the bitter components identified by standard methodologies (Fourier transform infrared (FTIR), MS and NMR).

Dunkel and Hofmann (2010) identified the components in chicken broth that provide its unique orosensory sensations to foods. Their work flow is presented in Fig. 3.12. They started by preparing a chicken broth and did an initial pentane extraction to remove lipids. They freeze dried the defatted broth and applied a size fractionation producing fractions of <1 kDa,

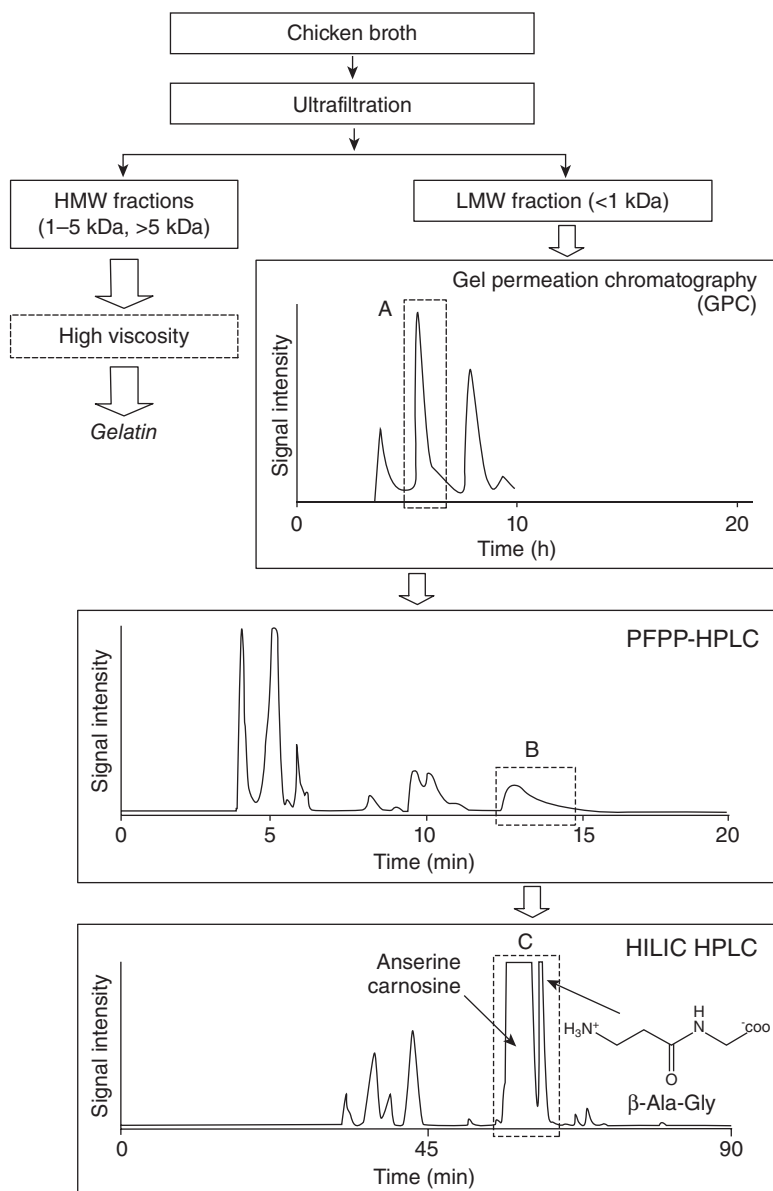


Fig. 3.12 Flow diagram depicting the activity guided isolation of taste modifying β -alanyl peptides from chicken broth (Dunkel and Hofmann, 2010).

1–5 kDa and >5 kDa. Only the lowest molecular weight (LMW) fraction provided significant sensory character. They identified ca. 50 compounds in this fraction but recombination studies suggested that some sensory components were not represented by this formulation. Thus, an effort was made to do a more thorough separation of the LMW fraction which would enhance identifications. They produced seven GPC fractions that on evaluation, one (marked A in Fig. 3.12) had the desired sensory character. Further analysis of that fraction by reversed phase LC-MS yielded the chromatogram (labeled B) and chromatography on a hydrophilic interaction liquid chromatography (HILIC) column (labeled C, for polar substances) produced a peak with good chromatographic properties and sensory activity. The active compounds in this peak were identified as anserine-carnosine and β -alanyl glycine.

A recent paper presented information on the pungent and tingling components of black pepper using a taste dilution (TD) approach (Dawid *et al.*, 2012). It is interesting that the primary pungent compound in black pepper was identified nearly two hundred years ago (Oersted, 1820; Landenburg and Schaltz, 1894) (both as cited by Dawid *et al.* 2012). This study began with an ethanol extraction of ground pepper, filtration, and ethanol removal under vacuum. The resultant oily residue was dissolved in methanol/water (70/30, v/v), filtered, and then fractionated by preparative reversed phase high-pressure liquid chromatography (RP-LC). After the purity of each fraction was checked by means of analytical RP-HPLC, single compounds were directly analyzed by LC-MS and NMR spectroscopy, while the fractions containing mixtures of individual substances were purified by rechromatography using semipreparative HPLC. The solvent was removed in vacuum and freeze-dried twice, and then used for sensory experiments as well as for structure determination by means of UV-vis, LC-MS/MS, ultra-performance liquid chromatography (UPLC)-TOF-MS, and 1D/2D NMR. As a result, 25 pungent and tingling compounds were identified, of these compounds, eight amides had not been reported in the literature.

A last paper to be discussed is on determining the bitter components of whole wheat bread (Jiang and Peterson, 2013). The work flow (analytical protocol) used in this study is shown in Fig. 3.13. The techniques applied are fairly standard with the exception that 2D LC was used in the final stages of the separation protocol. The first two steps were solvent extractions while step 3 was a fractionation based on SPE. Step 4 was a fractionation based on molecular size. The final two steps, 5 and 6 are preparative LC and reversed phase HPLC, respectively. Basically, molecular size and hydrophobicity were key properties used in the fractionation of the sample.

Sensory evaluation guided the work and traditional methods were used in compound identifications (MS and NMR). Eight bitter compounds were identified: Acortatarins A and C, 5-(hydroxymethyl)furfural (HMF), 2,3-dihydro-3,5-dihydroxy-6-methyl-4(H)-pyran-4-one (DDMP), N-(1-deoxy-D-fructos-1-yl)-L-tryptophan (ARP), tryptophol (TRO), 2-(2-

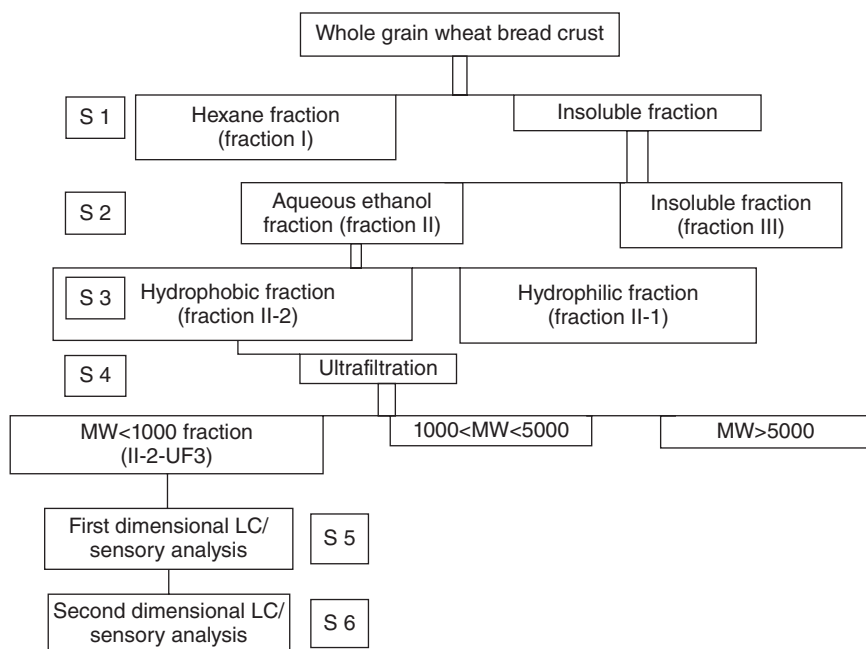


Fig. 3.13 Analytical scheme for bitter compounds identification from whole wheat bread crust (Jiang and Peterson, 2013).

formyl-5-(hydroxymethyl-1H-pyrrole-1-yl)butanoic acid (PBA), and L-tryptophan (TRP).

While the techniques used in this study are fairly commonly applied to this type of work, the application of 2D HPLC is fairly unique (Jiang *et al.*, 2010). Most other studies on non-volatiles have used less effective sample separations and thus, have been left with a mixture of compounds to be evaluated for sensory impact. While other protocols can identify which fractions from HPLC have the sensory notes they want, they typically are mixtures. The researchers have then had to identify components in a mixture, often do synthesis and then evaluation of components originally in the mixture: this is very tedious. The use of high-efficiency 2D HPLC generally provides adequate sample resolution such that pure compounds can be isolated greatly simplifying the subsequent tasks of determining what compounds are causing a given sensory note.

3.4.6 Summary of taste analysis

While the analysis of taste compounds in foods does not have as long a history as aroma analysis, the nature of the task results in taste analysis not lagging greatly behind aroma analysis. The advantages of monitoring

primarily non-volatile, water soluble compounds reduces one of the most problematic tasks, that of isolation of the components of interest for subsequent analysis. Also, working with non-volatiles simplifies collection for subsequent sensory analysis.

The most challenging parts of taste analysis are generally separation of taste components for sensory evaluation, and subsequent compound identification of taste active components. Fortunately, separation techniques in LC have been advancing rapidly with two-dimensional HPLC-MS now available to us. The task of compound identification remains as a significant hurdle but advances in metabolomics are helping to provide public libraries for reference. Also, advances in NMR instrumentation are reducing the amount of unknowns needed for analysis also facilitating compound identification.

3.5 The analysis of compounds contributing to chemesthesis

3.5.1 Introduction to the analysis of chemesthetic compounds

As noted in the section on flavor perception, chemesthetic compounds (irritants) are responsible for oral and nasal chemosensory sensations (chemesthesis). They generally have a molecular weight in the range of volatile compounds (<400 Da), thus, methods used to analyze volatile compounds are appropriate for the analysis of most chemesthetic compounds. However, some of these compounds fall into the non-volatile range and if mouthfeel sensations, such as astringency, are considered part of chemesthetic sensations, then one has to also consider methodologies appropriate for the analysis of non-volatiles. The relevant point is that methods developed for the analysis of aroma compounds or taste compounds will also include the chemesthetic compounds: new, unique methodologies are not required.

It is relevant that the food and flavor industry has developed a very strong interest in this group of compounds (primarily cooling compounds). The concept of adding cooling to a refreshing beverage has commercial interest, or perhaps adding a 'special' effect of tingling from a compound such as spilanthol. Recently there have been a substantial number of publications and patents appearing in this area (e.g., Furrer *et al.*, 2009; Bom, 2009; Kazimierski and Kraut, 2011; Klein *et al.*, 2011; Starkenmann *et al.*, 2011; Kazimierski, 2012).

3.5.2 Examples of the analysis of chemesthetic compounds

The methodology used in this type of analysis will be demonstrated by an example. Starkenmann *et al.* (2011) were searching for cooling compounds and their strategy was to look for compounds found in nature that were structurally similar to menthol. They found a structural similarity between menthol and dihydroumbellols (reported to be found in *Hyptis pectinata*

– but they could not find it on examination). They then chose to study *Umbellularia* since it had been reported to contain (–)-(R)-umbellulone – again a compound closely related to menthol. They obtained *Umbellularia* leaves and prepared an essential oil from it by steam distillation under vacuum followed by pentane extraction of the distillate. The oil thus obtained was fractionated by flash chromatography (SiO₂, toluene/tetrahydrofuran eluent) with fractions one and two having the compounds of interest. They further purified this fraction by flash chromatography and finally by preparative GC to obtain enough material for positive identification by NMR.

A second example is work done by Hiserodt *et al.* (2004) again on cooling compounds. The objective of their work was to find monomenthyl succinate, monomenthyl glutarate, and dimenthyl glutarate in nature such that these known cooling compounds could be classified as natural or nature identical. Their strategy was to investigate plants that were known to contain both precursors of the compounds of interest, e.g. menthol and succinic acid, hoping that the menthyl esters may be formed. They selected *Lycium chinensis* for this combination and lychee for the glutamic acid/menthol precursors. Leaves of both plants were obtained, frozen and finely ground. The leaf powders were extracted in a Soxhlet for 13.5 hr with 95% ethanol. The ethanol extract was concentrated (800 g to 50 g), filtered and then analyzed by triple quad LC-MS. The extracts were found to contain the compounds they were searching for.

The last example to be presented is one where Ottinger *et al.* (2001) were searching Maillard reaction products for compounds of desirable taste character: they were not necessarily looking for cooling compounds or known compounds. This study started with preparing a glucose/L-proline reaction product (heated 190°C for 20 min) that was diluted in water and then extracted with dichloromethane. The dichloromethane extract was taken to dryness and then reconstituted in methanol and an aqueous ammonium formate solution to permit separation via GPC (Sephadex LH 20). They collected 10 fractions from the GPC column which were freeze dried and then used in taste dilution analysis to locate regions of sensory interest. One fraction (V) contained a cooling note that was of particular interest. They further fractionated this material by HPLC to yield 30 additional fractions. Ultimately, they identified three new compounds that had a significant cooling effect. The methodology used by Ottinger *et al.* (2001) was very consistent with that used for the general analysis of taste compounds.

3.6 Non-targeted flavor analysis

3.6.1 Introduction to non-targeted flavor analysis

As noted earlier, flavor perception from food is a complex phenomenon resulting from multiple sensory inputs. However, flavor chemists

traditionally have minimized this fact and tended to ignore the contributions that the senses other than olfaction make to perception. Also, the vast majority of research in recent years has used sensory driven techniques to determine drivers of sensory properties. As discussed earlier, this approach completely ignores the potential influence of compounds that have no taste or smell on overall flavor perception.

A relatively new approach for flavor research (Flavoromics [Reineccius, 2008] aka metabolomics or chemometrics) is emerging which takes advantage of developments in metabolomics, i.e. links the chemical composition of foods with sensory attributes (flavor) using chemometrics. While some studies are appearing in the literature that relate sensory data to only volatiles, or only non-volatiles, we prefer to consider all measurable, LMW compounds in food systems as candidate chemical stimuli in human flavor perception. This includes the study of volatiles and non-volatiles *in food systems* and *investigates associations between all compounds* and flavor perception. By enlarging the spectrum of compounds studied, this research strategy offers the potential to investigate and identify compounds that were not previously considered as contributors to flavor perception and also to understand better the relationships between the chemical composition and the flavor attributes of a given food product. (See de Vos *et al.*, 2008, for a brief but thoughtful discussion of this topic.)

This approach is challenging from a sample preparation and analytical point of view because of the expected diversity of compounds studied (i.e., physicochemical properties and ranges of concentration; de Vos *et al.*, 2006). A combination of methods must be used to get the most comprehensive view of the sample being analyzed. Also, one must limit the number of preparation steps as each step is a potential source of change in the original sample and limits sample analysis throughput. There are currently no standardized methods established for metabolomic investigations because of the diversity in composition of biological samples studied (e.g., urine, saliva, blood, plasma, and tissue); similarly, food systems are very diverse in composition and their study should be considered on a case-by-case basis. While numerous analytical platforms have been used for metabolomics (e.g., GC-MS, LC-MS, NMR, FTIR spectroscopy and capillary electrophoresis-MS), MS interfaced with a separation technique such as gas or liquid chromatography has been the method of choice in most metabolomic studies because of their ability to separate, identify, and to quantify metabolites with high selectivity and sensitivity. Thus, these analytical platforms have been selected for use in related studies on food flavor.

3.6.2 Data collection

Chemical data

As mentioned earlier, there is no perfect (or standardized) analytical protocol for this type of research. The requirements for speed (i.e. automation

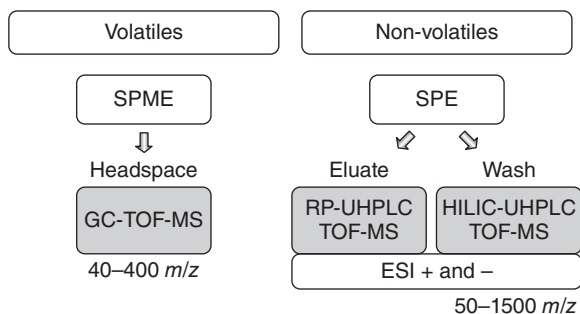


Fig. 3.14 Analytical protocol used by Charve (2010) in a non-targeted study of mandarin juice flavor.

facilitating the analysis of four or five replicates of a large number of samples), and obtaining reproducible and comprehensive analysis of both volatiles and non-volatiles result in compromises in methodology. To illustrate some choices in approaches, two published studies will be discussed below.

Our choices for the non-targeted analysis of mandarin juice flavor are shown in Fig. 3.14 (Charve, 2010). Although SPME has many weaknesses and is seldom our method of choice, we used it in this study due to speed and breath of analysis. (At that time we did not have an automated stir bar methodology or we would have used it.) For non-volatiles, we chose to do an initial centrifugation and filtration of the juice followed by fractionation on an SPE cartridge. The SPE cartridge wash and eluate were separated on both polar and non-polar LC columns using both positive and negative ion MS. This combination of volatile and non-volatile analyses maximized the data on potential chemical stimuli we had to work with later in modeling efforts.

A study by Lindinger *et al.* (2011) focused on relating non-targeted analysis of coffee flavor (both volatiles and non-volatiles) to sensory quality. This group used real time PTR-MS to monitor volatiles released during espresso brewing with an associated Tenax trapping and GC-TOF-MS to determine what was being measured by the PTR-MS. Non-volatiles were isolated by first dissolving the coffee in water:methanol (25:75, 0.1% formic acid) which was centrifuged and then filtered (0.2 µm). This filtrate was directly analyzed by liquid chromatography photodiode array quadrupole time of flight mass spectrometry (LC-PDA-QTOF-MS) in the electrospray ionization (ESI) positive ion mode. Polar non-volatiles were analyzed by first dissolving the coffee in 80 °C water, centrifuging and then adding chloroform to the supernatant. The aqueous phase was then dried under vacuum and derivatized using *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide. The sample was analyzed by GC-TOF-MS. The chemical data used were thus PTR-MS for volatiles, LC-MS for non-polar non-volatiles, and GC-MS for polar non-volatiles.

Sensory data

We will not discuss techniques to collect sensory data since these methods are well established. Our only point here is to collect the right information (addresses research goals) using proper methods. There is little question that sensory requirements are a limitation in this type of research. One can run an instrument 24 hours per day and using fast GC with peak deconvolution, thus, one can collect a lot of data. However, one cannot operate a sensory facility in the same manner. Sensory work is costly in money and time.

3.6.3 Data management

Pre-processing of the raw chromatographic-MS data is a critical step before comparing samples and is done with specialized software packages. The commercial and free packages currently available were reviewed by Pierce *et al.* (2008). With non-targeted data analysis, the main objective is to convert the instrumental data into organized matrices (list of all mass spectral signals detected during the entire chromatographic runs across samples and their intensity) in order to be explored with mathematical tools.

Pre-processing of the data includes noise and background reduction, chromatogram alignment, deconvolution of co-eluting peaks, and peak picking (Fig. 3.15). The data matrix obtained contains information

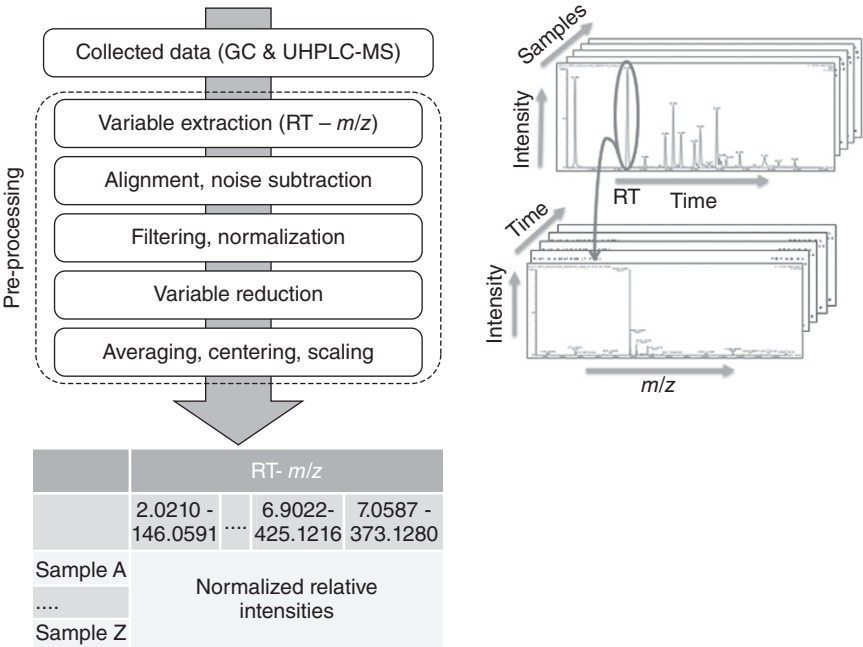


Fig. 3.15 Required data pretreatment. (Adapted from Boccard *et al.*, 2010, and Chen *et al.*, 2007, by Charve, 2010.)

on retention time (RT), associated m/z values (nominal or accurate) and corresponding intensity. RT- m/z pairs are referred to as variables or features. All RT- m/z detected by the software are extracted for each chromatogram (sample) and compiled in a table with y rows (samples) and x columns (variables). Following this, some mathematical transformations (scaling, normalization, transformation) may be required so that the biological meaning of the data is improved. It has been shown that the choice of the data treatment will greatly affect the results (and conclusions) and therefore should be carefully decided based on some biological criteria (van den Berg *et al.*, 2006). For instance, differences in concentration between compounds are not necessarily proportional to their biological relevance. Logarithmic transformation of concentrations has been mentioned when dealing with chemical compounds and sensory perception, arguing that concentrations are not necessarily proportional to their sensory relevance. This suggestion is supported by Steven's power law which states that the perceived intensity of a given stimulus grows as a power function of the intensity of the stimuli (concentration) and, therefore, is not linear in magnitude (Dravnieks, 1976; Meilgaard *et al.*, 2007; Lindinger *et al.*, 2008).

3.6.4 Data analysis

Multivariate techniques are part of the chemometrics toolbox and constitute primarily a graphical tool to visualize the entire data set and to highlight any correlations between variables. They are commonly used in comprehensive research because of their ability to extract the most relevant information by reducing the massive amount of instrumental data into visual graphics, thereby making their interpretation easier in terms of biological meaning (Trygg *et al.*, 2007). In fact, data generated by comprehensive research cannot be analyzed through conventional univariate statistics due to the nature of the data. In contrast, multivariate methods can handle incomplete, noisy, and collinear data (variables are not independent); further, conventional assumptions (normality and variance homogeneity) are not necessary (Eriksson *et al.*, 2006). The use of software packages and computer power are critical for data handling; such calculations would be highly time-consuming and tedious if done otherwise.

The most common multivariate techniques used in non-targeted studies are principal component analysis (PCA) and partial least-squares regression (PLSR) (Sumner *et al.*, 2007). PCA is the basis of multivariate data analysis, and is often used as a diagnostic tool before applying other techniques. It provides a graphical overview of the variation in a data matrix $\mathbf{X}$ with N rows (observations) and K columns (variables). This is useful to understand the structure of the data and to detect outliers. The relationships between observations and variables, and between variables themselves, are revealed through plots: a score plot and a loading plot. The score plot

represents the grouping of observations (samples) while the loading plot assists in identifying the influential variables (e.g., signals measured by the instrument) responsible for the patterns seen in the score plot (Eriksson *et al.*, 2006; Trygg *et al.*, 2007). Examining the score plot reveals how observations are related to each other (similar samples will be nearby) and also allows detecting any outliers.

PLSR combines features from PCA and multiple regression. PLSR is a modeling method that finds a linear multivariate model to link two data matrices, **X** (predictor variables – instrumental data) and **Y** (dependent variables – sensory data), to each other. Once the mathematical relationship is established, the model is tested on new samples and if good performance is found, it can be used for predictions of future samples (i.e., to predict sensory scores using instrumental inputs). Historically, quite good sensory predictions have been obtained in this manner.

3.6.5 Data mining and value

This research approach has value in two ways: first to be able to more accurately predict sensory scores due to more complete data on the chemical stimuli in a food, and second, in the discovery of chemical stimuli influencing the perception of specific flavor attributes or overall liking. The latter application is of the greatest interest to most so it will be discussed further.

As noted, our current sensory driven methodologies do not permit us to find any compounds that might contribute to a sensory response if they do not have a character of their own, when evaluated individually and out of context (the food). This non-targeted approach provides the tools to discover if, for example, there are components in a food that enhance salty perception even if they are not salty themselves, or sweet if not sweet, etc. This is likely to prove extremely valuable.

To do this, one considers the regression coefficients of the developed models since they indicate the importance and direction of the variable (a measured chemical component) to the selected response (e.g., sensory property). These coefficients are not overly important if one is predicting sensory response based on the chemical data: one gets the prediction simply from the sample data without any knowledge of chemical identity. However, if one wishes to know what chemical components are potentially influencing a given sensory attribute, then one will want to identify those compounds with the highest amplitudes: those with a positive amplitude *may be* responsible for giving that sensory attribute and those with the largest negative amplitude *may be* inhibitors of that attribute. *One cannot forget that these are correlations and not causative.* Herein lies one of the biggest challenges of this method, one may get many chemical markers associated with a given sensory note: which are important as opposed to simply related? Fortunately, with today's tools (e.g., 2D GC or LC), one can isolate single, pure

components from foods for sensory testing. One does not necessarily have to identify a component to test its contribution to flavor. When identification is simple, one can identify, purchase, or synthesize and sensorially evaluate. When identification is complicated as it often is for non-volatiles, one can isolate, evaluate, and then identify/synthesize only if valuable. There is no question that this technique has challenges. Yet there is no methodology currently available to us that has the potential to provide such new learning for us.

3.7 References and further reading

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4

Principles of solid food texture analysis

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Abstract: Destructive force/deformation methods are widely used for objective measurement of the textural properties of solid food because they often mimic or emulate the sensory evaluation by humans. This chapter gives a brief introduction to the basic engineering principles and concepts for determining the mechanical properties of solid food. It then describes the principles and applications of different destructive force/deformation techniques for measuring the texture of solid food. Finally, the chapter discusses the challenges and future needs in research for objective measurement of food texture.

Key words: food, texture, force/deformation, mechanical properties, solid food.

Note: This chapter is a revised and updated version of Chapter 5 ‘Force/deformation techniques for measuring texture’ by R. Lu and J. A. Abbott, in *Texture in Food: Volume 2: Solid Foods*, ed. D. Kilcast, Woodhead Publishing Limited. 2004, ISBN 978-1-85573-724-2.

4.1 Introduction

Food texture is a major factor in the sensory evaluation of food quality, and it is critical or important in the quality grading and marketing of solid food. For instance, firmness is important for apple and many other fresh fruits. Apples that do not meet the minimum firmness requirement, as measured by the standard destructive penetrometric test, may be at risk for being downgraded or even rejected by the retailer. Tenderness is an important textural attribute in determining the quality, and hence the price, of meat and meat products. It is important that food producers and processors provide consistent, high-quality food with desirable food textural characteristics to the market.

Mention of the names of commercial products is solely for providing factual information for the reader and does not imply the endorsement of the United States Department of Agriculture.

Food texture is a term that is difficult to define because it is the human's aggregate perception of a food item when it is acted upon by force or deformation in a complex form to cause changes or breakdown in the structure of the food. Bourne (2002) stated that

the textural properties of a food are that group of physical characteristics that arise from the structural element of the food, are sensed primarily by the feeling of touch, are related to the deformation, disintegration, and flow of the food under a force, and are measured objectively by functions of mass, time, and distance.

The difficulty in defining food texture also arises from the fact that there are a large variety of foods with vastly different textural characteristics and different people may have different descriptions or expectations for different types of food. Consequently, diverse terminologies have been used to describe the textural characteristics and it is hence important that objective, standard methods be adopted for measuring the textural properties of food.

Objective measurement of the textural properties of food at various stages of production and/or postharvest processing is needed to assure that the food product to be marketed will meet or exceed consumer expectations. Force/deformation methods are widely used for objective measurement of the textural properties of solid foods because they often mimic or emulate the sensory evaluation by humans in measurement. These methods measure either single or multiple (composite) mechanical properties of food that are important to the sensory perception of texture by humans in the hand or mouth and to the resistance to mechanical damage during handling. Since there is a vast range of foods with vastly different textural and mechanical properties, it is not surprising that a large variety of force/deformation methods and techniques are available for different types of foods. These force/deformation methods, based on their measurement principles, may be classified into fundamental, empirical, and/or imitative (Bourne, 2002). Fundamental force/deformation methods are developed based on the engineering theory of materials and measure well-defined mechanical properties of food. On the other hand, empirical methods measure those mechanical properties that are not well defined and/or are poorly understood but have been found to correlate with the sensory evaluation of the food.

There are two schools of approaches to force/deformation measurement of food texture: *destructive* versus *nondestructive*. Destructive force/deformation methods are considered by many to be a preferred means of measuring the texture of food because they are usually better related to the sensory evaluation than are nondestructive methods. Destructive measurements are often used as the standard against which a new nondestructive technique is compared. Destructive techniques are useful for providing information about the average quality for a batch of food items. However, they suffer a major shortcoming in that the food samples are destroyed in

the process of measurement. Many foods, particularly those fresh raw or unprocessed food products, are inherently variable in texture among individual items. Measurements of 'average' texture are not sufficient to guarantee the quality and consistency of individual items of the food. Nondestructive sensing would allow us to better manage the harvest time for optimal food quality and to monitor, grade, and sort food products to ensure their consistency and superior quality. Consequently, considerable research activities for the past two decades have been focused on nondestructive techniques for quality evaluation of fresh, raw food products.

This chapter primarily focuses on the principles of destructive force/deformation techniques for measuring or analyzing the textural properties of solids food. A brief introduction is first given to the basic engineering methods for determining the mechanical properties of solid foods, followed by different destructive force/deformation techniques for measuring the texture of food.

4.2 Mechanical characterization of solid foods

4.2.1 Basic concepts

Texture is a quality attribute that is closely related to the structural and mechanical properties of a food. It is, therefore, essential to understand the mechanical properties of food in studying their textural properties and measurement techniques. The study of mechanical behavior, i.e., deformation and flow, of foods under applied forces falls within the scope of food rheology, which is a broad research area covering both solid and liquid foods. A number of textbooks and monographs have been written about the rheology of agricultural and food products with various degrees of mathematical requirements (Mohsenin, 1989; Rao and Steffe, 1992; Steffe, 1996). Those who are interested in the topic are recommended to read one or more of these textbooks to gain a better, more comprehensive understanding of this important subject area.

The force/deformation relationship for most food materials is dependent on time or loading rate. Force (F), deformation (D), and time (t) are three basic variables used in studying the mechanical properties of foods. Force, often expressed in N (newton), is considered an external variable because it is acting and/or measured at the surface (or at the surface point) of an object (gravitational and magnetic or electric forces are exceptions, which act on the entire body of the object). In engineering applications, the force and deformation on a plane in the interior of an object are of considerable interest in quantifying the mechanical response of the object subjected to external loading. Corresponding to the force is *stress*, expressed in force per unit of area [N/m^2 or Pa (pascal)], which has the same unit as pressure. Stress is caused by, and accompanied by, external forces and/or other factors such as temperature (thermal stress) and humidity (hygroscopic stress).

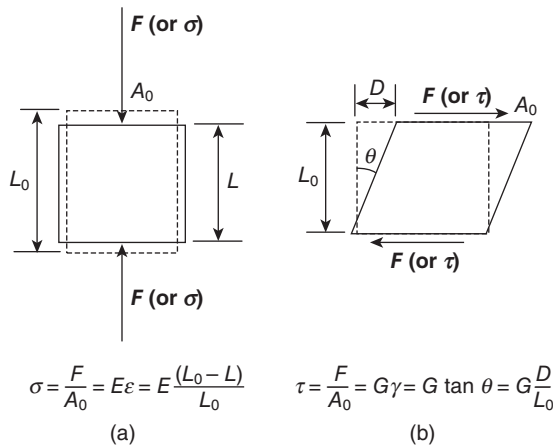


Fig. 4.1 Uniaxial compression (a) and shearing (b) of a specimen. (a) Uniaxial compression of a specimen with an original length L_0 and area A_0 and the Young modulus E . (b) Shear stress τ acting on the opposite planes causing the distortion of the specimen with the shear modulus G and the area of A_0 .

Strain is the measure of deformation at a point on a plane in an object; it measures the unit change, due to force, in the size or shape of an object with respect to its original size or shape and is a dimensionless quantity.

There are two basic types of stresses; one is the normal stress, designated as σ , that acts in a direction normal (perpendicular) to the plane of an object and the other is the shear stress, τ , tangential to the plane on which the forces act (Fig. 4.1). For example, twisting of a rod by applying torsional force at the two opposite ends induces pure shear stresses on the transverse cross-section of the rod, whereas uniaxial compression or tension causes the normal stress either toward or away from the plane perpendicular to the direction of the applied force. As a sharp knife cuts through a food sample, shear stress is created along the two shearing surfaces of the food sample, whereas forces used to bend a beam create both normal compressive and tensile stresses as well as shear stress on the cross-section of the beam. Most destructive force/deformation measurements involve a complex form of loads, which often induce both normal and shear stresses in a food sample. Corresponding to the two types of stresses are normal strain (ϵ) and shear strain (γ). Normal stresses are primarily responsible for the expansion or contraction, i.e., the size change, of an object (Fig. 4.1a) and shear stresses cause distortions or the angle change between two planes in the object (Fig. 4.1b).

Foods come from biological origins, and they, whether raw or processed, constantly change with time due to chemical reactions (such as oxidation), microbial actions, and physical interactions with the environment (such as heat and moisture). Consequently, the mechanical properties of foods also

change with time and are influenced by external or ambient conditions such as temperature, humidity, air composition and pressure, and the supply and consumption of energy. Accurate description of the mechanical behavior of agricultural and food materials is an extremely difficult task and fortunately, for many applications, it is not required. The engineering characterization of solid materials is based on some basic assumptions and simplifications – such as linearity in the stress/strain relationship, and homogeneity and/or isotropy in the material properties – which may or may not be valid for biological materials and food products. The theory of rheology describes the basic mechanical behavior of many food products with reasonable accuracy, especially when deformation is small. For many engineering materials, a small deformation would mean having the strain level less than 0.2%. For solid foods, a deformation of as high as 5% may still be considered small. During the masticating process (chewing), food items undergo extremely large deformation, well beyond the limit of elasticity or the normal ‘small deformation’ level. No adequate theory is available for describing the mechanical response of food under large or extremely large deformation that takes place during mastication. Despite this, the theory of rheology can help us better understand the underlying principles and processes of measuring the textural properties of food. And it offers a useful guide for designing a better and more efficient texture measurement method or system, which is especially true for nondestructive sensing of food texture.

Solid foods, depending on their mechanical responses, may be classified into *time-independent elastic materials* and *time-dependent inelastic materials* (Fig. 4.2). For time-independent elastic materials, their mechanical response is independent of time or the rate of loading. Upon removal of applied loads (that did not exceed the elastic limit), the deformed body will recover to its original size and shape. Time-independent elastic materials

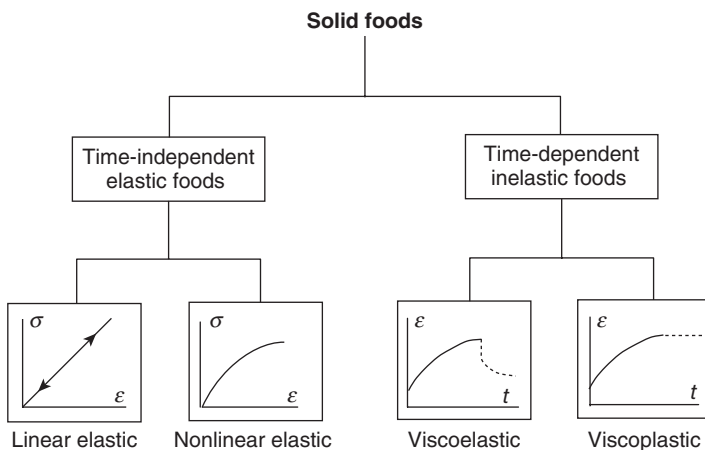


Fig. 4.2 Classification of solid foods based on their mechanical properties.

may be further divided into linear elastic materials whose stress/strain relationship is linear and the nonlinear elastic with the nonlinear stress/strain relationship. Time-dependent materials can be classified into *viscoelastic* and *viscoplastic*. Viscoelastic materials exhibit both solid-like (elastic) and liquid-like (viscous) behavior in which the stress/strain relationship is time-dependent. Upon removal of the applied loads, the deformed body of a viscoelastic material will recover partially, whereas a viscoplastic material retains its deformation without recovery. Most biological and food materials behave viscoelastically at small and/or intermediate levels of deformation. The viscoplastic theory may be needed for describing the mechanical behavior of food at large deformation. When the time-dependent response is not critical and can be ignored, the elastic theory offers considerable simplification in analyzing the mechanical responses of food when they are subjected to different forms of loading.

4.2.2 Elastic materials

Different foods can exhibit very different mechanical behaviors under uniaxial compressive or tensile loading (Fig. 4.3). The force/deformation response for a cylindrical apple tissue specimen under compressive loading (Fig. 4.3a) may be divided into three phases. During the first phase of deformation, the relationship between force (or stress) and deformation (or strain) is linear and elastic. (Nonlinearity at the beginning of the force/deformation curve is mainly caused by the imperfect contact between the loading device and the specimen and is therefore ignored in the discussion.) Since most food materials are not truly elastic, they often cannot recover completely to their original size and shape upon removal of the load even under small deformation. Despite this, the theory of linear elasticity applies to this phase of deformation with reasonable accuracy. There are three material constants or parameters for characterizing a linear elastic material: the modulus of elasticity (also called the Young's modulus), designated as E ; the shear modulus, G ; and the Poisson ratio, μ . Since the three parameters are interrelated, once any two of the three parameters are determined, the third one can be calculated using an appropriate equation. The elastic modulus is the ratio of the normal stress to normal strain, which can be determined from the slope of the linear portion of the force/deformation curve in Fig. 4.3a using the following equation:

$$E = \frac{\sigma}{\epsilon} = \frac{F / A_0}{\Delta L / L} \quad [4.1]$$

where E has the unit of N/m^2 or Pa; F is the applied force in N; A_0 is the original, undeformed cross-sectional area of the specimen in m^2 ; ΔL is the net deformation of the specimen in m; and L is the original length in m. The Poisson's ratio is the absolute value of the ratio of transverse strain to the corresponding axial strain under uniaxial loading. The Poisson ratio

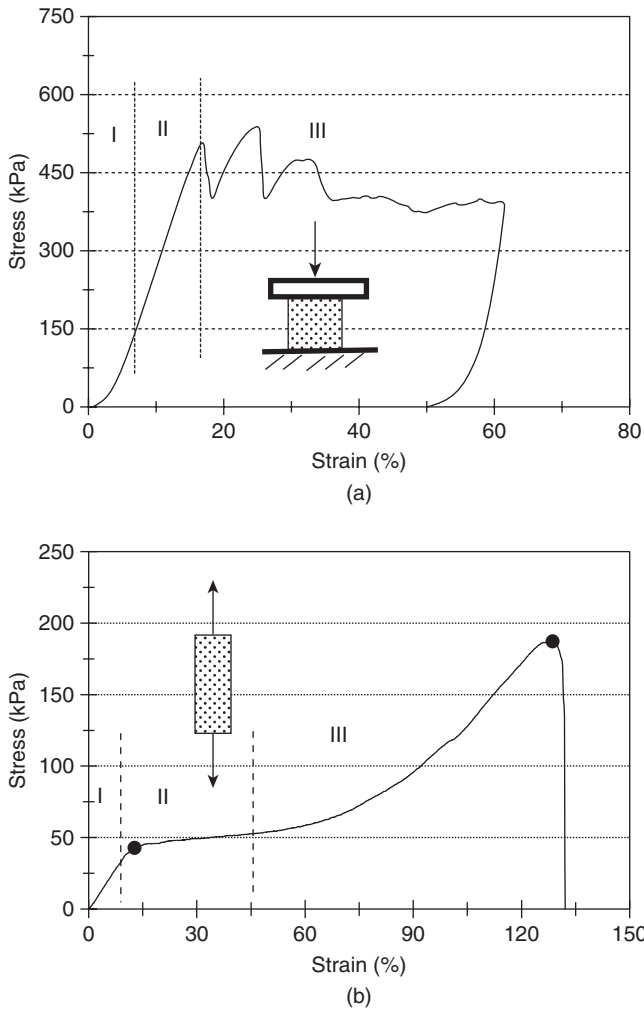


Fig. 4.3 Stress-strain (or F - D) curves of a cylindrical apple tissue specimen under uniaxial compression (a) and a raw beef specimen of rectangular cross-section under uniaxial tensile loading (b) (Lu *et al.*, 1998). The stress-strain curves may be approximately divided into three phases of deformation: elastic, yielding, and post-yielding (or stiffening for beef).

ranges from 0.0 for completely compressible materials (i.e., no lateral expansion under uniaxial compression of a constant cross-sectional specimen) to 0.5 for completely incompressible materials. The Poisson ratio for most food materials is between 0.2 and 0.5 (Mohsenin, 1989). For example, apple flesh has a Poisson ratio of 0.25–0.35 whereas the Poisson ratio of potato tissue is as high as 0.49, close to that for incompressible materials.

The Poisson ratio can be directly determined by simultaneously measuring both axial and lateral deformations from a constant cross-sectional specimen under uniaxial loading. The measurement error associated with this method can be great due to the difficulty in accurate measurement of the small lateral deformation during uniaxial compression. A better approach is to prepare two same-size specimens; one is used for uniaxial loading with no lateral constraints and the other measured under uniaxial loading with lateral constraints (i.e., no lateral deformation). From the force/deformation responses of the two specimens, the Poisson ratio can be determined using the following equation (Gyasi *et al.*, 1981; Hughes and Segerlind, 1972):

$$\mu = \frac{1}{4}(R + \sqrt{R^2 - 8R}) \quad [4.2]$$

in which

$$R = \frac{E_u}{E_c} - 1 \quad [4.3]$$

where E_u and E_c are the Young moduli determined using Equation [4.1] from the unconstrained and constrained uniaxial loading test, respectively.

Beyond the first phase of deformation, the theory of elasticity is no longer valid. During the second phase of deformation, the force/deformation relationship starts to deviate from linearity and becomes increasingly nonlinear as the load increases. When the load is removed during this phase of loading, the specimen will only recover partially. As deformation continues to increase, a noticeable drop or sometimes no increase in the force occurs to many biological and/or food materials. The point at the force/deformation curve where a drop or no increase in force takes place with an increase in deformation is called the bioyield point. The bioyield point indicates the initial cell rupture in the cellular structure of the specimen (Lu and Tipper, 2009). For some food materials, the bioyield point does not show clearly on the force/deformation curve. For example, the bioyield point may appear merely as a change in slope. Beyond the bioyield point, the third phase of deformation starts; the force/deformation relationship becomes irregular and jagged, with numerous peaks and valleys until complete breakdown of the specimen. Fresh fruits and vegetables often undergo all three phases of deformation under compressive loading. Other food products such as grains at the normal storage moisture content or even lower often only go through the first and second phase of deformation, followed by a sudden, complete failure of the specimen during the second phase of deformation (Wouters and de Baerdemaeker, 1988).

Many muscle foods, when subjected to tensile loads, exhibit a mechanical behavior (Fig. 4.3b) that is significantly different from the one shown in

Fig. 4.3a. Muscle foods can withstand large deformation and exhibit a prominent nonlinear behavior under uniaxial tensile loading. The force/deformation behavior of raw beef under tensile loads may also be divided into three phases of deformation: linear elastic at small deformation, stress yielding at intermediate deformation, and work hardening (or stiffening) at large deformation (Lu *et al.*, 1998). Depending upon muscle type and post-rigor treatment, raw muscle meats may exhibit all or some of the three phases of deformation.

4.2.3 Viscoelastic materials

Although many foods can be approximated as elastic materials, most are in fact viscoelastic. The mechanical response of viscoelastic materials is time-dependent; it not only depends on the current loading level but also the rate and/or history of loading. Two types of test are often used to characterize the viscoelastic properties of food materials (Fig. 4.4). One is the creep test, which measures the deformation of a specimen with time when a constant load is instantaneously applied to the specimen (Fig. 4.4a). The second type of test is the stress relaxation, which measures the change in stress over time when the specimen is subjected to a constant deformation (Fig. 4.4b).

Mechanical models are often used to help us understand or visualize the mechanical behavior of linear viscoelastic materials. A large number of mechanical models can be constructed to describe the viscoelastic material; a vast majority of them consist of two basic elements: the spring (E) representing the elastic component of the material and the dashpot (η) for the viscous component to account for the time effect in the material. The

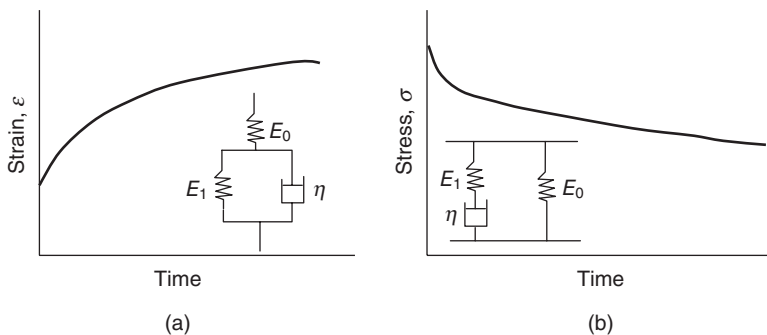


Fig. 4.4 Creep under constant load (a) and stress relaxation under constant deformation (b) response curves and two simple mechanical models for describing the viscoelastic behavior of solid foods, where E_0 and E_1 are the springs representing the elastic components, while η is the dashpot for the time-dependent viscous component.

viscosity η is related to the shear stress τ and shear strain rate $\dot{\gamma}$ for the Newtonian liquid by the following equation:

$$\eta = \frac{\tau}{\dot{\gamma}} \quad [4.4]$$

Figure 4.4 shows two simple mechanical models that may be used to describe the creep and stress relaxation behavior of food materials. To better describe a viscoelastic material, it often requires a more complex model with additional spring and dashpot elements. As more mechanical elements are added to the model, the mathematical equation becomes increasingly complicated.

For more detailed discussion about characterizing the viscoelastic properties of foods and other solid materials, readers are recommended to consult the textbooks of Mohsenin (1989) and Steffe (1996).

4.2.4 Quasi-static versus dynamic measurement

Since viscoelastic material is time or loading-rate dependent, its mechanical behavior will be different for different loading rates. For example, the elastic modulus of apple tissue generally increases with loading rate (Petrell *et al.*, 1980). To completely describe this rate-dependent behavior, the test specimen may have to undergo an extended time period (e.g., from many minutes to days) under the creep and/or stress relaxation tests. This is not only time-consuming but also impractical, as food specimens can experience a significant change in their mechanical properties over time due to chemical and/or physiological activities, and loss and gain of moisture. In addition, it is often difficult to apply a truly instantaneous load or deformation to the specimen. Dynamic tests allow the elastic modulus (to be exact, the complex modulus consisting of the storage modulus and loss modulus) to be determined for a range of frequencies over a short time period. With the commonly used dynamic test, a food specimen is subjected to a sinusoidal varying stress (or force) given by the following equation:

$$\sigma = \sigma_0 \sin(\omega t) \quad [4.5]$$

The resulting strain will be a sinusoidal response of the same frequency as the stress, but out of phase by a lag phase φ :

$$\varepsilon = \varepsilon_0 \sin(\omega t + \varphi) \quad [4.6]$$

The complex modulus $E^*(i\omega)$, also called dynamic modulus, is determined from the following equation:

$$E^*(i\omega) = \frac{\sigma_0}{\varepsilon_0} (\cos\varphi + i \sin\varphi) = E'(\omega) + iE''(\omega) \quad [4.7]$$

where E' is called the storage modulus representing the elastic component of the material, E'' is the loss or imaginary modulus representing the viscous

component or loss of mechanical energy as in a dashpot, and i is the imaginary unit equal to $\sqrt{-1}$.

In dynamic tests, the applied loads or deformations are generally very small and confined to the elastic limit. The loading rate can vary greatly over several scales of magnitude, depending on the vibration amplitude and frequency used. The test often requires a number of specimens to cover a desired range of frequencies, which is prone to experimental errors due to the variability among the test specimens. Lu and Abbott (1995) proposed a method for fast measurement of the dynamic properties of solid foods. They showed that under certain constraints (i.e., specimen size and frequency range), the dynamic viscoelastic properties of solid foods can be measured over a range of frequencies from one single specimen using an impulse load (or transient load). This transient test makes it faster and easier to obtain the dynamic properties of solid foods.

In measuring basic mechanical properties of solid foods, several important issues need to be considered. First, many food products exhibit significantly different force/deformation behaviors under compressive and tensile loads. For example, apple flesh exhibits the force/deformation relationship shown in Fig. 4.3a under compressive loading. When subjected to tensile or bending loads, the apple flesh behaves more like a brittle material (Vincent *et al.*, 1991). Secondly, many foods exhibit different mechanical properties when measured in different directions. Abbott and Lu (1996) studied the anisotropic property of apple fruit and found that the mechanical properties of apple tissues, as measured by the elastic modulus and failure strength, are significantly different in three perpendicular directions. Third, the mechanical properties of raw food products such as fresh fruits and vegetables and muscle meats are variable from location to location on the same food sample. Care must be taken in experimental design and reporting results to clearly indicate the location and direction from which specimens are taken.

4.3 Destructive measurements

There are a wide variety of methods and/or techniques for destructive measurements of solid foods. Destructive methods can be empirical or fundamental. Empirical destructive methods are often somewhat imitative of methods used in sensory analyses or in the preparation of the food such as spreading butter on bread, slicing meat, or cutting asparagus. They involve a complex form of loading with the stress and strain levels well beyond the initial failure, and the quantity measured often cannot be adequately interpreted in terms of basic engineering parameters or properties. Fundamental destructive methods, on the other hand, measure basic mechanical properties including the Young modulus, Poisson ratio, and shear modulus as well as yield strength, failure strength, and others; but these often cannot be

adequately interpreted in terms of human perception of 'texture.' When the time- and/or loading rate-dependent effect is important, the creep test, the relaxation test, and/or the dynamic test is required to measure the visco-elastic properties. Fundamental tests may be performed directly on original food samples that have well-defined dimensions but often require using specimens of specific dimensions excised from food samples. Therefore, the tests are more time-consuming compared to many empirical tests and sometimes can be difficult in preparing specimens and mounting them onto the testing device in such tests as tension and torsion. Fundamental tests are usually conducted using a universal testing machine. Overall, empirical methods tend to correlate better with sensory textural properties of foods, whereas fundamental methods can help us better understand the mechanical behavior of a food, its structural features, and their changes. Based on the pattern of loading, destructive methods may include puncture, compression, shear, twisting/torsion, tension, bending, and so on.

4.3.1 Puncture

The puncture test measures the force required for a probe to penetrate into a food sample for a pre-specified depth. The test involves both compression and shearing of a food sample; it is an empirical technique that is somewhat imitative of the biting of a food item in the mouth. Puncture measurements depend on a number of factors such as probe size and shape, type of food, speed of loading, and number of probes on the tester. Bourne (1966) proposed the following empirical equation relating the puncture yield force to the area and perimeter of the probe:

$$F_s = K_c A + K_s P + C \quad [4.8]$$

where F_s is the force in N acting on the probe; A and P are the cross-sectional area (mm^2) and the perimeter (mm) of the probe, respectively; K_c is the compression coefficient in N/mm^2 and K_s is the shear coefficient in N/mm ; and C is a constant in N. The first term in Equation [4.8] represents the contribution of compression to the puncture force and the second term represents the shear contribution. Equation [4.8] suggests that as the ratio of area to perimeter increases, the proportion of compression contributing to the overall puncture force will increase. Conversely, as the ratio of area to perimeter decreases, the relative contribution of shear to the overall puncture force increases. Therefore, by using different probe geometries, such as circular, square, star, and polygonal, we can manipulate the relative contributions of compression and shear to the overall puncture force measurement. However, the edge effect (or stress concentration) with different probe geometries on texture measurements should also be considered. Most commonly used puncture probes are of circular shape, which gives the maximum area to perimeter ratio among all geometries, and thus measures the puncture force with the maximum compression to shear ratio. At the

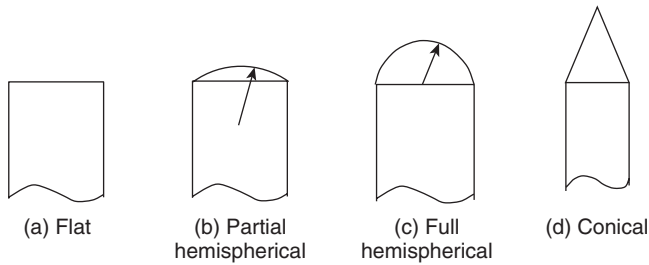


Fig. 4.5 Different probe tips used in puncture tests. The Magness–Taylor (MT) probe, represented by (b), is popular for fruits and vegetables.

other extreme is the thin blade probe, which gives the minimum ratio of area to perimeter, and the test in fact becomes a shear test.

Different foods have different compression and shear properties. The ratio K_c/K_s may be useful for comparing the relative contributions of compression and shear to the puncture force among different foods. For example, the ratio K_c/K_s is approximately five for apples, two for potatoes, and one for bananas (Bourne, 1966). The different ratios of K_c/K_s for different foods may explain why the puncture test works well for some foods but not for others. Equation [4.8] also suggests that in order to optimize the texture measurement, different probe geometries should be considered for different types of foods.

The geometry of the probe tip is also important in the puncture test. Commonly used probe tip geometries include flat end, hemispherical (both full and partial), and conical (Fig. 4.5). In measuring the firmness of many intact fresh fruits and vegetables, a partial hemispherical probe is widely used (Fig. 4.5b), as exemplified by the two standard Magness–Taylor (MT) probes. On the other hand, the flat-end probe has been suggested for testing fresh-cut commodities (cut slices or chunks), which would ensure constant contact area between the probe and flat cut surface of the piece during the test (Wu and Abbott, 2002). Multiple conical probes have been used for measuring the tenderness of meat, such as the Armour Tenderometer, which uses ten 3.2 mm (1/8 inch) diameter probes with sharp points (conical probes) (Hansen, 1972).

The MT firmness tester, which uses a partial hemispherical tip probe (Fig. 4.5b), is the best-known puncture method for measuring the texture of foods. The MT tester is widely used for estimating the harvest maturity or postharvest firmness of many fruits and vegetables (Mendoza *et al.*, 2012). There are several variations of the MT tester for measuring different fresh fruits and vegetables. A number of reviews have given detailed discussion of various forms of the MT tester (Abbott, 1999; Bourne, 2002). There are basically three types of MT tester available: the handheld MT tester with a mechanical force gauge, the portable MT tester equipped with an electronic

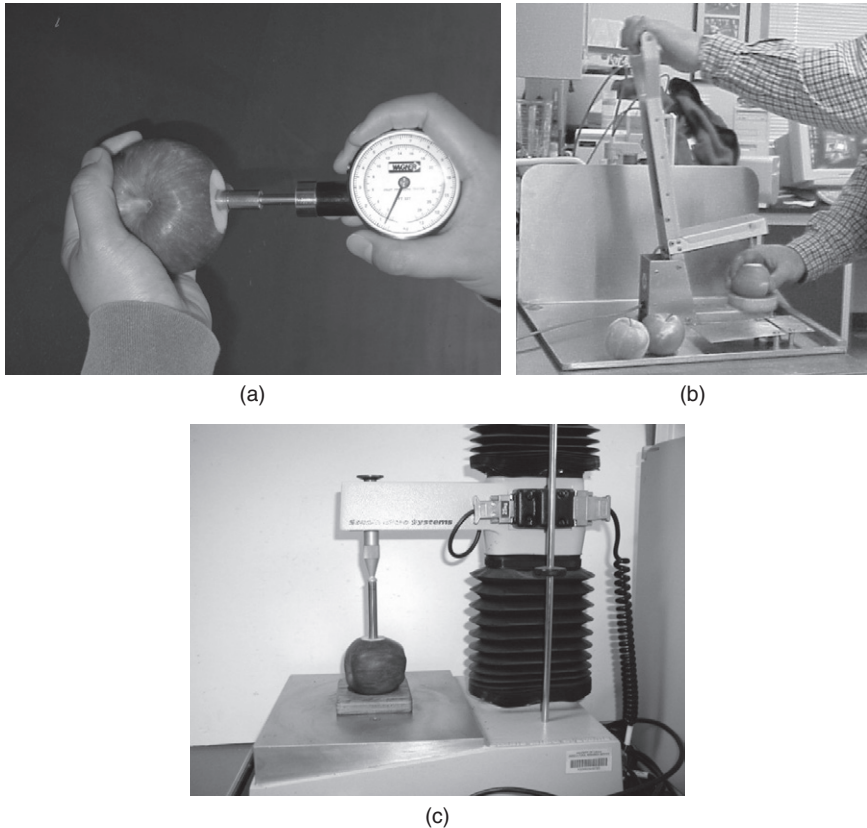


Fig. 4.6 Three Magness–Taylor (MT) firmness testers: (a) a handheld mechanical MT tester; (b) a portable MT tester with an electronic gauge; and (c) an MT probe mounted on a standard laboratory material testing machine.

force gauge, and the MT tester that uses a universal testing machine with an MT probe attached to it (Fig. 4.6). Two probe diameters, 11.1 and 7.9 mm, are used for different commodities. There are two types of MT testers with mechanical force gauges – the original MT with a helical spring and the Effe-gi with a spiral spring (Effe-gi testers may be marketed under the names McCormick, Wagner, R. Bryce, and others) – but the testers are fundamentally the same. Mechanical MT testers are popularly used for measuring the firmness of fruit in the orchard and at the packinghouse or the shipping point as well as in some laboratories. They are low in cost but prone to operational error because MT measurements are affected by the rate of loading and operator (Harker *et al.*, 1996; Lehman-Salada, 1996). MT testing with a universal testing machine, such as Instron or Texture Analyzer, can accurately control the loading rate and record the force/deformation curve for each food sample. Thus, the measurements are more

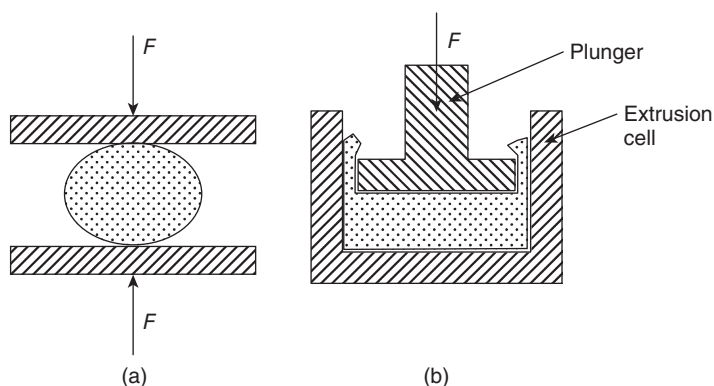


Fig. 4.7 Two types of compression tests: (a) the uniaxial compression test between two plates and (b) the simple compression-back extrusion test.

reliable and reproducible. But these machines are expensive and only suitable for laboratory uses. Many portable MT testers with an electronic force gauge are low in cost and can accurately record the force during the puncture test, and therefore they provide a good alternative to either the mechanical MT tester or the universal testing machine-based MT tester. One problem with these testers is that the loading rate is usually not controllable, although some reject excessively fast or slow measurements.

4.3.2 Compression

Compression is widely used for measuring the basic mechanical properties of a large variety of solid foods including fruits (Abbott and Lu, 1996; Khan and Vincent, 1993), vegetables (Alvarez and Canet, 2000), grains (Wouters and de Baerdemaeker, 1988), and processed foods (Moiny *et al.*, 2002). These tests are often conducted on cylindrical specimens excised from food samples under uniaxial loading with a universal testing machine. Compression tests may also be used to measure the basic mechanical properties of intact food samples with well-defined geometry. The American Society of Agricultural and Biological Engineers (ASABE) has provided a standard for conducting compression tests with intact food samples and the method for calculating the elastic modulus from the compressive force/deformation curve (ASABE, 2009).

In texture measurement of intact foods, two types of compression are often used: the uniaxial compression test of food samples between two plates and the confined compression test, such as extrusion (Fig. 4.7). During uniaxial compression, force is applied to the sample in one direction and the sample is allowed to expand freely in the other two directions. The sample is compressed until it breaks or is completely crushed. The initial portion of the force/deformation curve can be used to estimate the modulus

of elasticity of the food sample (Mohsenin, 1989). Uniaxial compression is easy to perform and is useful for such foods as grains. The single kernel wheat characterization system developed by the US Department of Agriculture (Martin *et al.*, 1993) measures the hardness of individual wheat kernels by crushing them between two surfaces that move closer together through a rotor and a crescent. The crushing force profile is recorded and analyzed for predicting wheat kernel hardness. Gaines *et al.* (1996) reported a high correlation between predicted softness equivalent (SE) values from the single kernel wheat characterization system and actual SE milling values.

In the simple compression–extrusion test shown in Fig. 4.7, force is applied through a plunger to compress the food sample in the test cell until it is crushed and flows through the gap (or annulus) between the plunger and the test cell. The force required to compress the food sample not only depends on the properties of the food but also the size of the annulus. This type of test is often used for measuring viscous liquids, gels, fats, and some fresh and processed fruits and vegetables. A variation to the simple compression–extrusion tester is to have slits or a grid of holes in the bottom of the extrusion cell so that some of the food will be extruded forward through the slits or holes during compression by the plunger. The Kramer Shear Press (Kramer, 1951) and the Ottawa Texture Measuring System (Voisey, 1971) are two popular compression–extrusion testers for measuring the texture of many solid foods (Cavitt *et al.*, 2005; Chen and Marks, 1998; Harker *et al.*, 2002; Rodrigo *et al.*, 1997; Strange and Whiting, 1998). The measurement process involves a complex form of mechanical loading, including shear, compression, and extrusion. For this reason, these testers are more often considered as a shear device rather than a compression device. The Kramer Shear Press is generally more popular for processed products than for fresh fruits and vegetables for several reasons: (1) it is intuitively appealing since each test can measure many pieces of cut-up food; (2) the user does not need to be concerned about the choice of free or constrained compression, tension etc.; probe geometry; sample size; and measurement parameters to report; and (3) many practitioners come from a food science rather than horticultural background.

4.3.3 Shear

Shear often refers to the action of applying force to cut an object into two separate pieces. This loose definition is different from the strict engineering definition of pure shear, which is difficult to conduct experimentally, except under torsion and some special loading conditions. Shear tests are especially useful for measuring the textural properties of muscle foods. The Warner–Bratzler (WB) shear tester is the standard device recommended by the American Meat Science Association for measuring the tenderness of meat. The device (Fig. 4.8) consists of a thin blade with a triangular opening and

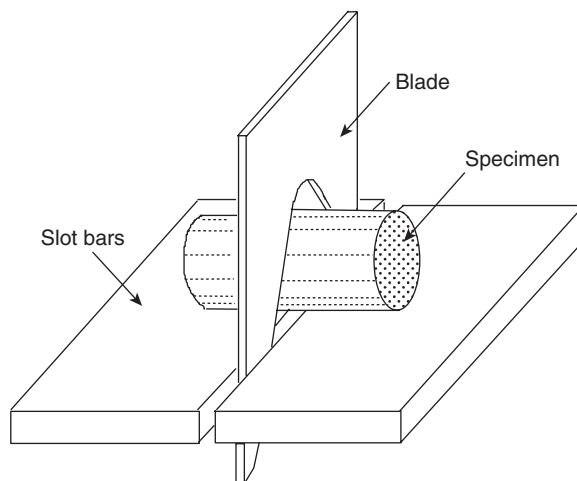


Fig. 4.8 Schematic of the Warner-Bratzler shear tester for measuring the tenderness of meat.

slotted base (of specified geometry and dimensions) (Bratzler, 1949; Voisey, 1976). Cylindrical specimens of 13 mm diameter are excised from cooked meat. As the blade moves through the slot, the meat specimen is compressed and changes the cross-sectional shape to conform to the restriction imposed by the triangular opening of the blade until it is eventually sheared into two pieces. The maximum force recorded during shearing is considered to be a measure of meat tenderness. The measurement process involves shear, tension, and compression. The conventional interpretation, as implied in the name, is that shear forces are primarily responsible for cutting the meat specimen during WB measurement. Other researchers (Voisey, 1976; Zhang and Mittal, 1993) argue that tensile strength is the primary contributor in WB shearing process. A number of different shear devices have been developed for measuring meat tenderness; they include Volodkevich tenderometer (Volodkevich, 1938), the MIRINZ tenderometer (McFarlane and Marer, 1966), the NIP tenderometer (Smith and Carpenter, 1973), and the razor blade shear method (Cavitt *et al.*, 2004).

Eckhoff *et al.* (1988) developed a rapid single-kernel wheat hardness tester. The tester consists of a rotary knife sitting in a groove. As the plate that contains kernels rotates, the knife slices through the kernels. The force required to cut the kernels over time is recorded and analyzed for differentiating hard wheat from soft wheat. Brusewitz *et al.* (1997) investigated a shearing technique to quantify the texture of several fruits: apples, peaches, and bananas. A cylindrical specimen was cut through with a wire probe. A frequency analysis method, i.e., fast Fourier transform or FFT, was used to analyze the force/displacement curves generated as the wire cut through

the specimen. They reported that FFT peak energies at frequencies below 4 Hz could be used to detect the change in the texture of fruits.

4.3.4 Torsion/twisting

Torsion/twisting is another form of measuring texture that is based on the shear properties of foods. In a torsion test, force is applied to an object to cause the rotation or twisting of one part relative to another part of the object. Torsion tests are less commonly used for solid foods because of special requirements in preparing and mounting specimens and the difficulty of applying torsional force to food samples (Hamann *et al.*, 2006; Truong and Daubert, 2001). Diehl *et al.* (1979) developed a torsion test to study the structural failure of selected raw fruits and vegetables. The test required preparation of specimens of special dimensions. Phillips (1992) developed a rotating pin shear device to measure the tenderness of meat. The device consisted of two sets of concentric pins that can be rotated relative to each other. The force required to rotate the inner set was measured against the angle of rotation and used as a measure of meat tenderness.

Studman and Yuwana (1992) developed a twist device for measuring fruit firmness. The device consists of a blade on a spindle, which is pushed into the fruit and rotated about the axis of the spindle. Fruit firmness is estimated by measuring the maximum moment (or rotational angle) required to crush the flesh. The twist test is quite different from a torsion test as the properties tested with the former are likely a combination of shear and compression. Hopkirk *et al.* (1996) suggested that puncture and twist tests may produce quite different firmness judgments. Harker *et al.* (1996) found the twist test to be more precise than several testers using the MT puncture probe. The twist test has the advantage of being able to measure strength of tissue zones at specific depths from the surface without requiring the excision of tissue samples.

4.3.5 Tension/bending

Tensile tests measure the force required to stretch a food specimen or an intact food sample apart. Tensile tests are useful for studying the tensile mechanical properties and, particularly, the structural failure characteristics of foods. Many foods have tensile properties that are quite distinct from those in compression. For example, the tensile properties of muscle foods are very different from those in compression (Lepetit and Culioli, 1994), and tensile tests are considered to be especially valuable for understanding the structural changes of muscle food during such processing treatments as aging and cooking (Dransfield *et al.*, 1986; Lu *et al.*, 1998; Mutungi *et al.*, 1995; Penfield *et al.*, 1976; Purslow, 1991). Researchers also used tensile tests to examine the structural and/or textural changes and the mode of fracture

in fruits (Hallett and Harker, 1998; Harker and Hallett, 1992, 1994; Stow, 1989) and in processed foods (Katagiri *et al.*, 2011).

Tensile tests are not widely used for measuring the texture of foods because: (1) the process of mastication primarily involves compression and shearing, not tension, of the food; and (2) tensile tests are more difficult to perform than other techniques discussed above; they present a special challenge in gripping or holding a food specimen without causing tissue damage or slippage during the test.

Bending tests provide an alternative of overcoming the specimen-gripping problem to study the tensile properties of foods. There are two types of bending tests available for measuring foods: the cantilever beam bending and the three-point bending. The latter seems to be preferred over the former due to the consideration of specimen mounting. Lu and Siebenmorgen (1995) used a three-point bending test to measure the bending strength of rough and milled rice and related bending strength to rice milling quality. Rice kernels were placed on a sample holding device with the distance between the two supporting points at 4.5 mm. Loads were applied to the middle section of the kernel through a loading head mounted on an Instron universal testing machine. Abbott and Buta (2002) used three-point bending to measure the firmness of fresh cut pear slices and found that the bending test was less suitable and convenient than the puncture test. Alvarez *et al.* (2000) determined the fracture toughness and fracture energy of fresh fruits and vegetables from rectangular cross-section beam specimens by using a standard engineering method known as the single-edge notched bend test (a form of three-point bending). Suhendro *et al.* (1998) developed a bending technique to measure corn tortilla texture. Tortilla strips were bent to a 40° angle and the force required to bend them was used to detect tortilla texture (i.e., rollability and flexibility). Using bending theory coupled with digital image analysis, Chauvin *et al.* (2010) and Pitts *et al.* (2008) proposed a three-point bending procedure to measure the elastic modulus of fruit and vegetables.

In conducting bending tests, the specimen length to thickness (or diameter for a circular beam) ratio is an important consideration. To minimize the shear and compressive stress, the specimen length should be sufficiently large in comparison with its thickness. Standard bending tests for many engineering materials often require the length to thickness ratio to be at least 16 (Van Hecke *et al.*, 1995). For many food products, this requirement can be difficult, if not impossible, to achieve and one has to balance various factors (such as sample geometry, the easiness of preparing test specimens, etc.) in selecting an appropriate length to thickness ratio.

4.3.6 Methods in data analysis

In most destructive methods discussed above, the data extracted from the force/deformation curves is maximum force or, sometimes, the slope or the

area up to the maximum force. This approach may not be good enough for certain applications since information embedded in the force/deformation curve, especially after initial failure, has not been effectively utilized or has even been totally discarded. A notable deviation from the conventional single parameter approach is the texture profile analysis (TPA) technique developed by Szczesniak and coworkers in the 1960s (Friedman *et al.*, 1963; Szczesniak *et al.*, 1963). The TPA test involves two complete cycles of compression and decompression of a food sample. The degree of compression of a food sample can be as high as 90%. The force/time or force/deformation relationship is recorded during the cycles of compression and decompression. From this force/time curve, a number of texture parameters such as fracturability, hardness, stringiness, and springiness are extracted. Szczesniak *et al.* (1963) reported that these parameters were closely related to sensory evaluation results. Bourne (1968, 1974) used an Instron universal machine to perform a modified TPA for pears and peaches. TPA tests have been used to quantify the textural properties of many other solid foods, including meat and meat products (Brady *et al.*, 1985; Chen and Trout, 1991; Herrero *et al.*, 2008), dairy products (Bryant *et al.*, 1995; Lakhani *et al.*, 1991), pecans (Ocon *et al.*, 1995), fruit and vegetables (Alvarez and Canet, 2000; Madieta *et al.*, 2011), and cereal products (Champagne *et al.*, 1999; Kim *et al.*, 2009; Lyon *et al.*, 2000).

Abbott *et al.* (1982) examined puncture and compression force/deformation curves of apple tissue and extracted numerous data including forces, areas, slopes, and locations of specific events, and estimated jaggedness after fracture. These data were compared to sensory evaluations of oral texture (crispness, hardness, toughness, mealiness, etc.) using stepwise regression (Abbott *et al.*, 1984). Maximum force was never the first-selected variable. Variables often selected were peak force of the first peak (not necessarily bioyield force), mean force near midcompression, force about the maximum compression (75% strain), and total work in compression. Slopes were seldom selected, indicating a weaker relationship of oral texture to modulus of elasticity than to strength variables in apples. They concluded that inclusion of two or more force/deformation variables in regression equations generally improved prediction of sensory textural attributes over maximum force alone.

Several other methods have been reported for analyzing the force/deformation (or stress/strain) curve to predict the textural properties of foods. These methods include the use of FFT, fractal analysis, principal component analysis, and stress/strain spectral analysis. One significant feature of these new data analysis methods is that they utilize the entire force/deformation curve or the portion of the curve after initial failure that shows considerable jaggedness and irregularity. Barrett *et al.* (1992) used FFT and fractal methods to analyze the jagged stress/strain curves to measure the crispness of food. The FFT method gave qualitative representation of the jaggedness of the force/deformation curve, but it lacked

quantitative interpretation of food texture such as crispness. Peleg (1997) recommended that the jaggedness of force/deformation curves be determined by at least two methods simultaneously for mutual verification. Meullenet *et al.* (1999) treated the force/deformation curves obtained from extrusion tests of cooked rice samples as if they were spectral data. Subsequently, a spectral analysis technique was used to develop a partial least square (PLS) model to predict eight sensory texture characteristics of cooked rice, including adhesion, hardness, cohesiveness, roughness, etc. Their results showed that the method has the potential to predict multiple sensory texture characteristics.

These new data analysis methods are useful for improving the prediction of specific textural properties of foods since the entire force/deformation curve is used in analysis. One major drawback with these methods is that they are more involved mathematically and often cannot provide meaningful interpretation on how textural attributes are predicted. However, once the methods and appropriate algorithms are developed, this should not present a great challenge to food technologists who may not have a good mathematical background.

4.4 Conclusion

The ability to measure texture objectively and quickly enables the food industry to set standards for quality and to monitor deterioration that occurs during storage and distribution. The study of chemical and physiological changes that determine texture has been underpinned by the development of methods for quantifying texture. Since texture can be defined as the human perception of the mechanical properties of the food, most commercial and research methods to measure texture have focused on the mechanical properties of the foodstuff. The diversity of foods, the variety of attributes required to fully describe textural properties, and the changes in these attributes as the product senesces (raw fruits and vegetables) or ages (processed foods) or undergoes microbial breakdown contribute to the complexity of texture measurement. The complexity of texture can still only be fully detected and described by sensory evaluation, which involves using a panel of people who have been trained to quantify defined attributes. However, instrumental measurements are preferred over sensory evaluations for both commercial and research applications because instruments are more convenient to use, widely available, tend to provide consistent values when used by different (often untrained) people, and are less expensive than sensory panels. We reviewed the principles of destructive instrumental texture measurements and provided some practical applications that have been reported to give guidance in selecting among the many methods that have been developed. These instrumental measurements are widely understood and can provide a common language among researchers,

producers, and customers (retailers or consumers). There are numerous empirical and fundamental measurements that relate to textural attributes. Mechanical methods measure functions of force, deformation, and time. Destructive mechanical methods generally relate more closely to sensory evaluations than do nondestructive measurements; but, by their destructive nature, they cannot be used for sorting products. Therefore, the commodity, the purpose of the measurement, sometimes tradition, and sometimes regulations guide the choice of the textural measurement method.

4.5 Acknowledgement

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Principles of food viscosity analysis

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Abstract: This chapter reviews key aspects of food rheology analysis. It begins by looking at the relationship between viscosity and the sensory attributes of food as well as processor requirements. After discussing rheological theory, the chapter reviews key fundamental and empirical test methods such as capillary and rotary viscometers.

Key words: food rheology, food viscosity, viscoelastic properties, capillary viscometers, rotary viscometers,

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5.1 Introduction

While food rheology is the study of deformation and flow of foods under well-defined conditions, it has been shown to be closely correlated with food texture (Bourne, 2002), in particular that of liquid and semi-solid foods (McKenna, 2003). There are many other areas (Escher, 1983; Bourne, 1992; Steffe, 1996) where rheological data are required by the food industry including:

- plant design: pumps and pipe sizing and selection, heat and mass transfer calculations, filler designs and other process engineering calculations involving extruders, mixers, coaters and homogenisers
- quality control: both of raw material and the product at different stages of the process (including ingredient functionality determination in product development and also shelf-life testing)

and, of course, the detailed evaluation of sensory attributes, quantitative measurement of consumer-determined quality attributes by correlating rheology measurements with sensory data and assessment of food structure and conformation of molecular constituents.

Food rheology literature normally concentrates on the behaviour of liquid foodstuffs, since this has developed into quite an exact science. However, there is an increasing tendency to consider the response of both solid and liquid materials to applied stresses and strains as being two extremes of the same science. There are in fact some foods that will exhibit either behaviour depending on the stress applied; molten chocolate, fat-based spreads, mashed potato and some salad dressings will exhibit a solid-like behaviour at low stresses and a liquid-like behaviour at high stresses (Mitchell, 1984). This tendency is increasing as more food products are developed that would be classed by the consumer as being semi-solid or semi-liquid. A more exact definition would therefore be the study of both the elastic and the plastic properties of foods.

In this chapter it is proposed, however, to place most of the emphasis on classic liquid rheology measurements, although elastic and viscoelastic properties will also be discussed in the context of semi-liquid foods. In addition, due to its inexact nature, sensory attributes and the contribution of viscosity measurement to its assessment will be largely confined to Section 5.2 below.

Examples of reviews of basic rheology include Borwankar (1992), Prentice (1992), Windhab (1995), Barbosa-Cánovas *et al.* (1996) and Rielly (1997). While the objective of this chapter is to review the influence of viscosity measurement on sensory attributes, it is nevertheless necessary briefly to consider some of the fundamentals. One should also justify the need for measurement given the wealth of published data already available. Some of these include Rao (1986), Kokini (1992), Rao and Steffe (1992), Vélez-Ruiz and Barbosa-Cánovas (1997) and the bibliography of McKenna (1990). The primary need for measurement was, and still is, as stated by Prins and Bloksma (1983): 'Rheological measurements have to be made under the same conditions as those which exist in the system studied.' In other words, there is limited use in carrying out measurements on a product or extracting values from the literature, if the stresses used and their rates of application during the measurement differ from those in the process calculation or assessment for which the measurement is required. In particular, the wide and varied range of stresses and shear rates found in the mouth will have significant effect on the sensory perception of the food.

5.2 Relevance of rheological properties of foods: the consumer's perception

The relevance of food rheology has been summarised above into the four categories of plant design, quality control, sensory attributes, and the research and development of food structure. Ultimately the food product must be eaten, so sensory attributes become most important. However, en route from the farm to the mouth the product may have to be pumped, heated, stored or subjected to other processes, and must be amenable to

flow when being placed in a container/package. Equally important is its ability to flow out of the container before consumption. Indeed, it is this ability (or the occasional lack of it) that first brings the consumer into a direct and sometimes frustrating contact with rheological principles. How often has the consumer experienced the dilemma of tomato ketchup refusing to flow from its bottle and found that the application of a sharp blow to the bottle base resulted in an excess amount being deposited on the plate? This provides an excellent example of a situation in which a product has a yield stress below which it will not flow, but flows perhaps too well once the consumer unknowingly provides the stimulus that exceeds it. Not only does this example illustrate yield stress, but it also shows the relationship between force and deformation and flow!

This simple example also gives emphasis to one of the basic rules of rheological measurements, namely that the product should be tested under a range of conditions of stress and shear rate that reflect those experienced during subsequent use, whether that use be tasting, pouring, shaking, stirring or any other action that requires movement of the material.

Of course, rheological relevance does not stop when a food reaches the plate but influences the sensory perception or ‘mouthfeel’ of the product. Matz (1962) defines mouthfeel as the mingled experience deriving from the sensations of the skin of the mouth after ingestion of a food or beverage. It relates to density, viscosity, surface tension and other physical properties of the material being sampled. These relationships between rheology and mouthfeel have been the subject of extensive research, as reviewed in the author’s bibliography on food rheology (McKenna, 1990). It will, however, be obvious that a change in the manner in which a food may move or flow in the mouth and throat will influence our perception of it as a desirable food.

There is a very significant literature and the relationship between sensory properties and rheology, with the viscosity being its simplest manifestation. Viscosity influences sensory perception in many ways, and in this chapter we will consider them in the order in which the consumer will experience them during eating. These consist of amount ingested (or bite size in the case of a solid food), mouthfeel, flavour perception and, finally, satiety.

5.2.1 Amount ingested

Two recent studies from the same team provide most of the information on this topic (Zijlstra *et al.*, 2007; de Wijk *et al.*, 2008). In their first paper they investigated the effect of viscosity on *ad libitum* food intake and the underlying mechanisms. These findings clearly show that products different in viscosity but equal in palatability, macronutrient composition and energy density lead to significant differences in *ad libitum* intake. In the later paper, the authors reported on two studies that investigated the effect of viscosity on bite size, bite effort and food intake by sipping from one of two products, a chocolate-flavoured dairy drink and a similarly flavoured semi-solid of equal energy density. They showed that the panellists needed 47% more

from the liquid than from the semi-solid to arrive at the same degree of satiation and that larger bite sizes were taken from the liquid than from the semi-solid. When the bite effort was removed through using a pump, ingestion for satiation was similar for both foods while the bite size for liquids started small but grew in size over successive bites with the opposite effect shown for the semi-solid. This led to the conclusion that bite size was smaller and intake lower from semi-solids than from liquids but the effect disappeared when bite effort was removed. This would seem to suggest that the higher the viscosity, the smaller the bite size and overall intake.

5.2.2 Mouthfeel

This area has been the subject of intensive research in recent years. In particular, the relationship between viscosity and texture in the mouth has been investigated. One study found that the establishment of casual relations is still hampered by poor physical definition of sensory texture terms together with insufficient knowledge of the deformations involving food in the mouth, the lack of homogeneity within many food products and insufficient development and understanding of relevant theoretical concepts in the field of rheology and fracture mechanics (Van Vliet, 2002). Other complications can arise from viscosity change due to mixing of the food liquid with saliva in the mouth which can increase the viscosity of a low viscosity food liquid and lower than of a high-viscosity one.

It is also worth noting that an early study in this field could not find a temperature effect when trying to correlate instrumentally measured viscosity with temperature effects (Sharma and Sherman, 1973). While the temperature range considered (20–40 °C) fell within the common range for food consumption, it is not the range in which major texture-changing effects such as phase change or starch gelation would be expected.

Of course, it is clear that the structure of the food liquid will have a significant influence on its sensory perception. One would expect this to be particularly true when the liquid in question is an emulsion or when the food had a gel structure. A study of the relationships between rheological and sensory attributes of acidified milk drinks (Janhøj, 2008) found that creaminess appeared to be largely determined by sensory viscosity (viscosity as perceived by the consumer) and could be manipulated by addition of thickeners. Unfortunately, sensory viscosity was not predicted with any great effectiveness by rheological measurements. They also found that the sensory perception of creaminess is, in fact, constituted of several underlying sensory descriptors and confirms the findings of Van Vliet (2002) above, that the science of relating sensory and rheological properties is hampered by poor physical definition of the sensory terms. Indeed, some researchers (Guinard and Mazzucchelli, 1996) showed that some sensory parameters such as creaminess and juiciness is quite complex with some researchers relating creaminess to viscosity and smoothness to physical frictional forces.

It is not surprising that studies on oil-in-water emulsions and their sensory attributes show that the sensory perception depends on a range of emulsion variables and ingredients (Vingerhoeds *et al.*, 2008). They found that perception of fat-related attributes, like creaminess, fattiness, satiation and after-feel coating, is affected by several factors, such as fat type and content, polysaccharide thickening agents and fat replacers. More recent studies from the same team (van Aken *et al.*, 2011) using oil-in-water emulsions had the remarkable conclusion that there was little direct effect on mouthfeel found by varying the oil viscosity by about a factor of 30. They concluded that any oil film deposited on the oral surfaces does not significantly contribute to sensory perception by viscous forces generated by shearing this film, suggesting that such a layer is either not formed or, if it is, it is sensed in a different manner. One nutritional outcome is the suggestion that increased viscosity caused by the oil droplets and its associated increase in thick and creamy mouthfeel could be achieved by replacing the oil droplets by other means of increasing viscosity such as polysaccharide thickeners. However, if the oil film mentioned above is found to form and have a significant sensory effect, such thickeners might not be able to provide a similar effect.

There are varying success rates reported in a wide range of studies trying to correlate viscosity and sensory attributes. Because of the complexities of the food liquids, the success rate is normally quite low. For one complex food, namely, soups using different thickeners and the complexity of before and after freezing, significant success has been reported (Lyly *et al.*, 2004). Good correlations were obtained between sensory texture attributes and viscosity ($r = 0.70\text{--}0.84$) while moderate correlations between flavour attributes and viscosity ($r = 0.63$ to 0.80).

With food gels the situation is even more complex. Firstly, we are moving from the classical liquid regime characterised by rheology and into the complex area of semi-solid foods. In addition, while possessing some liquid characteristics, such foods are not normally assessed using rheological terms such as viscosity. There are several significant studies in this field including Barrangou *et al.* (2006) but while finding some correlations between sensory and instrumental measurements, the measurements fall more into the field of 'solid' rheology rather than classical liquid measurements. Some success can be reported, however, with Tärrega and Costell (2007) showing that for semi-solid dairy desserts the yield stress correlated well with oral thickness and both the storage modulus at 1 Hz and the complex viscosity at 7.95 Hz (50 rad s^{-1}) were the viscoelastic parameters best correlated with this sensory property.

5.2.3 Flavour perception

It is obvious that any property that may lead to coating formation in the mouth may have significant effects on flavour perception. This may be an

enhancement if the flavour is concentrated in the coating or a masking of flavour if the flavours need to migrate through such a barrier to reach the flavour sensors. Here again there has been a significant volume of published work. However, only those studies that combine flavour effects with viscosity will be considered. Ferry *et al.* (2006) looked at viscosity effects on starch thickened liquids of intermediate viscosity. It was shown that for hydroxypropylmethyl cellulose thickened products a considerable decrease in perception was detected for both flavour and saltiness with increasing viscosity, while when thickened with different starches it was found that viscosity induced flavour and taste suppression was very much smaller. It is suggested that both flavour perception and mouthfeel can be related to the efficiency of mixing of the thickened solutions with water or with saliva in the case of ingestion. This would appear to be more affected by the physical structure of the starch granules than by the viscosity they induce.

Koliandris *et al.* (2010) studied the influence of thickeners on viscosity and saltiness perception. This is important as salt plays a major role in the diet as a tastant, flavour enhancer, nutrient, preservative and structuring aid. While salt is part of a healthy diet, in the developed world the vast majority of people consume salt at a very high level and may be at risk of developing diet-related illnesses. This study looked at whether careful choice of the viscosity behaviour of food thickeners can be used in Newtonian and shear-thinning aqueous solutions to enhance salt perception and allow for a salt content reduction of foods without flavour loss. It was found that saltiness perception correlated inversely with viscosity below 50 s^{-1} . In addition perceived thickness correlated with shear rates around 500 s^{-1} .

5.2.4 Sensory conclusions

From all of the above studies and the many others that are not reported here, it can be concluded that the major difficulties in trying to relate rheological properties to sensory ones are that of trying to relate an exact science to an inexact one or, more correctly, a historically well-developed science with a newer less well-developed one. From reading Sections 5.4 and 5.5, one can conclude that rheology and rheological measurements are based on basic underlying scientific and mathematical principles. On the other hand, while there is obviously a very scientific basis for sensory perception and reactions in the mouth, the principles are, as yet, not sufficiently developed to apply the same mathematical rigour to it as can be done for rheology. In its absence, sensory science is encumbered with a vast array of descriptive terms, few of which can be regarded as a basic property and, as many authors have suggested, are combinations of several scientific properties. To date, the relationships between rheology and sensory values have not progressed far beyond the area of empirical correlation. Until this is overcome, rheological measurements, and particularly viscosity, will not become a valuable tool in sensory perception.

5.3 Relevance of rheological properties of foods: the requirements of the processor

The processor requires rheological data for a range of activities. During plant design, it is necessary to select pumps, pipes, heat exchangers, stirrers, etc. The flow rate of a liquid in a pipe is highly dependent on these rheological properties (Singh and Heldman, 1993) (see Eqns 5.10 to 5.17). Another way of considering this is that for a given flow rate of a food liquid, a particular pressure drop will be required along the pipe length and this will influence the quantity delivered by the chosen pump. The process itself may further influence the behaviour. Heating of the food liquid will change the rheological properties and may lead to changes in the flow system since, for most liquids, viscosity is highly dependent on temperature. The dependency is usually that of a fall in viscosity as the temperature increases. In an extreme case, a large, heat-induced viscosity decrease can cause the velocity to increase so much that the residence time in the system is not sufficient for the desired processing effect to be achieved. This is especially the case when pasteurising or sterilising food liquids. Equally detrimental to achieving the desired processing effect may be a change in the flow or velocity profile in the system (rheology induced) that can alter the residence time distribution and again lead to an under-processed product. The opposite effect may be a heat-induced starch gelation or similar reaction that can lead to a thickening of the food liquid and, effectively, increase the severity of the heating process.

There are also many other rheological problems in processing. Yield stress, as is exhibited in the ketchup example above, may lead to more serious processing problems with significant economic relevance. This is also of significance in enrobing of food products, especially in the area of prepared consumer foods (Hillam, 2000). Coatings may range from chocolate-enrobed confectionery to batter-enrobed fish or meat products, all demand an enrobing material that exhibits a yield stress. If this yield stress is too low, the weight of enrobing liquid adhering to the sides of the product will induce a stress in excess of the yield stress, either on the vertical side of the product or on a plane parallel to this within the enrobing material, and will cause the material to flow off the product. Conversely, too high a yield stress will lead to excessive thickness of enrobing material possibly attractive to the consumer of a chocolate bar, but with adverse economic consequences for the processor.

Quality control is also an area of rheological significance for the processor. While there is the obvious need to induce the desired characteristics into the product and to test the product for these attributes, rheology can provide other quality control information by drawing on the wealth of correlations between rheological and other data that have been developed over many years. For example, Sharma and Sherman (1966) have shown that for ice cream there is a good correlation between rheological

measurements and fat droplet size, the volume of air incorporated (overrun), ice crystal size and product temperature. For chocolate, information on the hardness and consequently the fat composition of the major ingredient, cocoa butter, can be deduced (Lovegren *et al.*, 1958).

In the dairy industry there are many examples of the use of rheological control techniques. Many of the attributes controlled, while not within the usual range of defined sensory attributes, can be regarded as somewhere in the wide interface between physical and sensory properties. While the textural related rheological attributes of yoghurts, whether set or stirred, is an obvious example, there is an ever-increasing range of dairy-based spreads that demand that the successful product should have the correct viscoelastic properties for spreadability. So also in the case of soft and cream cheeses which have liquid properties that must be kept within chosen ranges and which are highly dependent on the ongoing microbiological activity, proteolysis and syneresis within the product as well as product temperature. Holsinger *et al.* (1995) emphasise the importance of rheology in providing an insight into the influence of composition and processing on cheese texture. A less obvious example is the need for rheological control of concentrated milk products during evaporation and drying since changes in the rheology will alter the drop size range produced by the atomisers in the drying process (McKenna, 1967), which will, at best, change the particle size distribution in the finished powder, not only altering its bulk density and ease of reconstitution but also leading to increases in powder losses in the final air-powder cyclone separators. A worst case scenario would see the droplet size increasing leading to incomplete drying and the larger, semi-dried particles adhering to one another to form a sticky mess. Further reviews on the influence of rheology on dairy products may be found in Vélez-Ruiz and Barbosa-Cánovas (1997).

Food ingredients, including dairy ingredients for soups and sauces, cereal ingredients and the aforementioned batters and coatings, constitute a sector that has seen a rapid expansion in sales over the past two decades. This expansion, largely in response to increasing consumer demand for convenience meals, has led to a significant demand in functional ingredients for their manufacture. While some functionality is driven by nutritional demands, rheological attributes contribute to a sensory functionality in the ingredients. Consequently, rheology is a major tool used in the development stages of both the ingredients and the final products. Cream sauces are an example of a component of many convenience meals, but the use of fresh cream is problematical owing to its perishability and poor process stability. Such sauces can be developed from dry ingredients but to ensure the manufactured sauces have appropriate rheological characteristics they can be compared with sauces formulated from fresh ingredients. Another area, which is continually developing, is extrusion cooked ingredients, which are used in the production of snacks, coatings and convenience meals. The expansion of these products as they pass through the extruder die is

dependent on the viscoelastic properties of the dough as is the flow behaviour of the paste within the screws of the system (Kokini *et al.*, 1992).

Probably the most extensively researched area of food rheology has been that of dough of various types. Not only does dough rheology influence the physical characteristics of the finished baked product, it also has a significant effect on sensory attributes. Typical of the many reviews of this topic are those of Bloksma (1990), Faridi and Faubion (1990) and Rasper (1993). Dough rheology will influence the texture of the bread crumb produced and also on the final volume of the baked product. The use of frozen dough has become an increasingly popular alternative to conventional dough processing both within in-store bakeries and domestically. Rheological measurements have been used to predict the baking performance of such products (Kennedy *et al.*, 1999). High-fat, microencapsulated powders are a healthy and convenient alternative to fats normally used in cereal products and rheological properties have been used to assess the impact of these powders on wheat flour doughs (O'Brien *et al.*, 2000). Indeed, the importance of dough rheology has led to the development of specialised instruments over the years to monitor these properties (e.g. farinograph and extensigraph). Unfortunately, while they are widely used, many of the properties measured are machine specific and are not the absolute properties defined in the next section.

5.4 Basic rheology

Food rheology, of which viscosity is its simplest manifestation, is concerned with the description of the mechanical properties of food materials under various deformation conditions. Under external force, food materials exhibit the ability to flow, or accumulate recoverable deformations, or both. According to the extent of recoverable deformation, the basic rheology concepts can be classified into viscous flow, elastic deformation and viscoelasticity (Barbosa-Cánovas *et al.*, 1996).

5.4.1 Viscous flow

Rheology is the study of deformation and flow of foods under well-defined conditions. These conditions could be defined in terms of their rate of deformation or in terms of the magnitude of the stress or the strain applied. Foods of differing internal structure and bonding react in different manners to these applied conditions. In the simplest case the shear stress developed in the fluid is directly proportional to the rate of deformation or the rate of strain. In such cases, the liquid is said to be Newtonian and obeys the relationship:

$$\tau = \mu \dot{\gamma} \quad [5.1]$$

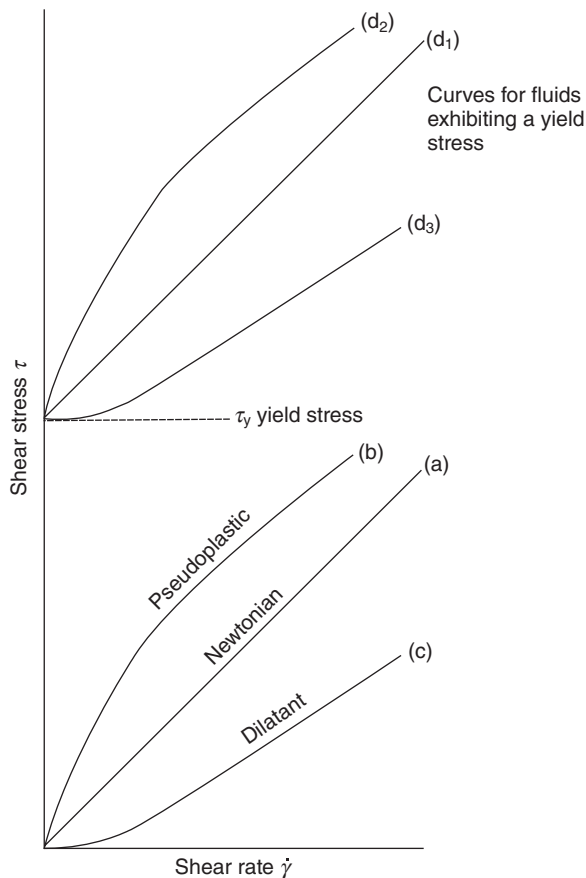


Fig. 5.1 Typical flow curves.

where τ is the shear stress and $\dot{\gamma}$ is the shear rate. Such a relationship is shown by line (a) of Fig. 5.1. In SI units, τ will normally be in pascals (Pa), $\dot{\gamma}$ in reciprocal seconds (s^{-1}) and μ in pascal seconds (Pa s). The constant of proportionality μ between the shear stress and the shear rate is termed the viscosity of the fluid, and from the 1663 definition of a fluid by Pascal can be viewed as a measure of its internal friction (i.e. ability to resist motion when a shearing stress is applied).

Equation 5.1 is representative of the Newtonian fluid line shown in Fig. 5.1. Background to the development of this simple model from force balances can be found in many reviews, including one by the present authors (McKenna and Lyng, 2003). In particular, the simple concept of two flat parallel moving surfaces is still relevant to a discussion of rheological concepts today. Of course, modelling of fluid behaviour has progressed significantly since the development of this model and many would dispute its

inclusion in any discussion on modern rheology. However, the basic principles of many instruments are still the two-surface concept, one moving and one stationary, with the fluid being characterised by force measurements at one of the surfaces.

Using the concept of a Newtonian fluid in which there is a fixed proportionality between shear stress and the applied shear rate and with a simple linear form of the flow curve, such liquids can be characterised by a single term, namely the constant of proportionality or the viscosity. More importantly, a single experiment such as the measurement of the shear stress at one surface at a single shear rate is sufficient to quantify the rheological characteristics of the fluid. However, few food liquids follow this simple relationship (water, unconcentrated milk, vegetable oils, some dilute solutions) and most foods may be classified as non-Newtonian and exhibit responses or flow curves such as those of (b), (c) and (d) in Fig. 5.1. Obviously, such fluids cannot be characterised by a measurement at a single shear rate as can the simple Newtonian fluid, and it is the ignoring of this requirement that produces the most common rheological measurement errors in the food industry. Furthermore, for many food liquids shear stress is not only determined by shear rate but is also time dependent, a factor which demands its own unique measurement system.

Many foods are termed 'pseudoplastic' and their response to an applied deformation varies with the rate of application of the deformation. Typically, plots or flow curves such as curve (b) of Fig. 5.1 represent such fluids. Because the slope of the curve decreases as shear rate increases, the term 'shear thinning' is often applied to such fluids (e.g. concentrated milk, solutions of concentrated molecules (xanthan and guar gum) and several fruit juices). Of lesser importance in the food industry are foods with curves of type (c), which are 'shear thickening' or 'dilatant'. Shear thickening behaviour of foods is only rarely observed (e.g. concentrated suspension of starch granules) and then over shear rate ranges normally not observed in practice (Van Vliet, 1999).

Rather than apply polynomial regression analysis to obtain equations for such behaviour, it has been found more convenient to plot the logarithm of shear stress against that of shear rate. For most pseudoplastic or dilatant fluids this results in a straight line and leads to the equation:

$$\tau = k\dot{\gamma}^n \quad [5.2]$$

which is normally termed the power law equation. In this equation, n is the power law exponent and k is the apparent viscosity or consistency index. While mathematically simple, there is a theoretical objection to its use, namely that the dimension of k is dependent on the value of n . A Newtonian fluid would of course have an n value of 1.0 and k would equal its viscosity. For pseudoplastic fluids, n will lie between 0 and 1.0, while for dilatant liquids the value will be greater than 1. Though widely used, the power law model is not the only available, and in some cases its two-parameter

equation represents an oversimplification (Launay and McKenna, 1983). Ree and Eyring (1958) proposed a three-parameter model:

$$\mu = \mu_0 + (\mu_0 - \mu_\infty) \frac{\sinh^{-1} \beta \dot{\gamma}}{\beta \dot{\gamma}} \quad [5.3]$$

where μ_0 and μ_∞ are the Newtonian viscosities at zero and infinite shear rate, while β is a characteristic relaxation time. Obviously, such a model facilitates consideration of time dependent behaviour. Cross (1965) proposed a four-parameter model:

$$\mu = \mu_0 + (\mu_0 - \mu_\infty) [1 + (t\dot{\gamma})^{1-n}] \quad [5.4]$$

where t is another relaxation time. However, while Eqns 5.3 and 5.4 give more precise modelling of the flow curves of many foods, the widespread use of power law values in engineering equations makes Eqn 5.2 the most useful, if not the most exact, model. Neither do three- or four-parameter models imply a better understanding of the structure of the food in question nor of the effect of rheology on the sensory properties.

Finally, one must consider the family of curves marked (d) in Fig. 5.1. Such foods exhibit a yield stress τ_y which must be exceeded before any deformation or flow can occur (i.e. these materials behave like solids under low stress and like fluids under high stress). For certain food processes (e.g. chocolate, confectionery and other coatings) the existence of a yield stress in the food is essential for application of the technology. Indeed, in the absence of rapid crystallisation or solidification of a coating, the magnitude of the yield stress will determine the thickness of the coating on a vertical surface. If the weight of coating divided by the vertical area (i.e. the shear stress exerted by the coating itself) exceeds the yield stress, then the coating will flow off the product. If not, it will neither flow nor deform and will remain to set on the product.

Equations, which describe such products mathematically, are those of Casson (1959) and Herschel-Buckley (see Charm, 1971):

$$\text{Casson: } \tau^{0.5} = \tau_y^{0.5} + k' \dot{\gamma}^{0.5} \quad [5.5]$$

$$\text{Herschel-Buckley: } \tau = \tau_y + k'' \dot{\gamma}^n \quad [5.6]$$

where τ_y is the yield stress and k' and k'' are constants. While the Casson equation is widely used (particularly in the chocolate industry, where it is generally accepted that molten chocolate can be modelled using the Casson equation), the Herschel-Buckley equation has the added attraction of merely adding a yield stress to the power law model.

Time-dependent behaviour of liquid foods is not considered in detail in this chapter and the reader is referred to texts such as Steffe (1996), Rielly (1997) and Van Vliet (1999). This is not because such aspects are unimportant for many foods but because, in steady state flow in pipes or channels in a food processing operation, little or nothing of time-dependent

behaviour is observed. However, in storage of foods these properties become increasingly important as the onset of undesirable change may limit the effective shelf-life of a product and they may play some role in the sensory attributes of the products if the residence time in the mouth exceeds the time dependencies.

Once again, the temperature dependency of rheological characteristics must be stressed. Since rheology is based on internal friction and internal friction is a molecular phenomenon, anything that alters molecular movement will influence internal friction. Consequently, the rheology of most liquid foods is highly temperature dependent. In particular, the viscosity of Newtonian liquids exhibits such a dependency, as does the consistency index or apparent viscosity of power law fluids. The power law exponent is, however, relatively unaffected. No attempt will be made to quantify this phenomenon mathematically or to give a thermodynamic explanation for its existence. It is merely highlighted here to stress the importance of temperature control on the accuracy of any of the experimental rheological techniques detailed in later sections. For example, since the viscosity of water at 20 °C (293 K) will change by 2.5% per kelvin temperature change, an accuracy of 0.1% in the measurement of this viscosity will demand temperature control to within 0.04 K. Many oils will change in viscosity by 10% for each kelvin temperature change at 298 K (25 °C), thus demanding temperature control to 0.1 K for a 1% accuracy. It should be assumed that close temperature control is an essential feature of any of the measurement systems described in the following section.

5.4.2 Elastic deformation

As was stated earlier, greater emphasis will be placed on classical liquid rheology in this chapter. However, it is necessary to mention briefly elastic deformation in solids before going on to discuss the concept of viscoelasticity, which can be observed in semi-liquid fluids. Certain types of solids, known as hookean solids, display ideal elastic (or hookean) behaviour. This particular behaviour occurs when a force is applied to a solid material and the resultant response gives a straight line relationship between stress and strain (Vélez-Ruiz and Barbosa-Cánovas, 1997). This relationship is known as Hooke's law and occurs in an ideal elastic solid (also called Hooke's body).

Based on Hooke's law the following relationship (Eqn 5.7) has been established for a Hooke solid subjected to distortion by shear stresses:

$$\tau = G\gamma \quad [5.7]$$

where G is the shear modulus (Pa), τ is the shear stress (Pa) and γ is the shear strain ($\gamma = (L'_0 - L_0)/L_0$, dimensionless, where L'_0 is the final length after deformation of the material and L_0 is the original length before deformation) (Barbosa-Cánovas *et al.*, 1996).

5.4.3 Viscoelasticity

Many complex structured foodstuffs display both viscous and elastic properties and are known as viscoelastic materials. The use of this term is often restricted to solids, with the term ‘elastico-viscous’ being used to describe liquids displaying similar characteristics. However, following on from Whorlow (1992) in this chapter we will use the term viscoelastic to describe both, because it is often not possible to establish whether a material is behaving as a solid or as a liquid. Linear viscoelasticity is the simplest viscoelastic behaviour in which the ratio of stress to strain is a function of time alone and not of the strain or stress magnitude, while non-linear viscoelastic materials exhibit mechanical properties that are a function of time and the magnitude of stress used. The theoretical complexity of non-linear viscosity makes it impractical for most applications (Steffe, 1996) and in this text we will focus on viscoelasticity in its simplest linear form. Such viscoelastic behaviour may be explained using models, examples of which include the Maxwell and also the Kelvin (sometimes called the Kelvin–Voight) models. Both of these models use an ideal spring to represent the elasticity, while viscosity is represented by an ideal dashpot. In the Maxwell model this spring and dashpot are joined in series (McKenna and Lyng, 2003). In the Maxwell model if the strain rate is kept constant and the sample is deformed at a known rate, the build up of stress can be calculated from:

$$\tau = \mu\gamma(1 - e^{-t/t'}) \quad [5.8]$$

where t' is the relaxation time.

In the Kelvin model, the spring and dashpot are joined in parallel and similar treatment for the Kelvin body gives rise to the following:

$$\tau = \gamma(\mu + Gt) \quad [5.9]$$

The Maxwell and Kelvin models may be used as building blocks in parallel or tandem to construct more sophisticated models (e.g. Burgers model) but these are beyond the scope of this chapter and the reader is referred to texts such as Muller (1973), Prentice (1992) and Steffe (1996) for further information.

5.5 Measurement systems

Rheology measurement, or in its simplest manifestation, viscosity, for sensory analysis has not seen the development of specialised instrumentation and the instruments used for such analysis are the same as those used for other rheological purposes. Below, the reader will find an updated version of the instrumental section of an earlier chapter by McKenna and Lyng (2003).

Instrumental food rheology measurement systems can be broadly categorised into fundamental or empirical tests. Fundamental methods are

conducted on a material by imposing a well-defined stress and measuring the resulting strain (or strain rate) or alternatively by imposing a well-defined strain (or strain rate) and measuring the stress developed (Barbosa-Cánovas *et al.*, 1996). Based on the geometry of the fixtures used, fundamental measurement systems can be divided into two groups: (a) capillary viscometers (Section 5.5.1) that make use of gravity (hydrostatic head) or pressurised (piston or pressurised gas) flow in capillary tubes for the measurement process; (b) rotary viscometers (Section 5.5.2) in which the sample is enclosed between rotating or oscillating surfaces. Empirical methods (Section 5.5.3) are also important in that they can give rapid results, but are arbitrary, poorly defined, have no absolute standard and are effective only for a limited number of foods. In general they measure rheologically affected phenomena from which it is possible to make a correlation to a desired variable. The main emphasis in this chapter will be on fundamental methods.

5.5.1 Capillary viscometers

Theory

Capillary viscometers are the simplest form of viscometer available from which it is possible to obtain absolute values of viscosity for Newtonian fluids and to obtain limited information on power law fluids. The basic measurement made is of the time t taken for a fixed volume V of the test fluid to pass through a length L of capillary tubing. Relative movement takes place between the axial part of the sample and that in contact with the tube walls. The driving force for fluid flow can come from gravity (as determined from the hydrostatic head difference between two liquid reservoirs in the viscometer) (glass (U-tube) viscometers) but pressurised gas or a piston (high-pressure capillary viscometers) can also be used (see Fig. 5.2).

From first principles it is possible to derive an equation for the flow rate of fluid through such a tube or pipe. For Newtonian fluids, this equation is known as the Hagen–Poiseuille law (Hagen, 1839; Poiseuille, 1841) and relates the flow rate to the driving pressure for flow, with many of the variables of such a system incorporated into the constants of the equation:

$$\frac{Q}{d^3} = \frac{\pi d \Delta p}{128 \mu L} \quad [5.10]$$

which can be rearranged to

$$\mu = \frac{\pi \Delta p d^4}{128 L Q} \quad [5.11]$$

where Q is the flow rate through the tube (m^3/s), d is the tube diameter (m), L is the tube length (m) and Δp is the pressure difference across the tube (N m^{-2}). For a given instrument d and L are fixed, so by measuring Q at a known Δp the coefficient of viscosity μ may be calculated. Indeed, since the volume processed in a given instrument is fixed at V , then Q may be

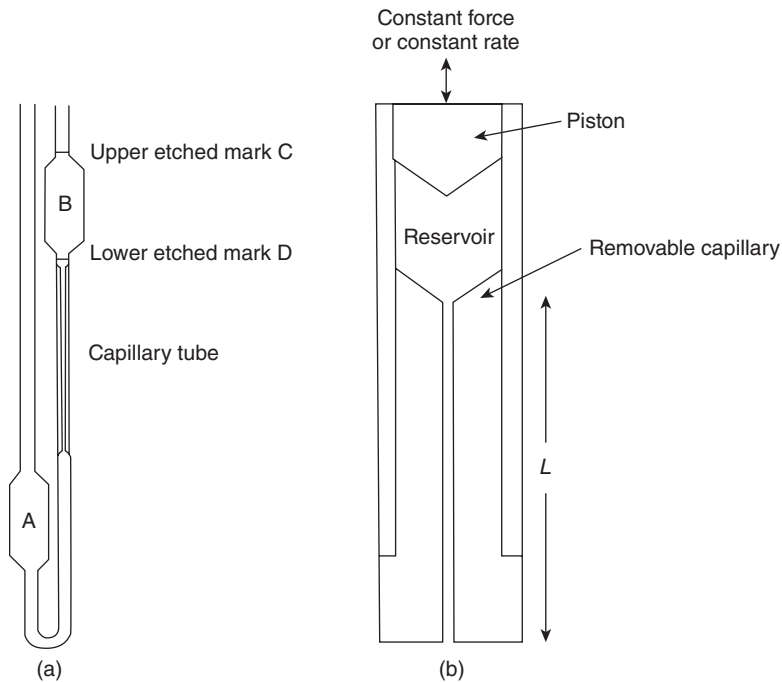


Fig. 5.2 Capillary viscometers: (a) Ostwald viscometer; (b) pressure capillary viscometer.

replaced by V/t , where t is the time required for the flow. Taking the glass capillary (U-tube) viscometers as an example, the driving force for flow will normally be the hydrostatic head within the system and will be equal to the product ρgh , where ρ is the liquid density, g is the gravity constant and h is the difference in liquid levels between the reservoirs of the system. For the U-tube viscometers it is then possible to simplify Eqn 5.11 and write it in the form:

$$\mu = K\rho t \quad [5.12]$$

where ρ is the density of the fluid under test, t is the time taken for the fluid to flow through the capillary tube, and K is a constant for the instrument given by:

$$K = \frac{\pi g h d^4}{128 L V} \quad [5.13]$$

This value is often supplied by the viscometer manufacturer. However, a common alternative approach is to use such capillary viscometers for comparative measurements against standard fluids of known viscosity. If the pressure difference causing flow is the same while measuring both fluids

(for the glass (U-tube) viscometers atmospheric pressure and gravity flow are usually applied), then the ratio of the viscosity of the food sample to that of the standard fluid will be equal to the ratio of the time required for equal volumes of the fluids to flow through the viscometer tube. Similarly, such standard fluids may be used to compute or to check the value of K given in Eqn 5.13. In the case of piston or gas pressure viscometers, the mean hydrostatic head due to the test fluid must be added to the measured applied pressure but the slight variation in hydrostatic head as the fluid leaves the upper bulb can usually be ignored (Whorlow, 1992).

The equations above have traditionally been used not only for viscometry but also to quantify the flow rate in a pipe system by monitoring the pressure drop along a section of the pipe. However, as the following section will demonstrate, this method should be used only as a rough estimate with food liquids as their generally non-Newtonian behaviour will demand that more complex relationships be used.

The flow of more complex fluids is governed by variations on the above equation. For laminar flow of power law fluids through a cylindrical tube under the influence of a pressure difference Δp , the following equation is obtained:

$$\frac{Q}{d^3} = \frac{\pi}{8(3+1/n)} \left(\frac{d\Delta p}{4kL} \right)^{1/n} \quad [5.14]$$

where n and k are the power law constants. At constant temperature, the apparent viscosity, k will be constant, so a plot of $\log Q$ versus $\log \Delta p$ will give a straight line of slope $1/n$, with the value of k being abstracted from the intercept value of the plot:

$$\log \frac{\pi d^{3+1/n}}{(4kL)^{1/n} [8(3+1/n)]} \quad [5.15]$$

However, with such simple equipment, facilities are seldom available to apply different pressure differences so as to obtain the points for such a plot. A more limited possibility is to take a range of viscometers of different capillary diameters but similar tube lengths and then to test the power law liquid in each using gravity flow. A plot of $\log Q$ versus $\log d$ should then give a straight line of slope $3 + 1/n$. Again, k could be abstracted from the intercept value:

$$\log \frac{\pi \Delta p^{1/n}}{(4kL)^{1/n} [8(3+1/n)]} \quad [5.16]$$

A food liquid that behaves as Newtonian once its yield stress value has been exceeded (curve (d₁) in Fig. 5.1) will have a characteristic behaviour equation as follows:

$$\frac{Q}{d^3} = \frac{\pi d \Delta p}{128 \mu_p L} \left(1 - \frac{16 \tau_y L}{3 d \Delta p} + \frac{256 \tau_y L}{3 d \Delta p} \right)^4 \quad [5.17]$$

where μ_p is the slope of the straight line plot of shear stress versus shear rate once the yield stress had been exceeded. For a more complete discussion of this equation see Leniger and Beverloo (1975); Prentice (1984) details the flow of Herschel-Buckley and Casson liquids in tubes or capillaries. As this is a more complex equation and post-yield stress linear behaviour is seldom experienced with food products, these simple viscometers cannot be recommended for examination of such products. They are, however, widely used, often in circumstances where their limitations are not fully understood. This is because they are relatively cheap and are easily available from most laboratory supply companies. Indeed, when one considers the equations involved and the multiple measurements required for all but simple Newtonian fluids, the use of such unsophisticated equipment presupposes knowledge of the basic behaviour of the fluid under test. In other words, these viscometers should be used only for known Newtonian fluids. This would confine their use to dilute solutions and vegetable oils. For other foods, they can provide only rough quality control tests.

The sizes of the food sample and of the constituents within the sample are important with viscometers of this type. As they rely on measuring the time taken for a given volume of sample to flow through the capillary tube, it is important to ensure that a homogeneous sample of the volume required can be obtained from the food. Difficulty may be experienced with foods containing large amounts of suspended solids. Indeed, suspended solids will contribute to large errors in the measured times if they are of a size that is significant when compared with the diameter of the capillary tube. Further, particles that affect laminar streamline flow within the capillary will change the time measurement. Of course, these comments are equally relevant to droplets within an emulsion as they are to solid particles. Care must also be taken to ensure that suspended particles within a food do not settle during the duration of a test. Nor should any separation occur within a food emulsion.

Examples have already been given that place general emphasis on the need for exact temperature control during measurements with this as with any type of viscometer. Prentice (1984) quotes an instance where temperature variations of ± 0.12 K will alter the linearity of the flow curves obtained.

Instruments

In this section (similar to Whorlow, 1992) capillary viscometers will be classified according to the method used to apply pressure. Glass capillary (U-tube) viscometers rely on a hydrostatic head to force the test fluid (generally a low-viscosity liquid) through the capillary tube, while in high-pressure capillary viscometers (generally used for more viscous liquids), air, gas or hydraulic pressure is applied or the fluid is forced through the tube by means of a piston. The distinction between pipe versus capillary type systems is also mentioned at the end of this section.

Instruments: glass capillary (U-tube) viscometers

Figure 5.2a shows the simplest glass capillary viscometer available known as the Ostwald viscometer. However, there are many variations of this on the market (e.g. Cannon-Fenske, Ubbelohde), each of which would claim a special advantage and may have its own specific name applied by its manufacturers. These glass capillaries rely on a hydrostatic head to induce fluid flow through a tube.

Operation is as simple as the design of the system. A standard volume of the test food liquid is pipetted into reservoir A of the viscometer and the U-tube below it. The instrument should preferably be held exactly vertical. If not, the support fixture should be such as always to hold the instrument at the same angle from the vertical. The instrument and the test liquid must now be equilibrated at the test temperature by immersing the viscometer in a controlled temperature water bath. Earlier sections have discussed the influence of the precision of this temperature on the accuracy of the results obtained. As these are related to the temperature sensitivity of the viscosity of the test liquid and this is often unknown before the measurements are undertaken, this author recommends that ± 0.1 K be taken as a target temperature variation. Equilibration may take up to 0.5 h, during which the earlier comments on sedimentation or separation become relevant. Suction is then used to raise the liquid through the capillary into reservoir B until the meniscus of the liquid is above the etched mark C. The liquid is now allowed to flow under gravity and the time is taken for the meniscus to pass between marks C and D. Generally reservoirs A and B should be of similar radius to minimise surface tension errors.

During this process the hydrostatic head will fall as the liquid level falls on the right hand side in Fig. 5.2a and rises in the left-hand leg. However, because the geometry of the system is arranged so as always to have test liquid within reservoir A with its large cross-section, the rise in the level in reservoir A will be very small. Consequently, the variation in hydrostatic head will be minimised. In addition, the shape of reservoir B is such that most of the measured flow will occur with the level central in this chamber and further reduce the variation in the head. A mean value will be quoted by the manufacturer. Examination of Eqns 5.10 and 5.14 shows that this variation has no effect on Newtonian fluid measurements, while its effect on power law fluids could be considerable if the power law exponent n varied significantly from 1.

As previously stated, variations in design of glass capillary viscometers are many in number. One common form involves bending both legs of the U-tube slightly so that the bulb of the lower reservoir A is directly below that of reservoir B. Another variation is the use of light sensors to note the passing of the meniscus across the etched marks C and D coupled to electronic timing, thereby ensuring more accurate measurement. As with most scientific instruments, corrections are necessary if a high level of accuracy is required. These include kinetic energy effects, end effects, turbulence and

wall effects, effects of time-dependent properties and thermal effects. Many authors cover these corrections in some detail and the reader is referred to Lapasin and Pricl (1995) for a complete discussion.

Instruments: high-pressure capillary viscometers

High-pressure capillary viscometers are also available and are constructed from glass or steel tubes. As earlier stated, these systems differ from the glass capillary viscometers mentioned above in that they rely on pressure from either compressed gas (air or nitrogen) or a piston to induce fluid flow through a tube. The gas pressure viscometers normally operate at a constant pressure whereas piston viscometers tend to operate at constant flow rate. In both the gas and piston systems the intake reservoir and capillary tube should be held in a thermostatically controlled environment for the duration of any measurements. These high-pressure systems are widely used in the plastics and lubricants industries but are less commonly used for rheology measurements on foodstuffs.

Whorlow (1992) outlines a number of different gas pressure viscometer designs. In general terms these systems consist of a straight length of capillary tube that connects two reservoirs (an intake and a receiving reservoir). The gas supply passes via a pressure regulator into an intake reservoir whereupon it forces the liquid through a capillary tube. The capillary tubes can be removed for cleaning and are interchangeable, with the possibility of being replaced by tubes of different diameter or length as required. Tubes range in diameter and length from 2.5 to 6 mm and from 25 mm up to 3 m, respectively. Other variations in design include a facility to prevent gas becoming dissolved in the test liquid, by housing the fluid in the intake reservoir within a plastic bag with pressure being applied to the outside of the bag forcing the liquid through the capillary tube.

Piston viscometers differ from a gas pressure viscometer in terms of the design of intake reservoir and also in the fact that they can be used at constant flow rates or constant pressures. The intake reservoir consists of a cylindrical barrel into which the fluid to be measured is placed. A piston head fitted with sealing rings is inserted into the barrel and is used to force the liquid through the capillary tube. Similar to the gas pressure viscometer, the intake reservoir and capillary tube are held in a thermostatically controlled environment. The reader is referred to Whorlow (1992) for a more complete description of these systems.

Instruments: pipe viscometers

Pipe and capillary viscometers differ in terms of tube diameter but there are no clearly defined sizes at which a tube should be called a capillary rather than a pipe. Commercial capillary instruments range in diameter from 0.1 to 4 mm. Pipe viscometers vary widely in diameter with some systems having diameters as small as 7 mm but values of greater than 12 mm and up to 32 mm are not uncommon in food applications (Steffe, 1996).

5.5.2 Rotary viscometers

In rotary viscometry the product is enclosed between two surfaces, one of which subsequently undergoes an applied rotary motion. The geometry of these surfaces can be in the form of concentric cylinders (or Couette viscometers) while other possibilities include a cone and plate or a pair of parallel plates. Depending on how the rotating surfaces are controlled these viscometers can be classified as rate-controlled or stress-controlled. In rate-controlled instruments the velocity of rotation of the one of the surfaces is the controlled quantity and the transmitted torque is recorded on the measuring surface, while for stress-controlled instruments a controlled torque is applied to one surface and the resultant rate of rotation is subsequently recorded (Lapasin and Priel, 1995).

Traditionally a rheometer was designed to measure under controlled-stress or controlled-rate conditions but combined units, which offer measurement under both conditions are now available. Although we use the term viscometer in this section many instruments are generically called rheometers (versus viscometers) since they measure other properties in addition to viscosity. In the more sophisticated microcomputer-controlled systems, several operating modes are generally possible, examples of which include creep measurements, controlled stress flow and oscillatory mode.

Concentric cylinder viscometers: theory

The concentric cylinder type is shown schematically in Fig. 5.3 and owes its development to the pioneering work of Couette (1890). These instruments consist of a cylindrical bob positioned concentrically in a hollow cylinder. In Searle-type viscometers the bob rotates while in Couette-type viscometers the cylinder can be rotated. In rate-controlled instruments the measured variable is either the torque transmitted through the liquid to the stationary cylinder or the torque required to keep the moving cylinder rotating at a given velocity. In stress-controlled systems the rate of rotation induced in the measuring surface is recorded as controlled torque (or shear stress) is applied. The shear-stress/shear-rate relationship is the same with each system of rotation. Continuous measurements may be made and time-dependent effects studied. Continuous or step variation over a wide range of torques or velocities is normally available. Because of this, a range of shear stresses or shear rates may be readily obtained, thus permitting analysis of Newtonian or non-Newtonian behaviour. However, a major disadvantage is that the liquid is not subjected to a spatially uniform shear rate even if it is a simple Newtonian liquid.

Owing to their versatility these systems are, without doubt, the most widely used in rheological measurements, and fluid behaviour within the annular gaps of these instruments has been the subject of intensive investigation. Consequently, there is a wide range of analytical equations

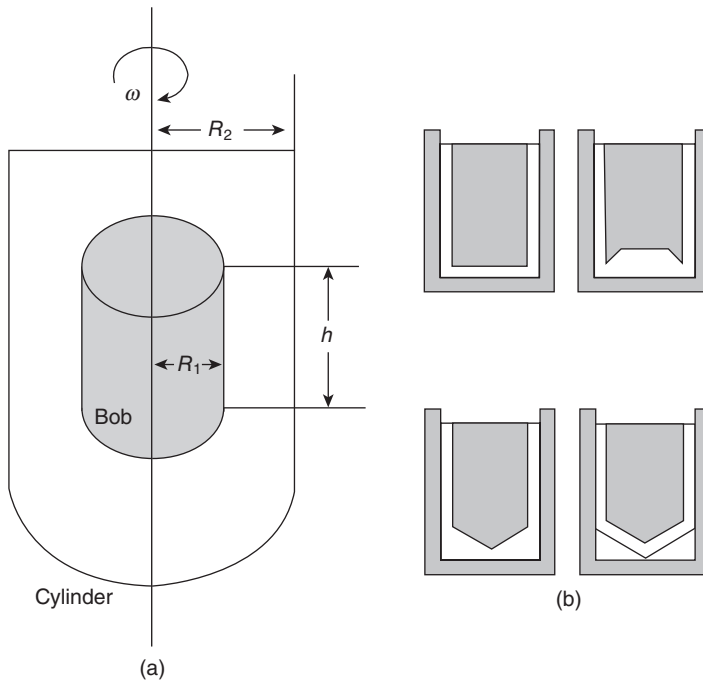


Fig. 5.3 Concentric cylinder viscometer (a) dimensions and (b) side profiles illustrating flat, angled and recessed bottoms.

available for assessing their results and for modifying the readings obtained to correct for a wide variety of error sources.

For Newtonian fluids, the simplest relationship is the Margules equation (1881):

$$\mu = \left(\frac{T}{4\pi h' \omega} \right) \left(\frac{1}{R_1^2} - \frac{1}{R_2^2} \right) \quad [5.18]$$

where μ is the viscosity, T is the torque on the cup or bob (measured in rate-controlled and fixed in stress-controlled), ω is the angular velocity of the rotating cup or bob (measured in stress-controlled and fixed in rate-controlled), h' is the height of the bob, R_1 is the radius of the bob and R_2 is the radius of the cup.

For non-Newtonian materials, Van Wazer *et al.* (1963) derived the general equations for flow in the annular space between the concentric cylinders and provided solutions for Newtonian fluids, power law fluids, power law fluids with a yield value (Herschel-Buckley), Eyring model fluids and several others. For simple power law fluids the following relationship is available for shear rate $\dot{\gamma}$:

$$\dot{\gamma} = \frac{\omega(R_2^2 + R_1^2)}{(R_2^2 - R_1^2)} \cong \omega \left(\frac{R_2}{\Delta R} \right) \quad [5.19]$$

where ΔR is the width of the gap between the cylinders, and the shear stress at the bob τ_b is calculated from the following equation:

$$\tau_b = \frac{T}{2\pi R_1^2 h'} \quad [5.20]$$

In the case of rate-controlled systems ω will be fixed and T will vary while for stress-controlled systems T will be fixed while ω will vary. Plotting the logarithms of the values derived from Eqns 5.19 and 5.20 should give a straight line of slope n and intercept k . It may, however, prove more convenient to calculate from Eqn 5.19 (stress-controlled systems) or τ_b from Eqn 5.20 (rate-controlled systems). These values calculated from these equations can then be used in further calculations, for example a plot of $\ln \omega$ versus $\ln \tau_b$ should give a curve that fits the equation:

$$\ln \omega = (1/n) \ln \tau_b + \ln \{ (n/2)(\sqrt[n]{k}) [1 - (R_1/R_2)^{2/n}] \} \quad [5.21]$$

There are a number of potential sources of error in concentric cylinder viscometers. The major ones include inertial effects, differences in shear rate distribution, edge and end effects and thermal effects (Lapasin and Prici, 1995; Steffe, 1996). Inertial effects manifest themselves in localised circulation instabilities known as Taylor vortices. Data analysis equations developed for concentric cylinder viscometers assumed that laminar flow occurs. However, the outward movement of liquid under the influence of centrifugal force can give rise to secondary flow or Taylor vortices. These vortices occur at lower Reynolds numbers in Searle-type relative to Couette types where the rotation of the outer cylinder helps in stabilising the flow of liquid.

End effects are the most common source of error and occur due to the fact that the cylinders have finite dimensions, instead of being infinite, as the theory requires. In rate-controlled systems the torque response imposed by the bottom of the cylinder was not accounted for in the development of the fundamental theory. These end effects can, however, be corrected by taking torque readings at several different immersion depths of the cylinders in the test fluid. If T is then plotted against h , the resulting graph will intersect the h axis at a negative value h_c that corresponds to the correction to be added to h in any of the above equations. Alternatively, this may be calculated from the following equation of Oka (1960):

$$h_c/R_1 = (R_1/8e)[1 - (R_1/R_2)^2] \left\{ 1 + (4e/R_1) \sum_{n=1}^{\infty} A_n I_2(n\pi R_1/e) + (8e/\pi R_1) \sum_{n=1}^{\infty} B_n [\sinh(K_n h)]/K_n R_1 \right\} 1 \quad [5.22]$$

where e is the distance between the bottom of the bob and the cup, I_2 is a modified second-order Bessel function, K_n is the n th positive root of a derivation of the Navier–Stokes equation for incompressible fluids, and A_n and B_n are functions of the variables R_1/R_2 , h/R_2 and e/R_2 . However, if the immersion system is such that the gap between the end of the bob and either the cup or the fluid container is large, then the end effects become negligible and the difficult application of eqn 5.18 is avoided. In rate-controlled systems, the end correction can also be calculated using an equivalent torque (T_e) using the method described by Steffe (1996). In addition to adjusting calculations to account for end effects, various cylinder designs have been developed to minimise the occurrence of end effects. A number of these cylinders have been designed one of which has a slightly angled bottom (Mooney Couette bob) while another has a recessed bottom and top (Fig. 5.3).

Another source of error is shear rate variation across the sample. Equation 5.19 gives a mean value for shear rate. However, for power law fluids this can be corrected by the relationship:

$$\dot{\gamma}_{\text{eff}}/\dot{\gamma}_{\text{meas}} = (1/n)(11/2)^{1/n-1}[1 - (R_1/R_2)^2][1 + (R_1/R_2)^2]^{1/n-1}[1 - (R_1/R_2)^{2/n}]^{-1} \quad [5.23]$$

where $\dot{\gamma}_{\text{eff}}$ is the effective shear rate and $\dot{\gamma}_{\text{meas}}$ is the measured value. The reader is referred to a correction table available in Prentice (1984), which obviates the need to carry out this detailed calculation.

Temperature rises can occur in concentric cylinder viscometers where some of the work done is dissipated as heat. Many viscometers have temperature control systems, which are designed to remove excess heat generated during testing. Although these temperature increases can potentially affect rheological properties it is possible to accommodate them in some substances, while for others it is possible to adjust the results appropriately to account for them (Whorlow, 1992; Lapasin and Prici, 1995; Steffe, 1996).

Concentric cylinder viscometers: instruments

There is a large range of concentric cylinder viscometers available from many different manufacturers. All use the same basic configuration, but they vary significantly in their degree of sophistication. Systems with dial displays are still available but digital displays of rotational speed and torque are more or less standard. Many systems have their own microprocessor incorporated and have the capacity to be operated from a PC, which also serves as a data acquisition and analysis system. It is impossible to make specific recommendations in this general chapter other than to emphasise the guidelines of Prins and Bloksma (1983) to which reference has already been made in Section 5.1. However, it is essential that when selecting an instrument, consideration be given to the range of shear rates required in the case of rate-controlled or the range of stress rates required in the case

of stress-controlled systems. The fluids must be subjected to the same shear or stress rates as those in the application for which the rheological characteristics are required. In particular, processors of fluids such as chocolate, which have a yield value, must select an instrument capable of accurate measurement at very low shear rates. Systems differ in the method that is used to detect torque, some are fitted with mechanical transducers (i.e. torsional bar), whereas other systems use a non-mechanical force transducer (electronic force sensor). Another feature of sophisticated modern-day systems is that many are fitted with air bearings, which lubricate and minimise the friction of the measuring shaft. The reader is referred to Ma and Barbosa-Cánovas (1995) for more information on the range of viscometers currently available.

Cone and plate viscometers: theory

A much recommended system for rotary measurement is the cone and plate viscometer (Fig. 5.4). This consists of a cone of shallow angle, normally of less than 3° (up to 5° are possible but edge effects can distort the flow field) and possibly with a truncated tip, that almost touches a flat plate. The sample for assessment is placed in the intervening space and different angular velocities (in a rate-controlled instrument) or torques (in a stress-controlled one) are applied to either the cone or the plate (most commonly the cone). While in theory it is possible to rotate either the cone or the plate and measure the torque transmitted through the intervening liquid, the normal procedure is to rotate the cone and measure either the transmitted torque on the plate or the torque required to rotate the cone at a constant angular velocity. In rate-controlled instruments the velocity of rotation of the cone (or plate) is controlled and the transmitted torque on the plate (or cone) is measured, while for stress-controlled instruments the opposite situation occurs where a controlled torque is applied and the resultant rate of rotation is measured.

The major advantage of this measuring system is that the shear rate is constant at all points in the fluid. This feature is true only when small conical angles are used and makes the system particularly useful when characterising non-Newtonian fluids, since the true rate of shear may be determined

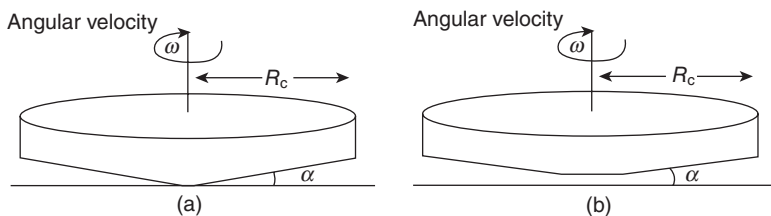


Fig. 5.4 Cone and plate viscometers: (a) normal; (b) truncated cone.

without the need for detailed corrections as in the concentric cylinder type. This constant shear rate may be determined from the relationship:

$$\dot{\gamma} = \frac{\omega}{\alpha} \quad [5.24]$$

where ω is the angular velocity (rad s^{-1}) and α is the angle of the cone (rad).

The shear stress may be calculated from the following equation where R_c is the radius of the cone:

$$\tau = \frac{3T}{2\pi R_c^3} \quad [5.25]$$

For a Newtonian fluid these may be simply combined to give:

$$\dot{\gamma} = \frac{1.5T\alpha}{\pi\omega R_c^3} \quad [5.26]$$

Indeed, even when measurements are performed on more complex systems, the analysis of results simply requires the substitution of the above equations for $\dot{\gamma}$ and τ into the relevant behaviour model for the fluid. Carrying out this substitution for power law (Eqn 5.2), Casson (Eqn 5.5) and Herschel-Buckley (Eqn 5.6) fluids leads to the following set of relationships:

$$\text{Power law: } 3T/2\pi R_c^3 = k(\omega/\alpha)^n \quad [5.27]$$

$$\text{Casson: } (3T/2\pi R_c^3)^{0.5} = \tau_y^{0.5} + k'(\omega/\alpha)^{0.5} \quad [5.28]$$

$$\text{Herschel-Buckley: } 3T/2\pi R_c^3 = \tau_y + k''(\omega/\alpha)^n \quad [5.29]$$

While these instruments are much recommended, particularly for transient measurements, care must be exercised when treating any fluid containing suspended solids. The gap between the plate and the truncated cone is normally 50 μm or less. The problems and errors that would be encountered with particles of this size or larger need not be stressed. There is a general recommendation that particles should be at least ten times smaller than the size of the smallest gap between the cone and plate.

Potential sources of error in cone and plate viscometers, include inertial effects, edge and end effects and thermal effects (Whorlow, 1992; Lapasin and Pricl, 1995; Steffe, 1996). Inertial effects give rise to secondary flows, which can affect the torque and can occur in a number of different forms as illustrated by Whorlow (1992) and Lapasin and Pricl (1995). However, for sufficiently small gap angles and fluids with low Reynolds numbers the effects of secondary flow can be ignored. Edge effects manifest themselves as edge failure in thick fluids. Edge failure occurs in rate-controlled systems where as the rotation speed is increased the fluid at the edge of the cone and plate breaks up and gives rise to a sharp drop in torque. These edge effects limit the maximum shear rates that can be used in cone and plate systems. Temperature effects can also occur and the reader is referred to

Whorlow (1992) and Lapasin and Prici (1995) for more detailed information. However, Steffe (1996) claims that temperature rises caused by viscous heating are rarely a problem when testing biological materials.

Cone and plate viscometers: instruments

Similar to the concentric cylinder viscometers, a range of cone and plate rheometers is available. There are a number of options regarding the choice of cone including its angle and diameter. Increasing shear stress is encountered with decreasing cone diameter, while increasing shear rate is encountered with decreasing cone angle. As stated above, the apex of the cone is often truncated by a small amount so that it does not touch the plate and as a consequence there is a small region near the tip where the opposing faces are parallel. This truncation also prevents wear on the cone tip and also the indentation of the plate. Many cone and plate rheometers are fitted with autozero and autogap controls which allow the operator to control and standardise the gap between the cone and plate and assist in ensuring reproducible data are obtained from the system.

Parallel plate viscometers

The parallel plate viscometer is similar in operation to the cone and plate device outlined above. However, unlike the cone and plate, and concentric cylinder geometries where the gap separating the two surfaces is fixed, the parallel plate system has the advantage of flexible gaps. This is useful for materials such as coarse dispersions, which are intolerant of the narrow gaps in cone and plate and concentric cylinder viscometers. Another difference between the parallel plate and cone and plate system is the uneven distribution of shear rate, which varies from zero at the centre to a maximum ($\dot{\gamma}_{\max}$) at the outer edge (i.e. the rim) of the plate, the value for which can be calculated from the formula:

$$\dot{\gamma}_{\max} = \frac{\omega R_p}{H} \quad [5.30]$$

where R_p is the radius of the plate and H is the separation of the two plates.

The shear stress at the outer edge of the plate can be calculated from the following equation:

$$\tau_{\max} = \frac{3T}{2\pi R_p^3} \left(1 + \frac{1}{3} \frac{d \ln T}{d \ln \dot{\gamma}_{\max}} \right) \quad [5.31]$$

Owing to the large variation in shear rates this method is not that commonly used in steady shear measurements. The limitation of parallel plate geometry is that the shear rate has to be below 500 s^{-1} . The major sources of error associated with parallel plate viscometers are similar to those outlined for cone and plate systems. However, parallel plate systems may also be subject to slippage, although slip correction methodology, which allows for correction of this phenomenon, is available (Steffe, 1996).

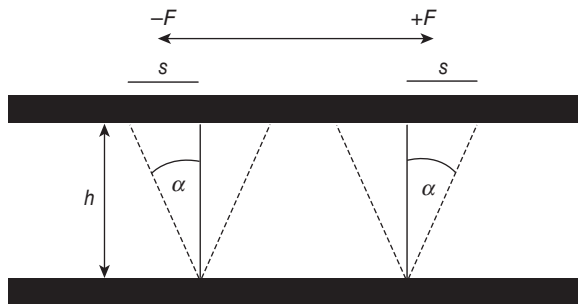


Fig. 5.5 Oscillatory strain between rectangular plates.

Dynamic rheology

Dynamic rheology is a form of rheology which uses the same applicator geometries as those described for rotary rheometers (i.e. concentric cylinder, cone and plate and parallel plate). However, unlike rotary rheometry where the sample is subjected to an applied rotary motion, dynamic rheology is a form of rheometry where samples are subjected to small sinusoidally varying loads in which either the shear stress τ or strain γ is controlled (i.e. control stress or control strain, respectively). The magnitude of these deforming loads is small and they are chosen (e.g. by an amplitude sweep test) such that the material structure is not destroyed. Under such conditions the viscoelastic properties of the sample become evident. To illustrate dynamic rheology, imagine a slab-shaped volume between two parallel rectangular plates (Fig. 5.5) in which the lower plate is fixed and the upper plate is allowed to move backwards and forwards in a horizontal direction. In a control strain test the strain γ is applied by presetting the path and the volume is submitted to a force ($\pm F$) or shear stress. In control stress systems, the oscillating stress from the force ($\pm F$) means that the volume element undergoes a strain. With controlled strain instruments the strain curve as a function of time is given by:

$$\gamma = \gamma_0 \sin(\omega t) \quad [5.32]$$

where γ_0 is the amplitude of the strain equal to L/h (L is the distance from centre moved by the plate and h is the distance separating the plates), ω is the frequency expressed in rad^{-1}s and can be calculated from $2\pi f$, where f is the frequency (Hz) (Steffe, 1996). Thus the magnitude of the strain is governed by the amplitude and frequency. Corresponding to the strain curve the strain rate can be calculated from:

$$\dot{\gamma} = \gamma_0 \omega \cos(\omega t) \quad [5.33]$$

which is the derivative of Eqn 5.32.

For controlled stress instruments the stress curve as a function of time can be calculated from:

$$\tau = \tau_o \sin(\omega t) \quad [5.34]$$

τ_o being the stress amplitude (Pa). The measured result from a controlled strain system is a shear stress curve the equation for which is:

$$\tau = \tau_o \sin(\omega t + \delta) \quad [5.35]$$

where δ is the phase displacement angle. In rheometers, which operate as controlled stress systems, the following equation characterises the strain curve produced by the sinusoidally varying stress input:

$$\gamma = \gamma_o \sin(\omega t + \delta) \quad [5.36]$$

Regardless of whether a controlled stress or controlled strain system is used, in perfectly elastic substances the strain and stress waves will be in phase with each other (i.e. $\delta = 0^\circ$) while in purely viscous fluids the strain and stress waves will be exactly 90° out of phase with each other (i.e. $\delta = 90^\circ$). For viscoelastic substances the phase angle will lie in the range $0^\circ < \delta < 90^\circ$. From the recorded sinusoidal curve the storage modulus (G') and loss modulus (G'') can be calculated. The storage modulus represents the elastic behaviour of a sample as its magnitude represents the strain energy, which is reversibly stored in and recoverable from the substance:

$$G' = \left(\frac{\tau_o}{\gamma_o} \right) \cos \delta \quad [5.37]$$

As the name suggests the loss modulus represents the quantity of energy irreversibly given off by the substance to its environment and thus lost. This modulus characterises the viscous behaviour of the sample.

The storage modulus and loss modulus can be combined to give a single figure called the $\tan \delta$ which gives a ratio between the amount of energy lost and stored per cycle and hence a relationship between the viscous and elastic portions of the sample. $\tan \delta$ can vary from zero to infinity with highest values in Newtonian fluids and lowest values in substances, which resemble hookean solids (Steffe, 1996):

$$G'' = \left(\frac{\tau_o}{\gamma_o} \right) \sin \delta \quad [5.38]$$

$$\tan \delta = \frac{G''}{G'} \quad [5.39]$$

Dynamic testing is not the only method that can be used to gather information on the viscoelastic properties of substances. Other non-oscillatory methods are available including step strain (stress relaxation), creep and recovery and startup flow (stress overshoot), which differ from dynamic testing in that the sample, is subjected to a constant load (shear stress τ or shear rate $\dot{\gamma}$). These methods are widely used and the reader is referred to Whorlow (1992) and Steffe (1996) for further detailed discussion on their theory and application.

5.5.3 Empirical rheology methods

The emphasis of this chapter is on fundamental versus empirical measurements. However, empirical measurement methods are widely used in areas such as quality control, correlation to sensory analysis results and even as official identification standards. They are suitable for foods with non-homogeneous complex structures where measurement by fundamental means is not possible but empirical methods can be used to obtain an index of product rheology. Empirical methods include dough testing equipment (farinograph, mixograph, extensigraph, alveograph), cone penetrometers, Warner-Bratzler shear devices, Bostwick consistometers, Adams consistometers, Zhan viscometers, viscoamylographs, rapid visco analysers, falling ball viscometers, Hoesppler viscometers, compression extrusion cells, Kramer shear cells and texture profile analysis systems, each of which are outlined in detail by Steffe (1996). Some of these methods are more suited to the measurement of solids and as stated earlier, they measure rheologically affected phenomena from which it is possible to make a correlation to a desired variable.

To illustrate empirical systems we will take an example of rotary viscometers in which cylinders, bars or agitator paddles rotate in a test fluid. Analysis of such systems is difficult because of their geometric complexities. Consequently, their use depends on the existence of empirical relationships, which relate measured variables, normally the torque required to rotate the instrument at a known speed, to the rheological characteristics. However, it must be stressed that many such instruments provide correlations with Newtonian viscosity only and consequently may have limited use when one is considering the more complex fluids normally found in the food industry.

Two instruments are worthy of mention because of their widespread use in the food industry. In the Brookfield Synchro-Lectric Viscometer a series of cylindrical spindles and horizontal disks are rotated at fixed speeds while the torque required to overcome the viscous drag of the fluid is recorded. Conversion tables are available to convert this into Newtonian viscosity. It is, however, very difficult to estimate accurately the shear rates being used and consequently its use for non-Newtonian fluids is limited. Some work has, however, been carried out (Mitschka, 1982) to enable calculation of some of the basic power law (Eqn 5.2) variables from Brookfield readings. In repetitive quality control use, many processors find its rugged simplicity useful and happily use its readings for comparative purposes. For more precise rheological evaluations, an optional attachment converts it into the more useful concentric cylinder geometry.

The Brabender Viscocorder measures the torque imparted to a paddle by the viscous drag of the test fluid in a rotating cup. Various forms of the instrument are available and a version capable of heating the test fluid in the rotating cup has found widespread use in the starch industry. Again it is difficult to relate the results obtained to the fundamental rheological

properties, and the instrument, while widely used in quality control in the baking industry, has not seen extensive use in other areas. Details of both the Brookfield and the Brabender instruments are widely available in reviews including those of Matz (1962) and Sherman (1970).

5.5.4 On-line measurement systems

On-line systems are finding ever increasing applications in process control. An excellent comprehensive review of the instruments available was published by Cheng *et al.* (1984) of the Warren Springs Laboratory, UK, while a more recent review has been published by Davidson *et al.* (1989) and also Steffe (1996). Roberts (2003) also provides an excellent update on in-line systems.

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6

Food colour measurement using computer vision

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Abstract: Colour is the first and basic quality attribute of food for consumer perception. Computer vision is a rapid, objective and economic inspection technique that could provide a detailed characterization of colour uniformity at pixel-based level. The chapter first reviews the principles of colour measurement, including the concept of food colour, visual colour measurements and instrumental colour measurements. The chapter then introduces computer vision measurement, including imaging systems and colour spaces. The chapter also presents some applications of food colour measurement using computer vision. Finally, the advantages and disadvantages of computer vision for colour measurement and its future are proposed.

Key words: computer vision, colour measurement, colour space, imaging system, food.

6.1 Introduction

Colour is one of the most important object measurements for image understanding and object description of food products. It provides the basic quality information of food products for human perception. As a primary determinant for consumer satisfaction, colour affects consumers' decisions to purchase food products. The colour of food products should be 'right' when consumers are purchasing foods. To ensure food conformity to consumer expectations, it is critical for the food processing industry to develop effective colour inspection systems to measure the colour information of food product during processing operations and storage periods. Since colour is an important attribute of food quality, the research on colour measurements of food products is an expanding field. With the aim of measuring food colour rapidly and non-invasively, new objective and consistent methods are required for the effective quality control of foods. Among numerous new sensing technologies for information assessment of

agricultural and food products, computer vision is a novel technology for recognizing objects, which include colour measurement. For a digital image measured using computer vision, colour is elementary information stored in pixels. With the help of image processing and analysis, computer vision can extract quantitative colour information from digital images in order to provide objective, rapid, non-contact and non-destructive colour measurement. Driven by intensive efforts of development in electronics, hardware, image processing techniques, and software, computer vision has attracted much research and development attention, and their applications have been extended to the colour measurement of diverse and processed food agricultural products.

6.2 Principles of colour measurement

6.2.1 Colour and food

Colour is the result of visible radiation producing a stimulus on the retina, which is then transmitted to the brain via the optical nerve. It is affected by several major factors: the spectral radiant energy of a light source, the physiology of vision, and the observer's own psychological perception, with the last of these constituting a highly complex phenomenon. Although colour perception is in part dependent on the composition of the object reflecting or transmitting light, colour cannot be said to be an inherent property of the object, because the perceived colour of the object is altered if the source of light changes (Melendez-Martinez *et al.*, 2005).

Colour is an important quality parameter for foods throughout the agricultural and food industries. It is the first contact point of a food for the consumer, even before it enters the mouth. The measurement of colour allows the detection of certain anomalies or defects that food items may present (Leon *et al.*, 2006). Because colour has a close association with quality factors such as freshness, ripeness and desirability, and food safety, its prominent role is unquestionable for the consumer acceptability of food products. It is an important grading factor for most food products including meats and crops such as peas, corn, canola, rice and wheat for both human and animal consumption (McCaig, 2002). When the colour of food product alters, consumers' reactions to the product are likely to be affected. Increased requirements for quality by consumers require the colour evaluation of food products to be more rapid, objective and quantifiable. Therefore, the food industry has paid numerous efforts for a long time to measure and control the colour of their products.

At present, colours are created, represented and visualized using colour spaces and numerical values in two- and three-dimensional space (Trusell *et al.*, 2005). Different colour spaces are used for measuring colour in food, and the most used one is the $L^*a^*b^*$ colour space due to the uniform distribution of colours, and because it is very close to human perception of

colour. The $L^*a^*b^*$, or CIELab, colour space is an international standard for colour measurements, adopted by the Commission Internationale d'Eclairage (CIE) in 1976 (CIE, 1986). L^* is the luminance or lightness component, which ranges from 0 to 100, and parameters a^* (from green to red) and b^* (from blue to yellow) are the two chromatic components, which range from -120 to 120 (Segnini *et al.*, 1999a; Papadakis *et al.*, 2000; Yam & Papadakis, 2004). The $L^*a^*b^*$ space is perceptually uniform, i.e. the Euclidean distance between two different colours corresponds approximately to the colour difference perceived by the human eye (Leon *et al.*, 2006). The principles of $L^*a^*b^*$ colour measurement can be found elsewhere (Francis & Clydesdale, 1975; Clydesdale, 1978; Hunt, 1991; Francis, 1994).

6.2.2 Manual visual measurements

Colour measurements are mainly carried out in two ways: manual visual evaluation and instrumental analysis (Melendez-Martinez *et al.*, 2005). Many operations in existing food colour inspection systems are done through qualitative visual inspection by trained inspectors and sometimes with the aid of colour atlases or dictionaries. Manual visual analyses must be carried out in well-illuminated rooms, although further overhead artificial illumination should be supplied in each cabin to promote reflection of light by the samples (Melendez-Martinez *et al.*, 2005). Hutchings (1994) examined the sensory evaluation of food colour, and provided comprehensive guidance on panel selection, the physical requirements for visual assessments and the types of sensory tests to be used. The study by MacDougall (2002b) provided a number of very useful guidelines for carrying out visual analysis of food colour, looking at optimal illumination, correct presentation of samples, and accurate processing of data, among other key factors. Visual analysis allows a particular description of colour to be obtained, which is described using a specific vocabulary (Melendez-Martinez *et al.*, 2005). However, colour perception differs from person to person, and depends upon lighting and numerous other factors (McCaig, 2002).

An alternative to this type of visual analysis is to carry out a visual assessment using colour scales or atlases containing comparative standards, a method widely used in several industries in the manufacture of cars, foods, paints, and many other products. However, although the human eye is able to effectively distinguish different colours, the human brain is much less effective at remembering them. In the paint and textile industry, stable colour standards can be stored for easy comparison, meaning that the brain's poor capacity for remembering colour does not pose a problem (Melendez-Martinez *et al.*, 2005); in the food industry, though, storing standards in this way is not usually possible, meaning that samples must be matched to a colour chip from a colour order system (Hutchings, 1994). To be effective, such a system must include all possible hues and intensities of different colours, with each point in the scale given a number. Samples are

then visually compared with the corresponding scales and are assigned to the appropriate number (Melendez-Martinez *et al.*, 2005). Unfortunately, the use of colour standards implies that the inspection is slow and requires more specialized training of the observers (Leon *et al.*, 2006). Moreover, the human inspections are laborious, time-consuming, costly and tedious. For these reasons it is recommendable to determine colour through the use of colour measuring instrumentation (Leon *et al.*, 2006).

6.2.3 Traditional instrumental measurements

When instrumental measurement is used for colour determination, colour coordinates are generally used to express colour. These instruments attempt to simulate the manner in which the average human eye sees the colour of an object, under specified illumination conditions, and provide a quantitative measurement (McCaig, 2002). The basic types of instrument for the colour measurement include colorimeters, spectrophotometers and spectroradiometers (Wyszecki & Stiles, 1982).

Colorimeters are commonly used instrument for the measurement of colour, such as Minolta chroma meter; Hunter Lab colorimeter, and Dr. Lange colorimeters (Leon *et al.*, 2006). Take the Minolta chroma meter as an example. It is a hand-held colorimeter commonly used for measuring the average colour of a food sample area by providing controlled illumination, either average daylight illuminant C with a colour temperature of 6774 K or average daylight illuminant D65 with a colour temperature of 6504 K (Yagiz *et al.*, 2009). It is widely applied for measuring the colour of food products such as seafood (Choubert *et al.*, 1997; Olsen & Mortensen, 1997; Buttle *et al.*, 2001; Jaczynski & Park, 2003; Oliveira & Balaban, 2006), beef (Rodas-Gonzalez *et al.*, 2011), chicken (Garcia *et al.*, 2010; Sirri *et al.*, 2010), apple (Iglesias *et al.*, 2008; Iglesias & Alegre, 2009; Toebe *et al.*, 2011), orange (Cao *et al.*, 2011) and wheat (McCaig *et al.*, 2006). The measurement is fast and simple, and accuracy is kept high by calibration with standard tiles at the beginning of the operation (Oliveira & Balaban, 2006).

Near infrared (NIR) instruments were developed approximately 15 years ago that had an extended spectral range including the visible region: these are known as VNIR instruments, and allow pigments to be measured (McCaig, 2002). One VNIR instrument widely used in the food and agriculture industries is the spectrophotometer, which measures the spectral distribution of transmittance or reflectance of the sample. Colour is then calculated under different conditions on the basis of the measurements obtained through spectrophotometry. The transmittance measurements are the ratio between the two responses: one when the sample is in the optical pathway of the instrument and one when the sample is not present (Melendez-Martinez *et al.*, 2005). The values are dependent on the illuminant, the measurement geometry and the psychology of the observer (Hutchings, 1994). While both transmittance and reflectance are inherent properties of

the objects, colour is not, and depends instead on illumination and/or the observer.

Another useful instrument in this respect is the spectroradiometer, designed to measure radiometric quantities as a function of wavelength (Wyszecki & Stiles, 1982). It also allows tristimulus values to be mathematically obtained. The components of the spectroradiometer are the same as those of the spectrophotometer, except that in this case the light source is external. Like spectrophotometers, spectroradiometers can be used to measure the transmittance or reflectance of any object (Melendez-Martinez *et al.*, 2005).

However, although simple colour measurements can be achieved using traditional instrumental measurements, instrumental measurement has some limitations: the surface to be measured must be uniform and rather small, which makes the measurements obtained quite unrepresentative and furthermore the global analysis of the food's surface becomes more difficult (Segnini *et al.*, 1999a; Papadakis *et al.*, 2000; Mendoza & Aguilera, 2004). The sampling location on the food and the number of readings for obtaining an accurate average colour are also important (Oliveira & Balaban, 2006). Moreover, if the sample cannot fill the sample window, e.g. a shrimp, colour readings may be inaccurate (Oliveira & Balaban, 2006). In order to carry out a detailed characterization of the image of a food item and thus more precisely evaluate its quality, it is necessary to know the colour value of each pixel of its surface. However, at present available instrumental measurements obtain $L^*a^*b^*$ only over a very few square centimetres, and thus their measurements are not very representative in heterogeneous materials such as most food items (Leon *et al.*, 2006). This in turn has increased the need for developing automatic pixel-based colour measurement process in the food industry to replace traditional method of human subjective evaluation and instrumental measurements.

6.3 Computer vision measurements

Computer vision is the science of developing theoretical and algorithmic basis to automatically extract and analyse useful information about an object or scene from an observed image, image set or image sequence (Gunasekaran, 1996). As a rapid, consistent, objective and economic inspection and evaluation technique, the aim of computer vision is to emulate the function of human vision by electronically perceiving and evaluating an image (Sonka *et al.*, 1999). Computer vision technique works by acquiring the image of an object, analysing the image to extract the desired image attributes, comparing these attributes with predefined criteria, and then helping to make decisions or taking some types of corrective operations on the object or the manufacturing process (Gumus *et al.*, 2011). A digital monochrome image can be defined as a two-dimensional (2D)

light-intensity function of $I(x, y)$, which reflects spatial representation of an object or scene. The intensity or amplitude I at spatial coordinates (x, y) has proportional relationship with the radiant energy received in the electromagnetic band to which the sensor or detector is sensitive in a small area around the point (x, y) (Gunasekaran, 1996). The intensity of the monochrome image is generally known as the grey level. However, the grey level of a digital image has limitations such as being positive and finite. The interval of grey level from low to high is called a grey scale, which is numerically represented by a value between 0 and L in common practice. Specifically, pure black is represented by the lowest value 0 and white is represented by the maximum value L . All values in between 0 and L are shades of grey, varying continuously from black to white. For example, grey levels ranging from 0 to 255 (i.e. $2^0 - 1$ to $2^8 - 1$) are stored by an 8-bit integer (Gunasekaran, 1996). Therefore, an acquired image from computer vision is a matrix of numeric values. Each spatial coordinate (known as a pixel) represents a quantified image-intensity value. The total number of pixels within an image is determined by the size of the 2D array used in the camera (Gunasekaran, 1996). The core steps of computer vision include image acquisition and image analysis. The former requires scrupulous design of the image capturing system and careful operation to obtain digital images with high quality, while the latter includes numerous algorithms and methods available for classification and measurement (Krutz *et al.*, 2000). Because of its advantages of being rapid and non-destructive, computer vision based on the analysis of digital images has attracted much attention for research and development and has been considered as a potential solution for quality and safety inspection of a wide range of food and agricultural products (Gunasekaran, 1996; Brosnan & Sun, 2004), including shrimp (Luzuriaga *et al.*, 1997), pork (Lu *et al.*, 2000), oysters (Diehl *et al.*, 1990), bananas (Yoruk *et al.*, 2004), sturgeon (Oliveira & Balaban, 2006), rainbow trout (Yagiz *et al.*, 2007) and mahi mahi (Yagiz *et al.*, 2007).

Among numerous applications of computer vision for inspecting different quality attributes of food products, computer vision has been used to objectively measure the colour of different foods since they provide some obvious advantages over a conventional colorimeter, namely, the ability of analysing of each pixel of the entire surface of the food even when it is of non-uniform shape and colour, the ability to calculate the average and standard deviation of colour, quantifying surface characteristics and defects, identifying the colours present in a sample, and providing a permanent record by keeping the picture (Leon *et al.*, 2006; Yagiz *et al.*, 2009). Computer vision has been applied to providing a more versatile and less expensive way of measuring the colour of many foods than traditional colour-measuring instruments (Yam & Papadakis, 2004). Successful applications of measuring the colour of food products using computer vision include salmon fillets (Misimi *et al.*, 2007; Yagiz *et al.*, 2009; Quevedo *et al.*, 2010), ham (Valous *et al.*, 2009), fruits (Balaban, 2008; Balaban *et al.*, 2008), pizza

(Du & Sun, 2005), potato chips (Pedreschi *et al.*, 2011), cheese (Everard *et al.*, 2007), beef (Zheng *et al.*, 2006a), pork (Lu *et al.*, 2000; O'Sullivan *et al.*, 2003), paddy rice (Liu *et al.*, 2005), oil palm (Abdullah *et al.*, 2001) and bell peppers (Shearer & Payne, 1990). Owing to its advantages of superior speed, consistency, accuracy and cost-effectiveness, the automatic colour measurement using computer vision can not only optimize quality inspection but also help in reducing human inconsistency and subjectiveness. That is why automatic quantitative computer vision measurement of colour is preferable in both research and industrial applications.

6.3.1 Image acquisition systems

The first step in using computer measurement is to acquire images of the objects to be measured. The digital images are acquired by incident light in the visible spectrum falling on a partially reflective surface with the scattered photons being gathered up in the camera lens and converted to electrical signals either by vacuum tube or by CCD (charge-coupled device). The hardware configuration of computer vision system generally consists of an illumination device, a solid-state CCD array camera, a frame-grabber, a personal computer and a high-resolution colour monitor. Figure 6.1 shows a typical set-up of a computer vision system that can be found in many food laboratories, mainly for research and imaging applications. Although a computer vision system is easy to operate, a developer needs to understand the characteristics of the part and sub-assemblies of the machine system in order to ensure successful implementation of a machine vision application.

Illumination

Illumination is an important prerequisite of image acquisition, which can greatly affect the quality of captured image. It is absolutely decisive for

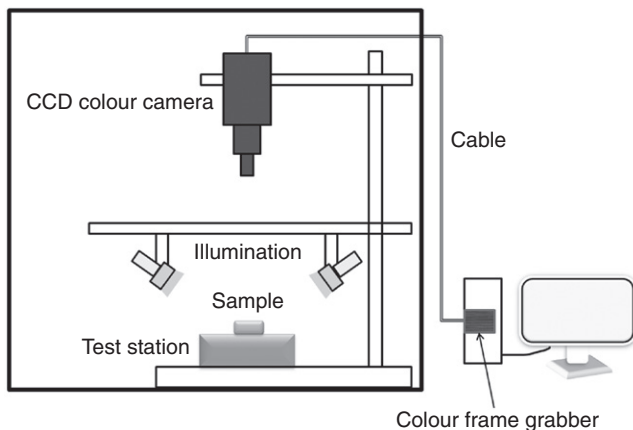


Fig. 6.1 Schematic diagram of a typical computer vision system.

lighting system to provide correct and high-quality illumination for computer vision applications. With a high-quality image, the time and complexity of the subsequent image processing steps can be reduced, resulting in decreasing the cost of an image processing system (Du & Sun, 2004). A well-designed illumination system can improve the accuracy and lead to success of image analysis (Gunasekaran, 1996). The sensor response of a standard imaging device is given by a spectral integration process (Matas *et al.*, 1995), showing that different illuminants may yield different stimuli using the same camera. Therefore, the illuminant is an important factor for integrating a computer vision system.

Fluorescent and incandescent bulbs are two most common used illuminants, while there are some other useful light sources, such as light-emitting diodes (LEDs) and electroluminescent sources. The comparison in relative spectral energy distribution among day, incandescent and cool white fluorescent light shows that the only difference between daylight and electric light is the amount of energy emitted at each wavelength (Wyszecki & Stiles, 2000). The fluorescent source has sharp peaks in some regions, while the incandescent source has a fairly normal distribution over the visible spectrum. An image produced by objects under an incandescent source has a lower signal-to-noise ratio, which is not acceptable in some cases, especially for those that are concerned with colour-image processing (Daley *et al.*, 1993). In contrast, fluorescent bulbs have more efficient inheritance to produce more intense illumination at specific wavelengths. Because fluorescent light provides a more uniform dispersion of light from the emitting surface, it is not necessary to use diffusing optics to disseminate the light source over the field of view, as is the case with incandescent bulbs. For these reasons, fluorescent bulbs have been widely used by many computer vision practitioners (Luzuriaga *et al.*, 1997; Abdullah *et al.*, 2001; O'Sullivan *et al.*, 2003; Pedreschi *et al.*, 2006; Blasco *et al.*, 2007).

Besides the illuminant, the surface geometry is also important in the illumination design, which includes specular surface and diffuse surface. Light striking a glossy surface is reflected at the angle of incidence and striking a diffuse surface is scattered. Therefore, in order to achieve high contrast in an image, it is important to determine the position of an illuminant. Two geometries are commonly used for the illuminators, which are the ring illuminator and the diffuse illuminator (see Fig. 6.2). The ring illuminator has a simple geometry and is widely applied for general purpose applications, especially for the objects with flat surfaces. The diffuse illuminator delivers virtually 180° of diffuse illumination and is well suited for the imaging application of food products with sphere shape.

Electronics

In capturing the image, the camera and the frame-grabber are two key elements responsible for this. The camera is used to convert photons to electrical signals and the frame-grabber then digitizes these signals to generate a

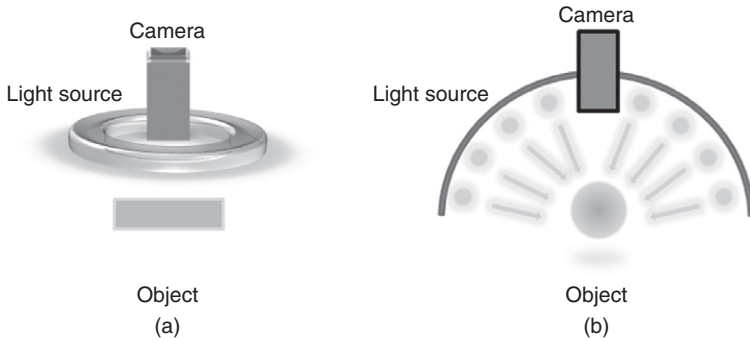


Fig. 6.2 Two possible lighting geometries: (a) the ring illuminator; (b) the diffuse illuminator.

bitmap image. Two major types of camera are CCD and CMOS (complementary metal-oxide-semiconductor) cameras, which are both solid-state imaging devices. A CCD camera consists of mass photodiodes (called pixels) that convert radiation energy to electrical signal, which is proportional to total light exposure. Each photodiode is an individual spot detector that is made of light-sensitive materials such as silicon (Si) or indium gallium arsenide (InGaAs). CCD detectors can be categorized into a line detector or area detector based on the arrangement of pixels in the sensor as 1D or 2D arrays, respectively. A CMOS camera is another common type of solid state camera that is considered to have the potential to replace CCD in the consumer electronics market since it is quieter and has, higher sensitivity and a greater dynamic range. Because a CMOS camera has the wires inside, which can transfer signal very fast, it is especially suitable for the requirement of high-speed imaging for online industrial inspection.

Frame-grabber is another electronic device for capturing an image. In general, the frame-grabber basically comprises signal-conditioning elements, an A/D converter, a look-up table, an image buffer and a PCI bus interface. It should be kept in mind that the modern and state-of-the-art frame-grabbers have more complex internal working circuitry for high-speed applications. There are three criteria in deciding on the frame-grabber: the camera, the speed requirements and the computer.

6.3.2 Colour spaces

In using computer vision measurements, there are different colour spaces available. The trichromatic theory shows that colour can be discriminated by the combination of three elementary colour components (Young, 1802). Therefore, every pixel of a colour image has three digital values. For each value from image acquisition, there are two typical statistical colour measurements, namely the mean and variance. The measurements of colour are

dependent on colour spaces. Different colour spaces have different types of values stored for the three colour components and different colour reproduction methods using these three values. Generally there are three types of colour spaces, namely hardware-orientated space, human-orientated space and instrumental space.

Hardware-orientated spaces

Hardware-orientated spaces are used to facilitate hardware processing, such as capturing, storing and displaying. RGB (red, green, blue) space is the most popular hardware-orientated space, because it is the way in which cameras sense natural scenes and display phosphors work (Russ, 1999). Colour in the RGB space is defined by coordinates on three axes, i.e. red, green, and blue. Besides RGB space, YIQ (luminance, in-phase, quadrature) space, is another popular hardware-orientated space, which is mainly used for television transmission. RGB space can be transformed into YIQ space by separating the luminance and the chrominance information in order to facilitate compression applications (Katsumata & Matsuyama, 2005). CMYK (cyan, magenta, yellow, black) is also a hardware-orientated colour space, which is however mainly employed in printing and copying output.

Colour can be effectively measured by combining values from each component in the hardware-orientated spaces. The hardware-orientated spaces can sense even a very small variation in colour and are therefore popular in evaluating colour changes of food products during processing (Lana *et al.*, 2005). However, hardware-orientated spaces are not suitable for measuring the colour of food products, because they are non-linear with regard to the visual perception of human eyes. For this reason, human-orientated colour spaces are developed.

Human-orientated spaces

With the aim of corresponding to the concepts of tint, shade and tone, which are defined by an artist based on the intuitive colour characteristics, human-orientated spaces have been developed, which include HSI (hue, saturation, intensity), HSV (hue, saturation, value) and HSL (hue, saturation, lightness). Hue is a measurement of the distance of the current colour position from the red axis, which is manifested by the difference in colour wavelengths (Jain, 1989). Saturation is measured by the amount of colour, i.e., the amount of white light that is present in the monochromatic light (Jain, 1989; Russ, 1999). As the last component, intensity, value or lightness refers to the brightness or luminance, which is defined as the radiant intensity per unit projected-area by the spectral sensitivity associated with the brightness sensation of human vision (Hanbury, 2002). Unlike the cuboidal coordinate which is used to define colour in RGB space, HSI, HSV and HSL are defined by cylindrical coordinates. The visual significance of food surfaces has a better relationship with the colour measurements obtained from HIS. Great correlation is between the colour measurements from

human-orientated spaces and the sensory scores of food products, which has been clarified by a study for the evaluation of acceptance of pizza toppings (Du & Sun, 2005). The shortcoming of human-orientated spaces is that they are not sensitive to a small amount of colour variation, resulting in being not suitable for evaluating changes of product colour during processing.

Instrumental spaces

Instrumental spaces are used for colour instruments, such as the colorimeter and colorimetric spectrophotometer. The CIE standardized these spaces under the specifications of lighting source, observer and methodology spectra (Rossel *et al.*, 2006). *XYZ* is an early used space in defining colour, where *Y* represents the lightness while *X* and *Z* are two primary virtual components (Wyszecki & Stiles, 1982). However, *XYZ* is not ideal to describe colour perception in human vision. For this reason, CIE *L*a*b** and CIE *Lu*v** colour spaces, which are the non-linear transformation of *XYZ*, are brought out and adopted in many colour measuring instruments. These instrumental colour spaces are commonly used for evaluating the performance of computer vision systems in measuring object colour, such as beef (Larrain *et al.*, 2008), pork (O'Sullivan *et al.*, 2003), fish (Quevedo *et al.*, 2010), juice (Fernandez-Vazquez *et al.*, 2011), potato chip (Pedreschi *et al.*, 2006) and wheat (Zapotoczny & Majewska, 2010).

Colour space transformations

In general, computer vision registers the colour of each pixel within the image of the object using three colour sensors (or one sensor with three alternating filters) per pixel (Segnini *et al.*, 1999a; Forsyth & Ponce, 2003). The resulting signals of these three sensors are nonlinearly transformed and combined. Because of the camera apparatus, RGB model is the most often used colour model in which each sensor captures the intensity of the light in the red (R), green (G) or blue (B) spectrum, respectively (Leon *et al.*, 2006). However, the RGB image acquired by a computer vision system is device-dependent and not identical to the RGB intensities of the CIE system. The differences between colours (i.e. Euclidean distances) in RGB space do not correspond to colour differences as perceived by humans (Wyszecki & Stiles, 1982). Therefore, RGB is not a perceptually uniform space. For this reason, the CIE has defined several perceptually uniform colour spaces, such as *L*a*b**, *L*u*v**, *L*C*H**, and HVC (Paschos, 2001), and a linear transform that defines a mapping between RGB signals from a computer vision camera and a device-independent system such as CIE *XYZ* was previously determined to ensure the correct colour reproduction according to the CIE system and International Telecommunication Union (ITU-R 1998) (Mendoza & Aguilera, 2004). However, traditional computational approaches that convert RGB into *L*a*b** units use an absolute model with known parameters (Segnini *et al.*, 1999a; Paschos, 2001; Mendoza & Aguilera, 2004), which vary

from one case to another because RGB is a non-absolute colour space, i.e., the RGB colour measurement depends on external factors (sensitivity of the sensors of the camera, illumination, etc.). These works did not consider using a calibration process to estimate the parameters (Leon *et al.*, 2006). Considering that most cameras (even of the same type) do not exhibit consistent responses (Ilie & Welch, 2005), the conversion from RGB to $L^*a^*b^*$ cannot be done directly using a standard formula, like a conversion from centimetres to inches (Leon *et al.*, 2006). For this reason, Leon *et al.* (2006) presents a methodology for obtaining accurate device-independent $L^*a^*b^*$ colour units from device-dependent RGB colour units based on modelling the transformation of coordinates of the RGB colour space into coordinates of the $L^*a^*b^*$ colour space, so that the values delivered by the model are as similar as possible to those delivered by a colorimeter over homogeneous surfaces. The best results were achieved with the quadratic and neural network model, both of which show small errors (close to 1%). However, although the methodology presented is general, i.e. it can be used in every computer vision system, it should be noticed that the results obtained after the calibration for one system (e.g. system A) cannot be used for another system (e.g. system B) (Leon *et al.*, 2006).

6.4 Applications

Nowadays, computer vision has gained extraordinary interests as a non-destructive and fast inspection method for food colour measurement, which includes beef (Larrain *et al.*, 2008; Sun *et al.*, 2011), pork (O'Sullivan *et al.*, 2003), fish (Yagiz *et al.*, 2009; Quevedo *et al.*, 2010), juice (Fernandez-Vazquez *et al.*, 2011), potato chips (Pedreschi *et al.*, 2006), wheat (Zapotoczny & Majewska, 2010) and wine (Martin *et al.*, 2007).

6.4.1 Beef

Freshness is ranked as the most important factor by consumers for buying meat (Maguire, 1994). 'Red' and 'bright red' lean is associated by consumers with freshness of the raw beef and dislike is expressed for brownish colour which is perceived to be an indicator of stale or spoiled beef (Larrain *et al.*, 2008). Objective colour measurements are required for establishing correlations to visual colour rather than pigment concentrations. Many works have done for detection of fresh meat colour differences using various CIE colour expressions, such as lightness (L^*), redness (a^*), yellowness (b^*), hue angle and chroma (Liu *et al.*, 1996; Hunt *et al.*, 1999; Von Seggern *et al.*, 2005). However, these works are conducted based on colorimeters, which have limitation of scanning a small surface area.

Gerrard *et al.* (1996) applied computer vision technique to the assessment of muscle colour of beef ribeye steaks. Sixty steaks with various

degrees of colour were subjected to sensory evaluation and image processing. Colour scores were assigned to each steak with USDA lean colour guide. The same conditions were used for image acquisition and sensory analysis for each steak. Features characterizing colour of the entire LD muscle were extracted from the muscle image, which were means ($= \mu_R, \mu_G$ and μ_B) and standard deviations ($= \delta_R, \delta_G$ and δ_B). The results indicated that red and green (μ_R and μ_G) were significant for colour prediction, whereas blue mean (μ_B) was not significant. Statistical analysis indicated that μ_R and μ_G were significant for colour scores. Image processing effectively predicted the lean colour ($R^2 = 0.86$), showing that computer vision was an effective tool for determining USDA quality attributes of fresh meat.

Later, Larrain *et al.* (2008) evaluated the use of computer vision to estimate CIE colour coordinates of beef as compared to a colorimeter. Loin samples from 21 steers finished with high-grain diets were displayed under retail-simulated conditions. Colour readings were obtained from 63 cores covering the full spectrum of discoloration in beef: from fresh samples having a bright-red colour to stale samples with a green-brownish tint. CIE L^* , a^* , and b^* were measured near the centre of each core using a colorimeter (Minolta Chromameter CR-300, Osaka, Japan) with a 1 cm aperture, illuminant C and a 2° viewing angle. Red, green and blue (RGB) values were obtained from computer vision and sequentially transformed to XYZ and CIE $L^*a^*b^*$ colour spaces. RGB was converted to XYZD65 using the matrix transform (Pascale, 2003):

$$\begin{bmatrix} X_{D65} \\ Y_{D65} \\ Z_{D65} \end{bmatrix} = \begin{bmatrix} 0.4125 & 0.3576 & 0.1804 \\ 0.2127 & 0.7152 & 0.0722 \\ 0.0193 & 0.1192 & 0.9503 \end{bmatrix} \times \begin{bmatrix} R \\ G \\ B \end{bmatrix} \quad [6.1]$$

Then XYZD65 was converted to XYZC using the Bradford matrix transform (Pascale, 2003):

$$\begin{bmatrix} X_C \\ Y_C \\ Z_C \end{bmatrix} = \begin{bmatrix} 1.0095 & 0.007 & 0.0128 \\ 0.0123 & 0.9847 & 0.0033 \\ 0.0038 & -0.0072 & 1.0892 \end{bmatrix} \times \begin{bmatrix} X_{D65} \\ Y_{D65} \\ Z_{D65} \end{bmatrix} \quad [6.2]$$

Finally, the following equations were used to convert XYZC to CIEC $L^*a^*b^*$ (Konica Minolta, 1998):

$$\begin{aligned} L^* &= 116 \times (Y/Y_n)^{1/3} - 16 \\ a^* &= 500 \times [(X/X_n)^{1/3} - (Y/Y_n)^{1/3}] \\ b^* &= 200 \times [(Y/Y_n)^{1/3} - (Z/Z_n)^{1/3}] \end{aligned} \quad [6.3]$$

where X_n , Y_n and Z_n are the values for X , Y and Z for the illuminant used, in this case 0.973, 1.000 and 1.161 respectively. Also, $(X/X_n)^{1/3}$ was replaced by $[7.787 \times (X/X_n) + 16/116]$ if X/X_n was below 0.008856; $(Y/Y_n)^{1/3}$ was replaced by $[7.787 \times (Y/Y_n) + 16/116]$ if Y/Y_n was below 0.008856; and

$(Z/Z_n)^{1/3}$ was replaced by $[7.787 \times (Z/Z_n) + 16/116]$ if Z/Z_n was below 0.008856 (Konica Minolta, 1998).

Hue angle and chroma were calculated from a^* and b^* values. Regressions of colorimeter on computer vision for a^* , hue angle and chroma had R^2 values of 0.96, 0.94 and 0.93, while only R^2 values of 0.58 and 0.56 were obtained for L^* and b^* . Results show that colour readings from digital images could be used to accurately predict colour coordinates measured by colorimeter, especially a^* , hue angle and chroma. Therefore, computer vision is a practical tool to detect changes in beef colour.

Recently, Sun *et al.* (2011) investigated the usefulness of colour image features of fresh lean beef for predicting official Chinese beef colour scores. Cross-section images of about 160 beef *longissimus thoracis (ribeye)* were collected. The 12 colour features were extracted and one feature was calculated using stepwise multiple regression analysis. Multiple linear regression (MLR) and support vector machine (SVM) models were respectively established for estimating the grade of beef muscle colour based on inputs of colour features and outputs of 4–7 colour scores. MLR analysis had the correct percentage of 86.8% of beef colour muscle scores and SVM improved the performance to 94.7%, showing that computer vision technique can provide an effective tool for predicting colour scores of beef muscle.

6.4.2 Pork

Colour is also a main quality attribute of pork, which plays a major role in consumer evaluation of pork quality. Early work on the measurement of pork colour was carried out by Lu *et al.* (2000). They investigated the potential of computer vision technology for evaluating fresh pork loin colour. Forty-four pork loins randomly picked were cut at the 10th rib, and the muscle colour of each sample was evaluated by a trained seven-member sensory panel. The colour was scored using a five-point scale, where 5 = dark and purplish red, 4 = purplish red, 3 = reddish pink, 2 = greyish pink and 1 = pale-purplish grey. Image acquisition was immediately performed after sensory analysis by the sensory panel under similar lighting conditions. Captured images were then segmented into background, muscle and fat. Colour image features, including mean and standard deviation of red, green and blue bands of the segmented muscle area, were extracted from segmented images, which were then used to predict the colour scores by using both statistical models and neural network (NN). The partial least squares (PLS) technique was used to derive latent variables, which were further analysed by MLR and NN using a back-propagation learning algorithm. The correlation coefficient between the predicted and the sensory colour scores was 0.75 in NN modelling, with 93.2% of the 44 pork loin samples having prediction errors lower than 0.6; for the statistical model, 84.1% of the samples had prediction errors of 0.6 or lower, which was considered negligible from a practical viewpoint. The overall results of this study

showed that computer vision combined with NN is an efficient tool for measuring sensory colour of fresh pork.

In another work, Tan *et al.* (2000) applied a computer vision system to predict colour scores of fresh pork visually assessed by a ten-member untrained panel in three separate studies. There were 73, 79 and 51 pork loin chops fabricated from different loins adjacent to the 10th and 11th ribs of carcasses in each of three studies, and the panel performed sensory evaluation. After training using pork images classified by the panel, the computer vision system had a capability of predicting fresh pork colour, with up to 86% agreement with visually assessed colour scores. By combining with an efficient tracking system, computer vision could sort retail meat cuts into uniform quality/colour groups before shipping to retail merchandisers, and operate at on-line speeds with accuracy and repeatability similar to that of sensor evaluations.

Later, O'Sullivan *et al.* (2003) compared the instrumental colour measurements of a Minolta colorimeter and a computer vision system and determined their respective effectiveness in predicting the sensory visual quality of pork meat patties (*M. longissimus dorsi*). Three dietary treatment groups were considered, each of them had seven pigs fed either on a low vitamin E diet, supplemental iron, or on supplemental vitamin E. Visual colour evaluation was conducted by a trained sensory panel ($n=8$) and an untrained panel ($n=8$) on days 0, 1, 3 and 5. A computer vision system was used to measure RGB (red, green and blue) and Hunter L^* , a^* and b^* values on each day of analysis, and a Minolta colorimeter was used to measure instrumental hunter L^* , a^* and b^* values. Computer vision had a higher correlation with the sensory terms determined by both trained and untrained sensory panelists, particularly for the red, brown and L value descriptors, which was due to the fact the entire surface of samples could be measured by computer vision and therefore a more representative measurement was taken compared to the colorimeter.

6.4.3 Fish

Studies on fish products have shown that consumers associate colour with the freshness of a product having better flavour and higher quality (Gormley, 1992). However, in the fish industry, colour assessment is achieved by using visual inspection aided by the use of colour charts, such as SalmonFan™ card (Hoffmann-La Roche Basel, Switzerland; Quevedo *et al.*, 2010), which is, however, laborious, tedious, subjective and time-consuming. Quevedo *et al.* applied computer vision technique to assign colour score in salmon fillet according to SalmonFan™ card. The same illumination conditions in ten independent sets (20 salmon fillets in each one) of experiments were used to measure colours by a computer vision system and a sensorial panel with eight panelists. The calibration to transform RGB to $L^*a^*b^*$ colour space was conducted by using a methodology suggested by Leon *et al.* (2006) with

30 colour charts and 20 SalmonFan cards. Calibration errors for transforming RGB to $L^*a^*b^*$ values were 2.7%, 1% and 1.7%, respectively, with a general error range of 1.83%. On the basis of the calibrated transformation matrix, RGB values of each pixel within the digital image of salmon fillet acquired by computer vision were transformed to $L^*a^*b^*$ values, and then matched with other $L^*a^*b^*$ values that represent a SalmonFan score. In general, similar SalmonFan scores were obtained from computer vision and the sensory panels, which had a high correlation coefficient of 0.95. There were no differences in the measurements of the SalmonFan score between both methods from the statistical analysis using t test ($t_c = 1.65 \leq t = 1.96$ at $\alpha = 0.05\%$). Good results obtained in the study by Leon *et al.* (2006) show the potential of using computer vision technique to qualify salmon fillets based on colour according to the SalmonFan card. However, it is important to highlight that the calibrated parameters of transformation matrix need to be recalculated when the computer vision system or the illumination is modified.

In another study, Oliveira and Balaban (2006) compared a computer vision system and Minolta CR-200 colorimeter in their abilities to measure changes of colour over 15 days of iced-storage of Gulf sturgeon fillets from fish fed on three different commercial diets. The $L^*a^*b^*$ values were measured at days 0, 5, 10 and 15 using both the computer vision system and the Minolta CR-200 colorimeter, and ΔE values calculated to allow comparison of results. The ΔE value measures the 'total' colour change, which was calculated by the following function:

$$\Delta E = \sqrt{(L_0 - L_i)^2 + (a_0 - a_i)^2 + (b_0 - b_i)^2} \quad [6.4]$$

where the subscript 0 refers to the values at time 0, and i refers to values at 5, 10 or 15 days.

No significant differences were found from statistical analysis in ΔE values from the hand-held colorimeter or machine vision between either treatments or storage days ($P < 0.05$). ΔE values were significantly different ($P < 0.05$) between instruments, except for day 0. Later, Yagiz *et al.* (2009) measured the colour of irradiated Atlantic salmon fillets using a hand-held Minolta colorimeter and a machine vision system and compared their performance. Both a computer vision system and a Minolta CR-200 Chroma Meter were used to measure the L^* , a^* , b^* values of Atlantic salmon fillets subjected to different electron beam doses (0, 1, 1.5, 2 and 3 kGy). The L^* value obtained using both methods increased with increasing irradiation dose and the a^* and b^* values decreased. Significantly higher readings were obtained by the computer vision system for L^* , a^* , b^* values than the Minolta colorimeter. Because of this difference, visual comparison was conducted to illustrate colours that were actually measured by the two instruments. The colour represented by the Minolta readings was purplish based on average L^* , a^* , b^* values, while that measured using the machine vision system was much closer to the average real colour of Atlantic salmon

fillets (Plate III between pages 242 and 243). The results of this study (Yagiz *et al.* (2009) were similar to those of Oliveira and Balaban (2006), and the difference could be due to the different average daylight illuminants used, namely D65 with a colour temperature of 6504 K for the Minolta colorimeter and D50 with a colour temperature of 5000 K for the machine vision system. However, Yagiz *et al.* (2009) used the same illuminant, i.e. D65 with a colour temperature of 6504 K, for both instruments. In addition, the known L^* , a^* , b^* values of the standard red plate measured using the Minolta colorimeter and the machine vision system were very close. Hence, Yagiz *et al.* recommended caution in reporting colour values measured by Minolta, even when the 'reference' tiles are measured correctly. There are various factors that can affect the colour readings, such as the surface roughness and texture, the amount of surface 'shine', and the geometry of the measuring instrument. It is recommended that the colour formed by the L^* , a^* , b^* values read from any device is compared visually with the observed colour of the sample.

6.4.4 Orange juice

The colour of orange juice has the correlation to the consumer's perception of flavour, sweetness and other quality characteristics (Pangborn, 1960; Tepper, 1993), and is considered as one of their main advantages over other juices (Fernandez-Vazquez *et al.*, 2011). Colour is also found to influence sweetness in orange drinks and affects intensity of typical flavour in most fruit drinks (Bayarri *et al.*, 2001). The validity of using colorimetric for the objective colour evaluation of orange juice colour has been demonstrated (Eagerman, 1978). New advances in computer vision offer the possibility of evaluating colour in terms of millions of pixels with low cost. Fernandez-Vazquez *et al.* (2011) characterized the colour of the juice from five orange varieties and explored the relationship between computer vision and sensory evaluation of the colour attributes (lightness, chroma and hue) quantified by 18 trained panelists. Fresh hand-squeezed orange juice from five varieties of oranges was evaluated by image analysis (DigiEye System, VeriVide Ltd, Leicester, UK). Correlation coefficients of 0.96, 0.069 and 0.92 were obtained for lightness (L^*), chroma (C_{ab}^*) and hue (h_{ab}) between panelists' colour evaluation and the image values, showing that hue and lightness were well correlated with the DigiEye image measurement but not chroma. The poor measurement of chroma was probably because of the fact that it is not an intuitive attribute. Therefore it was not well evaluated, resulted in no correlation with computer vision measurement observed.

6.4.5 Potato chips

In the case of potato chips, colour is an extremely important criterion that is strictly related to consumer perception and has to be controlled during

processing (Orr & Janardan, 1990; Scanlon *et al.*, 1994). However, the subjective manual measurements usually do not give any reproducible results due to the complicating factors relating to human perception (Segnini *et al.*, 1999b). Therefore, a good objective method is required for measuring colour of potato chips.

As an early research, Scanlon *et al.* (1994) evaluated whether potato chip colour could be quantified by computer vision analysis. Mean grey level values from specific regions of potato chips were used to characterize the colour of chips, which were fried three times. On the basis of mean grey level values, it was feasible to distinguish differences in chip colour from potatoes stored at the two temperatures and to discriminate different chip frying times for potatoes that had been stored at 5 °C. There was good relationship between colour assessed by mean grey level and colour measured by the Agtron M31A colour meter and Hunterlab D25L-2 colorimeter. However, it was found that the colour based on image analysis was not as repeatable as colour measured by the two other techniques. Later, Segnini *et al.* (1999a) developed a computer vision-based image analysis system to quantify the colour of potato chips in $L^*a^*b^*$ colour space. The system was not sensitive to light intensity and had less influence from the undulating surface of the chips. A clear relation was found between the obtained L^* , a^* or b^* and the scale by human eyes. The sensitivity of the technique to separate 'colours' had a good correlation with the capability of the human eye. In another study, Segnini *et al.* (1999b) investigated the relationship between computer vision and sensory analysis for measuring colour of commercial potato chips. Sensory evaluation of colour, which included 'yellow colour', 'burnt aspect', 'sugar coloured aspect' and 'transparency', was performed by a sensory panel especially trained in evaluating potato chips. L^* showed a good ability ($R > 0.79$) to predict most of the sensory colour attributes, which include 'yellow colour', 'burnt aspect' and 'sugar coloured aspect'. A good prediction of 'burnt aspect' was also obtained by a^* , while the b^* attribute did not significantly correlate with any of the sensory parameters ($p > 0.05$).

In addition, Pedreschi *et al.* (2006) designed an inexpensive computer vision system for measuring the colour of a highly heterogeneous food material in colour such as potato chips in $L^*a^*b^*$ units from RGB images. The system consisted of a digital colour camera for acquiring the images in a digital format, a computer for storing the images, and image analysis routines integrated into a software programmed in Matlab that had functions of digital pre-processing, segmentation, feature extraction, and colour transformation from RGB to $L^*a^*b^*$ units. By implementing this computer vision system, the kinetics of colour formation of potato slices during frying at four different oil temperatures was analyzed based on the parameter total colour change (ΔE) which was calculated from $L^*a^*b^*$ values. The system implemented results showed that it was an easy, precise, representative, objective and inexpensive way to determine the colour of potato slices

from RGB images into $L^*a^*b^*$ units. The system allowed the measurements of the colour over the entire surface of a potato chip or over a small specific surface region of interest.

6.4.6 Wheat

Computer vision technique was also used for colour measurement of wheat kernels. Zapotoczny and Majewska (2010) analysed the correlations between the colour of the seed coat of wheat kernels, measured with a spectrophotometer and by digital image analysis, and the colour on their cross-section. Spring and winter wheat with 17 varieties were analysed, which had a high proportion in the cropland structure and seed reproduction in Poland. The colour of the endosperm and seed coat was saved in RGB space after image acquisition, and was then transformed into XYZ and $L^*a^*b^*$, which enabled the computation of the hue and saturation of colour. After image analysis, it was found that there was a high linear correlation ($p < 0.05$) between the colour of the seed coat measured with a spectrophotometer and by computer vision analysis, which means that the colour of the seed coat of wheat kernels can be determined by computer vision instead of spectrophotometry.

6.4.7 Wine and beer

Colour is one of the main parameters of the quality of wines and has an important influence on the overall acceptability by consumers (Martin *et al.*, 2007). The interaction study among various sensory shows that colour of wine affects the determination of aroma (Williams *et al.*, 1984), odour (Pokorny *et al.*, 1998) and variety (Morrot *et al.*, 2001; Parr *et al.*, 2003). Compared with traditionally used visual assessment and instrumental colour measurement using spectrophotometer, computer vision offers the possibility of measuring colour of wine within inhomogeneous colour areas in terms of millions of pixels with relatively low cost. Martin *et al.* (2007) quantified the relationships between visual observations and physical measurements with a Minolta CS-1000 tele-spectroradiometer and a calibrated computer vision camera of colour for various wines with reference to the change of depth. Four commercial wines with different colour and appearance properties were selected: table red, oloroso, tawny port and rose. Samples of the wine were poured at different depths in Petri dishes and in cocktail glasses. All samples were assessed by a panel of eight volunteer observers (three females and five males aged between 24 and 41) in terms of lightness, colourfulness and hue. There are good agreements between the tele-spectroradiometer and the digital camera with visual estimates. Computer vision had many advantages for quantifying colour appearance of liquid food products, such as time saving in sample preparation, fast measurement of more than one sample at a time, and most importantly, reliable

readings agreeing with visual and conventional tele-spectroradiometer results (Martin *et al.*, 2007).

In another study, Sun *et al.* (2004) applied computer vision for measuring beer colour, which was compared to the result of the European Brewery Convention (EBC) colorimetry. Experimental samples comprised 76 beer samples differing in location of production, categories of beers, strength of wort, alcohol content and storage time (within and outside shelf-life). There was a high positive correlation between colours measured by computer vision and those determined by using spectrophotometry and colorimetry, which shows that it was feasible to determine beer colour using computer vision analysis. A standard deviation of zero showed the computer vision technique had a high repeatability for measuring the colour of beer.

6.5 Quantification of colour nonhomogeneity

Most food products have non-uniform colours. Colour nonhomogeneity is an important colour attributes and its quantitative measurement is required. Traditional colour measurement using sensory panels is subjective and time-consuming, and is difficult to convert to reproducible numerical values. Colorimeters typically measure the 'average' colour of food products and are not available for non-uniform colours. For this reason, Balaban (2008) and Balaban *et al.* (2008) used a computer vision technique to quantify uniform or non-uniform colours of food products. In their studies, experimental samples included three mangoes of different colours, three pieces of rabbit meat (a hind leg, a foreleg and a torso), and a banana in different stages of ripening. For uniform colours, average colour was used, which is similar to the averaging performed by a colour meter. For non-uniform colours, several image analysis methods were applied, which include colour blocks, contours and 'colour change index' (CCI).

The colour blocks calculation had three steps: firstly, reducing the number of colours in the RGB colour space by dividing each colour axis into either 4 ($4 \times 4 \times 4 = 64$ colour blocks) or 8 ($8 \times 8 \times 8 = 512$ colour blocks) or 16 ($16 \times 16 \times 16 = 4096$ colour blocks); secondly, counting the number of pixels that fall within a colour block, and calculating the percentage of that colour based on the total view area (total number of pixels) of the object; and finally, an appropriate threshold was set to consider only those colour blocks that have percentage areas above that threshold. Based on the set threshold, the higher the number of colour blocks, the more nonhomogeneous the colour is.

The contours calculation had two steps: firstly, identifying colour attributes lower than, or higher than a given threshold, or attributes between two threshold values; secondly, calculating the percentage of pixels within contours based on the total view area of an object. Contours

calculation can be used to quantify the colours of defective areas, such as dark spots.

Finally, CCI was calculated based on colour primitives, which are several continuous areas of an image where the 'intensity' of any pixel is within a given threshold value. The more colour primitives in an image, the more nonhomogeneous the colour of that object is. The calculation function of CCI was proposed as follows:

$$\text{CCI} = \frac{\sum \Delta I \text{ for all neighbouring pixels}}{\sum \text{distances between equivalent circles}} \times \frac{\text{number of neighbours}}{\text{object area}} \times 100 \quad [6.5]$$

The results of the study by Balaban (2008) showed that the colour blocks method can give a quantitative measure of non-uniformity if the range of hue values is large as in the case of mangoes; and the colour primitives method and the CCI value can quantify the non-uniformity of colour well if the hue range is narrow as in the case of rabbit samples.

Furthermore, although much research has been conducted in the comparison and correlation of homogeneous colour measurements using instrumental and visual colour analysis, since no method is known to quantify these colours by sensory panels, the correlation between computer vision and sensory panel evaluations has not been made. Balaban *et al.* (2008) proposed a method to quantify the perception of nonhomogeneous colours by sensory panellists and compared the differences in colour evaluation between a computer vision system and sensory panels. Three colours of 15 reference colours and their perceived percentage of the total sample area were selected by untrained panellists. The colour difference error ΔE was defined as the differences between the average colours perceived by panellists and those from the computer vision. Generally, the more non-uniform the colour of a sample, the higher the error of a panellist was in quantifying the colour of a sample, therefore panellists had more difficulty in evaluating more nonhomogeneous colours. Moreover, the effects of colour nonhomogeneity were evaluated in the sensory panels on ΔE using real samples or their images. Results showed that no significant difference in ΔE values existed between panelists' errors based on evaluating the real fruit and evaluating its image (Balaban *et al.*, 2008). Therefore, images can be used to evaluate colour instead of the real samples, which may be significant, since a well-acquired digital image of a food sample is a good representation of the visual attributes of the food itself. Visual evaluation of images eliminates temporal and geographical restrictions, especially for the evaluation of perishable foods. In addition, images can be sent electronically to distant locations and last much longer than the food, which allows much more flexibility in the analysis of visual attributes of food products.

6.6 Advantages and disadvantages of using computer vision

The advantages and disadvantages of computer vision were assessed by many researchers (Sistler, 1991; Heinemann *et al.*, 1995; Brosnan & Sun, 2004; Du & Sun, 2004; Gumus *et al.*, 2011). The main advantages of applying the computer vision technique in food colour measurement include the rapid, precise, objective, efficient, consistent and non-destructive measurement of colour data with no sample pretreatment, high spatial resolution and low cost, reduction of tedious and subjective human involvement, automation of mass labour-intensive operations, rapid generation of reproducible results, the ability to analyse each pixel of the surface of a food product, extracting more colour features with spatial information, generating the distribution map, the possibility of analysing the whole food even it is of small or irregular shape and of nonuniform colours, the flexibility of selecting a region of interest, and the availability of a permanent storage of colour data for further analysis by keeping the picture.

Although computer vision has the aforementioned advantages, it does have some disadvantages, such as the requirement of well-defined and consistent lighting (such as a light box, where the light intensity, spectrum and direction are all controlled), difficulties encountered with overlapping objects, objects that are difficult to separate from the background, or both sides of a food need to be evaluated (Brosnan & Sun, 2004; Gumus *et al.*, 2011), and careful calibration and setting requirements because different camera and light box settings may result in different measurements. Moreover, the intensity and the spectrum of light bulbs may change over time (Balaban & Odabasi, 2006).

6.7 Conclusion and future trends

Computer vision is a science-based automated food inspection technique that has been proved to be efficient and reliable for simultaneous colour measurement without monotonous sample preparation, and therefore offering the possibility of designing inspection systems for the automatic grading and quality determination of food products. On the basis of digital image process algorithms, computer vision had the ability of reducing industrial dependence on human graders, increasing production throughput, reducing production cost, improving product consistency and wholesomeness, and enhancing public confidence in the safety and quality of the food products. Nowadays, the computer vision technique had been used on a production line or in the quality control lab, and several commercial systems are already available for food colour measurement, such as QualiVision system (Dipix Technologies, Ottawa, Ontario, Canada), Lumetech Optiscan system (Koch Lumetech, Kansas City, Mo.), Model L-10 Vision Weigher (Marel, Reykjavik, Iceland), Parasensor system (Precarn, Ottawa, Canada),

Prophecy 550 system (Imaging Technology, Bedford, Mass.), and SINTEF system (SINTEF, Oslo, Norway) (Balaban & Odabasi, 2006).

On the other hand, although colour measurement of food products using computer vision has made excellent progress, challenges still remain, which also create many future research opportunities. Owing to the complex nature of food images, a big challenge in computer vision is to develop efficient segmentation algorithms. This is a prerequisite to the success of all subsequent operations leading to successful computer vision-based colour measurement, because no existing algorithm is totally effective for food-image segmentation. Automatic segmentation should be a reliable and consistent process to segment region of interest, without human intervention. Unsupervised learning techniques, such as clustering and self-organizing maps, will be the key to effective and robust image segmentation. Further research is also required to develop efficient and robust calibration for computer vision systems to reduce the influences of a change of camera, illumination and environment.

Developments in electronics, hardware, computing and software are also critical for computer vision to measure colour of food products rapidly and accurately. Hardware used in computer vision system should be faster, lighter/smaller and less expensive, resulting in decreasing image acquisition and analysis time, improving speed and storage space for measuring larger and more detailed images, and increasing the resolution (number of pixels) of cameras for detailed colour measurement. Faster and simpler interfaces should also be considered, such as Apple's FireWire standard, which eliminates the image-grabber card between the camera and the computer to convert visual information to digital data (Balaban & Odabasi, 2006). System robustness, real-time capability, sample handling and standardization are also among the issues that require further in-depth research, as there are still major challenges to design a computer vision system that has sufficient flexibility and adaptability to handle the biological variations in food products. However, the issues seem not to be insurmountable with further research and development.

On the basis of the development of hardware for image acquisition and efficient software for image analysis, computer vision can be a viable method for measuring the colour of foods with capabilities that are not possible with other methods, especially the ability to analyse samples with nonhomogeneous colours, shapes and surfaces. Moreover, on the basis of combining colour information with shape, size, orientation, defects and nutrition, computer vision allows evaluation of total visual quality of foods. Compared with traditional visual inspection or colorimeter measurement, computer vision technology not only provides a high level of repeatability and flexibility at a relatively low cost, but also, and more importantly, it permits fairly high plant throughput without compromising accuracy. With the help of computer vision, human visual inspectors can be free from undertaking

tedious, laborious, time-consuming and repetitive inspection tasks, and can focus on more demanding and skilled jobs.

6.8 Sources of further information and advice

The first book on using computer vision technology for the evaluation of food quality including colour was published by Sun (2007), which gives very comprehensive information from computer vision principles to various applications. In addition, numerous reviews are also published on computer vision for measuring colour and other quality attributes of food products and on image process techniques (Gunasekaran, 1996; Brosnan & Sun, 2002, 2004; Du & Sun, 2004; Zheng *et al.*, 2006b; Valous *et al.*, 2010; Costa *et al.*, 2011; Gumus *et al.*, 2011; Jackman *et al.*, 2011; Mathiassen *et al.*, 2011). In addition, there are some books on colour measurements which are also relevant (Hunter & Harold, 1987; Hutchings, 1995; McDonald, 1997; MacDougall, 2002a; Mathworks, 2005; Culver & Wrolstad, 2008; Gulrajani, 2010).

Some international universities and companies working at colour measurement in food applications are as follows:

- University College Dublin, Ireland, UCD Food Refrigeration and Computerized Food Technology Center, <http://www.ucd.ie/refrig/>
- Universidad de Sevilla, Spain, Food Colour & Quality Group, <http://www.color.us.es/w-index.htm>
- The University of Derby, UK. Colour and Imaging Institute, <http://colour.derby.ac.uk/>
- Rochester Institute of Technology, USA. Munsell Colour Science Laboratory, <http://www.cis.rit.edu/mcsl/>
- The University of Leeds, UK. Department of Colour Chemistry, <http://www.leeds.ac.uk/ccd/>
- The Colour Group (Great Britain), <http://www.city.ac.uk/colourgroup/index.html>
- The National Physics Laboratory, UK, <http://www.npl.co.uk>

Multinational colour measurement companies include Minolta, HunterLab, DataColour and GretagMacBeth.

In addition, some useful websites related to food colour and digital image processing are listed below:

- www.easyrgb.com – this site provides information and services related to colour technology, such as a colour calculator, creating colour harmonies, from RGB values to commercial tints, from commercial tints to RGB values, monitoring colour calibration, managing software and colour database, providing colour tutorials and the ability to freely submit your tints
- www.prenhall.com/gonzalezwoodseddins – this site provides support to the book entitled *Digital Image Processing Using Matlab*, such as

downloadable M-files, including all M-files in the book, tutorials, projects, teaching materials, links to databases, including all images in the book, book updates, and background publications

- www.prenhall.com/gonzalezwoods – the site of the Gonzalez-Woods book offers additional support on instructional and research topics
- www.couleur.org/index.php?page=rgbcube – this site displays the RGB cube transformation in different colour spaces (RGB, XYZ, xyY, I1I2I3, UVW, LSLM, $L^*a^*b^*$, $L^*u^*v^*$, LHC, HSV, HSV Polar, CMY, HSI, HSI Polar, LHS, YUV, YIQ)
- <http://www.aic-color.org/> – the website of International Colour Association

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	Treatment dose (kGy)	Minolta	Machine vision	Picture
(a)	0			
(b)	1			
(c)	1.5			
(d)	2			
(e)	3			
(f)	Standard red plate			

Plate III (Chapter 6) Colour representations of Minolta and machine vision reading results and actual pictures of differently treated salmon fillets and standard red plate (Yagiz Y, Balatan MO, Kristinsson HG, Welt BA and Marshall MR, 2009. Comparison of Minolta colorimeter and machine vision system in measuring colour of irradiated Atlantic salmon. *Journal of the Science of Food and Agriculture*, **89**(11), 728–730).

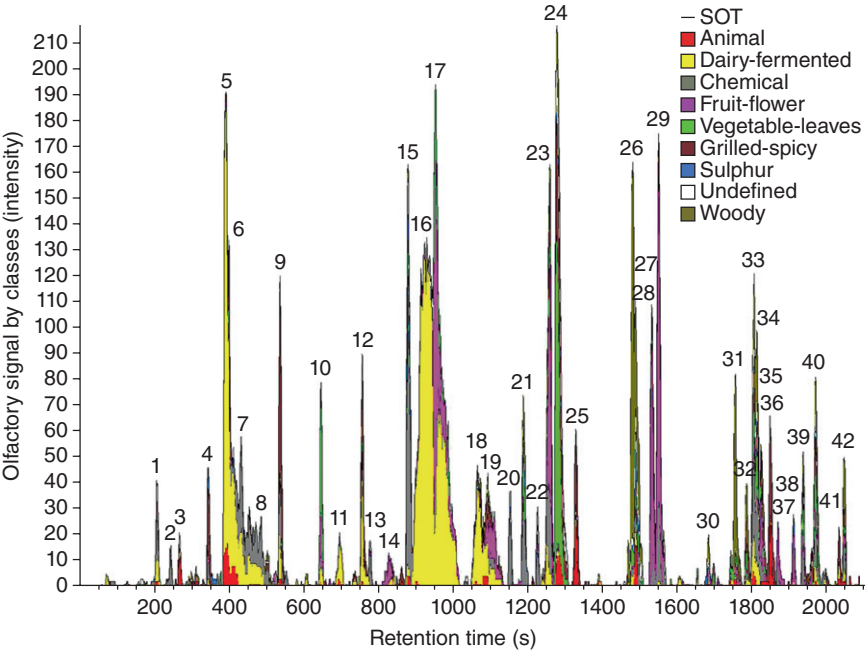


Plate IV (Chapter 10) Odorous volatile compounds in Cantal type cheese according to 8 different assessors and 8 different cheese treatments. (Reproduced with permission from Cornu A, Rabiau N, Kondjoyan N, Verdier-Metz L, Pradel P, Tournayre P, Berdague JL and Martin B, 2009. Odour-active compound profiles in cantal-type cheese: effect of cow diet, milk pasteurization and cheese ripening. *International Dairy Journal*, **19**(10), 588–594).

Gas chromatography-olfactometry (GC-O), electronic noses (e-noses) and electronic tongues (e-tongues) for *in vivo* food flavour measurement

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Abstract: The flavour of food products is undoubtedly one of the most important parameters influencing acceptance of particular products by consumers. Therefore, evaluation of taste and smell of food products is important in different areas of food industry. This chapter reviews two types of artificial olfaction instrumentation, i.e. gas chromatography-olfactory (GC-O) and chemical sensor technologies (electronic nose and tongue – e-nose and e-tongue), combined with multivariate data processing methods as promising approaches for rapid analysis of food. The chapter first discusses the working principles of each system and gives a brief description of instrumentation. Further discussion concerns comparison of the GC-O system with sensor technologies, especially by considering the advantages and disadvantages of both type of instrumentation. The chapter also includes examples of specific applications for the detection of food flavour and volatile components.

Key words: food flavour, machine olfaction, gas chromatography-olfactometry (GC-O), electronic nose (e-nose), electronic tongue (e-tongue), odour, taste.

7.1 Introduction

The flavour of food products is important as a characteristic sensory feature and for the quality of the product. Flavour frequently influences food acceptance and is the decisive factor for the choice of particular product. Therefore, flavour is of interest both to food manufacturers and consumers to evaluate taste and smell (the entirety of taste-aromatic sensations) of food products, raw materials and semi-products during the different technological processes (Seo and Hummel, 2012). The analysis of compounds responsible for taste and smell is usually carried out through classic

sensorial analysis (Ashurst, 1999; Lopetcharat and McDaniel, 2012, p. 309; Zawirska-Wojtasiak, 2012, p. 439) or with the use of instrumental methods (machine olfaction) (Cayot, 2007; Cho and Kang, 2011) delivering a valuable information about the quality of a given product.

Classic sensory analysis is still the technique used most often and is the determinant of food quality analysis, especially of semi- and final products. This method is characterized by many limitations (Shurmer and Gardner, 1992; Ashurst, 1999), such as: the fallibility of the human factor (the evaluation perception depends on panellists' training and the sensorial sensitivity of the evaluating person, his/her state of health, comfort or fatigue), low reproducibility and repeatability of results, as well as the infeasibility of identifying compounds affecting taste and no possibility of performing a quantitative analysis. Furthermore, this approach is time consuming, expensive and impossible for on line monitoring.

Chromatographic techniques, especially gas chromatograph coupled with mass spectrometry (GC-MS), are popular techniques for the identification of volatile compounds in food analysis. In GC-MS the high separation power of the GC system can be complemented by MS with high sensitivity. GC-MS systems are able to identify the compounds on the basis of their fragmentation patterns at one part per billion sensitivity ($1:10^9$). A breakthrough in aroma research was the introduction of combination of olfactometry and gas chromatography (GC-O, Fuller *et al.*, 1964), a technique that associates the resolution power of capillary GC with the selectivity and sensitivity of the human nose (Plutowska and Wardencki, 2008, 2012).

As alternatives to the GC-O technique, two types of equipment based on electronic sensors are increasingly being employed. Depending on the type of analytes, electronic nose (e-nose) and electronic tongue (e-tongue) instruments are regularly utilized (Deisingh *et al.*, 2004; Ciosek *et al.*, 2004, 2006; Apetrei *et al.*, 2010). The first performs an entirely aromatic analysis (volatile compounds) in the gaseous phase, without separating the aroma into individual aromatic components. The second allows for the determination of components of medium and low volatility in the liquid phase and complements the first (Leake, 2006). Both types of equipment consist of arrays of non-selective gas or liquid sensors and are provided with a pattern recognition system, capable of identifying simple or complex taste and aromatic profiles (Apetrei *et al.*, 2010). Such equipment is quick-acting, easy to operate and does not influence the analysed sample (Haugen *et al.*, 2006).

In this chapter, a concise overview of two types of artificial olfaction instrumentation, GC-O and chemical sensor technologies (e-nose and e-tongue), is provided. At the beginning, a brief history of each system is presented followed by a description of their principle of operation. Next, data measurements in the GC-O technique and data analysis and pattern recognition in electronic systems are characterized and the GC-O system

is compared with sensor technologies, especially by considering the advantages and disadvantages of both type of instrumentation. Finally, specific applications for detection of food flavour and volatile components will be given.

7.2 Artificial olfaction: gas chromatography-olfactometry (GC-O)

The combination of olfactometry and gas chromatography into food analysis was a breakthrough in aroma research, enabling the differentiation of a multitude of volatiles in odour-active and non-odour-active, related to their existing concentrations in the matrix under investigation. The GC-O technique combines the resolution power of capillary GC with the selectivity and sensitivity of the human nose. This combination (instrumental and olfaction) allows the method to be treated as artificial olfaction. It should be mentioned that human nose is able to detect and distinguish some volatile compounds at the amount of 10^{-17} g, while the detectors commonly used in chromatography require, as a rule, at least 10^{-13} g to identify a compound.

Many factors influence the correct detection and assessment of odour-active compounds using GC-O technique, i.e. extraction procedure, the method of data collection and separation capability of the GC column (Falque Lopez and Fernandez Gomez, 2000; Nonato *et al.*, 2001; Bonino *et al.*, 2003).

The choice of an appropriate sample preparation method becomes crucial because aroma profile is closely related to the isolation procedure, which should yield a product that is representative of the sample. Many extraction techniques have been developed to ensure representative nature of extracts but the best way to attain an optimum recovery of the flavour chemicals and to achieve a complete reproduction of a flavour's profile is the employment of more than one extraction technique. The choice of the GC-O data measurement method is of extreme importance for the correct characterization of a matrix, since the application of different methods to the same real sample can distinctively select and rank the odour-active compounds according to their potency and/or intensity (Plutowska and Wardencki, 2007, 2008).

A limitation of GC-O is the co-elution of odour-active compounds, which results in the perception of odour clusters. Using a different stationary phase may allow the intensity and quality of the odorants to be evaluated individually without co-elution. Another possible solution for identifying active odour when co-elution occurs is to use comprehensive, multidimensional GC (GC $\times$ GC). The application of this technique in conjunction with the identification capabilities of time-of-flight mass spectrometry (TOF-MS) facilitates the identification of character-impact odorants in very complex samples (Chmiel *et al.*, 2011).

Since the first description of the GC-O system by Fuller *et al.* in 1964, many papers presenting different techniques for designing GC-O experiments, recording the data and estimating the sensory combination of aroma compounds have been published. The general aspects of food aroma analysis using GC-O techniques may be found in several books (Blank, 1996; Deibler *et al.*, 1999; Grosch, 2007; Plutowska and Wardencki, 2012) and in the reviews published in journals (van Ruth, 2001; Delahunty *et al.*, 2006; d'Acampora Zellner *et al.*, 2008; Plutowska and Wardencki, 2008).

7.2.1 Overall characteristic of the GC-O technique and instrumentation

GC-O uses the human nose as a detection device, generally parallel to standard chromatographic detector (i.e. flame ionization detector, FID, or, more frequently, a mass spectrometer) and thus permits rapid identification of so-called odorant zones in a chromatogram. An analysis using the human sense of smell is carried out by an appropriately trained person or a group of evaluating persons who in the course of the test sniff the eluate from column and relate the aromatic impressions to the retention times.

Many improvements have been made to GC-O devices after first description of a gas chromatograph modified for the sniffing of effluent to determine volatile odour activity in 1964 (Fuller *et al.*, 1964). In most of the presently used systems an organoleptic estimation of odour is facilitated with the use of a specially constructed attachment, the so-called olfactometric port, in most cases a poly(tetrafluoroethylene) (PTFE) or glass cone fitting the shape of human nose, connected in parallel to conventional detectors (Fig. 7.1).

Simultaneous detection is realized by dividing the eluent stream in an appropriate ratio so that it reaches both detectors. The most favourable is the simultaneous use of an olfactometric detector and mass spectrometer. Such an approach allows for both the description of odours and an identification of the compounds responsible for them, followed by a determination of which of them is characterized by the most intense aroma. The possibility of divergence between the retention times for analytes detected both by means of the mass spectrometer and the olfactometer (in the case in which the retention times are longer) can be overcome by installing a restrictor (thin capillary tube) and adjusting the flows of the carrier and auxiliary gas (Hochereau and Bruchet, 2004). When the tested extracts are sufficiently concentrated, they can be separated into a few streams which are simultaneously applied to separate olfactometric ports and analysed by several evaluators operating in parallel. The results obtained in such situations are the most representative (Debonneville *et al.*, 2002). The eluate arriving at the olfactometric port may cause drying the mucous membrane in the nose of the evaluating person, particularly during long-lasting analyses. To exclude this unfavourable factor, which affects the final results,

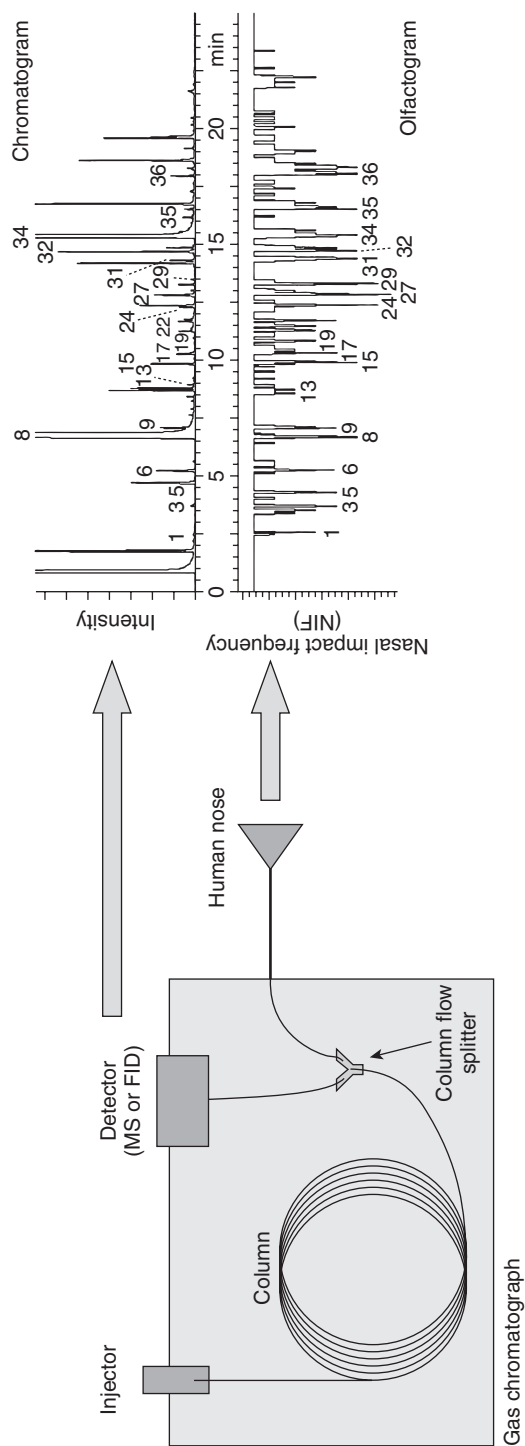


Fig. 7.1 Schematic of GC-O analysis.

an appropriate addition of an auxiliary gas, usually moist air, is used. The eluate from the column is fed into the olfactometric port through an transfer line. Its length must be adjusted so that the evaluating person assumes a comfortable position during the detection procedure, to avoid any discomfort resulting from the proximity of hot equipment. Moreover, the transfer line should be heated in the course of analysis, to prevent the condensation of the medium or hardly volatile analytes on capillary walls.

7.2.2 GC-O data measurement methods

By applying GC-O methodology, information on the olfactory impact of compounds in a sample can be obtained. Several quantitative techniques have been developed for collecting and processing GC-O data and to evaluate the intensity of odours and their relative influence on the odour of sample. These methods can be classified into three groups based on the method of the determination:

1. Detection frequency methods (nasal impact frequency (NIF) and surface nasal impact frequency (SNIF)).
2. Dilution to threshold methods (aroma extract dilution analysis (AEDA), combined hedonic of aromatic response measurement (CHARM)).
3. Direct-time intensity methods (odour-specific magnitude estimation (OSME), finger span cross-matching method (FSCM) and posterior intensity).

The choice of the GC-O method is of extreme importance for the correct characterization of a matrix, since the application of different methods to the same real sample can distinctively select and rank the odour-active compounds according to their potency and/or intensity.

Dilution to threshold methods are the most frequently used in the analysis of the odours of alcoholic beverages. In dilution analysis, an extract is diluted, usually as a series of dilution of odour compounds (1:2, 1:3, 1:5 or 1:10), and each dilution is sniffed until no significant odour is detected. Several injections are required to reach dilution of the aroma extract in which odorous regions are no longer detected. The odour potential can be described as so called aroma values or odour values, as well as odour units or flavour units. The most frequently counted are so-called odour activity values (OAVs), which represent the ratio of the concentration of a given substance in the sample to the sensory detection threshold. Both AEDA and CHARM are based on odour-detection threshold principle. In AEDA, the dilution factor (FD value) is simply the last dilution at which an odour-active compound is detected. The results are usually presented as the logarithm of the factor of dilution (log FD) versus the retention time or by listing the FD values. The difference between AEDA and CHARM is that in CHARM analysis the duration of perception is taken into account

together with the final dilution (dilution value) in which compound is detected. This dilution value is analogous to the FD value in AEDA. In fact, the dilution value at the peak maximum in a CHARM chromatogram is identical to the FD factor calculated when the data are plotted on an AEDA basis.

The main difference between both dilution methods is that CHARM analysis measures the dilution value over the entire time the compounds elute, whereas AEDA determines the FD, which is simply the last dilution at which an odour-active compound is detected. Generally, CHARM is recommended for the determination of the significance of the individual odour compounds in a given sample, at the cost of lower precision.

One of the limitations of the dilution to threshold methods is the yet questionable necessity of only two sniffing assessors. This factor can be controlled by realizing aroma recombination experiments with the obtained GC-O data. Another disadvantage is that analyses using these approaches are time-consuming due to the numerous dilution steps, and individual responses vary between assessors. Therefore, a panel of at least eight judges is strongly recommended for GC-O.

Time-intensity methods and frequency of detection methods use a panel of 8–12 assessors, overcoming the limitations of small number of assessors and the use of detection thresholds. The methods measure the odorant compounds' intensity (time-intensity) or detect a given odour in one single sample (detection frequency) in non-diluted extracts, without any dilution step. They are thus not based on individual detection thresholds and detect all odorant compounds present in the given sample.

Direct intensity methods use different kinds of quantitative scales to measure the intensity of the odour of the eluting compound. Depending on the method, the measurement can be performed in different ways. These include a single, time-averaged measurement, a measurement registered after the elution of the analyte (posterior intensity evaluation methods), or, most frequently, a dynamic measurement, where the appearance of an odour, its maximum intensity and decline are registered in a continuous manner (OSME, fingerspan method). The posterior intensity method is quite similar to the OSME method, except that the perceived odour intensity of each odorant compound is rated in a memorized five-point intensity interval scale after a peak has eluted from the olfactory port. Compared with methods utilizing dilution techniques, the posterior intensity method, since it uses more sniffers, gives more representative results.

A critical comparison between GC-O methodologies may be found in different reports (d'Acampora Zellner *et al.*, 2008; Delahunty *et al.*, 2006; Plutowska and Wardencki, 2008; van Ruth, 2001). Despite the fact that GC-O is quite a mature technique, further investigations are still conducted in order to improve its capability, i.e. to achieve a higher sensitivity and better repeatability of the results.

7.3 Electronic nose (e-nose) and electronic tongue (e-tongue) systems

Numerous drawbacks of sensory analysis help explain the development of alternative methods for the evaluation of the sensory quality of food, mainly electronic nose (e-nose) and tongue (e-tongue) instruments, also termed as humanlike artificial sensors (Jamilah *et al.*, 2012). Because e-nose technology is today better developed, attention in this chapter is mostly focused on e-nose instruments. A short history of the development of such devices, their construction and operating principles will be presented, as will be a concise review of the sensors and data analysis methods employed in these systems. Finally the major applications in food analysis, including possibility of using them in food industry will be considered.

7.3.1 Principle of operation of e-nose

The e-nose has many synonyms: artificial nose, mechanical nose, odour sensor, flavour sensor, aroma sensor (Mielle, 1996), odour-sensing system (Gardner and Bartlett, 1994), multi-sensor array technology (Shiers, 1995), and electronic olfactometry (Martí *et al.*, 2005). It is an instrument which mimics the sense of smell (Fig. 7.2). The device is designed to detect and discriminate among complex odours using an array of chemical sensors. The sensor array, under the influence of an odour stimulus, generates a characteristic fingerprint or smellprint. Using a database constructed on the basis of patterns or fingerprints from human odours and trained pattern recognition systems it is possible to classify and identify unknown odours. In recent years, the classic sensor types used for e-noses have been enhanced and complemented by other technologies introduced in this field. In the last decade, besides chemical sensors, e-nose systems based on mass spectrometry or fast gas chromatography have also been introduced (Wilson and Baietto, 2009).

The term 'electronic nose' was introduced by J. Gardner in 1988 as the informal name of a device consisting of a set of chemical sensors connected to a pattern recognition system, thereby distinguishing and discriminating simple and complex aromas (Gardner and Bartlett, 1994; Stetter and Penrose, 2001; Rajamäki *et al.*, 2006). However, the history of this technology is much older and goes back 40 years before the term had been introduced. The first e-nose instruments were very primitive (Pearce, 1997; Schaller *et al.*, 1998). The first report on the subject of an intelligent model of an artificial nose was published by Persaud and Dodd in 1982. The great interest in developing sensing systems led to significant progress and a breakthrough in the field of sensor design, and was confirmed by numerous patents on sensor arrays suitable for the control of safety of food and beverages, microbiological measurements and medical applications (Keller *et al.*, 1995; Schaller *et al.*, 1998). Along with the technological progress and new

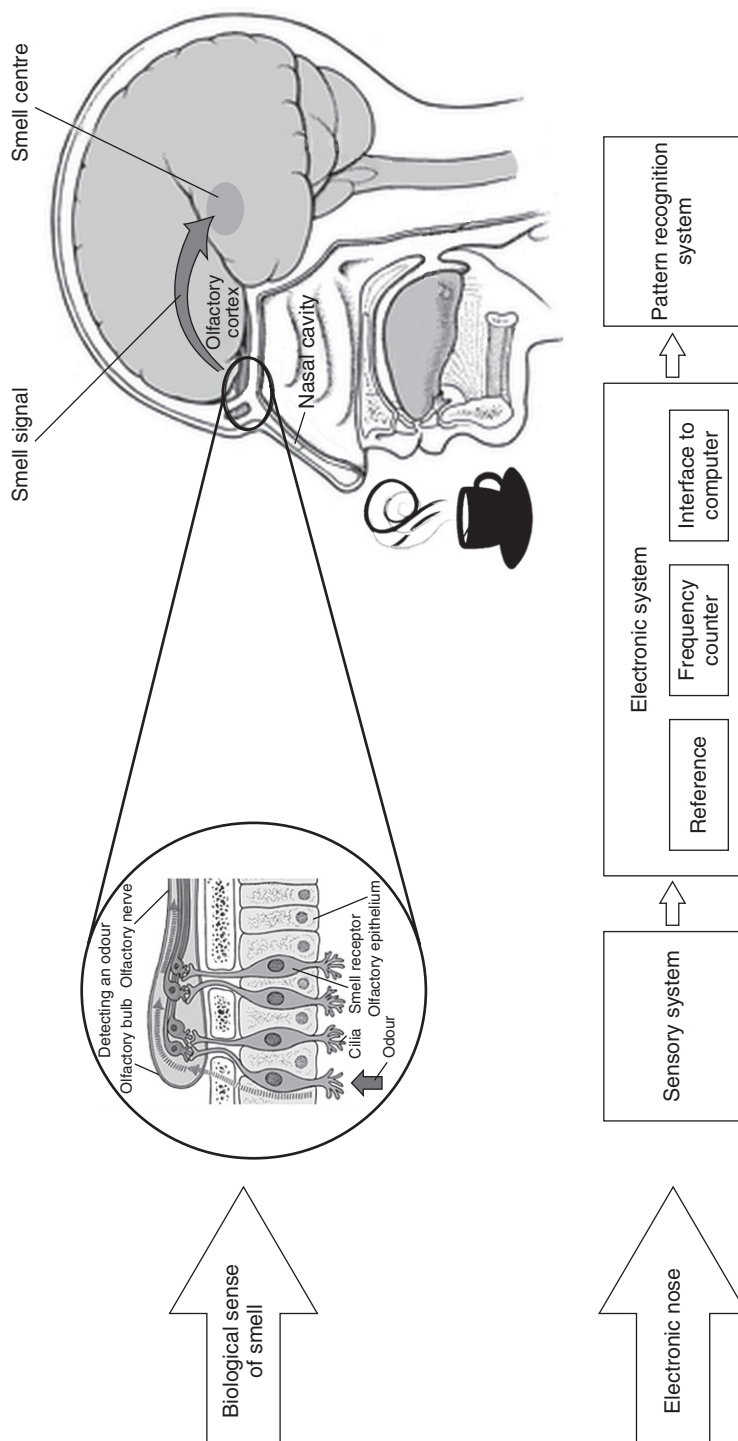


Fig. 7.2 Typical block diagram of human olfaction and e-nose.

possibilities for applications of the e-nose instrument, the first commercially available e-nose instrument appeared in the early 1990s (AlphaMOS 1993, Neutronics and Aromascan 1994, Bloodhound and HKR Sensorsysteme 1995). Afterwards, a new type of artificial olfactory system – an MS-based e-nose instrument – was developed at the end of the 1990s (Dittmann *et al.*, 1998; Nitz *et al.*, 1999; Martí *et al.*, 2005). The rapid development of these systems has focused on reduction of time of volatile fraction analysis, increasing sensitivity and the simplification of instrumentation.

Previously, classic sensor-based e-nose instruments used static headspace (SHS) as isolation technique of aroma compounds, which is certainly not sensitive enough to deal with complex food matrices (Dirinck *et al.*, 2009). In addition, gas sensor array systems have problems with drift, stability (as a result of humidity or influence of CO₂), frequent calibration, sensor poisoning, profile masking by some major components of the sample (e.g. ethanol), low sensor-to-sensor and instrument-to-instrument reproducibility and high power consumption (e.g. metal oxide semiconductor (MOS) sensors operates at high temperature). Current work concerning MOS sensors has focused on micromachining to reduce power consumption, optimization modelling and sample pre-treatment to avoid poisoning (Lee *et al.*, 2003; James *et al.*, 2005). In the case of metal oxide semiconductor field-effect transistor (MOSFET) sensors, recent development of these devices is directed to the appliance of new construction materials allowing to operate in higher temperatures (possibility of detection of high boiling compounds and reduction of recovery time). The other group of sensors introduced by Curie brothers is that of piezoelectric sensors (James *et al.*, 2005), especially, quartz crystal microbalance (QCM) and surface acoustic wave (SAW) sensors. Actual work is concentrated on extending the variety of coatings for these sensors for different applications, miniaturization of sensor array and searching for more reproducible QCM and SAW sensors. E-nose instruments based on MS can solve typical problems found with conventional e-noses, such as sensor poisoning, profile masking, the strong influence of moisture and the non-linearity of signals. Examples of commercially available e-nose instruments, models and technologies may be found in recent papers (Dymerski *et al.*, 2011; Noh, 2011).

7.3.2 Types of sensors and other detectors for the e-nose

The classic e-nose consists of an array of gas sensors as the detection system. Recently, other detection systems, such as MS and ion mobility spectrometry (IMS) enter the field (Peris and Escuder-Gilabert, 2009).

The sensors on an electronic nose should be selectively sensitive to odours which may be present in a given kind of tested sample (Di Natale *et al.*, 1997) (for a specific application, e.g. samples of air, food, explosives). Depending on the measured physical intermediate quantity they can be

divided into four categories: conductive, piezoelectric, electrochemical and optical (smell-sensing) sensors. The fifth category of sensors is based on GC and MS. The first two groups of sensors substantially differ from the others, so that they may be classified in a further another way, as 'hot' and 'cold' sensors (Schaller *et al.*, 1998). The first can operate at higher temperatures, but they cannot be excessively utilized. Their advantage is reduced susceptibility to humidity (Shiers, 1995).

Conductive sensors

The operating principle of these sensors relies on changes in some properties of the material from which they are made, as a result of an action of a gas or odour (e.g. volatile organic compounds, VOCs), which leads to a change in their resistance. Generally, three types of conductive sensors are distinguished: polymer (conducting polymer, CP), made from metal oxides (MOS) and field-effect transistors with a metal oxide semiconductor (MOSFET). The mechanism causing the resistance change is different in each type of sensor, but the construction and placement of individual elements in conductive sensors are basically the same (Arshak *et al.*, 2004).

CP sensors

The conducting layer in this type of sensor was heretofore mostly made from organic conducting polymers such as polypyrrol, polythiophen, polyalanine and polyacetylene and their derivatives (Korel and Balaban, 2009). Currently, electrical conductors diffused in an organic insulator are used. Conductance decreases with a reduction of conducting paths through which charges are transported, but the resistance of this layer rises (Panigrahi *et al.*, 2006). The detecting layers (of sensors) may also be created from composites of a conducting polymer in which both the conducting material and the insulating one consist of organic polymers (Korel and Balaban, 2009). In this case, the conducting layer is most often an inorganic conductor such as carbon black and the insulating phase is formed by an organic material capable of expansion, usually consisting of 80% (w/w) insulating polymer and 20% (w/w) of carbon black (Arshak *et al.*, 2004; Kim, 2010).

The sensitivity of a single sensor to a given odour is described by the measurement of the so-called gas-polymer partition coefficient. Sensors made from conductive polymers can be used in various conditions. Usually they are employed at low temperatures, such as room temperature, thus they do not need heating and are simple in construction. In addition, they are stable and their regeneration and response times are short (especially for polar compounds) and inversely proportional to the thickness of the polymer (Matthews *et al.*, 2002; Bai and Shi, 2007). They are also handy, portable devices (Wilson and Baietto, 2009). Their only disadvantage is high susceptibility to humidity, which may mask their response to VOCs (Pearce *et al.*, 2003; James *et al.*, 2005).

MOS sensors

MOS sensors have many advantages, and are therefore the most popular sensors using e-noses on the market. They are relatively inexpensive, stable in time (it is possible to carry out many analyses without significant changes of basic parameters, which ensures high repeatability of results), have high sensitivity (from 5 to 500 ppm) and chemical resistance, and are easy to operate (James *et al.*, 2005; Emelin and Nikolaev, 2006a, 2006b). They are usually produced from a thin layer of tin, zinc, tungsten and iridium oxides doped with noble metals such as platinum or palladium, which change the response characteristics of the semiconductors. MOS sensors operate at high temperatures, between 300 and 500 °C, which averts the effect of humidity on the results of the analysis and shortens the response and regeneration times (Gardner and Bartlett, 1999; Korel and Balaban, 2009) and is connected with the provision of an additional element – a heater – to the e-nose. On the other hand, the use of high temperatures is the source of many disadvantages, such as high consumption of electrical energy, which excludes the use of e-noses with sensors of this type in portable devices. For these reasons, it is more advantageous to use sensors with a thin layer of metal oxides, as they are smaller, simpler to construct and, in addition, consume less energy (Homer *et al.*, 2004; James *et al.*, 2005; Wilson and Baietto, 2009).

Catalytic reactions of molecules on the surface cause the transfer of charges, which leads to a change in the electrical resistance of the sensors. Strictly speaking, when an odour compound molecule finds itself in the vicinity of the sensor, the resistance at the contact boundary between two metals where the oxide material is, changes in proportion to the concentration of the odour substance. Unfortunately, MOS sensors exhibit low sensitivity to fragrant sulphur compounds (sulphur-based odours); furthermore, they are susceptible to poisoning by these compounds because they form durable, irreversible combinations with metal oxides (Schaller *et al.*, 1998; Kośmider *et al.*, 2002).

MOSFET sensors

MOSFET sensors are similar to MOS sensors, but they operate at far lower temperatures (100–200 °C). Their response mechanism is proportional to the concentration of odour compounds in the mixture. They are produced by the standard micro-production technique which binds a sensitive catalytic metal in the form of atomized gas with a silicon oxide layer (the gate). The operation of this type of sensor is as follows: in the result of odour compound contact with metal, a reaction follows which causes a change in the physical or chemical property of a sensor element and, consequently, a change in the electrical signal. At the instant that a mixture of odour compounds reacts with the catalytic (active) surface, a change in potential occurs, which initiates the output signal. In order to initiate such changes, the gas sample should have easy access to the area of contact of the metal with the insulator.

The sensitivity and selectivity of sensors of this type ought to be adapted to concrete requirements (applications) through an appropriate choice of the type and thickness of the catalytic metal, similar to the choice of the temperature of the determination. For example, sensors with thick metals are effectively used for the detection of hydrogen sulphide, as opposed to sensors with more porous thin layers, which are designed to determine amines, alcohols and aldehydes (Kalman *et al.*, 2000). The production techniques of MOSFETs permit the obtainment of small and inexpensive sensors (Pearce *et al.*, 2003), allowing a relatively high repeatability of results of the analyses in which they are employed. In spite of the high sensitivity and selectivity of these sensors, their operating conditions (the environment) have to be under constant control, which excludes their application in portable equipment. The constant lack of proper types of MOSFET sensors precludes their use in commercial e-nose systems.

Piezoelectric sensors

The piezoelectric sensors include two basic types: QCM or bulk acoustic wave (BAW) devices and SAW devices. They are used in an e-nose as devices detecting changes in mass, although they can measure temperature, mass changes, pressure, force and acceleration (D'Amico and Verona, 1992; Nagle *et al.*, 1998).

The main difference between SAW devices and QCM equipment is that the wave created by the former propagates along the surface of the sensor, while in the latter it propagates through the whole volume of the sensor (Arshak *et al.*, 2004). In turn, the operating principle of both types of devices is similar and is based on a change in the mass of the piezoelectric sensor occurring during its exposure to odorous compounds (adsorption/absorption of the compound on/in the layer), which causes a change in the resonant frequency of the sensor (Kumar *et al.*, 2010).

BAW sensors

Quartz microbalances (BAW sensors or QCMs) are the simplest type of piezoelectric sensors. They are built from a single quartz crystal (several millimetres in diameter) and two disks covered with sputtered gold, acting as electrodes and connected with wires (Korel and Balaban, 2009). The mass of the gas molecules being absorbed on the surface of the sensor is measured by the change of resonant frequency and the signal's frequency varies between 5 and 30 MHz. A three-dimensional wave is created which travels through the whole volume of the crystal.

The membrane which covers the crystal absorbs molecules of the odorous compounds which come into contact with the vapour (gas). At that instant, the mass of the polymer disk (on the surface of the crystal) increases, the resonant frequency decreases – it is an inversely proportional relation. Therefore the frequency changes allow for the identification of odours. After performing a measurement, an appropriate reference gas should be

passed through the e-nose so that the resonant frequency of the QCM sensor returns to its initial state (Albert *et al.*, 2000).

QCM devices are very sensitive and can operate effectively in analysing compounds at the ppb level. Moreover, QCM sensors exhibit linear responses in a broad range. They are characterized by high sensitivity to vapours of organic compounds, for example for pairs such as ethanol and n-heptane in the range of 7.5–48.2 Hz/mg/l. They show no susceptibility to humidity and temperature changes, but at the same time they perfectly absorb analytes from gas samples. There is also the possibility of matching a QSM polymer sensor to a given application. It is worth mentioning that the commonly used packing of chromatographic columns can also be used as absorbing material in sensors of this type.

SAW sensors

A number of differences between SAW and BAW sensors exist, in spite of the fact that they belong to the same group of sensors – piezoelectric sensors. In a SAW sensor, an acoustic wave travels along its surface, not through the whole volume of the sensor. Also, the operating frequencies of SAW sensors are considerably higher (in the range 100 MHz to 1 GHz), which does not affect their sensitivity, even with the smallest mass changes. In turn, this favourably influences the signal-to-noise ratio, which increases slightly with an increase of the area to sound intensity ratio.

A SAW device is built from a piezoelectric substrate with an input (transmitting) and output (receiving) transducer placed on its surface. The membrane, which is selective to volatile compounds, is placed between these transducers, and the membrane is most often made from polymers, lipids, Langmuir–Blodgett films or self-assembled monolayers. At the instant when the alternating current is applied to the input transducer (the transmitter), a two-dimensional acoustic wave is emitted, which travels along the surface of the crystal (at the depth of one wavelength). The substrate must be made from a material with piezoelectric properties, therefore it is most often made from zinc oxide (ZnO), lithium niobate (LiNbO₄) and quartz. Furthermore, SAW devices operate like QCM devices. A membrane absorbs the odour compound molecules and as a result, its mass changes, as does the frequency of the wave.

The sensitivity of SAW sensors depends on the type of membrane used. The layers in sensors of this type can be made from various substances, earlier used as packing in GC columns (Nieuwenhuizen and Nederlof, 1992; Schaller *et al.*, 1998). The broad choice of such layers ensures that there is a broad spectrum of odours possible for identification. This, in turn, means that more than one SAW device is needed to analyse a mixture of odour compounds.

The quantity sensitivity is low, at the ppm level. The sensitivity varies from 0.5 to 12 Hz/mg/m³, depending on the employed polymer layer. The response time is relatively short (Korel and Balaban, 2009).

A disadvantage of both types of devices – SAW and QCM – is their complicated and expensive electronics, as well as their susceptibility to humidity and temperature changes. What is more, replacing a faulty SAW sensor is accompanied by many problems.

Electrochemical sensors

Electrochemical sensors are built from electrodes immersed in an electrolyte (Collier *et al.*, 2003). Analyte molecules react and are reduced or oxidized on the active (hot) electrode. Voltage changes or resistance are caused by charge mobility. Electrochemical sensors do not age and the relation between the concentration of a given odour compound and the obtained signal is linear. Moreover, they are characterized by moisture resistance (Korel and Balaban, 2009).

Smell-seeing (optical) sensors

Optical sensors, commonly known as optical waveguide sensors, are built from glass fibres covered with a thin, chemically active material. This material contains a fluorescent dye in the polymer matrix.

The sensitivity of such sensors depends upon the type of dye used or the mix of dyestuff, and on the type of polymer matrix (Arshak *et al.*, 2004). The availability of a broad range of dyes which may find application in sensors, leads to low cost and a simple production procedure (Aernecke and Walt, 2009). This is conducive also to the high selectivity of these sensors. Moreover, it is possible to use dyes to which one specific volatile compound can be attributed. All this results in optical sensors with a very wide range of sensitivities, which are impossible to attain in other types of sensors used in e-noses.

An advantage of optical sensors is their short and, at the same time, linear response time (Kim, 2010; Ryan *et al.*, 2004). In contrast to many sensors employed in e-noses, they are characterized by resistance to the influence of toxic compounds, and therefore can be used to detect such compounds.

It is obvious that optical sensors have their defects. The lifetime of fluorescent dyes is limited due to the effect of light (the photo-bleaching process) (Nagle *et al.*, 1998; Arshak *et al.*, 2004). Therefore periodic calibration of sensors is necessary with respect to the repeatability of results. An additional disadvantage is the high complexity of the supporting electronics connected to the sensors, which leads to a considerably higher cost of the e-nose.

GC and MS-based e-noses

Sensors based on the operating principles of GC and MS constitute a new generation of sensors for an electronic nose. In these systems chromatographic columns distribute volatile constituents which are subsequently identified by a SAW or FID.

The principle of operation of the GC/SAW system consists of measurement of the concentration of volatile constituents. This concentration is proportional to the frequency of the wave travelling in the SAW sensor (Staples and Viswanathan, 2008; Mah and Thurbide, 2006). The result of the analysis is in the form of a profile of volatile constituents.

The SAW sensor is very sensitive and stable; it is well fitted to determine compounds found in potable water through headspace analysis of the water sample (Staples, 1999; Staples and Viswanathan, 2008). The main advantage of an electronic GC/SAW nose is the very short time needed for analysis (about 10s), high precision and accuracy (relative standard deviation within 1–2% limits) (Staples, 1999; Oh *et al.*, 2008). Moreover, it can operate over a wide range of concentrations, which allows for the analysis of solid, liquid and gaseous samples. It is also easy to operate and to calibrate. An additional benefit of this technique is the possibility of carrying out not only a qualitative analysis, but also a quantitative one (Oh *et al.*, 2008).

MS-based e-noses

Devices based on MS constitute an alternative to conventional e-noses employing sensors (Ampuero *et al.*, 2002; Pavón *et al.*, 2006; Peris and Escuder-Gilabert, 2009). They are often called mass sensors, or, sometimes, new-generation e-noses. This relatively new instrumentation concept involves the introduction of the volatile compounds into ionization chamber of a MS instrument without prior chromatographic separation (Llobet *et al.*, 2007; Dirinck *et al.*, 2009). The advantage is that the analyser of mass does not exhibit problems typical with a sensor array, such as poisoning by some compounds and profile masking by some major constituents of the sample (ethanol for example), susceptibility to humidity and nonlinearity of the response signal in some operating ranges.

When using devices of this type, the headspace analysis of a sample is performed in two ways: static (SHS-MS) or dynamic (DHS-MS) (Yamazoe *et al.*, 2003; Yu *et al.*, 2005; Capone *et al.*, 2000). The first stage is proportioning of the gas sample, which subsequently is ionized in the mass spectrometer chamber. Afterwards ions are separated according to the different ratio of mass-to-charge (m/z). In such a way a mass spectrum is obtained, which is characteristic for given kinds of samples. It constitutes a profile of the sample constituents, often called the ‘signature’ or the ‘fingerprint’.

Most e-noses available on the market are used for qualitative analysis of everyday products or substances which could pollute the environment. It has been observed, however, that these devices can also be used for quantitative analysis. The advantage of this technique in comparison with GC-MS is the very much shorter response time. On the other hand, it provides much less information than GC-MS (Fenaille *et al.*, 2003; Pavón *et al.*, 2006; Dirinck *et al.*, 2009).

The e-nose based on MS has an unquestionable advantage over commonly used e-noses equipped with a sensor array, especially when

adaptability and sensitivity are taken into account (Martí *et al.*, 2005; Llobet *et al.*, 2007; Biasioli *et al.*, 2011). In a classic e-nose, the number of sensors has to be specified according to the requirements of a concrete application. E-noses based on MS have a wider application than GC-MS (Cynkar *et al.*, 2007, 2010). These systems can be used not only to determine the differences between samples, but also for their diagnosis. They are, however, relatively expensive, as their price exceeds that of classic e-noses based on sensors. Another inconvenience is the inability to use them for online measurements and as portable equipment.

7.3.3 Comparison of GC-O and artificial noses

The GC-O method allows a simultaneous qualitative and quantitative evaluation of the odour of each analyte separately (after separation in a chromatographic column) (Plutowska and Wardencki, 2008). In other words, it provides the possibility of determining whether the given compound appears in the sample above the threshold of sensory detection, what its odour is, and of determining the time of sensory activity and the intensity of the odour (van Ruth, 2001). In spite of these advantages this instrumentation often does not provide reliable results, mainly due to the complexity of different food aroma – food samples cover a wide range of physical matrix types and range of potential chemical constituents is equally diverse – and the subjectivity of human response to odours. Hence, there is still a need for an instrument such as an e-nose that combines high sensitivity and correlation with data from human sensory panels in food control. Other advantages of e-nose instruments such as mobility, short analysis times, low price and ease of use, have caused them to spread in increasing numbers to industrial enterprises for control and improvement of food quality far away from well-equipped chemical laboratories and trained specialists. A comparison of the GC-O system with an e-nose is presented in Table 7.1.

7.3.4 Principle of operation of e-tongue

The e-tongue, also known as an artificial tongue and taste sensor, is an analytical instrument comprising an array of non-specific, low-selective chemical sensors with partial specificity (cross-sensitivity) to different components in solution (Deisingh *et al.*, 2004; Leake, 2006; Scampicchio *et al.*, 2008; Chen *et al.*, 2009; Deisingh, 2010). An e-tongue is mainly based on potentiometric, voltammetric, ion-selective field-effect transistor (ISFET), piezoelectric and optical sensors with pattern recognition tools for data processing. Ciosek and Wróblewski (2007) have reviewed recent developments of a multisensor array-based e-tongue for food analysis. An e-tongue can be used for recognition (identification, classification, discrimination), quantitative multicomponent analysis and artificial assessment of taste and

Table 7.1 Electronic nose vs gas chromatography olfactometry

Electronic nose	GC-O
Advantages	
• Short time of analysis • Mixtures do not have to be separated • Short recovery time • Portable version available • High sensitivity and reproducibility • Objective identification of odours • Simple or complex odours recognized	• The relevance of single compounds for the aroma assessed • Selection of odour active compounds from complex mixtures
Disadvantages	
• Sensors suffer from ageing • Poisoning of sensors possible • Moisture-sensitive sensors • Less sensitive than human olfactory system • Sensors are partially specific	• Time consuming • Lack of information about behaviour of compounds in the mixture • The analytes must be isolated, enriched and separated before analysis • Difficulties with detection of the end of the odour region • Possibility to connect with other detectors for qualitative and quantitative analysis reasons • <i>In situ</i> analysis is impossible • Decrease in alertness influences results • The reproducibility is lower than in the case of an e-nose • Training of human personnel is necessary • The number of measurements is limited – comparison of results from different laboratories extremely difficult • Data could be affected by different chromatographic behaviour of compounds

Source: van Ruth (2001); Korel and Balaban (2009); Dymerski *et al.* (2011).

flavour of various liquids. Liquid samples are directly analysed without any preparation, whereas solids require a preliminary dissolution before measurement (Winquist *et al.*, 1999; Leake, 2006; Apetrei *et al.*, 2010).

The mechanism involved for taste recognition in human and electronic noses is similar (Fig. 7.3). Chemical compounds responsible for taste are perceived by human taste receptors (taste buds), and the sensors of electronic instruments (lipid membrane) detect the same dissolved organic and inorganic compounds. Like human receptors, each sensor has a spectrum of reactions different from the others. The information given by each sensor is complementary and the combination of all sensor results generates a

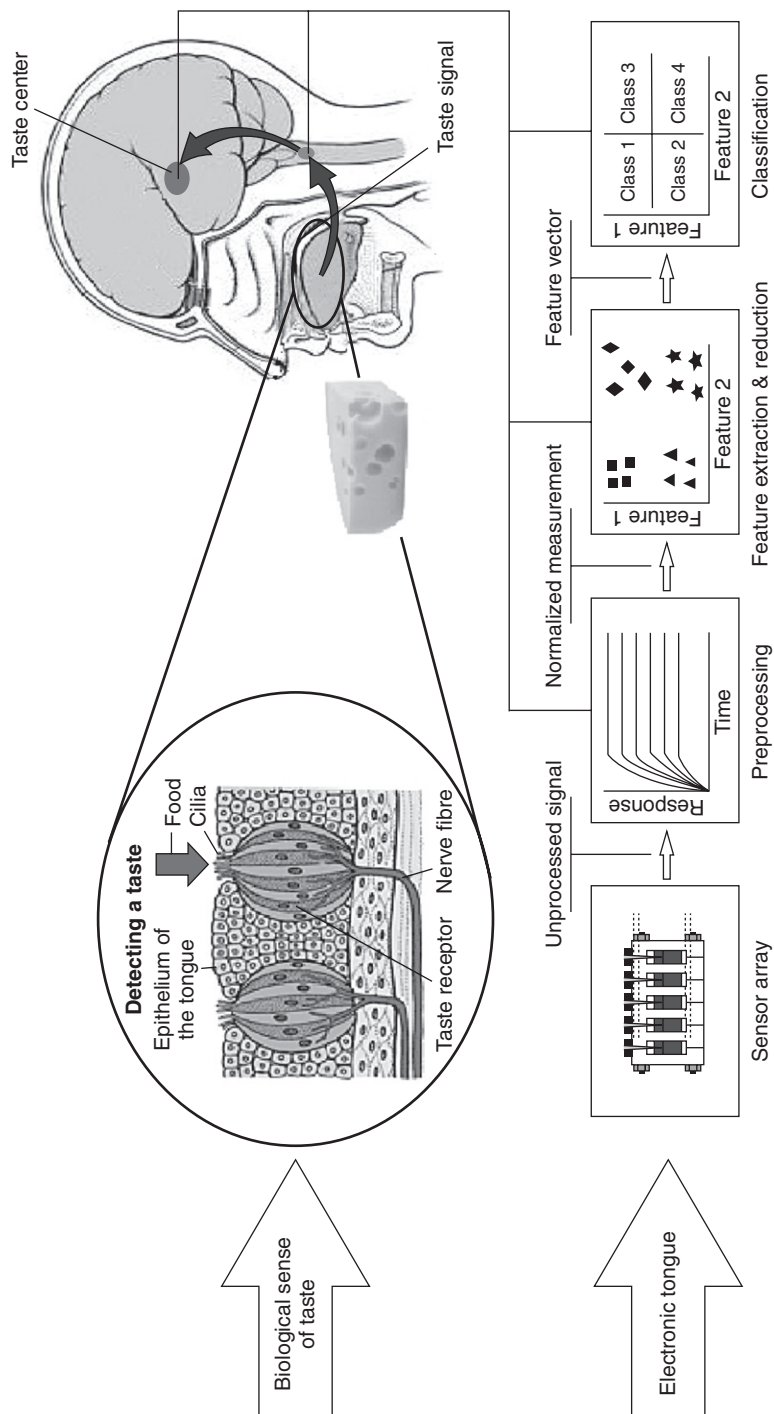


Fig. 7.3 Electronic tongue concept.

unique fingerprint characteristic for the liquid matrix. Most of the detection thresholds of sensors are similar or better than those of human receptors. In the biological mechanism, taste signals are transduced by nerves in the brain into electric signals whereas tongue sensors generate electric signals as potentiometric variations. Taste quality perception and recognition is based on building or recognition of activated sensory nerve patterns by the brain and on the taste fingerprint of the product. This step is achieved by the e-tongue's statistical software which interprets the sensor data into taste patterns.

The typical e-tongue system consists of an array of sensors (as in the e-nose) that are not combined into the single body. This permits the composition of the array to be rapidly modified, making it flexible and precisely adjustable to various tasks. Of primary importance is the stability of sensor behaviour and enhanced cross-sensitivity, which is understood as a reproducible response of a sensor to as many species in solution as possible. If properly configured and trained (calibrated), the e-tongue is capable of determining the quantitative composition (the content on multiple components) and of recognizing complex liquids of different natures. A unique feature is the possibility of maintaining a correlation between the output of the e-tongue and human perception. After an appropriate calibration the e-tongue can produce results in the same way as a human sensory panel: as marks or assessments of various simple and complex features of taste and flavour of different products. The e-tongue can easily 'taste' raw substances, semi-products and also new entities that are not yet allowed for human consumption.

7.3.5 General characteristic of e-tongue instrumentation

E-tongue systems are available in different versions, i.e. as desktop and mobile, static (dipped in a liquid) and flow (with the liquid pumped through a sensor cell). The typical e-tongue system consists of the following parts:

- the sensor array comprising non-specific sensors;
- multi channel electronic interface device, a specially designed high-impedance hardware, which reads the sensor output, converts them to digital form and sends it to a computer;
- a personal computer for data acquisition, storage and processing.

Electrochemical sensors, especially potentiometric ones (Gallardo *et al.*, 2005; Mimendia *et al.*, 2010), are the most frequently applied sensors for solution analysis in e-tongue systems. These sensors are the widest, best known, and profoundly developed and studied class of chemical sensors. Many of earlier available sensors (conventionally called ion-selective electrodes) for solution analysis were insufficiently selective. For this reason their application for analytical purposes has been significantly restricted. On the other hand it is possible to develop a very wide range of materials,

which can display reproducible sensitivity to multiple substances in liquids. The idea of the e-tongue approach is to develop and employ the sensors with broad sensitivity – cross-sensitivity to as many species in solutions as possible. Now it is possible to prepare and apply unique sensors displaying a response to inorganic and organic substances, to ionic and non-ionic species, to the groups of chemically similar substances, etc. These sensors are stable and robust; their response is highly reproducible.

Sensor array and reference electrode are dipped in a beaker containing a test solution for certain time (e.g. 120 seconds). A potentiometric difference between each sensor and a reference electrode is measured and recorded by the e-tongue software. These data represent the input for mathematical treatment that will deliver results.

The sensor for e-tongues may be used for a single component medium, both to inorganic and organic substances. The sensor responses of carefully developed and selected materials can be very reproducible (typically $\pm 1\text{--}3\text{ mV}$, while the scale of measured effects is often 20, 50 even 100 mV and more) and really long lasting (the typical lifetime for a solid sensor is several years: but up to 1 year for an organic polymer one). Such sensors are put together in sensor sets combining up to 10–40 different sensors.

In a multi-component medium (e.g. food stuffs and beverages) the response of such sensors in an array is usually no longer linear, but it is still reproducible and the differences between samples are usually very significant. The sensor array of the electronic tongue mainly consists of two types of sensors – chalcogenide glass (Schoning *et al.*, 2000) and polymer-based (Legin *et al.*, 2002). The system architecture is intrinsically open, thus other sensors and sensor devices can be incorporated and other methods can even be put together with the e-tongue to produce wider complementary information about an analyte.

7.4 Methods of data analysis and pattern recognition

The response of multisensory e-nose and e-tongue systems is very complicated. Thereby, proper utilization of such seemingly chaotic information is not as easy as reading the indications of a classic single-sensor analyser. Thus, it is necessary to employ special data analysis techniques that provide a way of presenting the data in an understandable format. Adequate data processing is the essential part both of e-nose and e-tongue approaches but even advanced mathematical methods cannot improve the results. The data produced by the sensor array must be reproducible and reliable. This is ensured by the responsible design of the sensor array and thoroughly elaborated measuring procedure.

The analysis of the signal obtained through an artificial nose and tongue includes signal processing and pattern recognition – comparison with a standard. All successive stages can be divided into four processes:

preliminary analysis, choice of features (variables), classification and decision making (Nagle *et al.*, 1998; Pearce *et al.*, 2003).

Preliminary analysis includes smoothing the signals from the sensors, averaging of instantaneous responses from the sensor array and limiting the effects of a previous measurement upon the successive one (Ortega *et al.*, 2000). Information on the measured value provided by the sensors is an unprocessed response, full of disturbances which are removed during the feature extraction process. This leads simultaneously to a reduction of dimensions of the measurement space. The techniques of statistical analysis controlling the feature extraction stage can be divided in two groups: quantitative methods and pattern analysis methods (Nagle *et al.*, 1998; Röck *et al.*, 2008).

Quantitative methods are being verified and on this basis a database of known samples is being constructed. In case of pattern analysis techniques, their verification is possible as in the case of cluster analysis (CA) and in the principal component analysis (PCA), or its lack, e.g. discriminant function analysis (DFA) or canonical correlation analysis (CCA) (Ortega *et al.*, 2000; Pearce *et al.*, 2003; Fu *et al.*, 2007; Scott *et al.*, 2007; Röck *et al.*, 2008). A better-known classification of commercially available signal analysis techniques from the sensors can be presented in the form of three main groups:

- graphical analysis;
- multi-variable analysis;
- network analysis.

The simplest method of data analysis is a graphical representation of a raw data histogram, i.e. a 'smell fingerprint' or polar diagram. Such histograms are used mostly for detection of samples which differ considerably from the remaining ones (Pearce *et al.*, 2003; Scott *et al.*, 2007; Chmiel *et al.*, 2008).

A somewhat more complicated method of data interpretation on the distribution of action of a sensor array is the realization of statistical calculations. Here, various analysis techniques of many variables can be used, such as PCA, CA or multidimensional scaling (MDS) (Röck *et al.*, 2008; Scott *et al.*, 2007).

Multi-variable analysis is generally based on reduction of data to such a degree that they could be linked together by one or two relations ($z = f(x)$ or $z = f(x,y)$). Due to this, sheets with calculation results can be checked using a graphical presentation on two- or three-dimensional spot diagrams. The distances between spots representing the compared 'objects' are a measure of mutual similarity/dissimilarity. Odour maps provide the possibility of separation of spaces or areas containing mixtures which create similar olfactory sensation (Capone *et al.*, 2000).

In many cases, the use of more complex methods of data collection and analysis is justifiable. Systems which closely resemble the human odour

analyser are neurocomputers – electronic models of neuron networks, artificial neural networks (ANN) (Keller *et al.*, 1995; Benedetti *et al.*, 2004; Bermak *et al.*, 2006). A similar role is played by computer models simulating such networks.

Both approaches permit an analysis of data whose distribution is totally unpredictable. There is, however, lack of appropriate mathematical models which would allow algorithms to be formulated and computers to be programmed. The program of an ANN is contained in its structure (in connections between elementary network units, whose strength changes in the result of ‘learning’). Through training, the networks gain the ability to classify information sets on various objects even when the differences between them have not been discerned and indicated by humans. In the case of the e-nose, a specific set of signals coming from a sensor array can be linked with a similar set which occurred earlier during an exposure of the sensors to a standard (e.g. a specific sort of coffee or perfume, an aromatic trace of a delinquent).

The elementary units of the technical model of a neural network – ‘neurons’ – are very simple electronic devices (their common links are synapse equivalents) (Giordani *et al.*, 2008). Neurons in the network are situated in layers, forming a hierarchic structure. The layers are parallel to each other and they can appear in different numbers (generally a three-layer network is sufficient to process a signal with good efficiency). Each of the neurons has many ‘inputs’ and one ‘output’. The electrical signal arriving at each of the inputs is multiplied by a numerical value – the so-called ‘weight’ (Linder *et al.*, 2005; Scott *et al.*, 2007). The magnitude of the output signal depends upon the input signals, their weights and the ‘input–output’ function.

7.5 Applications

7.5.1 Application of GC-O in food flavour measurements

GC-O investigations on the odour of some food products usually have the goal of determining the relationship between the composition and the content of volatile compounds and the organoleptic properties (Plutowska and Wardencki, 2008). Thus, this technique has frequently been used to assess different alcoholic beverages such as beer, wines, agriculture distillates and spirit beverages. Another aim is to identify and compare the compounds entering the aroma of different alcoholic beverages. Yet another goal might be the determination of compounds responsible for undesired odours.

The GC-O results are often correlated with the results of conventional sensory evaluation conducted in parallel, usually using typical methods. Sensory analysis of the individual samples yields descriptors characterizing their smell. Chromatographic analysis with olfactometric detection makes

it possible to determine which compounds are responsible for the individual descriptors.

The typical applications of GC-O technique in the alcoholic beverages sector of the industry are as follows (Plutowska and Wardencki 2012):

1. Identification of the impact aroma compounds in alcoholic beverages.
2. Reconstitution studies.
3. Monitoring of the production process in spirits industry (e.g. investigation of the influence of raw materials on the development of aroma, monitoring of ageing process and storage conditions).
4. Evaluation of alcoholic beverage quality.
5. Investigation of the organoleptic properties of alcoholic beverages.

The general aspects of food aroma analysis using GC-O techniques may be found in several books (Blank, 1996; Deibler *et al.*, 1999; Ebeler, 1999; Grosch, 2007; Leland *et al.*, 2001) and in reviews published in journals (d'Acampora Zellner *et al.*, 2008; Delahunty *et al.*, 2006; Plutowska and Wardencki, 2008; van Ruth, 2001).

7.5.2 Most important applications of e-nose in food flavour measurements

The areas of application of an e-nose are those in which the odour plays an essential role or signifies the quality of analysed products. The majority of scientific publications devoted to the application of the e-nose concerns food (Di Natale *et al.*, 1997; Schaller *et al.*, 1998; Ampuero and Bosset, 2003; Zhang, 2003; Haugen *et al.*, 2006; Haugen and Kvaal, 1999; Korel and Balaban, 2009; Peris and Escuder-Gilabert, 2009). The food industry is the largest and most promising market for such systems. Application of the e-nose to the food industry includes quality assessment in food production, quality control of food through evaluation of its smell (Börjesson *et al.*, 1996), control of the cooking process, nondestructive qualification of ripeness, control of the fish-processing industry (Du *et al.*, 2002), monitoring of fermentation processes (Pinheiro *et al.*, 2002), checking the degree of rancidness of mayonnaise, verification of the origin of fruit/vegetable juices (Goodner *et al.*, 2001; Boilot *et al.*, 2003; Zhang, 2003; Zhang and Suslick, 2007; Reinhard *et al.*, 2008), classification of alcohol products (e.g. wine, vodka, beer) (Ragazzo *et al.*, 2001; Martí *et al.*, 2005; Cynkar *et al.*, 2007; Lozano *et al.*, 2008; Ragazzo-Sanchez *et al.*, 2008), detection and identification of chemical impurities coming from packages (Shiers, 1995; Rajamäki *et al.*, 2006). In some cases, e-noses can complement or replace totally the sensory analysis performed by a team of specialists.

A search of the recent, relevant literature shows that there are five major categories of use for electronic noses in food control. These are:

1. process monitoring (product consistency) (Collier *et al.*, 2003; Pani *et al.*, 2008);

2. shelf-life investigation (quality deterioration of products) (Benedetti *et al.*, 2005, 2008; Labreche *et al.*, 2005);
3. freshness evaluation (quality evaluation of raw materials) (Dutta *et al.*, 2003; Lebrun *et al.*, 2008);
4. authenticity assessment (quality evaluation of raw materials) (Cerrato Oliveros *et al.*, 2002; Penza and Cassano, 2004); and
5. other quality control studies (Cosio *et al.*, 2006, 2007; Cheli *et al.*, 2009).

Some of the most important contributions to each of these in the present century include the following:

- Process monitoring: in the brewing industry especially in fermentation stage, aroma production in grape must fermentation, volatile organic compounds in fruit ripening, belly cavity odours in fish spoilage.
- Shelf-life investigation: odour evolution in cheese, oxidation rate in extra virgin olive oils, ageing in milk.
- Freshness evaluation: analysis of fruits, bacteria and histamine in salmon, freshness in soy curd.
- Authenticity assessment: aroma composition in alcoholic drinks, aroma composition in vinegar.
- Quality control: fast and accurate analysis of beer, quality control of olive oils, defects in fruits, adulteration in olive oils.

Detailed information concerning the applications of e-noses in food analysis can be found in the literature (Dymerski *et al.*, 2011; Noh, 2011).

7.5.3 Major applications of e-tongue in food flavour measurements

The e-tongue, similar to the e-nose, has been widely exploited for food quality assessment, food authenticity estimation, food freshness evaluation, food shelf-life investigation and food process monitoring. For example the e-tongue appears to be capable of distinguishing between various liquid foods and beverages: natural and artificial mineral waters, fruit juices, soft drinks and edible oils. Some specific applications include analysis of different apple varieties (Rudnitskaya *et al.*, 2006), classification of different grades of black tea (Palit *et al.* 2010), analysis of tomato taste (Beullens *et al.*, 2008), identification of milk adulteration (Dias *et al.*, 2009), prediction of wine age (Rudnitskaya *et al.*, 2010) and screening of beer quality (Polshin *et al.*, 2010). The e-tongue has also been used to analyse flesh food, fruits and vegetables such as: fish, pork and beef, liver, etc., apples, oranges, onions and shallots, tomatoes and so on.

Typical examples of taste sensors are partly similar to e-nose applications and comprise:

- analysis of flavour ageing in beverages (for instance fruit juice, alcoholic or non-alcoholic drinks, flavoured milk);

- quantification of bitterness or 'spicy level' of drinks or dissolved compounds (e.g. bitterness measurement and prediction of teas);
- quantification of taste masking efficiency of formulations (tablets, syrups, powders, capsules, lozenges);
- analysis of medicines stability in terms of taste;
- assessment of benchmark target products.

Many more applications of electronic tongue for the analysis of foodstuffs are possible and foreseen (Jamilah *et al.*, 2012).

7.6 Conclusion

All three discussed instrumental techniques play very important roles in food industry for measuring flavour (aroma and taste) of different food products. The introduction of the combination of olfactometry and gas chromatography into food analysis was a breakthrough in aroma research, enabling the differentiation of a multiple of volatiles in odour-active compounds and non-odour compounds, related to their existing concentrations in the matrix under investigation. In spite of the fact that GC-O is known and has been used for many years there is still need for improvement of familiar techniques and the investigations on using them for quantitative analysis of odour compounds. Further research is also needed because studies on the optimization of working parameters and on the quality and reliability of the obtained results, which are very important considering feasibility of implementing GC-O technique to industrial practice, have not been taken on board very well.

E-noses and e-tongues are high-class devices which are being increasingly used by industrial enterprises for control and improvement of quality of food products. This is, above all, ascribed to their many advantages and the ever-increasing range of sensors available. It should be kept in mind, however, that the expenditure of work, time and financial outlay necessary to build, train and adapt (program) such equipment to the appropriate applications are initially still very high. Nevertheless, costs borne at the production stage are returned quickly during the practical exploitation of such a system. This is due to the superiority of e-noses over the traditional sensory analysis and chromatographic techniques, resulting from: lack of sample preparation, nondestructive influence on the analysed sample, very short time of analysis and objective and repeatable results of analyses. Further utilization of the e-nose is related to the relatively low operational costs, and therefore the financial expenditure incurred is returned after a relatively short time.

E-tongues as relatively young instruments have proved to be a valuable tool for assessment and prediction of the taste. The system could potentially assist, or even replace, a sensory panel in a certain type of routine analysis in different food sectors. E-tongue measurements can be performed as

often as analytically needed without the regulatory hurdles or expense of human testing. E-tongue systems seem to be very useful for process monitoring and as a quality control tool in the food industry and in research laboratories.

Simultaneous utilization of e-nose and e-tongue sensors increases the amount of information extracted from a specific sample (Di Natale *et al.*, 2000; Prieto *et al.*, 2011).

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7.8 References and further reading

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7.9 Appendix: glossary of terms

AEDA – aroma extract dilution analysis
 ANN – artificial neural networks
 BAW – bulk acoustic wave
 CHARM – hedonic and aromatic response measurement
 CA – cluster analysis
 CCA – canonical correlation analysis
 CP – conducting polymer
 DFA – discriminant function analysis
 DHS – dynamic head space
 FSCM – finger span cross-matching method
 FD – factor of dilution
 FID – flame ionization detector
 GC – gas chromatography
 GC-O – gas chromatography-olfactometry
 GC × GC – two-dimensional gas chromatography
 IMS – ion mobility spectrometry
 LDA – linear discriminant analysis
 MDA – multiple discriminant analysis
 MDS – multidimensional scaling
 MOS – metal oxide semiconductor
 MOSFET – metal oxide semiconductor field effect transistor
 MS – mass spectrometry
 MVA – multivariate analysis
 NIF – nasal impact frequency
 OAVs – odour activity values

OSME – odour-specific magnitude estimation

PCA – principal component analysis

PTFE – poly(tetrafluoroethylene)

QCM – quartz crystal microbalance

SAW – surface acoustic wave

SCA – spectral clustering analysis

SHS – static headspace

SNIF – surface nasal impact frequency

SPME – solid-phase microextraction

SPR – supervised pattern recognition

TOF-MS – time-of-flight mass spectrometry

VOCs – volatile organic compounds

Non-destructive methods for food texture assessment

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Abstract: Over the past 15 years, considerable research activities have been reported on non-destructive texture evaluation of food. This chapter reviews non-destructive sensing techniques, with the emphasis on the latest advances in mechanical and optical techniques, for measuring the texture of solid food. The principles and applications of various non-destructive techniques are described and their merits and shortcomings are discussed. The chapter ends with concluding remarks about research challenges and future directions in non-destructive sensing of food products.

Key words: food texture, force/deformation, impact, acoustics, optical.

8.1 Introduction

Food texture reflects the sensory perception of humans when taking actions on a food item, primarily in the form of biting, chewing, grinding, etc., to destroy or change its overall structural form, so as to make it suitable for transfer to the stomach. The texture of food is thus closely related to the physical structures and mechanical properties, and it directly affects the acceptability and repeat purchase of a product by the consumer. Consumers generally have varying expectations of texture for different types of food. For instance, they would prefer apples to be crispy and firm, while demanding peaches and mangos to be juicy and melting. For animal products like beef and chicken, tenderness and juiciness are critical to consumer acceptance and satisfaction. Consequently, many different terminologies, such as firmness, juiciness, mealiness, toughness or tenderness, hardness, chewiness, stickiness and gumminess, are used to describe various textural characteristics for different food products. Bourne (2002) has provided an excellent

Mention of commercial products in the chapter is only for providing factual information for the reader, and it does not imply endorsement by the USDA.

review about the science and measurement of food texture, mainly from the perspective of a food scientist.

Since food texture is so important to the marketing and profitability of many food products, it has become an active field of research over the past half century. Conventional sensory evaluation of food texture by humans is subjective, imprecise and time-consuming. Hence objective instrumental techniques are preferred or even required. Nowadays, objective instrumental methods have been firmly established for assessing the texture of food products, even though many of them still fall short of expectations in performance. For instance, the Magness–Taylor (MT) penetration test is widely used as a standard method to measure the firmness of fresh fruits and vegetables. The Warner–Bratzler shear test, on the other hand, provides tenderness measurements for meat products. Most of these standard methods are, however, destructive and, therefore, are only suitable for measuring a few samples. As competition for the market share and consumer demand for superior quality and consistent food products are increasing in both domestic and global marketplaces, it has become increasingly important or even necessary for the food industry to inspect individual product items for food quality, including the texture, before they are being delivered to the consumer. This requires sensing techniques that can be deployed inline or online for rapid assessment, sorting, grading or even monitoring of individual food items. Non-destructive quality evaluation, thus, has been, and is continuing to be, an important multidisciplinary field of research for engineers, food scientists and practitioners.

Over the past decades, many non-destructive techniques have been developed for food texture assessment; most of them are based on either mechanical or optical principles. Mechanical techniques rely on measuring force, deformation, and elastic or viscoelastic parameters at small deformation. Instrumentation and data analysis for mechanical measurement vary greatly, depending on how load is applied to the sample (i.e., quasi-static, dynamic, vibration, etc.). In recent years, optical techniques have gained great attention as a new means for food texture measurement due to the rapid developments in cost-effective optical sensing technology and also because optical measurements can provide rich spatial and/or spectral information about the structural and physiological characteristics of a food. Imaging and spectroscopy are two primary forms of optical measurement. In recent years, we have also seen increased research activities in hyperspectral imaging or imaging spectroscopy, which combines the main features of imaging and spectroscopy, for the texture evaluation of food products. A large array of optical imaging and spectroscopic techniques are available now, which cover different spectral regions and meet different application needs. Unlike mechanical techniques that directly measure specific food textural attributes, optical techniques, in most cases, only provide indirect measurement of food texture, and they rely on calibration models relating the optical properties or features to textural attributes.

This chapter reviews non-destructive sensing techniques, with the emphasis on the latest advances in mechanical and optical techniques, for measuring the texture of solid food. Various techniques, grouped by their respective measurement principles, are described and their merits and shortcomings are discussed. The chapter ends with concluding remarks on research challenges and future directions in non-destructive sensing of food products.

8.2 Mechanical techniques

8.2.1 Force/deformation measurement

Force/deformation measurements are performed by applying small deformation or force commonly with a metallic probe so that no damage will be caused on the sample. The displacement of the probe needs to be well controlled to assure reliable force measurements during the test. Force/deformation measurements can be performed under either quasi-static or dynamic loading mode, which has led to different instrumentation designs. Over the past 60 years, many force/deformation techniques or devices have been developed, and Abbott *et al.* (1997) provided a comprehensive review of earlier research on the development of these techniques or devices.

In assessing the texture of fruits and vegetables, most quasi-static force/deformation techniques measure force, deformation or modulus of elasticity (or Young's modulus) as an indicator of fruit firmness. Mohsenin (1989) reported that many plant materials exhibit the bioyield phenomenon, in which they show a sharp drop in force during the compressive loading, due to the sudden cell failure within the contact area of the plant tissue. Compared to the non-destructive force/deformation methods that measure the elasticity of the sample, the bioyield point reflects the failure strength of plant tissue, which causes small bruising to the fruit but would not degrade it, and hence could correlate better with MT firmness. However, Lu *et al.* (2006) reported that when the bioyield test was carried out on apples using a rigid probe, some fruit exhibited a series of minute bioyield points that were difficult to measure accurately and consistently from the force–deformation curve. This is because the stress resulting from the compression of the rigid probe within the contact area is not distributed uniformly; as a result, the tissue within the contact area would fail gradually. After performing finite element analysis for various bioyield probes with different elastic moduli, sizes, and thicknesses, Lu *et al.* (2006) concluded that a soft tip with an elastic modulus comparable to or less than that of the fruit and a thickness greater than 2 mm would generate uniform contact pressure within the contact area, thus enhancing consistent and reliable measurements of the bioyield point. Lu and Tipper (2009) further developed a portable bioyield tester for measuring the firmness of apple fruit (Plate V between pages 242 and 243), and they reported that force at the bioyield point correlated well with MT firmness for 'Golden Delicious', 'Delicious' apples and the pooled

data with the determination coefficients (r^2) of 0.835, 0.654 and 0.751, respectively.

Besides the numerous works on the firmness measurement of fruits based on the quasi-static force/deformation method, much research has also been reported for other food products. Botta (1991) developed a portable instrument to non-destructively determine the firmness and resilience of raw Atlantic cod fillets. A 2.5 cm diameter probe with an initial force of 10 g was first applied to the surface of the fillet. The force was then increased to 500 g to press the fillet in one second to measure the deformation. The rebound distance was measured by removing the force to allow the fillet to rebound for one second. The texture indexes related to the firmness and resilience were calculated from the ratio of the deformation distance and the rebound distance.

Several commercial instruments are available for measuring the firmness of fruit, which include FirmTech (BioWorks, Stillwater, Oklahoma, USA) and AGROSTA[®]100 (Agro Technologie, France) testers. The FirmTech tester was designed for measuring small fruits like berries and cherries (Ehrlenfeldt and Martin, 2002; Peterson *et al.*, 2003; Prussia *et al.*, 2006). Fruits are placed on shallow pockets of a turntable that automatically rotates and aligns each fruit periodically under the probe, and compressed by a pre-determined force. The force–deformation curve for each fruit is recorded; average firmness, maximum and minimum firmness, standard deviation of firmness, and a frequency distribution of firmness are also obtained. AGROSTA[®]100 (formerly Durofel DFT 100), a portable electronic durometer with its automatic depth limitation, has been widely used for firmness measurement of soft fruits like tomatoes (Camps *et al.*, 2012), cherries (Clayton *et al.*, 1998), peaches (Lurol and Emery, 2007), apricots (Camps and Christen, 2009), and strawberries (Khanizadeh *et al.*, 2000), and different tips are also available for other soft products.

Quasi-static measurements discussed above require physical contact between the probe and the sample. Prussia *et al.* (1994) developed a non-contact laser air-puff device for fast measurement of mechanical deformation. The method involves sending a sharp puff of pressurized air onto the sample surface and recording the deformation with a laser displacement sensor. McGlone and Jordan (2000) used the laser air-puff method to measure the firmness of kiwifruit and apricot. A fruit stiffness value was calculated from the maximum deformation, as a measure of the firmness. Maw *et al.* (2003) applied a laser air-puff tester to differentiate non-melting-flesh from melting-flesh peaches over time. Their study showed that the laser air-puff tester could be used for segregating peaches with non-melting flesh from ones with melting flesh. The laser air-puff method was also used for assessing the tenderness of broiler breast meat (Y. S. Lee *et al.*, 2008). The classification accuracy for tender fillets ranged from 82% to 88% using the tenderness-related parameters extracted from the air-puff system, but the result for identifying tough meat was not as good as that

for the tender meat. These studies indicated that the laser air-puff method is more suitable for measuring the texture of soft fruit or tender food products than that of firm or tough products. The technique could be used for different food products by adjusting the air puff pressure, but it may not be suitable for translucent tissues due to excessive light scatter (Lu and Abbott, 2004).

8.2.2 Impact

Considerable research has been reported on the evaluation of fruit firmness using the impact technique. The impact response is governed by the impacting velocity and the elastic modulus, mass, size and shape of the object. Hence impact force response can be used to assess the texture of food products. Different parameters, including peak force, ratio of peak force to time-to-peak, coefficient of restitution, contact time and frequency spectrum, obtained from the impact measurement were used to evaluate the firmness of food products. Impact firmness measurement is commonly implemented in either drop or probe test mode. In the drop test, a sample is dropped onto an impacting sensor that records the force–time or force–frequency spectrum, while the probe impact test uses a probe with a sensor to impact the sample.

The drop test was studied by a number of researchers for fruits and vegetables. The impact indices proposed by Delwiche *et al.* (1987) were used in many studies, which include time-domain [peak force/(time to peak force)²] and frequency-domain characteristics (e.g., 295 Hz spectrum magnitude). Delwiche *et al.* (1989) developed an automatic firmness sorting system based on the analysis of impact responses, which achieved 74% accuracy in sorting peaches into the correct firmness categories at a sorting rate of five fruit per second. Yen and Wan (2003) developed a non-destructive method using an impact pendulum for the textural analysis of guava fruit (Fig. 8.1). The impact tester used a digital oscilloscope to record the impact force signal produced by the swing of an impact pendulum sensor when the sample lightly impacted the motionless force transducer. Using the combined impact indices (i.e., force–time and amplitude spectrum, and amplitude spectrum and imaginary part spectrum), the technique achieved more than 80% classification accuracies for the maturity of guava fruit, higher than the accuracies obtained using single impact parameters. Lien *et al.* (2009) investigated the drop impact test for the assessment of tomato maturity, and they reported that the level of tomato maturity was related to the peak force (F_p), time-to-peak (t_p) and total contact time (t_c ; Fig. 8.2). An overall accuracy of 82% was obtained for classifying tomatoes into unripe, half-ripe, and ripe classes using nine impact indices.

Jaren and Garcia-Pardo (2002) investigated the impact probe test system developed by Chen *et al.* (1985) and improved by Garcia *et al.* (1988) for the firmness assessment of apples and pears. The system used a 50 g steel

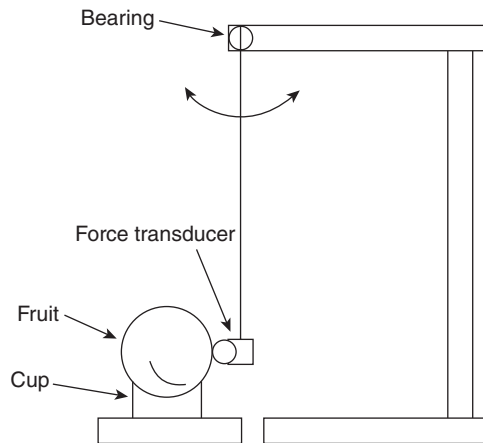


Fig. 8.1 A pendulum impact test device (Yen and Wan, 2003).

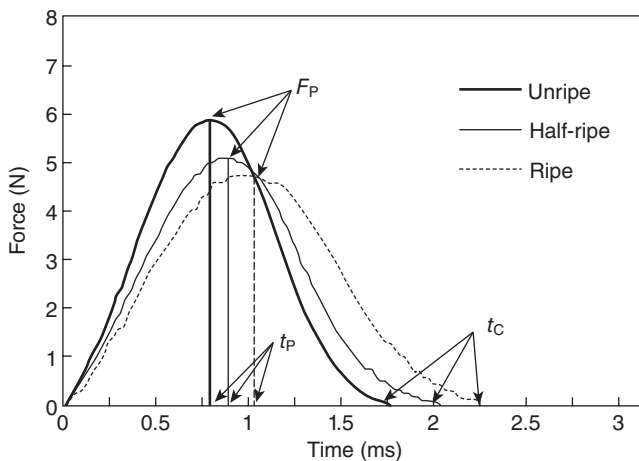


Fig. 8.2 Impact responses of three tomatoes at different levels of maturity (Lien *et al.*, 2009).

rod with a spherical tip of 0.98 cm radius to drop from a height of 4 cm onto pear fruit and 3 cm for apple fruit. Conde *et al.* (2007) designed a probe impact device for the cheese texture measurement. The test was implemented by releasing the impact probe from its upper position until it impacted on the cheese surface, and the maximum impact force and the maximum force for the spherical probe were highly correlated with the cheese texture parameters, with the average correlation coefficient of 0.97. Homer *et al.* (2010) modified and installed a laboratory impact sensor in an experimental fruit packing line with a commercial sizing chain to measure

the fruit firmness. The system consisted of an optical sensor to detect the fruit, a 10 g spherical low-mass probe with a piezoelectric accelerometer to impact the fruit, a spring to release the impacting mass, and an electromagnet to hold the impacting mass. The system was tested for apples, peaches and pears, and the best result was achieved for the two-class firmness classification of peach fruit with 88% accuracy. Sinclair International Ltd. (Norfolk, United Kingdom) developed a commercial on-line firmness sorting system based on a low-mass impact sensor (Sinclair IQ^T firmness tester or SQ-FT), which measures the firmness by using the sensing element to hit the fruit at a speed of 10 fruit per second. Valero *et al.* (2007) tested the SQ-FT system for peaches, nectarines, and plums in a commercial packing line environment, and reported correlations of 0.77–0.84 with MT firmness. GREEFA in Geldermalsen, Netherlands also developed a firmness sensor called iFD, which has a large wheel equipped with multiple sensor tips to impact the fruit moving on the packing lanes at a speed of seven fruit per second.

Both drop and impact probe techniques showed good results in the firmness measurement of select fruits (i.e., pears, peaches, and some tropical fruits), but not in apples (Homer *et al.*, 2010; Khalifa *et al.*, 2011; Kupferman, 2007). The drop impact test is relatively easy to implement, but the mass and shape of the fruit will affect the accuracy in firmness measurement since the impact force response is also a function of these two parameters. The probe impact technique, on the other hand, eliminates the effect of fruit mass and the impact response is also less sensitive to the curvature of the fruit. However, the probe design is critical for accurately and reliably measuring the impact response of the fruit, as it can affect the acquisition of impact signals and cause potential damage to fruit. Compared with force/deformation methods, impact technique provides a faster way of measuring the texture of food, and it has good potential for online sorting and grading of fruits and vegetables.

8.2.3 Vibration

Vibration tests are conducted by applying either a periodic force at certain frequencies or an impulse force to generate free vibrations in the test sample. The response of food products to vibration depends on the mechanical and physical properties of the food (i.e., modulus of elasticity, mass, and shape) and vibration frequency. Different types of vibration can be used for the food texture assessment, and the most commonly used are sonic (or acoustic) and ultrasonic. Sonic vibrations refer to those occurring at the audible frequencies ranging between 20 Hz and 15 kHz, whereas ultrasonic vibrations are above the audible frequency range (>20 kHz). Sonic and ultrasonic waves can be reflected, transmitted, refracted, or diffracted as they interact with the tissue of a food product, which are directly related to the mechanical properties and geometry (i.e., size and shape) of the food.

Sonic vibration techniques have been studied extensively for fruit firmness or stiffness measurement (Chen and De Baerdemaeker, 1993; De Belie *et al.*, 2000; Diezma-Iglesias *et al.*, 2004; Wang *et al.*, 2006). Sonic firmness or stiffness, expressed as f^2m or $f^2m^{2/3}$, is calculated based on the mass of the fruit (m) and the first, second, third, or higher resonance frequency (f), or any other combination depending on the application and/or sensor setup (Abbott and Liljedahl, 1994; Pathaveerat *et al.*, 2008; Taniwaki *et al.*, 2009). Fruit shape also affects the sonic firmness measurement and needs to be considered (Langenakens *et al.*, 1997; Lu and Abbott, 1997). Armstrong *et al.* (1997) reported a poor correlation between the frequency parameters generated from the fruit resonance and Effe-gi penetrometer firmness (a 7.8 mm diameter probe, Effe-gi, Alfonsine, Italy), when sonic vibrations in peaches were detected using a microphone. The sonic method showed the potential for sorting out excessively soft or hard fruit from desirable fruit. Wang *et al.* (2006) investigated the sonic impulse response for monitoring the mandarin firmness change during storage (Fig. 8.3), and a correlation of $r = 0.88$ was obtained between the stiffness coefficient and MT firmness. The sonic method was also applied for the hollow heart detection in potato tubers (Elbatawi, 2008), almond nuts sorting (Ebrahimi and Mollazade, 2010), and texture measurement of cabbages and persimmons (Taniwaki *et al.*, 2009; Taniwaki and Sakurai, 2010). Commercial sonic systems, such as AFS by Aweta in Nootdorp, Netherlands, are currently available for evaluating the firmness of apples, peaches, and tomatoes (Cen *et al.*, 2012b; De Ketelaere *et al.*, 2006; Mendoza *et al.*, 2012). The Aweta AFS detects the vibration pattern of the sonic waves travelling across the fruit generated by gently tapping the fruit by a plastic probe. In addition, the sensor also measures the impact response generated by the probe.

Mizrach (2008) reviewed recent advances in ultrasonic technology for monitoring the quality of fruits and vegetables. The technology has been

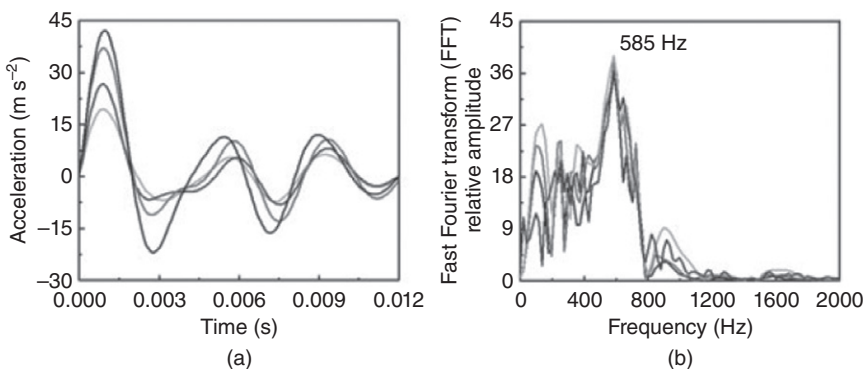


Fig. 8.3 The response of a mandarin fruit to four impulses around the equator line: (a) time domain and (b) frequency responses (Wang *et al.*, 2006).

used for measuring firmness and mealiness of some fruits, such as avocado. Different ultrasonic sources are needed for different fruits in order to achieve good signal penetration (Bechar *et al.*, 2005; Mizrach *et al.*, 2003). Compared with the sonic technique, the ultrasonic technique is more difficult to implement due to strong attenuation when the ultrasonic waves travel through the plant tissue (Abbott, 1999).

8.3 Optical techniques

8.3.1 Visible/near-infrared (Vis/NIR) spectroscopy

The electromagnetic spectrum of light from the shortest to longest wavelengths includes gamma rays, X-rays, ultraviolet, visible, infrared, and radio waves. Light at different wavelengths carries different levels of energy and, therefore, can have different patterns of interaction with matter, depending on its chemical composition and structural characteristics. Absorption and scattering are two basic phenomena when light interacts with a turbid biological medium or a food product. Absorption is related to the chemical composition of the product, while scattering is dependent on the density and structural characteristics. Hence optical techniques based on the electromagnetic radiation for a specific spectral range could be useful for non-destructive measurement of the texture of food products.

Color, which covers the spectral region of approximately 400–750 nm, is directly associated with pigments (e.g., chlorophylls, carotenoids, and anthocyanins). Surface color is the basis for sorting many agricultural and food products into commercial grades, because it can be indicative of their maturity or quality, which is often related to the structural and textural characteristics of food products. However, in most cases, color alone is not sufficient for accurate measurement of food texture.

Visible/near-infrared (Vis/NIR) spectroscopy, which measures the light reflected back from or transmitted through the sample over either the entire spectral region of approximately 400–2500 nm or a narrower section of the region, is widely used for chemical composition analysis, quality inspection, and process monitoring and control of food products. The NIR region involves the responses of the molecular bonds O—H, C—H, C—O, and N—H. These bonds are subject to vibrational energy changes when irradiated by NIR frequencies, and two vibration patterns exist in these bonds, including stretch vibration and bent vibration. The energy absorption of organic molecules in the NIR region occurs when the molecules vibrate or are translated into an absorption spectrum (Cen and He, 2007; Williams and Norris, 2001). Vis/NIR spectra provide rich information about a large set of overtones and their combinations that are related to the chemical composition and physical structures of the product. In practice, Vis/NIR measurement may be implemented in three sensing modes: reflectance, transmittance and interactance (Fig. 8.4). Transmittance measurement

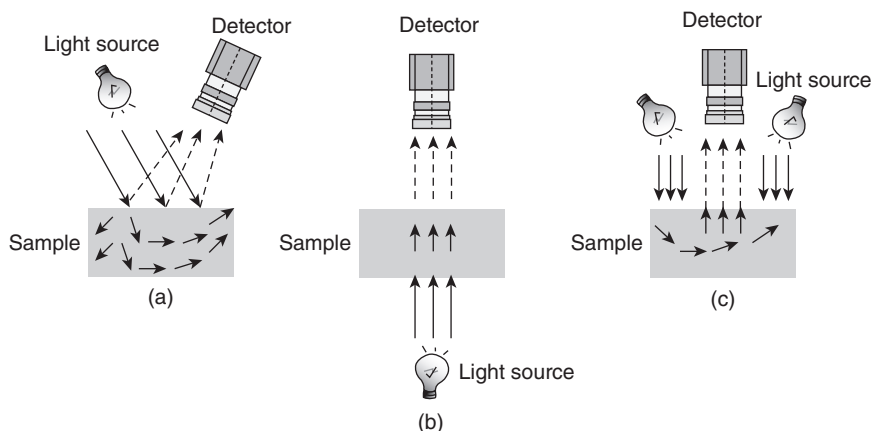


Fig. 8.4 Three sensing modes commonly used for visible/near-infrared (Vis/NIR) spectroscopic measurement: (a) reflectance, (b) transmittance, and (c) interactance.

is often applied for liquid samples, such as fruit juice, using a glass or quartz chamber with different sizes, while diffuse reflectance and interactance measurement are popular for solid food samples (Abbott *et al.*, 1997; Lu *et al.*, 2000; McGlone *et al.*, 2003). Interactance provides a compromise between the reflectance and transmittance modes, and it allows good penetration of light into the sample, while maintaining better control of the light pathlength. Vis/NIR measurements often involve four main steps: (1) spectrum acquisition; (2) data pre-processing to reduce or eliminate the noise; (3) development of a calibration model; and (4) model validation and prediction.

Over the past two decades, considerable research has been reported on the Vis/NIR technique for non-destructive determination of texture and other quality attributes of food (Carlomagno *et al.*, 2004; Lammertyn *et al.*, 1998; Pedro and Ferreira, 2005; Sirisomboon *et al.*, 2007, 2012). Lammertyn *et al.* (1998) used Vis/NIR spectroscopy (380–1650 nm) to measure the firmness and other quality attributes of ‘Jonagold’ apples. They reported that Vis/NIR gave good predictions of the flesh stiffness with the standard error of prediction (SEP) of $2.5 \text{ Hz}^2 \text{ kg}^{2/3}$ and a correlation coefficient (r) of 0.90, but it had poor predictions for the elastic modulus with $r = 0.75$. Lu (2001) used the NIR spectral region of 800–1700 nm in reflectance mode to measure the firmness of sweet cherries (Fig. 8.5), and reported correlations of 0.80 and 0.65 for two varieties of cherry. Vis/NIR spectroscopy was also used to measure the texture and rheological parameters of wheat kernels (Arazuri *et al.*, 2012; Williams, 1991), texture of cooked potatoes (Thybo *et al.*, 2000), tenderness of beef (Liu *et al.*, 2003), and sensory characteristics of lamb meat (Andres *et al.*, 2007). Several recent reviews have been published on

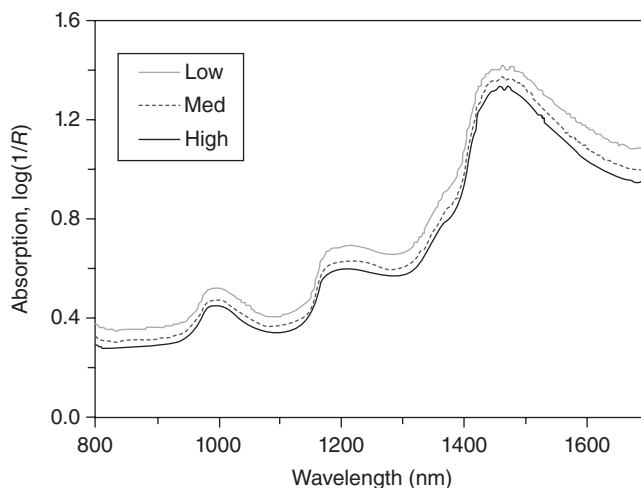


Fig. 8.5 Average absorption spectra of 'Hedelfinger' sweet cherries for three firmness classes (Lu, 2001).

using Vis/NIR spectroscopy to measure the texture and other quality attributes of fruits and vegetables and other food products (Cen and He, 2007; Nicolai *et al.*, 2007).

Spectral analysis and calibration model development are critical for successful application of Vis/NIR technology. Multivariate statistical analysis methods, such as partial least squares, principal component analysis, multilinear regression, and artificial neural networks, are widely used for Vis/NIR spectral analysis. Attention must be paid to the development of robust calibration models to prevent over-fitting problems (Gowen *et al.*, 2011). A reliable calibration model is usually built based on a large data set that takes into consideration operational conditions (e.g. temperature), sample specifications (cultivar, growing season, and location), and climate condition. Another topic of importance is the transfer of multivariate calibration models among different instruments, which has been reviewed by Feundale *et al.* (2002). Zamora-Rojas *et al.* (2012) investigated three standardization algorithms, including direct standardization, piecewise direct standardization and spectral difference by wavelengths, to transfer the meat quality database from an online NIR instrument to a handheld NIR device.

Due to the advances in optics and computer during the past two decades, a large variety of Vis/NIR sensors of different sizes (from benchtop to miniature handheld units) are currently available, with the prices ranging between \$1000 and \$100 000. The availability of low-cost miniature Vis/NIR spectrometers has led to the increased R&D activities in quality and texture evaluation of food products in recent years. Commercial spectrometers generally consist of an entrance slit, a grating-based dispersive design or an acousto-optical tunable filter-based design, a fixed array detector (e.g.,

linear silicon or indiumgallium-arsenide photodiode array and linear or two-dimensional charge-coupled device (CCD) array), and an analog-to-digital converter (8 to 16 bits). When selecting a Vis/NIR spectrometer for online or offline applications, several factors need to be considered, which include the signal-to-noise ratio (SNR), spectral range, response and resolution, sensitivity, and tolerance to vibration and dust. Moreover, spectral acquisition speed is also an important consideration for online applications, where the inspection speed can be more than 10 items per second for each sorting lane.

Vis/NIR technique is non-invasive, fast, and easy to implement for offline and online applications. However, Vis/NIR evaluation of food texture and quality relies on statistical methods to establish empirical relationships between spectral measurements and specific textural characteristics or chemical compositions. The performance of a Vis/NIR system is thus dependent on how the original samples are collected and how the calibration model is originally built and subsequently upgraded with new samples. Since many users do not have special knowledge or skills for performing the model calibration and upgrading, they have to rely on the original equipment manufacturer to provide these services. This has remained as one major hurdle in the adoption of Vis/NIR technology for quality and texture assessment of food and agricultural products.

8.3.2 Imaging technology

Imaging technology (or computer vision) is widely used for the quality assessment and safety inspection of food and agricultural products. Numerous review articles have been published on this topic (Brosnan and Sun, 2004; Costa *et al.*, 2011; Elmasry *et al.*, 2012; Fagan *et al.*, 2008; Tan, 2004). This section reviews several emerging imaging techniques for the texture assessment of food products.

Early applications of imaging technology were primarily for classification or sorting of food products based on the recognition of shape, size, and surface color and/or defect (Brosnan and Sun, 2004; Chao *et al.*, 1999; Chinchuluun *et al.*, 2009; Singh and Delwiche, 1994). Imaging technology was used to evaluate the texture of beef steaks by quantifying the marbling area percentage (Chen *et al.*, 1989) and the beef tenderness by determining the skeletal maturity of beef carcasses (Hatem and Tan, 1998). Skin color information is considered to be one of the maturity indices for horticultural products like peaches, bananas, and tomatoes, which may also be related to the fruit texture. Hence color imaging is also used indirectly for evaluation of the texture of horticultural products.

The information obtained from the broadband images (i.e., color and black/white) generally cannot achieve accurate assessment of food texture because it cannot effectively detect some subtle or minor features that are only sensitive to specific wavelengths or wavebands. Hence spectral imaging

techniques like multispectral and hyperspectral can be more appropriate for the texture evaluation of food products. Multispectral imaging usually generates a set of images at fewer than 10 discrete wavelengths or narrow wavebands, which can be obtained either by positioning a bandpass filter wheel in front of a monochrome camera (Park *et al.*, 2004), or by capturing spectral images at selected wavelengths using an acousto-optic tunable filter (AOTF) or a liquid crystal tunable filter (LCTF) (Peng and Lu, 2006; Tran, 2000). The technique was used for the evaluation of fruit firmness (Lleo *et al.*, 2009; Lu and Abbott, 2004; Peng and Lu, 2006). Peng and Lu (2007) developed a LCTF-based multispectral scattering system to evaluate the firmness of apple fruit. The system used a high performance CCD camera with a point light beam to acquire the scattering images at several selected wavelengths. Light scattering is influenced by the density, cell structures, and cellular matrices of fruit tissue, and therefore it can be used for measuring the fruit firmness. A good correlation ($r = 0.90$) between the modified Gompertz function parameters extracted from the scattering images and MT firmness of apple fruit was obtained.

Hyperspectral imaging combines conventional spectroscopy and imaging techniques to acquire both spectral and spatial information from an object simultaneously. The technique would thus enable us to analyze product properties or characteristics more reliably and accurately than either imaging or spectroscopic technique. Hyperspectral imaging may be implemented by acquiring a sequence of narrow-band spectral images (filter-based imaging mode) or by capturing line scanning images (line scanning mode) with a full spectral range for each pixel of the scanning line to create three-dimensional spatial-spectral data cubes or hypercubes (Bernhardt, 1995; Park *et al.*, 2002). Line scanning mode is preferred for online applications since it is much easier to implement compared with the filter-based imaging mode (Ruiz-Altisent *et al.*, 2010). A typical hyperspectral imaging system consists of a high-performance camera with a large dynamic range, low noise level, and good quantum efficiency, an imaging spectrograph, and a stable light source. Spectral and spatial calibrations are needed in order to achieve an accurate measurement. The hyperspectral imaging technique was used for measuring or classifying fruit firmness (Lu and Peng, 2006; Noh and Lu, 2007; Peng and Lu, 2008; Rajkumar *et al.*, 2012), meat tenderness (Cluff *et al.*, 2008; Elmasry *et al.*, 2012), fish freshness (Chau *et al.*, 2009), and the endosperm texture of maize (Manley *et al.*, 2009).

Absorption and scattering coefficients are two fundamental optical properties for turbid biological materials like food, and they are dependent on the chemical composition and physical structures. Hence fast, accurate measurement of absorption and scattering coefficient spectra can provide an effective means for evaluating such quality attributes as texture. A hyperspectral imaging-based spatially-resolved instrument, called the optical property analyzer, was developed in our laboratory for measuring the optical absorption and scattering properties of horticultural and food

products over the spectral region of 400–1000 nm (Cen, 2011; Qin and Lu, 2007). This general-purpose optical instrument (Plate VIa between pages 242 and 243) consists of a line scanning hyperspectral imaging system with an electron-multiplying CCD sensor and two separate light sources (Cen *et al.*, 2012a). A continuous-wave point light source is used to generate spatially-resolved diffuse reflectance images for the optical property measurement (Plate VIb), whereas a line light source is for general hyperspectral imaging applications (Plate VIc). The instrument was used to measure the absorption and scattering spectra of peaches and apples, and it showed promising results in predicting the fruit firmness using the optical property spectra (Cen *et al.*, 2012a, 2012b).

Figure 8.6 shows the absorption and reduced scattering coefficients spectra of peach fruit with different skin colors (hue value, $h^\circ = 23.7, 21.9$, and 20.4) and firmness levels ($F_M = 72, 47$, and 21 N) over the spectral region of 515–1000 nm. The reduced scattering coefficient, in general, decreased with the decrease of fruit firmness.

Because of its capability of acquiring both spectral and spatial information, hyperspectral imaging has emerged as a promising technique for the

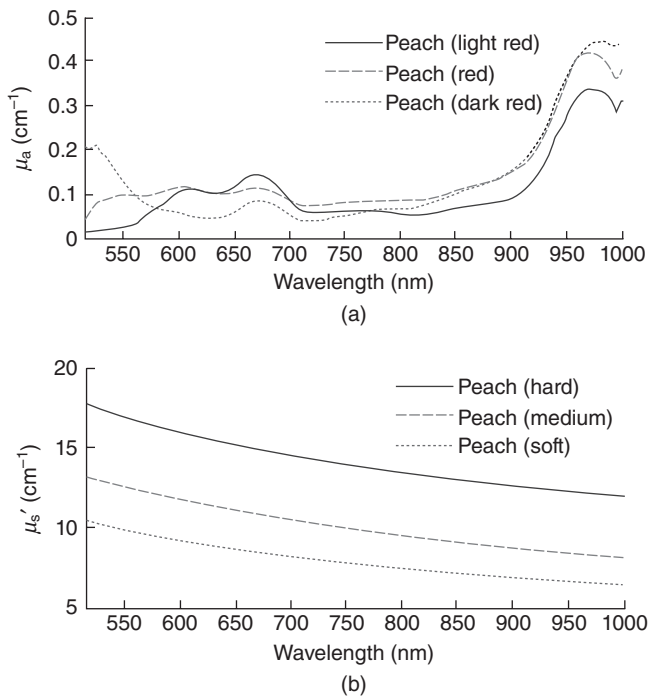


Fig. 8.6 Spectra of (a) absorption coefficient for three peaches with different skin colors (light red, red, dark red), and (b) reduced scattering coefficient for three peaches at different firmness levels (hard, medium, soft) (Cen *et al.*, 2012a).

texture evaluation of food products. However, hyperspectral imaging acquires a large amount of data, which presents a major hurdle for fast online applications. Hence, many studies were focused on developing effective algorithms for extracting useful information, reducing the number of image features, identifying the most useful wavelengths, and minimizing the model classification and prediction errors. Different machine learning and image analysis techniques were used to extract relevant information from the large-scale hyperspectral image data (Plaza *et al.*, 2009). Waveband selection is one of the most important approaches for this purpose; some of the commonly used methods are correlation analysis (K. Lee *et al.*, 2008; Qin *et al.*, 2011), principal component analysis (Cheng *et al.*, 2004), genetic algorithm (Kawamura *et al.*, 2010), sequential forward selection (Serpico and Bruzzone, 2001), as well as relatively new methods like minimum redundancy-maximum relevance (Peng *et al.*, 2005). Combined with these efficient waveband selection techniques, hyperspectral imaging can be implemented as a multispectral imaging solution for rapid, real-time online inspection of food products (Chao *et al.*, 2008; Mendoza *et al.*, 2011).

8.3.3 Other optical/non-optical techniques

In addition to the spectroscopic and imaging techniques described above, many other optical techniques have been researched for food texture evaluation over the years. Fluorescence is widely used in the food chemistry analysis, and also has the potential for food texture analysis because it can provide information about the structure of food products. Fluorescence spectroscopy and imaging provide a fast, sensitive, and non-destructive measurement of luminescence responses to the presence of fluorescent molecules and their environment in biological and food materials (Dufour *et al.*, 2001). Fluorescence excitation of a molecular is achieved by high energy (or shorter wavelength) light, mostly in the UV or visible range. Most applications of fluorescence in fruits and vegetables involve the determination of chlorophyll activity (Abbott, 1999; Ruiz-Altisent *et al.*, 2010). Since chlorophyll is considered to be an indirect marker for the ripeness of fruit, it is thus related to the fruit texture, such as firmness. Chlorophyll fluorescence was applied for measuring the fruit firmness and mealiness (Bron *et al.*, 2004; Moshou *et al.*, 2003; Noh and Lu, 2007). In these studies, either a laser or ultra-bright LEDs (light-emitting diodes) were used as a fluorescence inducer, and a hyperspectral imaging system, a plant efficiency analyzer, or a fluorometer was used to record the fluorescence emitted from the plant products.

Fluorescence has also found applications for other solid foods, such as meat (Allais *et al.*, 2004) and dairy products (Dufour, 2011; Kulmyrzaev *et al.*, 2005). Dufour *et al.* (2001) used fluorescence spectroscopy to evaluate the sensory texture of soft cheese. Yao *et al.* (2004) reported on using fluorescence polarization spectroscopy for characterizing fiber formation in

meat analogs. Despite all these studies, fluorescence technique has not been established as a viable alternative to other optical or mechanical techniques for the food texture measurement.

Time-resolved spectroscopy (TRS) (Zerbini *et al.*, 2006) is another optical technique for the texture evaluation of food products. TRS is based on the measurement of attenuation, broadening and delay of a short light pulse, which are caused by the absorption and scattering events during the photon propagation in highly scattering media. Photon propagation measurements in the time domain depend on the ability to extract photon information encoded in the temporal distribution of the re-emitted light, following the injection of a short monochromatic pulse in a diffusive medium. Temporal resolution and high sensitivity thus become two critical factors in designing a time-resolved spectroscopy system (Cubeddu *et al.*, 2001). TRS was reported for determining the fruit firmness and mealiness, but the measurement accuracy was low (Nicolai *et al.*, 2008; Valero *et al.*, 2004, 2005).

X-ray covers the wavelength region from 0.01 to 10 nm, which is shorter than UV rays and longer than gamma rays. X-ray can easily penetrate into food products, and the ability of X-ray transmitting through the food products depends on the incident energy and the absorption coefficient, density and thickness of the sample. X-ray can detect food texture changes caused by the water redistribution and binding, cell breakage, and agglomeration of chemical compositions. X-ray is especially useful for detecting some physiological disorders of plant products as well as evaluating the maturity or ripeness of fruit that is associated with changes in the tissue density and water distribution (Barcelon *et al.*, 1999; Brecht *et al.*, 1991; Haff *et al.*, 2006; Schatzki *et al.*, 1997; Tollner *et al.*, 2005). X-ray measurements for food applications may be performed using two-dimensional radiography, linescan radiography, X-ray computed tomography (CT), and X-ray fluorescence. With the recent advances in fast and powerful computers, new detector technologies and high-performance X-ray tubes, X-ray techniques, particularly three-dimensional X-ray CT, are gaining more attention in food applications.

Nuclear magnetic resonance (NMR) is another versatile non-destructive technique for measuring the internal features of a product on the basis of the magnetic properties of the nuclei of atoms making up a matter. NMR signals are related to the chemical composition and physical structures of products, and hence can be used to indirectly evaluate food texture. NMR spectroscopy generates a frequency-dependent spectrum, in which the energy transfer takes place at a wavelength that corresponds to radio frequencies. Magnetic resonance imaging (MRI), on the other hand, creates a two- or three-dimensional image by applying a graded magnetic field to the sample. NMR/MRI has been shown to be a useful non-destructive technique for the determination of food internal quality since it is sensitive to the chemical structure, density of certain nuclei, molecular mobility,

chemical reaction, and diffusion, among other phenomena (McCarthy, 1994). Applications of NMR/MRI for the texture evaluation of food products have been reported, including the mealiness assessment of apples and peaches (Barreiro *et al.*, 2000), firmness determination of pears and tomatoes (Tu *et al.*, 2007; Zhou and Li, 2007), and textural analysis of dairy products (Karoui *et al.*, 2003). These studies demonstrated that NMR/MRI is a promising method for the food texture assessment. However, proper signal interpretations present a challenge for the application of NMR/MRI in food texture evaluation. As the cost of NMR/MRI instruments continues to fall, we should expect more research activities in the application of the technology for food texture evaluation.

8.4 Conclusion

This chapter has reviewed non-destructive sensing techniques, mainly based on mechanical and optical principles, for the texture evaluation of solid foods. While earlier research was largely focused on mechanical techniques, we have seen increased R&D activities in optical techniques in recent years. This changing trend mainly resulted from the rapid advances in optical detector technology and computer and wireless technology, and it is also attributed to the increased capability of optical techniques, either in spectral or imaging mode, to provide a large amount of information about the structural characteristics and composition of food products.

Over the years, many promising non-destructive sensing techniques have been developed. But most of them have remained, or are still remaining, in the research laboratory. There are many factors affecting the slow adoption or even rejection of these techniques by the end user, among which are performance, cost, and market size. Unlike such quality attributes as taste and flavour, food texture often remains a vague term that cannot be well defined using standard engineering parameters. It is quite common that different researchers or practitioners use a different definition, procedure or technique in the texture evaluation of the same food product. For instance, firmness is such an important quality attribute for many horticultural products, but there are different definitions and techniques for the firmness measurement. A lack of consensus among the researchers and practitioners on the standard measurement procedure for fruit firmness has hindered the progress in research as well as the adoption of sensing technologies that have been developed in the past. Clearly, there is a critical need to develop a unified vocabulary and definition of firmness for fruits and vegetables. Engineers, food scientists, and practitioners need to take concerted efforts to establish standard methods and procedures that are based on the well-established measurement principles and engineering theories for determining the food texture for different types of food products.

Foods are such complex and diverse biological materials whose textural properties are influenced by both biotic and abiotic factors. There is still a lack of sufficient understanding of how these factors interact in influencing the texture of food. But such understanding is critical for developing effective non-destructive sensors. There are diverse food products with diverse textural attributes. This means the need of different sensors for different types of food. Many existing sensors for food texture measurement still do not perform satisfactorily, cost too much, or are too slow or inconvenient for online or onsite applications. Hence a new generation of sensors with substantially improved capabilities for rapid and reliable measurement of food texture is needed. These new sensors should be cost effective, easy to operate, and reliable or robust for offline or online inspection of food texture.

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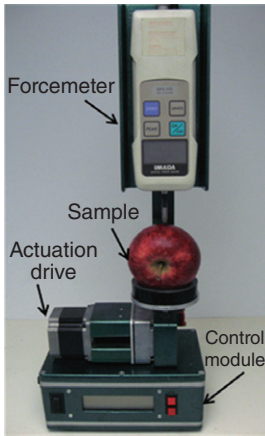
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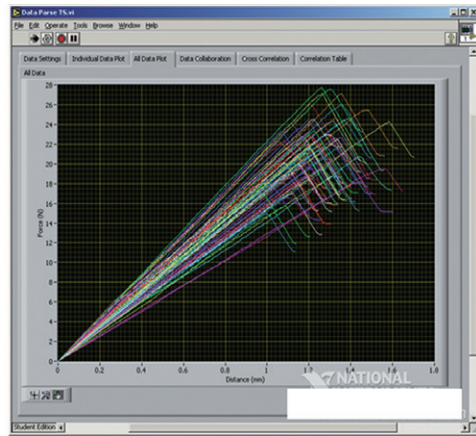
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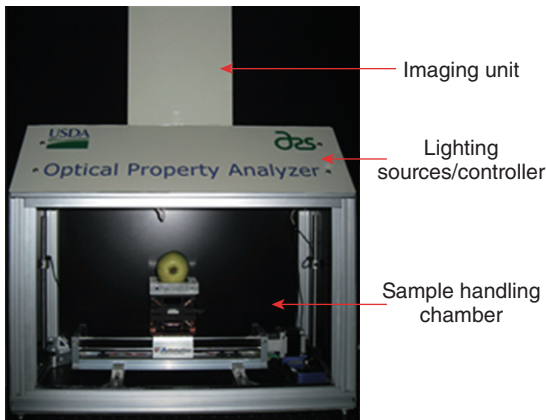


(a)

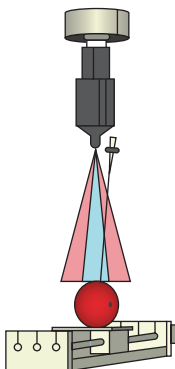


(b)

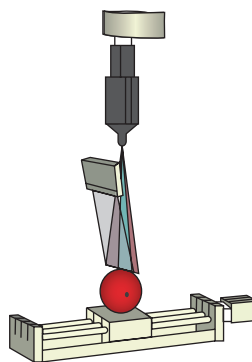
Plate V (Chapter 8) A portable bioyield tester (a) for measuring the firmness of fruit and a display window (b) showing the force/displacement curves up to the bioyield point. (Lu R and Tipper NC, 2009. A portable device for the bioyield detection to measure apple firmness. *Applied Engineering in Agriculture*, **25**(4), 517–523).



(a)



(b)



(c)

Plate VI (Chapter 8) A general-purpose hyperspectral imaging-based optical property measurement instrument, called the optical property analyzer (a), and its two operation modes for optical property measurement using a point light source (b) and for general hyperspectral image acquisitions using a line light source (c).

In-mouth measurement of food quality

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Abstract: The interactions between biological surfaces (in the mouth) and product materials under physiological oral processing conditions play an important role in generating sensory attributes and determining sensory perception. This chapter reviews measurement techniques that probe oral food processing either directly *in vivo* or *ex vivo* (including artificial mouths), discusses the techniques in context of the in-mouth process or mechanism that they probe (break down and fracture mechanics, food–saliva and food–biological surface interactions) and describes the results in relation to sensory perception. Some aspects of flavour release in relation to use of artificial mouths are also described.

Key words: measurement techniques of oral food processing, food material properties, food behaviour and sensory perception, food–saliva interactions, artificial mouths.

Note: This chapter was first published as Chapter 10 ‘Measuring the oral behaviour of foods’ by I.A.M. Appelqvist, in *Designing functional foods: Measuring and controlling food structure breakdown and nutrient absorption*, eds. D.J. McClements and E.A. Decker, Woodhead Publishing Limited, 2009, ISBN 978-1-84569-432-6.

9.1 Introduction

Studying and elucidating the interactions between biological surfaces (in the mouth) and product materials under physiological oral processing conditions has been difficult and until recently has not been very systematic. This difficulty is partly due to the complex nature of both physical and mechanical ‘measurement’ within such physiological environments, and because the physical/mechanical responses of food are highly time dependent through both the ‘openness’ of the product materials (swelling, dissolving, etc.) and the dynamic nature of biological surfaces and fluids. It is, however, through these complex domains that our products interact, change physically and chemically, and eventually elicit the sensory response that consumers perceive.

The interaction between food materials and physiological surfaces and fluids, which plays an important role in generating sensory attributes, is still

not well understood in terms of the physics and biophysics of the interfacial properties between product materials and biological surfaces and fluids. This aspect of measurement and the inclusion of knowledge about structural changes that occur after processing in the mouth are important to progress our understanding of sensory behaviour and to predict and quantify the stimuli generated during eating (van Aken *et al.*, 2007). The technical challenges for the food material scientists are to be able to measure the dynamic nature of material changes, which for many breakdown mechanisms can be very fast, and to measure these changes under relevant physiological conditions. Added to this is the complexity in which a number of changes to the materials properties of food during mastication occur simultaneously, and as yet what mechanisms dominate in terms of sensory perception are not fully known. Further complexity is in how multimodal features of food are integrated and is a fundamental neuroscientific question in food perception (Verhagen and Engelen, 2006).

The aim of this chapter is to outline and discuss a range of techniques that have been used by food material scientists and engineers to try to probe some of the aspects of oral processing identified above. The chapter will attempt to review the techniques in context of the in-mouth process or mechanism that they probe and how this information has been used to relate to sensory perception. The techniques and measurement approaches identified fall into a number of categories: those that use *in vivo* techniques that measure and visualise changes in food during mastication directly; those that use the mouth as a 'rheometer', the analysis of which is directed on the expectorated food; and those that measure *ex vivo*, but have taken into account some of the physiological and mechanical characteristics relevant to the mouth under which food gets processed. The type of technique selected has largely been determined by the 'texture' of the food, so that solid foods such as meat, cheese and nuts have been measured in terms of their breakdown and fracture mechanics whereas soft-solids such as cream cheese, yoghurt and mayonnaise are measured primarily through their interaction with saliva and/or the biological oral surfaces.

Conceptually, it is clear that the product dynamics and mouth interaction must also have a role to play in the delivery of small molecules and the release of ingredients at specified target sites, which offer novel routes to control texture and taste. Some aspects of flavour release will be briefly covered in Section 9.6 on artificial mouths.

9.2 *In vivo* visualisation measurements for understanding food behaviour in the mouth

The importance of measuring the impact of oral processing on the structural changes of food in the mouth, how these dynamic aspects of food interact with sensory receptors and finally the relationship to sensory perception

has long been recognised. Understanding the various mechanisms involved with oral processing of food and quantifying the in-mouth structuring and disassembly of food materials, as well as their interaction with oral surfaces, will support the establishment of engineering rules to control these mechanisms by the design of the structure and composition of food.

Recently food research studies have focused on investigating *in vivo* the product interaction with the structures of the oral apparatus and surfaces. A range of visualisation techniques has been used to gain a better understanding of the mouth movements, to visualise the surface oral structures and to monitor the complex interactions of food materials during eating. The most relevant techniques for measuring oral behaviour of food are described in the next section.

9.2.1 X-ray-based imaging for visualising oral movements

Attempts have been made to quantify tongue movements using videofluorography of lead markers glued to the tongue (Hiimeae and Palmer, 2003). Limitations of this technique are that only a small number of markers can be used and the radio-opacity of the mandible and teeth obscure the tongue, so that the complexity of the tongue movement is not captured. In addition having to use a radio opaque contrast medium in the food and because exposure to X-rays must be kept to a minimum, this technique has been replaced by more modern visualisation techniques. However, X-ray imaging has been used to visualise chewing action and swallowing of solid and semi-solid foods in real time (Hiimeae *et al.*, 2002), and has contributed to our understanding of the sequences of activity and processes that are undergone during eating of food. Videofluoroscopy, a dynamic X-ray technique, has been applied to observe the process of swallowing food and fluids using a fluid medium contrast (Buettner *et al.*, 2001) and results were analysed according to the stages of swallowing (bolus uptake, beginning of bolus transport, velopharyngeal closure, triggering of the swallowing reflex, propulsion of the bolus towards the oesophagus and original position) defined by Hannig (1995). The X-ray images showed that, during mastication of solid food, intermittent opening of the connection of the oral cavity to the naso- and dorsal oropharynx can be observed and that food residue is left on the back of the tongue after swallowing, which provides an after-taste and a reservoir for aroma molecules to be detected long after the food bolus has left the oral cavity.

9.2.2 Echo-sonography for measuring tongue movement

Ultrasonic echo-sonography has been used extensively in speech research (Green and Wang, 2003; Stone, 2005) and in studies of dysphagia (Casa *et al.*, 2003) as it has an adequate rate of data capture and it is non-invasive. Two forms of display are commonly used: B-mode imaging which shows a

two-dimensional slice through the body (De Wijk *et al.*, 2006b) and M-mode which depicts movement as a function of time (Hashimoto and Shinoda, 1999). The sonograph is based on the time of flight of high-frequency pulses emitted from the probe and is sensitive to changes in the acoustic impedance of the tissues. Thus the interface between the tongue surface and air generates a strong reflection but when the tongue touches the hard palate some energy passes through the interface, allowing the hard palate to be visualised and tongue palate contacts to be identified. De Wijk *et al.* (2006b) recently explored the use of ultrasonic imaging for the quantification of oral movements, especially tongue movements, during oral processing of semi-solid foods. Their interest was in determining the effects of specific food properties, such as taste (sweetness and bitterness) and texture (viscosity and creaminess) on the oral movements. These movements were characterised into three time periods corresponding to different processing phases, the bulk phase when the food is taken into the mouth and eaten, the swallow phase when the food is being swallowed and the clearance phase when the food is being cleared. The ultrasound study demonstrated that oral movements were determined by food properties, such as taste and texture as well as the rated attribute.

9.2.3 Magnetic resonance imaging (MRI)

Magnetic resonance imaging (MRI) and functional MRI (fMRI) have a number of advantages over other imaging techniques in that they provide direct quantitative and non-invasive measurements of body tissues such as the brain, skin, oral cavity and the gastrointestinal tract (Marciani *et al.*, 2001a,b; Fillery-Travis *et al.*, 2002). fMRI has had most success in producing *in vivo* high-resolution images of static tissues such as the brain to measure for example, the response as a bold signal in a specific part of the brain to sensory stimulus like taste (O'Doherty *et al.*, 2001; De Araujo *et al.*, 2003a; Schoenfeld *et al.*, 2004; Rolls 2005), smell (Marciani *et al.*, 2006a), pleasant touch (Francis *et al.*, 1999) and texture (Zald and Pardo, 2000; De Araujo *et al.*, 2003b, De Araujo and Rolls, 2004; Miyamoto *et al.*, 2006; Alonso *et al.*, 2007). MRI has been used to determine morphological characteristics of skin tissue (Mirrashed and Sharp, 2004) and skin hydration/drying (Ablett *et al.*, 1988; Loden, 1992; Zemtsov, 1993). However, most MRI studies have been conducted for the diagnosis of pretherapeutic stages of tumours of the oropharynx, the oral cavity and the floor of the mouth (Lenz *et al.*, 2000) and it is in the clinical setting that the technique of MRI and that of computed tomography has developed as non-invasive methods for diagnostic evaluations.

The process of eating and drinking has been observed *in vivo* by application of time resolved MRI (Buettner *et al.*, 2001). The study was aimed at elucidating the timing and performance of the physiological organs involved in mastication and swallowing. It was shown that, during the sequence of

mastication, the mouth protects the airways from obstruction by food particles by setting up a physiological barrier that is capable of retaining volatile molecules within the oral cavity. These barriers allow access of odorants to the nasal cavity only at certain times during the eating process, which is controlled by the texture of the food and the amount of food present in the mouth. Although potentially ideal, MRI is currently too slow to resolve some of the more rapid movements of the tongue made during mastication.

MRI is being used extensively to measure real-time processes in the gastrointestinal (GI) tract (Marciani *et al.*, 2001a, 2006b; Fillery-Travis *et al.*, 2002; Hoad *et al.*, 2006, 2007) and the first basic engineering models for digestion (Stoll *et al.*, 2000; Spratt *et al.*, 2005) are starting to appear. Results show that the stomach behaves as a poorly mixed system in which the walls oscillate, causing some surface mixing and erosion. *In vivo* imaging of inter-gastric gelation of model food (Hoad *et al.*, 2004) and gastric emptying (Marciani *et al.*, 2001b) have demonstrated that the time taken for complete digestion depends on the original food structure, with more highly structured food taking longer to digest.

9.2.4 Video rate endoscopy for visualisation of surface structures

Adams and co-workers (2007) applied video rate endoscopy for the visualisation of food materials in the mouth and reported on the distribution of food material in the oral cavity and clearance behaviour due to salivary flow. They were able to collect sub-millimetre resolution, real-time images of the oral mucosal surfaces from around the mouth from people both before and after eating, with the advantage that the subject could sit in a natural sitting position, which is more realistic. The principles of video rate confocal endoscopy have been described by Watson *et al.* (2002), who also verified the safety and flexibility of the technique to probe soft tissues in the body. Both fluorescent imaging/spectroscopy and reflectance imaging are possible and, as the soft tissues can generate contrast, it is also possible to use confocal imaging. Fluorescent labelling of the mouth structures was found to produce greater differentiation between the features of interest, and the images revealed information on both the structures within the oral cavity and on the product residue after eating. Analysis of the fluorescence emission spectra could give quantitative information about the food residue left after eating. Fluorescent images collected from the surface of the tongue after processing different viscosity oils (Adams *et al.*, 2007) showed evidence of emulsification and indicated that droplet break-up is a function of viscosity.

White light reflectance imaging was used to visualise the near-surface aspects of arterioles and capillary loops supplying blood to the mouth. The response of the blood flow to cold thermal stimulus (Adams *et al.*, 2007) and to trigeminal stimulus from a cooling agent such as menthol (Appelqvist,

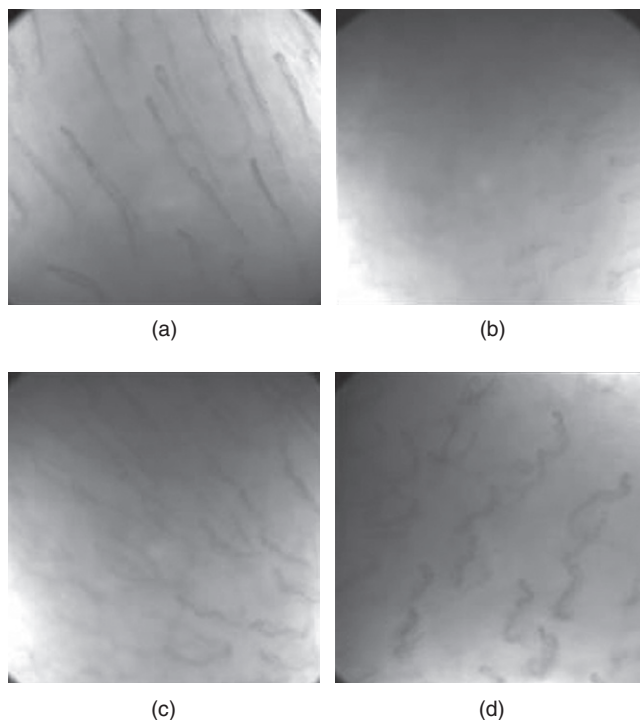


Fig. 9.1 Incoherent epi-illumination white light images from the inner cheek before (a) and after (b) application of ice, and after (c) direct application of a menthol mint and 5 min (d) after application. The capillary network is clearly visible under the oral mucosa in image (a) whereas the capillary structures virtually disappear due to reduction in blood flow after the ice was applied in image (b). Image (c) shows a slight reduction in contrast and a change to the capillary characteristics which increase as shown in image (d) where the structures appear more dilated probably because the oral mucosa has been slightly irritated by the menthol in the mint. The image width is about 1.5 mm. (Images based on Adams *et al.*, 2007, modified from Appelqvist, 2005.)

2005) was observed (see Fig. 9.1). This imaging mode was used to compare the response of blood flow on the cheek after placing either a block of ice or a menthol mint against the cheek surface. It can be seen that the contrast of the blood capillaries diminishes in Fig. 9.1(b) after the ice has been applied compared with pre-treatment (Fig. 9.1a) because the capillaries constrict and the blood flow reduces, which returns to normal shortly (Fig. 9.1a). In comparison, the capillaries after having a menthol mint against the cheek surface initially show a reduction in contrast associated with reduced blood flow (Fig. 9.1c), but then increase in contrast beyond the initial state, suggesting slight irritation of the oral mucosa (Fig. 9.1d). For highly detailed tissue structural information and to collect data on the food residues

remaining on the tissue surfaces, non-confocal fluorescence imaging in combination with a strong contrast agent such as sodium fluorescein was used, which gave good spatial and temporal resolution. The results from model biopolymer solutions that were swirled and spat out indicate that there is inhomogeneous coverage of the mouth, that this is related to the underlying structure of the tongue and that the rate of material clearance is dependent on the surface contact that occurs.

Adams *et al.* (2007) also confirmed that an increase in viscosity also increased the amount of residue left (Goulet and Brudevold, 1986) and that the rate the mouth processed the residue and cleared it from the oral cavity was influenced by salivary flow rates and rheological properties. Previous results have reported a bi-phasic clearance for solutions (Bashir *et al.*, 1995); however, Adams *et al.* (2007) found that they could fit a single exponential to their clearance data. As techniques improve in resolution and can measure the processes that occur directly, more accurate quantitative data will be obtained, which will challenge both some data in the literature and conclusions that have been accepted in the past.

Direct measurement of the food left in the mouth after eating without further disrupting it will be important to increase our understanding of food material behaviour in use and its impact on sensory perception. The potential for this technique is that it will be able to examine a wide variety of food materials ranging from aqueous solutions to solid foods, including multicomponent food. Dual channel imaging and different fluorescent labels will enable simultaneous investigation of two or more components in complex food. The technique also allows the determination of the spatial distribution of residue and the visualisation of structures that make up the mouth coating.

Imaging modalities currently used to diagnose disease in the oral cavity could potentially be used to visualise the oral processing of food. These include plain radiography (panoramic radiography and intraoral radiography), nuclear medicine scintigraphy, ultrasound (US), computed tomography (CT), MRI and positron emission tomography (PET) (Rumboldt *et al.*, 2003; Ng *et al.*, 2005). It will be the advancement in the visualisation techniques in the medical sciences that will ultimately be used by the food researchers to understand the mechanisms of oral processing and food behaviour.

9.3 Measuring solid food fracture breakdown upon oral processing

The effect of oral processing on solid food materials and the elucidation of the mechanisms by which food such as chocolate, nuts, meat and hard cheese fracture and breakdown during mastication has been widely

investigated (Szczesniak, 1963; Lucas *et al.*, 2002; Öhgren *et al.*, 2004; Loren *et al.*, 2007; Van den Berg, 2007; Brink *et al.*, 2007). Models such as the three-dimensional model for the processing of solid and semi-solid food, based on analysis of expectorated food, have been proposed (Hutchings and Lillford, 1988). The breakdown model based on meat products has three principal dimensions, which were loosely defined as the 'degree of structure', 'degree of lubrication' and 'time'. The model describes the processing of a solid food through these dimensions as a food-specific 'breakdown path' in which the bolus travels until a physiological trigger is met, which determines swallowing or not. The nature of the mechanical breakdown of these solids is dependent on the microstructure and the oral movements of the mouth in response to the changes in physical structure of the food. Having the ability to measure these variables from the mastication process, the fracture behaviour and the change in food microstructure is important to improve our understanding of oral behaviour of food and its relation to sensory perception.

9.3.1 Electromyography (EMG) for texture characterisation

Electromyography (EMG) is the recording of the electrical phenomena that determine muscle health, muscle excitation and contraction. Electromyographic and jaw movement (kinematic) recordings are commonly used to study mastication, from which several variables can be measured, such as amplitude of jaw opening during chewing and speed of jaw movements. Other factors that can be measured are the mastication force needed to break down solid food and the duration of mastication and rate of chewing (Perlman, 2006). Surface EMG is a technique based on attaching bipolar electrodes onto the four main masticatory muscles (masseter and temporal) and measuring the activity of the muscles during mastication of solid-like food where considerable activity from chewing using large mandibular movements can be generated in the jaw closing muscles. This technique has been used to compare activity in the jaw in subjects to evaluate oral processing of model foods with different hardness (Veyrune *et al.*, 2007) and tenderness of meat (Veyrune and Mioche, 2000). Since mastication is a physiological process controlled by the central nervous system and modulated by inputs from the mouth, information gained from EMG has shown that the mastication process adapts to changes in characteristics of the food. For example, the masticatory forces measured during chewing of solid food decreased as the food started to break down and the initial force required fracturing solid foods increased with increased hardness. A number of studies using EMG have been conducted to assess texture characteristics such as tenderness, hardness, crunchiness and juiciness, on products such as carrots, biscuits, cooked meat, cooked rice and cheese, and close-fitting correlations have been found between texture sensory attributes and EMG measurements (Gonzalez *et al.*, 2001).

9.3.2 Microscopy and large deformation measures to probe structure–mechanical relations

Techniques that have been used to determine the effect of oral processing on soft-solids such as mixed protein–polysaccharide gels have included large deformation measures and visualisation techniques simultaneously to allow structural changes such as failure points and crack propagation in composite food to be followed directly under dynamic conditions (Loren *et al.*, 2007).

Confocal laser scanning microscopy (CLSM) and large deformation measurements

Commercially, tensile stages have been developed with sensitive load cells, which can be placed under the confocal laser scanning microscope (CLSM) because of their small size. Analyses of solid materials (George *et al.*, 2003) and visualisation of stress concentration in polymers (Olmos *et al.*, 2002) can be conducted and time resolved measurements of structure and consistency can be used to explore material failure characteristics in both tension and compression.

Van den Berg (2007) used CSLM imaging under uniaxial compression and showed that protein–polysaccharide phase separated gels fractured through the protein network and only at a few places, whereas stranded gels fractured in many places, which was accompanied by coarsening of the protein network. The number of fracture sites and the rate of crack propagation in a food material have great impact on the perceived sensory properties. The way a structure deforms and breaks under stress is crucial for properties such as flow and fracture behaviour, sensory perception of structure, water release and mobility and release of active compounds. The perceived texture of foods is largely determined by structural rearrangements during the deformation process (Lillford, 2000, 2001) and having the ability to measure food failure behaviour under relevant physiological parameters such as time, temperature, stress and shear is important, since it mimics to some extent the breakdown of a food during consumption. Understanding the mechanism by which food deforms and breaks down at the microstructure length scale gives the food industry important knowledge about the structure–mechanical relationship of their ingredients so that better prediction of food sensory behaviour can be determined and foods can be designed with the desired texture and functionality.

Analysing fractures in food systems by means of microscopy techniques is a relatively new approach and has been useful in demonstrating the influence of the microstructure on material failure properties. Plucknett and co-workers (Plucknett and Normand, 2000, Plucknett *et al.*, 2001) carried out some of the first dynamic experiments on the fracture behaviour of gelatin/maltodextrin and gelatin/agarose gels using CLSM in combination with a tensile stage. They observed that these materials fractured differently because of the interfacial properties such as the bonding energy between

the two components and the mechanical strength of the networks in the two phases which determine their ability to deform and flow. For example, Rizzieri and co-workers demonstrated ductile behaviour in a gelatin/maltodextrin system using a specially built tensile stage in combination with an environmental scanning electron microscope (ESEM) (Rizzieri *et al.*, 2003).

Hermansson and co-workers have extensively used CLSM to follow transient changes in natural food that have been manipulated directly through, for example, applying a notch in complex structured foods such as cheese, in order to monitor the fracture line through the food microstructure and the rate at which the crack tip moves (Loren *et al.*, 2007). For practical reasons a small notch is made in the sample, so that the start of the crack can be induced and the microscope can be focused directly on that part of the sample. As the material is deformed, the notch widens and starts to move through the sample following the weakest part of the structure. The tensile stage makes it possible to simultaneously measure the microstructure and the stress–strain properties on the micron scale. CLSM combined with time-dependent image analysis allows data from the tensile stage to be related to the individual sequential micrographs, which capture the progress of the crack tip, which allows comparison of the required deformation for the same crack length. The importance of investigating the effect of microstructure on the breakdown behaviour of food materials is that it will provide information on the relationship between the sensory perception of texture and the mechanical behaviour of the food.

Øiseth and Lundin (2008) have used CLSM in combination with deformation measurements to investigate the fracture mechanics of plant tissues such as carrots both raw and after processing to determine the effect of processing on failure performance of whole pieces of vegetable. Figure 9.2 shows the force–distance curves from raw and blanched carrots during tensile testing and indicates that the raw carrot withstands more stress before it fractures compared to the heat treated sample. The CLSM micrographs (Fig. 9.2b–d) show how cracks propagate through the heated carrot tissue and the last two images (Fig. 9.2e and f) show CLSM 3D representations of the fractured tissue of (e) raw and (f) blanched carrot strips. In the raw sample, the crack has propagated through the cell walls as can be seen by ruptured cells, whereas in the heat-treated samples the tissue has separated mostly between the cells due to the weakening of the middle lamella after heating. These studies of microstructure-failure behaviour for plant materials provide the potential to determine the relationship between agro-nomic environment, processing and mechanical behaviour on sensory perception of texture and breakdown.

In order to fully map the structural dynamics of composite foods leading to failure and breakdown there is a need for sophisticated measurement techniques for fracture dynamics at the microscopic level in addition to traditional large-scale rheological methods. It is clear that there are advantages in combining large deformation and/or rheological measures while

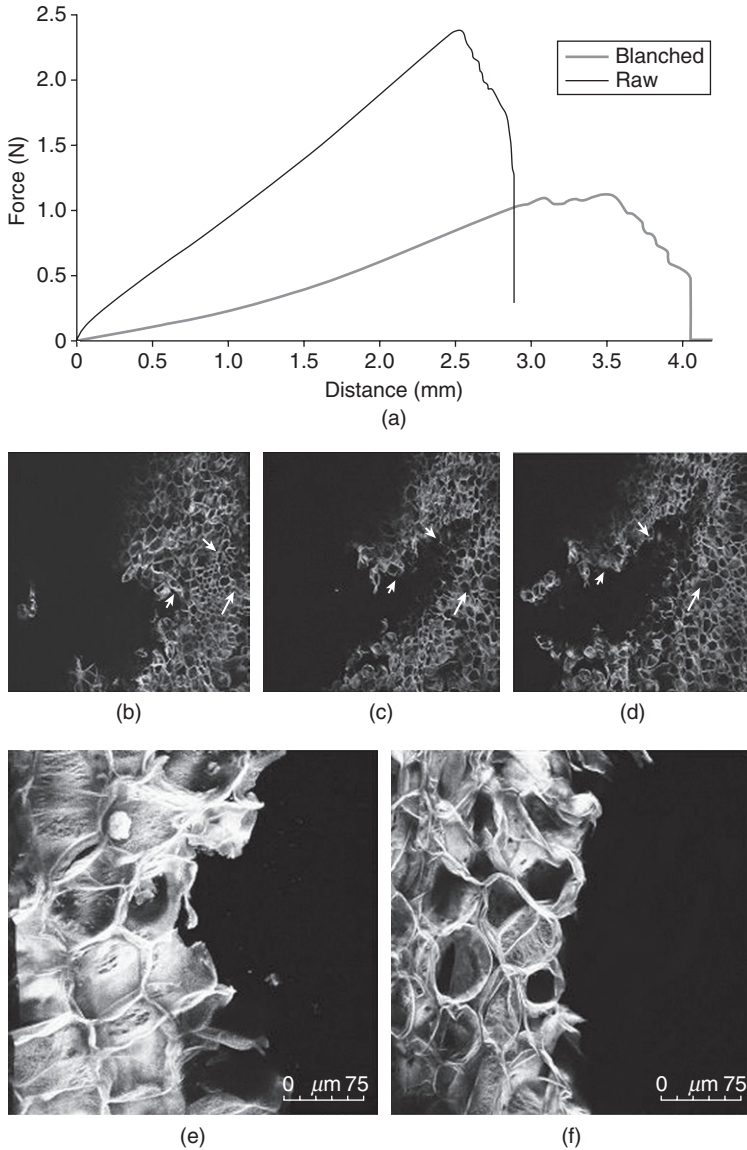


Fig. 9.2 Tensile testing of carrot strips. (a) Force–distance curves from raw and blanched carrots during tensile testing. The raw carrot withstands more stress before it fractures compared with the heat-treated sample. (b)–(d) Confocal laser scanning micrographs showing how the crack propagates through the heat-treated carrot tissue. The arrows serve as viewing guidance to three different cells during the testing process. Image size $1.55 \times 1.55 \text{ mm}^2$. The last two images show CLSM 3D representations of the fractured tissue of (e) raw and (f) blanched carrot strips. In the raw sample, the crack has propagated through the cell walls as can be seen by ruptured cells, whereas in the heat-treated samples the tissue has separated mostly between the cells. (Unpublished results, Sofia Kihlman Øiseth, with permission from CSIRO.)

viewing the behaviour of food materials directly under such stresses in real time, to simulate oral processing conditions. It should be noted that for very rapid fracture mechanics, CLSM may not be sufficient to follow the structural changes because of the limitations in scanning rate and so other microscopy techniques such as optical, video and new techniques such as multifocal multiphoton microscopy, which allows fast acquisition of 3D data may need to be considered (Egner *et al.*, 2004; Egner and Hell, 2005). For an excellent review of the modern CLSM and how it can be used to examine and control the microstructure of complex foods, including measuring mass transport and analysis of CLSM images, the reader is referred to Loren *et al.* (2007).

9.3.3 Chew and expectorate analysis

Chew and expectorate experiments have been used to measure the process of diminution of solid food materials such as peanuts and characterised visually using light microscopy. Figure 9.3 shows light micrographs of five peanuts

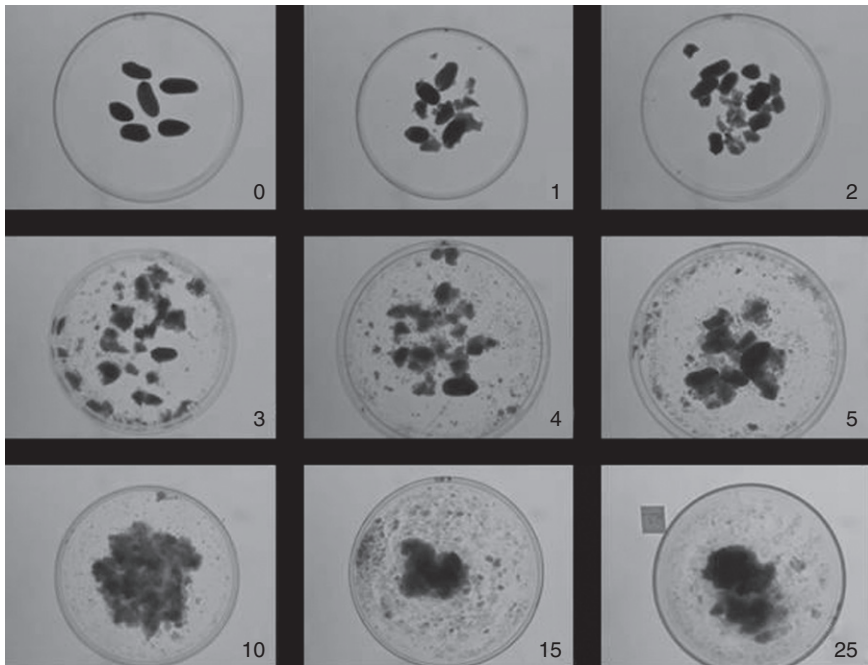


Fig. 9.3 Spit out samples of five peanuts with increased levels of mastication ranging from 1 chew to 25 chews. It is clear that the peanuts are fragmented into small pieces by chew 4 and thereafter a bolus starts to form with the saliva from chew 5 up to chew 10, which indicates time to swallow. Forced chewing beyond time to swallow causes the peanut fragments to disperse. (Zerdin and Kelly, 2008 with permission from CSIRO.)

having been chewed to different degrees in the mouth before being spat out. It is clear that, as the number of chews increases to about 5, the peanuts are continually being fragmented into smaller pieces, after which further chewing starts to produce a bolus between the peanut fragments and saliva (chews 5 to 10) and at this point the food is ready to be swallowed. Chewing beyond this point seems to cause increased dispersion of the peanut pieces from the main bolus mass and was reported to be 'uncomfortable' (Zerdin and Kelly, 2008). The advantage of this approach is that all the mouth physiology, mechanics and dynamics are inherent in the characterisation of the spat-out bolus, which provides direct information on the impact of oral processing on the fracture and breakdown of solid foods and on the changes in soft material structures due to mixing with saliva and shearing by oral movements. The latter is discussed further in the next section.

9.4 Measuring soft-solid food breakdown and interaction with saliva

Crucial in the oral processing of soft-solid foods such as sour cream, yoghurt, custard and soft cheeses are the structural and phase changes that occur from heating or cooling to mouth physiological temperature, mixing with saliva, the contact with the oral surfaces and the flow involved in squeezing and shearing between palate and tongue. In general the oral behaviour important for mouthfeel in soft materials is the rate of breakdown delivered primarily through tongue movement and mixing with saliva (De Wijk *et al.*, 2006a) and film-forming and binding on the oral surfaces delivered through squeezing the food between surfaces (Sala *et al.*, 2008). In the following sections the measurements used to determine structural changes of key food components (starch and emulsions) after oral processing and mixing with saliva and the film-forming and binding of food emulsions on oral surfaces will be discussed.

9.4.1 Measurement of physiological effects of saliva on food components

Human whole saliva (HWS) is a complex physiological secretion that performs essential functions such as protection of oral health and lubrication of mouth tissues, as well as pre-digestion of food through dilution, enzyme hydrolysis and ionic interaction (buffering) which impact on food structure and breakdown rates. The interaction between saliva and food components both *in vitro* by adding saliva to food or *in vivo* by mixing the food in mouth and spitting out has been extensively studied particularly for starch-based (Appelqvist *et al.*, 1995; De Wijk *et al.*, 2003, 2006a) and emulsion-based foods (van Aken *et al.*, 2005, 2007; Vingerhoeds *et al.*, 2005; Silletti *et al.*, 2007a,b,c).

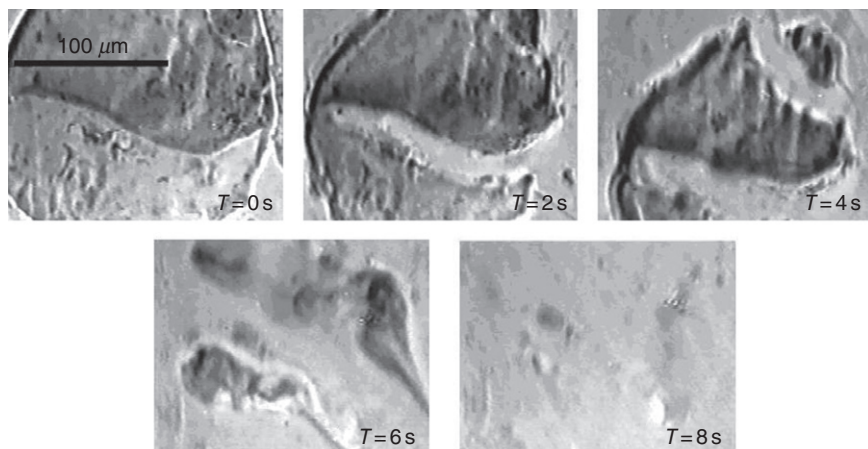


Fig. 9.4 Light micrographs of cooked starch being digested by human saliva on an optical slide in real time. The micrographs clearly show the starch granule being hydrolysed as the saliva imbibes from right to left in each image. (Adapted with modification from Appelqvist *et al.*, 1995.)

Saliva and starch

Mixing of saliva with food that contains amylase-sensitive starches often induces fast breakdown of food. When starch is heated, the granules hydrate and swell to provide texture, and become highly sensitive to the enzyme alpha amylase found in human saliva. This leads to fast digestion of starch in which the granule structure breaks down and disappears as shown in Fig. 9.4, resulting in a loss in viscosity of the product in the mouth (Engelen *et al.*, 2003a,b; De Wijk *et al.*, 2004). The rate of hydrolysis of starch granules can be altered by the type of starch selected (e.g. chemically modified) and the food matrix that surrounds it, by reducing the accessibility of the amylase enzyme to the starch granule (Appelqvist *et al.*, 1995). The importance of amylase action on controlling texture attributes such as creaminess was demonstrated in a range of custards where slowing the enzymic breakdown increased the creaminess ratings in a sensory test (Janssen *et al.*, 2007).

Saliva and emulsions

Saliva has been shown to interact with other food components through shear induced mixing generated by the tongue moving against the hard palate, which can induce shear thinning and can cause coalescence of oil droplets (van Aken *et al.*, 2005, 2007; Vingerhoeds *et al.*, 2005; van Aken, 2007). The oil droplets in unflocculated emulsions mixed with saliva either in the mouth or *in vitro* were found to aggregate (Vingerhoeds *et al.*, 2005; Silletti *et al.*, 2007a) and the flocculation mechanism (Silletti *et al.*, 2007b) characterised by CLSM and turbidity measurement is believed to be

regulated by a balance of forces including depletion, van der Waals forces and/or electrostatic interactions between emulsion droplets and salivary proteins. It is possible that the large bulky proteins such as the glycoproteins in saliva could cause depletion between the oil drops driving the droplets together and flocculation. On the other hand, salts in the saliva could act as a charge screen reducing the interaction between salivary proteins and the oil drops. Finally, displacement of emulsifier at the oil surface could potentially take place, causing destabilisation of the emulsion. Having a better understanding of these potential mechanisms at play in the physiological environment gives potential for food scientists to design the emulsion interface by selecting, for example, the appropriately charged emulsifier to stabilise the emulsion so that the tendency to flocculate can be controlled (Silletti *et al.*, 2007c).

To gain better understanding of the mechanisms underlying food performance under oral conditions, a number of researchers have started to integrate *in vitro* and *in vivo* instrumental measurements with sensory studies on a systematic set of food samples (De Wijk *et al.*, 2006b). These workers used *in vitro* and *in vivo* instrumental tests to determine the surface and bulk-related properties of the food bolus that relate to the perceived oral texture of products such as starch-containing custards. Measurements included the food's infrared reflectance to determine oil distribution, turbidity measures of spat-out rinse water to assess precipitation and flocculation effects, digital imaging of spat-out foods, which were analysed with respect to homogeneity and the friction (lubrication) between the food and the oral tissue, using tribology (De Wijk and Prinz, 2005, 2006; De Wijk *et al.*, 2006a). A model for custard products was proposed based on the results characterised by three main sensory dimensions: roughness to creaminess driven by lubrication due to fat, melting to thickness driven by viscosity due to starch hydrolysis, and airiness to heterogeneity primarily driven by mixing with saliva. The approach of integrating 'instrumental-sensory-ingredient' relationships firstly will facilitate an understanding of what instrumental techniques actually measure and secondly will provide the product developer with information on ingredient functionality to optimise specific product ranges that have been investigated.

9.5 Measuring soft-solid food interactions with oral surfaces: lubrication and binding

Friction at the interface of the food with the oral mucosa is detected by mechanoreceptors in the oral mucosa. Friction forces depend on factors such as the rheology and lubricious properties of the saliva (Bongaerts *et al.*, 2007b), the food and mucosa and the speed with which the two surfaces, e.g. the food bolus and tongue, move across each other. The force exerted by the tongue on the food bolus also contributes to the friction

coefficient of the food. Oral friction also depends on the difference between sliding and rolling motions as well as on the shear rate. It is likely that during mastication there are different rolling and sliding motions dependent on the food eaten.

9.5.1 Tribology measurement

Friction between two sliding surfaces in general depends on (a) the properties of the lubricating substance in the contact zone separating the surfaces (Cassin *et al.*, 2001) and the presence of an adsorbed layer, (b) the pressure exerted pushing the two surfaces together which is dependent on the relative speed and (c) the initial roughness and mechanical properties of the surfaces in contact. Friction has been measured over a wide range of entrainment speeds and Stribeck curves have been obtained spanning the boundary, mixed and hydrodynamic lubrication regimes (Cassin *et al.*, 2001; Butt *et al.*, 2003). The boundary regime is representative of where the surfaces are in contact with each other and the entrainment speed of fluid into the contact zone is slow and depends on the fluid constituents to form boundary films. In this regime the coefficient of friction is not dependent on the speed and the load. Boundary lubrication is characterised by an ‘immobile layer’ adsorbed to the surface that does not take part in the hydrodynamic flow of the bulk fluid (Georges *et al.*, 1993; Spikes, 1996). At high speed the hydrodynamic regime operates where a lubricant film entrained into the contact zone produces sufficiently high fluid pressure to fully separate the surfaces. The film thickness and friction generated in this type of lubrication are dependent on the viscosity of the fluid at high shear rate present in the lubricant film. Between the boundary and hydrodynamic regime lies the mixed regime of lubrication. Here the asperities on the surfaces are on the same length scale as the thickness of the lubricant film, so the contact load is borne in part by fluid pressure and in part by asperity contact pressure. In this regime the coefficient of friction decreases with increasing speed.

9.5.2 Rheology and tribology of saliva

The rheology and lubricating properties of saliva are highly important owing to its influence on oral health and physiochemical processes within the oral environment. Rheological measurement of HWS until recently has often been performed erroneously and/or limited to the viscosity at a single shear rate.

Stokes and Davies (2007) have re-examined the rheological measurement of whole saliva and have demonstrated the importance of controlling the environment of the rheological measurement and the timing. The recommendation is that saliva is tested immediately after expectoration and that a thin layer of surfactant solution is applied around the rim of the rheometer plates to reduce protein migrating to the air–liquid interface.

Stokes and Davies showed that the viscosity and viscoelasticity of HWS depended on the method of stimulation where mechanical action stimulated slightly shear-thinning and relatively inelastic saliva, while acidic solutions (e.g. citric acid) stimulated saliva that was highly elastic and shear-thinning. For acid-stimulated saliva, the results showed a high ratio between the primary normal stress and the shear stress which, for such a low viscosity fluid, indicated that saliva's elastic properties dominated its flow behaviour and indicated its lubrication role within the oral cavity. The boundary lubrication properties of mechanically stimulated HWS were demonstrated by Bongaerts *et al.* (2007a), who measured the lubricating properties of saliva in a soft hydrophobic contact and investigated the impact of applied load, entrainment speed and surface roughness on lubrication. It was found that an increase in surface roughness decreased the lubricating properties of saliva while it was increased for water. They also showed that the boundary lubricating properties of HWS did not change with time, whereas the bulk viscoelastic properties degraded over time. This suggests that the large bulky glycoproteins responsible for the rheology of saliva are not responsible for its boundary lubricating properties, being too large to fit the gap.

Recently, interfacial shear rheology was used to probe the relationship between surface shear elasticity of saliva and loss of lubricity when compounds such as polyphenols are added, resulting in astringent mouthfeel (Rossetti *et al.*, 2008). The glycoproteins contained in saliva are highly surface active and tend to migrate to the air–liquid interface, forming a solid-like surface film that is responsible for the lubricating properties of saliva (in the mixed regime) during mastication. The addition of citric acid and polyphenols appears to disrupt this surface film reducing the interfacial shear elasticity G' of saliva and thereby reducing lubrication of oral tissues. Another explanation by the investigators was suggested in which the added food components cause the glycoproteins to aggregate and thereby reduce the interfacial shear elasticity. However, in general, aggregation of protein often results in an increase in G' and therefore what might be occurring is that the proteins in the saliva are being displaced by surface active components in the food which provide a new surface with different lubricating properties.

9.5.3 Tribology measurements of soft-solid food emulsions *ex vivo*

Tribological measurements have been used as a tool to predict the lubrication and sensory mouthfeel of model biological fluids (Cassin *et al.*, 2001; de Vicente *et al.*, 2006a,b; Bongaerts *et al.*, 2007a) and model food emulsions (Dresselhuys *et al.*, 2008a,b). In general, the sensory perception of soft-solid food during mastication is dependent on its frictional behaviour as a thin film under high shear rates in the gap between the tongue and hard palate. Sensory attributes such as slipperiness, smoothness, fattiness, astringency and creaminess have been shown to correlate with the frictional behaviour of emulsions between oral surfaces (Malone *et al.*, 2003a; Lee

et al., 2004; De Hoog *et al.*, 2006) and certain rheological parameters such as viscoelasticity (Jellema *et al.*, 2005).

The lubricating behaviour of foods has commonly been analysed with standard tribological equipment such as the pin-on-disc or ball-on-disc set-up (de Vicente *et al.*, 2006a,b). However, the problem has been to select a suitable surface that will mimic the key characteristics of oral mucosa for generating relevant friction measurements *ex vivo*. Important characteristics of the oral surface, which are thought to influence the behaviour of lubricating substances, are the roughness of the surface, the deformability and the wetting characteristics (De Hoog *et al.*, 2006; Dresselhuis *et al.*, 2008a). It has been shown by De Hoog *et al.* (2006) that the load-dependent behaviour of the kinetic friction measured for food emulsions changed depending on the surfaces used when they compared hard glass-metal smooth surfaces with mucosal surfaces from pig tongue or oesophagus (Prinz *et al.*, 2007). They found that the friction decreased with increasing load for the mucosal surfaces, but was constant for glass-metal surfaces.

In the lubrication of oral surfaces, such as the tongue and palate, it has been suggested that the boundary and mixed regime dominate (Malone *et al.*, 2003a) and that the lubricating nature of saliva is most effective in the mixed and boundary regime (Bongaerts *et al.*, 2007b). While this can be demonstrated *ex vivo* on artificial mucosal surfaces, it has been questioned whether the boundary regime in the mouth can be obtained (Lundin, 2008) since the mastication process involves the tongue compressing food between surfaces rather than having food entrained into the soft contact zone as in the tribological experiments. In addition, it is important to note that in the oral cavity the surfaces are mostly soft and deformable, and the food fluids are often non-Newtonian (van Aken *et al.*, 2005) so that the shear viscosity is high at low shear rates, unlike water in which the shear viscosity remains low for most shear rates relevant to oral processing of soft food and therefore does not act as a good lubricant.

Typically, the oral regime is characterised by a mean contact pressure and a low traction speed during eating (Chojnicka *et al.*, 2008). The contact pressure in the mouth is estimated to be approximately 30 kPa and the shear rate applied in the mouth during mastication varies between 1 and 1000 s⁻¹ (Sharma and Sherman, 1973). Recent studies on lubricating properties of emulsions (Dresselhuis *et al.*, 2008b) and protein aggregates (Chojnicka *et al.*, 2008) have tried to mimic more closely the relevant oral conditions in which lubrication due to the oral processing of soft-solid foods occurs and have tried to take into account the changes that occur to saliva during eating (Bongaerts *et al.*, 2007b), and the amount and properties of the adhered food. The surface roughness changes as a result of abrasion during eating and the traction speed that is relevant for the oral regime (Malone *et al.*, 2003a; Lee *et al.*, 2004). Eating itself also induces changes both in the structure of the food (Malone *et al.*, 2003b; de Wijk and Prinz, 2005) and on the oral tissue that can have an effect on the lubrication properties sensed.

9.5.4 Evanescent wave spectroscopy (EWS)

Evanescent wave technology has been used to investigate specific and non-specific adsorption of biomolecules to surfaces without the need for labelling (Jonsson *et al.*, 1991; Knoll, 1991). Optical biosensors based on this technology allow *in situ* observations of real-time kinetics of the processes of molecular physi- or chemisorption. Information gained from this measurement includes association equilibrium constants, the timescales of the deposition processes and the adsorbed amount per unit surface, so that binding isotherms of biomolecules to specific surfaces can be obtained. Cassin *et al.* (2001) investigated the adsorption properties of polymers onto biomimics of oral mucosa using evanescent wave spectroscopy (EWS) and showed that the polymer that adsorbed onto solid surfaces was able to reduce friction in the boundary lubrication regime from tribology measurements. Biopolymers such as guar, which does not adsorb on surfaces, showed high friction in boundary lubrication but still promoted the onset of mixed lubrication; thus friction started to fall from its boundary values at low speeds.

Further studies by Appelqvist *et al.* (2004) using EWS demonstrated that the residence time of food coatings could be controlled by the level of binding to the oral mucosa, which was dependent on the overall charge of the surface of the food component. Emulsions were designed with a stabiliser that interacted with the oral mucosa to enhance surface deposition and increase the residence time of the oil droplets. The interfacial interactions between various emulsions and a mucin-covered solid substrate were investigated using EWS, and the influence of different emulsifiers on the extent of oil deposition onto oral mucosa was evaluated (see Fig. 9.5). The data showed that the chitosan emulsion adsorbed to the greatest extent onto the mucin film surface due to electrostatic interaction between the positively charged emulsifier and the negatively charged mucin film. The egg yolk stabilised emulsion which was slightly positively charged adsorbed to the mucin surface to a lesser extent and tween 60, which is non-ionic, did not interact with the mucin film. Increased binding increased the residence time for food films and impacted on the sensory properties perceived. Specifically, it was shown that emulsions that remained longer in the mouth increased the after-taste and flavour release after swallowing, which was measured directly in the headspace of the nasal cavity of subjects using atmospheric pressure chemical ionisation mass spectroscopy (APCI-MS).

9.6 Artificial mouth models

A review of the literature reveals that a wide range of artificial mouths have been developed to mimic some aspect of oral processes during eating (van Ruth *et al.*, 2000; Salles *et al.*, 2007) and to measure the flavour released

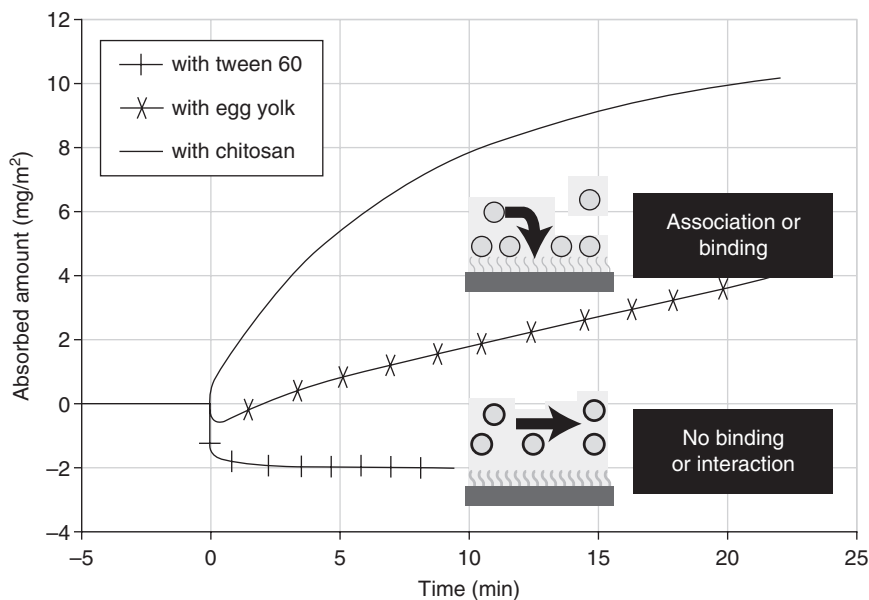


Fig. 9.5 Deposition of oil droplets in emulsions on mucous glycoprotein surface stabilised with different emulsifiers that adsorb to a mucin-covered surface to different degrees. Chitosan is the most positively charged emulsifier and is electrostatically attracted to the negatively charged mucin film. The proteins in the egg yolk are slightly positively charged and interact with the mucin surface to some degree, while the tween 60, owing to its non-ionic nature, did not show any adsorption onto the mucin covered surface. Measurements carried out at 35 °C. (Adapted and modified from Appelqvist *et al.*, 2004.)

from food components as a function of mastication and food breakdown (Roberts and Acree, 1995; van Ruth and Roozen, 2000; Rabe *et al.*, 2002).

It is clear that, while these artificial mouths have had some success in replicating specific areas of oral processing, there are no systems as yet available that can mimic the complex nature of the real mouth and therefore there will still be reliance on methodologies such as time intensity, descriptive analysis and discrimination tests in which key attributes can be extracted from real-time sensory data based on real consumption of food by subjects. The quantification of sensory perception is, however, difficult since oral processing of food is strongly coupled to changes in food texture and taste via a feedback mechanism. Sensory perception is also dependent on a hedonic aspect, which relates to cultural background and experience and the interpretation and integration of information from receptors via complex neural pathways in the brain provide a challenging investigation.

The focus in this section will be on the artificial mouth designs that are commonly used by a number of researchers and have had some validation in their simulation of real mouth conditions and processes. The most

commonly used artificial mouth is that developed by van Ruth and Roozen (2000). This is based on a plunger that is hemispherical, which crushes food against the bottom of a glass test tube and is mostly suitable for soft-solid food. The device allows for the introduction of saliva and the breakdown of soft foods at mouth temperature and head space sampling of aroma compounds. Results of aroma release from a range of foods (Hansson *et al.*, 2003; Mayr *et al.*, 2003) using this device appear to be similar to those measured *in vivo* monitored by proton-transfer-reaction mass spectroscopy. Roberts and Acree (1995) developed an artificial mouth based on a blender, which incorporated a purge and trap and allowed the addition of saliva and temperature control at 37°C. The blender speed was controlled to reproduce shear rates reported to occur during eating. The retronasal aroma simulator is a flavour analysis method that more closely simulates mouth conditions than existing headspace methods. The feature of the device (saliva addition, temperature regulation and shearing) allowed foods in different formats to be analysed and indicated different headspaces from dynamic headspace trapping.

Prinz *et al.* (2007) used an apparatus that simulated oral mixing that consisted of an electric motor that rotated the sample (semi-solid food). Also attached, which remained fixed, were a mixing vane, video camera, laser, optical sensor and temperature probe. This system was developed to measure changes in the viscosity of food due to physiological changes that are relevant in the mouth such as temperature, shear, dilution and structure breakdown. Samples recovered from the *in vivo* mouth were compared with those from the mouth simulator using an image processing technique which demonstrated that the simulator could mimic oral processing of soft-solids in real time. Finally, the most sophisticated artificial mouth is the one based on a design by Salles *et al.* (2007) in which they developed a chewing simulator that faithfully reproduces most of the functions of the human mouth. The active part of the system is a special cell that is fully actuated and computer controlled and can accurately reproduce shear and compression strengths and tongue function in real time. *In vivo* measures using the breakdown of peanuts in the mouth were compared with the results produced from the chewing simulator and found to be super-imposable.

9.7 Conclusion

Clearly, the structural changes in food that occur in the mouth and how they interact with the sensory receptors are important for delivering sensory perception. However, the challenge will be to both gain a deeper understanding of the various mouth processing mechanisms and to integrate this knowledge with the neural bases on which the multimodel aspect of food perception is delivered. The development of techniques that can probe and quantify in-mouth structuring and breakdown of food materials in either a

realistic biological mouth environment *ex vivo*, or measure non-invasively food–mouth interaction *in vivo*, will lead to establishing engineering rules that can be used to design food. The starting point for the design of structured foods is an understanding of the science underpinning the various performance functions, which is driven by the food microstructure. The response of structural changes in food to oral processing is also very dependent on the mouth conditions, such as the composition of the saliva and the mouth movements which are different for individuals, so that, within the context of food design models, these variations will need to be considered. Finally it will be the integration between our understanding of oral processing and neuroscience that will progress our understanding of how food is perceived, since interaction within and among the multisensory modalities is common and is highly relevant to food perception.

9.8 Future trends

For the future, direct quantitative and non-invasive measurement of oral tissues and the breakdown of food in real time will be possible. A framework of techniques used in an integrated manner to elucidate the mechanisms of food–oral tissue interactions and mechanical breakdown under a physiological environment will be possible, which will allow complete analysis of food behaviour and the ability of food technologists to design food more effectively with the desired performance and consumer benefits.

Already hand-held I-scan pressure mapping systems are available (from Tekscan) that use a laminated plastic film with up to 2288 pressure sensing areas. The sensor is very thin and flexible and can be designed to different shapes including the shape of the oral soft surfaces or the teeth. The technique will generate 2D and 3D pressure maps and can record as a movie up to 100 frames per second. This potentially can capture the changes in pressure induced as food is processed in the mouth, giving the investigator a real-time sequence of pressures produced during eating, and can identify the pressures required for solid food to fracture and components such as encapsulated actives to be broken, releasing their contents in the mouth.

Innovative techniques such as capsule endoscopy, which involves a tiny imaging camera (Hartmann *et al.*, 2004) have opened up new horizons for early diagnosis of disease but the potential for such technology to measure and visualise in real time the inter-oral and digestive processes in humans is possible. The SmartPill (SmartPill GI monitoring system, Anon. 2008) is a wireless capsule which measures pressure, pH and temperature as it transits the GI tract and has already been tested in clinical trials to determine the effect of food structure and composition on gastric emptying and ileal/caecal transit time. This technique also has potential for monitoring the physiological changes that occur in oral processing of food.

9.9 Sources of further information and advice

The review article by van Aken *et al.* (2007) on 'Food colloids under oral conditions' gives a very comprehensive overview of the structural and rheological changes of food during eating that are important in sensory perception. The review covers key aspects of the oral cavity, the physiology of saliva and, the interaction of food with the oral surfaces and salivary components, and introduces the reader to modern techniques for measuring oral processing. It discusses a selection of oral processing mechanisms relevant for soft solids, with an emphasis on oil-in-water emulsions.

Understanding and Controlling the Microstructure of Complex Foods published by Woodhead Publishing in 2007 (McClements, 2007) provides a review of the current understanding of significant aspects of food structure and methods for its control. It focuses on the fundamental structural elements present in foods such as proteins and fats and the forces that hold them together. The book discusses novel analytical techniques that provide information on the morphology and behaviour of food materials and examines how the principles of structural design can be employed to improve the performance and functionality of foods.

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Emerging flavour analysis methods for food authentication

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Abstract: This chapter starts with an overview of established flavour analysis methods for assessing food authenticity. Secondly the advantages and disadvantages of each method in combination with their application are discussed. Thirdly an integration of emerging methods with combined analytical techniques for validation of food authenticity will be reviewed. Finally data analysis techniques for determining relationships between instrumental flavour analysis, product authenticity and sensory quality are summarised.

Key words: food authentication, analytical flavour analysis.

10.1 Introduction

The impact of fraudulent activity on food authenticity has generated enormous public and political concern. As a consequence, verifying authentic from adulterated and/or contaminated food is often central to consumer acceptability, recognition of product quality and safety. According to European Union regulations, authentic foods must comply with the producer's declaration of ingredient composition, place of geographical and botanical origin, method of production/processing/preparation, genetic integrity, date of production and shelf-life (OJEC, 2002).

In principle consumers share the common notion that food products from a specific geographical origin reflect distinct methods of production, ingredients and sensory qualities that are often unique. From this aspect, consumer demand for high-quality products with clear regional identity in the form of Protected Designation of Origin (PDO), Protected Geographic Indication (PGI) and Traditional Speciality Guaranteed (TSG) will continue to increase in priority. In addition, indication of these regulations allows legitimate producers preferential market protection. In 2006 PDO, PGI and TSG regulations 2081/92 and 2082/92 were amended and replaced

by regulations 510/2006 and 509/2006 with the addition of regulation 1898/2006 (EC, 2006). According to the EU definition, PDO foods are distinguished by *terroir*, which is a sense of place expressed in the flavour of the food. Typical examples of PDO foods that are strictly produced, processed and prepared in a specific geographical area include Roquefort (cheese, France), 'Boeren-Leidse kaas met sleutels' (cheese, The Netherlands), Trentingrana (cheese, Italy), Olives noires de Nyons (table olives, France), Opperdoezer Ronde (potato, The Netherlands) and Altamura (bread, Italy). In the case of PGI foods the geographic association must occur in at least one stage of production, processing or preparation with examples consisting of Pachino (cherry tomatoes, Sicily Italy), Lechazo de Castilla y Leon (Lamb, Spain), Westlandse Druif (grapes, The Netherlands), New Season Comber Potatoes (Potatoes, England) and Miód kurpiowski (honey, Poland). For TSG foods the tradition and authentic method of preparation or composition must be represented in the character of the food, typical examples include Faro (Beer, Belgium), Falukorv (sausage, Sweden) and Boerenkaas (cheese, The Netherlands). Evidently additional means to provide regulations for emerging foods that are of organic, fair trade, environmentally friendly, sustainable and/or support animal welfare have received more attention.

Extensive literature exists on the detection and characterisation of authentic foods by sensory evaluation (Elortondo *et al.*, 2007; Nathalie, 2007). In particular, odour and flavour impressions have been commonly relied upon to distinguish authentic foods by geographical origin (Castro-Vázquez *et al.*, 2010; Esti *et al.*, 2010; Fischer *et al.*, 1999; Green *et al.*, 2011; Kallithraka *et al.*, 2001; Maitre *et al.*, 2010; Phillips *et al.*, 2010; Ryffel *et al.*, 2008; Stolzenbach *et al.*, 2011) and organic production (Callejon *et al.*, 2010; Jahan *et al.*, 2005; Kihlberg *et al.*, 2004; Talavera-Bianchi *et al.*, 2011; Thybo *et al.*, 2006; Walshe *et al.*, 2006). Other studies have applied sensory analysis to investigate food authenticity on the basis of botanical composition for honeys (Castro-Vázquez *et al.*, 2009), olive oil (Rotondi *et al.*, 2011), lamb (Lind *et al.*, 2009) and cheese (Carpino *et al.*, 2004). Nevertheless distinguishing foods using descriptive sensory analysis can be limited to individual assessor variability, sensitivity and concept of the perceived flavour quality (Maitre *et al.*, 2010). Thus individual sensory assessors may judge the same sensory quality differently resulting in poor product discriminability. For example, Frank and Kowalski (1984) showed that sensory data did not produce sufficient information to separate wines from different regions of France and the USA, with only 20% of assessors providing correct responses. Franke *et al.* (2005) reviewed the literature on analytical approaches to authenticate the origin of meat and concluded that no sensory studies exist which showed clear separation of meats on the basis of geographical origin. On the other hand, sensory assessments lack the possibility of identifying chemical compounds having an influence on flavour and the analysis of harmful substances. In turn, sensory data alone may not be

adequate to classify foods on the basis of product authenticity. Alternatively, when descriptive sensory assessors agree on perceived sensory concepts for a set of products, then analytical measurements are useful to characterise the sensory space. Particular emphasis has been placed on specifying sensory perceived flavour qualities by objective instrumental measures of volatile composition (Plutowska & Wardencki, 2007). From this aspect instrumental characterisation of food flavour profiles can act as a chemical 'fingerprint' of the product and can be applied to help determine product authenticity and quality.

10.2 Established flavour analysis methods for food authentication

10.2.1 Gas chromatography (GC) procedures used in food authenticity

Typical analytical approaches to determine food adulteration and guarantee quality have often involved wet chemistry for the quantification of specific marker compounds that distinguish a particular chemical component from another of known origin (Karoui & De Baerdemaeker, 2007). While quantifying specific chemical markers has been promising, the relative range of analytes required to be measured can be time consuming, and the limited knowledge of the variation in compounds from known origin is problematic. Thus usually no key chemical compound can be measured to substantiate product authenticity. On the other hand, traditional flavour analysis techniques that include compound separation such as chromatographic techniques with the addition of flame ionisation detectors (GC-FID), mass spectrometry (GC-MS), olfactometry (GCO), and high-performance liquid chromatography (HPLC), have been applied separately and in combination with sensory analysis to help characterise food authenticity. Liquid chromatography (LC), principally HPLC, is suited more to the detection of non-volatile compounds such as proteins, amino acids, phenolic compounds and carbohydrates, while gas chromatography (GC) is more appropriate for the detection of volatile and semi-volatile compounds. In principle, volatile compounds isolated from food are injected into the GC analyser and subsequently pass through the GC column where separation takes place due to specific compound interaction and absorption with the liquid stationary phase on the surface of the thin fused-silica capillary tube.

Of the various detectors FID offers good sensitivity, large linear response, reproducibility and is capable of measuring a wide range of volatile organic substances. This detector is simple and easy to operate, but following detection the sample is unfortunately unavailable for recovery. Other detectors consist of flame photometric detectors, thermal conductivity detectors, electron capture detectors, photo-ionisation detectors, thermionic detectors, flame photometric detector, atomic emission detectors, and ozone or fluorine-induced chemiluminescence detectors. These detectors give an

electrical response to the amount of analyte eluting from the column. Enhanced compound recognition, identification and quantification can be achieved by coupling GC with a mass spectrometer. In this technique analytes are subjected to high-energy electron impact ionisation when eluting from the column, which allows for mass analysis to then take place. Despite good identification of compounds this technique is time-consuming and expensive as a result of the purification steps required for analytes prior to injection. Thus volatile compounds from foods have been described as being short lived, where flavour isolation by absorbent trapping or distillation and concentration is difficult.

Extensive reviews of the literature have addressed GC analysis of aroma active compounds that contribute to food authenticity (Aznar *et al.*, 2001; Cayot, 2007; Luykx & van Ruth, 2008; Plutowska & Wardencki, 2007). Additionally there are reviews that have focused specifically on analysis of volatile flavour compounds and their contribution to characterising food authenticity in dairy products (Ferreira *et al.*, 2001; Karoui & De Baeremaeker, 2007), honeys (Cuevas-Glory *et al.*, 2007), olive oil (Acree, 1997; Grosch, 1993), meats (Ullrich & Grosch, 1987), vegetable oils (Aparicio & Aparicio-Ruíz, 2000) and wine (Arvanitoyannis *et al.*, 1999). In addition, Table 10.1 depicts GC approaches that have been applied to characterise food authenticity by headspace volatile composition.

As indicated in Table 10.1 GC flavour-derived techniques provide sufficient classification of products on the basis of volatile compounds emitted from foods. According to Plutowska and Wardencki (2007), volatile aroma compounds originate at nearly every stage of food production, from raw materials to intended and unintended production during processing and storage. Furthermore metabolic metabolism of amino acids, fats and carbohydrates will result in groups of odorous compound carboxylic acids, alcohols, carbonyl compounds, lactones, terpenes, esters and ethers, which are characteristic of a given raw material and its botanical origin. As for processing and geographical origin it is also possible to apply volatile profiling to determine product brands such as tea (Togari *et al.*, 1995) coffee (Costa Freitas *et al.*, 2001) and cheese (Carpino *et al.*, 2004).

10.2.2 Combined GC procedures used in food authenticity

While the flavour and aroma compounds present in and released from various food products have been well documented, limited information exists in the literature that characterises the odour quality of specific compounds as markers for identifying food authenticity. However GC-MS and GC-FID techniques can be coupled with GCO analysis providing the possibility of determining sensory descriptions of eluted analytes, while being quantified and identified simultaneously. Thus GCO relies on human assessors to evaluate the eluting compounds from the end of the column by providing a measure of perceived intensity and description of the odour

Table 10.1 Instrumental volatile gas chromatography (GC) techniques used in food authenticity

Instrumental technique	Product category	Volatile extraction	Analysis detail	Reference
GC-MS	Bread sourdough (Altamura bread)	Dynamic headspace sampling by volatile trapping onto Tenax	Determination of volatile composition of traditional and commercially produced bread during storage	Chiavaro <i>et al.</i> (2008)
GC-MS	Bread sourdough (Altamura bread)	Dynamic headspace sampling by volatile trapping onto Tenax	Characterisation of volatile compounds for Italian protected designation of origin (PDO) Altamura bread	Bianchi <i>et al.</i> (2008)
GCO, GC-MS	Cantal-type cheese	Dynamic headspace trapping onto Tenax	Cheese aroma characterization and secondary identification of odour active compounds for determining effect of cow diet, milk pasteurisation and ripening on PDO Cantal-type cheese.	Cornu <i>et al.</i> (2009)
GC-MS	Chestnut honeys	Direct trapping onto styrene-divinylbenzene cartridges	Effect of geographical origin on chemical and sensory characteristics of chestnut honeys	Castro-Vázquez <i>et al.</i> (2010)
GC-MS, LC/ESI-MS, MALDI-TOF-MS	Wines and non-aromatic grapes	Liquid/liquid extraction and headspace SPME	Identification of free and bound volatile compounds for determining wine typicality and authenticity	Nasi <i>et al.</i> (2008)
GC-TOF-MS	Coffee	Headspace SPME	Methodology development for determining the geographical origin and verification of coffee	Risticevic <i>et al.</i> (2008)
GC-MS	Fresh tomatoes, Tomato paste	Headspace SPME	Volatile profiling of fresh tomatoes and tomato paste to determine geographical origin	Feudo <i>et al.</i> (2011)
GC-FID	Red wine	Liquid/liquid extraction	Red wine classification by growing region in France	Sivertsen <i>et al.</i> (1999)
GC-MS	Natural fruit flavours	Headspace SPME	Authenticity assessment of natural fruit flavour compounds	Ravid <i>et al.</i> (2010)

GC-FID, HR-GCO	Olive oil	Dynamic headspace trapping onto Tenax	Authentication of virgin olive oils by volatiles, sensory attributes and consumers' attitudes	Aparicio <i>et al.</i> (1997)
GC-MS, MD-GC-MS	Olive oil	Headspace SPME, steam distillation-solvent extraction	Detection of adulterated olive oil	Flores <i>et al.</i> (2006)
GC-MS, FID, Electronic nose	Emmental cheese	Dynamic headspace trapping onto Tenax	Determination of geographical origin of Emmental cheese	Pillonel <i>et al.</i> (2003)
GC-MS	Milk	Dynamic headspace trapping onto Tenax	Characterisation of milk by analysis of its terpene fractions	Fernandez <i>et al.</i> (2003)
GC-MS	Honey	Dynamic headspace trapping onto Tenax	Botanical and geographic determination by volatile aroma compounds for authenticating honey	Radovic <i>et al.</i> (2001)
GC-MS	Ewes milk & cheese	Headspace SPME	Determining the influence of pasture type on the volatile profile of ewes milk and cheese	Povolo <i>et al.</i> (2007)
GC-MS	Cooked lamb	Headspace SPME	Product characterisation using volatile profiles	Elmore <i>et al.</i> (2000)
GC-MS	Cured hams	Dynamic headspace trapping onto Tenax and steam distillation-solvent extraction	Characterisation of hams cured by different methods of production	Dirinck <i>et al.</i> (1997)
GC-FID, GC-MS	Dried cured hams	Headspace SPME	Volatile characterisation of different hams on the basis of geographical origin and animal breed	Sánchez-Peña <i>et al.</i> (2005)
GC-MS	Pork meat	Headspace SPME	Meat characterisation for determining breed type, diet and geographical origin	Muriel <i>et al.</i> (2004)
GC-FID, GC-MS, GCO	Fish (sardines)	Headspace SPME	Determining fish freshness and identifying markers of freshness	Triqui and Bouchriti (2003)
GC-MS	Sausages	Headspace SPME	Product characterisation	Marco <i>et al.</i> (2004)

(Continued)

Table 10.1 *Continued*

Instrumental technique	Product category	Volatile extraction	Analysis detail	Reference
GC-MS, GC-FID	Apples	Dynamic headspace trapping onto Tenax, headspace SPME	Rapid screening of fruits and vegetable quality	González-Martin <i>et al.</i> (2000)
GC-MS, GC-FID, GCO	Bananas	Headspace SPME	Monitoring aroma during ripening and distinguishing bananas by geographical location	Boudhrioua <i>et al.</i> (2003)
GC-FID, GC-MS	Figs	Dynamic headspace trapping onto Tenax	Characterisation of volatile compounds from different fig varieties	Grison-Pigé <i>et al.</i> (2002)
GC/GC-FID	Strawberries	Headspace SPME	Distinguishing strawberries by variety and level of maturity	Williams <i>et al.</i> (2005)
GC-MS	Tomatoes	Headspace SPME	Classification of tomatoes by variety and storage time	Berna <i>et al.</i> (2004)
GC-FID	Olive oil	Headspace SPME	Detection of adulteration with vegetable oils	Webster <i>et al.</i> (2000)
GC-FD, GC-MS	Olive oil	Headspace SPME	Product characterisation by volatile profiling	Vichi <i>et al.</i> (2003)
GC-FID	Olive oil	Headspace SPME	Determining the quality of raw materials used in the production of olive oil	Jimenez <i>et al.</i> (2004)
GC-MS	Vegetable oils	Headspace SPME	Detection of impurities in vegetable oils	Page and Lacroix (2000)
GC-MS	Vegetable oils	Headspace SPME	Assessment of vegetable origin and degree of oxidation by volatile composition	Biswas <i>et al.</i> (2004)
GC-FID, GC-MS	Vegetable oils	Headspace SPME	Assessment of vegetable origin and degree of oxidation by volatile composition	Mildner-Szkudlarz <i>et al.</i> (2003)

GC-MS	Parmesan cheese	Dynamic headspace trapping onto Tenax, headspace SPME	Classification of parmesan cheeses on the basis of producer, geographical origin and time of ripening	Bellesia <i>et al.</i> (2003)
GC-FID, GC-MS	Brandy, whisky	Headspace SPME	Differentiating alcoholic beverages	Park <i>et al.</i> (1999)
GC-FID, GC-MS	Grape wine	Headspace SPME	Classification of wine types	Navarro <i>et al.</i> (2001)
GC-MS	White wine	Direct injection and headspace SPME	Classification of wine types	Garcia <i>et al.</i> (1998)
GC-FID	White wine	Headspace SPME	Wine characterisation by volatile esters	Rodriguez-Bencomo <i>et al.</i> (2002)
GC-FID, GS-MS	Wine	Headspace SPME	Wine characterisation by volatile phenols	Mejias <i>et al.</i> (2003)
GC-MS, GC-FID	Coffee	Headspace SPME	Coffee classification	Costa Freitas <i>et al.</i> (2001)
GC-MS, GC-FID	Coffee	Headspace SPME	Coffee classification	Ryan <i>et al.</i> (2004)
GC-MS	Honey	Headspace SPME	Honey classification by botanical and geographical origin to confirm honey authenticity	Soria <i>et al.</i> (2004)
GC-MS	Cupuassu liquor	Headspace SPME	Product characterisation	de Oliveira <i>et al.</i> (2004)
GC-FID	Mint confectionery	Headspace SPME	Assessing the menthol and menthone content	Ligor and Buszewski (1999)

Abbreviations: APCI – atmospheric pressure chemical ionisation, ESI – electrospray ionisation, FID – flame ionisation detector, FTIR – Fourier transform infrared, GC – gas chromatography, HR – high resolution, IR – infrared, MALDI – matrix-assisted laser desorption/ionisation, MD – multidimensional, MS – mass spectrometry, O – olfactometric analysis detector, SAFE – solvent-assisted flavour evaporation, SPME – solid phase microextraction, TOF – time of flight.

quality. Following analysis by these techniques, volatile and odour-active compounds are identified by comparing analyte retention times from mass spectra and odour descriptions with those of authentic standards and published literature.

For example, Cornu *et al.* (2009) applied GCO coupled with a mass selective detector to investigate odour-active compounds from PDO protected Cantal-type cheeses in accordance with cow diet, milk pasteurisation and cheese ripening. These authors determined major odorous peaks by summing the intensity scores and comparing descriptions from eight different assessors (Plate IV between pages 242 and 243). Subsequently odorous volatiles could be attributed to butanoic acid (peak number 16) described as vomit, 2,3-butanedione (peak 5) described as buttery, 3-methylthio-propanal (peak 24) described as cooked potato, hexanal (peak 17) described as apple, octanal (peak 29) described as grapefruit/citrus fruit, heptanal (peak 23) described as citrus and 1-octen-3-ol (peak 26) described as mushroom. Of the odorous compounds identified and described the authors concluded that the intensity of isobutanoic acid (peak 12) described as cheese, pentanal (peak 10) described as grass, E-2-hexanal (peak 39) described as cardboard, cyclohexanone (peak 22) described as glue, chlorine, tetramethylpyrazine (peak 35) described as grilled and γ -heptalactone (peak 38) described as fruit sweet were lower in cheeses aged for six months compared with cheeses aged for 3 months, while ethylbenzene (peak 20) described as solvent, paint was higher. The authors also established that cheeses derived from milk where cows were fed on a diet of pasture could be distinguished by lower levels of isopentanoic acid (peak 18), which was described as 'vomit'.

Ferreira *et al.* (2001) also showed good discrimination by identifying odorous volatile compounds for wines from different regions of Spain when combining GC-MS and GCO techniques. In this study 30 odour-active compounds from 69 identified and described compounds differentiated among the wines. On the basis of the description, important compounds that distinguished wines from different regions were reported for sotolon, described as raisin like, 2-methyl-3-mercaptofuran, described as smoky and 2,6-dimethoxyphenol and iso-eugenol, which were attributed to phenolic notes. Similarly Boudhrioua *et al.* (2003) observed a different aromatic profile for bananas sourced from different geographic locations of Martinique, Canary Islands, Ivory Coast and varieties *Cavendish*, Grande Naine and small Naine when using a combined GC-FID, GC-MS and GCO approach. Of the three geographical regions bananas obtained from Martinique contained the highest amounts of volatile compounds, which were attributed to two alcohols, nine esters and one phenol. These combined GC techniques provide a comprehensive understanding of not only discriminating volatile compounds capable of characterising food authenticity by geographical region or botanical origin but also the potential to identify perceived sensory qualities that can be attributed to specific distinguishing compounds.

10.3 Emerging volatile techniques used to characterise food authenticity and quality

The conventional techniques involving the use of GC rely on the fact that single compounds are detected and described, while in reality the distinctive flavours and aromas of foods are generated by a mixture of a number of volatile constituents (Reineccius, 2003). Similarly and as already mentioned no single or group of volatile compounds can be considered the key component responsible for distinguishing food authenticity. Furthermore these volatile constituents are likely to act in a synergistic way in relation to their relative proportions, which in turn can be modified by other substances present in the food (Noble & Ebeler, 2002). Thus GC-based approaches are limited when it comes to monitoring the temporal evolution of headspace volatile fingerprints in real time. However, advances in sampling techniques and instrumental sensitivity have enabled the detection and identification of thousands of compounds from food. From this perspective chemometrics is then often applied to help interpret the multi-component data structure of instrumental and sensory-derived data.

In principle, multi-measured volatile components with sufficient distinguishing power will give a characteristic profile that can be linked to both perceived sensory qualities and product authenticity. It is well recognised that direct rapid headspace techniques measured by MS without chromatographic separation can effectively represent a 'fingerprint' of the sample being analysed and can provide distinct chemical information in relation to product odour, flavour, shelf-life, geographical or genetic origin, processing, and presence of micro-organisms. In addition, the sensory perception of aroma is best determined by the concentration of the volatile compounds in the gas phase above the food. Thus headspace concentrations usually relate well to sensory properties of the food (van Ruth *et al.*, 2003). As a consequence, direct rapid headspace techniques are commonly used in relation to characterising the sensory properties of food (Pérès *et al.*, 2003). In the last decade, several non-chromatographic instrumental approaches that include, electronic nose with different types of chemical sensors (Hurst, 1999), direct coupling headspace-mass spectrometry (Pérès *et al.*, 2003), or real-time monitoring using more recently developed techniques such as Atmospheric pressure chemical ionization mass spectrometry (APCI-MS) (Taylor *et al.*, 2000), proton transfer reaction mass spectrometry (PTR-MS) (Lindinger *et al.*, 1998), selected ion flow tube mass spectrometry (SIFT-MS) (Davis & Mcewan, 2007) have been applied for volatile characterisation of food.

10.3.1 Applications of electronic nose (e-nose) for food authentication

In principle, an electronic nose (e-nose) consists of an array of semi-conducting sensors coated with tin, titanium or tungsten dioxide. These sensors have a low conductivity in clean air. In the presence of reducing

gases, oxygen is displaced from the surface and replaced with other gases, increasing the conductivity. The change in conductivity measures the concentration of total volatiles. As conductivity increases so does the sniffer response (mV) (James *et al.*, 2005). An e-nose provides a rapid nondestructive volatile fingerprint. However limitations of e-nose analysis include short-term sensor drift, resulting in poor signal reproducibility and lack of sensitivity (Hurst, 1999). Despite these limitations the e-nose has been highly successful for authenticating the geographical origin of a wide range of foods including: olive oil (Cosio *et al.*, 2006; Guadarrama *et al.*, 2001; Haddi *et al.*, 2011); wine (Berna *et al.*, 2009; Cynkar *et al.*, 2010; Penza & Cassano, 2004a, 2004b); fruit juice (Gobbi *et al.*, 2010; Steine *et al.*, 2001); onions (Ariyama *et al.*, 2004, 2007; Ariyama & Yasui, 2006); cheese (Pillonel *et al.*, 2003), honey (Ampuero *et al.*, 2004; Lammertyn *et al.*, 2004) and coffee (Costa Freitas *et al.*, 2001).

Considerable attention has focused on characterising food authenticity and origin by combining both electronic nose and conventional GC-MS techniques. In particular, Berna *et al.* (2009) investigated good predictions of wine origin for 34 Sauvignon Blanc wines obtained from France, New Zealand and Australia using GC-MS, metal oxide-based electronic nose (MOS-e-nose) and a relatively new type of e-nose technique based on direct MS (Cozzolino *et al.*, 2005). Similarly, Pillonel *et al.* (2003) demonstrated good discrimination of Emmental cheeses from different countries and regions within Europe using both GC-MS/FID and electronic nose. Moreover Ampuero *et al.* (2004) could distinguish the botanical origin of several Swiss honeys derived from dandelion, lime, acacia, chestnut, fir and rape using a direct MS nose technique and showed good correlations of specific ion masses with sensory descriptions. On the other hand, Lammertyn *et al.* (2004) separated honeys on the basis of botanical composition (buckwheat, clover, orange blossom, black locust, mint and carrot) and geographical origin using conventional e-nose coupled with an array of semi-conducting sensors. In addition, numerous reviews of the literature have address applications of e-nose in characterising food authenticity, highlighting the method as a fast aroma fingerprinting technique (Baldwin *et al.*, 2011; Escuder-Gilabert & Peris, 2010; Peris & Escuder-Gilabert, 2009; Schaller *et al.*, 1998).

10.3.2 The evolution of real-time MS techniques

Alternatively, direct headspace techniques that involve introducing volatile compounds from the headspace of samples without chromatographic separation into the ionisation chamber of a mass spectrometer have enabled fast economical methods of characterisation (Peres *et al.*, 2007; Pérès *et al.*, 2003). In most cases this type of analysis consists of electron impact ionisation MS. Subsequently, many ions show extensive fragmentation following electron impact, making compound identification difficult in

multi-component analytes (Blake *et al.*, 2009). Softer, more selective ionisation processes such as chemical ionisation are available to both eliminate the contribution of abundant inorganic gases and reduce ion fragmentation. Typical techniques that take advantage of chemical ionisation include PTR-MS, APCI-MS and SIFT-MS. What distinguishes PTR-MS from APCI-MS is the generation of the primary H_3O^+ and the chemical ionisation of the volatile compounds are individually controlled and spatially and temporally separated processes (Linthorpe & Taylor, 2010; van Ruth *et al.*, 2003). As a result of this separation, background noise in PTR-MS is low. In APCI-MS H_3O^+ ions are created and reacted with analytes in the same region. Thus PTR-MS can achieve high sensitivity when a longer dwell time is used for a specific ion, while increasing dwell time in APCI-MS does not increase sensitivity (Taylor & Linthorpe, 2000). On the other hand SIFT-MS relies on the selection of reagent ions using a mass filter (quadrupole). The most commonly used ion in SIFT-MS is H_3O^+ , but other ions such as NO^+ and O_2^+ , are often selected. Similar to PTR-MS the selection of reagent ions in SIFT-MS allows different proton transfer affinities to be established, which can facilitate the identity of isobaric and isomeric compounds when elucidating more complex mixtures. However, the advantage of using a mass spectrometer with H_3O^+ is that the proton transfer is specific to the volatile organic compound (VOC) being measured instead of air components (oxygen, nitrogen, argon, etc.).

It is important to note that variations to SIFT-MS technology led to the development of PTR-MS (Hansel *et al.*, 1995). In this case changes involved dispensing of the mass filter in SIFT-MS, that was used to select reagent ions prior to reaction with an analyte and replaced with a hollow-cathode discharge source in PTR-MS, which could generate H_3O^+ with high efficiency (>99.5%) without the need of a mass filter. In addition, the long flow tube used in SIFT-MS was replaced with short drift tube common in PTR-MS (Blake *et al.*, 2009). This addition for PTR-MS has increased ion-molecular collision energies, which reduce the likelihood of ion clusters and allow for a more compact version of the instrument. Collectively, these modifications have led to the improved sensitivity for PTR-MS that is some two orders of magnitude better than that for SIFT-MS (Lindinger *et al.*, 1997, 1998).

10.3.3 PTR-MS applications in food authenticity

In PTR-MS, protonated water molecules (H_3O^+) are introduced to a coaxial flow of headspace gas. The headspace gas consists mainly of air or inert gas that acts as a buffer in the reaction chamber, as volatile compounds are present in trace quantities. Volatile compounds that have a proton affinities higher than water (proton affinity of H_2O : 166.5 kcal/mol) are ionised by proton transfer from H_3O^+ , mass analysed in a quadrupole mass spectrometer and eventually detected as counts per second (cps) by a secondary

electron multiplier. In principle, a relationship exists between PTR-MS intensities (cps) and actual concentrations of the neutral compounds in the headspace. Thus headspace concentrations of volatile compounds are proportional to the ratio between measured count rates of protonated volatile compounds and of protonated water. A more detailed explanation of the PTR-MS and its application have been reviewed (Hansel *et al.*, 1995; Hewitt *et al.*, 2003). Advantages of PTR-MS include: (1) fast time-dependent variations of headspace measurements within a few minutes; (2) samples are not subject to previous treatments and nondestructive conditions are avoided, reducing the likelihood of artifacts, and allowing for an authentic representation of the volatile mixture; (3) mass spectral intensities can be transformed into absolute headspace concentration without the need for calibration of external standard; (4) soft ionisation that leads to low fragmentation, retaining chemical information; and (5) high sensitivity with a detection limit in the pptv range (Lindinger *et al.*, 1998).

Rapid analytical methods (APCI-MS and PTR-MS), in particular when coupled with descriptive sensory analysis, are becoming increasingly popular in determining food authenticity and quality (Cayot, 2007). This mixed method approach, which has the ability of evaluating numerous products in a short time, can act as a support to establishing links to the origin of foods, composition and fingerprints of quality. It should be emphasised that PTR-MS primarily has its strengths in fast monitoring of changes in concentration of compounds, rather than compound identification. Thus the capability of PTR-MS to monitor volatile compounds in real time, with high sensitivity and quantitatively, providing a tool for product authentication and process characterisation of food, has been very promising. In this capacity, PTR-MS has demonstrated the ability to classify products on the basis of geographical and botanical origin, food processing and method of production/cultivation (organic, conventional).

To date, this analytical technique coupled with multivariate statistical analysis has been used successfully to characterise and differentiate the volatile composition of cheese (Aprea *et al.*, 2007b; Biasioli *et al.*, 2006; Boscaini *et al.*, 2003; Fabris *et al.*, 2010; Galle *et al.*, 2011), butters (Maçatelli *et al.*, 2009; van Ruth *et al.*, 2007), custard desserts (van Ruth *et al.*, 2004), infant formulas (van Ruth *et al.*, 2006), truffles (Aprea *et al.*, 2007a), olive oil (Aprea *et al.*, 2006; Araghipour *et al.*, 2008; Ruiz-Samblás *et al.*, 2012), fats and oils (van Ruth *et al.*, 2010a, 2010b), whey (Gallardo-Escamilla *et al.*, 2005, 2007), orange juice (Biasioli *et al.*, 2003), breads (Heenan *et al.*, 2009), wine (Boscaini *et al.*, 2004; Spitaler *et al.*, 2007), cured ham (del Pulgar *et al.*, 2011), strawberries (Granitto *et al.*, 2006) and meat (Biasioli *et al.*, 2003; Mayr *et al.*, 2003a, 2003b).

In principle, PTR-MS analysis provides a volatile fingerprint of a food that is capable of distinguishing specific characteristics of the product. For example van Ruth *et al.* (2010a) classified the botanical origin of animal feed on the basis of PTR-MS finger printing (Fig. 10.1). The authors clearly

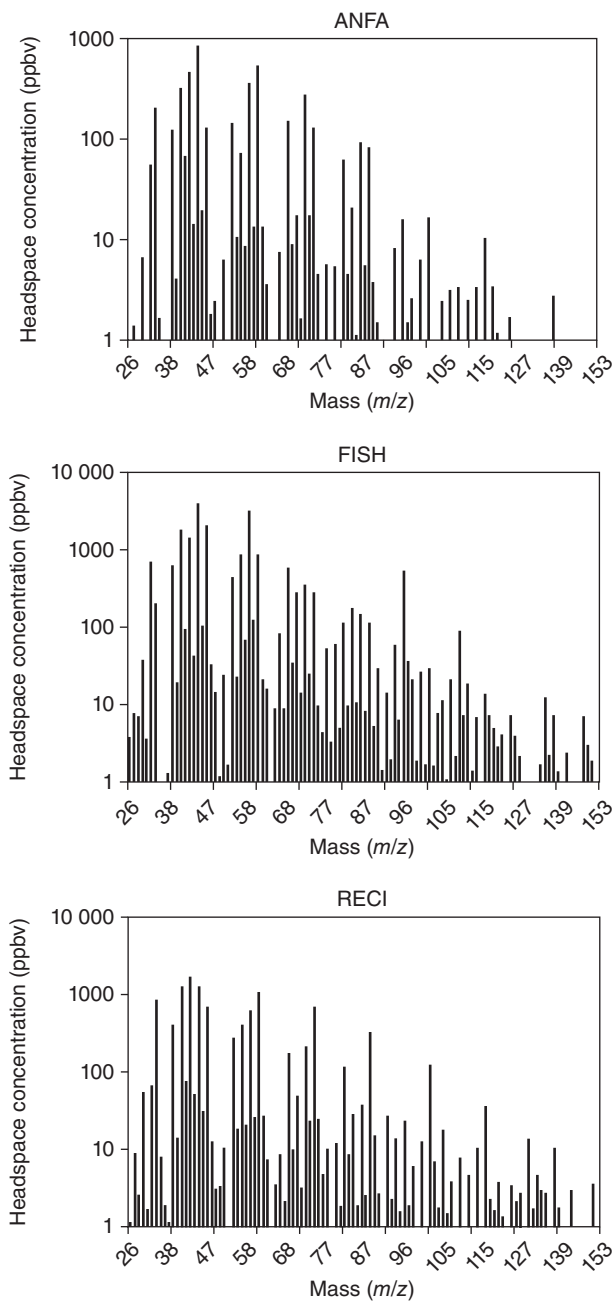


Fig. 10.1 Mean fingerprint mass spectra of volatile compounds measured by PTR-MS from animal fats (ANFA), fish oil (FISH), and recycled cooking oil (RECI) (reproduced with permission from van Ruth *et al.*, 2010a).

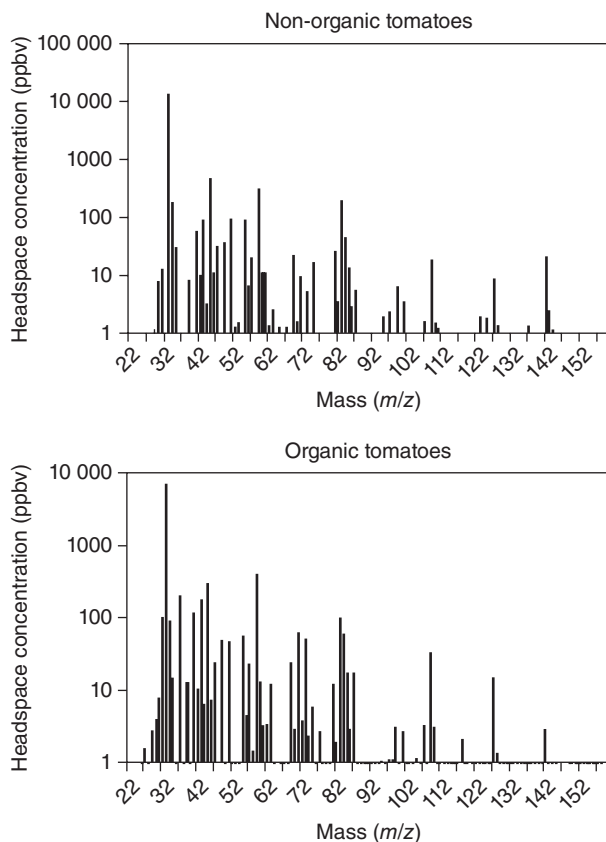


Fig. 10.2 Mean fingerprint mass spectra of volatile compounds measured by PTR-MS for non-organic and organic derived tomatoes.

illustrated differences between fish oils, animal fats and recycled cooking oil on the basis of the volatile signal intensities. In addition, similar studies by the same authors have been successfully applied to determine the correct classification of olive oils (Araghipour *et al.*, 2008) and butters (van Ruth *et al.*, 2007) from different geographical origins. Emerging unpublished applications by the same authors are showing promising opportunities to classify organic and non-organically grown tomatoes (Fig. 10.2) and characterisation of fair-trade coffee from different geographical origins: Indonesia, Ethiopia, Mexico and Peru (Fig. 10.3).

PTR-MS measurements consist of mass spectrometric data without chromatographic separation, which result in hundreds of mass ion signals that are often highly correlated. The application of multivariate techniques that include principal component analysis (PCA) or partial least squares regression (PLSR) has been used to extract and interpret volatile profile data

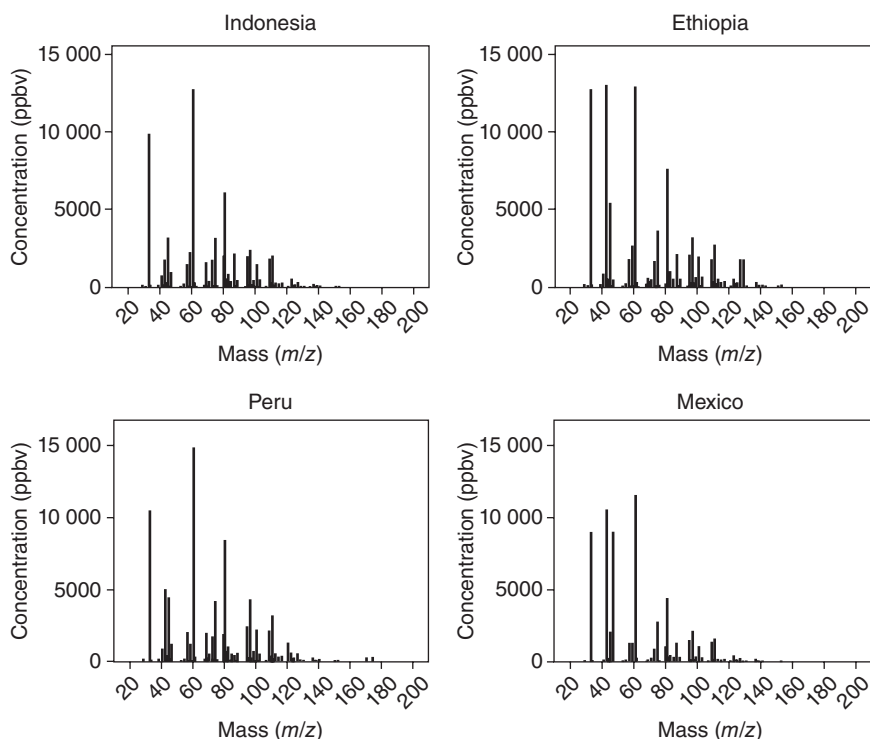


Fig. 10.3 Mean fingerprint mass spectra of volatile compounds measured by PTR-MS for fair-trade coffee from Indonesia, Peru, Ethiopia and Mexico.

generated from PTR-MS measurements for predicting the compositional or sensory properties of food (Aprea *et al.*, 2006, 2007b; Biasioli *et al.*, 2006; Heenan *et al.*, 2009; Lindinger *et al.*, 2008; Phillips *et al.*, 2010). In this approach mixed multivariate models capable of classifying foods by grouping products that have similar flavour attributes as perceived by a trained panel of assessors and volatile profiles measured by PTR-MS demonstrate good opportunities for determining product composition and geographical origin with links to explained sensory quality.

However, there exist some limitations for conventional quadrupole PTR-MS. In principle, quadrupole mass spectrometers are limited to analysing only one channel at a time. Thus resulting in the switching of the detector between the m/z (mass to charge ratios) when simultaneously analysing more than one mass. In this mode, there exists a compromise between the number of m/z monitored and the signal to noise ratio. Moreover, the analysis of complex food systems using quadrupole PTR-MS often results in difficulties in unequivocal compound identification by their mass-to-charge ratio protonated parent ion, on the basis of several species with the same nominal mass, cluster ion formation and compound fragmentation. To

address these limitations and improve mass resolution coupled with immediate data acquisition (complete spectrum with pptv sensitivity in less than 100 ms) proton transfer reaction time of flight mass spectrometry (PTR-TOF-MS) was developed (Ennis *et al.*, 2005; Tanimoto *et al.*, 2007) and commercialised (Jordan *et al.*, 2009). In this capacity, PTR-TOF-MS technique have proven capabilities in mass accuracy providing unambiguous determination of chemical formula leading to an improved interpretation of mass spectra (Cappellin *et al.*, 2010). Galle *et al.* (2011) applied PTR-TOF-MS to help identify volatile compounds detected by quadrupole PTR-MS that characterise PDO protected farmers' cheese from Leiden in the Netherlands. In this case, increased mass resolution using PTR-TOF-MS helped confirm differences between PDO farmers cheese from Leiden and similar non-protected cheeses from the Netherlands, while identifying strongly discriminating compounds that included diacetyl, 2-propanone, 2-butanone/butanal, 2,3-pentadiene, hexanoic acid *p*-cymene, formic acid and prop-2-enal. Evidently, this combined approach can provide more robust classifications of food, while allowing for an improved identification of potential volatile markers that distinguish differences between products.

10.4 Data analysis: determining relationships between instrumental data and food authenticity

From the current literature examined, it is evident that flavour analysis techniques used to study food authentication require advanced chemometric analysis of the data provided. For chemometric analysis of data obtained from volatile profiling, it is important to extract the interpretable and statistically reliable information. Thus several well-documented chemometric approaches that have included linear discriminate analysis (LDA), quadratic discriminate analysis (QDA), k-nearest neighbours (K-NN), soft independent modelling of class analogy (SIMCA), unequal dispersed classes (UNEQ), classification and regression trees (CART), support vector machines (SVM), artificial neural networks (ANN), principal component analysis (PCA) and principal component regression (PCR), partial least squares regression (PLSR) and partial least squares discriminate analysis (PLS-DA) have been reviewed (Oliveri & Downey, 2012). More detailed reviews on applications of these chemometric techniques are available for the analysis of dairy products (Arvanitoyannis and Tzouros, 2005; Karoui & De Baerdemaeker, 2007) and wine (Arvanitoyannis *et al.*, 1999).

10.4.1 Relationships between instrumental flavour and sensory analysis for determining food authenticity

Chemometric analysis techniques such as PCA and PLS have demonstrated that sensory characteristics defined by a trained panel can be related to

volatile profiles, enabling the sensory characteristics associated with volatile constituents of products to be described (MacFie & Hedderley, 1993; Martens & Martens, 1986). Often the discriminability of the data is first studied using PCA, where a limited number of products are described by one set of variables (X -data) consisting of either sensory characteristics or analytical readings defined by human assessors or instrumental measurements respectively. In this case, the basic form of the X -data consists of at least a two-way matrix of objects (i.e. samples) as rows and variables (i.e. sensory attributes or instrumental measurements) as columns. The variables are measurements of each object. Taken collectively, the variables characterise each and all of the objects. In this type of data matrix objects as rows and variables as columns can be represented in an orthogonal coordinate system of dimensions. From this aspect, PCA can be applied to study descriptive sensory data defined by product characteristics, or instrumental measurements defined by physiochemical product constituents. Subsequently, PCA allows for inspection of variables that are inter-correlated with each other (Luykx & van Ruth, 2008).

Secondly, the integration of volatile fingerprints and sensory profiles can be achieved by a bi-linear modeling method performed by PCR or PLSR, which consists of extracting the main patterns of relationships between a set of predictors (X -data) and a set of response variables (Y -data) (Martens & Martens, 1986). PCR relationships are created by explanatory variables (X -data) only, while the relationships of PLSR are created by the explanatory variables (X -data) and dependent variables (Y -data) jointly. In practice, PCR involves more relationships, which tends to complicate interpretation of the model and increases the risk of over fitting data. Simulated studies have established that PLSR is preferred over PCR in most practical approaches (Garthwaite, 1994). In PLSR analysis X -data often consists of product composition and/or instrumental measurements, while Y -data sets comprise sensory qualities or consumer preferences. The mathematical form of this relationship is illustrated in Fig. 10.4. For each analysis a plot of regression coefficients (β -coefficients) of the PLSR model illustrates the contribution of each of the X -variables (volatile compounds) in predicting Y -variables (sensory qualities). X -variables with a small negative or positive coefficient that contributed little information are removed and the model is recalculated. The intention of PLSR is to capture most information in the X -data that is useful for predicting the response in Y -data, while reducing the dimensionality of the regression problem by using

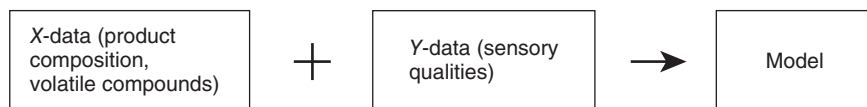


Fig. 10.4 Partial least squares regression and X - Y modelling.

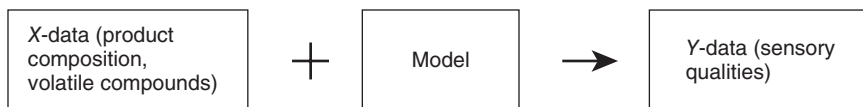


Fig. 10.5 Partial least squares regression predicting *Y*-data from *X*-variables.

fewer principal components than the initial number of *X*-variables (Garthwaite, 1994). Hence, PLSR is considered especially useful for modelling prediction when there are more explanatory variables (*X*-data) than samples. In addition, PLSR modelling is capable of handling highly correlated explanatory variables (*X*-data).

Optimisation of PLSR models is achieved by a procedure known as ‘Jack-Knifing’, where unrelated variables or variables that reveal high levels of uncertainty are removed (Martens *et al.*, 2001; Martens & Martens, 2000). The ‘Jack-knifing’ procedure combined with graphical interpretation of contributing *X*-variables improves the reliability of PLSR modelling. In PLSR the ‘Jack-Knifing’ procedure is then followed by recalibrating the model with the remaining *X*-variables left in. This procedure is repeated until a final optimum model, based on predictive ability, is obtained. In addition, the predictive validity can be assessed by full cross-validation, where the model’s predictive variance is calculated by leaving one sample out during the building of the model, and the remaining sample is used to test the model’s predictive performance; the process is repeated until all samples have been accounted for (Martens & Martens, 2000). From this type of analysis, optimum model predictive ability can be assessed by calculating the root mean square error of prediction (RMSEP). This value, expressed in the same units as the *Y*-variables (sensory mean scores), shows the average uncertainty that can be expected when predicting *Y*-values (sensory measures of product attributes) for new samples. As displayed in Fig. 10.5, the calibrated model can be utilised to predict the response of *Y*-variables from *X*-variables in completely new, unknown products of the same general type under the chosen experimental conditions.

10.5 Conclusion

As volatile compounds from foods have been described as being short lived, flavour isolation by absorbent trapping or distillation and concentration is difficult. In addition, subsequent analysis by separation techniques such as GC, where volatile compounds are separated from the headspace of a sample and detected one at time, makes for difficult correlations with perceived sensory sensations of food odour and flavour that are more often experienced as a mixture of compounds. However, as previously indicated, rapid headspace volatile measurements combined with sensory analysis and

studied with chemometrics are emerging as a fast, real-time, nondestructive method for characterising food authenticity and product quality. From this perspective chemometric analysis can provide an interdisciplinary understanding of relationships between instrumental volatile profiles, sensory qualities, and the botanical and/or geographical origin, processing or method of production for food.

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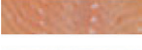
	Treatment dose (kGy)	Minolta	Machine vision	Picture
(a)	0			
(b)	1			
(c)	1.5			
(d)	2			
(e)	3			
(f)	Standard red plate			

Plate III (Chapter 6) Colour representations of Minolta and machine vision reading results and actual pictures of differently treated salmon fillets and standard red plate (Yagiz Y, Balatan MO, Kristinsson HG, Welt BA and Marshall MR, 2009. Comparison of Minolta colorimeter and machine vision system in measuring colour of irradiated Atlantic salmon. *Journal of the Science of Food and Agriculture*, **89**(11), 728–730).

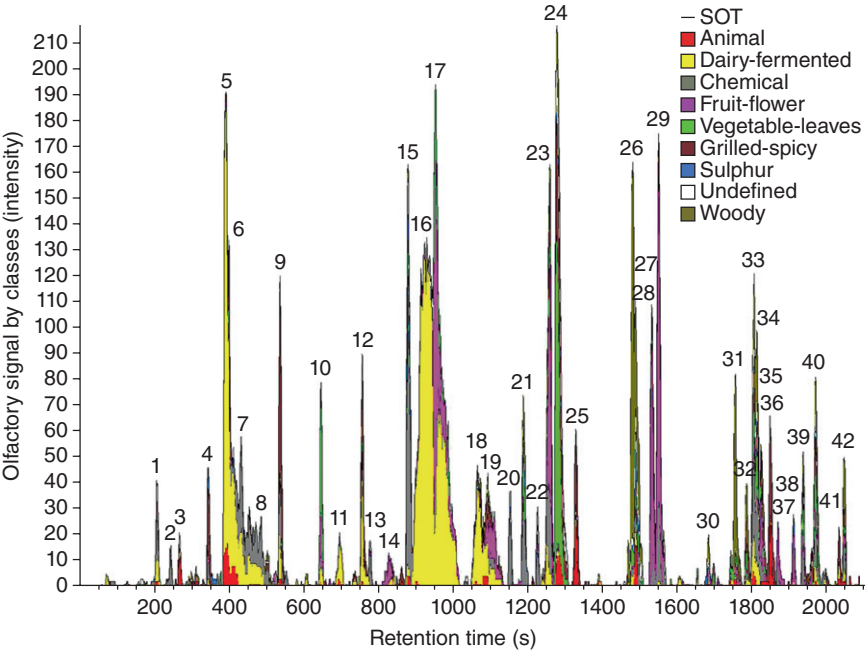


Plate IV (Chapter 10) Odorous volatile compounds in Cantal type cheese according to 8 different assessors and 8 different cheese treatments. (Reproduced with permission from Cornu A, Rabiau N, Kondjoyan N, Verdier-Metz L, Pradel P, Tournayre P, Berdague JL and Martin B, 2009. Odour-active compound profiles in cantal-type cheese: effect of cow diet, milk pasteurization and cheese ripening. *International Dairy Journal*, **19**(10), 588–594).

Advances in analysis of instrumental food sensory quality data

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Abstract: This chapter presents an overview of the main multivariate data analytical tools to deal with problems normally encountered in the instrumental evaluation of food quality (exploratory analysis, calibration and classification). Particular attention is given to the family of methods based on projection on a low-dimensional subspace of latent variables, as they have the advantages of allowing the analysis of data where many correlated variables are measured on a limited number of samples (which is often the case in instrumental assessment of food quality) and an easy and straightforward visualization of the results.

Key words: chemometrics, multivariate data analysis, exploratory data analysis, regression, classification.

11.1 Introduction

Food quality is a multifaceted issue, as it involves aspects as nutritional value, absence of alteration and/or adulteration, genuineness, safety of use, traceability and many more (Grunert, 1995; Moskowitz, 1995; Henson and Caswell, 1999). Accordingly, the analytical control of food quality requires, quite often, the holistic characterization of the product by means of multiple determinations and/or by the use of multichannel fingerprinting techniques. As a consequence, when dealing with the instrumental assessment of food quality, one is often confronted with so much data that the essential information may be not readily evident (Cozzolino *et al.*, 2009). For instance, in the case of chromatographic or spectroscopic analyses, many signals (intensity at different retention times or wavelengths) are collected on a single sample, which is then characterized by a multivariate profile. Additionally, many food quality analytical techniques are based on indirect measurements, in which the signal may not have a direct relationship with the

property of interest and a latent correlation is sought (Martens and Martens, 2000).

Therefore, there is a need to look at the samples in their entirety and not just at one or a few single components in order to untangle the complex interactions among the constituents and understand their combined effects on the whole matrix. From the standpoint of data analysis, this requirement translates into the fact that a multivariate mathematical and statistical approach, considering and processing multiple signals simultaneously, is necessary (Massart *et al.*, 1988; Martens and Naes, 1989; Naes *et al.*, 2002). Multivariate data analysis comprises a large number of methods, which can cope with different aspects related to food quality (Martens and Russwurm, 1983). In particular, there are techniques to define the proper experimental conditions for collecting good data and/or to identify whether some factors have an effect on the results; on the other hand, other methods allow to predict one or more properties of interest (Bro *et al.*, 2002). Furthermore, there are tools whose aim is to classify the samples into one of several categories (e.g. good *vs* bad, coming from origin A *vs* coming from origin B or C, ...).

In this chapter, the main multivariate mathematical and statistical methods that can be used to process instrumental food quality data will be presented in a simple and straightforward way, making extensive use of examples taken from the authors' own experience and from the literature. In detail, Section 11.2 provides an introduction to matrix algebra and multivariate geometric representation, Section 11.3 covers the methods to be used for exploratory data analysis, the multivariate regression methods are discussed in Section 11.4, while classification and class modelling are illustrated in Section 11.5; Section 11.6 contains some concluding remarks.

11.2 Essentials of matrix algebra and multivariate geometric representation

When a single property is measured on a sample, the result is usually a number, for instance pH, or concentration of Fe^{2+} , or absorbance at a specific wavelength; algebraically, this result is a scalar, which in the remainder of the chapter will be indicated using the notation x_{ij} , i.e. measurement of the property j on sample i . If more than a property is used to characterize a sample, then experimental profile takes the form of a vector, whose components are the results of the individual measurements on the sample itself:

$$\mathbf{x}_i = [x_{i1} \quad x_{i2} \quad \cdots \quad x_{iv}] \quad [11.1]$$

In Equation 11.1, $\mathbf{x}_i$ represents the vector collecting the v measurements performed on sample i . The direct consequence of the fact that a sample is described by a v -dimensional vector of measurements, is that it can also be represented as a point in a v -dimensional coordinate space, whose axes

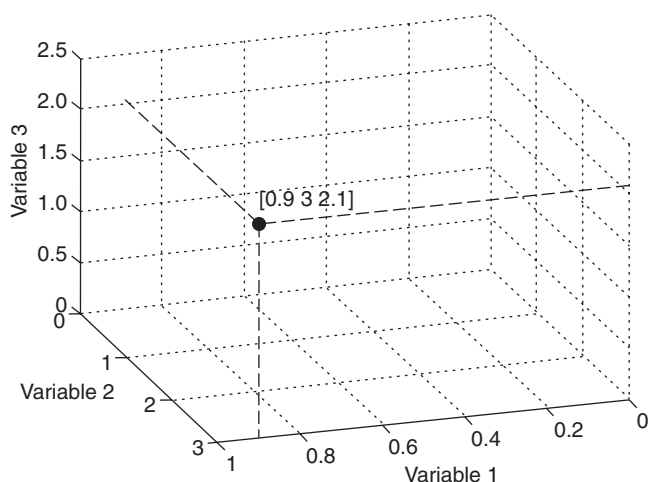


Fig. 11.1 Representation of the vector collecting the measurements of three variables on the i th sample as a point in the three-dimensional space.

correspond to the measured variables. For instance, if only three variables are measured on the i th sample, so that $x_{i1} = 0.9$, $x_{i2} = 3.0$ and $x_{i3} = 2.1$, then the sample can be represented as a point in a three-dimensional space having coordinates 0.9 along the first dimension, corresponding to variable 1, 3.0 along the second, corresponding to variable 2, and 2.1 along the third corresponding to variable 3 (Fig. 11.1).

If the same set of signals are measured on more than one sample, then a proper way of representing the experimental results is in the form of a data matrix $\mathbf{X}$:

$$\mathbf{X} = \begin{pmatrix} x_{11} & \cdots & x_{1v} \\ \vdots & \ddots & \vdots \\ x_{r1} & \cdots & x_{rv} \end{pmatrix} \quad [11.2]$$

Each row of the data matrix collects the measurements performed on a single sample, so that, analogously to that described above, it can be graphically represented as a point in the v -dimensional space defined by the variables. Therefore, if a data matrix contains the profiles measured on r samples, it corresponds to a distribution of r points in a v -dimensional space. On the other hand, each column is made of the measurements of an individual variable on all samples.

If experiments are properly designed, the matrix $\mathbf{X}$ contains variation which is supposed to be relevant for the food quality problem at hand. In the following sections, methods to investigate this variation depending on the purpose of the research and the problem definition will be presented.

11.3 Exploratory data analysis (EDA)

Exploratory data analysis (EDA) is an approach/philosophy for data analysis that employs a variety of techniques to summarize their main characteristics in easy-to-understand form, often with visual graphs, without using a statistical model or having formulated a hypothesis (Tukey, 1977). Its main purposes are to maximize insight into a data set, to uncover underlying structure, to extract important variables, to detect outliers and anomalies, to test underlying assumptions and to develop parsimonious models (Smilde *et al.*, 2004). Therefore, exploration is and should always be the first step in data analysis.

When many variables are measured on the samples, graphical inspection of the data in the original v -dimensional space described in Section 11.2 can be difficult or even unfeasible; therefore, to find patterns, relations, differences and similarities between objects and/or variables, methods which project the data on a relevant subspace (as principal component analysis (PCA) which will be described in Section 11.3.1) can be used to summarize the data conveniently and explore the data set using plots and figures.

11.3.1 PCA

As already stated, exploratory analysis relies extensively on graphs and figures to gain an insight into the underlying data structure. Unfortunately, human eyes can only see in three dimensions, so that when more than three variables are measured on each sample, visual inspection of the data is severely hindered by this limitation. Indeed, considering bi- and tri-dimensional plots of all the possible combinations of two or three variables, not only would be cumbersome, but still would not solve the problem, as in each plot only a minimum part of the overall variability would be represented. Therefore, the key issue in multivariate exploratory data analysis is how it is possible to condense v -dimensional information in few dimensions, ideally two or three. PCA (Pearson, 1901; Wold *et al.*, 1987; Jolliffe, 2002) addresses this issue by identifying a low-dimensional subspace, which best fits (i.e. approximates) the data in a least squares sense. This condition is mathematically equivalent to stating that PCA operates by projecting the samples onto directions along which the variance is maximal. Whatever the formulation, the main underlying aspects of PCA are:

- correlation among experimental variables results in data points not randomly spread in the multivariate space – in particular, fewer coordinates than the number of original variables can be needed to describe the greatest part of the variation among the samples;
- data compression can be achieved by describing the samples in terms of latent variables, i.e. by variables that cannot be measured directly but are computed as linear combination of the original ones.

Mathematical formulation of PCA

As anticipated, PCA operates by projecting the data onto a low-dimensional latent variable subspace. In mathematical terms, this transformation is defined by the equation:

$$\underset{r \times v}{\mathbf{X}} \underset{v \times F}{\mathbf{P}} = \underset{r \times F}{\mathbf{T}} \quad [11.3]$$

where $\mathbf{X}$ is the original data matrix, $\mathbf{T}$ is the matrix collecting the coordinates (*scores*) of the r samples onto the new set of latent variables (called principal components), $\mathbf{P}$ is the matrix containing the coefficients of the linear transformation (*loadings*), and F is the dimensionality of the principal component subspace. In principle, Equation 11.3 could describe an infinite number of linear transformations of the original data onto F -dimensional subspaces; uniqueness of the solution is achieved by imposing that the first principal component has the largest possible variance (that is, accounts for as much of the variability in the data as possible), and each succeeding component in turn has the highest variance possible under the constraint that it be orthogonal to (i.e. uncorrelated with) the preceding components. Explained variance is then an index of how much of the total information present in the data matrix is accounted for by each principal component. In PCA terminology, the variance along a principal component is also called an *eigenvalue* and it is usually indicated by the symbol λ ; mathematically, it is defined as:

$$\lambda_k = \frac{\sum_{i=1}^r t_{ik}^2}{r-1} = \frac{\mathbf{t}_k^T \mathbf{t}_k}{r-1} \quad [11.4]$$

where λ_k is the eigenvalue of the k th PC, t_{ik} is the score of the i th sample on the k th principal component, and $\mathbf{t}_k$ is the k th column of the score matrix $\mathbf{T}$, i.e. it is the column vector collecting the scores of all r samples on the k th PC. Accordingly, as the sum of all the eigenvalues represents the total variance of the data set, it is possible to define how much of this variation is explained by the single principal components:

$$\%EV(k) = 100 \times \frac{\lambda_k}{\sum_j \lambda_j} \quad [11.5]$$

where $\%EV(k)$ is the per cent variance explained by the k th principal component.

PCA in practice

To illustrate the use of PCA in exploratory data analysis, a data set from a paper on the discrimination of the botanical origin of honey samples will be used (Marini *et al.*, 2004). The data set comprises the results of 15 determinations – pH; free, lactone, and total acidity; diastase; water content; specific conductivity; dextrose, fructose and DP2; colour; specific rotation;

HMF content; and $\delta^{13}\text{C}/^{12}\text{C}$ (on the whole sample and on the proteic fraction only) – on 73 honey samples from six different botanical origins: chestnut, eucalyptus, heather, honeydew, sulla, and wildflower. Therefore the corresponding data matrix has dimensions 73×15 . The first three principal components computed on the matrix after autoscaling (see Appendix A, Section 11.8.1) account for more than 81% of the total variance (39.1%, 27.0% and 15.6%, respectively); therefore, most of the information in the experimental data is compressed along these directions. Accordingly, by plotting the coordinates of the samples along these principal components (the so-called *scores plot*), it should be possible to identify whether samples are clustered or not, and/or to see trends or other forms of systematic variation. In particular, the projection of the 73 honey samples onto the first two or three principal components is reported in Fig. 11.2.

The plots in Fig. 11.2 show how, using the first two principal components only, it is possible to highlight the presence of well-defined clusters. In particular, six of the seven groups that can be observed in the figure appear to be quite compact, the only one having a higher dispersion being that with low values of both PC1 and PC2. Adding up a third dimension (Fig. 11.2b) enhances the separation among the groups. It must be stressed that PCA is an unsupervised technique, so the information about the different botanical origin of the samples was not used in defining the projection: for this reason, all the points in Fig. 11.2 were given the same symbols. However, in cases where this additional information is known, as the one discussed in this section, one could use this information for a better interpretation of the results. Indeed, by labelling the samples according to their different botanical origins (Fig. 11.3), one could extend the considerations made previously about the nature of the observed clusters.

In particular, if the information about the different botanical origin of the samples is used for the interpretation of the results, one could affirm that unifloral and multifloral honeys are well separated along the second component and that, furthermore, multifloral honeys are more heterogeneous as heather samples are split into two subgroups, while wildflower ones correspond to the cluster with the higher dispersion. In general, it is evident that the clustering observed in PCA corresponds to the different origins of the samples.

Interpretation of the observed differences among the samples in terms of the measured variables is carried out by inspecting the loadings matrix **P**. Indeed, each column of the loadings matrix contains the weights of the experimental variables for the definition of a particular principal component. Under this respect, as the loadings represent the direction cosines of the linear projection PCA is based on, geometrically they are the coordinates of the variables onto the principal components. Therefore, by plotting their values in a bi- or tri-dimensional graph (*loadings plot*), it is at first possible to unravel the relation between the experimental variables and, by

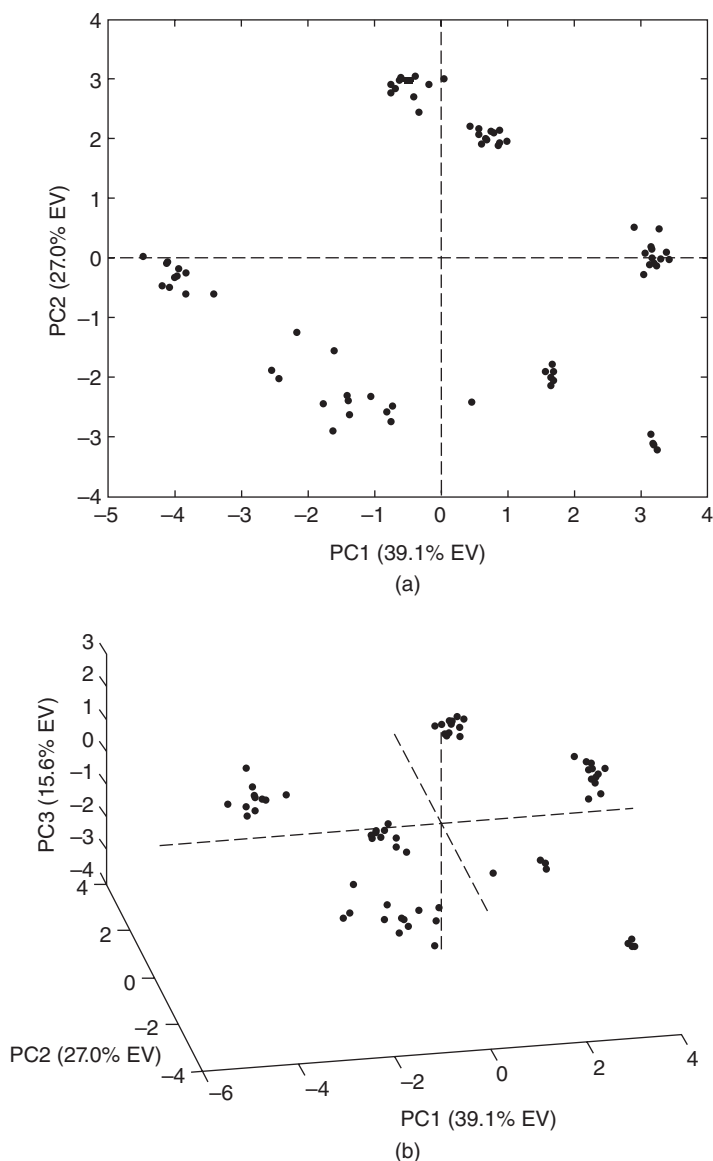


Fig. 11.2 PCA analysis of honey samples: projection of the samples onto the space spanned by the first two (a) or three (b) principal components (*scores plot*).

comparison with the scores plot, those between the variables and the samples.

The loadings plot showing the contribution of the 15 experimental variables to the definition of the first two principal components of the honey data set is shown in Fig. 11.4.

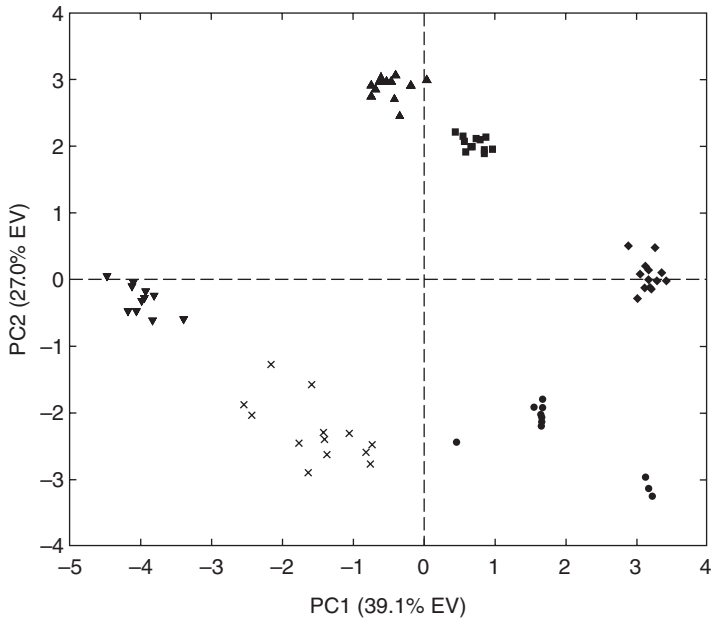


Fig. 11.3 PCA analysis of honey samples: projection of the samples onto the space spanned by the first two principal components, coupled to sample labelling according to their botanical origin. Legend: ◆ chestnut; ■ eucalyptus; ▼ heather; ● honeydew; ▲ sulla; × wildflower.

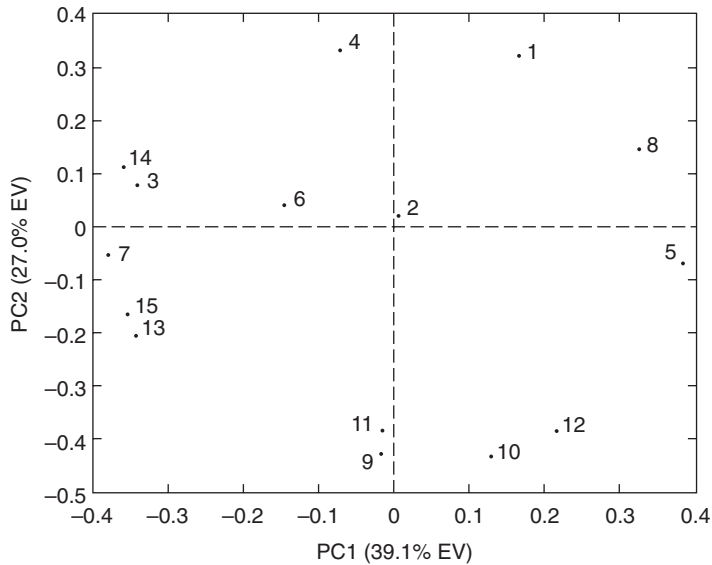


Fig. 11.4 PCA analysis of honey samples: loadings of the variables onto the first two principal components. Legend: (1) $\delta^{13}\text{C}/^{12}\text{C}$; (2) $\delta^{13}\text{C}/^{12}\text{C}$ on the proteic fraction; (3) dextrose; (4) fructose; (5) DP2; (6) water; (7) HMF; (8) diastase; (9) colour; (10) specific conductivity; (11) specific rotation; (12) pH; (13) free acidity; (14) lactones; (15) total acidity.

Interpretation of the loadings plot is relatively straightforward. At first, one can observe that the isotope ratio of the proteic fraction (labelled with number 2 in the figure) falls very close to the origin of the axis (i.e. it has loadings very close to zero for both principal components): this is an indication that this variable does not contribute to the definition of the first two components. In contrast, the variables having the highest absolute value of the loadings are the ones which contribute the most. In particular, %DP2 and HMF do contribute little to PC2 but are the variables contributing the most to PC1, the first with a very positive loading, the second with a negative one. Other variables contributing significantly to PC1 are diastase (positive loading) and dextrose, lactones, free and total acidity (negative loadings). As far as PC2 is concerned, variables contributing the most to its definition are: carbon isotope ratio and fructose (positive loadings), and colour, specific conductivity, specific rotation and pH (negative loadings). Furthermore, it is possible to identify which variables carry the same information, as they will fall quite close to one another onto the loadings plot: for instance, as far as the first two principal components are concerned, free and total acidity appear to be highly correlated and the same can be stated for dextrose and lactones, and for specific rotation and colour.

Lastly, it is possible to interpret the differences (and, in this case, the grouping) observed among the samples in terms of experimental variables, by comparing the scores and the loadings plots. As already discussed, multifloral and unifloral honeys are separated along the second principal component, the former having negative scores, the latter having positive ones. By coupling this information with that of the loadings plot, it is possible to affirm that unifloral honeys are characterized by a systematic higher value of fructose and carbon isotope ratio with respect to the multifloral ones, at the same time having lower values of colour, specific conductivity, specific rotation and pH. On the other hand, further differentiation among groups of samples occurs along the first principal component: heather, wildflower, sulla, eucalyptus, honeydew and chestnut are characterized by having increasing scores along PC1. Therefore, in moving from heather to chestnut, there is a systematic increase of diastase and %DP2, while at the same time HMF, dextrose, lactones, free and total acidity decrease.

PCA for outlier detection

As anticipated before, one of the aims of exploratory data analysis is the identification of outliers, i.e. of data, which deviate to a relevant extent from the typical range or pattern of observations exhibited by other samples (Rousseeuw and Leroy, 1996). Among its other uses, PCA can also be employed for the identification of outlying observations. To understand the way outlier detection by means of PCA works, it is first necessary to briefly mention about the use of PCA as a model of the data. In the paragraph describing the theoretical background of PCA, the projection leading to the principal component model was defined by equation 11.3:

this equation indicates that the score matrix $\mathbf{T}$ is the best F -dimensional representation of the original data in a least squares sense; accordingly, it would be possible to affirm that the F -component projection is a model of the systematic (relevant) variation present in the data matrix $\mathbf{X}$. If no measurement errors or other sources of undesirable variation affected the data, this representation would be exact, i.e. it would capture the total variability among the samples, so that projecting back the scores to the original variables, one would obtain a perfect reconstruction of the experimental data:

$$\mathbf{X} = \mathbf{TP}^T \quad [11.6]$$

However, as sources of variation other than the systematic (informative) ones are present in the data, especially noise due to measurement error, the principal component model describes only a part of the total variation in the data matrix (the relevant one), leaving the uninformative variation (residuals, $\mathbf{E}$) unmodelled:

$$\mathbf{X} = \hat{\mathbf{X}} + \mathbf{E} = \mathbf{TP}^T + \mathbf{E} \quad [11.7]$$

where $\hat{\mathbf{X}}$ represents the systematic variation in $\mathbf{X}$. This splitting of the experimental variability is crucial for understanding the way a sample can be outlying with respect to a PCA model. Indeed, when considering the model reported in equation 11.7, it is possible to define two ways according to which a sample can be identified as *abnormal* or *extreme*: on one hand, an observation could be outlying because it is distant to all the other samples in the model space, i.e. in the space spanned by the F principal components which account for the systematic variation; on the other hand, it can be anomalous because it is not fitted well by the principal component model (it has a high residual), and therefore it is said to be distant from the model space (Fig. 11.5).

Mathematically, this information is condensed into two statistical variables, which describe the distance of a sample to the centre of the principal component space (T^2) and its distance to the model space (i.e. its squared residuals, Q):

$$T_i^2 = \sum_{k=1}^F \frac{t_{ik}^2}{\lambda_k} \quad [11.8]$$

$$Q_i = \sum_{j=1}^v (x_{ij} - \hat{x}_{ij})^2 = e_{ij}^2 \quad [11.9]$$

where T_i^2 and Q_i are the values of the statistical variables T^2 and Q , respectively, for the i th sample, and e_{ij} is the element of the residual matrix $\mathbf{E}$ (Kourti and MacGregor, 1995). Statistical theory is then used to calculate threshold values at a defined confidence level (usually 95%) for these two statistics under the null hypotheses: if a sample presents either a T^2 or a Q value above their respective thresholds is considered to be an outlier. As the variation captured by the two statistical variables is orthogonal, outlier

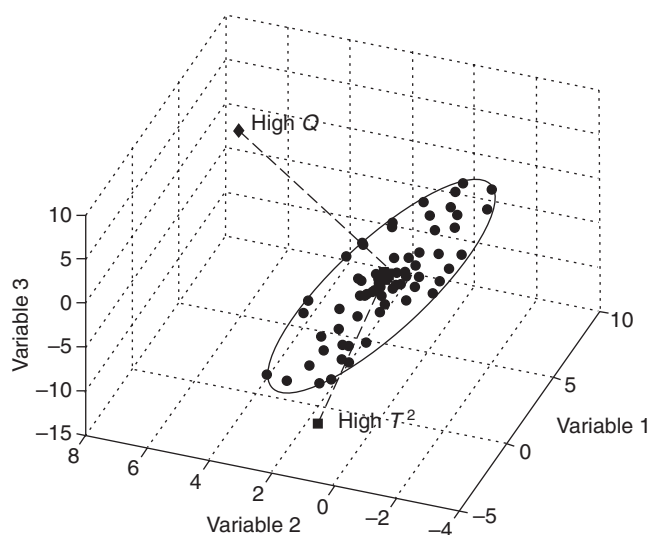


Fig. 11.5 Graphical representation of the meaning of T^2 and Q statistics to express distance to the model. The ellipse encloses the points with a small distance to the model ($\bullet$): the sample $\blacksquare$ lies in the same plane as the well-behaving points, so that its residual is practically zero; however, its distance within the model space is high (high T^2); on the other hand, the sample $\blacklozenge$, whose projection on the model plane falls in the middle of the normal observations, has a high residual, as it lies far away from that plane.

detection can easily be performed, by plotting the values of T^2 and Q for all the samples, together with their threshold values (Fig. 11.6).

The horizontal and vertical lines corresponding to the threshold values of T^2 and Q divide the plot in Fig. 11.6 in four regions: the normal samples fall in the lower leftmost part of the graph, having both T^2 and Q values below their respective thresholds; observations falling in the other three areas are all identified as extreme points even though, depending on their position on the plot, they can be further differentiated based on the different contribution of the two statistical variables.

11.3.2 Multidimensional scaling (MDS)

In some cases, the attention is focused on similarity (or dissimilarity) among the samples, so that rather than looking for a parsimonious representation of the distribution of points in the original variable space, one looks for a low-dimensional projection where the distances among the observation are as much preserved as possible. The resulting technique is called multidimensional scaling (MDS) and it is based on a projection similar to that described in equation 11.3; however, instead of using as input the experimental data matrix $\mathbf{X}$, the starting point is a square (usually symmetric)

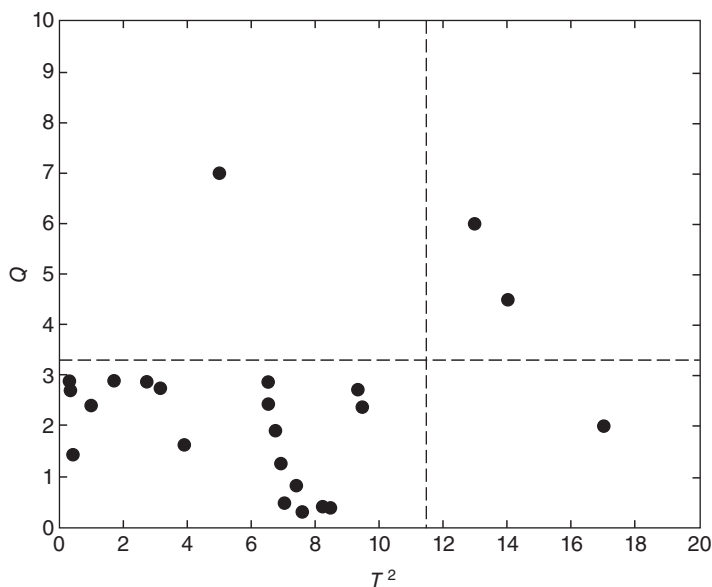


Fig. 11.6 Example of a T^2 vs Q plot for outlier identification.

matrix collecting a measure of dissimilarity (e.g. any kind of distance) among the samples (Kruskal and Wish, 1978).

11.4 Regression

Calibration is the use of empirical data and prior knowledge for determining how to predict quantitative information $\mathbf{Y}$ from available measurement $\mathbf{X}$ via some mathematical transfer function (Martens and Naes, 1989). In instrumental analysis, calibration plays a key role as the direct measurements of analyte concentration are rarely possible, so that quantification is usually based on secondary measures (e.g. chromatographic peak area, absorption or emission intensity, current, voltage and so on). It must be stressed that the relationship between the property (or properties) to be predicted and the secondary measurements does not always need to be linear: the importance lies in having a defined calibration equation in order to be able to make predictions on future samples. In this section, the most frequently used multivariate regression methods will be described; in particular, as most of the problems in instrumental assessment of food quality can be addressed by the use of linear methods, these techniques will be treated in greater detail. However, a brief survey of more complex non-linear methods will also be presented.

11.4.1 Multiple linear regression (MLR)

When one wants to predict the value of a property of the i th sample, y_i , based on the measure of v variables on the same individual, $[x_{i1} \ x_{i2} \ \dots \ x_{iv}]$, if a linear relation is assumed, the following equation holds:

$$y_i = b_0 + b_1x_{i1} + b_2x_{i2} + \dots + b_vx_{iv} + e_i \quad [11.10]$$

where b_0 is the intercept, the other b_i are the regression coefficients and the error term e_i indicates that the relation is not exact due to measurement errors. When multiple samples are analysed, the regression equation can be expressed by the formula:

$$\mathbf{y} = \mathbf{X}\mathbf{b} + \mathbf{e} \quad [11.11]$$

where $\mathbf{y}$ is the vector collecting the values of the measured properties for all the samples, $\mathbf{X}$ is the matrix containing the predictor variables, $\mathbf{b}$ is the vector of regression coefficients and $\mathbf{e}$ is the vector of residuals (Draper and Smith, 1998). It must be stressed that to account for the presence of the intercept b_0 in the model, the $\mathbf{X}$ matrix has to be augmented by including a column of ones; on the other hand, if both $\mathbf{X}$ and $\mathbf{y}$ are mean-centred (see Appendix A, Section 11.8.1), the model is without intercept. The calibration model is uniquely defined by the vector $\mathbf{b}$, which contains the regression coefficients. To calculate the value of the regression coefficient, it is necessary to have a set of samples for which both the $\mathbf{y}$ vector and the $\mathbf{X}$ matrix are known (*training set*); then, the regression vector $\mathbf{b}$ is defined as the set of coefficients which provides the best prediction of the $\mathbf{y}$ in a least squares sense:

$$\min_{\mathbf{b}} \|\mathbf{e}\|^2 = \min_{\mathbf{b}} \|\mathbf{y} - \mathbf{X}\mathbf{b}\|^2 \quad [11.12]$$

Solving equation 11.12, provides the set of MLR coefficients:

$$\mathbf{b}_{\text{MLR}} = (\mathbf{X}^T \mathbf{X})^{-1} \mathbf{X}^T \mathbf{y} \quad [11.13]$$

As already stated, the regression coefficient $\mathbf{b}$ univocally defines the functional dependence between the property to be predicted and the measured variables; therefore, once the values of the coefficient are calculated based on the training set, prediction of the y value for unknown samples, can be made according to:

$$\hat{\mathbf{y}}_{\text{new}} = \mathbf{X}_{\text{new}} \mathbf{b}_{\text{MLR}} \quad [11.14]$$

where $\mathbf{X}_{\text{new}}$ is the matrix collecting the experimental variables measured on the unknown samples, and $\hat{\mathbf{y}}_{\text{new}}$ is the vector containing the corresponding predicted values of the property.

Up to now, the case where only a single y has to be predicted for each sample was described; however, the mathematical model is easily translatable to the case when more than one dependent value is considered, i.e. when also $\mathbf{Y}$ is multivariate. When multiple responses have to be predicted for each sample, equation 11.11 changes to:

$$\underset{r \times m}{\mathbf{Y}} = \underset{r \times v}{\mathbf{X}} \underset{v \times m}{\mathbf{B}} + \underset{r \times m}{\mathbf{E}} \quad [11.15]$$

where $\mathbf{Y}$ is the matrix collecting the values of the m response variables for the r samples, $\mathbf{B}$ is the matrix of regression coefficients and $\mathbf{E}$ is the residual matrix. It should be stressed at this point that equation 11.15 indicates that each response variable is modelled independently on the others, i.e. the problem can be described as multiple single y regression models: the i th column of the matrix $\mathbf{B}$ represents the regression vector for the prediction of the i th column of $\mathbf{Y}$. As in the single y case, the least squares solution to the multiple linear regression problem can be calculated from the training data, according to:

$$\mathbf{B}_{\text{MLR}} = (\mathbf{X}^T \mathbf{X})^{-1} \mathbf{X}^T \mathbf{Y} \quad [11.16]$$

Analogously to that described in equation 11.14, prediction on new samples is computed by right multiplying the matrix of predictors by the regression coefficients:

$$\hat{\mathbf{Y}}_{\text{new}} = \mathbf{X}_{\text{new}} \mathbf{B}_{\text{MLR}} \quad [11.17]$$

The theory described in this section shows that MLR, being the natural multivariate extension of the classical univariate least squares calibration, is relatively simple and straightforward. However, in many real cases when instrumental assessment of food quality is concerned, there are severe limitations to its applicability. These limitations are a direct consequence of the mathematical form of the solution of the least squares problem: calculation of the regression coefficients requires the inversion of the matrix $\mathbf{X}^T \mathbf{X}$, and for many experimental data this inverse does not exist or it is ill-conditioned. In particular, in order to have a reliable estimate of the regression coefficients, two main conditions have to be met: the columns of the matrix $\mathbf{X}$ have to be linearly independent (i.e. the experimental variables have to be uncorrelated) and the number of training samples r must exceed the number of independent variables v . While the second condition could in principle be satisfied by analysing more samples or by variable selection, the first one is seldom met, as instrumental variables are quite often correlated by nature or by sampling. If the matrix $\mathbf{X}$ is ill-conditioned, the solution is governed by the noise part of the data resulting in high variance of the predicted y values for new samples (Martens and Naes, 1989).

Many alternatives exist for dealing with this problem, and the most frequently used will be discussed in the following sections.

11.4.2 Principal component regression (PCR)

In the end of Section 11.4.1, it was pointed out how the use of multiple linear regression becomes unfeasible in practice, when the independent variables matrix $\mathbf{X}$ is ill-conditioned due to the presence of correlations or

because there are more predictors than samples. One way of dealing with this problem is by substituting the characterization of the samples in terms of measured original variables with their description using an opportune number of principal components (see Section 11.3.1), and then regressing $\mathbf{Y}$ on the resulting score matrix $\mathbf{T}$ (Jolliffe, 1982). Indeed, PCA approximates the data using a low number of abstract variables (usually significantly lower than the number of samples), which are mutually orthogonal: therefore, the use of the score matrix $\mathbf{T}$ to describe the data, instead of the original matrix $\mathbf{X}$, allows all the problems of ill-conditioning to be overcome. The regression model is then obtained by substituting the independent matrix $\mathbf{X}$, with the scores matrix $\mathbf{T}$ in equations 11.15 and 11.16. Accordingly, in mathematical terms, the principal component regression (PCR) model can be expressed by:

$$\begin{aligned}\mathbf{X} &= \mathbf{TP}^T + \mathbf{E}_X \\ \mathbf{Y} &= \mathbf{TC} + \mathbf{E}_Y\end{aligned}\quad [11.18]$$

where $\mathbf{E}_X$ and $\mathbf{E}_Y$ are the residual matrices for the X - and Y -blocks, respectively, and the $\mathbf{C}$ matrix collects the regression coefficients of the MLR model relating the dependent variables $\mathbf{Y}$ to the scores matrix $\mathbf{T}$:

$$\mathbf{C} = (\mathbf{T}^T\mathbf{T})^{-1}\mathbf{T}^T\mathbf{Y}\quad [11.19]$$

By combining the two equations in 11.18, it is possible to obtain a matrix of regression coefficients relating directly the dependent variables to the measured $\mathbf{X}$:

$$\mathbf{B}_{\text{PCR}} = \mathbf{PC} = \mathbf{P}(\mathbf{T}^T\mathbf{T})^{-1}\mathbf{T}^T\mathbf{Y}\quad [11.20]$$

A crucial issue in building the PCR model is the selection of the optimal number of components to be used for describing the relevant variation in the $\mathbf{X}$ matrix. In this respect, the common practice is to select the first F components, i.e. the components associated to the F largest eigenvalues. However, some authors suggest using only those components, which correlate maximally with the dependent variables (Mason and Gunst, 1985), especially for data where most of the variation is known to be unrelated to the $\mathbf{Y}$. In general, there is a trade-off in selecting the optimal number of components: too few components do not fit $\mathbf{X}$ and do not predict $\mathbf{Y}$ well, whereas too many components overfit $\mathbf{Y}$ and $\mathbf{X}$, leading to unreliable and unstable predictions on new samples. Often, validation methods (see Appendix B, Section 11.8.2) are used to estimate the optimal number of components.

11.4.3 Partial least squares (PLS) regression

When describing the PCR model, it was mentioned that the principal components do not necessarily correlate maximally with $\mathbf{Y}$: indeed, the principal components are built so as to describe as much as possible of the

variation in $\mathbf{X}$; however, in cases where many sources of uninformative variation and/or a high level of noise are present, they can be poorly related to the $\mathbf{Y}$ (and, hence, not predictive). Based on these considerations, partial least squares (PLS) regression (Wold *et al.*, 1983; Geladi and Kowalski, 1986) finds components that compromise between fitting of $\mathbf{X}$ and predicting $\mathbf{Y}$. Indeed, as PCR, also PLS is a latent variable regression method, i.e. a method, which is based on projecting the samples onto a low-dimensional component space and then using this new set of abstract variables to predict the $\mathbf{Y}$. What is different in PLS with respect to PCR is that the information in $\mathbf{Y}$ is actively used for the definition of the component space. Mathematically, the PLS model is defined by the equation:

$$\begin{aligned}\mathbf{X} &= \mathbf{TP}^T + \mathbf{E}_X \\ \mathbf{Y} &= \mathbf{TQ}^T + \mathbf{E}_Y\end{aligned}\quad [11.21]$$

which shows that PLS regression tries to model both $\mathbf{X}$ and $\mathbf{Y}$ using the common components $\mathbf{T}$, which, as already stated, are constructed as a compromise between summarizing $\mathbf{X}$ and predicting $\mathbf{Y}$. In equation 11.21, $\mathbf{P}$ and $\mathbf{Q}$ are the X - and the Y -loadings, respectively, while $\mathbf{E}_X$ and $\mathbf{E}_Y$ are the residuals for the X - and Y -blocks. By comparing equations 11.18 and 11.21, it is possible to observe that the structure of PCR and PLS models is the same; however, the calculated components and the model coefficients are different in the two methods.

Mathematical formulation of PLS

As anticipated in the introduction to this section, PLS regression differs from PCR in using the Y -variables actively during the decomposition of $\mathbf{X}$, i.e. for the calculation of the scores matrix. The scores $\mathbf{T}$ are then defined in a way to be relevant both for interpretation and prediction; moreover, by balancing the X - and Y - information, the method reduces the impact of large, but irrelevant X -variation in the calibration modelling. To translate this concept in a mathematical formulation, a key role is played by the statistical concept of covariance. When two variables, a and b , are measured on the same r samples, resulting in the vectors $\mathbf{a}$ and $\mathbf{b}$, respectively, their covariance (assuming mean centring for both variables) is expressed as:

$$\text{Cov}(\mathbf{a}, \mathbf{b}) = \frac{\mathbf{b}^T \mathbf{a}}{r-1} \quad [11.22]$$

The covariance is a scale-dependent measure of linear interrelation between two variables, i.e. it takes into account not only the correlation between $\mathbf{a}$ and $\mathbf{b}$, but also the amount of variation in each of the two; therefore, it is a perfect measure for expressing in statistical terms the criterion for defining the PLS components. Indeed, the PLS latent variables are computed so that

the first PLS component is the direction of maximum covariance with the dependent variables, the second PLS component is orthogonal to the first and has maximal residual covariance, and so on. In the remainder of the section, the PLS algorithms for the cases of a single or multiple Y -variables will be described.

PLS algorithm for a single y variable

In order to illustrate how the PLS regression components are constructed, at first the case in which y is univariate will be discussed; for the sake of simplicity, $\mathbf{X}$ and $\mathbf{y}$ are assumed to be mean-centred (see Appendix A, Section 11.8.1). The first PLS component is calculated in a way that the scores along that direction, $\mathbf{t}_1$, have maximal covariance with $\mathbf{y}$:

$$\max_{\mathbf{w}_1} [\text{Cov}(\mathbf{t}_1, \mathbf{y})] = \max_{\mathbf{w}_1} (\mathbf{y}^T \mathbf{t}_1) \quad [11.23]$$

under the conditions:

$$\mathbf{t}_1 = \mathbf{X} \mathbf{w}_1 \quad \text{and} \quad \|\mathbf{w}_1\| = 1 \quad [11.24]$$

where $\mathbf{w}_1$ is the vector of PLS weights for the first component, i.e. the coefficients of the linear projection defining the first PLS direction, and, for convenience, the covariance is expressed without correction for the degrees of freedom (the denominator in equation 11.22). The first Y -loading q_1 is then calculated by regressing $\mathbf{y}$ on $\mathbf{t}_1$.

$$q_1 = \frac{\mathbf{y}^T \mathbf{t}_1}{\mathbf{t}_1^T \mathbf{t}_1} \quad [11.25]$$

After finding the first component based on equations 11.23 and 11.24, $\mathbf{X}$ and $\mathbf{y}$ are 'deflated' to calculate the successive ones: deflating means to remove the portion of variability accounted for by a particular component. Since the weights $\mathbf{w}$ describe the covariance between the dependent and the independent variables, deflation of the $\mathbf{X}$ matrix is carried out by a second set of coefficients, $\mathbf{p}$, which resemble the PCA loadings:

$$\mathbf{p}_1 = \frac{\mathbf{X}^T \mathbf{t}_1}{\mathbf{t}_1^T \mathbf{t}_1} \quad [11.26]$$

Accordingly, deflation of the $\mathbf{X}$ matrix is expressed as:

$$\mathbf{E}_1 = \mathbf{X} - \mathbf{t}_1 \mathbf{p}_1^T \quad [11.27]$$

where $\mathbf{E}_1$ is the residual matrix obtained after removing from the independent matrix the contribution of the first PLS component. In the univariate y case, the dependent vector could also be deflated, but this is not mandatory as deflating $\mathbf{X}$ makes the independent data orthogonal to the part of $\mathbf{y}$ already described. After deflation, the second component is calculated by solving a problem similar to equations 11.23 and 11.24, with $\mathbf{X}$ substituted by its deflated version $\mathbf{E}_1$: this results in a new set of scores

and weights $\mathbf{t}_2$ and $\mathbf{w}_2$, and a new Y -loading q_2 . The process continues with the computation of a new set of X -loadings $\mathbf{p}_2$ to operate the second deflation:

$$\mathbf{E}_2 = \mathbf{E}_1 - \mathbf{t}_2 \mathbf{p}_2^T \quad [11.28]$$

and goes on analogously until the desired number of components are extracted. As in PCR, the optimal number of latent variables is chosen on the basis of some kind of validation procedure (see Appendix B, Section 11.8.2). Defining this optimal number of components as F and grouping the single component vectors into the corresponding matrices:

$$\begin{aligned} \mathbf{T} &= [\mathbf{t}_1 \quad \mathbf{t}_2 \quad \cdots \quad \mathbf{t}_F] \\ \mathbf{P} &= [\mathbf{p}_1 \quad \mathbf{p}_2 \quad \cdots \quad \mathbf{p}_F] \\ \mathbf{W} &= [\mathbf{w}_1 \quad \mathbf{w}_2 \quad \cdots \quad \mathbf{w}_F] \end{aligned} \quad [11.29]$$

and the Y -loadings into a column vector:

$$\mathbf{q} = [q_1 \quad q_2 \quad \cdots \quad q_F]^T \quad [11.30]$$

the single y PLS model can be formulated as:

$$\begin{aligned} \mathbf{T} &= \mathbf{XW}(\mathbf{P}^T \mathbf{W})^{-1} \\ \mathbf{X} &= \mathbf{TP}^T + \mathbf{E}_X \\ \mathbf{y} &= \mathbf{Tq} + \mathbf{e}_Y = \mathbf{XW}(\mathbf{P}^T \mathbf{W})^{-1} \mathbf{q} + \mathbf{e}_Y = \mathbf{Xb}_{\text{PLS}} + \mathbf{e}_Y \end{aligned} \quad [11.31]$$

where the first equation relates the scores to the un-deflated $\mathbf{X}$ matrix, allowing the PLS regression vector to be defined in terms of the original independent variables as:

$$\mathbf{b}_{\text{PLS}} = \mathbf{W}(\mathbf{P}^T \mathbf{W})^{-1} \mathbf{q} \quad [11.32]$$

As stated above, the essential idea of PLS regression is the criterion of maximizing the covariance of $\mathbf{t}$ and $\mathbf{y}$. Since the covariance is the product of the variance of $\mathbf{t}$ and the correlation between $\mathbf{t}$ and $\mathbf{y}$, it follows that each component is characterized by (Smilde *et al.*, 2004):

- high variation in $\mathbf{t}$, meaning that noise is not modelled; and
- high correlation, ensuring that $\mathbf{t}$ has predictive relevance for $\mathbf{y}$.

PLS algorithm for multiple Y variables

In the case when more than one response variable has to be modelled, as $\mathbf{Y}$ affects the data compression of $\mathbf{X}$, the mathematical formulation discussed in the previous paragraph has to be slightly modified to account for the fact that the PLS components have to be optimal for the simultaneous prediction of all the dependent variables. Accordingly, the main difference with the single y PLS algorithm is that the systematic variation in the dependent matrix $\mathbf{Y}$ is also compressed into a set of scores, $\mathbf{U}$, and maximal covariance is sought between these scores and the scores of the X -block, $\mathbf{T}$.

More rigorously, the first PLS component in the case of multivariate $\mathbf{Y}$ is calculated according to:

$$\max_{\mathbf{w}_1, \mathbf{q}_1} [\text{Cov}(\mathbf{t}_1, \mathbf{u}_1)] = \max_{\mathbf{w}_1, \mathbf{q}_1} (\mathbf{u}_1^T \mathbf{t}_1) \quad [11.33]$$

under the conditions:

$$\begin{aligned} \mathbf{t}_1 &= \mathbf{X}\mathbf{w}_1 \quad \text{and} \quad \|\mathbf{w}_1\| = 1 \\ \mathbf{u}_1 &= \mathbf{Y}\mathbf{q}_1 \quad \text{and} \quad \|\mathbf{q}_1\| = 1 \end{aligned} \quad [11.34]$$

where $\mathbf{w}_1$ and $\mathbf{q}_1$ are the X -weights and Y -loadings for the first component, respectively. The two score vectors $\mathbf{t}_1$ and $\mathbf{u}_1$ are linearly correlated by means of the ‘inner’ regression coefficient c_1 , the term ‘inner’ indicating that it interrelates latent vectors:

$$c_1 = \frac{\mathbf{u}_1^T \mathbf{t}_1}{\mathbf{t}_1^T \mathbf{t}_1} \quad [11.35]$$

Given this difference, computation of the PLS model for the multiple Y case proceeds similarly to what has already been described, with the only exception that in this case it is mandatory to deflate also the $\mathbf{Y}$ matrix, yielding the residual matrix $\mathbf{F}_1$:

$$\mathbf{F}_1 = \mathbf{Y} - \mathbf{u}_1 \mathbf{q}_1^T \quad [11.36]$$

When the optimal number of component F is extracted, the resulting PLS model can be expressed as:

$$\begin{aligned} \mathbf{T} &= \mathbf{X}\mathbf{W}(\mathbf{P}^T \mathbf{W})^{-1} \\ \mathbf{X} &= \mathbf{T}\mathbf{P}^T + \mathbf{E}_X \end{aligned} \quad [11.37]$$

$$\mathbf{Y} = \mathbf{U}\mathbf{Q}^T + \mathbf{F}_Y = \mathbf{T}\mathbf{C}\mathbf{Q}^T + \mathbf{F}_Y = \mathbf{X}\mathbf{W}(\mathbf{P}^T \mathbf{W})^{-1} \mathbf{C}\mathbf{Q}^T + \mathbf{F}_Y = \mathbf{X}\mathbf{B}_{\text{PLS}} + \mathbf{F}_Y$$

where $\mathbf{C}$ is a diagonal matrix collecting the ‘inner’ regression coefficients and $\mathbf{Q} = [\mathbf{q}_1 \ \mathbf{q}_2 \ \dots \ \mathbf{q}_F]$. In the last equation, $\mathbf{B}_{\text{PLS}}$ is the matrix of regression coefficients defining the linear relation between the X - and Y -blocks in terms of the original variables:

$$\mathbf{B}_{\text{PLS}} = \mathbf{W}(\mathbf{P}^T \mathbf{W})^{-1} \mathbf{C}\mathbf{Q}^T \quad [11.38]$$

PLS in practice

To illustrate the use of PLS regression for multivariate calibration problems in the field of instrumental assessment of food quality, an example involving the quantification of proteins in oat samples based on visible-near infrared (NIR) spectroscopy will be discussed. The data set taken from Bellato *et al.* (2011) is made of the visible-NIR spectra (in the range 400–2500 nm) and the protein content (as quantified by a micro-Kjeldahl method) measured on 166 oat flour samples. In particular, 120 samples were selected as the training set, i.e. the set of observations used to build the calibration model,

while the remaining 46 were left out for the validation stage (see Appendix B, Section 11.8.2). As the reference method for the quantification of protein content in cereal samples (micro-Kjeldahl) is destructive and time-consuming, and requires the use of solvents and a long sample preparation step, the possibility of using a rapid, cheap and non invasive technique (visible-NIR spectroscopy in reflectance mode) to carry out the same determination is investigated. As the number of samples used for building the model (120) is significantly lower than the number of variables (1050), and since the spectral intensities at the different wavelengths are highly correlated, PLS regression is used to build the calibration model relating protein content to the visible-NIR fingerprint. Mean centring of both $\mathbf{X}$ and $\mathbf{y}$ was used to pre-process the data (Appendix A, Section 11.8.1). The optimal dimensionality of the model was evaluated as the number of PLS components resulting in the minimum error in 10-fold cross-validation (see Appendix B, Section 11.8.2), and was found to be six latent variables. A first evaluation of the quality of the model in terms of its accuracy can be obtained by calculating its bias (δ_C) and root mean square error in calibration (RMSEC), which are two figures of merit whose magnitude is inversely related to the trueness and precision of the model, respectively. They are defined as:

$$\delta_C = \frac{\sum_{i=1}^r (y_i - \hat{y}_i)}{r} \quad [11.39]$$

and

$$\text{RMSEC} = \sqrt{\frac{\sum_{i=1}^r (y_i - \hat{y}_i)^2}{r}} \quad [11.40]$$

where y_i and $\hat{y}_i$ are the measured and predicted y values for the i th training sample, and r is the total number of samples used for building the calibration model. For the data set discussed in this example, both values resulted to be quite low: in particular, calibration bias was practically absent (~ 0), while RMSEC was 0.32. Considering that the protein content ranged between 12% and 21% this indicates a high trueness and precision of the method in calibration. However, as also stressed in Appendix B, Section 11.8.2, calibration results can be over-optimistic, so that to estimate the performances of a supervised chemometric method, one should examine the figures of merit in validation. When the formulas in equations 11.39 and 11.40 are evaluated on an external validation set (test set), prediction bias (δ_P) and root mean square error in prediction (RMSEP) are obtained. In this study, 46 samples were left out as test set and used to validate the PLS model built on the training observations. When the optimal model is applied to the test samples, a prediction bias of 0.03 and an RMSEP of 0.28 are obtained. These results represent a more reliable estimate of the predictive ability of the method when unknown samples are analysed. Moreover, the fact that the bias and root mean square error values estimated on the

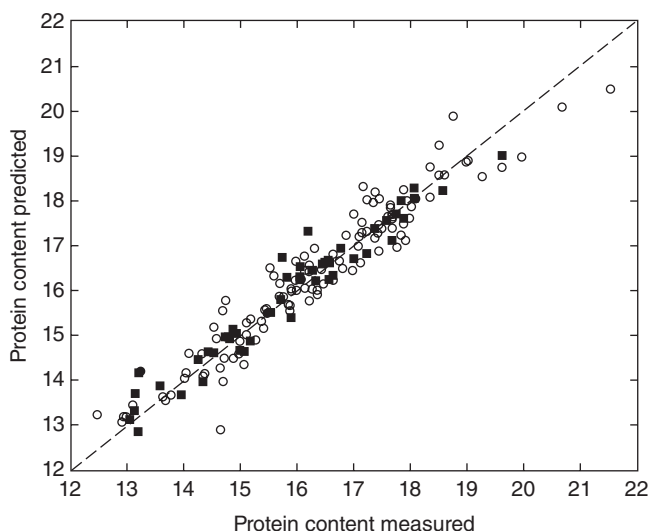


Fig. 11.7 PLS analysis of oat samples: comparison between the actual and predicted protein content for training ($\square$) and test ($\blacksquare$) samples.

training and on the test samples are of the same order of magnitude is an index of the absence of overfitting. The same information about the model's predictive ability on calibration and validation samples can be visualized in Fig. 11.7, where the predicted protein content is plotted as a function of the true (measured) protein content for both sets of observations.

In this graph, accurate calibration is represented by points lying as close as possible to the diagonal: it can be seen that the good predictive ability of the PLS model discussed above is reflected in the figure, as the observations are well aligned along the diagonal with a low scatter. Together with the figures of merit and the observed *vs* predicted plot described above, there are other graphs which can be useful for the interpretation and the diagnostics of a PLS model. A plot that is important for diagnostics is the one of $\mathbf{y}$ as a function of the different $\mathbf{t}_i$ (or of $\mathbf{u}_i$ *vs* $\mathbf{t}_i$ in the case of multiple dependent variables). Indeed, as the successive PLS components are extracted to account for as much as possible of the covariance with $\mathbf{y}$, linear trends have to be observed in these plots. The presence of curvature in the distribution of points in the graph is an index of a possible non-linear relation between the $\mathbf{X}$ and the $\mathbf{y}$. As an example, in Fig. 11.8, the scatterplot of $\mathbf{y}$ as a function of the scores along the first PLS component for the PLS model discussed in this section is reported.

Even if there is some scatter of the points, suggesting the possibility that other sources of uninformative variation are present, a clear linear trend can be observed in the figure, confirming the validity of the assumed linear model. On the other hand, from the interpretation standpoint, it can be

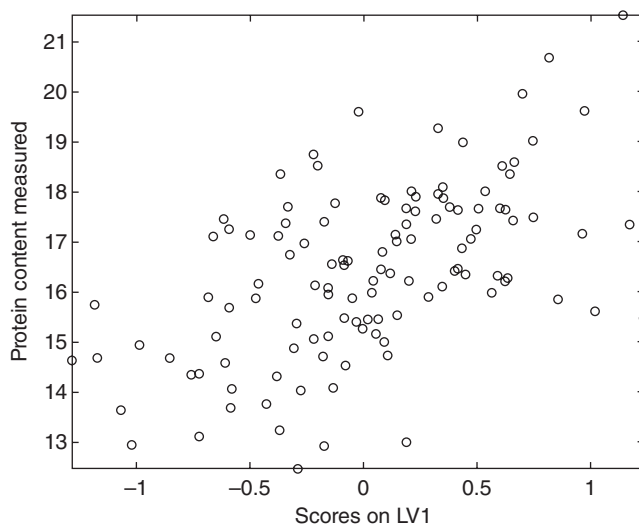


Fig. 11.8 PLS analysis of oat samples: plot of $\mathbf{y}$ vs $\mathbf{t}_1$ (i.e., protein content vs scores on the first latent variable) for training samples shows a linear trend, confirming the goodness of the model.

important to inspect the regression vector $\mathbf{b}$: indeed, the regression coefficients are the weights associated to the independent variables for the prediction of the $\mathbf{y}$, so that the larger their value the bigger the contribution of the corresponding predictor will be. In this respect, it must be stressed that their magnitude matches the units of both the independent and the dependent variable; consequently, the scaling of $\mathbf{X}$ must be kept in mind when interpreting regression coefficients. In cases when all the variables of the $\mathbf{X}$ -block are pre-processed in a way that makes them vary in the same numerical interval (e.g. by auto-scaling, see Appendix A, Section 11.8.1), interpretation can become more straightforward, as the regression vector may reflect the relative importance of the individual variables. However, care has to be taken when inspecting the regression coefficients. Indeed, with spectral data (or, in general, with other instrumental fingerprints), overlapping signals introduce phenomena in the regression vector that disturb interpretation (Seasholtz and Kowalski, 1990). For instance, when the signal of an interferent is overlapping (non-orthogonal) with the analyte pure spectrum, the regression vector no longer looks like the pure spectrum because negative parts and shifts in position of peak maximum are introduced. The presence of such phenomena may blur interpretation seriously. Similar problems occur for non-spectral data. For instance, a correctly estimated negative regression coefficient can easily be obtained for a variable that is positively correlated to the response (Kjeldahl and Bro, 2010). Figure 11.9 shows the regression vector for the PLS model described in this paragraph.

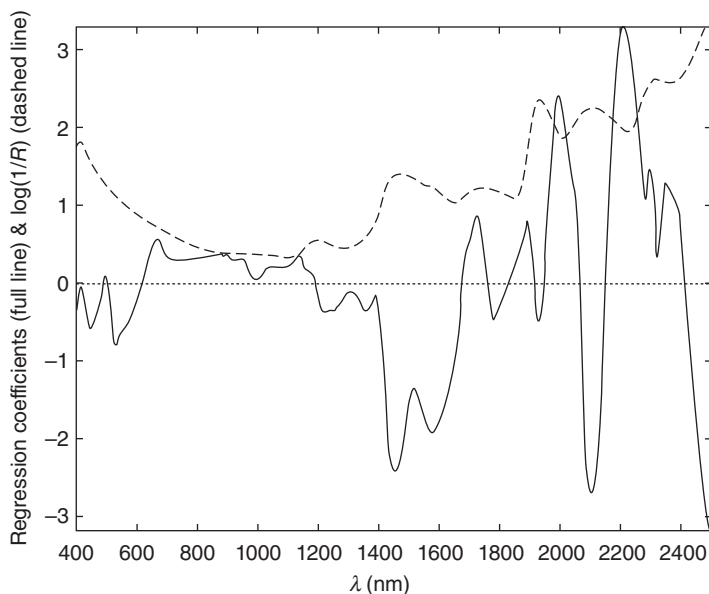


Fig. 11.9 PLS analysis of oat samples: representation of the regression vector **b** (continuous line) superimposed to the average NIR spectrum of the analysed samples (dashed line).

This figure provides a nice exemplification of the considerations and caveats discussed above: for instance, it is possible to observe that, in the case of the spectral bands at 1700–1900 nm and 2000–2200 nm, half peak is negatively and the other half is positively correlated with the dependent variable (protein content).

Accordingly, several other methods have been proposed in the literature for the identification of relevant predictors both for interpretation and variable selection: among these, the use of VIP scores (Wold *et al.*, 1993), target projection (Kvalheim and Karstang, 1989), selectivity ratio (Kvalheim, 2010) and orthogonal projections (Trygg and Wold, 2002) can be cited.

11.4.4 Non-linear regression

In complex situations, a linear model can be insufficient to describe the relationship between the dependent and the independent variables, so that a non-linear regression approach has to be followed. In this framework, to keep the advantages in terms of model parsimony and stability of the regression solution deriving from the use of latent variable-based methods, different modifications to the original PLS algorithm to accommodate non-linear relationship have been proposed. For instance, one non-linear version of the PLS algorithm introduces the non-linearity in the inner relation

between the X - and Y -scores of the i th component ($\mathbf{t}_i$ and $\mathbf{u}_i$), e.g. via a polynomial function (usually quadratic):

$$\mathbf{u}_i = c_{0,i} + c_{1,i}\mathbf{t}_i + c_{2,i}\mathbf{t}_i^2 \quad [11.41]$$

where $c_{0,i}$, $c_{1,i}$ and $c_{2,i}$ are the inner regression coefficients (Wold *et al.*, 1989). On the other hand, another very powerful way of dealing with non-linearities in the PLS framework is to take advantage of the ability of this algorithm to deal with situations where the number of variables is significantly higher than the number of samples. Indeed, if a suitable non-linear transformation to a high-dimensional feature space is found, there can be the case where the relationship between the dependent variable(s) and the transformed data becomes linear, and hence can be modelled by classical PLS. Moreover, it can be shown that there is no need to define the transformation function explicitly, as it is possible to take advantage of the so-called ‘kernel trick’: under some very loose conditions it is possible to estimate directly the covariance between the non-linearly transformed data and the dependent variables, without even defining the feature mapping. This constitutes the basis of radial basis function-PLS, which has been successfully applied to many non-linear real-world calibration problems (Walczak and Massart, 1996).

It should be stressed here that many more non-linear regression methods could be discussed here, such as neural networks (Marini, 2009), support vector machines (Vapnik, 2000), locally weighted regression (Centner and Massart, 1998), and so on, but such a complete and detail description would go beyond the scope of this chapter.

11.5 Classification

Classification is the operation of assigning one object (sample) to one category based on a set of experimental measurements performed on the sample itself. It can be thought as the process of finding a relationship between the matrix of independent variable $\mathbf{X}$ and a qualitative vector of responses, accounting for class belonging (Massart *et al.*, 1988). Problems involving classification are ubiquitous when dealing with instrumental assessment of food quality, as in many cases the authentication issues can be expressed in terms of categorical answers. For instance, one could be interested in whether a sample meets the production specification or not, or if a cheese sample was made with goat, sheep or cow milk, or again whether a wine bottle comes from a protected designation of origin (PDO) as declared or not. In all the aforementioned examples, each of the possible outcomes represents a class, so that the mathematical problem consists of finding a criterion to assign an unknown sample to one of the possible categories provided by the specific case. In this section, a general illustration of the two possible approaches to classification, discriminant

and modelling, will be presented, followed by a more detailed description of two of the most used techniques in this ambit, partial least squares-discriminant analysis (PLS-DA) and soft independent modelling of class analogies (SIMCA).

11.5.1 Discriminant classification vs class-modelling

As anticipated in the introduction to this section, by the term classification we mean the operation of assigning an object (sample) to a category based on the measurements performed on it. Geometrically, this corresponds to identifying regions in the hyperspace of the variables corresponding to the different classes. At present, there are many different classification methods in the chemometric literature, each one with different characteristics, so that several different subdivisions among them are possible. If, for instance, attention is focused on the mathematical form of the relation that characterizes the classification method and that corresponds to the shape of the geometric boundary of the classes in the hyperspace of the variables, one can speak of linear classification methods, when a linear functional dependence between the class boundaries and the measured variables is implied, whereas all the other cases fall within the family of non-linear classification tools.

On the other hand, another subdivision that is particularly important from an applicative viewpoint is that between pure classification (discriminant) and class-modelling techniques (Vandeginste *et al.*, 1998). Discriminant techniques are focused on the differences between samples coming from different classes and operate by dividing the hyperspace of the variables in as many regions as the number of available categories: for instance, if in the data set there are only samples from four categories, the whole hyperspace of the variables is divided into the same number of regions, so that if the coordinates of a sample are such that it falls in the region labelled as 'class 1' it is assigned to that class and so on (Fig. 11.10a).

When a discriminant technique is used, a sample is always assigned to one of the available classes. On the other hand, class-modelling techniques are rather focused on the similarities among samples of the same class than on the differences among the classes. Indeed, in a class-modelling technique every category is modelled separately irrespectively of the others (Fig. 11.10b). As a consequence a sample can be accepted or rejected by the specific category model and also cases of a single class can be analysed. When more than one category is present a sample can be accepted by only one category model (and in this case it is assigned to that category), be refused by all the category models (and hence it is considered as an outlier for all the models – this can be, for instance, the case of a sample coming from another category that was not modelled) – or be accepted by more than one class model (and in this case the sample is said to be 'confused'). In the latter case, classification is still possible as in the case of discriminant methods, by assigning the sample to the class to which it is more similar.

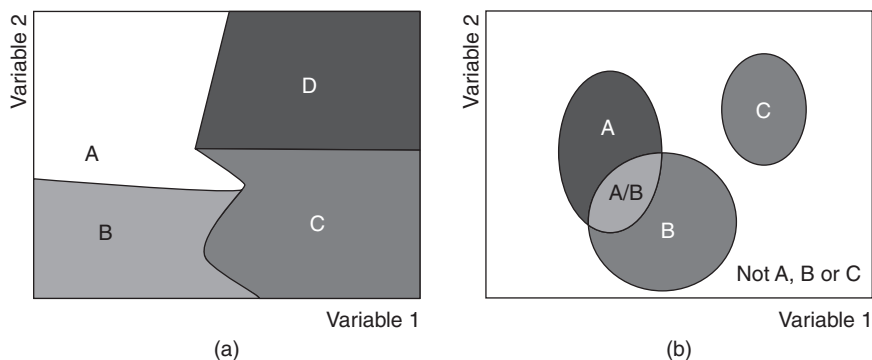


Fig. 11.10 Schematic representation of the difference between discriminant (a) and modelling (b) approach to classification. Discriminant techniques (a) partition the available variable space in as many regions as the available classes; modelling techniques (b) define the individual regions for a category allowing for overlap and unassigned areas.

11.5.2 PLS-DA

As explained in Section 11.5.1, discriminant classification techniques operate by dividing the multidimensional space of the variables in as many regions as the number of available categories, so that a sample is always assigned to one and only one class. Accordingly, the boundaries separating the different categories can be of increasing complexity, the simplest one being a surface that is linear in the original variables (a line in two dimensions, a plane in three, a hyperplane in the multivariate space). Notwithstanding the simplicity of the model, linear classification methods can provide accurate predictions in many real-world applications and therefore represent the family of discriminant techniques most often used. In this framework, historically the first method to be proposed was linear discriminant analysis (LDA), originally developed by Fisher (1937).

As all the linear classifiers, LDA assumes that the regions of the multivariate space corresponding to the different classes can be separated by linear surfaces (hyperplanes). However, from a mathematical statistical standpoint, it is a parametric technique, i.e. it assumes an underlying probability distribution for the experimental results measured on samples belonging to a specific category: as a consequence, building a classification model translates to evaluating the parameters of the distribution for the different classes, estimating the probabilities that the sample belongs to each of the categories and then assigning the object to the category for which it has the highest probability. In particular, the hypothesis behind LDA is that the data follow a multivariate Gaussian distribution, with the same scatter (represented by the covariance matrix $\mathbf{S}$) for all the classes, so that the probability that a sample characterized by the vector of measurement $\mathbf{x}$ belongs to class g can be expressed as:

$$p(g|\mathbf{x}) \propto \frac{2}{(2\pi)^{\frac{r}{2}}|\mathbf{S}|} e^{-\frac{1}{2}(\mathbf{x}-\mathbf{m}_g)^T \mathbf{S}^{-1}(\mathbf{x}-\mathbf{m}_g)} \quad [11.42]$$

where $\mathbf{m}_g$ is the centroid (a vector containing the means of each variable) of class g and r is the dimensionality of the variable space. As the decision boundary separating class g from class f is defined by the condition:

$$p(g|\mathbf{x}) = p(f|\mathbf{x}) \quad [11.43]$$

by substituting equation 11.42 into 11.43 and taking the logarithm of both sides, the following expression is obtained:

$$\begin{aligned} c_g + \frac{\mathbf{x}^T \mathbf{S}^{-1} \mathbf{x}}{2} - \frac{\mathbf{m}_g^T \mathbf{S}^{-1} \mathbf{m}_g}{2} + \mathbf{m}_g^T \mathbf{S}^{-1} \mathbf{x} &= c_f + \frac{\mathbf{x}^T \mathbf{S}^{-1} \mathbf{x}}{2} - \frac{\mathbf{m}_f^T \mathbf{S}^{-1} \mathbf{m}_f}{2} + \mathbf{m}_f^T \mathbf{S}^{-1} \mathbf{x} \\ \Rightarrow (c_g - c_f) + \frac{(\mathbf{m}_g - \mathbf{m}_f)^T \mathbf{S}^{-1} (\mathbf{m}_g - \mathbf{m}_f)}{2} + (\mathbf{m}_g - \mathbf{m}_f)^T \mathbf{S}^{-1} \mathbf{x} &= 0 \end{aligned} \quad [11.44]$$

where c_g and c_f are constant terms and it is shown that the assumptions result in linear separation boundaries, whose coefficients are functions of the centroids for the two classes and the pooled variance–covariance matrix. As the calculation of model parameters requires the inversion of $\mathbf{S}$, LDA suffers from the same drawbacks already discussed for MLR in cases where the matrix to be inverted is ill-conditioned. Therefore, this technique is not applicable when the number of samples is lower than the number of variables and/or when there is correlation among the signals. To overcome these limitations, a variant of PLS able to deal with classification problem has been proposed (Barker and Rayens, 2003). Indeed, if a suitably designed dummy response vector coding class belonging is introduced, traditional regression methods can be used also to tackle with classification problems. In particular, when dealing with a classification problem involving G categories, one can build a dummy binary-coded G -dimensional response vector, so that, if a sample belongs to class one, it will have a ‘1’ as the first component and the remaining components will all be zero, if it belongs to class two, it will have a ‘1’ as the second component, while the remaining will all be zero, and so on (equation 11.45).

$$\mathbf{Y} = \begin{bmatrix} 1 & 0 & \cdots & 0 \\ 1 & 0 & \cdots & 0 \\ \vdots & \vdots & \ddots & \vdots \\ 0 & 1 & \cdots & 0 \\ 0 & 1 & \cdots & 0 \\ \vdots & \vdots & \ddots & \vdots \\ 0 & 0 & \cdots & 1 \\ 0 & 0 & \cdots & 1 \end{bmatrix} \quad [11.45]$$

Under these assumptions, to compute a classification model corresponds to calculating the regression vector between the data matrix $\mathbf{X}$ and this dummy vector of responses $\mathbf{Y}$; as the name suggests, PLS-DA does so using PLS regression (see Section 11.4.3). Accordingly the values of $\mathbf{X}$ and $\mathbf{Y}$ for the training samples are used to build the PLS model, and in particular the regression coefficients $\mathbf{B}$ which are essential for predicting the values of the dependent variables on new samples. It is important to stress that while the dummy matrix $\mathbf{Y}$ for the training samples is binary coded, the corresponding predicted values $\hat{\mathbf{Y}}$ and any prediction made on unknown samples $\mathbf{Y}_{new}$ will contain real numbers. For instance, if a problem involves four categories, the 4-dimensional vector collecting the predictions for a generic sample could take the values $[0.87 \ 0.03 \ -0.08 \ 0.12]$. A sample is then assigned to the category corresponding to the component of the predicted response vector having the highest value (in this example, the first one). Accordingly, the error criterion employed in standard PLS (and in general in regression methods) to estimate the performances of the method, namely the root mean square error (described in equation 11.40), is not very useful when this algorithm is extended to classification issues. Indeed, as the aim is to assign a sample to one out of G possible categories, the only meaningful error criterion should be the one who considers whether this assignment is correct or not. Accordingly, to evaluate the performance of classification methods and, in particular, of PLS-DA, the percentage of correct classifications (%CC, also called non error rate) is introduced as:

$$\%CC = 100 \times \frac{n_{cc}}{n_{tot}} \quad [11.46]$$

where n_{cc} is the number of samples which are correctly classified by the model and n_{tot} is the total number of individuals. The correct classification rate can be computed either on the individual categories (and in that case the number of samples to be considered in equation 11.46 refers only to that class) or on the whole data set.

11.5.3 SIMCA

As anticipated in Section 11.5.1, discriminant techniques operate by univocally assigning an unknown sample to one and only one of the possible G classes represented in the training set. However, there can be cases where such an approach is not optimal to solve the problem of interest: for instance, when there are not enough samples for all the classes that are implied, or in those situations, such as the authentication of the origin of foodstuffs, when new categories are continually introduced, or again in the so-called asymmetric classification, that occurs whenever one is interested to assess whether a sample belongs to a specified class or not. Then, using a modelling approach could be more suitable to deal with the classification issue.

SIMCA (Wold, 1976; Wold and Sjöström, 1977) was the first class-modelling technique to be introduced in the chemometric literature. The main characteristics of this technique can be inferred by its name: each category is modelled independently of the others, and the definition of the model is based on capturing the similarities among the samples coming from the same category (contrarily to the discriminant approach, which focuses on finding what differentiates one class from another). In particular, in SIMCA, each class model is defined on the basis of a principal component model of opportune dimensionality. For instance, the model of class 1 can be assumed to be described by a F principal component model, according to:

$$\mathbf{X}_1 = \mathbf{T}_1 \mathbf{P}_1^T + \mathbf{E} \quad [11.47]$$

where $\mathbf{X}_1$ is the sub-matrix of the original data set obtained by selecting only the samples of category 1, $\mathbf{T}_1$ and $\mathbf{P}_1$ are the matrices containing the first F scores and loading vectors, respectively, and $\mathbf{E}$ are the residuals. Once the principal component model is computed, the class space is defined according to some statistically defined outlier detection criterion. Over the years, different criteria have been proposed, the simplest one focusing on the entity of the residuals only. At present, SIMCA is often performed using a distance-to-the-model criterion for outlier detection that is directly borrowed from multivariate statistical process control and is based on what has already been described in Section 11.3.1. In particular, the score matrix $\mathbf{T}_1$ and the residual matrix $\mathbf{E}$ are used to compute the probability distributions for the distances within the model space (T^2 statistics) and for the orthogonal distance to the model space (Q statistics) respectively. Accordingly, a threshold value corresponding to a predetermined confidence level (usually 95%) is chosen for both statistics so that the class space is defined by the equation:

$$d_i^g = \sqrt{\left(\frac{T_{i,g}^2}{T_{0.95,g}^2}\right)^2 + \left(\frac{Q_{i,g}}{Q_{0.95,g}}\right)^2} \leq \sqrt{2} \quad [11.48]$$

where d_i^g is the distance of the i th sample from the model of class g , $T_{i,g}^2$ and $Q_{i,g}$ are the values of the homonymous statistics computed for the i th sample with respect to the model of class g , while $T_{0.95,g}^2$ and $Q_{0.95,g}$ are their corresponding 95% confidence level threshold values for the same category. Once the model of a particular class (say class g) is defined, prediction on new samples is performed by first projecting the new observation $\mathbf{x}_{\text{new}}$ onto the principal component space for the category:

$$\mathbf{t}_{\text{new},g} = \mathbf{x}_{\text{new}} \mathbf{P}_g \quad [11.49]$$

where $\mathbf{t}_{\text{new},g}$ is the scores vector for the unknown on the model of class g , and $\mathbf{P}_g$ is the loadings matrix for the same category. Then, the vector of residuals for the unknown sample, $\mathbf{e}_{\text{new},g}$, is computed according to:

$$\mathbf{e}_{\text{new},g} = \mathbf{x}_{\text{new}} - \mathbf{t}_{\text{new},g} \mathbf{P}_g^T \quad [11.50]$$

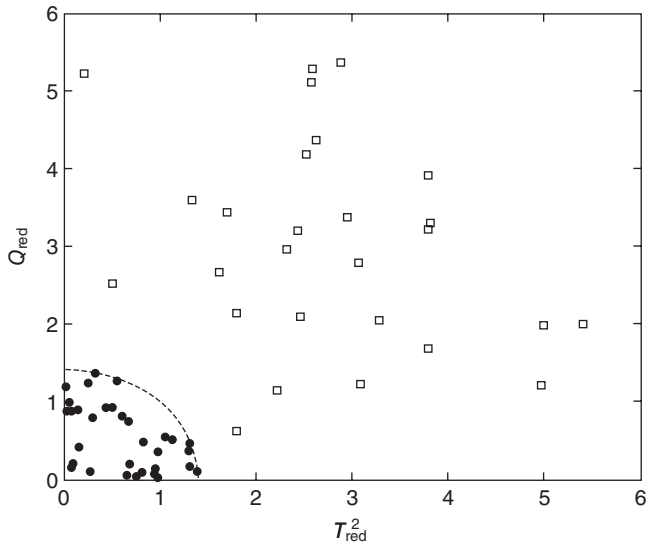


Fig. 11.11 SIMCA: illustration of the T^2 vs Q model plot. Samples falling below the dashed line are accepted by the class model, while samples above are rejected as outliers. In this example, as all the individuals of the modelled class (●) are accepted and all the objects from the other category (□) are rejected, the model has 100% sensitivity and specificity.

Lastly, the two vectors $\mathbf{t}_{\text{new},g}$ and $\mathbf{e}_{\text{new},g}$ are used to calculate the two statistics T^2 and Q which constitute the basis for the computation of the distance to the model:

$$\begin{aligned} T^2_{\text{new},g} &= \mathbf{t}_{\text{new},g}^T (\mathbf{T}_g^T \mathbf{T}_g)^{-1} \mathbf{t}_{\text{new},g} \\ Q_{\text{new},g} &= \mathbf{e}_{\text{new},g}^T \mathbf{e}_{\text{new},g} \end{aligned} \quad [11.51]$$

For the sake of an easier visualization of the results, the model space and the information about whether a sample is accepted or not by that specific category can be graphically represented in a T^2 vs Q plot, similar to the one reported in Figure 11.6. However, in this case the plot is divided in two regions by the dashed line corresponding to the thresholding criterion expressed by equation 11.48: samples falling below the line are accepted by the category model, while samples which are above are rejected as outliers (Fig. 11.11).

Since class-modelling techniques focus on assessing whether a sample belongs to a particular class or not, two different figures of merit are introduced to evaluate the performances of the method, sensitivity and specificity. Sensitivity is defined as the percentage of samples actually coming from the category that is modelled, which are correctly accepted by the class model. On the other hand, specificity is calculated as the percentage of

samples from other classes, which are correctly rejected by the model of the category.

When more than one class is modelled, the distance of an unknown sample to the different category models is computed, and three different situations can be encountered: the individual can be accepted only by one class, it can be accepted by more than one, or it can result an outlier for all categories. However, whatever the case, if a discriminant classification is sought anyway, it still can be performed by assigning the sample to the category it is less distant to.

11.5.4 Classification in practice

To illustrate the practical application of the two classification approaches described in the previous sections, an example of the use of PLS-DA and SIMCA for tracing the geographical origin of olive oil samples will be presented (Bevilacqua *et al.*, 2012). In particular, the aim of the study was to build a classification model for the authentication of oils coming from the PDO 'Sabina' (Lazio, Italy) using NIR spectroscopy as fingerprinting technique. To this purpose, a set of 20 oils from Sabina and 37 samples from other origins were collected and analysed; then 35 measurements (13 Sabina and 22 from other origins) were selected as training set and the remaining 22 (7 Sabina and 15 others) as independent validation set (see Appendix B, Section 11.8.2).

PLS-DA

At first, the application of a discriminant approach by means of the PLS-DA algorithm will be presented: in this framework, notwithstanding the heterogeneity of the oil samples from different provenances, the problem can be formulated as involving two categories, Sabina and oils from all other origins. In cases like this, where only two classes are present, due to the symmetry of the classification problem, it is not necessary to build a two-column matrix $\mathbf{Y}$ to codify the class belonging of the training samples, as the same information can be represented as a vector $\mathbf{y}$, assuming the value 1 for Sabina samples and 0 for all the others. Then, a PLS model relating the $\mathbf{X}$ matrix made of the spectral measurements performed on the samples to this binary $\mathbf{y}$ vector was built; its optimal complexity (i.e. the optimal number of latent variables) was chosen as the one leading to the highest correct classification rate in cross-validation (see Appendix B, Section 11.8.2) and resulted in 5 components. The PLS-DA model was able to correctly classify all Sabina samples and 95.5 of the individuals from other origins in the training set. Moreover, when it was applied to the independent validation observations, the origin of all the samples was correctly predicted. Since PLS-DA borrows the algorithmic structure from PLS, a rapid and intuitive interpretation of the results and model diagnostics can be obtained by means of the same plots already described in Section 11.4.3.

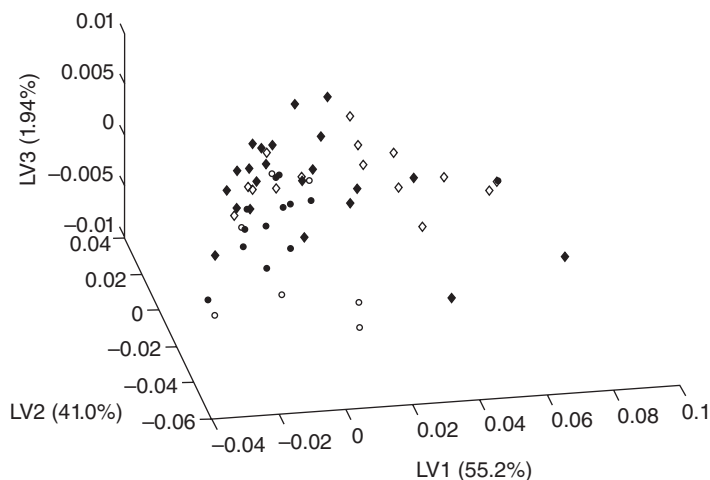


Fig. 11.12 PLS-DA on olive oil data: projection of the training and test samples onto the first three LVs of the PLS model. Legend: ● Sabina training, ○ Sabina test, ◆ other origins training, ◇ other origins test.

However, for classification purposes, inspection of other plots can provide further information. In particular, projection of the samples onto the first two or three PLS components (a scores plot analogous to the one described in the case of PCA, see Section 11.3.1) allows us to visualize to what extent the different categories are separated and how the samples are distributed within the individual class spaces (Fig. 11.12).

The plot in Fig. 11.12 shows the projection of the training and validation samples analysed in this study onto the first three PLS latent variables. It is possible to observe from the plot that the two categories are well separated and that the validation samples fall in regions of space spanned by the training samples of the corresponding categories: this very good separation is reflected in the optimal classification ability of the model both in the modelling and in the validation stages.

SIMCA

The problem illustrated in this example represents one of the cases in which a modelling approach to classification could be more appropriate than a discriminant one. Indeed, the aim is to assess whether an unknown sample comes from the PDO Sabina as declared or not: what in the case of PLS-DA was modelled as a single category, it is actually a heterogeneous collection of samples with different characteristics, having in common only the fact of not coming from the Sabina region. Accordingly, in this paragraph the same data set will be used as an example of how to apply modelling techniques, and, in particular, SIMCA, for classification purposes. The first obvious difference with the discriminant approach is that, as the interest is focused on

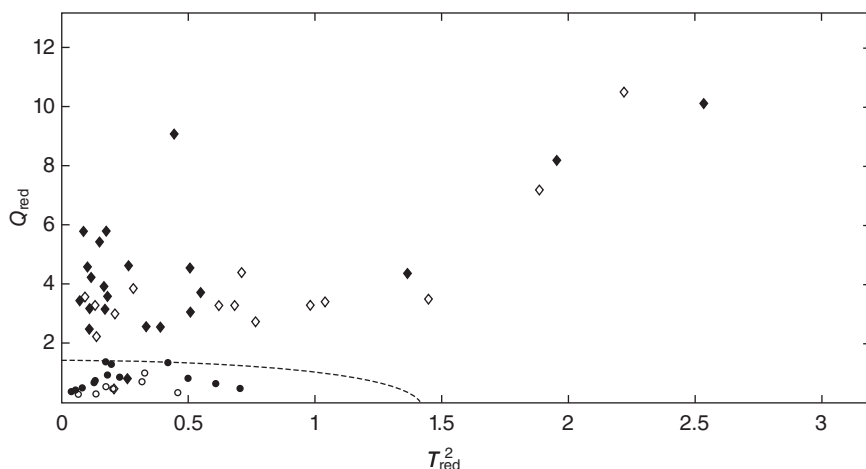


Fig. 11.13 SIMCA on olive oil data: Projection of the training and test samples onto the T^2 vs Q model space of category Sabina. Legend: ● Sabina training, ○ Sabina test, ◆ other origins training, ◇ other origins test.

authenticating the samples from the PDO ‘Sabina’, one can model this category only. Therefore, the training samples from the class Sabina were used to build a PCA model of the category, whose optimal complexity was chosen on the basis of the values of sensitivity and specificity in cross-validation (see Appendix B, Section 11.8.2) and resulted in four components. This optimal model had 100% sensitivity and 95.5% specificity on the training set and, when applied to the external validation measurements, showed comparable performances (100% sensitivity and 93.3% specificity). This information can easily be visualized in the T^2 vs Q model plot (see Section 11.5.3) of the class Sabina reported in Fig. 11.13.

Since this problem involved the authentication of samples from a specified category (asymmetric classification), only the model of class Sabina was originally built. However, to illustrate what happens with more than one class, the second category, oils from other origins, was also modelled. As explained at the end of Section 11.5.3, it is then possible to check whether samples are accepted by one, both or none of the modelled classes and, if desired, to turn SIMCA into a discriminant classifier by assigning each sample to the category it is closer to. This information can be easily visualized in the form of a Coomans plot (Coomans *et al.*, 1984), a graph where the two axes represent the distance of the samples to each of two class models. Accordingly, the horizontal and vertical lines corresponding to the threshold distances (in our case $\sqrt{2}$) will cut the plot in four different regions: the uppermost left and the lowermost right will correspond to unambiguous acceptance by a single category model, the lowermost left to acceptance by both classes while the uppermost right to rejection by both

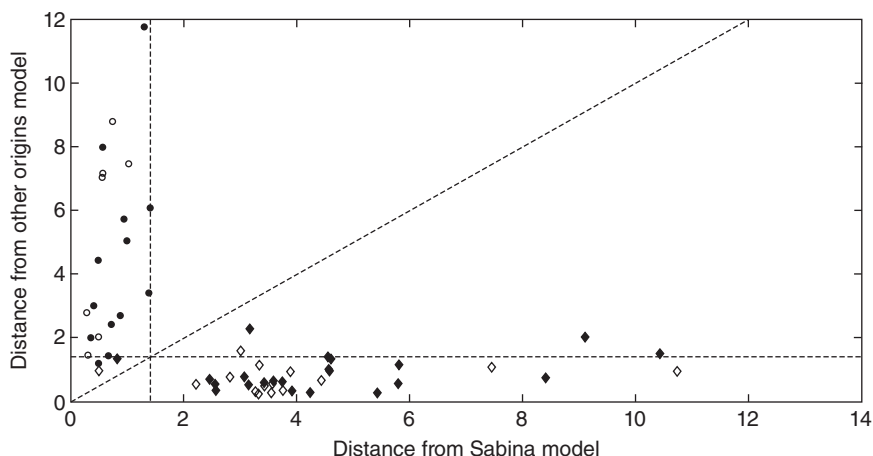


Fig. 11.14 SIMCA on olive oil data: Coomans plot. Legend: ● Sabina training, ○ Sabina test, ◆ other origins training, ◇ other origins test.

category models. The Coomans plot for the data analysed in this example is reported in Fig. 11.14.

Only a few samples are considered as outliers by both category models, while the greatest part of the samples falls in the two regions of unambiguous classification. The diagonal line bisecting the plot represents the discriminant classification boundary so that all the samples lying above are classified as being Sabina, while all the samples lying below are predicted as from other origins. Based on these considerations, if a discriminant classification is sought, inspection of the plot shows that very good performances can be obtained both in modelling (100.0% and 95.45% non-error rates for Sabina and other origins, respectively) and in external validation (100% and 93.3% non error rates for Sabina and other origins, respectively).

11.6 Conclusion

Modern instrumental techniques produce multivariate data, which require processing by suitable mathematical and statistical tools in order to have meaningful results. In this chapter, an overview of the main multivariate data analytical methods to deal with problems normally encountered in the instrumental evaluation of food quality (exploratory analysis, calibration and classification) has been presented and discussed. In all areas, particular attention has been given to the family of methods based on projection on a low-dimensional subspace of latent variables, as they have the advantages of allowing the analysis of data where many correlated variables are measured on a limited number of samples (which is often the case in instrumental assessment of food quality) and an easy and straightforward

visualization of the results. Therefore, principal component analysis (PCA) was illustrated as a tool for the exploratory analysis of data matrices and for the identification of outliers, principal component regression (PCR) and partial least squares (PLS) regression for calibration, and partial least squares-discriminant analysis (PLS-DA) and soft independent modelling of class analogies (SIMCA) as examples, respectively, of the discriminant and modelling approaches to classification. This choice was made, based on the consideration that the selected techniques are successfully involved in the majority of the application of multivariate data analysis to food chemical problems. However, it must be stressed that they represent only a part of the powerful toolbox available for the researcher interested in assessing the quality of foodstuff by instrumental methods coupled to statistical processing of the results, and that more complex or tailored techniques can be employed whenever needed. For instance, if multivariate signals are collected on samples prepared according to an experimental design, the effect of the controlled factors and of their interaction on the multidimensional response can be estimated using a recently proposed technique called ANOVA-simultaneous component analysis (ASCA), which operates by partitioning the variation in the data matrix $\mathbf{X}$ according to an ANOVA scheme and then uses PCA to explore the individual effect matrices (Smilde *et al.*, 2005). On the other hand, more complex problems in regression and classification, when the functional relationships implied cannot be expressed in a simple analytical form and non-linear dependencies have to be modelled, can be tackled with the use of support vector machines (Vapnik, 2000) or artificial neural networks (Marini, 2009).

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11.8 Appendices

11.8.1 Appendix A: Preprocessing

Preprocessing is a fundamental part of data analysis and is defined as any transformation of the original data prior to the principal processing step. Its aim is to remove or reduce the impact of irrelevant sources of variation for which the primary modelling tool may not account (Sharaf *et al.*, 1986). Selection of the kind of preprocessing to perform is strictly dependent on the problem: the objective/goals involved, physical or chemical factors, or even just common sense can concur in driving the decision (Beebe *et al.*,

1996). The choice can also depend on whether the data come from a single instrument or are *multi-source*. Many preprocessing techniques can be listed, but they can be divided into two basic types: those operating on samples and those operating on variables. Sample preprocessing tools operate on one sample at a time over all variables, while variable preprocessing techniques work on one variable at a time over all samples.

Sample preprocessing tools

The main sample preprocessing techniques are normalization and weighting: the former is used to remove sample to sample absolute variability (e.g. to put all objects on the same scale), while the latter emphasizes selecting observation at the expense of the others (modulates the influence of an individual in the model definition).

Normalization is carried out in order to remove the systematic variation generally associated to the total amount of a sample and it is mathematically performed dividing each variable by a constant. Generally, three different constants are used for the purpose: the 1-norm (normalization to unit area), the 2-norm (normalization to unit length) and the maximum intensity (infinity norm). If the value of the j th variable measured on the i th sample is indicated as x_{ij} , then, depending on the case, normalization is defined as:

$$\begin{aligned} x'_{ij} &= \frac{x_{ij}}{\sum_j^v |x_{ij}|} && \text{unit area} \\ x'_{ij} &= \frac{x_{ij}}{\sqrt{\sum_j^v x_{ij}^2}} && \text{unit length} \\ x'_{ij} &= \frac{x_{ij}}{\max_j(x_{ij})} && \text{maximum intensity} \end{aligned} \quad [11.A.1]$$

where the prime indicates preprocessed data.

Variable preprocessing tools

The main variable preprocessing techniques are mean centring and variable weighting: the former accounts for the presence of an intercept or offset in the model, while the latter is used to emphasize some variables over others or to modulate their influence in the definition of the model.

Mean centring is operated by subtracting the mean value of the column of the data matrix corresponding to the intensity of the same variable over all samples to all the values of the same column vector:

$$x'_{ij} = x_{ij} - \bar{x}_j \quad [11.A.2]$$

where $\bar{x}_j$ is the average value of variable j over all samples.

When centring is made on multiple variables, this corresponds to subtracting the mean sample vector from each of the sample fingerprints in the data set. It should be stressed that centring aims at estimating and removing

common offsets in the data, and therefore it should be used when eliminating such offsets provides an approximately reasonable model.

To explain the importance and the aim of variable weighting, it has to be considered that almost all the methods presented in this chapter are least-squares based: as least-squares model describes variation in the data, it holds that variables with large variation are implicitly assumed to be important. However, if the variation is mainly due to noise or to the use of different scales (e.g. in the *multi-source* case), it is necessary to preprocess the data in a way that makes this irrelevantly large variation not influence the model to a large extent. Variable weighting represents the preprocessing tool, which provides this step. Operationally, it consists in dividing all the element of a variable vector (a column of the original data matrix) by a constant, which represents the weight. The most common form of weighting is the so-called variance scaling, in which the standard deviation of the variable (s_j for variable j) is used as weighting factor:

$$x'_{ij} = \frac{x_{ij}}{s_j} \quad [11.A.3]$$

One of the main consequences of variance scaling is that the transformed variables have unit standard deviation. When variance scaling is coupled to mean centring, the corresponding pretreatment is called autoscaling:

$$x'_{ij} = \frac{x_{ij} - \bar{x}_j}{s_j} \quad [11.A.4]$$

11.8.2 Appendix B: Validation

Data analysis can be seen as the act of building mathematical and statistical models, which usually aim at providing a description (approximation) and/or predictions for a system under study. In this framework, it should be stressed that, given a problem, there can be a wide choice of models available for its solutions and their definition is based on real data: therefore, many factors can affect the quality of modelling, for instance the sampling, the intrinsic characteristics of the model itself, the algorithm used for fitting and so on. Validation can be described as the part of the analysis where it is verified whether valid conclusions can be formulated from a model (Harshman, 1984). Accordingly, issues that can be investigated in the validation phase include whether the model is able to generalize (i.e. to express the main variation in the data) parsimoniously (with the smaller number of components) or whether it predicts accurately. In general, whatever the specific purpose of validation, it is important to define a proper diagnostics for characterizing the quality of the solution. In particular, in this appendix attention will be focused only on those cases where diagnostics involves the calculation of some error criterion, usually based on the estimation of residuals. In fact, estimation of residuals (or of some analogous error

measure) can be used for different purposes: (1) assessing which model to use; (2) defining the model complexity in component-based methods; (3) evaluating the predictive ability of a regression (or classification) model; (4) checking whether overfitting is present (by comparing the results in validation and in fitting); (5) residual analysis (Smilde *et al.*, 2005). In all these areas, the use of fitted residuals (the residuals calculated on the same data used to build the model) would often suffer from the fact that their magnitude and structure are not similar to the ones that would be obtained if the model were used on new data. The use of test set validation or cross-validation, which provide error estimates that more correctly reflect the residuals obtainable on new data, is a way of dealing with this problem.

Test set validation is carried out by fitting the model to new data (which constitute precisely what is called the test, or validation, set). Then, the residuals are calculated by comparing the model of new data with the corresponding actual values. The use of an external test set represents a natural way of performing validation as it simulates the practical use of the model on future data. Ideally, the test set should be made as independent as possible from the calibration set, by collecting new samples and analysing them in different days (and better by different laboratories and/or technicians). However, when this is impractical, a representative portion of the total data set can be left aside as test set.

Cross-validation (Stone, 1974; Geisser, 1974) represents an alternative to test set validation and it is an internal resampling method, i.e. simulates test set validation by repeating a data splitting procedure where different objects are in turn placed in the validation set. Accordingly, it is particularly useful when a limited number of samples are available. Schematically, it consists of the following steps:

1. Leave out part of the data values.
2. Build the model without these data.
3. Apply the model to the left out values and obtain predictions.
4. Calculate the corresponding residual error.
5. Repeat steps 1–4 until each data value has been left out once and collect all the residuals.

Instrumental assessment of the sensory quality of meat, poultry and fish

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Abstract: The sensory parameters that define the quality of meat, poultry and fish products form the basis for instrumental methods such as those that measure colour, texture and aroma. This chapter will discuss these methods of instrumental measurement, their advantages, disadvantages and applications. It will discuss sensory-based instrumental colour methods such as colorimetry and those based on computer vision technology, traditional texture systems and odour-based instrumental methods such as gas chromatography–mass spectrometry (GC/MS) and the electronic nose. Near infrared (NIR) and Fourier transform infrared (FTIR) spectroscopy methods will be discussed as an emerging technology for meat, poultry and fish quality assessment.

Key words: instrumental, on-line, colour, aroma, texture.

12.1 Introduction

Colour, flavour and texture are all important factors affecting meat poultry and fish quality. Generally these modalities are assessed by human responses and it is important that reproducible and reliable methods are available to accurately quantify them (O'Sullivan and Kerry, 2008). Sensory-based instrumental methods are often employed by the meat, poultry and fish processing industries to measure sensory changes that occur in products. These methods may be quicker than sensory analysis, which traditionally can be quite expensive. The idea behind such methods is that sensory perceptions have chemical and physical counterparts in the substance under investigation (Dijksterhuis, 1995). Sensory quality-based programmes can be costly to maintain and limited in scope, but the most important feature of product quality in the marketplace is its direct relationship to consumer perception, satisfaction with, and ultimate acceptance of a product's sensory attributes (Muñoz, 2002).

Instrumental methods are usually quick and directly correlated to a sensory-based criterion. Instrumental and sensory limits can be assessed using survival analysis results to determine what instrumental limits correspond to the appropriate sensory limit of acceptability. A considerable amount of research has been undertaken to investigate the suitability of advanced sensor technology to simulate human sensory responses. The development of valid and relevant instrumental methods in concert with dynamic sensory methods has allowed for a more comprehensive analysis of human perception (Ross, 2009). Chemometrics, which uses multivariate data analysis to interpret sensory-based instrumental data, is an important research area which ultimately will determine the extent to which sensory-based instrumental technology is applied. This chapter discusses sensory-based instrumental colour methods based on computer vision technology, texture systems and odour-based instrumental methods such as gas chromatography–mass spectrometry (GC/MS) and the electronic nose. The use of near infrared (NIR) and Fourier transform infrared (FTIR) spectroscopy methods as an emerging technology for meat, poultry and fish quality determination is assessed.

12.2 Instrumental methods of colour and tenderness analysis

12.2.1 Instrumental methods of colour analysis

Colour stability of meats has been an important research area in meat quality assessment and improvement for many years. At the point of sale, colour and colour stability are the most important attributes of meat quality and various approaches have been used to meet consumer expectations that an attractive, bright-red colour indicates a long shelf-life and good eating quality (Hood and Mead, 1993). However, over time the cherry red colour of oxymyoglobin is oxidised to the grey-brown pigment of metmyoglobin. Oxymyoglobin is a haem protein in which iron exists in the ferrous form (Fe^{+2}), unlike metmyoglobin that possesses the ferric form (Fe^{+3}). The conversion of the ferrous to the ferric form is a result of oxidation (Liu *et al.*, 1995). Consumers relate this bright red colour to meat that is fresh, while discriminating against meat that has turned brown in colour, which they consider unsightly. Thus, it is important to quantify the colour quality, particularly of red meat products such as beef, lamb and pork.

Instrumental methods such as the ubiquitous Minolta colorimeter (Minolta Camera Co. Ltd., Osaka, Japan, Fig. 12.1) have been used by industry and research institutes to measure meat colour quality for decades. This device can use different measurement coordinates, but the principal ones used for meats are the CIE, L (white to black), a (green to red) and b (blue to yellow) values. However, these point-to-point colorimeters have certain drawbacks. Huselegge *et al.* (2001) in a study on 56 000 veal carcasses



Fig. 12.1 Instrumental methods such as the ubiquitous Minolta colorimeter (Minolta Camera Co. Ltd, Osaka, Japan) have been used by both the industry and research institutes to measure meat colour quality for decades.

found significant differences between Minolta CR300 devices and explained this to some extent by the fact that the individual Minolta instruments were operated by two different persons, who may not have placed the instrument on the same site.

Computer vision methods are a relatively recent innovation to the instrumental colour measurement area. Advantages of computer vision systems are non-invasive, objective, consistent and rapid estimations of meat palatability (Jackman *et al.*, 2009). O'Sullivan *et al.* (2003a) used a high-resolution digital camera and a custom algorithm (MATLAB, Massachusetts, USA) to determine the colour parameters of various fresh pork meat patties. They found that instrumental *a* values and *R* values (RGB colour measurement index, red, green and blue) as determined by the digital camera correlated with RED and negatively with the BROWN sensory terms in an experiment designed to correlate instrumental methods of colour analysis to sensory assessment as performed by a trained and an untrained sensory panel. Also, O'Sullivan *et al.* (2003a) showed that digital camera-derived *a* values correlated to a greater extent to the sensory RED term compared to the Minolta colorimeter for both the trained and untrained evaluation of the two different muscles, *M. longissimus dorsi* and *M. psoas major*. Similarly digital camera-derived *b* values correlated to a greater degree to the BROWN sensory term for both assessor groups and muscles compared to Minolta-derived *b* values (*M* = Minolta).

The digital camera-derived instrumental measurements had a high affinity for their respective sensory descriptors and instrumental measurements

taken with the digital camera were more highly correlated to and predictive of the sensory terms particularly for the RED, BROWN and *L* value descriptors when compared to Minolta colorimeter correlations. This is perhaps due to the fact that the camera took measurements over the entire surface of samples, resulting in a more representative measurement, whereas the Minolta colorimeter took point-to-point measurements, which missed out on some relevant information (O'Sullivan *et al.*, 2003a). These results agree with those of Lu *et al.* (2000) who found that computer vision can be used for predicting sensory colour scores of pork loin muscle. The sensory colour scores were non-linearly related to the colour features extracted from the loin images.

Both statistical and neural models resulted in satisfactory prediction, though the neural net model was better. Jackman *et al.* (2010) used computer vision methodology to correlate with consumer panel palatability data and found that it is possible for consumer opinion of beef likeability to be accurately modelled by using image colour, marbling and surface texture features. J.peg (Joint Photographic Experts Group, a standard for compressing digital photographic images) images taken by the digital camera allow direct conversion of the resultant data via an algorithm (MATLAB) to any of a number of colour measurement systems, i.e. Hunter Lab, CIE Lab, RGB, XYZ, etc. Also, only one picture need be taken as opposed to the multiple single point observations required by a colorimeter to obtain a representative colour profile (O'Sullivan *et al.*, 2003a). Meat is not a homogeneous material and variations in pigment concentration and therefore development of metmyoglobin occur on the surface of meat during display in commercial retail conditions. Thus, pork colour is difficult to assess because of this colour variation over a meat cut, even from within the same muscle (AMSA, 1991). Taking a picture of the entire surface of a meat sample will thus provide a more representative colour profile compared to the point-to-point measurements of traditional colorimeters (O'Sullivan *et al.*, 2003a).

12.2.2 Instrumental methods for measuring tenderness

Tenderness has often been described as the most important factor in terms of high eating quality, especially in beef. Consumers can distinguish between tender and tough beef steaks (Aaslyng, 2009). In addition to an acceptable flavour, the consumer desires meat to be palatable and, consequently, meat tenderness is another critical determinant used by the consumer to assess meat quality. In fact, it has been demonstrated that the consumer would be willing to pay a higher price in the marketplace for beef as long as it is guaranteed tender (Miller *et al.*, 2001). Tenderness can be evaluated by objective methods, instrumental or sensory (trained panels) or by subjective methods (consumer panel). Sensory methods, either analytical or affective, are expensive, difficult to organise and time consuming (Platter

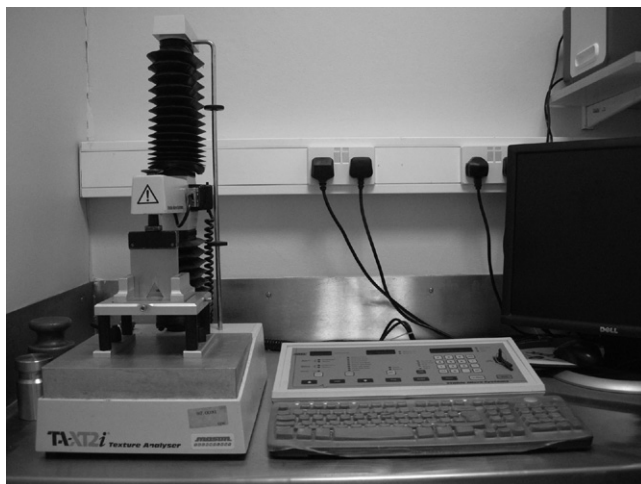


Fig. 12.2 The mechanical process of mastication has been simulated using texture profile analysis. Presented is a TA.XT2 Texture Analyser (Stable Micro Systems, England).

et al., 2003). Thus, there have been many attempts to devise instrumental methods of assessing the forces shearing, penetrating, biting, mincing, compressing and stretching of the meat whose results are a prediction of tenderness ratings obtained by taste panels (Lawrie and Ledward, 2006).

Shear tests measure the force to cut through fibres of cooked samples. They are the simplest and most common tests used to document cooked meat texture. However, information obtained from shearing devices that perform in a similar way may not be interchangeable (Lyon and Lyon, 1998). The mechanical process of mastication has been simulated using the first method, texture profile analysis (TPA, Fig. 12.2). This objective method measures the compression force of a probe and the related textural parameters of a test food during two cycles of deformation (Caine *et al.*, 2003). The main advantage of TPA is that one can assess many variates with a double compression cycle. Variates that can be assessed with this analysis are: hardness, springiness, cohesiveness, adhesiveness, resiliency, fracturability, gumminess, chewiness, etc. In meat the variates assessed are hardness, springiness and cohesiveness; the three altogether permit the calculation of chewiness (Ruiz de Huidobro *et al.*, 2001). However, the most widespread method normally used as an indicator of meat sensory hardness (tenderness) is the Warner–Bratzler (WB) shear test, almost the sole methodology used in raw meat (Bratzler, 1932; Warner, 1928), and which is referred to in most papers (Culioli, 1995). The basic concept and design of the WB shear device (Bratzler, 1932, 1949) have been subject to modification and improvement over the years. Yet, the familiar blade, with its triangular hole in the middle, remains one of the most widely used devices to provide

measurements of meat texture quality. Other machines built to measure rheological parameters of foods and materials usually include a version of the WB blade as a basic attachment to the system (Lyon and Lyon, 1998).

Shackelford *et al.* (1995, 1997) state that WB shear force is an imprecise predictor of beef tenderness characteristics determined by sensory trained panellists. However according to Destefanis *et al.* (2008) and also McMillin (2008) WB shear values of less than 42.87 N and greater than 52.68 N allow classification of tough and tender beef in a sufficiently reliable way to be highly related to consumer tenderness perception (Destefanis *et al.*, 2008; McMillin, 2008). The most widely used method for measuring meat texture is the single blade shear test of the WB type (Culioli, 1995). However, results obtained from using this method can be variable. This variability depends on many factors, such as muscle type, sample preparation, cooking method, shear apparatus, measurement procedure and panel type (Destefanis *et al.*, 2008).

Miller *et al.* (2001) reported that consumer perceptions of beef flavour and juiciness have a greater impact on consumer overall acceptability levels of New York strip steaks as the WB shear force and toughness levels increase. In short, as beef steaks become tougher, flavour and juiciness have a greater effect on consumer satisfaction. Zakrys *et al.* (2008) observed that WB shear force values had positive correlations to O₂ levels in modified atmosphere packed *M. longissimus dorsi* muscle samples, displaying that all samples appeared to become less tender with increasing O₂ level during the 15 days' storage. It appears that samples packed with 50% and 80% O₂ were tougher than low O₂ treated samples.

The width of the blades and the position of the triangle, the speed of the test, and the shape, mass and orientation of the test sample are important to interpret the results of shear tests. Yet these very specific conditions are often omitted from reports on testing protocols (Lyon and Lyon, 1998). Thus, it is sometimes difficult to replicate a previous workers studies due to this lack of information.

12.3 Sensory-instrumental methods of flavour analysis

12.3.1 GC/MS

To date, the analysis of characteristic food odours has been commonly carried out by human assessment and headspace/direct GC/MS (Fig. 12.3) (Grigioni *et al.*, 2000). Odour is among the first quality attributes registered when opening a package of sliced meat products and therefore volatile organic compounds (VOCs) have potential as early markers for consumer acceptability (Holm *et al.*, 2012). GC/MS in itself yields information on the concentration of volatiles present in a sample, but little is known about the relationship between these volatiles in a mixture and how they contribute to perceived sensory attributes (Hansen *et al.*, 2005).

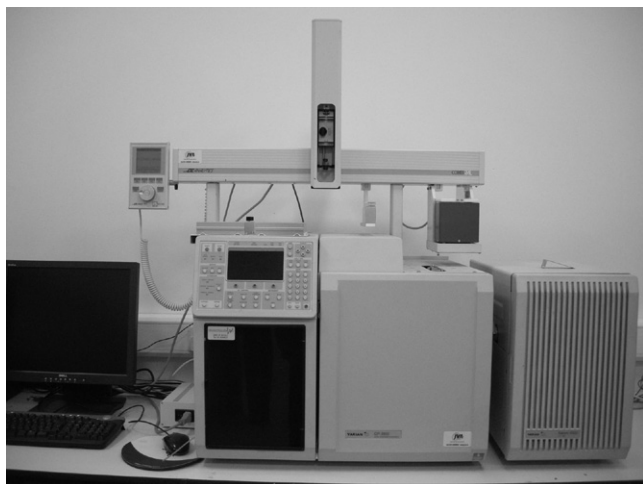


Fig. 12.3 Varian Saturn 2000 GC/MS with Combipal autosampler (CTC analytics, Basel, Switzerland).

Generally, GC/MS is used to identify chemical markers that can be used as indices of sensory quality. With respect to meat products much work has been undertaken in order to identify chemical markers of oxidation that can be directly correlated to human sensory responses. Hexanal has a distinctive odour described as being green or grassy (Gasser and Grosch, 1988) and is a secondary breakdown product formed during the oxidation of linoleic acid (C18:2). It has been identified and used to evaluate the oxidative state and correlated as an index to sensory scores of pork (Shahidi *et al.*, 1987) and beef (Drumm and Spanier, 1991) and also chicken (Byrne *et al.*, 2002). O'Sullivan *et al.* (2003b) found that the sensory descriptor *Green-O* (O = odour) as determined by a trained sensory panel covaried with hexanal levels obtained by GC/MS for warmed-over flavour (WOF) samples of cooked porcine *M. longissimus dorsi* and *M. psoas major* muscles. Also, pentanal and 2,4-decadienal could be used as marker compounds to follow the development of WOF and its associated rancid flavours in cooked meats (St. Angelo *et al.*, 1987). Siegmund and Pfannhauser (1999) determined that 2,4-decadienal was the most potent odourant and increased significantly with storage time of cooked chicken meat. The sensory properties of the two isomers (*EZ* and *EE*) of 2,4-decadienal were also described as fatty and fried and thus were sensory markers and not just a chemical index. These authors concluded that an increase of the influence of these compounds on the aroma of stored chicken meat would result in a more intense chicken aroma, and also in the undesired WOF of the meat. O'Sullivan *et al.* (2003b) found that WOF samples of cooked porcine *M. longissimus dorsi* and *M. psoas major* covaried with the sensory

descriptor *Green-O* and the GC/MS identified the compounds pentanal, 2-pentylfuran, octanal, nonanal and 1-octen-3-ol. Other authors have found these compounds to be indicative of lipid oxidation (Siegmund and Pfannhauser, 1999; Byrne *et al.*, 2002). O'Sullivan *et al.* (2003b) also showed that the compounds 1-octen-3-ol and 2-pentylfuran covaried with the sensory oxidative descriptors *Rancid-F* (F = flavour), *Fish-F* and *Rubber/Sulphur-Like-O* when samples of *M. longissimus dorsi* and *M. psoas major* with varying degrees of WOF were analysed. These results agree with those of Siegmund and Pfannhauser (1999) who found that the relative concentrations of the lipid oxidation products 1-octen-3-ol and 2-pentylfuran increased in cooked chill stored chicken meat as storage time increased.

GC/MS is often used in the analysis of meat, poultry and fish products with respect to fatty acid composition. The intramuscular fatty acid composition of the monogastric animals, and in particular the triacylglycerols are a reflection of the dietary fatty acids, while in ruminants the biohydrogenation in the rumen (i.e. saturation of the dietary unsaturated fatty acids) is responsible for the smaller variations in intramuscular fatty acid composition (Raes *et al.*, 2004). Poultry and pork muscle typically have higher levels of polyunsaturated fatty acids than lamb or beef. Pork muscle has more linoleic acid than beef or lamb which contributes to the higher polyunsaturated:saturated fatty acid ratio. However, beef and lamb commonly have more favourable n6:n3 fatty acids ratios than pork (Wood and Enser, 1997). Fat content and fatty acid composition of meat are of major importance for consumers due to their importance for meat quality and nutritional value (Wood *et al.*, 2004).

It is well known that the fatty acid composition of meat from ruminants differs from meat from non-ruminants. Polyunsaturated:saturated fatty acid (PUFA:SFA) ratio is lower in beef than in pork due to ruminal biohydrogenation. However, beef has a lower n-6:n-3 fatty acid ratio than pork, which is considered beneficial to human health (Enser *et al.*, 1996). Swine feeding has been formulated with a higher content of natural sources of unsaturated fatty acids (UFA), such as n-3 series or conjugated linoleic acid (CLA), due to human health concerns (Boselli *et al.*, 2008).

Also, diets enriched with vegetable oils (such as sunflower oil, soybean oil or corn oil) that contain an elevated UFA percentage should result in healthier products for consumers (Mitchaothai *et al.*, 2007). Beneficial effects of n-3 fatty acids have been shown in the secondary prevention of coronary heart disease, hypertension, type 2 diabetes and, in some patients with renal disease, rheumatoid arthritis, ulcerative colitis, Crohn disease and chronic obstructive pulmonary disease (Simopoulos, 1999). Also, the fatty acid composition of beef has been widely studied and fatty acids such as n-3 and n-6 PUFA as well as CLA are especially interesting to researchers or consumers considering their potential relationship to human health, but also fatty acid composition plays an important role with respect to beef eating quality (Jiang *et al.*, 2010). High n-3 PUFA levels in grass-fed beef

compared to traditional concentrate-fed beef (Vatansever *et al.*, 2000) can manifest fishy flavours in the resulting meat. Nuernberg *et al.* (2005), who investigated the effects of a grass-based and a concentrate feeding system on meat quality characteristics and fatty acid composition of *longissimus* muscle in different cattle breeds, found that fishy off-flavours were significantly higher and overall flavour liking scores were significantly lower in meat from grass-finished cattle with increased 18:1 *trans* isomers and, notably, CLA *cis*-9, *trans*-11. On the other hand (C18:1n9) is associated with favourable beef palatability attributes (Dryden and Marchello, 1970).

Holm *et al.* (2012) showed that the GC/MS derived microbial metabolites 2- and 3-methylbutanal, 2- and 3-methylbutanol, acetoin and diacetyl were closely related to the changes of the sensory attributes meaty and sour/old odour in saveloy, a Danish cooked sliced pork sausage or luncheon meat-type product. Thus, these aroma compounds could be used as chemical markers for the sensory shelf-life of sliced saveloy.

The usefulness of GC/MS is obvious, but as a technique it has certain drawbacks such as high operating costs and being time consuming (Pryzbylski and Eskin, 1995). However, the electronic nose may provide a practical advantage over other methods and may have an application in an on-line/at-line capacity for the quality determination of meat products with respect to WOF development (O'Sullivan *et al.*, 2003b).

12.3.2 Electronic nose

In contrast to these well-known analytical and sensory techniques that have been used for the analysis of flavour compounds, the electronic nose does not give any information about the compounds causing the investigated aroma; neither the identity of the compounds nor their sensory properties. Using the electronic nose the aroma is judged by the so-called 'aroma pattern', which should be characteristic to the investigated substrate (Siegmund and Pfannhauser, 1999). Thus, in food production plants there is a growing demand for on-line/at-line measurement of sensory relevant quality criteria. One promising technology in this capacity is the application of an electronic nose (Hansen *et al.*, 2005). The electronic nose may provide a practical advantage over other methods and may have an application in an on-line/at-line capacity for the quality determination of meat products (O'Sullivan *et al.*, 2003b).

In order to classify samples, an electronic nose combines the response profiles of the various sensors, which react to different types of volatile compounds in the sample gas (Rajamäki *et al.*, 2006). The electronic nose is an array of chemical gas sensors with a broad and partly overlapping selectivity for measurement of volatile compounds within the headspace over a sample combined with computerised multivariate statistical data processing tools (Gardner and Bartlett, 1994). The sensor array of an electronic nose has a large information potential and will give a unique overall

pattern of the volatiles. In principle, both the electronic and the human nose operate by sensing simultaneously a high number of components giving rise to a specific response pattern (Haugen and Kvaal, 1998). However, the electronic nose has both large differences in sensitivity and selectivity from the human nose (Haugen and Kvaal, 1998).

The electronic nose has been assessed in the analysis of a large variety of different meat, poultry and seafood products (e.g. Eklöv *et al.*, 1998, fermented sausage; Rajamäki *et al.*, 2006, chicken; Ólafsdóttir *et al.*, 1997; O'Connell *et al.*, 2001, fish; Hansen *et al.*, 2005, meatloaf; Tikk *et al.*, 2008, meatballs; Panigrahi *et al.*, 2006, spoilage beef strip loin) and in the warmed-over flavour analysis of various meat products (e.g. Siegmund & Pfannhauser, 1999, chicken; Grigioni *et al.*, 2000, beef; O'Sullivan *et al.*, 2003b, pork).

The above-mentioned electronic nose instruments all differed in the individual sensor array setup, numbers of sensors and the data analysis used to process the characteristic signature data of the various meat types. The data analytical techniques employed included AromaScan, Neural Networks, principal component regression (PCR) and partial least squares regression (PLSR) (O'Sullivan *et al.*, 2003b). One of the principal drawbacks to date of electronic noses is the large variation in data acquisition. In order to compare results over time (weeks, months or years) it is required that these instruments give the same signal when identical samples are being measured over time. However, due to dynamic processes taking place in the sensors over time, the signal from a sensor array may vary significantly (Haugen and Kvaal, 1998). O'Sullivan *et al.* (2003b) found that the electronic nose device used in a WOF experiment could clearly separate samples on the basis of muscle type, treatment and degree of WOF development. Also, the electronic nose data from two separate sample sets analysed in different laboratories and with a time separation of 11 months agreed with the sensory analysis and the device used in this experiment was effective in determination of the oxidative state of the samples analysed. O'Sullivan *et al.* (2003b) used level correction, a multivariate data analytical tool, to normalise the data from sample sets analysed at a 7 month interval prior to subsequent PLSR and this allowed their direct comparison.

Another potential drawback of the electronic nose is that sensors have a limited life – they must be replaced after a while – and new sensors from another batch will differ in performance (Haugen and Kvaal, 1998). One potential method of solving these fundamental problems is to use a reliable data analytical tool to correct for variations over time, possibly using a reference sample and secondly to use an electronic nose in which the sensors do not require replacement. This problem was overcome by O'Sullivan *et al.* (2003b) who employed an electronic nose in a WOF experiment which used sensor technology (MGD-1, Environics Ltd, Finland), which had an advantage over existing electronic nose devices in that the sensors do not wear because the molecules measured do not come in direct

contact with the sensors. The method of sensor operation is based on the principle of ion mobility and ionisation of gas molecules. The clusters formed through ion–molecule reactions are brought into different electrical fields perpendicular to the sample flow and detected. In effect the sensors used will not wear out, thus avoiding the requirement for replacement and the variations in sensor batch manufacture. This displayed the potential effectiveness of the electronic nose as an objective on-line/at-line quality control monitoring device.

If an electronic nose is to be used in quality assurance and quality control programmes for raw materials and/or end-products, there is a need to calibrate it against sensory assessment in order to determine the relevance of the measurements (Hansen *et al.*, 2005). However, the electronic nose has both large differences in sensitivity and selectivity from the human nose (Haugen and Kvaal, 1998). Tikk *et al.* (2008) concluded that a significant, positive correlation between the electronic nose gas sensor signals, the WOF-associated sensory attributes and the levels of secondary lipid oxidation products for pork meat balls, a very popular Danish dish. This also supports the potential of electronic nose technology as a potential future quality control tool in the meat industry. Hansen *et al.* (2005) demonstrated that an electronic nose could predict the sensory quality of porcine meat loaf, based on measuring the volatiles in either the raw materials or the meat loaf produced from those raw materials. They further stipulated that a strategy involving an operational and standardised methodology and vocabulary for in-house sensory evaluation of the raw materials was essential if the electronic nose was to be calibrated properly and used on-line in the future. GC/MS can provide a better understanding of what an electronic nose is measuring when both analyses are considered together and how they are related to the perceived sensory attributes as measured by sensory analysis (Hansen *et al.*, 2005).

12.4 Near infrared (NIR) and Fourier transform infrared (FTIR) spectroscopy

Over the past three decades, NIR reflectance spectroscopy has been proved to be one of the most efficient and advanced tools for the estimation of quality attributes in meat and meat products (Prieto *et al.*, 2009). NIR spectroscopy is a sensitive, fast and non-destructive analytical technique with simplicity in sample preparation allowing a simultaneous assessment of numerous meat properties (Osborne *et al.*, 1993).

NIR spectroscopy has been successfully applied to the quantitative determination of major constituents (moisture, fat and protein) in meat and meat products including: beef (Eichinger and Beck, 1992; Alomar *et al.*, 2003; Tøgersen *et al.*, 2003; Prevornik *et al.*, 2005; Prieto *et al.*, 2006), pork (Tøgersen *et al.*, 1999; Brøndum *et al.*, 2000; Ortiz-Somovilla *et al.*, 2007),



Fig. 12.4 Fourier transform infrared (FTIR) spectroscopy. Presented is an Agilent 670IR with 620 microscope.

poultry meat (Renden *et al.*, 1986; Valdes and Summers, 1986; Abeni and Bergoglio, 2001; Cozzolino and Murray, 2002; Berzaghi *et al.*, 2005; McDewitt *et al.*, 2005) and mutton (Viljoen *et al.*, 2007). Near infrared spectroscopy (NIRS) is an analytical technique that uses a source producing light of known wavelength pattern (usually 800–2500 nm) and that enables one to obtain a complete picture of the organic composition of the analysed substance/material (Van Kempen, 2001). It is based on the principle that different chemical bonds in organic matter absorb or emit light of different wavelengths when the sample is irradiated (Prevolnik *et al.*, 2004).

FTIR (Fig. 12.4) spectroscopy has substantial potential as a quantitative control method in the food industry (Van de Voort *et al.*, 1992). For lipid measurement it has the advantage of easy sample preparation, rapid measurements and no use of chemicals in contrast to traditional solvent methods accompanied by chromatographic techniques (Flåtten *et al.*, 2005). FTIR analysis is rapid, non-invasive, requires minimum sample pre-treatment, no specific consumables or reagents, and in conjunction with attenuated total reflectance (ATR) technology permits users to collect full spectra in a few seconds, allowing simultaneous assessment of numerous meat properties (Ellis *et al.*, 2002, 2004; Ammor *et al.*, 2009). For producers and manufacturers a rapid method to measure fat qualities would be useful; FTIR spectroscopy has proven to be a powerful tool in food research (Ripoche and Guillard, 2001). Flåtten *et al.* (2005) measured C22:5 and C22:6 marine fatty acids in pork fat with FTIR. Their results show that marine fatty acids and general fatty acid composition in pork fat can be measured with Fourier transform mid-infrared (FT-MIR) spectroscopy with good precision.

Classification of the samples on the basis of these measurements gives the opportunity for useful implementations of the method in commercial situations, with less labour and time required than alternative chromatographic methods (Flåtten *et al.*, 2005).

The data from NIR and FTIR analysis are processed using an appropriate chemometric or multivariate data analytical method. Essentially, results from test analysis are compared chemometrically to a database of similar known samples or standards. The model then best fits the current results to those that are available and thus present the concentration of the known variable of interest. Of course a suitable database of known compounds in specific samples must be available, either through the instrument vendor or created by the scientists undertaking the work in order to derive any significant data from NIR or FTIR. Adulteration in meat using FTIR-ATR and multivariate analysis has been studied and quantified by Al-Jowder *et al.* (1999, 2002), who identified adulteration of beef meat with heart, tripe, kidney, and liver. Similarly, Meza-Márquez *et al.* (2010) used mid-FTIR-ATR spectroscopy with multivariate analysis (soft independent modelling class of analogies, SIMCA) to successfully detect and quantify the adulteration of minced lean beef with horse meat, textured soy protein, or the addition fat beef trimmings in minced beef. At the same time, the model was capable of simultaneously determining the composition (water, protein, fat, ashes, and glycogen) of the meat samples with a 99% confidence limit (Meza-Márquez *et al.*, 2010).

FTIR combined with suitable multivariate data analytical methodologies will continue to be developed for other meat and poultry products and also has applications in fish, particularly fish oils.

12.5 Future trends

Many sensory-based instrumental methods are available as have been discussed above, but the industry will strive towards developing more reliable, cheaper and quicker methods. Rapid instrumental analysis that effectively monitors sensory changes in meat poultry and fish may present a future opportunity for on-line at-line analysis. FTIR appears to be an advantageous analytical method and coupled with multivariate data analytical tools offers considerable advantages over conventional methods which can be time-consuming, expensive and sometimes hazardous to health or the environment. Multivariate data analytical methodologies are continually being developed resulting in more powerful and user friendly tools for researchers to employ in process development and optimisation of on-line, at-line devices. The calibration of instruments to sensory-based criteria will ensure that objective measurements are taken to determine quality. Additionally, the inclusion of consumer acceptability data during calibration will increase the effectiveness of these instruments.

The most important feature of product quality in the marketplace is its direct relationship to consumer perception, satisfaction with, and ultimate acceptance of a products sensory attributes. Many new products fail because product production and development does not focus systematically on consumer preferences and perceptions of sensory properties (Pecore and Kellen, 2002; Weller and Stanton, 2002).

12.6 Sources of further information and advice

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12.8 References

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Instrumental assessment of the sensory quality of baked goods

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Abstract: Instrumental methods can be used to predict sensory properties of baked goods and to provide structural information which explains the product quality. Two types of sweet baked goods differing in their moisture content are the focus of this chapter: muffins and cake-like systems (high moisture) and biscuits (low moisture). The instrumental methods described include those for measurement of the rheological properties of the batters (flow and linear viscoelastic tests) and the texture of the baked goods, including texture profile analysis and elastic recovery tests in the high-moisture systems and three-point bending, bite jaw test and combination of fracture and acoustic tests in the low-moisture systems.

Key words: rheological properties, texture properties, muffins, cakes, biscuits, sweet baked goods.

13.1 Introduction

13.1.1 Muffins and cake-like systems

The matrix of muffins or cakes can be seen as a solid foam. Its characteristic aerated structure is achieved by incorporating bubbles into the batter during mixing and baking. These bubbles eventually become interconnected gas cells in the crumb of the final product. For most muffins or cakes, a desirable eating quality entails high volume and a light, open, fine texture.

Ingredients

Many muffins and cake-like products are made from rich recipes which typically have a moderate to high sugar content. Most muffins or cakes formulas also contain variable levels of flour, eggs and fat. Other commonly used ingredients are baking powders, emulsifiers and milk. A muffin or cake batter system is composed of these ingredients mixed in a way that causes

aeration (adds bubbles to the mixture). The quality of a muffin or cake largely depends on its crumb texture and final volume.

The initial rise in temperature in the oven gelatinizes the starch and coagulates the proteins. Both these effects increase the viscosity of the batter (Delcour & Hoskeney, 2010). Towards the end of the baking process, the starch granules and proteins form a solid structure which is strong enough to be self-supporting when the cake is taken out of the oven. The firm structure of the end product comes from the combined effect of the gelled starch granules and the continuous network of coagulated egg proteins. Donovan (1977) described starch granules as having two main functions: they swell to form the 'building bricks' of the final crumb and as they swell, they bind the excess water in the system. In this way, the starch is responsible for transforming a liquid, flowing batter into a solid, porous structure.

The most widely used flour (and consequently starch) is wheat. Nevertheless, a number of other starches are used in cake-like products. The way the batter behaves during baking depends on the size of the starch granules, their gelatinization temperature and the amount of water they are capable of absorbing. These properties also vary according to the moisture and sugar content. The sum of all these factors determines the final volume of the baked cake. However, it is the moment during baking when the batter sets that determines the type of cake and its final quality.

Baking

Baking consists of a transfer of heat from the oven to the surface of the food, both by radiation and by convection, combined with conduction of heat from the mould to the sides and bottom of the product. The differences in temperature control the transfer of heat into the interior of the cold batter. Simultaneously, baking also involves a transfer of moisture and entails loss of water from the product being baked. The migration of moisture to the surface is of vital importance. It depends on the temperature and concentration gradients, both of which are crucial for the development of the final crumb structure and, consequently, for its texture.

Bubbles

The amount of air that is incorporated in the form of bubbles during the mechanical mixing process and the changes in the bubbles that take place during baking have a vital effect on the cake texture. The batter needs to be sufficiently viscous to trap the bubbles during mixing and hold them during baking. The small air bubbles that are added in during the initial mixing stage act as nuclei and grow in size due to the generation of carbon dioxide (from the baking powders) and water vapour and the normal process of gas diffusion through the product during baking.

Any aerated system is inherently thermodynamically unstable, so the bubbles will tend to escape because they have a far lower density than the

batter. The amount of air incorporated into the batter depends not only on the beater design and speed but also on the viscosity of the batter and on how efficient it is at retaining the bubbles. The capacity to form bubbles with stable films surrounding the air depends on the surface tension of the batter, in other words, on the ingredients' capacity to act as emulsifiers.

Structure

The centre of the crumb contains small, evenly distributed gas cells, so it has a fine final structure. In this zone the temperature increases at first and then remains around 100°C because the availability of water is high. It is normal for all the starch in this region to gelatinize. The structure of the top zone is coarser, with a broader distribution of gas cell sizes. Some starch granules in this zone are still gelatinizing up to the final minutes of baking, so the gas cell walls are not rigid and continue to expand.

The bottom zone of the crumb is where the temperature rises fastest during baking, so the water migrates rapidly to colder zones. In this zone the starch gelatinizes at the start of the baking process because the availability of water falls considerably in a short time. The mean gas cell size is larger than in the rest of the product and the range of cell sizes is greater.

13.1.2 Biscuits

The term biscuit – or cookie in the USA – is used for a low-moisture product that is made of soft (low-protein) wheat flour, high levels of sugar and shortening; chemical leavening is used for gas production. All biscuits are produced using gentle mixing to produce a dough with almost no development of the gluten protein and with good machinability and sheeting properties. Water is a minor ingredient, but a very important one. The water added and the water content of the ingredients influence the consistency of the dough. Some authors consider that the initial moisture content of the flour is more important for the biscuit's surface properties than the water which is added. A higher water content produces biscuits with a smoother surface. The different types of sweet biscuit depend on the formulation, but in all of them the basic ingredients play the key roles described here below.

Ingredients: fat

Fat is one of the principal ingredients that affect biscuit texture. Fat is an essential ingredient in biscuit formulation because it performs shortening and textural functions. During mixing it acts as a lubricant and competes with water for the starch granule surface, preventing the formation of a gluten network and restricting the swelling of the starch granules. When the fat level is high the lubrication function in the dough is so efficient that very little water is required to form properly consistent dough. Normally this leads to a softer, more friable and less crispy biscuit.

Ingredients: sugar

Sugar is another essential ingredient. It contributes to the texture, flavour, sweetness and colour of the biscuits. The type of sugar and its granule size and quantity influence the quality of the final product. The amount of sugar that dissolves depends on the particle size and has a considerable effect on the spread and machining properties of the dough. Increasing the sugar level normally increases spread and reduces the thickness of the biscuits.

The procedure for mixing the ingredients is also important: in general, increasing the quantity of sugar increases dough spread when the 'all-in-one' method is used but the effect is smaller if the two-stage 'creaming' method is followed. The creaming method minimizes gluten development, but some gluten development is necessary to give sufficient cohesion for handling and shaping the dough. However, it should be remembered that a large part of the cohesion is due to the presence of plastic shortening.

Sugar also plays a role in limiting gluten development by competing with the flour for the available water. The quantity of water in a biscuit formulation is very small and its limited availability for proteins or for starch contributes to biscuit crispness.

Reducing sugars modify the dough consistency and hardness, but their effect depends to a large extent on the physical form in which these sugars are used. Syrups have a greater effect on dough rheology and on the final biscuit colour than those which are added in solid form. Generally speaking, doughs made with syrups are more cohesive, adhesive and sticky.

Ingredients: flour

Biscuit dough is very different from bread dough. Low-protein soft wheat flours are normally used and carbon dioxide gas is produced by the chemical leavening, mainly during baking. This gives the baked biscuit a much lower specific volume, thicker gas cell walls and a grainy texture. The gluten, when developed during mixing, becomes the continuous structure in the biscuit dough, providing a firmer eating quality. The use of a leavening helps to raise the pH high enough to prevent rapid gluten development during dough mixing. The mixing times and temperature of the dough should also be optimized for good sheeting properties, particularly when high levels of shortening are present.

13.2 Rheology of muffin and cake-like system batters

Rheology is a good tool for measuring the food structure and predicting the food quality properties of baked products. Rheology is the science that studies the deformation of a substance under the influence of a stress. The two extremes of rheological behaviour are 'elastic behaviour' and 'viscous behaviour'. Elastic behaviour is characteristic of solid substances which are deformed under the influence of an external force. When the force is

removed, the deformation is spontaneously reversed. A force applied to an ideal elastic substance is directly proportional to the deformation and independent of the speed at which this takes place.

At the other extreme, viscous behaviour is characteristic of Newtonian fluids, which deform when an external force is applied to them. The deformation continues as long as the force is maintained and ceases (without restoration) when it is removed. The force applied is directly proportional to the speed of deformation. Viscous fluids that show non-linear dependence between the force and the speed of deformation are known as 'non-Newtonian fluids'.

The majority of substances, including practically all food items, cannot be classified as purely elastic or viscous as they show an intermediate behaviour that falls between the two extremes. These substances are termed 'viscoelastic'.

13.2.1 Application of rheology to muffin and cake-like product batters

Batter viscosity is one of the important physical properties in cake baking. During baking, the velocity gradient in the batter as a whole will induce convection current at a given moment that depends on its viscosity, with low batter viscosity resulting in more convection flow (Frye & Setser, 1991). Retention of air and leavening gases by batters is clearly reflected in the specific gravity values of the batters and is also a function of batter viscosity (Bath, *et al.*, 1992). Although low-viscosity batters have been related to low cake volumes (Lakshminarayan, *et al.*, 2006; Lee, *et al.*, 2005), an excessive consistency could also diminish cake quality since it could impede the correct expansion of the batter (see further in the following section). Batter viscosity is also decisive in relation to the production process: changes in viscosity could lead to problems in handling the batter, in mould filling (metering) and in cleaning the machinery, or to greater energy expenditure on pumping in the case of high-viscosity batters.

Because of the structural complexity of batters, their viscosity is non-Newtonian. This implies that their viscosity depends on their shear rate. For this reason, viscosity needs to be measured at different shear rates and a batter viscosity value should always be accompanied by the shear rate value at which it was measured. For example, Fig. 13.1 shows flow curves for a standard Spanish muffin batter and a muffin in which a percentage of the wheat flour has been replaced by resistant starch (RS): a typical pseudoplastic behaviour (decrease in viscosity with the increase in shear rate) is found in this type of systems. The replacement of part of the flour by RS produced a decrease in viscosity over the shear rate range studied.

The simplest rheological model, which has been widely employed to define the pseudoplastic behaviour of batters, is the Ostwald de Waele model or power law model (Masoodi *et al.*, 2002):

$$\sigma = k \cdot \dot{\gamma}^n \quad [13.1]$$

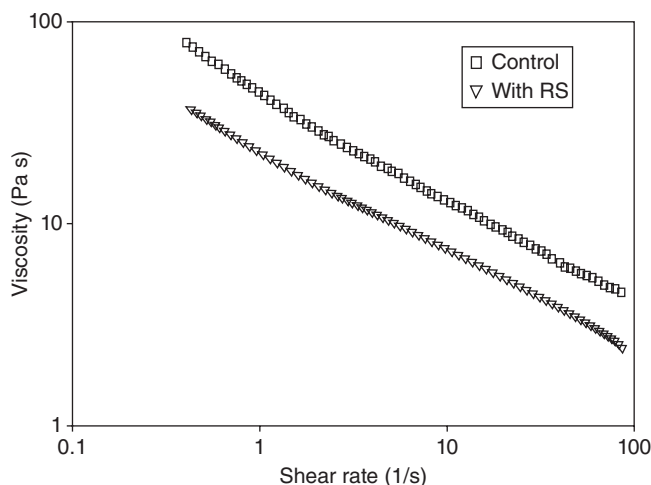


Fig. 13.1 Flow curves corresponding to a standard Spanish muffin batter formulation and one with 15% flour replacement by a resistant starch-rich ingredient at 20°C.

where k is the consistency index and n is the power law index. As $n = 1$ in this equation describes Newtonian behaviour, the difference between the value of n and 1 is a measure of the deviation from this behaviour. If $n < 1$, the rheogram represents a pseudoplastic fluid (Fig. 13.1). If $n > 1$, the rheogram represents a dilatant fluid, which is not very usual in food rheology.

Martínez-Cervera *et al.* (2011) studied the flow properties of a standard Spanish muffin batter and reported k and n values of 19 Pa s and 0.55 respectively. The k values were found to increase and the n values to decrease (values closer to 0) as the vegetable oil in the recipe was progressively replaced by cocoa fibre, in other words, as the viscosity and pseudoplastic properties of the batter increased. This reflects an increase in the complexity of the structure. Similar results were found by Masoodi *et al.* (2002) when apple pomace was added to a batter cake as a source of fibre and by Kim *et al.* (2001) when corn dextrins were used as fat replacers in yellow layer cakes.

Another empirical equation that could be employed to define the rheological behaviour of batters is the Herschel–Bulkley model, which includes a term that represents the yield stress, or the minimum stress that should be applied before the sample starts to flow.

13.2.2 Relationships between viscous properties and quality

Height and volume development during baking depend on a wide variety of factors, but viscosity is an important one. Low specific gravity is associated with better aeration of the batter (Kahlil, 1998), indicating a high

capacity of the batter to retain air bubbles during beating and, consequently, greater final volume and height in the baked product. In principle, a low specific gravity value could indicate a higher number of gas nuclei in the raw batter, which could encourage the formation of larger bubbles during baking and therefore result in greater height and volume. In turn, retention of air and leavening gases by batters during baking is also a function of batter viscosity (Bath *et al.*, 1992).

Batter consistency is important since it too is related to the capacity to retain air. Some authors have asserted that low consistency values are related to low cake volumes (Lakshminarayan *et al.*, 2006; Lee *et al.*, 2005). Baixauli *et al.* (2008a) found a decrease in batter viscosity and in muffin height when increasing proportions of the flour were replaced by resistant starch. However, excessive consistency could diminish cake quality since it could hinder correct dosing and the expansion of the batter.

Gómez *et al.* (2010) assessed the effects of fibre type (wheat bran, oat bran and microcrystalline cellulose), size (50, 80, 250 μm) and percentage of substitution (0%, 12%, 24%, 36%) on batter viscosity and layer cake characteristics. The lower fibre percentages caused a small increase in batter viscosity, which helps to retain gases and increased cake volume, whereas high fibre percentages produced a further increase in viscosity, which could impede expansion and diminish cake volume. The wheat bran that showed the highest viscosity also produced the lowest volume. The kind of fibre used did not affect the shape of the cake, but increases in fibre percentage resulted in flatter cakes. These changes could be related to batter consistency, since a 99% significant correlation was found between symmetry and the k value (consistency index) and symmetry and the n value (flow behaviour index). It was observed that the lower the consistency, the higher the symmetry.

Martínez-Cervera *et al.* (2011) found that the high consistency of the cocoa fibre muffin batter impeded final volume development. A similar result was found by Grigelmo-Miguel *et al.* (2001), who used peach dietary fibre as a fat replacer in muffins. Kim *et al.* (2001) found that the low specific gravity and relatively high viscosity of a cake batter prepared with maltodextrins as a fat replacer could have allowed more air to be incorporated, but the low cake volume obtained indicated that the membranes around the air cells were readily collapsed by gas expansion during baking. In general, the addition of any type of fibre to the ingredients of a bakery product causes a reduction in both the volume and the height of the final product (Peressini & Sensidoni, 2009).

13.2.3 Linear viscoelastic properties of muffin and cake-like product batters

The linear viscoelastic properties of muffin batters have mainly been studied through small amplitude oscillatory shear tests. In these tests the sample is

subjected to the oscillatory shear movement of a stress (or strain) low enough not to produce an irreversible alteration of the sample structure and the response of the sample to this variation is measured. As the relationship between stress and strain in relation to time can be described by linear differential equations with constant coefficients, their behaviour is termed linear viscoelastic. A relationship of this type implies that in an experiment, the force/deformation ratio will depend on time and not on the force or the deformation (Barnes, 2000). These tests provide important information on the original structure of the sample.

In small amplitude oscillatory shear tests there are two controlled variables: the frequency (ω) and the maximum amplitude of the deformation applied (γ^0). The response of the material is measured by the maximum amplitude of the force developed (σ^0) and the phase difference between the two waves (δ). It can also be measured in reverse by applying a sinusoidal stress and recording the deformation it causes.

In these tests, the material under study is subjected to a periodic sinusoidal deformation which can be expressed with respect to time:

$$\gamma = \gamma^0 \sin \omega t \quad [13.2]$$

where γ is the shear deformation, γ^0 is the maximum shear deformation or wave amplitude of the simple harmonic motion, ω is the frequency, and t is the test application time.

The shear stress can be expressed mathematically as:

$$\sigma = \sigma^0 \sin(\omega t + \delta) \quad [13.3]$$

where σ^0 is the amplitude of the shear stress wave (maximum stress value) and δ the phase angle difference in relation to the strain, also referred to as the mechanical loss angle (Dealy, 1982).

A further parameter used to study the dynamic test results is the complex modulus, G^* .

It is defined as the quotient of the force amplitude and the deformation amplitude ($G^* = \sigma_0/\gamma_0$) and represents the total resistance of a material to the deformation applied (Schramm, 1994). It should be pointed out that in viscoelastic materials, the G^* modulus also depends on the wave frequency. It can be broken down into two parts, one real and the other imaginary, as complex number notation is used to define it:

$$\begin{aligned} G^* &= G' + iG'' = |G|\cos\delta + i|G|\sin\delta \\ |G| &= \sqrt{(G')^2 + (G'')^2} \end{aligned} \quad [13.4]$$

where

$$G' = G^* \cos \delta = \frac{\tau_0}{\gamma_0} \cos \delta$$

and

$$G'' = G^* \sin \delta = \frac{\tau_0}{\gamma_0} \sin \delta$$

G' is called the storage or elastic modulus and represents the energy that the system stores temporarily and can later recover. G'' is called the viscous or loss modulus and represents the energy that the system uses at the start of flow, which is irretrievably lost in the form of heat.

In the case of an ideal purely elastic material, the response to a dynamic deformation does not exhibit any phase difference, in other words $\delta = 0$, so the relationship between the force and the maximum amplitude is $\sigma_0 = G' \gamma_0$. An ideal purely viscous fluid, on the other hand, presents a 90° phase difference, so the relationship between force and deformation is as follows: $\sigma_0 = G'' \gamma_0$.

In viscoelastic materials, which possess both elastic and viscous properties, the δ value is between 0° and 90° and the general expression of force in relation to deformation is

$$\sigma = G' \gamma_0 \sin(\omega t) + G'' \gamma_0 \cos(\omega t) \quad [13.5]$$

where γ_0 represents the maximum dynamic deformation amplitude.

The ratio of G' to G'' gives rise to another very important parameter which provides a direct index of the phase difference between force and deformation: $\tan \delta = G''/G'$. Tan delta, which shows the relationship between the loss modulus and the storage modulus, provides a measurement of the relative proportion of viscous and elastic properties in a viscoelastic material. If G' is much greater than G'' , the material will behave in a similar fashion to a solid and the strains applied will be elastic and recoverable. If G'' is far larger than G' , the energy employed in deforming the material will dissipate and will not be recovered.

Dynamic rheological methods are directly related to the molecular interactions and structure of the system studied. Depending on the frequency-dependence of the elastic modulus (G') and loss modulus (G''), three types of system can be described: macromolecular solutions ($G'' < G'$, where both parameters are highly frequency-dependent), weak gels ($G' < 10 G''$, where both are frequency-dependent) and strong gels ($G' > 10 G''$, where G' is independent of the frequency).

13.2.4 Relationship between the viscoelastic properties of the batter and the quality of muffins and cake-like products

The main criticism associated with testing small deformations is that they do not simulate real mixing, beating and baking processes, in which samples are subjected to quite large deformations. From this point of view, the data obtained from these tests should be interpreted as providing information on the structure of the sample in an unaltered state, which may be useful in predicting its behaviour when subjected to greater deformations.

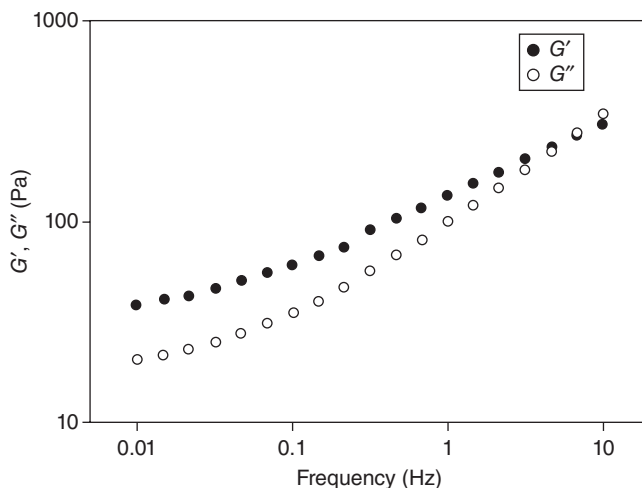


Fig. 13.2 Frequency dependence of a standard Spanish muffin batter at 20°C.

Nevertheless, the ability to predict the technological behaviour of a batter by rheological methods, using small or large deformations, requires a study of the possible correlation between them.

To study the linear viscoelastic properties of muffin batters, the dependence of G' and G'' on frequency (known as the mechanical spectra) has been studied (Sanz *et al.*, 2008). The results revealed the existence of a behaviour which is typical of soft gels, with G' values slightly higher than G'' values and both moduli slightly dependent on frequency within the available frequency range (from 10 to 0.01 s^{-1}). Figure 13.2 shows the frequency dependence of a standard muffin formula.

Like viscosity, the viscoelastic behaviour of doughs has been related to final muffin quality criteria. Lower batter specific gravity (SG) values, which imply a higher air content, have been associated with higher viscosity and elastic component values. This association has been related to higher resistance to bubble buoyancy and higher bubble stability in the batter. Higher viscosity and elasticity have also been associated with a greater height, volume and number of air bubbles in the baked muffins. However, a clear relationship between batter viscosity, elasticity, SG and baking performance has not always been found, which indicates that many other factors apart from the rheological properties of the batter contribute to appropriate baking performance (Sanz *et al.*, 2008).

The structural changes that occur during heating in the oven are also decisive factors in bubble formation and stability and in the development of the final baked structure. Studying the viscoelastic properties during heating by applying small amplitude oscillatory shear is a very suitable way of monitoring these changes. This test is carried out with an oscillatory shear

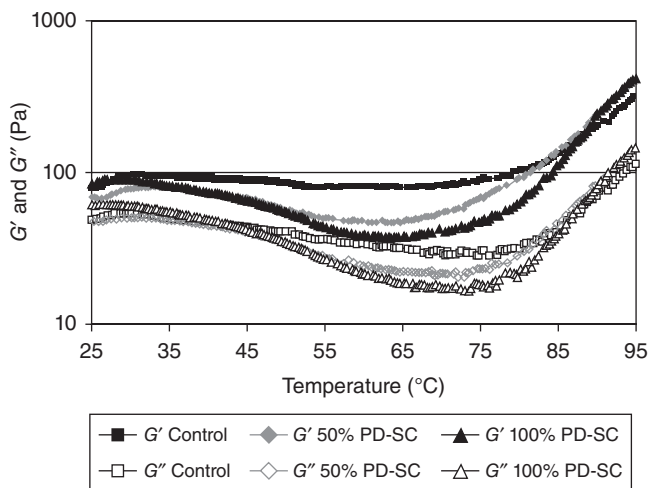


Fig. 13.3 G' and G'' as a function of temperature for a standard muffin batter and for the same batter with 50% and 100% sucrose replacement by a polydextrose-sucralose mixture (PD-SC).

amplitude value within the linear region. The strain control option of the rheometer should be used so that the stress applied is self-adapted and good sensitivity can be obtained throughout the experimental temperature range. Preliminary strain/stress sweeps should be carried out at different temperatures to select the appropriate value: the frequency most commonly selected for this test is 1 Hz (6.28 rad/s). Figure 13.3 shows a representative example of a temperature sweep in muffin batters.

The example corresponds to a standard Spanish muffin formula and to the same formula with different levels of sucrose replacement by a polydextrose/sucralose mixture (PD-SC) (Martínez-Cervera *et al.*, 2011). G' and G'' values during heating are shown. In all the batters, the initial increase in temperature produced a decrease in the values of both moduli, associated with CO_2 formation in the batter, diffusion into occluded air cells and expansion, which reduced the density of the batter. The moduli values then stabilized until an increase associated with starch gelatinization and/or protein coagulation took place. The initial decrease in the viscoelastic moduli was much more noticeable as the level of sucrose replacement with SC-PD increased. Another difference between the control and the sucrose-reduced batter was the lower temperature at which the moduli began to increase in the sucrose replacement formulations, which reflected earlier thermosetting of the structure. This finding may be associated with the sucrose's ceasing to exert its delaying effects and the PDs having a neutral effect on the protein denaturation temperature despite delaying starch gelatinization. As the sucrose was replaced, the viscosity and viscoelasticity of the raw batter decreased, as did the structure setting temperature. These circumstances do

not favour appropriate bubble expansion, even though the PD-SC batters had an appropriate initial number and distribution of air bubbles. The result was a muffin with less height and fewer final air cells as the sucrose was replaced, and with low hardness and springiness (see Section 13.3.1).

13.3 Determination of the texture of muffins and cake-like products using instrumental methods

The texture of baked products can be determined by instrumental or sensory methods. Instrumental methods offer some advantage over sensory analysis because they are rapid and objective. Baeva *et al.* (2000) made a study of sensory texture (a descriptive test for quantitative sensory profiling was used to establish the sensory textural characteristics, cell shape, size and uniformity and crumb tenderness) and instrumental texture (crumb springiness was determined with a relaxation test) to compare normal and energy-reduced sponge cakes. Mathematical and statistical methods were used to describe the physical and textural characteristics of the sponge cakes and their values were optimized. Sahi & Alava (2003) used instrumental texture profile analysis to study the crumb structure of sponge cakes in order to assess the effect of different emulsifiers. The same test was used by Gujral *et al.* (2003) to study the effect of sodium lauryl sulphate in a cake crumb and by Baixauli *et al.* (2008b) to compare the influence of replacing increasing proportions of wheat flour with different levels of resistant starch on the textural properties of muffins. Kamel & Rasper (1988) investigated the effect of sorbitol or polydextrose to replace sugar on the crumb firmness and elastic recovery of reduced-calorie cakes.

Of the different tests that have been used to assess the instrumental texture of muffins, the ones that are used most often are texture profile analysis (TPA) and the elastic recovery test.

13.3.1 TPA

Instrumental TPA was developed about 50 years ago and constitutes an interesting way of analysing a series of textural parameters in only one test (Szczesniak, 1963) because it provides a very quick calculation of parameters which are believed to correlate with sensory analysis. To apply instrumental TPA properly it is important to define the measurement parameters, such as the loading speed of the sample, sample shape and dimensions, degree of deformation and time interval between the two compression cycles (Pons & Fiszman, 1996). Regarding the sample dimensions and characteristics, Grigelmo-Miguel *et al.* (2001), Baixauli *et al.* (2008b) and Sanz *et al.* (2009) used 2.5 cm cubes of crumb cut from the centre of the muffin after removing the top and bottom, while Martínez-Cervera *et al.* (2011, 2012) used the 2.5 cm-high lower part of the muffins, cut

horizontally at the height of the paper cup, which was removed before the measurements were taken. In all these studies the samples were compressed with a 75 mm diameter aluminium plate probe (a greater diameter than that of the samples).

With respect to the degree of deformation applied, it is important to determine the compression percentage that will discriminate adequately between the different samples. Compression to 50% of the initial height has been used in some studies (Grigelmo-Miguel *et al.*, 2001; Kim *et al.*, 2001; Gujral *et al.*, 2003; Lee *et al.*, 2005), although other values such as 30% (Khouryieh *et al.*, 2005), 35% (Sahi & Alava, 2003), 40% (Baik *et al.*, 2000) or 60% (Arunepanlop *et al.*, 1996) have been reported, indicating that there is no clear criterion for this experimental condition because it depends on the nature of the sample.

In TPA the samples are compressed twice (the first and second 'bite') to give a curve from which the three primary textural parameters are obtained. These are hardness (the peak force during the first compression cycle), springiness (the height to which the food recovers during the time between the end of the first bite and the start of the second bite) and cohesiveness (the ratio of the positive force area during the second compression to the positive force area during the first compression). In cake products, measurements of resilience (the area during the withdrawal of the first compression divided by the area of the first compression) and the secondary texture parameter, chewiness (product of hardness $\times$ cohesiveness $\times$ springiness), are also of interest (Pons & Fiszman, 1996) (Fig. 13.4).

Springiness is a very important quality attribute of muffins and cake-like products. It reveals the ability of the sample to recover its height between

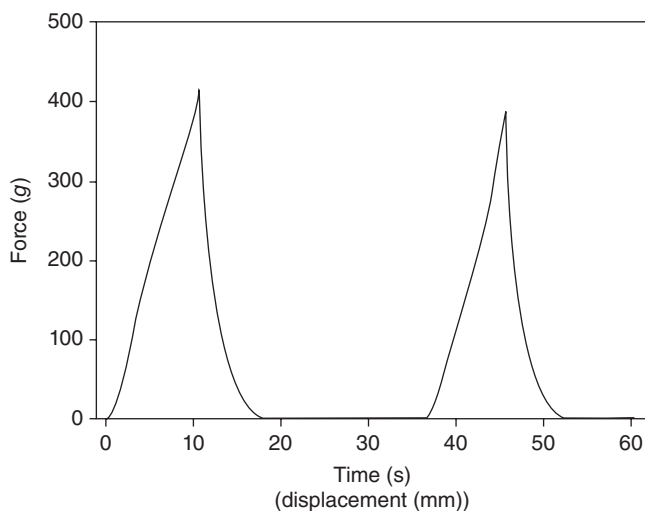


Fig. 13.4 Typical TPA curve for a muffin-type product.

the time before the end of the first compression cycle when the probe starts to rise and the beginning of the second cycle. Springiness is associated with a fresh, aerated product, so a high-quality muffin will be associated with high springiness values. Cohesiveness is another very important attribute, related to the energy required for the second compression. This parameter provides information about sensory crumbliness and perceptions related to denseness and the energy required to chew the piece of food.

In general, reformulation of bakery products is associated with changes in texture. For example, since one of the roles of fat is to coat the flour, reducing gluten hydration, fat reduction in bakery products causes an increase in hardness and chewiness due to greater gluten development. Grigelmo-Miguel *et al.* (2001) reported an increase in the instrumental hardness and chewiness of muffins when oil was replaced by dietary fibre in a reduced-fat muffin. In another study, low-fat muffins and low-fat muffins with xanthan gum also showed significantly higher instrumental hardness than the control (full-fat formulation) (Khouryieh *et al.*, 2005).

A different effect was found by Martínez-Cervera *et al.* (2011): the addition of soluble cocoa fibre as a fat replacer produced more tender and crumbly muffins with a more compact, less aerated crumb. These authors found that all the soluble cocoa fibre samples had significantly lower instrumental hardness values than the control (full-fat formulation). The hardness values increased as the soluble cocoa fibre content rose but even the highest fat replacement sample was significantly less hard than the control. With respect to springiness and cohesiveness, it was found that these values fell significantly as soluble cocoa fibre was increasingly substituted for fat, reflecting a more compact crumb and greater crumbliness of the muffins with lower fat levels.

Sugar reduction also causes changes in texture parameters. The retarding effect of sugar on the starch gelatinization temperature results in a tenderizing effect on muffin texture (Barndt & Antenucci, 1993). Sugar contributes volume, as well as humidification, which is linked to the sensory perceptions of moist mouthfeel and tenderness. Sugar also assists the formation of crystalline agglomerations of fat, improving air entrapment and air bubble stability during baking and giving rise to a more porous and spongy product (Hicsasmaz *et al.*, 2003). The replacement of sucrose by a polydextrose–sucralose mixture gave muffins a softer texture compared with a control (Martínez-Cervera *et al.*, 2012). Similar results were found by Akesowan (2009) in chiffon cake when sucrose was replaced by a sucralose–erythritol mixture. Ronda *et al.* (2005) also found a decrease in the firmness of sugar-free sponge cakes prepared with polydextrose.

The influence of fibre addition in terms of texture has also been studied. Baixauli *et al.* (2008b) studied the influence of replacing increasing proportions of wheat flour with different levels of resistant starch on the TPA parameters of Spanish muffins. They found that the hardness values of the muffins with RS were significantly lower than those of the control, but the

decrease in hardness was not linearly proportional to the flour replacement. The general effect of RS on muffin hardness may be explained by the dilution of the wheat flour gluten, as gluten development is an important determinant of hardness. The springiness of the muffins also decreased with RS replacement of flour; this effect was clear and significant for the highest concentration of RS. The decrease in springiness was associated with a decrease in the number of air bubbles and with a denser matrix. The cohesiveness values behaved in a similar manner to springiness, indicating that muffins with higher RS concentrations were very easy to crumble in the hand. Following these results, a study by Sanz *et al.* (2009) used a TPA test to evaluate differences in texture when part of the flour was replaced by different types of resistant starches. They found that although RS3 type (retrogradated) starches caused greater changes in the texture parameters than RS2 type ones (native resistant starch granules such as those in green bananas or raw potatoes), the RS3 type muffins also showed the lowest sensory acceptability in comparison with a control without RS.

13.3.2 Elastic recovery test

The elastic recovery test consists in applying a load to a food for a specific time and, when the load is removed, measuring the deformation recovered as elastic deformation. The elastic recovery of bakery products is measured by the Standard Procedure for Muffin Firmness and Elasticity (a relaxation test derived from the Novo Nordisk modified version of AACC method 74-09) (AACC, 2000). In this procedure, the muffin is compressed with a probe at a fixed test speed and percentage strain for several seconds, after which the probe is removed and the elastic recovery is measured from the relaxation curves (Fig. 13.5) by applying the following equation:

$$\% \text{ recovery} = (F_t / F_{\max}) * 100$$

where $F_{\max}$ is the maximum force and F_t is the force after ts .

Singh *et al.* (2006) studied the relaxation behaviour of some bakery items. They reported that angel cakes and pound cakes showed a percentage recovery in the range of 50–40 and that this may be due to the cross-linked protein structure formed during the baking process. The matrices of the baked products are viscoelastic in nature, as previously mentioned; the percentage recovery value indicates the ratio of the elastic and the viscous components, which were correlated with the gliadin and glutenin fractions present in the wheat gluten protein. Similar studies carried out by Kamel & Rasper (1988) to examine the effect of emulsifiers on the texture of reduced-calorie cakes showed that the percentage recovery values consistently fell when higher concentrations of emulsifiers were used. Shearer & Davies (2005) evaluated changes in freshly baked muffins with flaxseed oil or full-fat flaxseed meal. Freshly baked muffins prepared with flaxseed meal

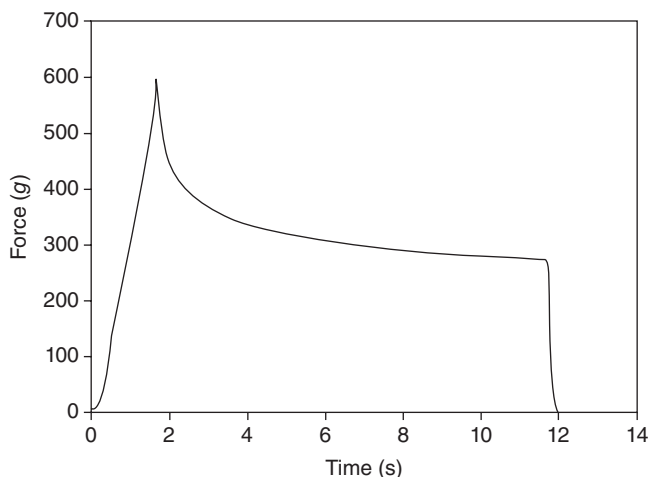


Fig. 13.5 Typical relaxation curve of a muffin-type product.

were less elastic (ability of a muffin to relax while compressed) than the control muffins. Baixauli *et al.* (2008b) studied the elastic recovery of muffins with RS as a dietary fibre enrichment. They found that the elastic recovery values in formulations with 5% and 10% RS were the same as for the control muffins, but at higher concentration of this ingredient this parameter decreased significantly, indicating a less elastic structure.

13.4 Biscuit dough rheology

13.4.1 Application of rheology to biscuit dough

Studies of the fundamental rheological properties of biscuit dough are very scarce. The existing studies are mainly related to tests that apply small deformations, such as small amplitude oscillatory shear and creep and recovery tests.

13.4.2 Application of small amplitude oscillatory shear in biscuit dough

The extension of linear viscoelastic response in biscuit doughs has been found to be limited to very low strain values, indicating a high sensitivity of the structure to the applied stress. Baltsavias *et al.* (1997) referred to strain values as low as 0.0002 that limited linear response in short dough. The frequency dependence of G' and G'' in the linear region reveals that biscuit dough at ambient temperature shows a typical rheological behaviour of a soft gel, with values of G' higher than G'' and some frequency dependence for both moduli. An example can be seen in Fig. 13.6, which shows the

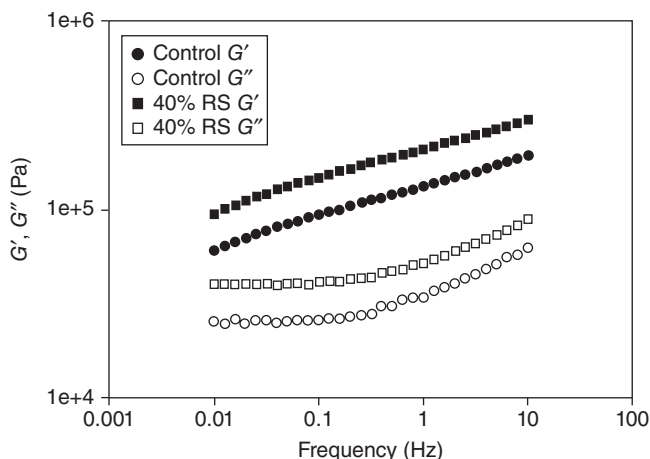


Fig. 13.6 Frequency dependence at 25 °C of G' and G'' in a standard short-dough and in the same dough with 40% wheat flour replacement by resistant starch.

frequency dependence of G' and G'' for a short-dough formula and for the same formula with 40% replacement of wheat flour by a resistant starch rich ingredient (Laguna *et al.*, 2013a).

Obviously, the rheological behaviour of biscuit dough depends on the dough composition. In Fig. 13.6 it may be observed that replacing flour with a resistant starch rich ingredient (RSRI) increased the values of G' and G'' . The different formulations showed a similar frequency dependence of both G' and G'' . No differences in $\tan \delta$ values were observed among the samples, indicating that although the incorporation of the resistant starch ingredient affected the G' and G'' values, this did not appear to affect the relationship between the two moduli.

Baltsavias *et al.* (1997) evaluated the linear viscoelastic properties of short dough with various compositions. They concluded that fat was a crucial structural component: decreasing the fat content or replacing solid fat with liquid oil caused a marked decrease in the stiffness of the dough and a greater dispersal of the fat in the dough. Replacing flour with native starch produced a decrease in the viscoelastic modulus. The absence of gluten in native starch affected water distribution and, thereby, the rheological properties of the non-fat phase. It was difficult to establish the contribution of other flour components like damaged starch, which increases short dough consistency, or pentosans, which decrease G' , as it was not possible to separate the magnitude of these different effects.

The effect of endogenous flour lipids on the structure of semi-sweet doughs and short doughs has been studied by oscillatory tests (Papantoniou *et al.*, 2003). Defatted flour gave higher viscoelasticity and the microstructure of the defatted short dough biscuits revealed that their gluten protein

was more hydrated and developed. It was suggested that polar lipids form bonds with the protein molecules and help to control their access to water (Papantoniou *et al.*, 2004). As for the role of sucrose, it increases the liquid-like properties of the dough because it modifies the properties of the non-fat phase via its influence on the amount of solvent available.

13.4.3 Application of creep and recovery tests in biscuit dough

Other tests used to study linear viscoelastic properties include creep compliance tests. In this case, as in oscillatory tests, small stresses that do not destroy the structure of the sample are used, so these are transient tests. In a creep test an instantaneous stress in the linear region is applied to the sample for a certain time. After removal of the stress, the sample is allowed to relax (recovery test). The system deformation per unit stress is called compliance (J) and is measured over time:

$$J(t) = \gamma(t) / \sigma_0 \quad [13.6]$$

where γ is the deformation and σ_0 the instantaneous stress applied to the sample within the linear viscoelastic region. The higher the value of J , the easier it is for the sample to become deformed under a given stress. In the linear viscoelastic zone, J is independent of the stress applied.

An ideal solid will display a totally elastic response: the deformation will be proportionate to the stress applied and will remain constant for as long as the stress is maintained (like an ideal spring). In a viscous liquid the deformation will increase continuously over time, so the slope of the deformation will be related to the viscosity (as in an ideal shock absorber). If the stress is removed, the elastic solid recovers instantly and the deformation suddenly drops to zero. In the case of the fluid, however, the deformation continues at the maximum value it has reached because no recovery takes place.

The behaviour of most substances lies somewhere between those of an ideal solid and an ideal liquid and the response presents a combination of their characteristics.

The mechanical models that reflect the deformation of a system in tests of this kind range from the simplest approaches (single spring or single dashpot) that represent purely elastic or purely viscous behaviour, respectively, and the association of both in series (Maxwell model) or in parallel (Kelvin–Voigt model), to generalized approaches including the association of a larger number of components. One of the most widely used models, due to its relative simplicity and the acceptable results obtained in many cases, is the four-components Burgers model, comprising the association in series of the Maxwell model and the Kelvin–Voigt model.

In the Burgers model, the system deformation per unit stress, called compliance, J , as a function of time, corresponds to the following equation (Barnes, 2000; Steffe, 1996):

$$J(t) = \frac{1}{G_0} + \frac{1}{G_1} \left[1 - \exp\left(\frac{-tG_1}{\eta_1}\right) \right] + \frac{t}{\eta_0} \quad [13.7]$$

where $J(t)$ represents the overall compliance at any time t , G_0 is the instantaneous elastic modulus of the Maxwell unit and G_1 is the elastic modulus of Kelvin–Voigt. The latter represents the contributions of the retarded elastic region to the total compliance. The dashpot of the Maxwell element represents the residual viscosity, η_0 , and the dashpot associated with Kelvin–Voigt is called the internal viscosity, η_1 . Obtaining the values of G_0 , G_1 , η_0 and η_1 makes it possible to compare the internal structure of different systems, yielding a mechanical model which reflects the behaviour of such systems.

13.4.4 Relationship between creep and recovery tests and biscuit quality properties

The Burger model has been employed successfully to characterize short dough behaviour during a creep test and assess the effect of flour replacement by an RSRI (Laguna *et al.*, 2013a). The compliance (J) values during the creep and recovery tests can be seen in Fig. 13.7. They reveal an increase in elasticity and in resistance to flow and a decrease in deformability with the incorporation of RS. The recovery results show that RS did not affect relative recovery (% R). The relative recovery is given by the difference between the compliance values at the end of the creep test (J_{MAX}) (maximum deformation) and the permanent deformation (J_{INF}), divided by the

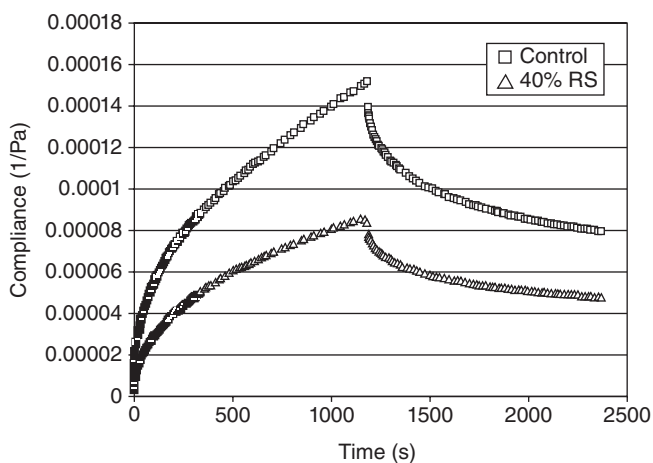


Fig. 13.7 Compliance values during a creep and recovery test at 25°C for a standard short dough and for the same dough with 40% wheat flour replacement by resistant starch.

maximum deformation, in other words $(J_{\text{MAX}} - J_{\text{INF}})/J_{\text{MAX}}$. The fact that %*R* was not affected revealed that no effect on the type of structure due to RS incorporation can be expected. Deformability was positively correlated with biscuit spread during baking (Laguna *et al.*, 2013a).

Pedersen *et al.* (2004) used oscillatory and creep-recovery tests to study the linear viscoelastic properties of semi-sweet biscuit doughs made with flours from eight different cultivars and related them to changes in biscuit dimension. The decrease in biscuit length was correlated to the phase angle, the flour mixing characteristics determined by the farinograph and the creep recovery parameters. The same authors (Pedersen *et al.*, 2005) evaluated the effect of adding sodium meta-bisulphite and a commercial protease to a biscuit dough. An increase in extensibility and a decrease in elasticity were observed. Biscuit contraction and spread were mostly correlated to percentage recovery of the dough and to protein and gluten content.

13.5 Instrumental texture properties of biscuits

The texture of biscuits can be interpreted in terms of the state of their principal ingredients. The process of baking short dough induces a large decrease in product bulk density, leading to a cellular solid with a thin coloured surface and a porous inner structure. Kulp *et al.* (1991) determined that most starch granules remained in their native condition during cookie baking and did not form a continuous structure. According to Slade & Levine (1994), biscuit sugar is in a concentrated solution that delays or prevents starch gelatinization/pasting during biscuit baking. Baltsavias *et al.* (1999) showed that irrespective of composition, starch gelatinization is slight due to the limited water content coupled with the low baking temperature, giving a crisp texture.

Moreover, proteins do not aggregate and hydrate enough to form a gluten network (Chevallier *et al.*, 2002). However, soft wheat flour proteins are not functionally inert in biscuit dough, especially during baking, when more associations of proteins probably occur due to increasing hydration and proximity, affecting cookie texture (Gaines, 1990). According to Eliasson & Larsson (1993), water plays a complex role, since it determines the conformational state of biopolymers, affects the nature of interactions between the various constituents of the formulation and contributes to dough structuring. If the proportion of water is too low, the dough becomes brittle, not consistent (Sai Manohar & Haridas Rao, 1999).

13.5.1 Three-point bending test

In this test the biscuits are fractured in tension. If the fracture takes place relatively close to the central zone, where the maximum stress is concentrated, this implies that the condition regarding the distance between

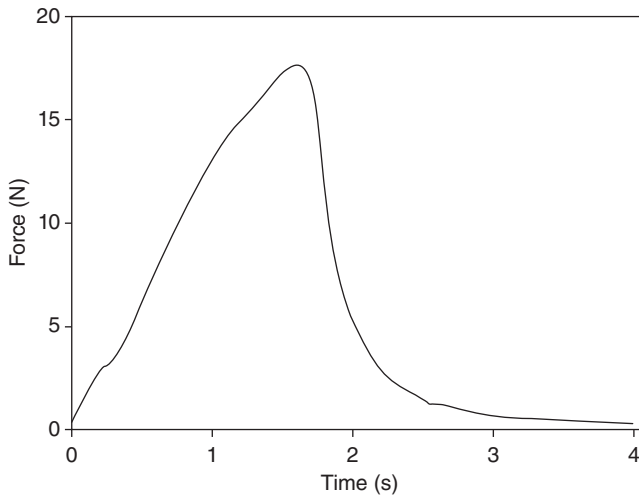


Fig. 13.8 Typical profile of biscuit breaking strength in a three-point bending test.

supports is satisfied. The control biscuit slope shown in Fig. 13.8 is typical of the force–deformation curves described for materials exhibiting brittle fracture, characterized by an essentially elastic response and a small fracture strain.

However, the somewhat shoulder-shaped part of the curve just before fracture could imply some plastic deformation in the vicinity of the crack. This behaviour is highly dependent on the proportion of the biscuit ingredients. Nevertheless, what is important is the comparison between the control and other formulations. For this reason, Laguna *et al.* (2011) studied the textural changes that take place in short dough biscuits when increasing proportions of the flour are replaced by an RSRI. They found that the highest force at break corresponded to the control biscuit, indicating a more interconnected, structured matrix, probably due to the higher level of wheat proteins in the control samples. The biscuits containing the RSRI showed curves characterized by a lower initial force gradient and lower peak force values, indicating lower resistance to snapping. Additionally, the force values did not drop as sharply as in the control sample after reaching the fracture stress values. This could imply that the crack propagated more slowly and the probe had to travel further to cause fracture. Obviously, during baking, the lack of a sufficient quantity of proteins to give the dough structure and cohesion became an important factor in the final texture of the biscuit, as it had a tenderizing effect. When fibre was incorporated into the biscuit formulation the quantity and type of both fat and sugar remained the same, so the changes in the properties of the doughs and the biscuits should be interpreted in terms of water content and distribution and the lower flour protein content of the formulations with the fibre. The

increasing proportions of RSRI had the effect of decreasing the total dough moisture level, because wheat flour is moister than RSRI. The RSRI also diluted the flour proteins. These factors need to be taken into account when interpreting the results.

On replacing 30% of the flour in a short dough biscuit formulation with native wheat starch, Baltasvias *et al.* (1999) found slightly lower fracture stress. Also, the stress–strain curve showed a small shoulder just before fracture, which they attributed to a stronger local permanent deformation as a result of a somewhat different fat distribution. Biscuits with increasing levels of different fibre sources become harder, as seen in their increasing breaking strength values, especially, as reported by Sudha *et al.* (2007), when 30% and 40% of rice and barley bran are added.

On the other hand, replacing part of the fat leads to an increase in hardness, associated with gluten development. Ghotra *et al.* (2002) noted that in the absence of shortening, the water interacts with the flour protein to create more cohesive and extensible gluten, while if the fat level is high, the fat surrounds the proteins, shortening their development. Pareyt & Delcour (2008) reported that in this type of biscuit, little, if any, gluten is formed, giving a very soft texture. Laguna *et al.* (2012) found that the higher the fat level, the softer the biscuits (lower breaking strength values).

13.5.2 Volodkevich bite jaw test

Volodkevich bite jaws imitate the force of biting the biscuit. The typical profile of a control biscuit shows a penetration curve with a maximum corresponding to the point when the probe penetrates the sample; the values then fall, indicating relaxation of the stress, and finally rise again as the increasing dimensions of the probe penetrate the sample. Laguna *et al.* (2011) found that the penetration force values of the samples containing the RSRI were lower than those of the control, indicating greater crumb tenderness. Gaines *et al.* (1996) used a puncture test on various biscuits and reported that the fracture force rose with increasing flour protein content.

13.5.3 Mechanical measurements coupled with acoustic measurements to evaluate crispness in biscuits

An acoustic envelope detector (AED) can be coupled to the texturometer for sound recording. An example of the setup is a Bruel and Kjaer free-field microphone (8 mm diameter), calibrated using a Type 4231 acoustic calibrator (94 and 114 dB SPL-1000 H) and placed in a frontal position in order to gain a better acoustic signal, at a distance of 4 cm and an angle of 45° to the sample, although this depends on the sample. The AED operates by integrating all the frequencies within the band pass range, generating a voltage proportional to the sound pressure level (SPL). Each sound graph is simultaneously displayed with the corresponding force/displacement

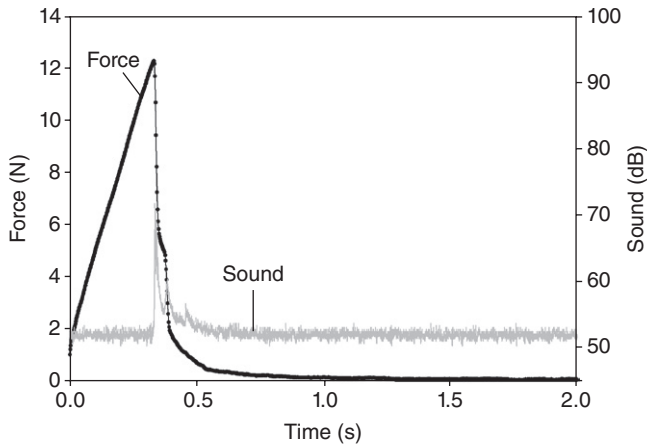


Fig. 13.9 Typical force–deformation and sound profiles of a biscuit breaking in a three-point bending rig.

graph (Fig. 13.9). The sound parameters are the number of sound peaks and SPL_{max} (dB) as an indication of crispness. The presence of numerous peaks could be understood as the breaking events that occur when the probe passes through the layers within the product structure. Therefore, a more compact structure will be reflected by fewer peaks and an airy or layered structure by a high number of peaks. Few works have combined mechanical and acoustic tests to evaluate biscuit crispness. Laguna *et al.* (2013b) measured the number of sound peaks as an indication of biscuit crispness to evaluate the influence of dose and fibre length when fibre replaces part of the flour. They found that the wheat fibre reduced crispness, which means that there was less microcracking and probably denotes a more compact biscuit matrix, whereas the apple fibre retained or increased biscuit crispness. This result is of great technological importance when deciding which fibre to use to enrich a biscuit, as it shows that a smaller particle size will give a crisper, crumblier texture.

13.6 Height and diameter of biscuits

The baking process transforms the biscuit dough into a cellular solid with a characteristic final texture (Chevallier *et al.*, 2000). Because of the CO_2 gases produced by the raising agents and water evaporation, biscuits undergo substantial expansion early in baking. The degree of spread is controlled by the spread rate and the set time which, in turn, depend on the level of water in the dough that is free to act as a solvent and the strength of the dough (Ram & Singh, 2004). Levine & Slade (1990) stated that ‘good’

biscuits facilitate expansion without significant functional network formation followed by structural collapse into a rubbery, thermoplastic polymer system. As indicators of biscuit quality, it is useful to measure the width (W), thickness (T) and W/T ratio (biscuit spread factor), which are controlled by the dough consistency and biscuit set time. Biscuit width is measured by placing 10 biscuits edge-to-edge (both vertically and horizontally) and biscuit thickness by stacking 10 biscuits. Measurements are expressed in mm as the mean value/10 of three different trials. Changes in the dimensions are expressed as gains (+) in comparison with the initial dimensions of the biscuits before baking (each biscuit is weighed individually before and after baking). Pareyt *et al.* (2008) and Kweon *et al.* (2009), among others, have recorded changes in biscuit geometry during baking by taking time-lapse photographs at intervals through a glass window in the oven door. These experiments have demonstrated the effects of the two-step method mentioned under 'Ingredients: Sugar' in Section 13.1.2.

Some reduction in biscuit dimensions due to fibre addition has been observed. Brennan & Samyue (2004) suggested that the fibres may act as biscuit dough mixture stabilizers at up to 10% of replacement, enabling the reformulated biscuit dough to retain its diameter during baking. Laguna *et al.* (2011) studied the physical changes that took place in short dough biscuits when increasing proportions of the flour were replaced by an RSRI. They found that in the RSRI biscuits, the higher the amount of RSRI the lower the spread, both in length and width, but found no change in the thickness of the biscuits. They argued that RSRI substitution had a diluting effect on the wheat proteins and that this behaviour was related to the lower gluten content. Pareyt *et al.* (2008) showed that the spread rate decreased linearly with the gluten level. Sudha *et al.* (2007) found that, depending on the type of fibre (oat, barley, wheat or rice bran) added to a biscuit formulation, the resistance to extension varied; they attributed this effect to interaction between the polysaccharides and the protein in the wheat flour. However, Laguna *et al.* (2013b) found no significant differences in biscuit height, width or length due to the addition of two different sizes of wheat fibre (200 and 101 μm) and an apple fibre.

13.7 Future trends

Many further factors also decide the texture of sweet bakery products and merit the attention of researchers and the efforts of manufacturers.

13.7.1 State of water in the systems

Greater knowledge is needed of the part played by water, in terms of both its quantity and state, in cake matrices and above all in biscuits before and after baking. It is essential to know the state of solution, dispersion,

crystallization or hydration of the components and, consequently, their contribution to the texture of the system as a whole.

13.7.2 Microscopy

Another field in which much research remains to be done is microscopy. Nowadays many techniques have been improved and, more recently, complemented by image analysis. These methods make it possible to quantify certain features of system structures that will assuredly improve our understanding of the relations between structural elements and texture.

13.7.3 Sensory aspects

Any change in the composition, preparation process, storage type and time or packaging can bring about sensory changes in texture that will need to be analysed through quantitative descriptive techniques and the study and definition of key descriptors. It is quite astonishing how many studies of these products report their sensory analyses only partially or confusedly, or even with major errors such as assessing the degree of liking with only a few people.

Still in the field of sensory analysis, the new techniques that analyse the food's passage through the mouth, such as temporal dominance of sensations (TDS), open up new areas of study that will allow interpretation of how a change in formulation can affect the processes of chewing, insalivation, bolus preparation and final swallowing and the predominant sensory attributes over the ingestion time. All these processes are intimately related to perception of texture.

13.7.4 Combined techniques

Last but not least, combination methods such as coupling mechanical measurements with acoustic techniques are proving encouraging. This type of study makes it possible to cast the spotlight on matters that have long been a subject of debate, like pairs of properties such as hard/crunchy or fragile/crisp.

13.8 Sources of further information and advice

Books:

- *Principles of Cereal Science and Technology*, 3rd Edition, J. A. Delcour and R. C. Hoseney, AACC International, 2010.
- *Bakery Food Manufacture and Quality: Water Control and Effects*, 2nd Edition S. P. Cauvain, & L. S. Young, Wiley, 2008.
- *Bakery Products: Science and Technology*, Y. H. Hui, Blackwell Publishing, 2006.

Web sites:

- <http://baking-management.com/>
- <http://www.bakeryandsnacks.com/>
- <http://www.bsimagazine.com/>
- <http://www.bakingbusiness.com/>

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Measurement of the texture of dry crisp products

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Abstract: Crispness is a textural attribute which is an important indicator of the quality of dry crisp products. In this chapter, product characteristics which contribute to crispness will be discussed. This discussion will include an overview of structural properties necessary for a product to be considered crisp as well as information related to moisture. Acoustic and force deformation techniques which have been used to characterize crisp products will also be outlined and parameters used to quantify crispness will be presented.

Key words: crispness, cellularity, critical water activity, acoustics, mechanical testing.

14.1 Introduction

Crispness is a textural attribute that is ‘universally liked’ by consumers (Szczesniak, 1988). It has been described as a ‘stimulant to active eating’ and, after consumption, as a ‘relaxing, satiable texture’ (Szczesniak and Kahn, 1971). From a quality perspective, crispness is indicative of ‘freshness and wholesomeness’ in foods and is associated with a high-quality product (Szczesniak and Kahn, 1971). Given that crispness is of such importance to quality, it is therefore, necessary to have a solid understanding of factors contributing to this textural property.

When asked to name foods that have crisp textures, consumers have identified products which can be classified into one of three categories: raw fruits and vegetables, fried products (such as bacon and chicken) and farinaceous products (such as crackers, cereals and potato chips) (Szczesniak, 1988). What differs among the foods in each category is the moisture content contributing to the perception of crispness. Raw fruits and vegetables are classified as ‘wet crisp’ and crispness is related to turgor pressure resulting from the presence of water within the cellular matrix (Vickers, 1976). As the product is bitten into, the release of pressure leads to the generation of a

sound. Farinaceous products are 'dry crisp' and, like 'wet crisp' products, are cellular. However, unlike 'wet crisp' products, farinaceous products contain gas. Crispness perception is the result of the breakage of the cells during crushing and therefore cellular morphology is important to this perception. 'Dry crisp' foods are often manufactured and therefore their structure, and resulting texture, are under the control of the manufacturer. As such, understanding the role of structure on product crispness and identifying instrumental techniques which can be used to characterize the texture of crisp products is necessary. These topics will be the focus of this chapter.

14.2 Product characteristics and crispness

'Dry crisp' products have been described as consisting of a solid foam structure with a water content of under 10%. This is equal to a water activity of less than 0.55 (Roudaut and Champion, 2011). From this description it is obvious that two key aspects of the product are important influences on crispness perception: product morphology and water content.

14.2.1 Product morphology

A dry cellular product is composed of rigid cell walls which enclose a gas (van Vliet and Primo-Martin, 2011). The size of the cells within the product and the thickness of the beams surrounding those cells are two product characteristics of importance to crispness. Cell size of importance for crispness has been estimated to lie between a minimum size of 120–200 μm and a maximum size of 270–350 μm (Luyten and van Vliet, 2006). When the product is broken, the breakage of smaller sized cells results in what has been described by Luyten and van Vliet (2006) as 'distinguishable events' while the breakage of larger cells produces an overlap of sound events. This overlap is required to produce enough sound for crispness to be perceived. Other researchers have also shown that extruded products with large cell sizes are perceived to be crisper than those with a smaller cell size (Barrett *et al.*, 1994; Vincent, 2004). Similarly, bread rolls with a coarse structure (i.e. large gas cells) are perceived to be crisper than those with smaller gas cells (Primo-Martin *et al.*, 2008).

Estimations of the thickness of the beams surrounding the cells of a crisp product have also been published. Beam thickness of a crisp product has been calculated to range from a minimum of 50–100 μm to a maximum thickness of 300–400 μm (Luyten and van Vliet, 2006). Beams below 50 μm are too thin to produce sounds upon breakage. Above the maximum value of 400 μm , the beams will result in a product that is perceived as hard (Luyten and van Vliet, 2006).

Given that the beam thickness and cellularity of a product will affect crispness, it is intuitive, therefore, that other product characteristics must also relate to crispness. There are currently few research papers, however,

directly linking other product characteristics with perception of crispness. Barrett *et al.* (1994) showed that the bulk density of extruded corn-based snacks was linearly correlated with sensory crispness (defined as the perceived horizontal force with which the product separates into two pieces), indicating that a more dense product is perceived to be crisper than a product which is less dense. However, this is one of the few papers published in this area. Combining material science approaches with sensory measures of crispness is imperative to ensuring quality of crisp products is at an optimum and should be the focus of future research.

14.2.2 Water and crispness

It is well documented that water affects crispness. As water activity increases, the crispness of a product decreases (Katz and Labuza, 1981; Seymour and Hamann, 1988; Sauvageot and Blond 1991; Duizer *et al.*, 1998) and more recently, it has been shown that water content has an effect on crispness (van Nieuwenhuijzen *et al.*, 2008). However, what is relevant for sensory quality is the water activity value at which the product can no longer be described as crisp and/or is not acceptable. This has been labelled as the critical water activity (aw_c) value. Critical water activity values for a number of dry crisp products have been determined and are outlined in Table 14.1. Depending on the product and the technique used to calculate aw_c , these values fall within a water activity range of 0.2 and 0.76.

A variety of techniques have formed the basis of critical water activity calculations and results collected from sensory testing, acoustic testing and mechanical testing have been used in these calculations. From a sensory perspective, critical water activity calculations have been based on measures of crispness intensity as well as liking scores for crispness (Katz and Labuza, 1981; Sauvageot and Blond, 1991; Peleg, 1994; Roos *et al.*, 1998; Hough *et al.*, 2001). The collected sensory data (liking or intensity) is plotted against product water activity and aw_c is identified from the plot. Katz and Labuza (1981) identified aw_c from the developed plot as the median value for liking of texture. Slightly higher aw_c values were observed by these researchers when median crispness intensity scores were used in place of liking. Sauvageot and Blond (1991) plotted crispness intensity values collected from a sensory panel and identified three distinct linear regions of crispness on the crispness–water activity plot. The first linear region on the plot was characterized by a high crispness intensity with little variation in crispness as water activity increased. The second region showed a rapid decrease in crispness intensity with increasing water activity while the third region was characterized by a slight decrease in crispness intensity as water activity increased. These researchers identified the aw_c as the water activity at which the second linear region began. In later analyses, Peleg (1994) refined this approach by plotting the data from Sauvageot and Blond's research as a continuous curve rather than three distinct regions. Using the assumption that crispness is the result of a continuous plasticization process

Table 14.1 Published critical water activity values for 'dry crisp' products

Product	Technique forming basis for a_{wc} determination	a_{wc}	Author
Popcorn	Sensory crispness	0.49	Katz and Labuza (1981)
	Texture liking	0.43	
Puffed corn curls	Sensory crispness	0.36	
	Texture liking	0.20	
Potato chips	Sensory crispness	0.51	
	Texture liking	0.47	
Saltines	Sensory crispness	0.39	
	Texture liking	0.37	
Rice crispies	Sensory crispness	0.56	Sauvageot and Blond (1991)
Corn flakes		0.53	
Extruded cereals		0.50–0.54	
Rice crispies	Sensory crispness	0.65	Peleg (1994)
Corn flakes	modelled using	0.62	
Extruded cereals	Fermi's equation	0.64–0.68	
Extruded snacks	Sensory crispness	0.708–0.762	Roos <i>et al.</i> (1998)
Commercial toasted sweet biscuit	Consumer acceptability modelled using Fermi's equation	0.60	Hough <i>et al.</i> (2001)
Commercial whole wheat cracker		0.51	
Commercial normal flour cracker		0.52	
Commercial sweet biscuit		0.43	Gondek and Lewicki (2006)
Corn flake cereal	Acoustic emission	0.555	
Wheat bran cereal	energy modelled using Fermi's equation	0.438	
Bread rusk roll – fine texture	Sensory crispness	0.57	Primo-Martin <i>et al.</i> (2008)
Bread rusk roll – coarse texture		0.59	
Crackerbread	Various acoustic and mechanical parameters	0.51–0.59	Arimi <i>et al.</i> (2010)

within the product as water activity increases, the data were fitted to a curve using Fermi's equation (equation 14.1).

$$Y(a_w) = \frac{Y_o}{\left(1 + \exp\left[\frac{a_w - a_{wc}}{b}\right]\right)} \quad [14.1]$$

where Y_0 is a product sensory score; a_w is water activity; a_{wc} is critical water activity; and b is a steepness parameter (Peleg, 1994).

Critical water activity is stated to be the point at which there was a 50% reduction in the perceived sensory property when plotted against water activity. Similarly, using Fermi's equation, Hough *et al.* (2001) ascertained that critical water activity was the point at which the Fermi's curve intersected ideal acceptability (defined as 0 on an acceptability scale ranging from -100 to 100). More recently acoustic and mechanical data have been modelled using Fermi's equation and critical water activity based on these parameters has been determined (Arimi *et al.*, 2010).

Water contributes to crispness through its activity as a 'structuring or plasticizing agent' for many of the chemical and physical properties of food (Roudaut and Champion 2011). However, the physical changes which are occurring to the product at its critical water activity are still under investigation. It has previously been stated that the loss of crispness at and above a_{wc} occurs due to an increase in the mobility of the macromolecules leading to structural reorganization (Katz and Labuza, 1981; Roos *et al.*, 1998; Hough *et al.*, 2001). This results in a shift of the amorphous regions within the product from a glassy state to a rubbery state resulting in a softening of the product and a loss of crispness (Katz and Labuza, 1981; Valles Pamies *et al.*, 2000). Others, however, have found that loss of crispness occurs while the product is still in a glassy state (Braga and Cunha, 2004; Marzec and Lewicki, 2006).

Water can also act as an 'anti-plasticizer'. It is proposed that while in the glassy state, water is adsorbed by a product, leading to a compact structure which is resistant to deformation (Pittia and Sacchetti, 2008). A number of researchers studying dry crisp products have attributed the hardness of their products to anti-plasticization (Valles Pamies *et al.*, 2000; Heidenreich *et al.*, 2004; Gondek and Lewicki, 2006). To date, the reasons for anti-plasticization occurring in dry crisp products have not been entirely elucidated; however, it may be dependent on the composition of the material being studied. For instance, it has been shown that the anti-plasticization effect differs between extruded breads made from rye in comparison to those made from wheat (Marzec and Lewicki, 2006). One hypothesis is, that at a molecular level, there is hardening within the glassy state due to the adsorbed water molecules filling low-density domains within the product; thus, increasing their density (Marzec and Lewicki, 2006; Roudaut and Champion, 2011).

14.3 Methods for characterization of the texture of crisp products

Crispness perception is the result of input from more than one sensory modality, with both an acoustic component and a fracture component

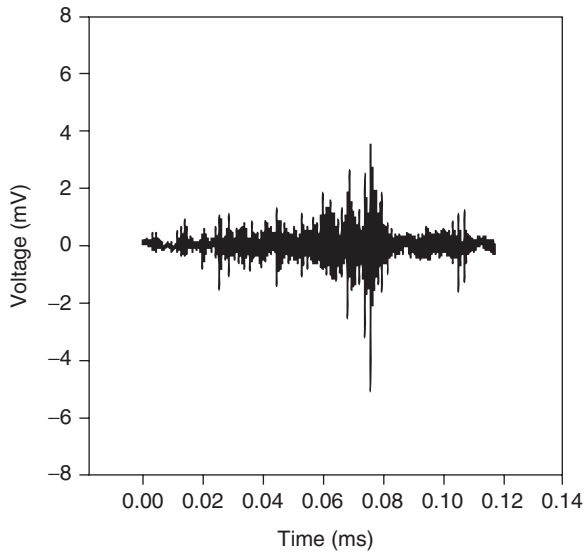
important for its manifestation. The importance of each of these components as they relate to crispness has been extensively studied using human and/or an instrumental measures and a wealth of information regarding the texture of crisp foods has been acquired. This section will outline approaches which have been used to measure the acoustic and fracture properties of crisp textured products. Parameters of importance for characterizing crisp products will be identified and discussed.

14.3.1 Measures of sound

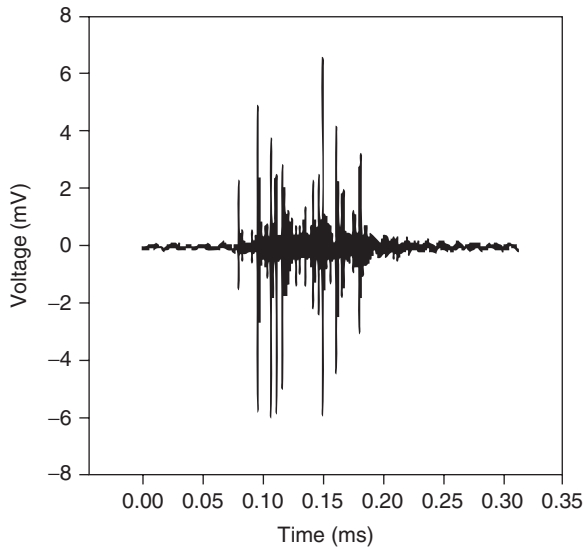
Sound is generated during biting or compression of a crisp food. With the application of a force, the stress within the product builds until a critical point is reached and cracking of the structure occurs (Chen *et al.*, 2005; Piazza *et al.*, 2007). Accompanying this cracking is a sound. In order for acoustic emission from the product to occur, the crack must develop at a speed above a critical velocity. Luyten and van Vliet (2006) have estimated that for most dry crisp products, the critical crack speed falls within the range of 300–500 m/s. The speed is dependent on the physical properties of the product, particularly the Young modulus of the product as well as its density (van Vliet and Primo-Martin, 2011). If crack speeds are too low, sound is not generated, with the end result being that a product is not crisp (van Vliet and Primo-Martin, 2011).

From a physics perspective, sound is the result of pressure changes (compression and rarefaction of molecules) generated by a vibrating source travelling through the surrounding medium (Moore, 1982). In the case of crisp textured products, this medium could be air, as is the case for air-conducted sounds, or it could be bone, as is the case for bone-conducted sounds. The resulting pressure changes are plotted against time in order to generate an acoustic waveform. The waveforms which occur as the result of breaking crisp products are complex and exhibit many jagged, irregular peaks, as shown in Fig. 14.1. Products which are perceived by a sensory panel to be more crisp result in a waveform that has many jagged peaks, such as that shown in Fig. 14.1a, while those which are perceived to be less crisp produce a less complex signal, as shown in Fig. 14.1b.

Within the literature two general approaches exist for collecting the sounds generated from biting of crisp products: recording the sounds produced during mastication by an individual or recording the sounds generated by mechanical breakage of the product. Recording of the acoustic emission produced during mastication and relating parameters from the waveform to sensory measures of crispness is the most direct method for understanding the contribution of acoustics to texture perception. Using the hypothesis that most chewing sounds perceived by an individual are the result of bone-conducted vibrations travelling through the jaw to the ear canal, early research concluded that placement of a microphone at the opening of an individual's ear canal resulted in acoustic recordings that



(a)



(b)

Fig. 14.1 Examples of acoustic waveforms for two crisp products: (a) is more crisp and (b) is less crisp.

were most representative of what is perceived during chewing (Drake, 1963). Other approaches have also been used and include inserting a needle probe onto the bony surface of the cheek (Kapur, 1971), the application of a contact microphone onto the cheek near the maxillar angle on the eating side of an individual (Drake, 1963; Dacremont *et al.*, 1991) or the placement of an accelerometer against the mastoid bone of an individual (de Liz Pocztaruk *et al.*, 2011).

Air-conduction however is also important for perception of texture. Dacremont (1995) have stated that crisp products generate high-frequency sounds, particularly via air-conduction, while bone-conduction is necessary for differentiating between textures such as crackliness and crunchiness. When compared with crisp products, those with crunchy and crackly textures generate low-frequency sounds when bitten. The contribution of air-conducted sounds to crispness perception has also been investigated by others through recording of sounds produced while individuals bite or chew the products, with the conclusion that air-conduction is an important contributor to crispness (Edmister and Vickers, 1985; Vickers, 1987; Lee *et al.*, 1988).

While testing using humans provides a direct link with sensory perception, it has been shown that sounds produced during instrumental testing of crisp products are strongly correlated with the bone-conducted vibrations occurring when an individual bites into the product (van der Bilt *et al.*, 2010). This research provides support for previously published research presenting sounds recorded during mechanical breakage of crisp products (Chen *et al.*, 2005; Varela *et al.*, 2006; Primo-Martin *et al.*, 2009; Salvador *et al.*, 2009; Saeleaw and Schleining, 2010). When correlated with sensory measures of crispness, strong relations have been shown to exist between mechanical acoustic signatures and sensory perception of crispness (Mohamed *et al.*, 1982; Seymour and Hamann, 1988; Liu and Tan, 1999).

Acoustic parameters of importance for characterizing crisp textures

Regardless of the origin of the sound, the resulting acoustic waveform has been processed to obtain a number of different parameters. These parameters can be broadly classified into three categories: those which characterize the amount of the sound, those which determine the frequency of sound and those which characterize the shape of the acoustic signature. A list of commonly used parameters is included in Table 14.2.

The amount of a sound generated during crushing of the sample is indicated by the number and size of the peaks of the acoustic waveforms generated during crushing. Perceptually, the amplitude of the peaks of an acoustic waveform is related to loudness of the sound, therefore, the mean size of the peaks of the waveform provides an indication of a sound's loudness. Sound pressure level and acoustic energy calculated from the waveforms are also an indication of loudness. There is much published literature supporting the relation between these parameters and crispness (Mohamed

Table 14.2 A summary of acoustic parameters for measuring sounds produced by crisp product

Parameters measured	Authors
Amount of sound	
Mean height of peaks	Edmister and Vickers (1985); Vickers (1987)
Duration of sound	Edmister and Vickers (1985); Vickers (1987); Castro-Prada <i>et al.</i> (2007)
Number of peaks	Edmister and Vickers (1985); Vickers (1987); Piazza (2007); Arimi <i>et al.</i> (2010); Saeleaw and Schleining (2010)
Sound pressure level	Mohamed <i>et al.</i> (1982); Seymour and Hamann (1988); Chen <i>et al.</i> (2005); Castro-Prada <i>et al.</i> (2007); Piazza <i>et al.</i> (2007); Primo-Martin <i>et al.</i> (2009); Arimi <i>et al.</i> (2010); Saeleaw and Schleining (2010)
Frequency of sound	
Fast Fourier transformation	Vickers (1987); Lee <i>et al.</i> (1988); Seymour and Hamann (1988); Dacremont (1995); Chaunier <i>et al.</i> (2005)
Shape of acoustic signature	
Fractal analysis	Barrett <i>et al.</i> (1992); Tesch <i>et al.</i> (1996); Duizer <i>et al.</i> (1998)

et al., 1982; Seymour and Hamann, 1988; Chen *et al.*, 2005; Castro-Prada *et al.*, 2007; Varela *et al.*, 2006; Primo-Martin *et al.*, 2009; Arimi *et al.*, 2010; Saeleaw and Schleining, 2010). The best predictor of perceived crispness has been shown to be the logged product of the number of peaks and the mean height of the peaks (Edmister and Vickers, 1985; Vickers, 1987). Duration of sound has also been shown to be related to oral crispness perception with a correlation coefficient of 0.87 (Vickers, 1987). These results indicate that a crisp textured product can be characterized by a sound that is loud and of long duration.

Sound frequencies are also important for characterizing crisp sounds and often fast Fourier transformation (FFT) of the acoustic waveform is conducted. During Fourier analysis, data from the acoustic waveform are transformed into underlying frequencies and these frequencies are plotted versus their amplitude. Frequencies of importance for characterizing the sound show a higher amplitude on the graph. Using FFT, it has been found that crisp products exhibit high-frequency sounds when broken or bitten into with predominant frequencies typically above 3 kHz (Lee *et al.*, 1988; Dacremont, 1995).

The final means of characterizing sound waves is through fractal analysis. Fractal analysis is a technique for assessing the overall ruggedness or jaggedness of irregular objects or lines through the use of a mathematical

algorithm (Barrett *et al.*, 1992). The outcome of the algorithm is known as the fractal dimension of the object.

While many different algorithms exist for determining the fractal dimension of an object, the algorithm that is used most often for characterizing acoustic signatures is the Kolmogorov algorithm. This algorithm is best described as a box counting technique whereby the object or line to be quantified is divided into a grid and the number of occupied squares is counted. The size of the boxes within the grid is halved and, again, the number of squares within the grid that contain a portion of the object or line is counted. This process continues through numerous iterations with the only condition being that the size of the boxes in the grid cannot be smaller than the resolution of the object (Russ, 1994). The slope of the line generated from a log–log plot of the number of filled boxes versus the size of the box indicates the value of the fractal dimension.

For acoustic signatures, the fractal dimension is not true and has been labelled as ‘apparent’ (Barrett *et al.*, 1992; Tesch *et al.*, 1996). This is due to the fact that a truly fractal object is self-similar which means that no one part of the object is distinguishable from the whole object or any other part of the object. In other words, it has the same scaling factor in all directions.

Fractal analysis has proven useful for measuring the jaggedness of acoustic signatures recorded during mechanical compression of products that make noise when fractured (Tesch *et al.*, 1996; de Belie *et al.*, 2002) as well as during biting of crisp products (Duizer *et al.*, 1998). Products which are crisp exhibit a higher apparent fractal dimension than those which are less crisp, as evaluated using a sensory panel (Duizer *et al.*, 1998).

14.3.2 Measures of force

While sounds contribute greatly to the perception of crispness, forces applied to break the product also play a significant role. Using a materials science approach, Vincent (1998) has pointed to a drop in applied force during deformation as the reason for crispness occurring in a product. At a critical point in product deformation, a fracture occurs. This results in a rapid drop in the applied force. Further deformation of the product leads to more fracture events, each with their own drop in applied force. These rapid drops in fracture force are detected by the muscles of the jaw and, combined with the loss of pressure on the teeth, result in a texture which is related to crispness (Vincent, 1998). The length of the fracture path through the product and the velocity with which the crack spreads throughout the product have also been shown to be important contributors to crispness (Vincent, 2004).

Once the initial fracture has occurred, sufficient energy is required for future crack propagation. When a food is being chewed by an individual, it is speculated that, physiologically, this energy is stored in the jaw muscles

(Vincent, 1998). Few researchers, however, have examined intra-oral bite forces, particularly as they relate to crisp products. One reason for this may be the difficulty in recording and collecting such data intra-orally. For this, pressure on the teeth must be measured reliably. In published literature, two groups of researchers have attempted this. Tornberg *et al.* (1985) implanted strain gauges in the upper dentures of one individual to measure bite forces while chewing meat samples. Results were related to a number of sensory properties with the conclusion that maximum load on the implanted strain gauge did not have a significant relationship with the perceived textural properties of the meat samples. Mioche and Peyron (1995) placed three miniature load cells in the mouth so that the maxillary incisors were in contact with the flat side of the load cell. A sample was placed on the surface of the load cell and in contact with the mandibular incisors. During biting, the subjects were instructed to place their incisors edge to edge so that the forces developed were perpendicular to the flat surface of the sample. Occlusal forces generated during biting of elastic, plastic and brittle non-food products (silicone elastomers, waxes and pharmaceutical pills) were found to be significantly correlated with hardness as well as to mechanical compression results.

While there have been few studies investigating bite forces generated while chewing, more published research exists which examines mechanical fracture forces generated during crushing of crisp products (Vincent 1998; Chen *et al.*, 2005; Primo-Martin *et al.*, 2008; Varela *et al.*, 2006; Salvador *et al.*, 2009; Saeleaw and Schleining, 2010). These tests mimic the human bite and involve the movement of a probe at a constant speed into a food product. Probes have also been designed to move into the product in a number of different ways which typically include either the action of shearing, penetrating or crushing of food samples. The data collected via mechanical means results in a force–deformation curve. An example of such a curve for crisp extruded products is shown in Fig. 14.2. From this curve, it is possible to visualize the multiple drops in fracture that Vincent (1998) has identified as being necessary for crispness to be perceived.

Mechanical parameters of importance for characterizing crisp textures

A number of parameters have been removed from the force–deformation curves in the study of crispness (Table 14.3). Based on the theory that crispness is the manifestation of a number of small drops in fracture force, many researchers have counted the number of force peaks present on the force–deformation curve (Chen *et al.*, 2005; Varela *et al.*, 2006; Primo-Martin *et al.*, 2008; Salvador *et al.*, 2009; Saeleaw and Schleining, 2010). Varela *et al.* (2006) found that the number of force peaks was positively correlated with the perceived crispness of almonds. Maximum force values from the force–deformation curve have also been correlated with sensory crispness using multivariate data analysis. Crisp corn flakes were found to have lower maximum force values than those which were evaluated to be less crisp

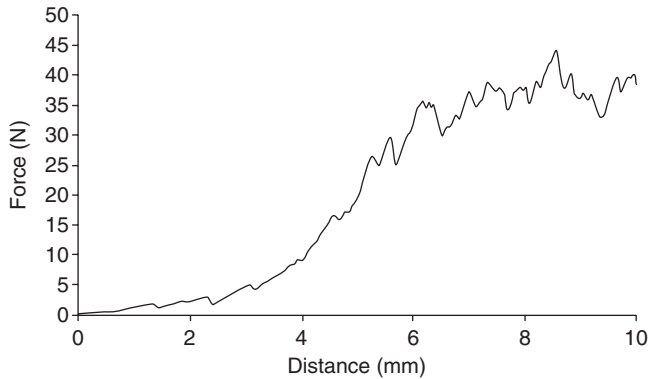


Fig. 14.2 Sample force deformation curve for crushing of crisp extruded snack (note: individual peaks indicate cracking and fracture of cell walls of the product).

Table 14.3 Instrumental parameters commonly used for characterizing crisp products

Parameter extracted from force-deformation curve	Authors
Maximum force/force at failure	Chaunier <i>et al.</i> (2005); Varela <i>et al.</i> (2006); Primo-Martin <i>et al.</i> (2008); Saeleaw and Schleining (2010)
Number of force peaks	Varela <i>et al.</i> (2006); Primo-Martin <i>et al.</i> (2008); Salvador <i>et al.</i> (2009); Sealeaw and Schleining (2010)
Area under the curve (total work)	Chaunier <i>et al.</i> (2005); Varela <i>et al.</i> (2006); Salvador <i>et al.</i> (2009)
Slope of line to first deformation	Chaunier <i>et al.</i> (2005); Varela <i>et al.</i> (2006); Salvador <i>et al.</i> (2009)

(Chaunier *et al.*, 2005). This agrees with previous results from Vickers (1987) who showed that hardness was a negative contributor to crispness perception of dry crisp products.

14.3.3 Combination measures of sounds and forces

While much of the research into texture of crisp products has focused on sounds and forces individually, there is a growing body of research which combines mechanical and acoustic measures (Chen *et al.*, 2005; Varela *et al.*, 2006; Primo-Martin *et al.*, 2009, 2010; Salvador *et al.*, 2009; Saeleaw and Schleining, 2010). The benefit of this approach is the ability to obtain a complete understanding of the interaction between the two parameters associated with crispness. During simultaneous testing, the microphone

used for recording sounds is attached to the instrument in such a way that the sounds produced during breaking or shearing of the samples can be recorded. Some researchers have suggested the use of an anechoic chamber to prevent extraneous noise produced by the machine being recorded by the microphone (Castro-Prada *et al.*, 2007; Primo-Martin *et al.*, 2009, 2010).

While some researchers have designed their own equipment for simultaneous recording of sounds and forces (Chaunier *et al.*, 2005; Taniwaki *et al.*, 2010), one piece of equipment sold commercially that is gaining in popularity for use in this area is the acoustic envelope detector (AED), which is sold as an attachment for the Texture Analyzer (Stable Microsystems, Surrey, UK). A number of researchers have used the AED and Texture Analyzer for collection of data related to crisp products (Chen *et al.*, 2005; Varela *et al.*, 2006; Primo-Martin *et al.*, 2009; Salvador *et al.*, 2009; Saeleaw and Schleining, 2010; de Liz Pocztaruk *et al.*, 2011).

The AED consists of a preamplifier and a Brüel and Kjær free-field microphone which are both attached to the base of the Texture Analyzer for data collection. Sounds are recorded without the need of an anechoic chamber and a filter function within the software allows for the filtering of extraneous background noise during testing. To ensure the smallest variation in the recorded acoustic signature, placement of the microphone at a 1 cm distance from the break point of the sample and at a 45° angle to the sample is required (Chen *et al.*, 2005). The speed of the probe moving into the sample can be controlled and Vincent *et al.* (1991) have suggested that a cross-head speed of 40 mm/s is adequate to mimic the human bite with the front teeth. The collected acoustic-force data is presented in the same graph so that sound production occurring as the product is being deformed can be observed. Acoustic events which occur during structural failure are documented and the data can be analysed to characterize the sound events (number of sound events, loudness of the sound) which occur because of fracture. Such an approach has been effectively used to study forces and sounds for biscuits (Chen *et al.*, 2005), potato chips (Salvador *et al.*, 2009), toasted sliced bread (Piazza *et al.*, 2007) and almonds (Varela *et al.*, 2006).

14.4 Future trends

Given the large body of knowledge which exists for crisp products, the future of research in the area of crisp textured products should involve an integration of research by material scientists, sensory scientists and oral physiologists. Studying the interactions occurring between the areas of product morphology, fracture behaviour, oral physiology and texture perception has recently been identified as a priority area (van Vliet and Primo-Martin, 2011). Such an interaction would ensure that crisp products which are of optimum quality are developed. The advent of simultaneous data collection using the Texture Analyzer and the AED will assist with this. The

ability to collect sound and force data generated during crushing of a crisp textured product will allow researchers to understand the effects of modifying production parameters (ingredient substitutions, process modifications) on crispness perception. However, care must be taken to ensure that the data collected via mechanical means is representative of what is perceived by an individual. For instance, a comparison of the acoustic signal generated during mechanical crushing with data from sensory panellists showed that the sounds produced by individuals biting the samples were not as loud as from the instrumental test, possibly due to damping by oral tissues (Chen *et al.*, 2005). Additionally, Mioche and Peyron (1995) suggested that instrumental measures of force are an overestimation of intra-oral bite force. In order to be able to validly predict crispness based on mechanical measures, it is important that sensory testing of the products of interest also be studied and correlated with mechanical data.

As there is a large amount of data generated during simultaneous acoustic-mechanical testing, attention must be paid to data analysis strategies for handling the datasets. Research investigating the use of multivariate data analysis techniques such as, principal components analysis and discriminant analysis has been undertaken (Varela *et al.*, 2006; Piazza *et al.*, 2007; Salvador *et al.*, 2009). These techniques have proven effective at understanding the parameters of importance from an acoustic and fracture perspective for characterizing crispness of a number of products.

More information about the AED designed by Stable Microsystems can be found at: <http://www.stablemicrosystems.com/frameset.htm?http://www.stablemicrosystems.com/acousticenvelopedetector.htm>.

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Instrumental assessment of the sensory quality of dairy products

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Abstract: Instrumental assessment of the flavour of dairy foods includes analyses of volatile compounds responsible for aroma, non-volatile compounds responsible for taste, and components perceived as mouthfeel and texture. The first part of this chapter is devoted to instrumental methods used to identify key aroma compounds that contribute significantly to the flavour of dairy products, i.e. mainly techniques that combine gas chromatography to olfactometry (GC-O). Dynamic *in vivo* methods used to obtain flavour release data that should reflect flavour perception are then described. Advantages and limits of the global approaches using the so-called ‘electronic noses’ for dairy products are then presented, and the description of some advances made to identify taste-active compounds, particularly in cheeses, is given. Some advances obtained in instrumental techniques used to measure rheological properties and developed to approach the texture of dairy products are also presented. Finally some concluding remarks emphasize the multidisciplinary approach that is necessary to develop in order to better understand the sensory perception experienced during food consumption.

Key words: dairy food, gas chromatography-olfactometry (GC-O), nose-space, electronic noses, cheese taste compounds, rheological methods.

15.1 Introduction

Flavour plays a prominent role in the sensory quality of food. Flavour may be defined as the combination of taste and aroma, sensations of pain, heat and cold, and tactile sensation, including possible cognitive interactions. Therefore, instrumental assessment of the flavour of foods when eaten should include analyses of volatile compounds sensed in the nose at the olfactory epithelium, non-volatile compounds sensed on the tongue, and compounds perceived as mouthfeel and texture.

The first part of this chapter will be devoted to a group of instrumental methods used to identify volatile key flavour compounds that contribute

significantly to the flavour of dairy products, i.e. techniques that combine gas chromatography with olfactometry (GC-O). Completed with gas chromatography–mass spectrometry (GC-MS), GC-O becomes a means of both identification and quantification, and can play a prominent role in determining individual key volatiles that are supposed to contribute significantly to the flavour of the food and numerous examples apply to dairy products (D'acampora Zellner *et al.*, 2008). However, it is important to remember that all GC-O methods are based on sensory evaluation of individual compounds separated in a GC experiment and sniffed sequentially at a GC sniffing port. This is not equivalent to sensory evaluation of foodstuffs themselves, where flavour is evaluated in a complex mixture context, where interaction with the food matrix occurs, and where masking or enhancing effects may influence the overall sensory perception. Therefore, a validation step where the key odorants determined via GC-O are incorporated in a suitable matrix in amounts equivalent to their measured quantities in the food and evaluated together in mixture is highly recommended (Grosch, 2001). Some applications to dairy products will be presented.

Poor correlations are often found between all the flavour compounds identified in a food and the sensory perception experienced by a consumer eating this food, as it is still not completely known how the various components combine to produce a sensory impression. Perception of flavour is a dynamic process, concentration of aroma compounds at the olfactory epithelium varying with time as they are progressively released from the food during consumption. The second part of this chapter will deal with techniques that measure flavour compounds directly in the nose or nose-space (Taylor and Linfoth, 1996) aimed at obtaining data that are supposed to reflect the pattern of flavour release in real time, supposed to be representative of perception. Examples using MS operated at atmospheric pressure (atmospheric pressure chemical ionisation, APCI, or proton transfer reaction, PTR) will be given for dairy products. *In vitro* measurements using model mouth systems will also be discussed.

Global approaches developed to evaluate the complete aroma emitted from food will be presented in a third part. The methods currently used to evaluate and control the quality of dairy products are still essentially based on sensory evaluation by panels of experts. These trained panels are able to handle quality monitoring through descriptive analysis, off-flavour detection and comparison of samples for classification purposes. It has been recognized that it would be interesting to substitute instruments for humans, which could give quicker answers at reduced costs. These include the so-called 'electronic noses' and the systems based on MS (Le Quére, 2004). Advantages and limits of these methods for dairy products will be discussed. Nevertheless, for these rapid instrumental methods, patterns or fingerprints are obtained for each sample and extensive data treatments, either by conventional multivariate statistics or artificial neural networks, are necessary. As some significant efforts have been made recently to

develop instrumental procedures to characterize non-volatile components responsible for cheese taste (Le Quéré, 2004), part of this chapter will focus on advances made in the *in vitro* and *in vivo* studies of taste-active compounds.

A variety of different instrumental techniques can be used to measure rheological properties of dairy products. This section will emphasize the rheological methods developed to approach the texture of the products. Finally concluding remarks will discuss the importance of texture in the relationships existing between flavour release and perception in dairy products. Notwithstanding huge inter-individual subject variability, physico-chemical mechanisms versus a cognitive mechanism based on aroma–taste–texture interactions are important determinants of such relations (Gierczynski *et al.*, 2011).

15.2 Identifying key aroma compounds

Volatile active flavour compounds that contribute to aroma can be localized in the gas chromatogram of flavour extracts of food products and determined on the basis of their odour activities by GC-O. The method aims at identifying key aroma compounds by simultaneously ‘sniffing’ the effluent from the GC column and identifying the eluted compounds by the nature of their odour in complementary GC-MS analyses. The method has received considerable attention and has been the subject of specialized treatises (see D’acampora Zellner *et al.*, 2008, Delahunty *et al.*, 2006, and Leland *et al.*, 2001, for recent ones).

There is no universal aroma isolation method available that would give a true picture of the food flavour; however, it has been claimed that the most reliable results would be obtained if the odour of the extract resembles closely that of the food itself (Etiévant *et al.*, 1994). To check the sensory representativeness of the food extract it is necessary to perform sensory tests with a trained sensory panel using a suitable sensory method (Etiévant *et al.*, 1994). This approach was applied for various foodstuffs (detailed in Mehinagic and Le Quéré, 2010), including butter (Guyot *et al.*, 1998), and specific applications for various types of cheese are detailed in Le Quéré (2004). A key point in these evaluations of representativeness is the choice of a suitable test matrix in which aroma extracts are incorporated. For fat-containing food, like cheese or butter, the best results have been obtained using an emulsion as test medium, that is, a matrix similar to food in terms of fat content (Etiévant *et al.*, 1994; Guyot *et al.*, 1998).

The first aim of GC-O is to discriminate the odorous compounds from the many background volatile components that may have little, if any, odour of significance in a food. The so-called ‘aromagram’, simply qualitatively constructed from smelling the GC effluent, results in odour descriptors that can be compared with the descriptors generated by a sensory panel. This

tool constitutes a potential interface with sensory analysis and is particularly efficient for identifying compounds responsible for off-flavours.

The second objective of the method is to select key odorants, i.e., character-impact compounds in a particular food, and quantitative approaches (the true GC-O) have been developed. GC-O methodologies based on odour detection thresholds, on odour intensities or on frequency of detection have received a significant attention (see D'acampora Zellner *et al.*, 2008, Delahunty *et al.*, 2006, and Leland *et al.*, 2001, for recent specialized reviews). The data can be generated using either dilution experiments based on detection thresholds determination such as Charm (combined hedonic aroma measurement) analysis (Acree *et al.*, 1984), aroma extract dilution analysis (AEDA) (Ullrich and Grosch, 1987) or its variant aroma extract concentration analysis (AECA) (Kerschler and Grosch, 1997; Kubickova and Grosch, 1997), or cross-modality matching methods based on perceived odour intensity such as odour-specific magnitude estimation (OSME) (McDaniel *et al.*, 1990), or detection frequency methods originally developed by Roozen and co-workers (Linssen *et al.*, 1993) and now referred to as nasal impact frequency (NIF) or surface of nasal impact frequency (SNIF) (Pollien *et al.*, 1997). Only a few studies have compared the methods for their performance and the results were found to be very similar and well correlated. Dilution techniques are time consuming, intensity methods give better results with a trained panel, and detection frequency methods are the least demanding but also the least precise. Comparative critical reviews are available (Etiévant and Chaintreau, 2001; Delahunty *et al.*, 2006).

Aroma recombination studies in model foods (Grosch, 1994) are the very last step for sensorially validating the potential impact odorants determined by GC-O and for quantification of key odorants of foods (Mistry *et al.*, 1997). Many examples of food models used in recombination studies may be found in the authoritative review of Grosch (2001). Liquid model foods appear easier to prepare than solid ones where the composition and the distribution of the non-volatile fraction of the matrix are not easy to handle (Grosch, 2001). However, for cheese models, either bland unripened cheese (Grosch, 1994; Preininger *et al.*, 1996; Kubickova and Grosch, 1998a) or especially designed odourless model cheeses (Salles *et al.*, 1995a; Smit *et al.*, 1995) have been successfully used.

The application of GC-O to dairy products, briefly reviewed by Friedrich and Acree (1998), enabled the identification of impact aroma compounds of several matrices including milk (Moio *et al.*, 1993a, 1994, 1996), butter (Schieberle *et al.*, 1993), yoghurt (Ott *et al.*, 1997), sweet cream butter (Peterson and Reineccius, 2003), milk powder (Karagul-Yuceer *et al.*, 2001, 2002, 2004), and cheeses with considerable efforts on Cheddar types (Singh *et al.*, 2003; D'acampora Zellner *et al.*, 2008; Kim *et al.*, 2011, and references therein). However almost all types of cheeses have been the subject of such an approach, e.g. Swiss-types (Preininger *et al.*, 1996; Rychlik *et al.*, 1997;

Rychlik and Bosset, 2001), goat cheese (Le Quéré *et al.*, 1996; Salles *et al.*, 2002; Carunchia Whetstine *et al.*, 2003), Camembert (Kubickova and Grosch, 1997, 1998a,b; Grosch *et al.*, 2001), Mozzarella (Moio *et al.*, 1993b), Grana Padano (Moio and Addeo, 1998), Gorgonzola (Moio *et al.*, 2000), Parmigiano-Reggiano (Qian and Reineccius, 2002a), Blue (Le Quéré *et al.*, 2002; Qian *et al.*, 2002), or more recently Circassian, an acid-coagulated cheese (Guneser and Yuceer, 2011).

A comprehensive list of cheese key odorants determined by different extraction procedures and GC-O methods has been published (Curioni and Bosset, 2002), a comprehensive list of Cheddar volatiles exists (Singh *et al.*, 2003) and a list of odour-active compounds most commonly detected by means of GC-O in dairy products may be found (D'acampora Zellner *et al.*, 2008). However, as outlined for Cheddar (D'acampora Zellner *et al.*, 2008), single volatiles eliciting typical cheese notes have not been identified, confirming that for each cheese type the global aroma results from a balance between the odorants present in different concentrations in the matrix. The same applies obviously for other dairy products and a standard list of a particular dairy product's odour-active molecules responsible for its overall aroma does not exist (D'acampora Zellner *et al.*, 2008).

The GC-O methods that have been developed during the past decades, combined with all types of aroma extraction procedures including solid phase micro-extraction (SPME) (Dufour *et al.*, 2001), have allowed the identification of potent odorants in various dairy products (D'acampora Zellner *et al.*, 2008). The most recent developments combine multidimensional GC (Delahunty *et al.*, 2006) to olfactometry (GC-GC-O) with the benefit of numerous coelution-resolving capabilities (Chaintreau *et al.*, 2006) and a multi-sniffing ports GC-O device that has found applications for cheese aroma analysis (Berdague *et al.*, 2007; Cornu *et al.*, 2009).

15.3 Other methods to characterize the aroma of dairy products

15.3.1 Dynamic methods for aroma release characterization

If GC-O constitutes a major breakthrough in the identification of odour-active compounds in food, as already outlined, relating aroma compounds composition to aroma perception by humans is not straightforward in many occurrences. Perception of flavour is a dynamic process (Piggott, 2000) during which the concentration of aroma molecules at the olfactory epithelium varies with time as they are released progressively from the food in the mouth. The release kinetics depends on the food matrix itself (see Guichard, 2002, for general aspects, and Chung, 2007, for specific aspects on frozen desserts like ice cream), but also on in-mouth mechanisms such as mastication pattern and bolus formation with saliva (Salles *et al.*, 2011), for which individual variations are markedly important (Tarrega

et al., 2008, 2011). Sensory methods, such as time-intensity (Piggott, 2000) or more recent temporal dominance of sensations (Pineau *et al.*, 2009), have been used to account for the dynamic- and time-related aspects of flavour perception.

Techniques for measuring volatiles release under conditions that are found when humans consume foods have been developed over the last two decades. Since the significant robust results obtained for sampling aroma from the nose (nose-space) using a collection of expired air samples on adsorbents (Linforth and Taylor, 1993; Taylor and Linforth, 1994), including important applications on Cheddar cheese (reviewed by Le Quéré, 2004), real-time *in vivo* flavour release analysis has been obtained using atmospheric pressure ionization MS (reviewed by Roberts and Taylor, 2000). The two main techniques – atmospheric pressure chemical ionization, APCI (Linforth *et al.*, 1996; Taylor and Linforth, 1996) and proton transfer reaction, PTR (Lindinger *et al.*, 1993) – use H_3O^+ as reagent gas. Volatile compounds that have in most cases higher proton affinities than water ionize by proton transfer from H_3O^+ and are accelerated into a mass spectrometer, generally a quadrupole instrument. APCI sources may also be connected to a tandem mass spectrometer (MS/MS) such as an ion-trap, providing selectivity and structural capability benefits of MS/MS (Sémon *et al.*, 2003) and a PTR source has been recently connected to a time-of-flight (TOF) instrument with the benefit of its high resolution power (Jordan *et al.*, 2009). The specificity of PTR-MS compared to APCI approaches is that the generation of the reactant ion and the PTR are spatially and temporally separated. A control of the ionization process is therefore possible and individual optimization and quantification are made easier (Yeretzian *et al.*, 2000).

With these techniques, air from the nose (nose-space) is sampled directly into the mass spectrometer through a heated interface, making real-time breath-by-breath analysis routinely possible (see Kühn *et al.*, 2009, for a recent PTR-MS study on flavour release from thickened milk protein gels). Combining time-resolved sensory studies with nose-space analysis, it is therefore possible to relate temporal parameters of aroma release to perception. Thus, it has been demonstrated for some French soft cheeses (pasteurized Brie, pasteurized and raw milk Camembert) that the perception scored by 15 assessors is consistent with their release of aroma compounds while eating the cheeses (Salles *et al.*, 2003), despite huge inter-individual differences in terms of some physiological factors (chewing force and rate, saliva flow). For instance, a perfect correspondence between the sulphury note determined by time-intensity scoring and the APCI-MS release curves of the main sulphur key compounds, previously identified in a GC-O experiment (*S*-methylthioacetate, dimethyldisulphide and dimethylsulphide), could be found (Le Quéré, 2004, Salles *et al.*, 2003). Despite significant inter-individual differences again largely due to differences in oral parameters (respiratory and masticatory values), the mouldy attribute described by a panel of assessors was clearly related to the in-mouth release of

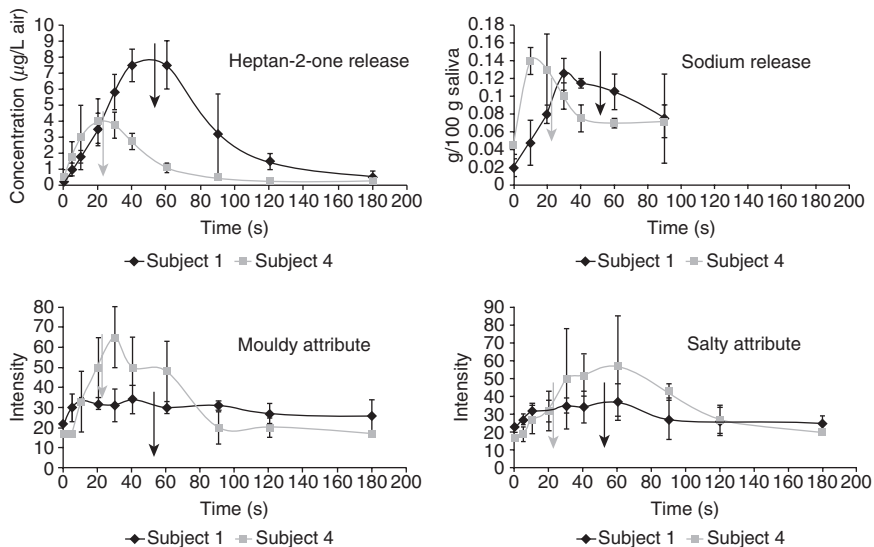


Fig. 15.1 *In vivo* flavour release patterns observed for heptan-2-one (APCI-MS) and sodium (off-line ionic chromatography) and corresponding mouldy and salty perceptions (time-intensity) for two subjects eating a model cheese (Pionnier *et al.*, 2004a,b,c, 2005). Vertical bars on mean data points are standard deviations (three replicates) and vertical arrows represent swallowing events for each subject.

2-heptanone determined with APCI-MS (see Fig. 15.1) in a study on model cheese aiming at establishing relationships between aroma release, oral parameters and flavour perception (Pionnier *et al.*, 2004a,c, 2005). The same study revealed a clear correspondence between the salty (see Fig. 15.1) and sour attributes and in-mouth release of sodium (see Fig. 15.1) and citric acid, respectively, evidencing again inter-individual differences in respiratory, masticatory and saliva parameters (Pionnier *et al.*, 2004b,c, 2005).

Studying whey protein gels with different hardness and water holding capacities, it was demonstrated that significant changes in flavour intensity between the gels were perceived, despite the fact that the APCI nose-space aroma concentration was found to be independent of the rheological properties of the gels (Weel *et al.*, 2002). It was concluded that the gel texture determines perception of aroma intensity, evidencing a texture–flavour interaction. A perceptive sweetness–aroma interaction, this time independent of the texture of the products, was also clearly demonstrated in APCI nose-space for sweetened flavoured dairy desserts varying in texture (Lethuaut *et al.*, 2004), confirming previous observations on chewing-gums (Davidson *et al.*, 1999). The influence of texture on aroma release and on perceived aroma intensity has been recently investigated on three types of dairy product varying in viscosity or firmness, i.e. yoghurts, custard desserts

and model cheeses (Saint-Eve *et al.*, 2006a,b; Gierczynski *et al.*, 2007, 2008; Guichard *et al.*, 2010). For yoghurts and custards (liquid to semi-liquid foods), *in vivo* aroma release, decreasing when viscosity increased, could explain differing perceived aroma intensity (Saint-Eve *et al.*, 2006a,b; Guichard *et al.*, 2010). For firmer dairy gels (model cheeses), *in vivo* aroma release increased with cheese hardness, but large inter-individual perception differences linked to chewing behaviour have been observed, and perceptual interactions between texture and aroma have been once more evidenced (Gierczynski *et al.*, 2007, 2008; Guichard *et al.*, 2010). The technique using PTR-MS is also the basis for studying aroma compounds transfer properties in cheeses (Doyennette *et al.*, 2011); moreover a protocol useful to study flavour release and perception in cheese bases has been recently proposed (Overington *et al.*, 2011).

To deal with the observed large inter-individual variations, a number of mechanical devices aimed at mimicking in more or less details eating events have been proposed (Le Quéré, 2004; Mehinagic and Le Quéré, 2010; Salles *et al.*, 2011, and references therein). These ‘model mouths’ are often variants of dynamic headspace analysis, even though adaptable shear conditions in a dimensioned closed vessel may mimic *in vivo* conditions for dairy gels and yoghurts (Decourcelle *et al.*, 2004). Though important flavour release parameters can be studied with these apparatus such as temperature, airflow, shearing, food destructure, effect of saliva and influence of fat, they are far from reproducing the complexity of the overall phenomena occurring during human food consumption, with particularly clear limits for the temporal dimension of the flavour release (Deibler *et al.*, 2001; Geary *et al.*, 2004; Gierczynski *et al.*, 2007). Swallowing events are not taken into account, even though a device developed to simulate swallowing and *in vivo* aroma release in the throat has been published (Weel *et al.*, 2004), with, however, restricted use for liquid and semi-liquid food systems.

15.3.2 Global assessment of aroma profiles

Sensory evaluations by panels of experts are still currently and widely used to evaluate and control the quality of food flavour. These trained panels are able to handle such difficult tasks as descriptive analyses, quality monitoring, off-flavour detection and comparison of samples for classification purposes. Sensory evaluation remains a prominent descriptive tool widely used in dairy science and industry. For a long time, it has been acknowledged that quicker responses and reduced costs should be obtained using instruments instead of humans. Electronic noses and systems based on mass spectrometry are those instruments developed to evaluate the global aroma release from food.

Evaluation of the complete headspace emitted from food using gas sensors, the so-called ‘electronic noses’ or e-noses, was demonstrated more than 15 years ago (Mielle, 1996; Hodgins, 1997; Schaller *et al.*, 1998).

Electronic noses are generally composed of arrays of non-specific gas sensors which are based on different physical principles, the most commonly used being semiconducting metal oxides and conducting organic polymers, sometimes associated in single instruments. All the arrays of sensors give rise to nonspecific responses with typical patterns. Therefore, data treatment for final presentation of the results requires pattern recognition software packages that use either conventional multivariate statistics or artificial neural network technology. Electronic noses appeared particularly attractive for quality control applications where conformity/nonconformity responses were expected. Applied to evaluation and categorization of cheeses varieties of Swiss types, the electronic noses presented some repeatability and reproducibility problems (Le Quéré, 2004, and references therein), due to instability, deterioration over time and/or poisoning. In a study conducted on the ripening of Danish Blue cheese, an electronic nose which contained 14 conducting polymer sensors gave results highly correlated with sensory analysis and GC-MS analysis of volatile compounds during the whole ripening period (Trihaas *et al.*, 2003). A close control of the experimental sampling conditions (quality of dry air and equilibration time at controlled temperature) might explain this success, as already suggested (Schaller *et al.*, 1998). Nevertheless, despite some success in some classification and authentication tasks (see Ampuero and Bosset, 2003, and references therein, for dairy applications), electronic noses can hardly be considered as instruments fulfilling the requirements of the food industry in terms of precision, reproducibility, sensitivity and stability.

To conduct classification tasks, analytical methods based on mass spectrometry seem more powerful and reliable than electronic noses, and they have been used quite regularly for dairy foods. The first consists of a global analysis of a headspace sample by a mass spectrometer operated in electron ionization mode, without any GC separation. The feasibility of the method was originally demonstrated for classification of four rather different French cheeses (Vernat and Berdagué, 1995), and dynamic headspace sampling can be replaced by SPME, as exemplified for off-flavours in milks (Marsili, 1999). This method is often referred to as a 'MS-based electronic nose' (Dittmann *et al.*, 1998; Schaller *et al.*, 2000). The mass patterns obtained, considered as fingerprints of the food products analysed, need also extensive data treatment. A review on the subject appeared in 2003 (Péres *et al.*, 2003), and examples obtained on various cheese samples may also be found in Le Quéré (2004). In a similar approach, headspace samples may be directly connected to a PTR-MS and the technique applied to Mozzarella cheese was found to deliver comparable discrimination power to sensory descriptive analysis (Gasperi *et al.*, 2001). Applied to process monitoring, i.e. lactic acid fermentation of milk (Soukoulis *et al.*, 2010), influence of milk storage conditions on the volatiles profiles of Trentingrana cheese (Fabris *et al.*, 2010), or to geographical origin of protected designation of origin (PDO) cheeses (Galle *et al.*, 2011) or European butters (Macatelli *et al.*,

2009), the PTR-MS technique is gaining in terms of potential applications and affordability (Biasioli *et al.*, 2011).

Another MS-based method developed for food applications, which delivers fingerprints as mass patterns, uses Curie point pyrolysis of tiny food samples as direct coupling method (Aries and Gutteridge, 1987). The volatile fraction resulting of pyrolysed samples at up to 530°C is analysed by mass spectrometry with low energy ionization and is characteristic of the flavour, but also of the food matrix breakdown. Powerful classification/authentication tasks are possible, generally after several data pre-processing steps in order to select a reduced number of mass fragments from the rather complex mass patterns obtained. Applied to cheeses, the main advantage of the method is that it provides not only flavour profiles but also specific fingerprints of the cheese matrices which could be potentially related to textural parameters (Pérès *et al.*, 2002). Authentication of dairy products, for instance varieties with PDOs for which the sensory properties are discriminative criteria, remains a challenge. The tools presented here, which combine analytical instrumentation for global assessment of flavour with multivariate data analyses, have demonstrated their usefulness for classification purposes. Systems based on MS seem more reliable.

15.4 Cheese taste and *in vivo* determination of non-volatiles

The taste of cheese has been considered for a long time as being mainly due to non-volatile components that may concentrate in the water-soluble extract of the product (McSweeney, 1997), which contains also some water-soluble volatiles responsible for aroma. It has also been recognized that bitterness, which can limit cheese acceptability if too intense, is due to excessive amounts of low molecular weight and mainly hydrophobic peptides, which accumulate during ripening as a result of proteolysis (McSweeney, 1997). Bitterness in Cheddar cheese, considered as an off-flavour, has been comprehensively reviewed (Singh *et al.*, 2003). No other clear sensory properties have been attributed to any other nitrogen-containing compounds (small peptides and amino acids), although they are probably contributing to the background flavour of cheese (McSweeney, 1997). However, in Comté cheese, the umami taste was clearly associated with a substantial amount of monosodium glutamate, found at a concentration ten times above its sensory threshold value, while other amino acids were confirmed to be present well below their sensory thresholds (Salles *et al.*, 1995b). The salty taste is essentially due to the sodium ion Na^+ present in the mineral salt NaCl , the higher molecular weight mineral salts (KCl , CaCl_2 , MgCl_2) imparting essentially bitterness rather than saltiness (McSweeney, 1997). Acid taste is due to H_3O^+ , and the principal organic acid in cheese is lactic acid. However, total lactate concentration does not reflect evaluated cheese acidity. Cheese pH increases during ripening caused by the production of

ammonia (McSweeney, 1997), and perceptive interactions were evidenced with NaCl and nitrogen-containing components (Le Quéré, 2004, and references therein). It has also been hypothesized that short- and medium-chain fatty acids might contribute to the acid taste of cheese (McSweeney, 1997). Although this hypothesis seems reasonable for the abundant short chain acids (e.g. formic, acetic or propionic), the principal contribution of fatty acids to cheese flavour is their aroma in their unionized form, as exemplified for goat cheese (Le Quéré *et al.*, 1996; Salles and Le Quéré, 1998) or Parmesan (Qian and Reineccius, 2002b).

The study of taste-impact compounds in cheese involves the study of water-soluble low molecular weight material (small peptides, amino acids, organic acids, minerals, etc.) dispersed in a very complex matrix. After a suitable extraction step (rationalized in Le Quéré, 2004) the contribution of individual components may be estimated on the basis of taste activity values (TAVs), a concept analogous to the odour activity values (OAVs) used for aroma compounds characterized in GC-O experiments, and defined as the ratio of actual concentration to taste threshold. As for aroma compounds, the final step consists of recombination experiments preferably conducted in a model matrix analogous to cheese. Thus, a study conducted on a model based on unripened Mozzarella-type cheese confirmed the importance of acetic, propionic, lactic, succinic and glutamic acids, and sodium, potassium, calcium, magnesium, ammonium, phosphate and chloride ions for the taste of Emmental cheese (Preininger *et al.*, 1996). The same approach applied to Camembert cheese identified acetic, butyric, 3-methylbutyric, caprylic and succinic acids, monosodium glutamate, ammonia and NaCl as major taste contributors (Kubickova and Grosch, 1998a). The biogenic amine, cadaverine, and the rare amino acids, ornithine and citrulline, when present, were suspected to contribute to the bitter taste of Camembert (Kubickova and Grosch, 1998a). Many experiments conducted in this context confirmed that small peptides do not seem to be key flavour compounds of cheese, apart from important contribution to bitterness for some of them, as was previously hypothesized (Le Quéré, 2004, and references therein). Thus it was recently concluded that hydrophilic glutamyl di- and tripeptides are not a precondition for savoury flavour in mature Cheddar cheese (Andersen *et al.*, 2008). The search for the sources of umami taste in Cheddar and Swiss cheeses confirmed the major role of glutamate, while succinic and propionic acids contributed significantly to umami in Swiss cheeses (Drake *et al.*, 2007).

Complementary to recombination experiments, omission tests (see Engel *et al.*, 2002a,b, and references therein, for a comprehensive review) allow precise sensory evaluation of single compounds in a mixture and investigation of additive or synergistic effects. Using omission tests in a model mixture that was sensorially validated, relative impact of non-volatile components on goat cheese taste was determined: saltiness was explained by additive effects of K^+ , Ca^{2+} and Mg^{2+} on Na^+ , sourness was due to synergism

between NaCl, phosphates and lactic acid, bitterness resulted entirely from CaCl_2 and MgCl_2 , and amino acids, lactose and peptides had no significant contribution to goats' milk cheese, all these observations confirming previous results cited above (Engel *et al.*, 2000). The same procedure was applied to a Camembert cheese specially selected for its bitterness. Sourness of this cheese was explained by an enhancing effect of NaCl on the acid taste due to H_3O^+ , saltiness was explained by NaCl, and bitterness was mainly due to small ($\text{MW} < 1000\text{Da}$) bitter peptides produced in the intense proteolytic activity of the *Penicillium camemberti* strain used in this case (Engel *et al.*, 2001a,b,c). A similar approach conducted more recently on 49 putative taste-active metabolites and minerals of Gouda cheese largely confirmed these observations: CaCl_2 and MgCl_2 induced bitterness, modulated quantitatively by various bitter-tasting free amino acids and qualitatively by some bitter peptides; sourness was essentially dependent on lactic acid and hydrogen phosphate, while umami taste was due to monosodium L-glutamate and sodium lactate; saltiness, induced by the sodium ion of sodium chloride and sodium phosphate, was enhanced by L-arginine (Toelstede and Hofmann, 2008).

A limited number of techniques have been developed to study the real-time release of non-volatile compounds in the mouth (Le Quéré, 2004, and references therein). One of the most applicable one consists in saliva sampling using cotton buds coupled to off-line liquid analyses, like high-performance liquid chromatography (HPLC) or LC-MS, performed after saliva weighing and extraction in a suitable solvent. The feasibility was first demonstrated for the release of sucrose from chewing gums (Davidson *et al.*, 1999). The technique was applied to a model flavoured cheese in order to try to relate the release of taste components in the mouth during consumption to time-intensity curves of target sensory attributes and to oral parameters (Pionnier *et al.*, 2004b,c, 2005). Time-course release curves for minerals (sodium, calcium, magnesium and potassium), amino acids (leucine, phenylalanine and glutamic acid), organic acids (citric, lactic, propionic and butyric) and phosphoric acid were obtained with off-line electrospray MS and/or ion chromatography (Pionnier *et al.*, 2004b). Large inter-individual differences were again noticed in the release behaviour, due to physiological parameters mainly related to mastication and salivation, but the results allowed the time-course of salty (see Fig. 15.1) and sour attributes to be clearly linked to the release of sodium (see Fig. 15.1) and citric acid, respectively (Pionnier *et al.*, 2004c, 2005). In a recent study on model processed cheeses of different composition and texture, it has been found that, again despite the fact that high inter-individual variations were observed for temporal sodium release and saltiness perception, mainly due to chewing behaviour, sodium release during mastication is mainly influenced by the matrix structure and composition, particularly enhanced by the water content, while saltiness perception is limited by increasing amount of fat (Phan *et al.*, 2008). This behaviour, which was demonstrated to depend on NaCl mobility

in the model cheeses (Lauverjat *et al.*, 2009), suggests that for such matrices, an increase of water and a decrease of fat contents could be a tool to reduce salt concentration without saltiness perception alteration (Phan *et al.*, 2008).

15.5 Instrumental assessment of the texture of dairy products

Dairy products cover a large spectrum of texture going from fluid fermented milk to hard ripened cheese through melting ice-creams. Texture properties of dairy products are related to: (i) the functional properties of milk proteins that are gelling properties, emulsifying properties and foaming properties, (ii) the plasticizing properties of water and fat, (iii) the gelling and/or viscosity properties of polysaccharides, and (iv) finally, properties resulting from interactions between the above-cited ingredients.

Rheological methods are generally used to assess texture properties of dairy products. Due to the extensive variety of the dairy products and the existence of traditional know-hows, specific empiric rheological methods were frequently developed for quality control purpose. Such methods often mimic the way products are handled during process or during food consumption. For example, Depypere *et al.* (2009) studied the rheology of dairy desserts containing kappa-carrageenan, skimmed milk powder (SMP), adipate cross-linked acetyl-substituted waxy maize starch, sucrose and water, and prepared in ultra-high treatment (UHT) pilot plant. The rheological measurements included large deformation penetration tests. The chosen methods were empiric tests known as texture profile analysis (TPA) tests, consisting of two consecutive penetration cycles. In such tests, parameters are calculated from the force–time curve obtained and are assimilated to sensory properties. For example, the area under the force–deformation curve was defined as the gel strength, and the ratio of the positive force areas under the first and second compression is defined as cohesiveness. Güven and Karaca (2002) studied vanilla and fruit (strawberry) frozen yogurts stored at -23°C . The physical properties and sensory characteristics of the products and the effects of sugar content and fruit concentration on these properties were determined. For that purpose, the simple use of a penetrometer equipped with a conical spindle provided good discrimination of the samples.

Texture properties of cheese are evaluated using large strain methodologies (compression, torsion or tension), which may be performed empirically (TPA) or fundamentally. Different types of rheological tests may be encountered to assess texture properties of cheese. Using a compression test, a cylindrical specimen is squeezed between two plates. The force to deform the material to failure is used to compute a fracture stress, while the degree of deformation determines fracture strain. With a tension test, the sample is extended until rupture, and similar parameters are calculated. Finally,

using a torsion test, the sample is twisted until fracture. The degree of rotation and magnitude of torque required to break the sample is monitored to calculate the strain and stress at fracture (Foegeding *et al.*, 2003).

Other rheological methods, such as small amplitude dynamic methods, were developed to better control major properties linked with meso- or macro-structure such as gel setting, emulsion stability or foam stiffness. More recently, rheological measurements were very often completed by other physical measurements such as particle size determination. Such studies may concern the sensory properties of final products. For example, creaminess, one important property of texture for semi-solid dairy desserts, was studied and was reported to be increased by (i) increasing the bulk-viscosity of the food, (ii) minimizing, but not eliminating, loss of bulk viscosity during oral processing by using starches that show limited mechanical and enzymatic break-down during oral processing, (iii) using small, stable fat droplets, and finally (iv) adding flavours associated with creaminess (De Wijk *et al.*, 2006). Others (Cayot *et al.*, 2008) found that fat-free stirred yoghurts could be perceived as more or less creamy depending on the viscosity, as for semi-solid dairy desserts, and on particle size: products perceived as creamy were firm; however, when particles size was above 150 μm , products could not be perceived as creamy.

Such studies may also be conducted to investigate some phenomena occurring during processes. For example, Martin *et al.* (2009) observed the structure of acid milk gels during storage for up to 28 days, under specific gaseous conditions. Viscoelastic properties and gel setting were determined by low-amplitude dynamic oscillation. During acidification of milk, elastic (G') and viscous (G'') moduli were followed as a function of time (until pH 4.6) on a controlled-stress rheometer equipped with coaxial cylinders. To complete rheological measurements on these gels, apparent viscosity was measured using a coaxial viscosimeter. Additionally, whey separation was measured and gel structure was observed using confocal laser scanning microscopy. Sandra *et al.* (2011) studied the renneting behaviour of milk concentrated by ultrafiltration. They were particularly interested in the effect of concentration on the stages preceding aggregation and on interactions between micelles. For that purpose, they studied the scattering properties of micelles and rennet coagulation time was assessed, using both diffusing wave spectroscopy and rheology (low amplitude dynamic oscillation and flow behaviour in a concentric cylinder viscosimeter).

Dickinson and Eliot (2003) studied the amount of additional ionic calcium required to enable a caseinate-stabilized emulsion system to gel on heating. They reported that 'the onset of gelation is attributed to a temperature-induced flocculation of casein-coated oil droplets, presumably as a result of strengthening hydrophobic interactions between protein molecules adsorbed on the different droplets'. Here again, the authors tried to correlate different measurements to precisely study the phenomenon: droplet-size distributions of emulsions using laser light-scattering analyser,

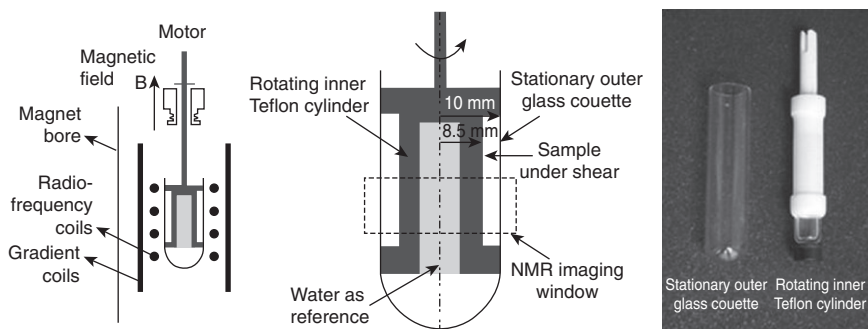


Fig. 15.2 'Rheo-NMR' device reported by Gabriele *et al.* (2009) allowing the evaluation of emulsion stability.

small-deformation oscillatory shear rheology and large-deformation steady-state shear rheology. Gabriele *et al.* (2009) followed rheological properties and stability of dairy emulsions typically used for whipped cream production. In emulsions, droplet size and distribution may affect stability, creaming-resistance, rheology and chemical reactivity. They chose to combine rheology and nuclear magnetic resonance (NMR) to study the material behaviour in flow conditions, producing relevant information on the structural changes induced by flow. In their study, commercial dairy emulsions were subjected to NMR diffusion study to determine droplet size and to 'rheo-NMR' (see Fig. 15.2) to evaluate wall slip and structure stability problems.

In addition to rheological analysis or other physical methods, descriptive sensory analysis may be used to explain the connection between material properties and sensory performance. Brown *et al.* (2003) investigated the sensory and rheological properties of young cheeses in order to better understand perceived cheese texture. Rheological methods were used to determine the linear and nonlinear viscoelastic and fracture properties. In parallel, a trained sensory panel developed a descriptive language and reference scales to evaluate cheese texture. All the used methods allowed discriminating among the cheeses. Principal component analysis (PCA) of both the instrumental and sensory parameters revealed that rheological properties were highly associated with rigidity and resiliency.

The study of oral processing, which occurs during food consumption to transform the food into a bolus ready to be swallowed, may also help to relate rheological properties and perceived texture. Drago *et al.* (2011) reported a study designed to relate food, saliva and bolus properties, by using model dairy products ranging from liquid to gelled samples. Spreading and rheological properties of bolus were studied using a texturometer. A bolus was compressed between two plates to mimic the displacement of the tongue and the compression of bolus between tongue and palate. The force–

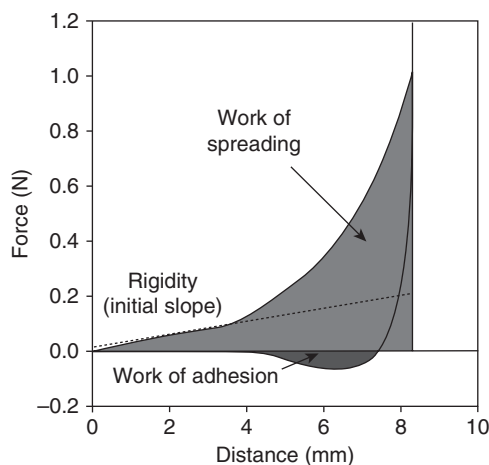


Fig. 15.3 The force–displacement curve obtained during compression of food bolus, showing the main parameters (rigidity, work of spreading, work of adhesion) that can be calculated (Drago *et al.*, 2011).

displacement curve obtained during compression was used to obtain information on bolus rheological properties (see Fig. 15.3).

15.6 Conclusion and future trends

Flavour is commonly defined as the combination of taste and aroma perception. However, this combined sensation that implies a first level of interaction, the taste–aroma interaction (Noble, 1996) is influenced by sensations of pain (i.e. pungency), chemical heat and cold, and tactile sensations on the dependence of food texture. If interactions within one modality, such as taste, were evidenced (Breslin, 1996), interactions between the different modalities clearly play an important role in flavour perception. Sensory enhancement due to congruent stimuli is well documented for taste–aroma interactions (i.e. enhancement of sweetness perception by fruity aroma or increase of fruity aroma perception on addition of sucrose) as are documented the impact of texture on taste or aroma perception (i.e. reduction of both perceived taste and aroma intensities on viscosity or hardness increase). The impact of flavour on texture perception has also been evidenced. Both physicochemical and cognitive mechanisms have been hypothesized to rationalize these interactions (Tournier *et al.*, 2007). For example, all possible reciprocal binary interactions for texture–taste–aroma perceptions have been recently examined for custard desserts varying in viscosity, sucrose level and aroma nature (Tournier *et al.*, 2009). The results suggested that cross-modal interactions for custard are both product- and

flavour-dependent. Not all possible binary interactions were observed; taste–aroma interactions were confirmed to be essentially on the cognitive part while interactions between texture and flavour were found to be less significant than previously reported. This unexpected result was interpreted as resulting from the way the process was used to vary the texture of the product (only mechanical treatment without any compositional change). Nevertheless, different impacts were obtained for *in vivo* flavour release and for flavour perception on varying the custard viscosity (Tournier *et al.*, 2009). This result highlights cognitive mechanisms and the recurrent difficulty in predicting sensory perception from physicochemical measurements only. It is still not well understood how the various flavour-active components combine to produce a particular sensory perception, which is moreover the result of a complex integration in the brain. Recent developments in dynamic instrumental methods are valuable tools to understand the changing balance of flavour compounds released during food consumption, even though the relationships between temporal flavour release and temporal perception remain generally difficult to establish, difficulty enhanced by huge inter-individual behavioural differences.

This illustrates a second level of interactions, implying food oral processing is linked to consumer physiology (Salles *et al.*, 2011). Chewing behaviour and saliva flow are important parameters that result in the formation of a cohesive food bolus, which is then swallowed. Texture influences mastication and salivation, which in turn influence texture perception and bolus formation. Flavour compound release is influenced by saliva impregnation of the bolus, but also probably by saliva composition and enzymatic activity (Salles *et al.*, 2011). Recently, it has been demonstrated that in-mouth air cavity volume change occurring during oral movements and particularly during deglutition constitutes a crucial parameter to characterize inter-individual variability (Feron *et al.*, 2011). For dairy products, the respective roles of texture, aroma release and consumer physiology on aroma perception have been recently reviewed (Gierczynski *et al.*, 2011). It appeared that physicochemical and cognitive mechanisms underlying aroma perception are strongly dependent on the type of texture of the dairy food (liquid, semi-solid or solid). For instance, a modification of aroma perception may result in certain circumstances of a direct impact of texture perception, the interaction being hypothesized to be due to a mechanism linked to attentiveness. In a recent study on aroma release during cheese consumption, relationships between the amount of saliva incorporated in the food bolus and aroma release parameters could not be clearly established because oral parameters and food characteristics have been found to be interdependent: cheese bolus texture was dependent on chewing behaviour and amount of saliva incorporated, which was dependent on both composition and initial food structure properties (Tarrega *et al.*, 2011). Understanding the complete mechanisms of flavour perception relies on links between physicochemical and cognitive parameters. A multidisciplinary approach including not only

flavour chemistry and sensory evaluation but also physiological, behavioural and psychological aspects is necessary to better understand the formation of a sensory image during food consumption. In this context, understanding the temporal perception of flavour in all its facets remains a challenge as a fundamental determinant of food acceptability and food choice by consumers, with direct consequences for their nutritional intake.

15.7 Sources of further information and advice

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Instrumental assessment of the sensory quality of fruits and vegetables

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Abstract: Increasing demands for improved quality of fruit and vegetables in the fresh-produce market and the food industry, as well as growers' expectations of high prices for premium quality produce drive the demand for fast, accurate, and objective methods for measuring and monitoring product quality. Such methods are required throughout the chain of preharvest and postharvest processes, from the field to the consumer. Several technologies are available for correlating specific quality-related indices and characteristics of fruits and vegetables with the stages of development during growth and maturation, and during the course of storage and shelf-life, through to consumption. This chapter will summarize the studies, adaptation, modification, and innovation of technologies and devices for determining the material properties of fresh fruits and vegetables as they pass along the processing chain from the field to the consumer. Included are descriptions of the various methods, measurements, equipment, principles and procedures for data processing and correlating the measurements with quality indices of fruits and vegetables as they pass through the various agricultural processes.

Key words: produce sorting, quality indices, fruit maturity, nondestructive methods, spectroscopy, ultrasonic, hyperspectral imaging.

16.1 Introduction

Quality assessment of agricultural products is a continuously developing subject, stimulated by the advances in vendor and customer requirements and increases in the volume of production that have occurred in recent decades. It is therefore continually necessary to improve quality measurement methods and to develop more efficient sorting lines and classification procedures, in order to enable farmers to supply higher-quality produce.

The revenue from high-quality, sorted produce for export markets is much higher than the average income. There is strong consumer demand for evaluation of fruit and vegetable quality during growth and maturation, and through the harvest period, storage, and shelf-life. Furthermore, there

is increased demand for fruits and vegetables that provide health benefits and also that are rich in nutritious ingredients that help to prevent health malfunctions. In many cases, mechanical and manual sorting is based on external attributes and indices and cannot determine essential internal properties of the produce. Nondestructive rapid determination methods are therefore increasingly demanded by growers, distributors and consumers to address this issue.

Over the years many methods and associated instrumentation, based on mimicking human sensory perceptions, have been developed to measure quality and quality-related attributes; they have been accompanied by a vast array of instrumental sensors for real-time and nondestructive testing (Abbott, 1999). There have also been numerous studies of technologies for nondestructive quality measurement of fruits and vegetables – visual, spectroscopic, acoustic, etc. and a literature survey by Butz *et al.* (2005) found that, in the context of food, about 20% of nondestructive or noninvasive techniques used acoustic methods. This multitude of techniques encompasses an enormous range, from traditional human-sensory evaluation by trained inspectors to sophisticated mechanical and electronic devices equipped with state-of-the-art sensors that measure and classify many different quality parameters of fruits and vegetables.

16.2 Quality indices and their determination

With regard to produce, the term ‘quality’ encompasses sensory properties, nutritive value, chemical constituents, mechanical properties, functional properties, and freedom from defects, each of which has been the subject of many studies (Abbott, 1999; Shewfelt and Bruckner, 2000). Generally, people base their quality evaluation of produce on sensory input. Since human judgments are usually subjective, such evaluations are liable to be inconsistent and to lead to erroneous quality assessments. Thus, there is an increasing need for improved quality monitoring techniques. In most agricultural products, both internal and external properties continue to change after harvest, so it is essential to accurately determine the optimal harvest time. Failure in this can negatively influence the quality of the final produce: either some properties may not reach their optimal level or, in case of over-ripeness, valuable components such as vitamin C could start to degrade. The shelf-life of the produce may also be shortened. Harvesting unripe produce may inhibit development of cultivar-specific properties such as color or taste; whereas over-ripeness can lead to accelerated softening and the produce becoming wrinkled and tasteless.

One example highlighting the need for better, more objective quality monitoring than can be carried out by the human eye alone is the case of peppers. Quality can be seen as a complex; it includes among other characteristic parameters of color, which are related to chlorophyll and carotenoid

contents, firmness, and contents of soluble solids, dry matter, and vitamin C, which all need to be accurately measured to determine the quality of the pepper (Dereje, 2003; Gomez-Ladron de Guevara and Pardo-Gonzalez, 1996; Zsom-Muha, 2008). Another example is apple, for which traditional destructive methods are used to measure several quality criteria, some of which are associated with maturity and some with the quality of the harvested and marketed product. Apples may have to be stored for long periods, therefore optimal harvest schedule determination is an important tool of quality management.

In general, criteria such as firmness, acidity and, of course, sugar content are examined. A special index, developed by Streif (1983) encompasses firmness and soluble solids and starch contents:

$$\text{StreifIndex} = \frac{F}{R \cdot S}$$

where F = firmness, R = SSC % Brix, and S = starch content.

Routine measurements of these indices are generally conducted in laboratories, and some of them involve chemical analyses, which are usually destructive and time- and labor-intensive.

16.3 Sorting and classifying principles

Several expressions are used for different aspects of product classification: separation, sorting, sizing, and grading. 'Separation' refers to the removal of unwanted material from the desired product, whereas 'sorting' indicates division of individual products into lots characterized by differing, pre-assigned quality attributes, which may be external or internal quality criteria. 'Sizing' is dividing the produce according to predetermined ranges of, e.g., weight or linear size, whereas 'grading' involves sorting according to established grade standards, and may refer to the final assigning of an overall grade that encompasses all aspects of quality. The last of these represents the classification of the produce according to its commercial value; therefore it requires evaluation of more attributes than are recognized when sorting is considered.

Several statistical methods have been developed to evaluate classifications, of which the most commonly used is based on a confusion matrix, with Cohen's kappa coefficient, which is calculated as:

$$k = \frac{\text{Pr}(\alpha) - \text{Pr}(e)}{1 - \text{Pr}(e)}$$

in which $\text{Pr}(\alpha)$ is the relative right classification and $\text{Pr}(e)$ is the hypothetical probability of the right classification; values of k above 0.9 are regarded as good results.

16.4 Routine destructive methods

As cited above many quality indices are evaluated during the pre- and postharvest procedures. For those indexes that involve internal properties, destructive analyses are generally used, and in practice these also need to be considered as reference methods for calibrating the nondestructive testing (NDT) methods.

16.4.1 Total soluble solids (TSS) determination

Total soluble solids (TSS) contents can be measured with a refractometer, either manual or digital such as the Model PR-1 (Atago, Tokyo, Japan). The TSS is expressed as percentage (w/w) in accordance with the Brix reading (°Bx). Instruments are calibrated with aqueous solutions of cane sugar which yields a ratio of 1:1 in Brix, i.e., 10°Bx for 10% sugar concentration. Test samples need to be cut, crushed, or squashed in order to extract the juice for TSS measurement.

16.4.2 Dry matter (DM) percentage determination

Samples of approximately 10 g should be taken from the examined product. Each sample is weighed to determine 'weight before' (Wb) and is then dried at a temperature ranging from 60°C – for samples containing sugars – up to 100°C. Generally, drying is for 72 h in a forced-ventilation oven, in some cases a vacuum oven is needed. After drying, the sample is weighed again to determine 'weight after' (Wa), and the percentage of DM is calculated as:

$$DM = (Wa/Wb) \times 100$$

16.4.3 Acidity

Total acidity (TA – titratable acidity) is a measure of the total acids present in produce; it is related to pH but the concepts are not identical. Whereas pH is a measure of acid strength, TA is a measure of the amount of acids present. TA is measured in g/(100 ml) in the US, and values for table wines generally fall somewhere between 0.4 and 0.9 g/(100 ml).

Acid–base titration is performed with a phenolphthalein indicator for strong-acid/strong-base titration, with a bromthymol blue indicator for weak-acid/weak-base reactions, and a methyl orange indicator for strong-acid/weak-base reactions. If the base strength is off the scale, i.e., a pH of >13.5, and the acid has a pH >5.5, an Alizarine yellow indicator may be used. On the other hand, if the acid is off the scale, i.e., a pH <0.5, and the base has a pH <8.5, a Thymol Blue indicator may be used.

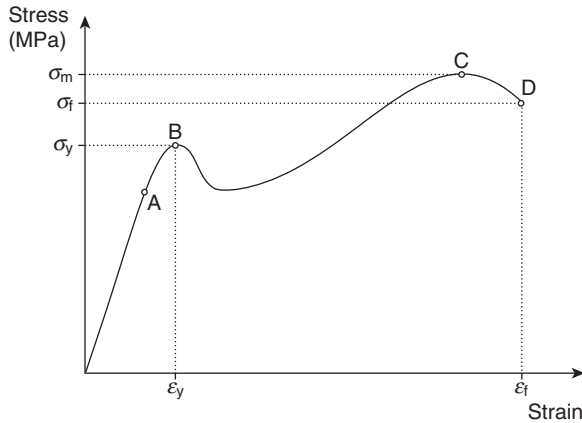


Fig. 16.1 Stress–strain curve. The portion of the initial slope (A) up to the inflection represents nondestructive elastic deformation. Beyond that portion, cells start to rupture and there may be a bioyield point (B) beyond which a noticeably changed slope continues until the rupture point (C), at which significant tissue failure occurs. Beyond the rupture point, as deformation increases the force may increase further, level off, or decrease.

16.4.4 Firmness

Puncture or compression tests are made at relatively low speeds. A typical instrument is the Magness–Taylor (1925) fruit firmness tester, and electronic universal testing instruments are considered quasi static. A stress–strain curve is shown in Fig. 16.1. The portion of the initial slope (A) up to the inflection represents nondestructive elastic deformation. Beyond that portion, cells start to rupture and there may be a bioyield point (B) beyond which a noticeably changed slope continues until the rupture point (C), at which significant tissue failure occurs. Beyond the rupture point, as deformation increases the force may increase further, level off, or decrease.

Puncture force vs. deformation curves appear similar to compression curves. Firmness of horticultural products can be measured by compression or by puncture, with various probes applying diverse levels of force or deformation, depending on the purpose of the measurement and how the quality attributes are defined. Horticulturists tend to define firmness as the maximum force the product can sustain. Whereas materials engineers often use the slope of the force/deformation curve, which reflects the apparent elastic modulus, as an index of firmness. Bourne (1982) found that the best relationships to sensory firmness, hardness and crispness were obtained with forces or deformations up to or beyond those that caused tissue damage. Penetrometer testers such as the Magness–Taylor instrument (Magness and Taylor, 1925), which was developed primarily as an objective means of determining picking maturity, are widely used for testing fruits and vegetables. Penetrometer measurements are moderately well

correlated with the human perception of firmness and with storage life, so that this technique has been accepted for testing a number of horticultural commodities, such as apple, cucumber, kiwifruit, pear and peach. However, because of their low speeds and often destructive nature, compression and penetration techniques are not very adaptable for on-line sorting of horticultural produce.

16.4.5 Oil content

Crude fat content can be determined using the Soxhlet method. A solvent is used to extract the fat from the sample, which can take up to 6 hours. The recovered fat is then weighed. The sample is comminuted as finely as possible and placed into a porous thimble, allowing thorough penetration by the solvent. The extraction apparatus used, of which the thimble is a component, allows for repeated reuse of the solvent. This maximizes the contact time between sample and solvent, allowing all of the fat in the sample to be dissolved.

Moisture and fat analyses of the sample are usually required before extraction. This involves accurate weighing of the sample using an analytical balance before and after it is dried and extracted. The sample can be weighed in a pre-dried extraction thimble or filter paper to avoid moisture loss during the weighing process. The dried extraction thimble or filter paper used should also be weighed before drying and extraction. If for any reason it is not necessary to carry out a moisture analysis, the sample need only be weighed after each of the drying and extraction processes (CSIRO, 1998).

16.4.6 Protein

Tests for crude protein, or Kjeldahl nitrogen, can be used to determine the level of organic nitrogen and ammonia in a sample. This test includes only small fractions of nitrite and nitrate. A preliminary digestive step is used to convert nitrogen (in the form of amino acids, peptides or proteins) to ammonia and to convert any carbon compounds to CO₂ by oxidation. This is followed by the traditional digestion phase, which uses sulphuric acid as well as combinations of metallic catalysts and salts, and lasts for a minimum of two hours. Sodium hydroxide is then added to the digest, and finally the ammonia is distilled into a boric acid or buffer solution. Nesslerization, or back titration, is then used to measure the level of ammonia in the distillate. Overall, the process takes several hours (www.hach.com).

The method for this process begins with the specimen being heated with sulfuric acid. Potassium sulfate is also added to raise the boiling point from (169 to 189°C) (337 to 373°F). The heating process results in oxidation and decomposition of the organic substance and liberation of ammonium

sulfate (the reduced nitrogen). The medium starts out a very dark color, and gradually becomes clear and colorless, indicating that the chemical decomposition process is complete. Sodium hydroxide is then used to distill the colorless solution by converting the ammonium salt to ammonia. Back titration is carried out to determine the amount of ammonia and therefore the amount of nitrogen present in the sample. This involves dipping the condenser into a boric acid solution, which reacts with the ammonia. The rest of the acid is then titrated with a sodium carbonate solution, using a methyl orange pH indicator (McClements, 2007).

16.4.7 Vitamin C: ascorbic acid measurement

Several kits are available for determination of ascorbic acid content, which is routinely based on the official method of the American Association of Analytical Chemists (AOAC) (AOAC, 2000). A sample of a few grams of the product is needed, which can be frozen and kept in a closed tube pending examination, if it is not to be measured immediately. For example, in determination of vitamin C content, frozen tissue is macerated in 25 ml of 3% metaphosphoric-acetic-acid ($\text{HPO}_3\text{—CH}_3\text{COOH}$) extracting solution in an Ultra-Turrax homogenizer and the extracted solution is vacuum filtered through a Whatman fiberglass filter disk. Residues remaining in the homogenizer and on the filter disk are washed with extracting solution, and the final volume of filtrate is measured in a graduated cylinder. Part of the solution is transferred to an Erlenmeyer flask and metaphosphoric-sulfuric-acetic-acid ($\text{HPO}_3\text{—CH}_3\text{COOH—H}_2\text{SO}_4$) is added to maintain the appropriate acidity (pH about 1.2) and to prevent auto-oxidation of ascorbic acid. The solutions should be titrated with 25% 2,6-dichlorophenolindophenol (DCIP) standard solution until a light but distinct rose-pink color persists for more than 5 s. The blue dye DCIP is reduced to a colorless form on addition of ascorbic acid, whose content is then determined by comparison with a preprepared standard ascorbic acid calibration solution set, and expressed as milligrams of ascorbic acid per 100 g of fresh sample. It is recommended that the indophenol dye reagent be standardized each time a new stock solution has been prepared.

16.4.8 Chlorophyll and carotenoid measurement

Total chlorophyll and carotenoid contents are determined by extraction in absolute ethanol followed by spectral determination of absorbance at wavelengths of 470, 648.6 and 664.2 nm. It is recommended to use quartz cuvettes and to carry out all the processes from extraction to spectral measurement in dim light, to avoid degradation of the chlorophyll and carotenoids in the sample. Calculation of total chlorophyll and carotenoid contents is based on Lichtenthaler's (1987) equations:

$$Ca = (13.36 \times A_{664.2}) - (5.19 \times A_{648.6}) \quad [16.1]$$

$$Cb = (27.43 \times A_{648.6}) - (8.12 \times A_{664.2}) \quad [16.2]$$

$$Ca + b = (5.24 \times A_{664.2}) + (22.24 \times A_{648.6}) \quad [16.3]$$

$$Cc = [(1000 \times A_{470}) - (2.13 \times Ca) - (97.64 \times Cb)] / 209 \quad [16.4]$$

in which A represents the absorbance of the sample at the respective wavelengths, as measured by spectrophotometer.

Calibration is conducted against pure chlorophyll *a* and *b* components dissolved in ethanol (Lichtenthaler, 1987). The total chlorophyll and carotenoid concentrations are expressed in milligrams per gram of fresh weight.

16.5 Nondestructive testing (NDT) methods

16.5.1 Color measurements

The external appearance of fruits and vegetables, particularly their color, is of prime importance among the various attributes that determine quality, especially in produce destined for fresh consumption. A visual impression that does not conform with the established standard easily leads to rejection.

Color is, by definition, a human perception. The standards for color spaces representing the visible spectrum were established in 1931 by the Commission Internationale de l'Eclairage (CIE) – the International Commission on Illumination, which also specifies lighting conditions for measurements. These standards were intended to provide an approximately unified scale for describing color. The three curves, $\bar{x}$, $\bar{y}$, and $\bar{z}$, when combined with the input stimulus and integrated, generate three signals that relate closely to perceived color. These signals, called tristimulus values, and denoted as *X*, *Y*, and *Z*, respectively, form the basis of most popular and useful color descriptions.

There are two forms of color-measurement devices: those that measure spectral reflectance (spectrophotometers) and those that measure only tristimulus values (colorimeters). The main difference is that spectrophotometers measure physical properties (spectral reflectance, spectral transmittance, and spectral absorbance) from which tristimulus values are calculated. Colorimeters typically pass the light through specially designed filters and enable tristimulus values to be calculated directly from detector output levels.

There are many CIE color spaces; they serve diverse purposes, and are all 'device independent', unlike RGB and CMYK color spaces, which are related to specific devices. These RGB and CMYK spaces usually do not cover the entire visible color spectrum.

A CIE LCH color space or color model, generally applied, is essentially in the form of a sphere Plate VII (between pages 242 and 243). There are three axes; L^* , C^* , and H° . The L^* axis represents lightness. It is vertical and ranges from 0, which represents zero lightness (i.e. perfectly black), at the bottom, through 50, to 100 which represents maximum lightness (i.e.

perfectly white) at the top. The C^* axis represents chroma or ‘saturation’. This ranges from 0 at the center of the circle, which is completely unsaturated (i.e. a neutral gray, black or white) to 100 or more at the edge of the circle for very high chroma (saturation) or ‘color purity’. Around the circumference of the colored circle can be seen every possible saturated color, or hue. This circular axis is divided into hue angles or degrees (H°), which range from 0° (red) through 90° (yellow), 180° (green), 270° (blue) and back to 0° . LCH is device-independent.

Once the perceived color of a fruit has been measured and determined using one of the techniques described above, it can be used to assess its quality. Within the visible range of wavelengths for the absorption of light energy, the major absorbers are pigments: chlorophylls, carotenoids, anthocyanins and other colored compounds. The pigment content of a commodity can be determined from the colors perceived by the human eye, which depend on the reflective properties of the product within the visible region (approximately 400–750 nm). Color perception is a major aspect of product appearance (Abbott, 1999), and links have been established between factors such as the VIS reflectance fingerprint or the ripening process in fruits, and pigment content. Components that contribute to the quality attributes of fruit such as antioxidant properties, aroma and taste are often synthesized in genes, chromoplasts or chloroplasts (Barry, 2009).

One example of this is the use of chlorophyll (and therefore color) to assess fruit quality. As fruits mature their chlorophyll content decreases. This process has traditionally provided a criterion for visual assessment of fruit maturity (Crisosto *et al.*, 2007). Average surface color can be used as a quality indicator in fruits whose skin surface is one homogeneous color, for example oranges. Early computer vision systems, which used monochrome cameras, were utilized to assess the color and therefore the quality or grade of homogeneously colored fruits. However, these systems could not detect defects. In fruits with a secondary surface color, such as some peach and apple cultivars, the changes in color can be used as an indicator of maturity, although this is not always reliable.

By contrast, modern packinghouses use RGB color video cameras to sort produce. A color image is made up of pixels, each of which three intensity values – the red, green, and blue (R, G, and B) primary color components that form a large part of the visible spectrum. Due to the extreme variability in produce color, continuous training is required to successfully use those techniques (Blasco *et al.*, 2007; Lleo *et al.*, 2009). Three-chip cameras (CCD and CMOS) use dichroic prisms to direct light, which provides a great improvement in image resolution and color accuracy.

16.5.2 Visible and near-infrared (NIR) spectral measurements

Chemical bonds absorb light energy at specific wavelengths, and therefore some compositional information can be extracted from spectra measured

by spectrophotometers or spectrometers. Water, carbohydrates, fats, and proteins have absorption bands in the near-infrared (NIR) region (Abbott, 1999).

NIR radiation covers the part of the electromagnetic spectrum between 780 and 2500nm (Sheppard *et al.*, 1987). In NIR spectroscopy, the product is exposed to NIR radiation and the spectrum of the reflected or transmitted radiation is measured. The spectral characteristics of the incident beam are modified as it passes through the product, because of wavelength-dependent absorption and scattering processes. These changes depend on both the chemical composition and the physical properties of the produce (Nicolai *et al.*, 2007). The short-wave infrared region is that part of the electromagnetic spectrum lying between 750 and 1900nm, associated with vibration and combination overtones of the fundamental O—H, C—H, and N—H bonds, which are the primary structural components of organic molecules (Williams and Norris, 2002).

Chemometric statistical techniques, such as partial least squares (PLS) regression, multilinear regression (MLR) and principal component analysis (PCA) are then applied, to correlate the NIR spectrum with quality attributes such as sugar content, acidity, firmness, or storage period of the product (Schmilovitch *et al.*, 2000).

NIR measurements have been successfully used to nondestructively quantify and characterize ingredients of fruits and vegetables, and these techniques have been used successfully for rapid analysis of multiple components, such as oils, proteins (Schmilovitch *et al.*, 2001; Shenk *et al.*, 1992), dry matter (Schmilovitch *et al.*, 2000), firmness (Penchaiya *et al.*, 2009; Schmilovitch *et al.*, 2000), and total soluble solids (Penchaiya *et al.*, 2009; Schmilovitch *et al.*, 2000; Zude *et al.*, 2006) in a wide variety of agricultural produce. Blanco *et al.* (1993) used NIR diffuse reflectance spectroscopy to determine ascorbic acid in pharmaceutical products. Microstructure of the fruit and vegetable tissue affects the propagation of NIR, therefore NIR spectroscopy has been successfully applied in measuring microstructure-related attributes such as internal damage (Clark *et al.*, 2003) and stiffness (Lammertyn *et al.*, 1998).

As well as NIR, Vis-NIR (visible and near-infrared) techniques can be employed, which use light and radiation to obtain a more complete picture of fruit properties. Ortiz *et al.* (2001) related Vis-NIR spectral information to soluble solids and acidity contents and firmness of peach fruits, and Zude *et al.* (2006) used Vis-NIR spectroscopy to examine soluble solids contents in apples. Wang *et al.* (2011) estimated vitamin C content in chilies with a quantitative analysis technique based on Vis-NIR diffuse reflectance spectroscopy. Zude (2003) found significant correlations between the chlorophyll content of apple fruits and their spectral transmittance, by using the red-edge values as well as various indices used in remote sensing and PLS regression in the spectral range of 600 to 750nm. Merzlyak *et al.* (2003) studied the diffuse reflectance of apple fruits in the spectrum range from

400 to 800nm: they used five apple cultivars, all picked at maturity, and obtained significant correlations between various reflectance indices and fruit chlorophyll content. Xudong *et al.* (2009) used the Vis-NIR spectral range to nondestructively measure quality indices (soluble solids contents, titratable acidity, vitamin C content, and color) of intact Nanfeng mandarins.

More widespread use of these technologies depends on several factors. The most important technical factor is the prediction model's robustness: the accuracy of the NIR calibration models should be maintained in application to unknown samples that were not used in building the calibration model. Calibration models should be based on large data sets, including samples from diverse origins, taken under varied climate conditions, and during various seasons. The issue of temperature sensitivity of NIR measurements also should be considered (Roger *et al.*, 2003), as should transfer of a calibration model to a different spectrophotometer (Greensill *et al.*, 2001).

16.5.3 Hyperspectral imaging

Multi- or hyperspectral cameras enable image acquisition at many wavelengths. 'Multispectral imaging' refers to image acquisition at fewer than 10 wavelengths, whereas for more than 10 wavelengths the procedure is designated as 'hyperspectral imaging'. The acquired images can be visualized in a hypercube with the *X* and *Y* dimensions being the length and width of the image and the *Z* dimension representing wavelengths. The data set also could be visualized as a set of single-wavelength pictures of the object, with as many pictures as the number of wavelengths used. Such imaging can provide information about the spatial distribution of constituents (pigments, sugars, moisture, etc.) near the product's surface (Ruiz-Altisent *et al.*, 2010).

Hyperspectral imaging integrates both imaging and spectroscopy techniques. The process is particularly suitable for inspecting food products because it simultaneously acquires spatial and spectral information about the product (Gowen *et al.*, 2007; Kim *et al.*, 2001). Hyperspectral imaging can be carried out in one of two modes: line-scanning (push-broom) or filter-based-imaging (Lu and Chen, 1999). The line-scanning mode involves scanning the moving item to create a three-dimensional hyperspectral image or hypercube. Line-scanning is used in on-line applications as it is relatively easy to implement. In filter-based imaging, the stationary product item is spectrally imaged using either a liquid crystal tunable filter (LCTF) or an acousto-optical tunable filter (AOTF). Filter-based systems are not suitable for online applications, as they need complex calibration (Ruiz-Altisent *et al.*, 2010).

Recent studies have demonstrated the successful use of hyperspectral imaging technology in measuring fruit qualities such as maturity, firmness and soluble solids contents (ElMasry *et al.*, 2007; Lu and Peng, 2007; Noh

et al., 2007). It has also been used to detect bruises and bitter pits on mushrooms and apples (Gowen *et al.*, 2008; Nicolai *et al.*, 2006) and internal defects and chilling injury in cucumbers (Ariana and Lu, 2010; Cheng *et al.*, 2004).

16.5.4 Mechanical methods for firmness measurement

Vegetables and fruits exhibit viscoelastic behavior under mechanical loading. The viscoelastic behavior of the product depends on both the loading rate and the force applied. Viscoelastic measurement involves functions of force, deformation, and time, so for practical purposes the loading rate is often ignored and the products assumed to be elastic, because measurement of a product's elastic properties requires only the force and deformation to be taken into account. However, fruits and vegetables do have a viscous component to their force vs. deformation behavior. When conducting instrumental tests, therefore, the loading rate (test speed) should be kept constant and recorded and reported. The viscous component contributes only minimally to the perceived texture of firm produce such as root crops; however, it is significant in softer produce such as citrus fruits or tomatoes. Relaxation or creep measurements are therefore often more suitable than puncture tests for soft produce (Abbott, 1999).

When measuring qualities such as firmness, nondestructive mechanical techniques provide an alternative to destructive instruments such as the Magness–Taylor penetrometer (García-Ramos *et al.*, 2005; Nicolai *et al.*, 2006). Measurement of acoustic responses to vibrations, variables extracted from quasi-static force-deformation curves and impact forces are some examples of major mechanical techniques (Fekete and Felföldi, 2000; Felföldi, 1996; Felföldi and Ignát, 1999). Most nondestructive mechanical methods measure elastic properties at very low levels of deformation. One such property is the modulus of elasticity, which is a measure of the capacity of the material to withstand elastic deformation. The modulus of elasticity can be characterized as the stress–strain ratio (or the slope of the force vs. deformation curve prior to rupture) for a tissue specimen with a constant cross-sectional area (Abbott, 1999).

The nondestructive force–deformation curve can be created by application of a low deformation force to the fruit (to cause minimal damage) using a metallic plunger, or by calculating the required force to a preset level of deformation (Fekete and Felföldi, 2000). The variables of the curve can then be measured.

One example of a nondestructive force/deformation device used to generate such a curve is the durometer, which is used for soft fruits such as cherries (Clayton *et al.*, 1998). Other devices include the analogue and digital firmness meters (Macnish *et al.*, 1997), which use a combination of a V-shaped holding area and a 40 mm disc to press against the fruit so that the level of deformation can be measured. Hung *et al.* (1999) developed the

'laser air-puff', a non-spectroscopic laser instrument which measures the mechanical deformation of fruits subjected to a short, forceful blast of air (69 kPa in 100 ms). In 2009, Lu and Tipper developed a device to measure apple fruit firmness that worked by measuring the applied force at the point of bioyield. The devices described above and other firmness testers have a variety of applications, and some can be used for on-line sorting (Ruiz-Altisent *et al.*, 2010).

16.5.5 Ultrasonic vibration

Ultrasound technology has been known for many years. Its main areas of application are medical diagnostics, industrial processes and inspection procedures. At high frequencies and low power it can be used as an analytical and diagnostic tool, and at a very high power it can assist processing. Ultrasonic vibrations are above the audible frequency range, i.e. >20 kHz. Ultrasound is generated by a transducer containing a ceramic crystal that is excited by a short electrical pulse with a typical form of several sinusoidal oscillations. Through the piezoelectric effect, this electrical energy is converted to a mechanical wave that is propagated as a short sonic pulse at the fundamental frequency of the transducer. The energy is then transferred into the material or body under analysis and propagated through it (Krautkramer and Krautkramer, 1990). The ultrasound signal emerging from the test specimen is sensed by a piezoelectric element that acts as a receiver, converting any ultrasonic vibrations impinging on it back to electrical energy. When the system operates in 'pulse-echo' mode, the same piezoelectric element acts as a transmitter and a receiver alternately; in 'through-transmission' mode a second piezoelectric element acts as a receiver.

Ultrasonic energy will propagate through a material until the sound wave encounters an impedance change, caused by some change/s in the material density and/or the velocity of the sound wave (Kuttruff, 1991). The energy attenuation of the ultrasound beam and the speed of wave propagation depend on the nature and structure of the material (Kuttruff, 1991), and most physical or chemical changes in the material cause the attenuation and velocity of the propagated beam to be altered.

The potential for using ultrasound in the food industry has been recognized since the 1970s (Povey and Wilkinson, 1980), and developments related to the technology have progressed rapidly over the years (Povey, 1998). However, development of ultrasound technology as a means of evaluating food quality has not progressed as fast in the fresh fruit sector as in the food processing industry; lack of appropriate equipment – sufficiently powerful to penetrate but, at the same time, sufficiently gentle to avoid damage to the sensitive tissues of fruits and vegetables – has been an important obstacle (Mizrach *et al.*, 1989; Porteous *et al.*, 1981). However, some advances in equipment design, and availability of new instruments and sensors, mainly designed for industrial use with new composite materials, have facilitated

progress and have stimulated more studies and development of ultrasonic methods and techniques for the fresh fruits and vegetables market (Mizrach *et al.*, 1989). Recently, ultrasonic techniques have been investigated for the sensory analysis of various quality parameters in agricultural produce: devices and measuring techniques, based on ultrasonic waves, have been developed for nondestructively monitoring some of the changes in physico-chemical, biochemical, and mechanical properties that occur in fruit tissues during the various stages of their pre- and postharvest existence.

These stages include growth and maturation (Chivers *et al.*, 1995; Gaete-Garretton *et al.*, 2005; Mizrach *et al.*, 1999a,b; Self *et al.*, 1994), storage under various conditions (Flitsanov *et al.*, 2000; Mizrach *et al.*, 2000; Verlinden *et al.*, 2004), and shelf-life (Johnston *et al.*, 2002; Mizrach, 2000; Mizrach and Flitsanov, 1999). Such changes are expressed differently in the course of the various periods, and are mostly reflected in the final quality of the produce. Textural attributes are among the factors mainly considered in quality assessment (Peacock *et al.*, 1986), and are regularly used to determine the stage of maturity of various fruits and vegetables (Abbott, 1999). Firmness is considered as one of the main indices of maturity, and its changes during the ripening and softening process start on the tree and continue during harvesting, handling, and storage. Chemical contents and concentrations in fresh tissues are also important factors in determining maturity of fruits and vegetables, but firmness is the factor most closely related to the stage of maturity (Peacock *et al.*, 1986).

Many studies have addressed the difficulties and limitations in applying ultrasound technology to quality evaluation in the various pre- and postharvest stages, and it seems that the technology is not yet ripe for commercial use and that there is a lot yet to be done in order to bring it into a widely used sorting tool (Mizrach, 2007).

When acoustical measurements are used in conjunction with measurements of other physicochemical properties, such as firmness, mealiness, dry weight (DW) percentage, oil contents, TSS, and acidity, a link between acoustical parameters and physicochemical indices enables the indirect assessment of the proper harvesting time, storage period or shelf-life (Abbott, 1999; Butz *et al.*, 2005; Mizrach *et al.*, 1989). The ultrasound technique has been adopted mainly for nondestructive, rapid, and accurate assessment of the changes involved, but has also been used to monitor additional natural and environmental factors that cause changes in quality-related parameters (Mizrach *et al.*, 1989, 1999a, 2000).

16.6 Sorting machines

16.6.1 Weight sorting

Weight-sorting machines use an electronic weighing system that can weigh a succession of passing cells containing the produce at a high rate. For

example, 'Compac' carriers feature four individual weighing points, in order to compensate for variations in fruit shape or position. Two load cells per lane gather weight information from each weighing point and process approximately 250 readings, taking less than 0.1 s for each fruit. The fruit carriers and load cell runners are designed so that each carrier is being measured for over 95% of the weighing cycle, giving maximum time for precision weighing, even at high speeds of 10 to 15 fruits per second per lane.

16.6.2 Visual and color sorting

A color sorting and grading system provides detailed, accurate sorting according to color, as well as size or shape; a few machines can also detect blemishes or external defects. Some of these machines sort with respect to surface marks, stains, insect damage, cuts, punctures, and bruising; usually the software captures multiple images (up to 25). For grading they generally evaluate diameter, elongation, flatness and symmetry and, sometimes, the produce volume. All the camera operations – for color, brightness, and field-of-view settings, and corrections for lighting changes – are computer controlled. In some cases density sorting had been applied, based on accurate diameter and weight readings for each item.

16.6.3 Internal contents

Several machines based on NIR technology are available for nondestructive measurement of contents of internal components, such as sugars, dry weight, and moisture. Companies such as Griffa, Sacmi Compac, and others offer NIR sorting equipment for incorporating into each lane of a sorting machine. As the fruit is being graded, the NIR sorting equipment uses an NIR light source focused to illuminate the fruit as it passes under the NIR system. The fruit reflection or transmittance is measured by a spectrometer and spectrally precalibrated models are used to determine the contents of the produce. Other machines using MRI or X-rays are commercially available, but are not utilized on a large scale.

16.7 Conclusion

This chapter presents some concepts, technologies, developments, modifications, and applications associated with instrumental quality evaluation of fruits and vegetables during pre- and postharvest processes. It surveys various measurement methods and how they have been adapted to measure physicochemical changes and quality indices of various tissues, specimens and whole fresh fruits during the course of growth, maturation, harvesting, storage, shelf-life and consumption. The public demand for a higher level

of quality in agricultural produce is likely to intensify in the future, which could lead to increased availability of sophisticated techniques, sensors and user-friendly, noninvasive devices for measuring quality indices. Some of these instruments will certainly incorporate the technologies described in this chapter. The nondestructive nature of such techniques will also help to boost their attractiveness for application to monitoring pre- and postharvest processes. However, not all the technology described here is currently commercially available for field or laboratory use, and a great deal of study is still needed. Continued development of equipment and techniques will ensure increasing implementation and adoption of new technologies to meet the expanding requirements of the fresh and processed agricultural produce sector.

16.8 References and further reading

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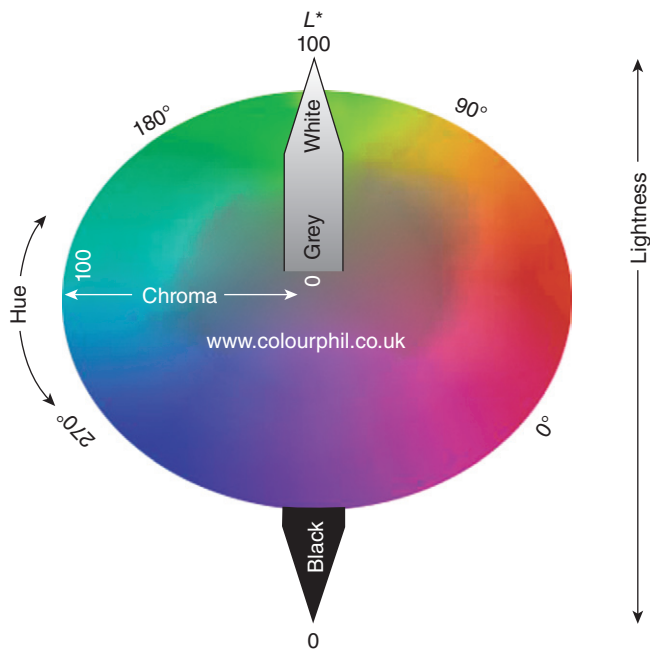


Plate VII (Chapter 16) LCH colour space (http://www.colourphil.co.uk/lab_lch_colour_space.html).



(a)



(b)

Plate VIII (Chapter 19) Background effect in colour measurement. Different orange juice dilutions over white (a) and black (b) backgrounds for visual and spectroradiometric analysis.

Instrumental assessment of the sensory quality of wine

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Abstract: Over the years, the application of analytical chemistry in the wine industry has done much to improve the level and consistency of wine sensory quality. Nowadays, instrumental analysis, particularly chromatography and spectroscopy, is applied to all aspects of wine production, from vineyard to bottle. Key sensory parameters or components are determined directly – or indirectly using rapid methods such as Fourier transform infrared (FTIR) spectroscopy and chemometric/statistical analysis. The structures of many complex sensory components have been elucidated by techniques such as mass spectrometry (MS) and nuclear magnetic resonance spectroscopy, the former usually being combined with gas chromatography (GC) or high performance liquid chromatography (HPLC). Over the past few decades, sample preparation techniques, especially extractive/focusing methods, have greatly facilitated instrumental analysis.

Key words: wine, grape must, instrumental analysis, chromatography, spectroscopy, electrochemistry, sample preparation, chemometric/statistical methods.

17.1 Introduction

In the wine industry, as in other areas of the food industry, instrumental analytical methods, along with organoleptic analysis, are crucial for the achievement and maintenance of high sensory quality. The major areas of application (with some overlap) are described in Table 17.1.

As grape must and wine contain an exceedingly complex mixture of components (of a very wide range of chemical complexity and character: see Fig. 17.1) many methods need preliminary sample preparation before analysis can be performed effectively. Sample preparation ranges from simple dilution to the application of one or more extractive or separative techniques, in order to minimise interferences and matrix effects, and to

Table 17.1 Major general applications of instrumental methods to the analysis of must and wine

General application	Comments
1. Process monitoring and quality assurance/control (QA/QC) (including satisfaction of regulatory requirements)	Routine quantitative analysis sometimes uses rapid direct methods or indirect methods (e.g. Fourier transform infrared (FTIR), nuclear magnetic resonance (NMR), or electrochemical methods) and chemometric analysis; reference methods are used for confirmatory or official analysis.
2. Determination of authenticity/detection of fraud	Profiles of sensory parameters (e.g. acidity, sugar content, polyphenol content) or quantitation of certain components are used to discriminate between samples, using chemometric/statistical analysis.
3. Identification of specific components and investigation of reactions or interactions with other components (including 'time evolution' studies)	Chromatographic, electrophoretic and spectroscopic techniques are most important here (especially NMR and mass spectrometry (MS)), the latter often requiring some sample pretreatment to remove interferences.
4. Development of new analytical methods and improvement of established methods	New methods should be always compared with contemporary methods, which could include reference/official or standard methods of, for example, the Organisation Internationale de la Vigne et du Vin (OIV) and the Association of Official Analytical Chemists (AOAC).

increase sensitivity by 'focusing' the analytes of interest from the original sample volume into a much smaller volume. The major focusing techniques are liquid-liquid extractions (including countercurrent chromatography, CCC), distillation methods, headspace methods and sorptive extraction methods.

Of paramount analytical importance are instrumental methods, particularly gas chromatography (GC), high-performance liquid chromatography (HPLC), UV-visible spectroscopy, Fourier transform infrared (FTIR), atomic spectroscopy, electrochemical methods, nuclear magnetic resonance (NMR) and mass spectrometry (MS). Chemical and physical methods (such as titrations, distillations, optical methods, density measurements, and measurement of foam parameters,) are still used in certain contexts. A brief overview of the application of these methods is given in Table 17.2.

Some techniques are applied to the analysis of just one component or of a family of like components, but most others, particularly chromatographic techniques, NMR and FTIR, can be used to analyse several different components simultaneously (Table 17.2).

Odour (aroma) (Section 17.3)

Alcohols, carbonyl compounds, esters (inc. lactones), heterocyclic compounds, hydrocarbons, norisoprenoids, sulphur compounds, terpenoids, volatile phenols

Ethanol aids release of odour compounds, proteins retard it

Colour (Section 17.2)

Red wine: anthocyanins, derivatives and condensation polymers
White wines: polyphenols and others

Taste (Section 17.6)

Acids, sugars, polyphenols, salts

Mouthfeel (Section 17.6)

'Body' related to amount of dissolved substances
Astringency given by polyphenols, viscosity mainly by glycerol, prickliness by dissolved CO₂

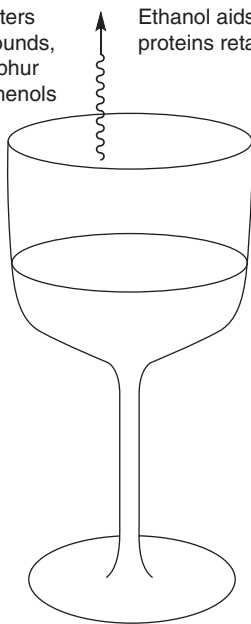


Fig. 17.1 Sensory quality factors of wine.

The majority of methods are invasive and/or destructive, requiring the sacrifice of a volume of sample (though often a small one); the major non-destructive method being NMR, when used with external reference (Table 17.2). Apart from direct determinations, many of these techniques, especially FTIR, NMR, UV-visible spectroscopy and atomic spectroscopy, as well as electrochemical methods, can be used as rapid indirect methods for the prediction of important sensory quality parameters (acidity, sugar content, phenolic content, etc.) of unknown samples. This is done by construction of a database (often using data from conventional or reference methods) of optimised calibration models. Statistical analysis (often partial least squares regression, PLSR) is then used to correlate these data with FTIR or NMR (for example) data for the same set and to compute regression models and errors for the chosen parameters. Validation is carried out on a different set of samples.

Sections 17.2–17.6 deal with instrumental analytical methods used to determine the sensory quality of colour (Section 17.2), odour (Sections 17.3–17.5) and taste/mouthfeel (Section 17.6). Sections 17.7 and 17.8 give brief overviews of trends and developments, and some sources of useful information, respectively.

Table 17.2 Major instrumental methods for the analysis of grape must and wine

Method	Main sensory components or parameters analysed	Contexts, general comments and selected references (for general reference, see Buglass and Caven-Quantrill, 2011a)
UV-visible ¹ spectrophotometry and colorimetry	Colour, total anthocyanin content (TAC), total phenolic content (TPC), acids ² , carbohydrates ² , aminoacids ² , some metal cations ² and anions ²	QA/QC ³ . For determination of specific components, such as glucose, malic acid and others, enzymic methods are probably most widely used.
Gas chromatography (GC)	Most volatile components (odour)	QA/QC ³ , identification/structure determination. Less volatile components, such as many phenols, acids and amino acids, can be analysed by GC after derivatisation. Flame ionisation detector (FID) and mass selective detector (MSD) most common detectors.
High-performance liquid chromatography (HPLC)	Most components, especially non-volatile ones (taste/mouthfeel)	QA/QC ³ , identification/structure determination. Sensitivity to weakly absorbing components can be increased by derivatisation. Mato <i>et al.</i> (2005); de Rijke <i>et al.</i> (2006).
Capillary zone electrophoresis (CZE)	Ionic and ionisable compounds (taste/mouthfeel)	QA/QC ³ . Mato <i>et al.</i> (2005).
Fourier transform infrared spectroscopy (FTIR)	Most components	QA/QC ³ . FTIR data frequently used with data from other sources and statistical analysis as rapid indirect method for confirmation of origin and discrimination amongst types.
Atomic spectroscopy	Metal and nonmetal elemental species, especially Cu(II) and Fe(II/III) ⁴	QA/QC ³ . atomic absorption or atomic emission data frequently used with data from other sources and statistical analysis as rapid indirect method for confirmation of origin and discrimination amongst types. Grindlay <i>et al.</i> (2011).
Mass spectrometry (MS) ⁵	Most components	Most often used as detector for GC or HPLC. Major method of identification/structure determination, including of aroma compounds, phenols, carbohydrates and proteins. March and Brodbelt (2008); Lin and Harnly (2007) ; de Rijke <i>et al.</i> (2006).

(Continued)

Table 17.2 *Continued*

Method	Main sensory components or parameters analysed	Contexts, general comments and selected references (for general reference, see Buglass and Caven-Quantrell, 2011a)
Nuclear magnetic resonance spectroscopy (NMR) ⁶	Most components	Non-destructive. Can test condition of unopened bottle (by CH ₃ CO ₂ H and C ₂ H ₅ OH quantification) (Weekley <i>et al.</i> , 2003) and a 3D diffusion-ordered method has been used to follow maturation (Nilsson <i>et al.</i> , 2004). Alcoholic and malolactic fermentation has been monitored using NMR (Avenova <i>et al.</i> , 2006). Also NMR can be used to link fermentation character with yeast strain (Son <i>et al.</i> , 2009) and to build data-sets for statistical analysis (Conte, 2008).
Electrochemical methods ⁷	Metal and nonmetal ions; also oxidisable inorganic components (like SO ₃ ²⁻ and O ₂) ⁸ and organic components (like ascorbic acid and glucose)	Non-specific (but cross-sensitive) electrochemical sensors (arrays of electrochemical cells) are used in 'electronic noses' and 'electronic tongues', and other miniaturised devices. These give a 'fingerprint' which can be matched to sample type, using chemometric/statistical software, leading to confirmation of origin or discrimination amongst types. Other sensors are specific and are often used in conjunction with flow injection analysis (FIA). Biosensors use enzymes in the redox process at the working electrode.

¹ Not including use as major HPLC, CZE and flow injection (FI) detector.

² Using chemical or enzyme reagents to create chromophore.

³ Includes compliance with regulations.

⁴ In conjunction with proteins and phenols, can cause visual sensory defects.

⁵ Electrospray ionisation (ESI) is most commonly used ionisation mode in LCMS; electron ionisation (EI) in GCMS. The most common analysers are quadrupole (Q), quadrupole ion trap (QIT, which allows MSⁿ), and for tandem MS, QqQ and QTOF.

⁶ Two-dimensional techniques, like homonuclear correlation spectroscopy (COSY), homonuclear multiple bond coherence (HMBC), heteronuclear multiple quantum coherence (HMQC) and total correlation spectroscopy (TOCSY), predominate. Solvent (water) signals can be suppressed using a '2D' WET shaped pulse sequence, or similar.

⁷ Includes many variants, including ion selective electrodes and other potentiometric methods, as well as voltammetric methods.

⁸ Sulphites are added as preservatives, but levels should be below regulatory maximum values; dissolved oxygen is a potential spoiler of sensory quality – it can be estimated using the Clarke oxygen electrode.

17.2 Assessment of colour

17.2.1 Importance of colour

Colour is one of the major contributors to wine quality: indeed, it is the first characteristic to be perceived when wine is poured from bottle to glass. The colour of a wine imparts information to the consumer regarding its age or condition, its body (concentration of dissolved substances) and possible defects. In effect, perception of colour prepares the consumer for what is to follow – odour, flavour, taste and mouthfeel.

During the winemaking process, pigments are released from grape skins (to a greater or lesser extent, depending on the desired wine style), where they mix and many react gradually with other components during fermentation and subsequent maturation, causing significant changes in colour. Hence there is great interest in wine colour, its correlation with winemaking processes (or styles) and wine development (including spoilage). At the same time, there is also great interest in pigment structures and their gradual chemical transformations, many of which have a definite influence on wine quality. For reviews and summaries, see Boulton (2001), Cheynier *et al.* (2006) and Buglass and Caven-Quattrill (2011a).

17.2.2 Origins of wine colour: grape pigments

In black grapes, the major pigments are anthocyanins (Fig. 17.2), which are located in skin vacuoles. Proanthocyanins – anthocyanin dimers and trimers – are thought to be located in different skin vacuoles (Vidal *et al.*, 2004a). Other polyphenolic compounds are found in both skins and pericarp (pulp) (Fig. 17.3). The concentration of anthocyanins in black grape skins, must and new wine is so high as to allow copigmentation via stacking of molecules in their flavylum forms (as in Fig. 17.2) or quinoidal forms at pH ~3.5, thus giving rise to intense colour (Asenstorfer and Jones, 2007).

The colour of ‘white’ grapes is thought to be due to the presence of chlorophyll (green) and its yellow-green degradation products, nonfluorescent chlorophyll catabolites (NCCs) and possibly pheophytins and carotenoids, as well as polyphenols in the skins and flesh (Fig. 17.3) (Mendes-Pinto *et al.*, 2005; Müller *et al.*, 2007).

17.2.3 Factors influencing wine colour

Once black grapes are crushed and the winemaking process begins, the constituents mix and π – π interactions between stacked anthocyanins and polyphenols (copigmentation) cause the fermenting must to become purple. Very soon, however, reactions between anthocyanins and other components (especially polyphenols and carbonyl compounds) occur at varying rates, eventually giving a large number of new pigment molecules (Fig. 17.4), each of which contributes its own colour characteristics to the overall wine

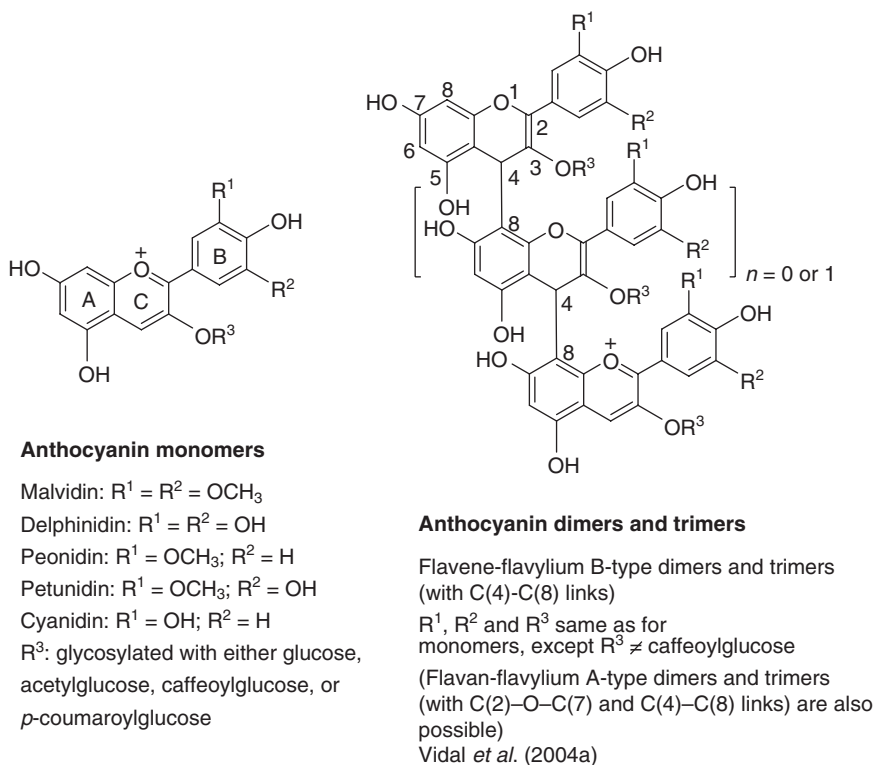


Fig. 17.2 Some pigments of black grapeskins. Malvidin is the major monomer. These pigments also contribute to the colour of must and new red wine.

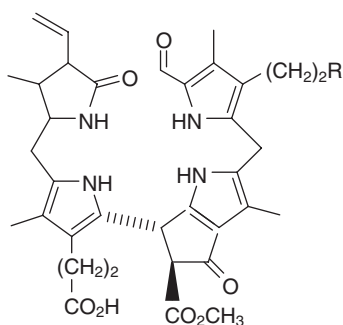
colour. Many of the new pigments are condensation oligomers formed from anthocyanins or vitisins and flavan-3-ols, but other polyphenols can be involved (Fig. 17.5).

In general, anthocyanins and vitisin type derivatives have relatively sharp absorption maxima ($\lambda_{\max}$ values) at ~500–560 nm, with $\lambda_{\min}$ or low absorptions at ~420 nm, corresponding overall to purple-red. Many oligomeric pigments, on the other hand, have broader, less intense maximum absorptions at ~500 nm, but higher absorptions at ~420 nm, giving a greater orange component. Hence, as red wine ages, its colour changes from purple-red, through brick red to orange-red, red-brown or even brown (Fig. 17.6).

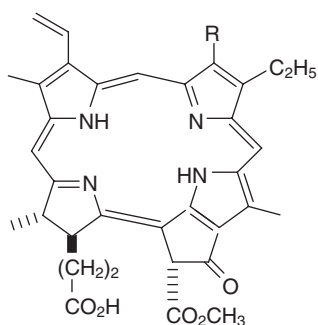
The colour of young or unoxidised white wine is believed to be due to polyphenols, such as flavan-3-ols and their condensation oligomers (Fig. 17.3), along with a contribution from chlorophyll and/or chlorophyll degradation products and carotenoids. All of these are present in low concentrations and most absorb predominantly below 350 nm, thus giving rise to the characteristic pale gold colour. Enzymic and nonenzymic browning are

Phenolic substances: procyanidins, tyrosol, caffeic acid, *p*-coumaric acid, gallic acid, quercetin, flavan-3-ols

Carotenoids: (all *E*)- β -carotene, (13*Z*) - or (13'*Z*)-lutein, (13*Z*)- β -carotene

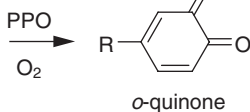
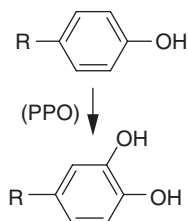


Nonfluorescent chlorophyll catabolites NCC (R = H or Oglc)
Müller *et al.* (2007)



Pheophytins a, b (R = H or Oglc)
Mendes-Pinto *et al.* (2005)

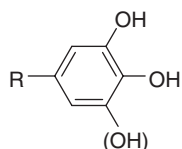
Monophenol (e.g. coumaric acid, B ring of kaempferol)



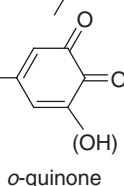
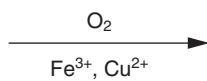
Enzymic browning
(white grape must)

Polyphenols and
other nucleophiles

Catechol phenol (e.g. caffeic acid, B ring of quercetin)



Catechol or galloyl phenol
(e.g. gallic acid, B ring of flavan-3-ols)



Nonenzymic browning
(white must and wine)

Brown polymers

Oliveira *et al.* (2011)

Fig. 17.3 Some pigments of white grape skins, flesh, must and wine, and 'browning' mechanisms.

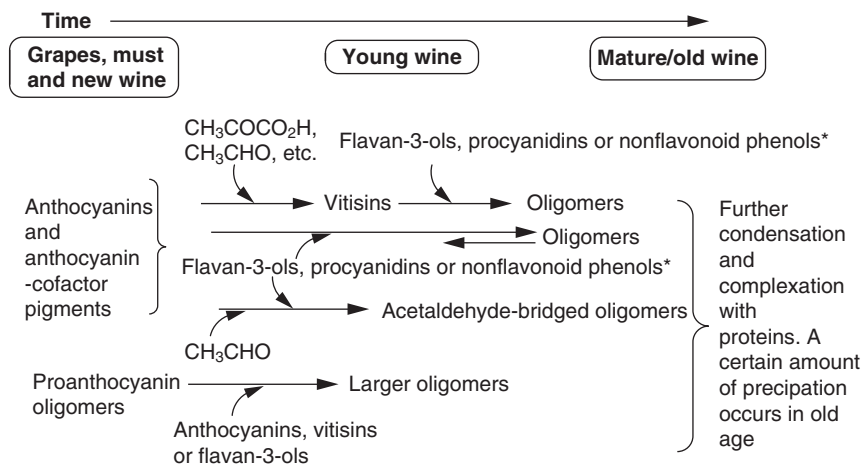


Fig. 17.4 Simplified summary of possible processes leading to colour modification in red wine. It shows tendencies: some original pigments survive into mature wine and some simple pigments are produced by cleavage of oligomers (e.g. acid-hydrolysis of procyanidins, flavan-3-ol oligomers). Many of these processes are accompanied by changes in mouthfeel (Section 17.4). * Some derived via prolonged contact with oak.

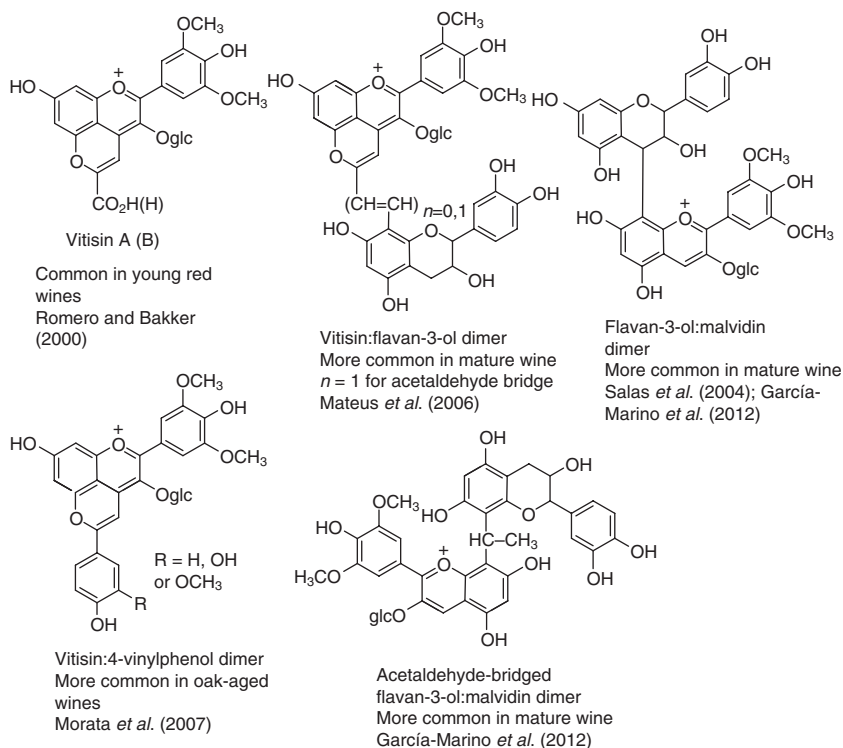


Fig. 17.5 Some pigments in red wine. Trimers and higher oligomers are also formed, and there are many other combinations.

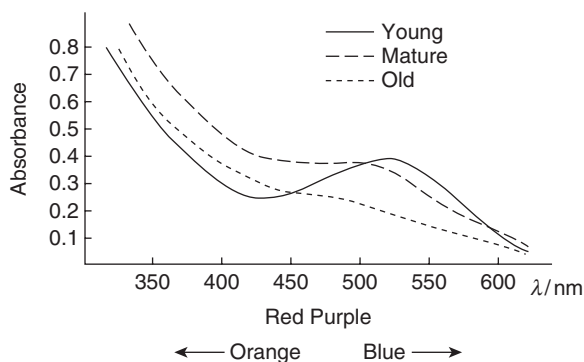


Fig. 17.6 Typical UV-visible spectra of red wines (diluted 100-fold).

spoilage processes that give white must and wine a brown colour (Fig 17.3) (Oliveira *et al.*, 2011).

Wine colour can be influenced by many factors, the most important of which are summarised in Table 17.3.

17.2.4 Colour determination and description

Red wine colour can be described satisfactorily by measuring absorbance (A) at 620 nm, 520 nm and 420 nm (on a 1 mm optical path length) using a colorimeter or UV-visible spectrophotometer (Ribéreau-Gayon *et al.*, 2000b). Four important aspects of red wine colour, intensity, hue, composition and brilliance, are explained in Table 17.4. Thus colour intensity can be related to variety and winemaking process (see Table 17.3), hue and composition can be used as indicators of age, and brilliance is related to the shape of the spectral peak at 520 nm. This relatively simple colour analysis is effective and is still used (see, for example, Cadahía *et al.*, 2009).

Additionally, the parameter ionisation value I (Fig. 17.7) can be used to estimate the ratio between monomeric anthocyanin species and various oligomeric co-pigments (Ribéreau-Gayon *et al.*, 2000b). The method is based on the fact that the latter are much less susceptible to sulphite bleaching than the former. Young red wines typically have I values of 10–30%, whereas more mature wines give higher values, reaching ~90% for very old wines.

Probably the most comprehensive system of colour measurement is that known as CIE $L^*a^*b^*$ space, devised by the Commission Internationale de l'Éclairage (Buglass and Caven-Quantrill, 2011c). Transmittance is measured under a standard set of conditions (D65 illuminator, CIE 1964 10° observer and 10 mm pathlength) at three wavelengths, 450 nm, 550 nm and 600 nm, corresponding to maximum response of human retinal cone cells for blue, green and red, respectively. The derived CIE colour-matching

Table 17.3 Some factors influencing wine colour

Factor*	Comments
1. Identity (i.e. varietal type) and ripeness of grapes	Concentration of pigments and co-pigmentation factors in grape skins/pulp depend on variety (e.g. Cabernet Sauvignon skins are richer in pigments and co-pigments than those of Grenache or Pinot Noir). Fully ripened, sound grapes give deeper coloured wines. Over-ripe white grapes (e.g. Auslese type) give deeper gold. Over-ripe or dried grapes (e.g. Ripasso or Amarone types) give browner coloration to wine.
2. Infection of ripe grapes by <i>Botrytis cinerea</i>	Bad for black grapes: gives wine of less intense brown/red colours. Good for some white grapes: gives deep golden wine (e.g. Sauternes, Tokaji, Trockenbeerenauslese).
3. Standard maceration techniques for red wine production	Grapes crushed, fermented on skins, pressed. Prolonged skin contact gives wine of deepest colour. Not extensively used for white wine production.
4. Carbonic maceration (macération carbonique)	Grapes fermented whole, then pressed. Fewer pigments extracted from skins: light colour, body, great fruitiness (e.g. Beaujolais Nouveau). Can be used for white wine production (e.g. Mâcon-Villages Nouveau).
5. Fortification of fermenting must	Spirit components (esp. aldehydes) can stabilise purple colour of young wine (e.g. ruby Port). Generally helps extract maximum colour (e.g. vintage Port, Australian liqueur Muscat).
6. Fermentation/maturation in small oak casks (1–3 years)	Red wines have more brown hues to red-purple colour. White wines have deeper gold. Colour changes due to slow air ingress, leaching of oak phenols and subsequent reactions. Too long in cask (>3 years) gives definite brown hues. See 7.
7. Ageing	<i>Cask ageing</i> is usually a slow oxidative process – see 6. Extended oxidative ageing (using partially filled casks, frequent racking, etc., as for tawny Port, Marsala, some vins doux naturels) and heat processing (as for Madeira and some vins doux naturels) gives brown hues. <i>Bottle ageing</i> . Tendency for red wines is slow, gradual browning, sometimes with loss of colour intensity. Tendency for white wines is for colour to turn deeper gold, then brown. Ageing is accelerated by leakage (ullage, which allows air ingress) and poor cellarage.

*Factors 1 and 2 relate to the status of the grapes that produce the wine, factors 3–6 refer to production methods and 7 refers to ageing processes, some of which are related to methods of production.

Table 17.4 Aspects of red wine colour from the visible spectrum

Aspect	Definition and comments
Colour intensity CI	$CI = A(620\text{ nm}) + A(520\text{ nm}) + A(420\text{ nm})$ Typical variation: 0.3–1.8
Hue (or taint, T)	$T = \frac{A(420\text{ nm})}{A(520\text{ nm})}$ Hue gives colour development toward orange. $T = 0.5\text{--}0.7$ for young wines; ~ 1.3 for oldest wines
Colour composition CC(%)	$CC\ 420\text{ (}\%) = \frac{A(420\text{ nm})}{CI} \times 100\text{ (orange)}$ $CC\ 520\text{ (}\%) = \frac{A(520\text{ nm})}{CI} \times 100\text{ (red)}$ $CC\ 620\text{ (}\%) = \frac{A(620\text{ nm})}{CI} \times 100\text{ (blue)}$
Brilliance dA(%)	$dA(\%) = \left[1 - \frac{A(420\text{ nm}) + A(620\text{ nm})}{2 \times A(520\text{ nm})} \right] \times 100$ Brilliance should be $\sim 40\text{--}60\%$ for young wines; lower for older wines

Source: Ribéreau-Gayon *et al.* (2000b).

pH = ~3.5 (normal wine pH)	pH = ~1
10.0 ml wine + 2.0 ml water Absorbance at 520 nm = A_1	1.0 ml wine + 7.0 ml 0.1 M HCl + 2.0 ml water Absorbance at 520 nm = A_1'
Absorbance at 520 nm = A_2	1.0 ml wine + 7.0 ml 0.1 M HCl + 2.0 ml NaHSO ₃ soln ($d = 1.24\text{ g/ml}$)
$\Delta A(\text{pH } 3.5) = 1.2(A_1 - A_2)$	Absorbance at 520 nm = A_2'
	$\Delta A(\text{pH } 1) = \frac{100(A_1' - A_2')}{95}$
$I = 1.14 \frac{\Delta A(\text{pH } 3.5)}{\Delta A(\text{pH } 1)} \times 100$	

Fig. 17.7 Method for determination of anthocyanin:oligomer ratio of red wine (Ribéreau-Gayon *et al.*, 2000b). Absorbance measurements (A) on 1 mm optical pathlength.

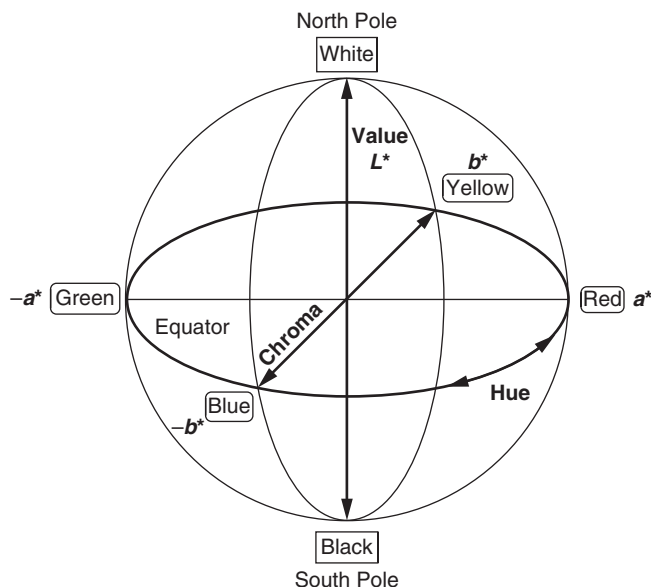


Fig. 17.8 The CIE $L^*a^*b^*$ colour space. Hue is a continuum of colours around the equator. Value describes brightness and chroma (C^*) relates to colour strength. $C^* = (a^{*2} + b^{*2})^{1/2}$ and the hue angle is defined as $h^* = \arctan(b^*/a^*)$ (Pissarra *et al.*, 2003). It describes the colour nuance. Young red wines, with characteristic deep purple, almost black colour (e.g. of Ch. Latour) should have blue-red hue, value (L^*) in the southern hemisphere and a chroma measurement close to the circumference. Lighter young red wines (e.g. of Valpolicella) will have a chroma measurement closer to the N–S axis. Figure adapted from Buglass and Caven-Quantrill (2011c), by kind permission of J Wiley and Sons Ltd, Chichester, UK.

functions ('tristimulus values') are usually transformed into three colour parameters L^* , a^* and b^* , which, when plotted in three-dimensional space, describe the hue, strength and brightness of red wine colour (Fig. 17.8). This requires specialist spectrophotometric equipment, standard conditions and computer software, and forms the basis of the Organisation Internationale de la Vigne et du Vin (OIV) standard method (OIV, 1990).

CIE $L^*a^*b^*$ measurements have been used to characterise vitisins in young Port wines (Bakker and Timberlake, 1997; Romero and Bakker, 2000, and references to earlier work therein) and, more recently, for the assessment of pulsed electric field (PEF) treatment of black grapes prior to conversion into wine and maturation in small oak casks (Puértolas *et al.*, 2010). PEF, combined with oak ageing, was found to improve chromatic characteristics.

It is felt by some that the CIE $L^*a^*b^*$ method of assessing red wine colour is rather too complex for the small winery, so simpler alternative methods have been proposed (Ayala *et al.*, 1997, 1999; Pérez-Magariño and

González-San José, 2002; Pérez-Caballero *et al.*, 2003). However, it has been proposed that a better assessment of colour can be obtained by measurement of refractive index and turbidity, as well as transmission, using a multifunctional reflectometer (Mutanen *et al.*, 2007). Here, CIE $L^*a^*b^*$ evaluation, coupled with principal component analysis (PCA), refractive index analysis and turbidity analysis can be used to give an 'optical fingerprint' to individual wines.

More recently, an analysis of various dilutions of phenolic fractions obtained by gel permeation chromatography (GPC) of single variety and blended wines (García-Marino *et al.*, 2012) has shown that spectroradiometry, rather than transmission spectrophotometry or diffuse reflectance spectrophotometry, gives results that are more akin to comparative interpretations by the human eye (visual sensual interpretation). It was suggested that this is because spectroradiometry is a non-contact reflectance method that relies on the interaction of environmental light (from a light source at a specific distance from the sample) with the sample.

Some of the applications of UV-visible spectrophotometry to wine colour involve chemometric or statistical analysis of the spectra of a large number of known samples to build a model, with which it is possible to predict various parameters relating to origin (origin, grape variety, age, winemaking method, vintage, etc.) or quality (acidity, total anthocyanin content, total phenolic content, free SO₂ content, etc.) of new samples. This methodology has been reviewed recently (Saurina, 2010) and some examples are given in Table 17.5.

Description of white wine colour is rather more difficult, since there is a lack of strong absorption in the visible region (Ribéreau-Gayon *et al.*, 2000b). Nevertheless, it is possible to employ chemometric/statistical analysis (usually using multivariate techniques) of corrected spectra to predict wine age, and hence quality. Chemometric/statistical analysis of corrected UV-visible spectra of known Madeira (fortified) wine samples gave a rapid, reliable and inexpensive method for long- and short-term prediction of wine age (Pereira *et al.*, 2011; see Table 17.5).

17.2.5 Determination of total and individual colour components

Total 'free' anthocyanin content (TAC) of red wine can be determined directly by the colorimetric pH shift or bisulphite bleach methods (Fig. 17.9) (Amerine and Ough, 1980b; Ribéreau-Gayon *et al.*, 2000b), the latter being thought to be preferable, since it is insensitive to the presence of free 'sulphite' in the wine (which is present in quantity in all but organic and very old wines). It relies on the fact that 'free' anthocyanins are bleached by sulphite, whereas most derivatives and condensation products are not. Indirect measurement of TAC can be made by combining (often routine) TAC data, for example obtained as in Fig 17.9, usually with FTIR spectral data (rather than UV-Vis data) for known samples and using chemometric/

Table 17.5 Some examples of chemometric/statistical analysis of wine colour using UV-visible spectra

Example/ reference	Aim	UV-vis data preprocessing*	Selection of variables [#]	Chemometric/ statistical methods
Pereira <i>et al.</i> (2011)	Prediction of age of Madeira wines	1st and 2nd order differentiation of smoothed data	WT-GA	PLSR
Acevedo <i>et al.</i> (2007)	Classification of Spanish red and white wines	–	SFS to select a maximum of 4 wavelengths	SVM
Urbano <i>et al.</i> (2006)	Classification of Spanish wines	1st order differentiation of data	PCA to select 300–400 nm range	PCA (exploratory) SIMCA

* Needed to eliminate undesirable systematic variation in data.

[#] Needed to optimise data with greatest predictive power.

Key:

PCA = principal components analysis.

PLSR = partial least squares regression.

SFS = sequential forward selection.

SIMCA = soft independent modelling of class analogy.

SVM = support vector machines.

WT-GA = wavelet transformation combined with genetic algorithm.

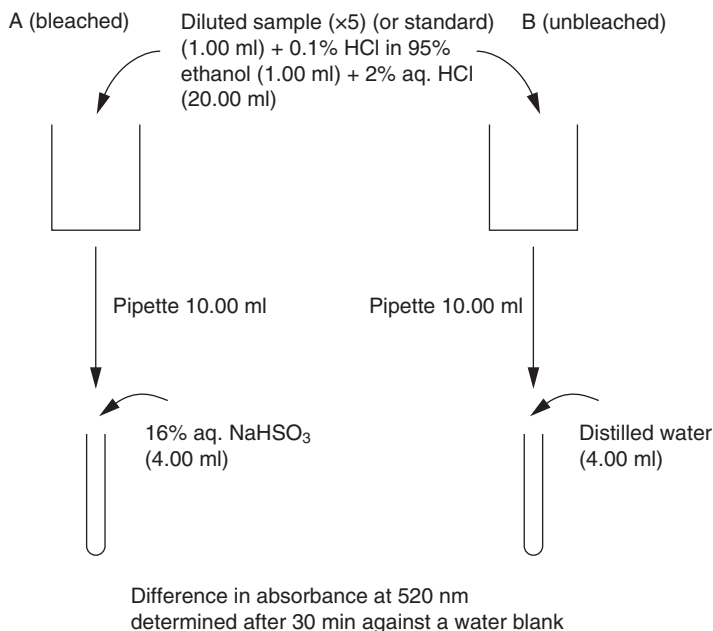


Fig. 17.9 Summary of bisulphite bleach method for estimation of 'free' anthocyanin content of musts and beverages. Cyanidin hydrochloride standards (e.g. 100–10 mg/l) are used to draw a calibration plot: the results are quoted as mg/l of cyanidin, taking into account the $\times 5$ dilution factor. Based on Buglass and Caven-Quantrill (2011c) and reproduced by kind permission of John Wiley and Sons Ltd, Chichester, UK.

statistical methods to build a model to predict TAC values for unknowns belonging to the same or similar class (see, for example, Jensen *et al.*, 2008).

Total anthocyanins in black grape skins can be determined by a non-destructive method that measures chlorophyll fluorescence excitation spectra by reflectance from the skin (Agati *et al.*, 2007). Likewise, the ripeness of white (actually green-gold) grapes can be estimated by measurement of chlorophyll fluorescence (Kolb *et al.*, 2006).

Individual anthocyanins in wine, must or skin can be determined (quantified and/or identified) directly by HPLC, usually with UV-Vis (especially diode array detection, DAD) and/or MS detection. Reversed phase (RP) mode with C18 standard analytical columns is favoured, but mini- and microcolumns are also useful when MS is the detection mode. Elution is usually performed with a two-solvent gradient containing formic acid. The most favoured MS analysers are quadrupole (Q) (or QqQ for MS² tandem MS) or quadrupole ion trap (QIT). Electrospray ionisation (ESI) is the most widely used ionisation technique, operating in positive ion mode for anthocyanin analysis and negative ion mode for polyphenols (Section 17.6). Some examples are given in Table 17.6.

Table 17.6 Selection of HPLC methods for the quantification and identification of anthocyanins and other pigments in grape skins and red wines

Example/ reference	Wines/analytes	Stationary phase	Eluent/flow rate	Detection method(s)	Comments
Dugo <i>et al.</i> (2004a)	Sicilian Carbernet Sauvignon and Merlot wines	Waters Symmetry C18 (3.5µm) 150mm × 1mm (i.d.) (micro column) Supelco Discovery Biopeptide C18 (3µm) 100mm × 0.32mm (i.d.) (capillary column)	A – H ₂ O:HCOOH (9:1)B – H ₂ O:HCOOH:MeCN (4:1:5) 40µl/min Gradient elution programme as above	UV-Vis at 518nm; ESI-MS (Q)	Accurate flow-splitter between pump and injector used for micro HPLC.
Heredia <i>et al.</i> (2010)	Syrah wines of SW Spain	Agilent Zorbax C18 (5µm) 250mm × 4.6mm (i.d.)	A – H ₂ O:MeCN:HCOOH (0.3:1:8.7)B – H ₂ O:MeCN:HCOOH (5:1:4) 0.8ml/min Gradient elution programme	UV-Vis (diode array), with quantification at 525nm	Direct injection of filtered wines. Investigation of pre-fermentative cold maceration winemaking technique.
Salas <i>et al.</i> (2004)	Reaction products of Mv3glc and B2-3'OG proanthocyanidin in wine-like medium	Merck Lichrospher 100-RP18 (5µm) 250mm × 2mm (i.d.)	A – H ₂ O:HCOOH (9.5:0.5)B – MeCN:A (8:2) 250µl/min Gradient elution programme	ESI-MS (QIT for MS ⁿ)	SPE used for fractionation of hemisynthesis reaction products. Oligomers formed by acid hydrolysis.
Morata <i>et al.</i> (2007)	Products of reaction between malvidin 3-O-glucoside, vitisin A and added carbonyl compounds	Waters Novapak C18 (4µm) 250mm × 4.6mm or 3.9mm (i.d.)	A – H ₂ O:HCOOH (9:1)B – MeOH	UV-Vis (DAD; 400–700nm); ESI-MS (Q)	Acetaldehyde or pyruvic acid added to young Tempranillo wines.

González-Paramás <i>et al.</i> (2006)	(epi) Catechin-anthocyanin oligomers in grape skins of Tempranillo	Phenomenex AQUA C18 (5 µm) 150 mm × 4.6 mm (i.d.)	A – 0.1% TFA in waterB – CH ₃ CN Gradient elution programme	UV-Vis at 520 nm; ESI-MS (QIT for MS ⁿ)	Anthocyanins extracted from grape skins using 5% 1 M HCl in MeOH; acids removed by column chromatography.
Vidal <i>et al.</i> (2004a)	Anthocyanin oligomers in skins of Shiraz (Syrah) grapes after fractionation from monomers using multilayer coil counter current chromatography	Phenomenex Synergi Hydro-RP 80 Å (4 µm) 150 mm × 2.1 mm i.d.	A – H ₂ O:HCOOH (9.5:0.5)B – MeCN:H ₂ O:HCOOH (8.0:1.5:0.5) Gradient elution programme	UV-Vis at 280 and 520 nm; ESI-MS (QqQ)	A T-piece split the flow between MS (22.5%) and UV-Vis (77.5%) detectors.
Pomar <i>et al.</i> (2005)	Anthocyanins in skins of Galician black wine grapes (50 varieties)	Eka C18 Kromasil 100 (4 µm) 250 mm × 4 mm (i.d.)	A – MeCN:H ₂ O:HCOOH (4.5:1:4.5)B – H ₂ O:HCOOH (9:1) 1.0 ml/min Gradient elution programme	UV-Vis at 313 nm and 546 nm; DAD 270–600 nm	Combination of ANOVA and PCA was used to build model and predict identity of grape variety from anthocyanin determination.
de Villiers <i>et al.</i> (2011)	Anthocyanins and condensation products in wine	Waters Acquity BEH C18 (1.7 µm) 100 mm × 2.1 mm (i.d.) and Waters XBridge C18 (5 µm) 250 mm × 4.6 mm (i.d.)	A – H ₂ O:HCOOH (9.25:0.75)B – MeCN:HCOOH (9.25:0.75)	UV-Vis at 500 nm; ESI-MS (Q-TOF)	Partial loop with needle overfill mode used for LCMS.

The powerful structure elucidatory value of MS is demonstrated by the determination of new anthocyanin vitisin-type derivatives (Morata *et al.*, 2007) and condensation products (Salas *et al.*, 2004; Vidal *et al.*, 2004a; González-Paramás *et al.*, 2006).

Samples may be analysed directly by these methods or fractions may be provided by, for example, column chromatography or solid phase extraction (SPE) (González-Paramás *et al.*, 2006), CCC (Vidal *et al.*, 2004a, 2004b), GPC (García-Marino *et al.*, 2012), preparative HPLC or solvent extraction (including using supercritical water – Ju and Howard, 2005).

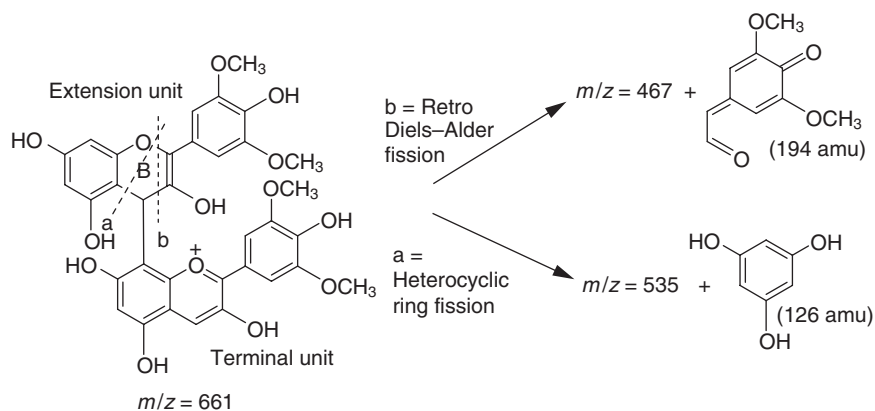
In general, the positive ion mass spectra of anthocyanins and condensation derivatives show initial fragmentations involving loss of sugar unit or water from the molecular ion. Subsequent fragmentations occur by dehydration, retro Diels-Alder fission or heterocyclic fission of ring C of the non-flavylium moiety (the 'extension unit') (Fig. 17.10).

Recently, indirect determination of individual monomeric anthocyanins in freshly bottled young red wine has been achieved by a chemometric/statistical method that combines HPLC and Fourier transform mid-infrared (FTMIR) data (Romera-Fernández *et al.*, 2012). Reference values for anthocyanin concentration were obtained using RP HPLC-DAD data, which were used to calibrate the model. A calibration model using PLSR was built from 153 Rioja wines, the prediction of anthocyanin content being validated by internal and external validation sets.

For the determination of phenolic substances (see Section 7.6) (including anthocyanins and derivatives) in wine, capillary zone electrophoresis (CZE) rivals solvent gradient HPLC in effectiveness. Accuracy and precision are similar, but CZE experimental time is shorter and there is no need for a solvent gradient. An applied potential of ~25 kV is common, Sodium tetraborate is often used as background electrolyte, along with 10–15% added methanol and UV detection is common (Sáenz-López *et al.*, 2003).

More recently, reactions between malvidin 3-glucoside and hydroxycinnamic acids in a model wine solution have been studied by CZE using 50 mM sodium tetraborate buffer, with 10% methanol (pH = 9.4), with UV detection, although liquid chromatography–mass spectrometry (LC-MS) was used to identify the products (Sáenz-Navajas *et al.*, 2009).

Along with MS, NMR spectroscopy has played a major role in the determination of the structures of anthocyanins, their derivatives and condensation oligomeric pigments (as well as colourless oligomeric species – see Section 17.6) in grape skins, must and wine. One-dimensional NMR data is still sometimes used, but generally the more complex structures also require study by two-dimensional techniques, such as COSY-90, HETCOR, HMBC, HMQC, INADEQUATE, INEPT, TOCSY and others. These methods give information on short- or long-range connectivity (through-bond couplings; usually ^{13}C — ^1H) and generally involve polarisation transfer between groups of coupled nuclei. NOESY is a double irradiation technique that utilises the through-space Overhauser effect in order to give information on the spatial proximity of non-bonded nuclei. Selected examples are given in Table 17.7.



B-Type flavene-flavylium dimer of malvidin (Vidal *et al.*, 2004a)

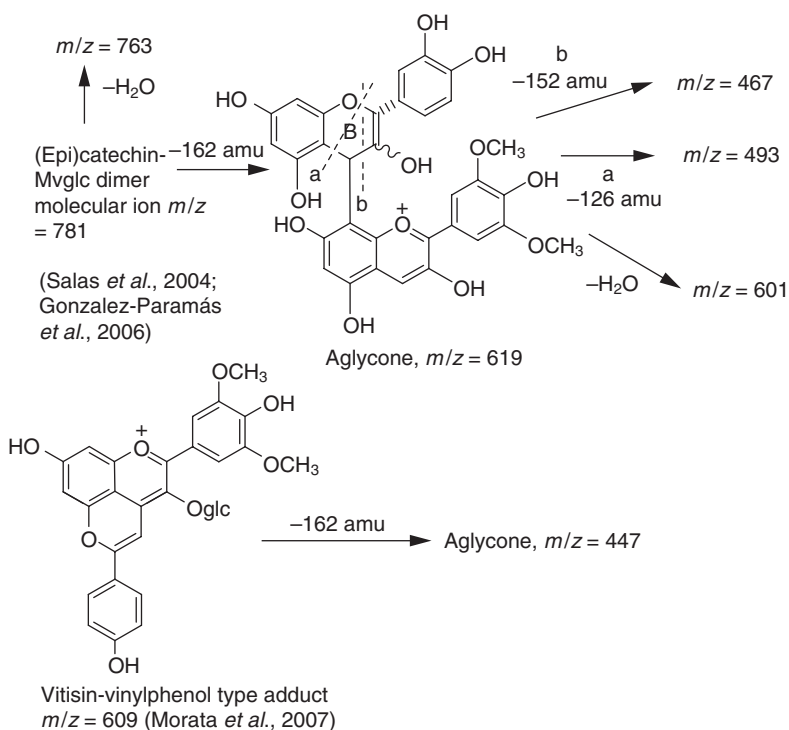
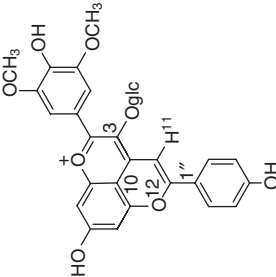
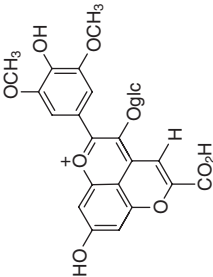
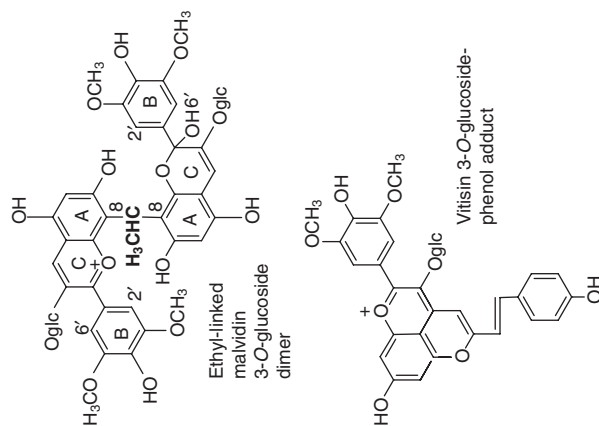


Fig. 17.10 Some fragmentation pathways from mass spectra of oligomeric anthocyanin pigments from grape must and wine.

Table 17.7 Structures of selected anthocyanin and polyphenol based pigments determined by NMR spectroscopy

Structure and name	Reference	Technique	Comments
 Malvidin 3-O-glucoside 4-vinylphenol adduct	Schwarz <i>et al.</i> (2003)	HMBC	From wine of pinotage, isolated using HSCCC. Two- and three-bond correlations were observed between the proton at C11 and C1'', C3, C10 and C12.
 Vitisin A	Fulcrand <i>et al.</i> (1998)	HMBC	A common pigment of young red wine. Correlations were noted between the proton at C11 and C3, C4, C10, C12 and CO ₂ H.



Atanasova *et al.* (2002)

NOESY

Through-space couplings were observed between the ethyl moiety (in bold) and H2', H6' and OCH₃ of rings B.

Mateus *et al.* (2006)

1-D NMR, *g*COSY, *g*HMBC and *g*HMQC

Found in 2-year old Port wines. Protons were assigned using 1D and *g*COSY; Carbons were assigned using *g*HMBC and *g*HMQC.

HSCCC: high-speed counter current chromatography, *g*COSY: gradient-selected correlation spectroscopy, *g*HMBC: gradient-selected homonuclear multiple bond coherence, *g*HMQC: gradient-selected heteronuclear multiple quantum coherence.

17.3 Analysis of odour (aroma): an introduction

17.3.1 Explanation of odour

The human olfactory system contains millions of receptors located high in the nasal cavity and it is capable of detection and discrimination of thousands of different odour molecules. In humans, the sense of smell is generally considered less critical to survival than the other special senses; however, the potential importance of this sense has been given new consideration recently in industrial and academic research due to its enormous impact on the quality of life (Youngentob, 1999).

The sensory combinations of colour, taste and smell (and occasionally sound) are routinely used to appreciate wine quality. The word 'flavour' is usually employed to indicate the combination between smell (or odour) and taste (Clarke and Bakker, 2004b).

When wine is taken into the mouth, smell or odour is firstly perceived via the nose during normal 'orthonasal' olfaction. This is followed by swallowing whereby flavour is perceived via 'retronasal' olfaction. Some aroma chemicals also interact with the trigeminal nerve endings of the olfactory system to produce a sensory perception that is a mixture of odour and trigeminal stimulation (such as acetic acid in wine). Therefore, flavour is often a complex perception that integrates three different sensory systems: odour, taste and the chemosensory receptors responsible for hot, cool, dry, irritant or pungent, etc. (Ferreira, 2010). For more information regarding olfaction and taste of wine, see Buglass and Caven-Quantrill (2011a) and Jackson (2000).

17.3.2 Summary of volatile organic compounds in wine

It is now widely known that numerous aroma compounds, some of which occur only in trace amounts, can be distinctive to the aroma and hence flavour of wine. In the last five decades, the introductions of state of the art aroma isolation and analysis techniques have enabled wine flavour chemists to study wine with respect to the volatile aroma compounds present; more importantly the varietal impact volatile organic compounds.

Fusel alcohols, acids and fatty acid esters quantitatively form the greatest part of flavour (aroma). Also important are carbonyl compounds, phenolic substances, sulphur and nitrogen compounds, lactones, acetals and numerous other compounds, such as terpenoids and norisoprenoids (Table 17.8). Varietal impact aroma compounds have been discussed in depth by a number of researchers (Clarke and Bakker, 2004a; Ribéreau-Gayon *et al.*, 2006; Ferreira, 2010, and references therein). Examples are summarised in Table 17.9.

17.3.3 Analysis problems

A majority of the organoleptic important components of wine are present at very low levels (parts per billion, ppb or $\mu\text{g/l}$) or even trace levels (parts per trillion, ppt or ng/l) and therefore require highly sensitive methods of

Table 17.8 Summary of volatile organic compounds in wine

Chemical class	Examples with some typical concentrations or ranges of concentration ($\mu\text{g/l}$ or ppb wine) ^a
Alcohols	Ethanol (1×10^8) Isobutyl alcohol (1×10^5) 3-Methyl-1-butanol (Isoamyl alcohol) (2×10^5) 2-Phenethyl alcohol (5×10^4)
Carboxylic acids	Formic acid (5×10^4) Acetic acid (5×10^5)
Esters	Ethyl acetate ($4500\text{--}1.9 \times 10^5$) ^b Diethyl succinate ($100\text{--}1400$) ^b Isoamyl acetate ($40\text{--}6100$) ^b 2-Phenethyl acetate ($200\text{--}5100$) ^b
Aldehydes & ketones (carbonyls)	Acetaldehyde (1×10^5) Acetoin (Acetyl methyl carbinol) (1×10^4) Diacetyl ($20\text{--}5400$) ^b
Acetals	1,1-Diethoxyethane (acetal) ($4.5 \times 10^4\text{--}6.0 \times 10^4$ in sherry)
Terpenoids	Linalool ($6\text{--}473$) α -Terpineol ($3\text{--}87$) Citronellol ($2\text{--}12$) Nerol ($4\text{--}135$) Geraniol ($5\text{--}506$) Ho-trienol ($25\text{--}127$)
Thiols/thioesters	4-Mercapto-4-methyl-pentan-2-one ($0\text{--}0.12$) 3-Mercaptohexyl acetate ($0\text{--}0.5$) 3-Mercaptohexanol ($0.15\text{--}3.5$) 4-Mercapto-4-methyl-pentan-2-ol ($0.015\text{--}0.15$) 3-Mercapto-3-methyl-butan-1-ol ($0.02\text{--}0.15$) Benzenemethanethiol ($0.005\text{--}0.02$)
Phenolics	Vanillin (4.5×10^4) ^c 2-Methoxyphenol (3600) ^c 2-Methoxy-4-vinyl phenol ($4500\text{--}2.5 \times 10^4$) ^c
Lactones	γ -Butyrolactone (1000) β -Methyl- γ -octalactone ((<i>Z</i>) & (<i>E</i>) Oak lactones) Wine lactone (100) ^c (<i>Z</i>)-6-Dodeceno- γ -lactone ($140\text{--}270$) ^c
Pyrazines	2-Methoxy-3-isobutyl pyrazine ($0.0005\text{--}0.05$) 2-Methoxy-3-isopropyl pyrazine 2-Methoxy-3- <i>sec</i> -butyl pyrazine 2-Methoxy-3-ethyl pyrazine
C ₁₃ Norisoprenoids	β -Damascenone ($0.005\text{--}6.5$) β -Ionone ($0\text{--}2.5$)

^aRibéreau-Gayon *et al.* (2006) and references therein.^bClarke and Bakker (2004a) and references therein.^cGuth (1997b).

instrumental analysis for their detection (qualification) and quantification from the wine matrix (all of the components making up the sample containing an analyte), or need to be concentrated by an extractive sample preparation technique to achieve a representative aroma concentrate prior to analysis.

The quantitative analysis of the volatile compounds present in wine is extremely demanding owing to the complex chemical composition of the volatile fraction and that the individual volatile compounds can be present in a wide concentration range. For example, Rapp *et al.* (1978) estimated that the concentrations of volatile flavour compounds in wines range between 1 g/l and 1 ng/l (a factor of 10^9).

Table 17.9 Varietal impact aroma compounds

Impact compound ^a	Aroma characteristics ^b	Perception threshold (ng/l or ppt) ^d	Example wine source	Reference
Linalool	Citrus, floral, bois de rose, woody	50000 15000 ^e	Muscat	Ribéreau-Gayon <i>et al.</i> (2006)
(Z)-Rose oxide	Green, red rose, spicy, fresh geranium	200 ^e	Gewürztraminer	Guth (1997b)
β-Damascenone	Woody, sweet, fruity, earthy, green floral	40–60	Pedro Ximénez	Campo <i>et al.</i> (2008)
4-Mercapto-4-methylpentan-2-one	Sulphurous, meaty, cat-urine, black currant	0.6 ^e	Scheurebe/ Gewürztraminer	Guth (1997b) Darriet <i>et al.</i> (1995)
3-Mercaptohexan-1-ol	Sulphurous, fruity, tropical	60	Sauvignon blanc Cabernet-Sauvignon Merlot	Bouchilloux <i>et al.</i> (1998)
3-Mercaptohexyl acetate	Floral, fruity, pear, tropical, passion fruit	4	Verdejo	Campo <i>et al.</i> (2005)
Rotundone	Spicy, peppercorn ^e	16 ^c	Shiraz	Wood <i>et al.</i> (2008)
Diacetyl	Strong butter, sweet creamy pungent caramel	6500 – 15000 ^f	Chardonnay	Bartowsky <i>et al.</i> (2002)
Isoamyl acetate	Sweet, fruity, banana, solvent, ethereal		Tempranillo	Ferreira <i>et al.</i> (2000b)
(E)-Whisky lactone	Coconut, woody, maple, lovage, toasted, nutty	490000 ^f	Oak aged wines	Ferreira (2010)
Sotolon (3-hydroxy-4,5-dimethyl-2(5H)-furanone)	Extremely sweet, strong caramel, maple, burnt sugar, coffee	8000 5000 ^e	Pedro Ximénez Scheurebe/ Gewürztraminer	Campo <i>et al.</i> (2008) Guth (1997b)

Furfurylthiol (furfuryl mercaptan)	Roasted coffee, sulphurous, burnt match, savoury, meaty	0.4 ^g	Champagne	Tominaga <i>et al.</i> (2003)
Benzyl mercaptan	Sharp, sulphurous, onion, garlic, horseradish, mint, coffee	0.3 ^g	Champagne	Tominaga <i>et al.</i> (2003)
Dimethyl sulphide (DMS)	Sulphurous, onion, sweet corn, vegetable, cabbage, tomato, fishy, berry fruity	10000 ^e	Syrah Grenache Noir Scheurebe/ Gewürztraminer	Segurel <i>et al.</i> (2004) Guth (1997b)
Methional (3-(methylthio) propanal)	Vegetable oil, creamy tomato, potato skin, French fry, yeasty, breadly, savoury, meaty, brothy	–	Chardonnay	Ferreira (2010)
Phenylacetaldehyde	Honey, floral rose, sweet, powdery, fermented, chocolate	–	Sauternes Pedro Ximénez	Sarrazin <i>et al.</i> (2007) Campo <i>et al.</i> (2008)

^aFerreira (2010).

^bwww.thegoodscentscompany.com.

^cWood *et al.* (2008).

^dRibéreau-Gayon *et al.* (2006) and references therein.

^eGuth (1997b).

^fClarke and Bakker (2004a) and references therein.

^gTominaga *et al.* (2003).

Wine extracts typically contain high concentrations of fusel alcohols, fatty acids and fermentation esters (more than 99%), whereas the remainder of the extract, which contributes significantly to the bouquet of a wine (very often containing significant flavour compounds with extremely low odour threshold values (OTV), i.e. a concentration of odourant just barely sufficient to achieve sensory recognition) is usually composed of hundreds of compounds present at concentrations about 10^6 – 10^8 times lower than the fusel alcohols (Maarse and Visscher, 1989; Etievant, 1991). The human sensory organs display sensitive and variable reactions to these amounts of aroma compounds. Boeckh (1972) and Guadagni *et al.* (1963) reported that threshold values differ considerably and could vary between 10^{-4} and 10^{-12} g/l. For example, Demole *et al.* (1982) quoted the odour threshold value for 1-*p*-menthen-8-thiol as 0.1 ng/l, whereas Guth (1997a) gave the value of 0.00001–0.00004 ng/l for wine lactone.

Extraction techniques for wine analysis generally fall into four categories: (i) liquid extraction (or solvent extraction including supercritical fluid extraction or SFE, Section 17.3.4) (ii) distillation methods (Section 17.3.5), such as simultaneous steam distillation-extraction (SDE) and solvent-assisted flavour evaporation (SAFE), (iii) the sorption techniques (Section 17.4.3) SPE, solid-phase micro extraction (SPME), stir bar sorptive extraction (SBSE) and headspace sorptive extraction (HSSE), and (iv) headspace methods (including static headspace and purge and trap, Section 17.5.1).

Solvent extraction is historically the most commonly adopted sample preparation technique for the analysis of wine volatiles (Buglass and Caven-Quantrill, 2011b). However, recent environmental concerns over the use of certain solvents (such as chlorofluorocarbons) have resulted in limitations in the availability of these solvent varieties for research purposes. Increasing costs for solvent disposal, as well as safety and environmental concerns are further prompting analytical chemists to search for sample preparation methods that minimise or, as a perfect scenario, eliminate the use of organic solvents. Because of this, the sorptive extraction techniques have gained popularity mainly due to the advantages in sensitivity, speed, robustness, safety and 'greenness' over previously established and widely accepted methods.

Although only the basics are covered in this chapter, for more information the reader is directed to a recent extensive review of the last five decades, concerning the sample preparation and analysis techniques used to study the organoleptic compounds of wine and other alcoholic beverages (Buglass and Caven-Quantrill, 2011b).

17.4 Extraction techniques for analysis of odour (aroma)

17.4.1 Liquid extraction techniques

Liquid extraction relies on the distribution of a solute between two immiscible solvents; usually an aqueous solution of analytes (for example aroma/

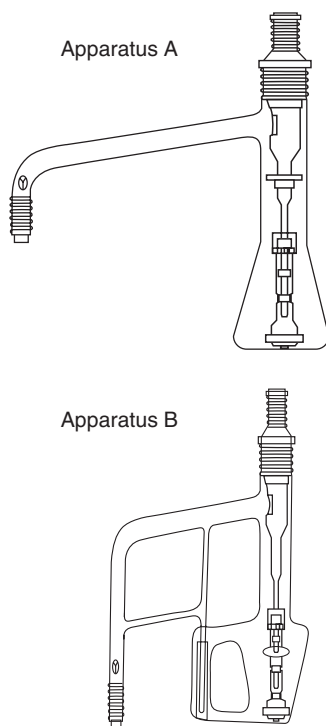


Fig. 17.11 Normag[®] liquid-liquid extractors (A) for extraction with solvents of lower density than water (B) for extraction with solvents of higher density than water. Reproduced with permission of Normag Labor-und Prozesstechnik GmbH, Ilmenau, Germany.

flavour components) and an organic solvent. An analyte which is soluble in both water and solvent phases will distribute itself between these two phases and equilibrium will be achieved when the free energy of the solute is the same in each phase. An extract is obtained by stirring, mixing or agitating a wine sample with a suitable organic solvent and then separating the resulting solvent phase. This can be carried out manually with a standard laboratory separating funnel (batch extraction) or automatically with a continuous liquid-liquid extraction apparatus (see Fig. 17.11).

The choice of extraction solvent is extremely important as it must have a low enough boiling point so that it can be easily removed from the extract (without a significant loss or change of the original sample aroma volatiles) and ideally be able to extract polar (such as alcohols and carbonyl compounds) and non-polar (such as terpenoids and esters) wine components, to yield a final extract with an aroma/flavour profile reminiscent of the original sample (an important prerequisite of any isolation technique applied to the study of aroma/flavour profiles).

Many continuous extraction devices operate on the same general principle (Fig. 17.11). This consists of distilling the extracting solvent from a round-bottomed flask that serves as a boiler/receiving flask, condensing the solvent and passing the condensate through the solution to be extracted that is held and mixed in an extractor vessel. During operation when the extracting solvent is lighter than the aqueous phase (Fig. 17.11, apparatus A) the solvent rises to the surface of the aqueous phase and eventually the extracting liquid flows back into the receiving flask (and boiler), from where it is evaporated and recycled. The extracted analytes remain in the receiving flask. For extraction solvents heavier than water (Fig. 17.11, apparatus B) the condensed solvent is allowed to drop down through the phase being extracted and then it flows to the outer part of the extractor. Spent solvent rises in the outer cylinder of the extractor and exits into the boiler for stripping and recycling. Therefore, the equivalent of several hundred batch extractions with fresh solvent can be achieved with the same starting solvent in a few hours and requires minimal attention once underway.

Hardy and Ramshaw (1970) found they could isolate and quantitatively analyse minor volatile constituents of Riesling white table wine, using the solvent trichlorofluoromethane (freon 11). This solvent does not extract water or ethanol, and was reported to extract other alcohols, carbonyls and esters with recoveries of between 70 and 100% in 17 hours by continuous liquid–liquid extraction.

The analysis of the wine extract was carried out by GC and GC-MS. Forty-five compounds were successfully identified. This enrichment technique has also been used for numerous studies and also recently for the comparison of odour active compounds in Sherry wines processed from ecologically and conventionally grown Pedro Ximenez grapes (Moyano *et al.*, 2009).

Ferreira and coworkers (1993) developed a fast and quantitative determination of wine flavour compounds using microextraction with freon 113 as extraction solvent. It was concluded that the proposed method allowed quantification of 26 wine flavour compounds with detection limits in the $\mu\text{g/l}$ range. Precision, linearity and accuracy of the method was tested using different wines and synthetic mixtures. Relative precision was shown to be better than 3%.

Many alternative solvents and solvent mixtures have been utilised to study the volatile composition of wines using the liquid–liquid extraction technique. Examples include: dichloromethane (Hernanz *et al.*, 2009) pentane/dichloromethane mixtures (Sánchez Palomo *et al.*, 2007) and hexane/diethyl ether 1:1, v:v (Rogerson *et al.*, 2002). Diethyl ether/pentane (1:1, v:v) has also been utilised to study the volatile composition of Mencia wines (Calleja and Falque, 2005).

A comparative study of the ability of different solvents and adsorbents to extract aroma compounds from alcoholic beverages has recently been

conducted (Ferreira *et al.*, 2000a). Seven liquid solvent systems – dichloromethane, dichloromethane/pentane (1:1, v:v), freon 113, diethyl ether/pentane (1:1 and 1:9, v:v), ethyl acetate/pentane (with and without an additional salting-out effect) (1:3 and 1:20, v:v) – were comparatively studied along with seven SPE systems. It was concluded that the best liquid extraction solvents were dichloromethane and freon 113 with salt (ammonium sulphate). Tominaga and co-workers (Tominaga *et al.*, 1998a, 1998b) have also reported a method for the specific analysis and identification of volatile thiols from *Vitis vinifera* L. Cv. Sauvignon Blanc wines using dichloromethane as extraction solvent.

A simple and rapid method has also been described for the extraction of wine volatile compounds based on ultrasonic assisted extraction (UAE) using pentane/diethyl ether (1:2, v:v) as extraction solvents (Vila *et al.*, 1999). Performance of the method was evaluated, and the procedure applied to the analysis of aroma compounds in white wines from ‘Condado de Huelva’ (Spain). It was concluded that the method had advantages over other extraction methods, such as higher reproducibility and the possibility of the simultaneous extraction of several samples. The proposed method allowed the quantification of 24 wine flavour compounds.

A similar UAE, using dichloromethane as extraction solvent, has been optimised for the extraction of volatile compounds from wines (Cabredo-Pinillos *et al.*, 2006).

In SFE the extraction phase (usually carbon dioxide, CO₂) is above its critical temperature and is therefore in a supercritical state. Supercritical carbon dioxide is known to have solvent polarity properties similar to diethyl ether and to be particularly selective for esters, aldehydes, ketones and alcohols.

The primary advantage of optimised SFE is that a highly selective extraction can be conducted if necessary. Exploitation of the selectivity aspects allows the preparation of extracts that can be easily analysed using chromatographic methods. Other benefits of this methodology are short extraction times and lower reactivity of thermally labile and oxygen sensitive compounds.

Kárasek *et al.* (2003) compared direct SFE of wines with CO₂ and conventional indirect extraction by SFE of the sorbent used for SPE (Section 17.4.3) of the same wine samples (SPE-SFE). It was concluded that the direct continuous SFE of wine resulted in a more specific and representative fingerprint of the sample, as revealed by the GC analysis of the extracts. Direct SFE was preferable because it was more straightforward than SPE-SFE. The additional analyte–sorbent interactions and sorption/desorption steps involved in SPE-SFE resulted in unfavorable alteration of the GC fingerprint, reducing both the peak number and the information obtained. In addition, the study showed the feasibility and expediency of direct SFE for the purpose of wine analysis with the goal to classify the samples according to the respective wine varieties. The superior robustness of direct SFE

compared with SPE-SFE of wines was also apparent from multivariate statistical processing of the analytical results.

17.4.2 Distillation methods

One of the most often cited sample preparation methods for the isolation of volatile compounds from foodstuffs and beverages is simultaneous steam distillation–extraction (SDE) as invented by Likens and Nickerson (1964); (Nickerson and Likens, 1966) for the analysis of hop oil (Fig. 17.12).

Typically with this technique, the sample (an aqueous solution or solid aqueous slurry) is boiled in a round bottomed flask (with stirring – connected to the left arm). The steam generated during the extraction procedure increases the vapour pressure of the extraction system and lowers the boiling point of the water and volatile compounds which are steam distilled through the upper part of the left arm. Simultaneously extraction solvent is refluxed and the subsequent solvent vapours distil through the upper part of the right arm of the apparatus. Vapours then condense on the cold finger, and the extraction process begins in the vapour–liquid film that forms. The proposed construction of the apparatus is such that high-density extraction solvents (such as dichloromethane) and the low-density aqueous layer de-mix and return via the correct return arm back to the appropriate flask to fulfil continuous steam distillation extraction (as shown in Fig. 17.12). Inverting the apparatus accommodates the use of low-density extraction solvents such as pentane or diethyl ether.

Since the original design, various modifications to the SDE apparatus have been made, such as that reported by Godefroot *et al.* (1981). This microscale apparatus allowed the concentration of volatiles into 1 ml of extraction solvent, thus achieving a high concentration factor, reducing problems of artefact build-up and allowing direct gas chromatographic injection of the organic extract without the need of a further concentration step (Fig. 17.13). Within 1 hour, quantitative recoveries were obtained for a wide range of aroma compounds.

Vacuum versions of the SDE technique have also been well discussed in the literature to theoretically reduce the thermal decomposition of sample analytes (resulting in artefact formation, i.e. creation of aroma/flavour volatiles in the analytical extract that were not initially present in the original sample). However, it has been reported that operation of SDE under vacuum conditions had a negative effect upon recoveries of analytes when directly compared to atmospheric use (Leahy and Reineccius, 1984). Vacuum operation is also complex since the boiling of the two flasks must be balanced, as well as keeping the solvent from evaporating and holding the pressure constant (Parliment, 1997). Chaintreau (2001) gives an excellent and extensive review of the SDE technique.

The aroma volatiles of aromatic grape juice have been successfully studied with a 1 h distillation time and a further 30 min of solvent extraction

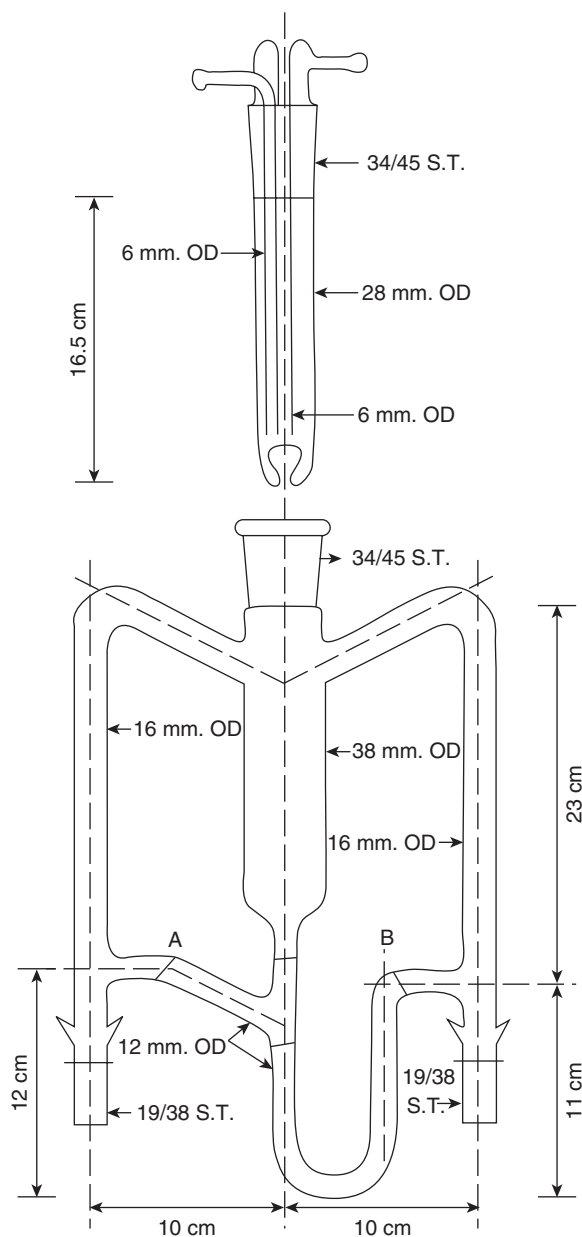
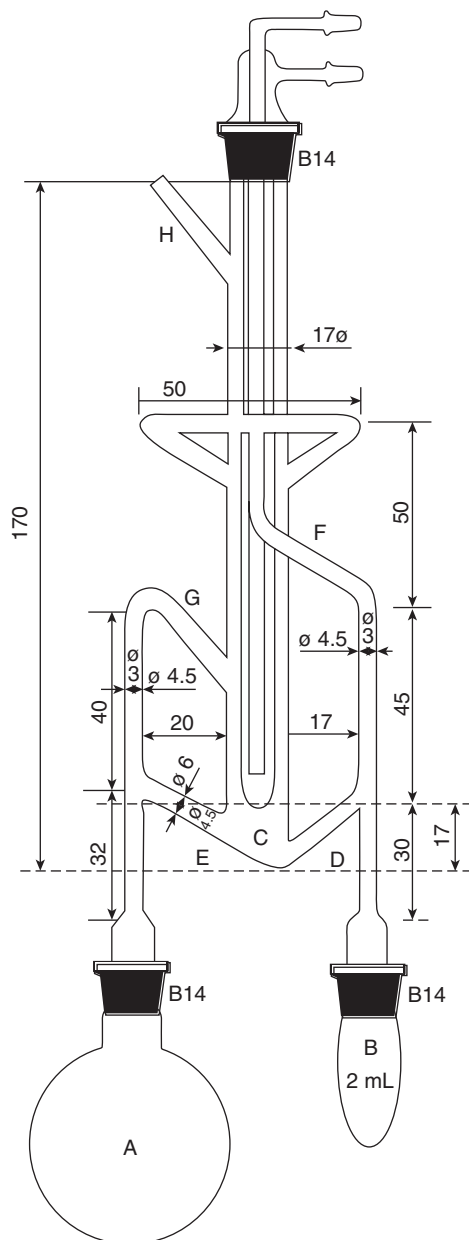


Fig. 17.12 Likens and Nickerson's apparatus for solvent denser than water (Likens and Nickerson, 1964). Reproduced with permission of the American Society of Brewing Chemists, St. Paul, USA.



Key: A – flask (50 ml or 100 ml) containing material to be extracted, distilled water and boiling chips; B – flask (2 ml) containing dichloromethane (1 ml) and boiling chips; C – reservoir containing water (1.5 ml) and dichloromethane (1.5 ml) prior to start of distillation; D and E – demixing-return arms; F and G – evaporation arms; H – open arm.

Fig. 17.13 Micro-SDE apparatus (Godefroot *et al.*, 1981). Reproduced with permission of Elsevier Ltd, Oxford, UK.

with dichloromethane (Blanch *et al.*, 1991; Caven-Quantrill and Buglass, 2005; 2006). These studies indicated that the SDE method with dichloromethane as extraction solvent should be adopted for carrying out an accurate and precise quantitative analysis of aromatic grape juices used to make quality wines.

The potential of the micro-SDE technique for the rapid enrichment of wine aroma compounds has also been investigated (Blanch *et al.*, 1996). However, the use of dichloromethane as the extracting solvent was not successful if a 100 ml volume of wine was placed in the sample flask. This led to ethanol from the sample flask getting into the de-mixing section of the SDE apparatus, thus hindering the equilibrium between the two solvent layers (corresponding to water and dichloromethane) in the separation chamber. This problem was reduced by dilution of the wine sample with water (1:1, v:v). Further dilution of the sample (i.e. 1:3, v:v) made the experimentation more convenient when unattended processing was required, although lower enrichment factors were obtained.

Following on from SDE, a novel, compact and versatile distillation unit for the careful isolation of volatiles from complex food matrices has been developed (Engel *et al.*, 1999). The new technique named solvent assisted flavor evaporation (SAFE) – is able to successfully isolate volatiles from either solvent extracts or directly from aqueous foods and beverages yielding aqueous distillates free from co-extracted non-volatile matrix compounds (Fig. 17.14).

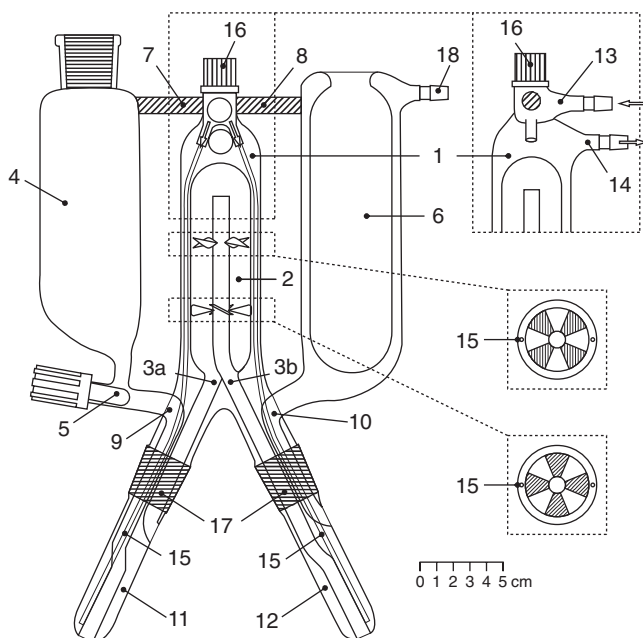


Fig. 17.14 Solvent-assisted flavour evaporation (SAFE) apparatus designed by Engel *et al.* (1999). Reproduced with permission of Springer-Verlag.

This apparatus (size, $40 \times 25 \times 7$ cm) consists of a dropping funnel (Fig. 17.14, no. 4), a cooling trap (no. 6) and a central head (no. 2) bearing two 'legs' (nos. 11 and 12), both equipped with ground joints NS 29 (no. 17) to fix distillation vessels of various volumes. The outlet of the dropping funnel leads to the bottom of the left 'leg' (no. 11). The vapour inlets to the head (no. 3a) and the inlet to the trap are mounted on the sides of each 'leg'. To ensure a constant temperature during distillation and to prevent condensation of the volatiles, the head and the two 'legs' are completely thermostated with water. From the water inlet (no. 13), two flexible polyethylene tubes (no. 15) guide the water flow to the bottom of both legs to afford effective temperature regulation by avoiding the formation of air bubbles.

Figure 17.15 shows the entire SAFE equipment required for performance as outlined by the inventors. Before commencement of distillation, the head and legs of the apparatus are thermostated at 20 to 30°C via the outlets 13 and 14 (Fig. 17.14). The distillation vessel (typically 250 ml to 1000 ml) is heated in a water bath at 20 to 30°C. A diffusion pump supplying a high vacuum (10^{-3} Pa) is applied to the apparatus via outlet no. 18 (Fig. 17.14) while the high vacuum stopcock of the dropping funnel is carefully closed. Liquid nitrogen is applied to the trap (Fig. 17.14, no. 6) prior to the start of the distillation which is achieved by dropping aliquots of the sample from the dropping funnel into the left vessel at which point, the volatiles and solvent are transferred via tube 3a (Fig. 17.14) into the distillation head no. 2. Here, two propeller shaped 'barriers' remove non-volatile material from the vapours and the distillate enters a flask, cooled with liquid nitrogen, via tube 3b on the right side of the apparatus. Volatiles, water or other solvents condense along the walls of the vessel.

Since its creation, the SAFE technique has been quickly adopted for the isolation and analysis of aroma compounds from various alcoholic beverages. For example 1000 ml of Oregon Pinot Noir wine was extracted with diethyl ether:pentane (1:1, v:v) three times in a separating funnel (extracts totalled 750 ml) before the aroma volatiles were isolated using SAFE, dried with sodium sulphate and concentrated to 10 ml under a stream of nitrogen. This aroma extract was further separated into acidic/water soluble and neutral/basic fractions by adding 10 ml of distilled water, adjusting the pH of the aqueous phase to pH 11 (with sodium carbonate solution) then separated in a separating funnel and retained. The organic phase was further washed with 10 ml dilute sodium hydroxide solution (pH = 11) three times and the washings combined with the aqueous phase. The organic phase was dried over sodium sulphate, filtered and concentrated to 200 µl for GC-O analysis. The aqueous solution was adjusted to pH 1.7 with sulphuric acid, 10 g of salt was added and the solution extracted three times with 50 ml diethyl ether:pentane (1:1, v:v). These extracts were combined, dried with sodium sulphate and concentrated to 500 µl for further GC-O analysis (Fang and Qian, 2005).

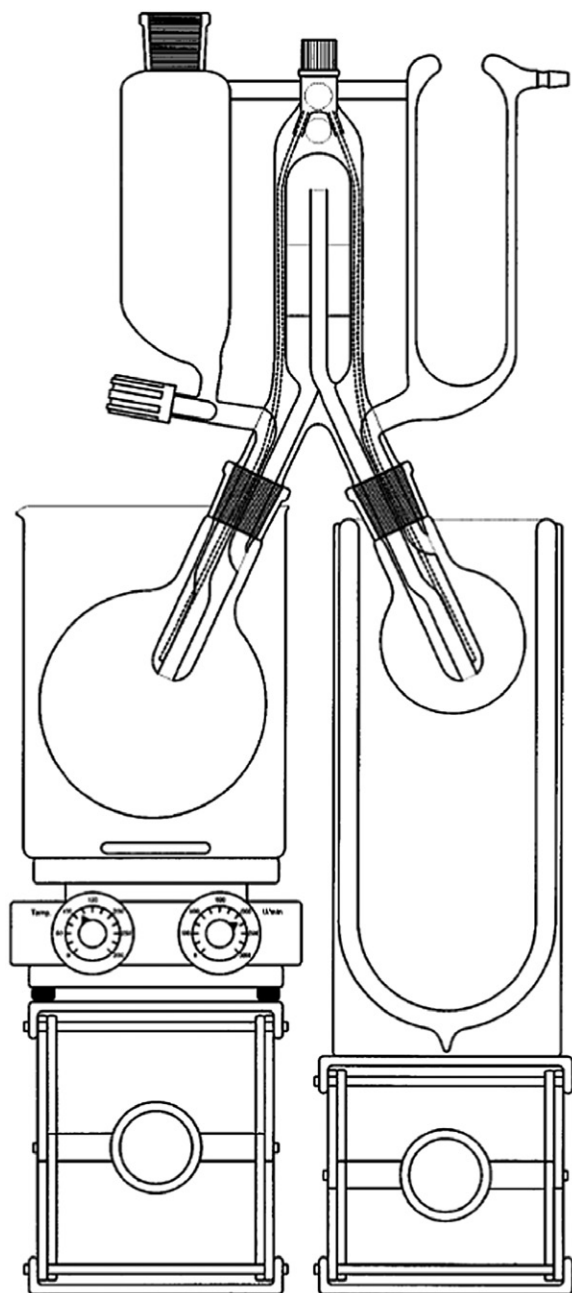


Fig. 17.15 Solvent-assisted flavour evaporation (SAFE) apparatus assembly. Temperature of water bath, 30 °C; temperature of the trap, -196 °C (Engel *et al.*, 1999). Reproduced with permission of Springer-Verlag.

17.4.3 Sorption techniques

SPE was introduced in the late 1970s and has since become a well-known and cited sample preparation technique. Now widely commercially available, SPE products contain milligram quantities of a sorbent phase which is supported between two fritted disks in a plastic syringe type cartridge. Analytes present in an aqueous/ethanolic wine matrix can therefore be selectively retained and subsequently eluted (removed) by an appropriate solvent. SPE has several well-reported advantages over the previously conventional techniques (e.g. liquid extraction) in that it is generally faster, requires less solvent (hence is more 'green' and economical), reduces the need for large concentration steps and is easily automated. SPE also eliminates the use of expensive speciality glassware.

The SPE process involves four key steps; (i) conditioning of the column, (ii) application of the sample, (iii) washing of the column to remove unwanted components and finally (iv) elution and collection of the analyte(s) of interest (Fig. 17.16).

The SPE cartridge is conditioned by passing through three to four times the sorbent bed volume of a high-purity (HPLC grade) organic solvent. Reversed phase sorbents are usually conditioned with a water miscible (polar) solvent such as methanol, followed by water or an aqueous buffer. In the second step of the SPE process (Fig. 17.16), the sample is transferred to the column/cartridge and allowed to flow through the sorbent bed. Sample volumes can range from microliters (μl) to litres. Once the analytes of interest have been retained on the sorbent phase, any unwanted sample matrix materials are washed away with the same solvent with which the matrix materials originated – in the case of wine, an aqueous ethanolic solution. The final step of the SPE process is to elute (rinse) the analyte(s) of interest from the sorbent phase and collect the eluent for analysis. This step should remove only the compounds of interest and leave any impurities on the sorbent phase that were not removed previously in the wash step.

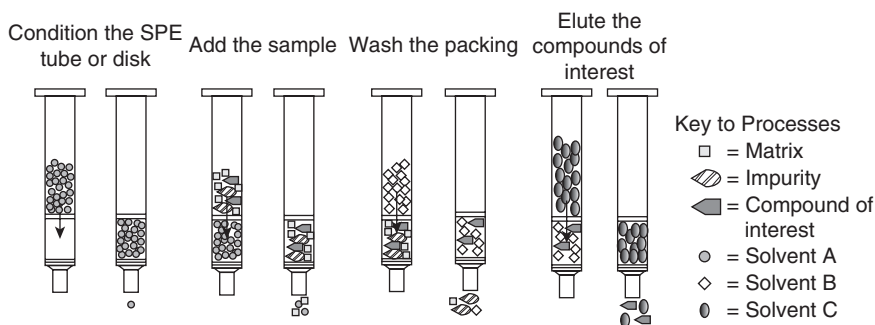


Fig. 17.16 Key steps of the SPE process (Supelco, 1998). Reproduced with permission of Sigma Aldrich, St. Louis, USA.

Wada and Shibamoto (1997) investigated the recovery efficiencies of the main volatile components of wine from porous polymer Porapak Q (an ethylvinylbenzene-divinylbenzene copolymer), using dichloromethane, diethyl ether and pentane as solvents. Dichloromethane showed the highest recovery efficiency. Volatile components from a commercial wine were trapped on Porapak Q (suspended in a glass column), and subsequently recovered using an organic solvent. This method exhibited satisfactory results on isolation of volatile compounds although relatively low recovery percentages were observed. Overall, this SPE method demonstrated the simplicity of the technique with rapid recovery of volatile compounds from aqueous alcohol samples.

In 1985, a method of extraction and determination of free and glycosidically bound grape aroma components was suggested (Gunata *et al.*, 1985). These compounds were adsorbed on the non-ionic resin, Amberlite® XAD-2, followed by elution with various selective solvents. Free forms were directly determined by GC; glycosidically bound forms were first enzymatically hydrolysed. The method was applied to a number of mature grape varieties and it was found that these could be classified in two groups, those rich in free and bound forms which give aromatic wines (Muscat varieties and Gewürztraminer), and those which contain small amounts of these compounds. This technique has since been used in many studies right up to present-day research (Sánchez-Palomo *et al.*, 2006; Botelho *et al.*, 2010).

Other adsorbents have also been adopted to study the volatile aroma compounds of wines by solid phase extraction. These include Amberlite® XAD-4 (Ferreira *et al.*, 2002), Merck's Extrelut resin (Gerbi *et al.*, 1992), LiChrolut EN resin (Loscos *et al.*, 2010), C-18 (Villena *et al.*, 2006), Analytichem's Extube CHEM ELUT (Gelsomini *et al.*, 1990) and IST Isolute ENV+ (Boido *et al.*, 2003).

The notorious off-flavour (cork taint) components 2,4,6-trichloroanisole (TCA) and 2,4,6-tribromoanisole (TBA) have recently been studied using SPE (Insa *et al.*, 2006). It was demonstrated that chlorophenols could be detected in corks contaminated at the nanogram per gram (ng/g) level, and therefore it was suggested that the technique could be successfully applied as a quality control measure in the cork industry.

A miniaturisation of the conventional SPE technique has recently been developed and optimised as a novel method of sample preparation for the quantitative determination of TCA and TBA in wine (Jönsson *et al.*, 2008). Microextraction in packed syringe (commercially known as MEPS) was optimised for the extraction and preconcentration of the analytes using extremely small sample volumes (0.1–1 ml).

SPME was first described for the analysis of environmental chemicals in water by Berlaridi and Pawliszyn (1989). Following its commercial introduction by Supelco (Bellefonte, PA), SPME rapidly gained popularity for the extraction of volatile and semi-volatile organic compounds from a wide variety of sample matrices. Since this technique does not require the use of

an organic solvent, extractions (in either immersion or headspace modes) can generally be performed with small sample volumes hence as with SPE, the process is 'green', economical, and is easily automated.

The stages of the SPME process are shown in Fig. 17.17. A small fibre of fused silica (1 cm length and 0.11 mm internal diameter) coated with a polymeric phase is bonded to a spring-loaded stainless steel plunger which allows movement of the silica fibre in and out of a hollow needle. For extraction of volatiles by liquid (immersion) sampling, each sample is placed, along with a small magnetic stirring bar, in a glass vial crimp sealed with a septum and cap. The SPME fibre is then drawn into the needle of the syringe, which in turn pierces the septum and the fibre is extended and brought into contact with the liquid sample. Used in the headspace mode, the fibre of the SPME device is extended into the vapour phase above the sample. In either mode, over time with stirring of the sample, aroma/flavour analytes of interest from wine adsorb to the polymeric phase of the fibre. Adsorption equilibrium is usually achieved using sampling times ranging anywhere between 2 and 30 min after which the fibre is drawn back into the needle, which is then removed from the septum and inserted immediately (whether manually or automatically) into the heated injection port of a gas chromatograph for 1–2 min for subsequent thermal desorption and analysis.

As with other sample preparation techniques such as liquid–liquid extraction and simultaneous SDE, extraction efficiency with SPME is also affected by contact time, immersion depth (in liquid sampling), efficiency of mixing, pH, salt concentration, temperature and phase ratio (ratio of sample volume/adsorbent volume). These factors can all affect the partitioning in SPME extractions (Harmon, 1997). Therefore, extraction selectivity can be manipulated by altering the type and polarity of the polymer coating (adsorbent) on the fibre, or the coating thickness to match and subsequently extract analytes of interest from wine.

As headspace SPME relies on the equilibrium partitioning of an analyte between the fibre coating and the vapour phase above a liquid sample, the sample headspace should be kept as small as possible.

Recently, SPME has been optimised for analysis of wine aroma compounds (Peña *et al.*, 2005; Tat *et al.*, 2005). Also an SPME-GC method with a poly(dimethylsiloxane) (PDMS) fibre for the analysis of volatile compounds in wines has been validated (Pozo-Bayon *et al.*, 2001). Headspace solid phase microextraction and gas chromatography (HS-SPME-GC) has been used for the determination of major aroma compounds in sweet wines (Rodriguez-Bencomo *et al.*, 2003).

The volatile profiles of many other wine varieties, from different origins, have been studied using the SPME technique. Some recent examples include: the rapid analysis of flavour volatiles in apple wines (Satora *et al.*, 2008), for the profiling of free volatile compounds in Cabernet Sauvignon grapes (during ripening) and wines (Canuti *et al.*, 2009) and for the analysis

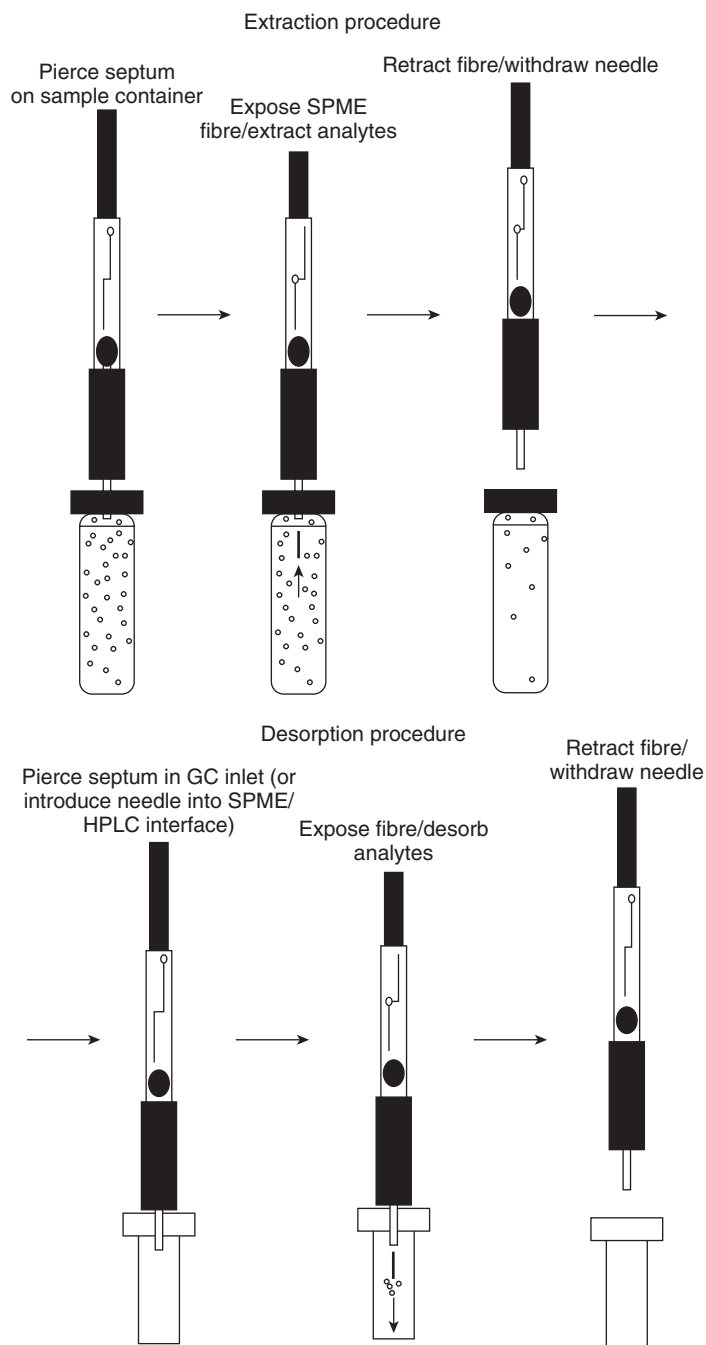


Fig. 17.17 Stages of the solid phase microextraction process Reproduced with permission of Sigma Aldrich, St. Louis, USA (Supelco, 2004).

of volatile compounds for the analytical classification of Chinese red wines from different varieties (Zhang *et al.*, 2010).

It has also been used in combination with principal component analysis (PCA) as a rapid tool for distinction of wines based on the global volatile signature (Rocha *et al.*, 2006).

The technique has also been investigated extensively for the analysis of specific aroma components such as sulphur compounds (Mestres *et al.*, 2002; Lopez *et al.*, 2007a), pyrazines (Ryan *et al.*, 2005), esters (Rodriguez-Bencomo *et al.*, 2002), 2-aminoacetophenone (Fan *et al.*, 2007), 'brett' character (ethylphenols) (Romano *et al.*, 2009), assessment of C13 norisoprenoids (Vinhos *et al.*, 2009) diacetyl (Hayasaka and Bartowsky, 1999) and the 'cork taint' compound TCA (Vlachos *et al.*, 2007).

One major drawback of SPME is the amount of extraction medium coated on the fibre. Consequently, the extraction efficiency (recovery) for solutes that are partially water soluble can be quite low.

Following these observations, a novel extraction method was developed whereby standard laboratory magnetic stir bars were coated with a layer of PDMS and then used to stir aqueous samples, thereby extracting and enriching analytes into the PDMS coating. The technique was named stir bar sorptive extraction or SBSE (Baltussen *et al.*, 1999). With SBSE, the extraction phase is identical to that used on PDMS-coated SPME fibres, although the coating typically uses 50–250 times greater amounts of extraction phase.

Sorptive extraction is an equilibrium technique and for water samples, the extraction of solutes/analytes from an aqueous matrix into the PDMS extraction phase is controlled by the distribution coefficient of the solutes partitioned between the PDMS phase and the aqueous phase. This is identical in principle to a standard liquid–liquid extraction of an aqueous or aqueous ethanolic sample with an immiscible organic solvent. Recently this partitioning coefficient theory has been correlated with octanol–water distribution coefficients ($K_{o/w}$).

Figure 17.18 shows the influence of $K_{o/w}$ and phase ratio on extraction efficiency. For SPME, the volume of PDMS coated to the fibre is approximately 0.5 μl . This results in poor recoveries for solutes with lower $K_{o/w}$ values (less than 10 000 or with a log $K_{o/w}$ value less than 4). In SBSE, greater quantities of PDMS (typically 25–125 μl) coatings are adopted, hence the sensitivity is increased by a factor of 50 to 250 when directly compared with SPME as a sample preparation method.

The theoretical extraction efficiency reaches 100% for solutes with $K_{o/w}$ values larger than 500 (log $K_{o/w}$ greater than 2.7). The theoretical recoveries can be calculated for a given sample volume, selected stir bar dimensions and a solute using the $K_{o/w}$ Win software program (Meylan and Howard, 2000).

Poly(dimethylsiloxane) coated stir bars are manufactured and available from Gerstel GmbH (Mülheim/Ruhr, Germany) under the trade name TwisterTM. These stir bars have three essential parts (Fig. 17.19). Previously,

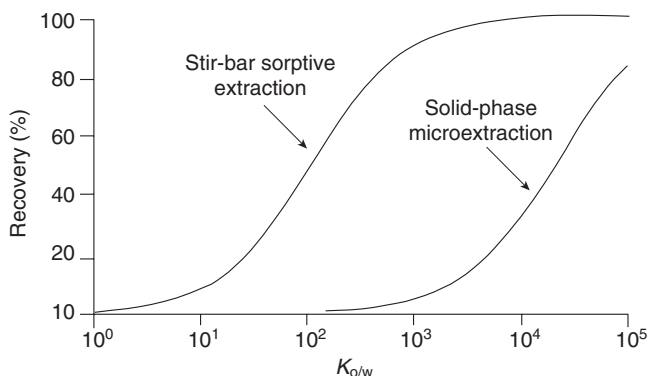


Fig. 17.18 Recovery for solutes in function of the octanol-water partitioning coefficient $K_{o/w}$ for SPME (10ml sample, 100 μ m PDMS fibre) and for SBSE (10ml sample, 10mm $\times$ 0.5mm PDMS-coated stir bar) (David *et al.*, 2003). Reproduced with permission of F. David.

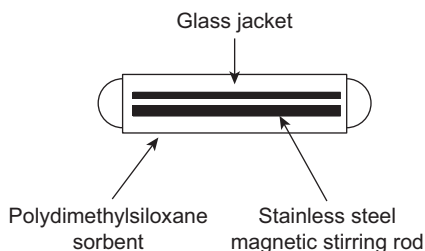


Fig. 17.19 Stir bar sorptive extraction device (Twister™). Reproduced with permission of Lisa Caven-Quantrill.

only stir bars with PDMS as extraction phase were commercially available (i.e. a non-polar phase); however, at the time of writing (2012) Gerstel has introduced to the marketplace an 'EG-Silicone' stir bar consisting of a PDMS/ethylene glycol (EG) copolymer on an inert metal grid for mechanical stabilisation. Research papers utilising this more 'polar' novel extraction phase are expected in the future.

An SBSE of a liquid sample can be easily carried out by placing a suitable amount of sample in a headspace vial along with a stir bar. The sample is then typically stirred for 30–240 min. The extraction time is controlled kinetically, determined by sample volume, stirring speed and stir bar dimensions, and must be optimised for a given application (David *et al.*, 2003).

On completion of the extraction, the stir bar is removed, rinsed with a small aliquot of analytical grade water (to remove adsorbed co-extracted matrix components) and blotted dry on a clean, lint free tissue to remove water droplets. Finally the stir bar is introduced into a preconditioned empty glass tube for thermal desorption on a GC-MS instrument. Desorption temperatures are application dependent, but typically between 150 and

300 °C. Desorption can be accomplished in 5–15 min under a 10–100 ml/min helium flow (David *et al.*, 2003).

SBSE has been used recently for the analysis of volatile phenols in wine by GC-MS (Díez *et al.*, 2004). It was concluded that the technique was an easy, fast and reliable analytical method for the quantitative determination of volatile phenols in wines. The procedure was reported to be simple and allowed 15 samples to be extracted simultaneously using a very small sample volume. A low detection limit with good sensitivity was also obtained.

Recently, SBSE coupled with GC-MS was used to analyse wine samples for three applications: flavour and compositional analysis, TCA, a common off-aroma in wine, and agrochemicals (Hayasaka *et al.*, 2003). SBSE was found to be several orders of magnitude more sensitive than modern conventional methodology, allowing for lower detection and quantitation levels and improved confirmation of identity.

Caven-Quantrill and Buglass (2006) compared traditional microscale simultaneous SDE and SBSE for their effectiveness in the extraction of volatile organic compounds from a synthetic grape juice and a real grape juice (Huxelrebe) from an English vineyard. The novel immersion mode SBSE method, using stir bars with PDMS sorbent, was optimised using a synthetic grape juice. Although mean percentage relative recoveries and reproducibilities (%CV) of the SBSE method were inferior to SDE, the stir bar method proved to be significantly more sensitive. This allowed the identification of a number of volatile components that had not been reported previously in the juice or wine from the grapes with Muscat ancestry.

This method was then further adopted for the volatile organic compound analysis of English vineyard wine grapes (Caven-Quantrill and Buglass, 2007) and up to four vintages of these varieties to study the seasonal variation of flavour content (Caven-Quantrill and Buglass, 2008). This technique was further optimised for the analysis of volatile components of a model wine. The presence of ethanol in the model wine sample matrix resulted in decreased sensitivity of the method toward most of the volatile constituents. Similar sensitivities for the grape and wine sample matrices were achieved by changing the gas chromatographic split ratio from 20:1 (grape juice) to 5:1 (wine), thus allowing direct comparison of chromatograms of volatile components in the two matrices. This enabled direct comparisons of grape juices and the wines derived from them by alcoholic yeast fermentation (Caven-Quantrill and Buglass, 2011).

SBSE has also been used recently for the analysis of wine primary aroma compounds (Zalacain *et al.*, 2007), for the determination of volatile compounds in oak-aged wines (Garde-Cerdán *et al.*, 2008), for off-flavour profiling (Franc *et al.*, 2009) and for classification of South African wines according to the volatile composition (Tredoux *et al.*, 2008).

As an extension of SBSE, HSSE was first developed by Tienpont *et al.* (2000) and Bicchi *et al.* (2002) for the analysis of volatile compounds of

solid matrices. Recently, the HSSE method was developed for the analysis of alcohols, esters, carbonyls, acids, phenols and lactones in South African wine samples (Weldegergis *et al.*, 2007; Weldegergis and Crouch, 2008). The authors concluded that the optimised method was very sensitive and gave acceptable repeatability.

A further and most recent spin-off sorptive technique involves SBSE with liquid desorption (Coelho *et al.*, 2008; 2009; Perestrelo *et al.*, 2009). With this method, SBSE is performed as standard; however, on completion the stir bar is removed from the liquid sample extraction vial and back-extracted with organic solvent (for example, pentane). The resulting liquid extract is then subjected to large volume injection on the GC instrument. The advantage of this approach is that a dedicated (and often expensive) thermal desorption device is not required, thus lowering the initial financial outlay of this very useful and sensitive sorption technique.

17.5 Odour (aroma) analysis methods

17.5.1 Headspace methods

To carry out a static headspace analysis, the sample is placed into a sealed vial, left to equilibrate and the atmosphere above the sample drawn into a gas-tight syringe and injected into a gas chromatograph (Da Costa and Eri, 2004). It is easily automated as a technique and widely commercially available from analytical instrument manufacturers with the option of heating sample vials and injecting a known volume of the sample headspace directly into a gas chromatograph.

Two main types of sample introduction are commonly available. This can either be by the transfer of a measured sample loop of headspace to the chromatograph, often via a heated transfer line (that connects directly to the chromatograph inlet) or by simple automation of a headspace syringe.

The static headspace sampling technique has limited applicability to the analysis of wine due to high quantities of water and ethanol. Since the concentration of these matrix components in the headspace (during sampling) is in equilibrium with the concentration in the liquid beverage sample, the headspace is saturated, and subsequently this results in a lack of analytical sensitivity for the aroma/flavour compounds of interest, which are usually at concentrations several orders of magnitude lower than the water and ethanol (Shimoda *et al.*, 1993).

The theory of static headspace, including general considerations, advantages and disadvantages, with particular reference to the analysis of food volatiles has been reported by Wampler (2002) and hence this is not discussed further in this section.

In dynamic headspace, the volatiles above a sample are swept away by an inert carrier gas, usually helium or nitrogen, onto a trap (glass or glass lined stainless steel tube containing porous polymers such as Tenax™

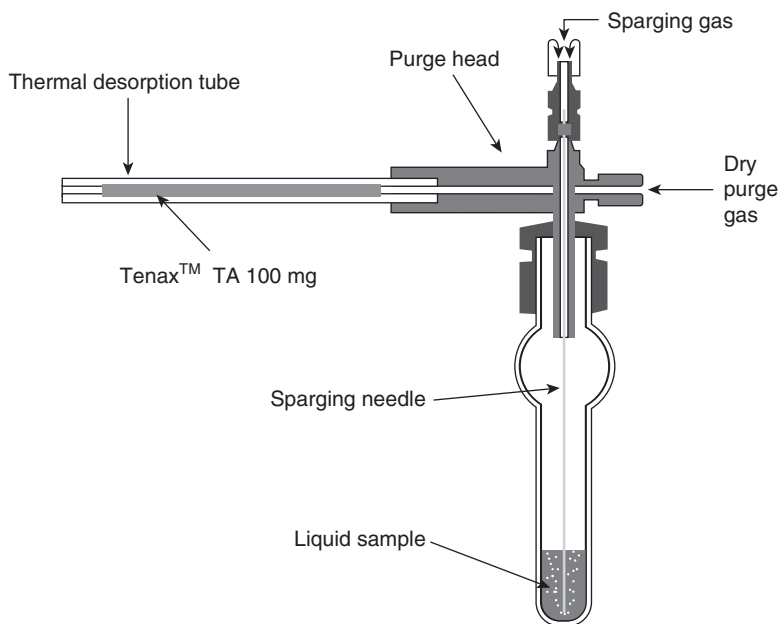


Fig. 17.20 Purge and trap system. Reproduced with permission of Scientific Instrument Services.

(poly-2,6-diphenyl-*p*-phenylene oxide), graphitised carbon sorbents (such as Carbotrap, Carboxpack), silica gel, PDMS and activated charcoal).

With liquid samples such as wine, the sample is purged with a flow of carrier gas and the analytes trapped, hence dynamic headspace analysis of liquid samples is usually referred to as purge and trap (the term dynamic headspace is generally used when the sample undergoing analysis is a solid). Figure 17.20 details the basic setup for carrying out a manual purge and trap analysis. Commercial instruments are also available for this technique, offering automated drying of moisture from the trap (Fig. 17.20 – dry purge gas), preconditioning and reconditioning of the trap in between a sequence of analyses and most include the ability to cryogenically cool the inlet of the gas chromatograph (for example with liquid nitrogen) to focus the aroma volatiles for sharper peaks during analysis. With careful attention to contaminants and system background, it has been reported that this technique is capable of routinely detecting analytes present in a sample in the parts per trillion (ng/l) range (Wampler, 2002).

The purge and trap technique has been utilised in recent studies for the prediction of the wine sensory properties related to grape variety from dynamic headspace gas chromatography-olfactometry (GC-O) data (Campo *et al.*, 2005), for the evaluation of an aroma similar to that of sparkling wine via sensory and GC analysis of fermented grape musts (Mamede *et al.*, 2005), for the analysis of red and white wines from different Spanish

regions (Aznar and Arroyo, 2007), for the identification of a previously unreported odourant (2-methyl-3-(methylthio)furan) in different mono-varietal red wines from the Canary islands (Culleré *et al.*, 2008), for modelling quality of premium Spanish red wines from GC-O data (Ferreira *et al.*, 2009) and for the study of methods for the extraction of volatile compounds from fermented grape must (Mamede and Pastore, 2006) where liquid–liquid extraction was compared with purge and trap. It was concluded by the authors that the liquid–liquid extraction method required more handling of the sample and was time consuming.

On the other hand, the purge and trap system was automated and practical, leading to minimal volatile loss, but low extraction of the high and medium boiling point compounds was observed. Overall, it was demonstrated that the purge and trap/dynamic headspace system and liquid–liquid extraction method were complementary in the determination of the aroma profiles of fermented grape musts and characterisation of the samples.

The aromatic profile as well as the off-odour of cork stoppers has recently been studied via the purge and trap technique. A method based on initial solvent extraction (with pentane) of either cork or wine samples followed by automated purge and trap GC-AED (atomic emission detection) was reported by Campillo *et al.* (2004) for the determination of TCA. Detection limits of 25 pg/g and 5 ng/l were obtained for corks and wines alongside reported recoveries from spiked samples ranging from 88.5–102.3%.

The sensory properties and aromatic composition of macerates of five synthetic and three natural corks were recently determined (Culleré *et al.*, 2009). Natural stoppers were reported to impart sweet, toasted, sweet wood and flowery Muscat aroma to the model wine extracts. Conversely, the synthetic stoppers included a sample with a clear rubber aroma and two samples with a cork/mushroom aromatic note. The results of the GC-O analysis confirmed that the natural cork samples had complex aroma profiles of 10–20 aromatic compounds, all well-known natural components of healthy wine, whereas, in contrast, the GC-O analysis of the synthetic stoppers was extremely simple and consisted of only a few odourants (the mushroom odour was successfully attributed to 1-hepten-3-one).

17.5.2 GC

As in other areas of science, chromatography, particularly GC has revolutionised the analysis of wines from all over the world.

With state of the art techniques such as two-dimensional GC along with sophisticated sample preparation techniques (as previously discussed) it is now possible to efficiently separate (by using a specific fused silica capillary column with a chemistry that suits the analytes to be separated – including chiral enantiomers) and identify many important sensory analytes from the complex wine matrix using a variety of detectors and methods. For more information the reader is directed to a recent extensive review of GC

analysis techniques used to study the organoleptic compounds of wine and other alcoholic beverages (Buglass and Caven-Quantrill, 2011b).

Although the sensitivity of GC and in particular GC-MS has greatly improved over the decades, sample preparation/aroma isolation still remains the important step for the successful instrumental assessment of the flavour and fragrance of wine – hence its emphasis in the previous sections of this chapter.

As an example, San Juan *et al.* (2012) used a myriad of sample preparation techniques to study the aroma chemical composition of red wines from different price categories and its relationship to quality. The aroma chemical composition of three sets of Spanish red wines belonging to three different price categories was studied using an array of GC methods. Significant differences were reported in the levels of 72 aroma compounds. Expensive wines were richest in wood-related compounds, ethyl phenols, cysteine-derived mercaptans, volatile sulphur compounds, ethyl esters of branched acids, methional and phenylacetaldehyde and poorest in linear and branched fatty acids, fusel alcohols, terpenols, norisoprenoids, fusel alcohol acetates and ethyl esters of the linear fatty acids; inexpensive wines showed exactly the opposite profile, being richest in (*E*)-2-nonenal, (*E*)-2-hexenal, (*Z*)-3-hexenol, acetoin and ethyl lactate. Satisfactory models relating quality to odourant composition could be built exclusively for expensive and medium-price wines but not for the lower-price sample set in which in-mouth attributes had to be included. The models for quality revealed a common structure, but they were characteristic of a given sample set.

As with other chromatographic techniques, GC uses a *stationary* phase and a *mobile* phase, whereby chemical components of a mixture are carried through the stationary phase (most modern state of the art systems contain a uniform thin film of phase that is affixed to the inner periphery of an open capillary tube) by a stream of gaseous mobile phase (carrier gas). However, in contrast to most other types of chromatography, the mobile phase does not interact with molecules of the analyte; its only function is to transport the analyte through the stationary phase. Separation is based on differences in migration rates among the sample components.

Modern GC systems consist of a carrier gas supply (and associated pressure regulators, gauges, flow controllers and gas filter system to remove impurities such as water, oxygen and hydrocarbons), injector (e.g. split/splitless or programmable temperature vaporizer (PTV) injector), column, oven, detector and a computer data system.

An ever-increasing number of analyses are now moving away from conventional GC analysis with 0.25 mm internal diameter (i.d.) columns. This is due to the recent commercialisation and hence availability of modern narrow i.d. columns (0.10 to 0.18 mm) which contain thinner films of stationary phase (typically 0.10–0.18 µm) and 10 to 20 m in length. This state of the art GC technique known commercially as ‘fast GC’ can provide typically a 3- to 10-fold reduction in analysis time, whilst still providing acceptable

resolution of target analytes during an analysis. This is achieved by manipulation of the crucial analytical variables, namely column length, column i.d. and film thickness, increased linear velocity of carrier gas, oven temperature and oven ramp rate during analysis.

With the modern popularity of fast GC and potential decrease of analysis times, the oven temperature ramp rate is also being increased faster than ever previously encountered in conventional analysis. In some cases, a conventional GC oven cannot provide the desired ramp rate for a fast method, therefore new oven inserts have appeared commercially. This type of system enables direct resistive heating of the capillary column using ceramic insulated heating wire with high precision temperature control across the entire length of the chromatographic column, and hence, rapid temperature ramping and cooling rates can be achieved with ease.

Plutowska and Wardencki (2008) recently reported an extensive review of the application of GC-O in the analysis and quality assessment of alcoholic beverages. They concluded that despite the fact that odour detectors have been in use for over 40 years, literature indicates that in recent years they have been used more frequently, finding numerous applications in the analysis of food and beverages (including wine analysis). There is, however, still need for improvement of GC-O techniques and the investigations on using them for quantitative analysis of odour compounds. Further studies are also required because past studies on the optimisation of working parameters and on the quality and reliability of the obtained results (which is very important considering feasibility of implementing the GC-O technique to industrial practice) have not been well accepted.

17.5.3 LC and other chromatographic methods

HPLC, in its many modes, is probably still the most widely used chromatographic method for the analysis of alcoholic beverages, especially for the determination of less volatile, more polar and hence more 'taste' as opposed to the volatile odour constituents discussed in this section.

The mode of HPLC used depends upon the stationary phase in the column, which in turn depends upon the nature of the analytes which influences the mobile phase used. Reverse phase (RP) is the most popular mode, but normal phase (NP), ion exchange (ion chromatography – IC), ion exclusion and size exclusion modes (often known as GPC, or gel filtration chromatography, GFC for polymers) are also used. Nowadays, many kinds of detectors are commercially available, but those most widely used in the analysis of wine are mass selective detectors (mass spectrometers) and UV-visible detectors. The former set up (HPLC linked to a mass spectrometer) is known as LC-MS.

Although terpenoids, norisoprenoids and other flavour compounds in alcoholic beverages are usually determined by GC (often GC-MS), β -damascenone has been determined in beers, wine and distilled spirits by

RP-HPLC with UV detection (Carneiro *et al.*, 2006), although a steam distillation/solid phase extraction preconcentration step was required before HPLC analysis.

Another flavour compound, rotundone (a sesquiterpene) in Shiraz (Syrah) grapes and wine was determined using NP-HPLC (Wood *et al.*, 2008). Rotundone is a major contributor to the peppery flavour of certain wine grapes (especially Shiraz) grown in relatively cool regions. Peppery fractions from column chromatography were analysed. Linalool, β -caryophyllene and caryophyllene oxide were used as standard markers and the peppery fractions were combined and studied using GC-MS and GC-MS-O to confirm the presence of (–)-rotundone.

Although wine aroma components are volatile, many exist in grape must and wine as glycosides and as such are nonvolatile and hence do not contribute to aroma until hydrolysed. Terpene glycosides were tentatively determined in must by LC-MS (Prosen *et al.*, 2007).

Countercurrent chromatography (CCC) can be regarded as a kind of automated liquid–liquid separation procedure or as a kind of partition chromatography in which the liquid stationary phase is not supported in any way. In either view, the analytes, originally in the mobile phase, are partitioned between that phase and an immiscible stationary phase. Although not used for direct analysis of aroma and flavour components of wine, it has been used for the fractionation and isolation of flavour precursors (often as glycosides).

Baderschneider *et al.* (1997), Roscher and Winterhalter (1993) and Winterhalter *et al.* (1997) used CCC, along with other techniques, to fractionate and identify a number of glycosidic components of Riesling grapes or leaves. The aglycones of many of these compounds are flavour compounds or possible flavour compound precursors. In the above, CCC was used to separate leaf glucosidic compounds into eight fractions, each of which was tested on its ability to generate 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN), an important flavour component of mature Rheinreising wines, giving rise to ‘petroleum’ notes. 3,4-dihydroxy-7,8-dihydro- β -ionone-D-glucopyranose was indicated as a major TDN precursor.

Baderschneider *et al.* (1997) used similar methods to isolate a new aroma precursor, 2-ethyl-3-methylmaleimide-*N*- β -D-glucopyranose, from Riesling wine. Similarly, the glucose ester of (*E*)-2,6-dimethyl-6-hydroxyocta-2,7-dienoic acid was isolated from Riesling wine by CCC and subsequent acetylation and purification by flash chromatography and HPLC. It is possible that the compound is a precursor of wine lactone, a powerful flavour compound found in many wines (Winterhalter *et al.*, 1997).

17.6 Analysis of taste and mouthfeel

17.6.1 Taste and mouthfeel

Wine taste is described by the sensations sourness (acidity), sweetness, bitterness and saltiness (minerality) and is a result of the presence of acids,

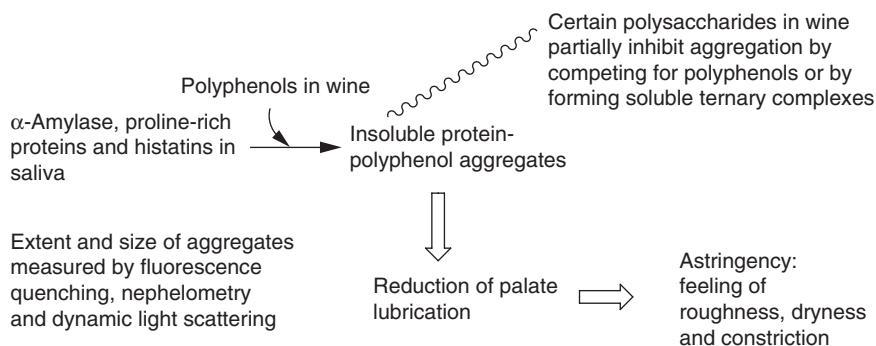


Fig. 17.21 Simplified mechanism for astringency in wine caused by phenols (Soares *et al.*, 2009).

sugars, polyphenols and salts (including those of aminoacids), respectively. Like aroma, taste and mouthfeel sensations are time-dependent. Also, taste characterisation can be performed by a dilution analysis technique (on de-aromatised wines) using LC-MS in conjunction with organoleptic analysis (Lopez *et al.*, 2007b).

Mouthfeel is the term used to indicate tactile (touch) sensations in the mouth area when wine is swallowed. It includes the dry, gritty sensation of astringency (caused by the precipitation of polyphenol-protein complexes – Fig. 17.21), the mouth-coating or viscous sensation (caused largely by the presence of glycerol) and the prickly sensation caused by carbon dioxide bubbles in sparkling and pétillant wines.

A good balance of these sensations depends on the relative concentrations of active components and is crucial to wine sensory quality: it is well known that a wine that lacks acidity (i.e. is ‘flabby’) or that is too acidic or bitter, or too astringent, for example, will be disappointing. The concentrations of some taste components, particularly acids and phenolic substances, also influence the colour, redox stability and biological stability (and hence flavour) of wine.

Many of the taste/mouthfeel components are already present in the must before fermentation, and although their concentrations change throughout the winemaking process, their contribution to the taste of the finished wine is important and hence their analysis in must as well as wine is necessary. Many other components are largely or entirely products of alcoholic or other fermentations (e.g. glycerol, lactic acids). Yet others are partially or wholly derived from other winemaking processes (e.g. salts from contact with metal or concrete, phenolic compounds from oak contact).

Table 17.10 lists the major must and wine components associated with taste and mouthfeel, and Table 17.11 summarises the main analytical methods of determination and identification of these components.

Table 17.10 Major taste and mouthfeel components of grape must and wine

Components	Examples with some typical concentrations or ranges of concentration (g/l must; g/l wine) ¹
Acids	Tartaric acid (4–8; 2–4) Malic acid (2–5; ~0–2) D-Lactic acid (wine only: usually not more than 0.5) L-Lactic acid (wine only: ~0–5, depending on occurrence of malolactic fermentation, MLF) Succinic acid (0.5–1; ~1) ² Uronic (sugar) acids (0.1–0.3; 0.2–1.4) ² Acetic acid (~0–0.5) ³ Sulphurous acid (total: ~0–0.45) ⁴
Sugars	Glucose, fructose (combined: 180–320; <1–12) ⁵
Polyhydric alcohols	Glycerol (~0 ⁶ ; 1–20). Butane-2,3-diol (0.3–1.4)
Salts	Sulphates (0.1–0.4), phosphates (red wines: up to 1; white wines up to 0.5), bisulphites ⁴ , ammonium salts, salts of K ⁺ (0.1–2), Na ⁺ (up to 0.06) ⁷ , Mg ²⁺ (0.02–0.17), Ca ²⁺ (0.08–0.14), Fe (0–0.1) Cu (~0–0.004) and organic acids. Total dry extract (red wine: 25–30; white wine: <25). Ash (inorganic only): 1.5–3
Amino acids	Arginine (0.4–>1), proline (0.3–>1), glutamine (0.3–0.7), alanine (0.2–0.6). Total amino acid content: 1.5–4;
Phenolic substances	Simple phenols, polyphenols: flavonoids (including anthocyanins), chalcones, stilbenes, oligomeric polyphenols (See Figs 17.2 and 17.3). Total phenolic content (TPC): 2–4; 0.5–4 for red must/wine, ~0.3 for white wine

¹ Data from Amerine and Ough (1980a) and Ribéreau-Gayon *et al.* (2000a). ~0 indicates a very low concentration, often less than 100 mg/l.

² Higher values are often found in botrytised grapes and corresponding wines.

³ Also an aroma compound: too high a concentration indicates spoilage.

⁴ Nearly all comes from added bisulphite (preservative).

⁵ Very sweet wines (e.g. Sauternes, Tokaji Aszu, Trockenbeerenauslesen) can have higher values.

⁶ Ripe grapes affected by *Botrytis cinerea* can have levels up to 5 g/l.

⁷ Higher values are possible for wines from coastal vineyards.

17.6.2 Acidity and acids

Total acidity of must and wine is a major sensory quality factor and is routinely analysed throughout the winemaking process, often by conventional acid-base titration with a visual indicator (e.g. see Buglass and Caven-Quantrill, 2011a), but pH titrations may be used instead. The reference procedures of the OIV or AOAC involve titration of the diluted decarbonated sample with standard NaOH up to a final pH of 8.2–8.4. Recently, a digital image based (DIB) method, using a webcam as a detector, was demonstrated to compare favourably with reference methods in the analysis of the total acidity of Spanish red wines (Tôrrres *et al.*, 2011). A miniaturised electrochemical electrode sensor device has been used for the rapid (~4 s) determination of acidity of must and wine via a ‘local’ electrochemical titration (a ‘flash’- or ‘nano-titration’) that does not affect the overall total acidity (Wen *et al.*, 2004).

Table 17.11 Major methods for the analysis of taste and mouthfeel components of must and wine

Components	Methods of determination/identification*	Contexts and comments
Acids	Total acidity: directly by acid-base titration; indirectly from chemometric/statistical methods (e.g. using acidity data and a rapid spectrometric method, such as FTIR) Individual organic acids: enzymic/UV-Vis spectrophotometric methods HPLC ¹ CZE, NMR, ICPAES. Sulphurous acid (from added sulphite): redox titration, distillation/redox titration, HPLC ¹ , CZE, electrochemical methods. Volatile acidity (as acetic acid, mainly): steam distillation/acid-base titration; NMR.	QA/QC; monitor of progress of malolactic fermentation (MLF: malic acid to L-lactic acid conversion), where appropriate.
Sugars	Total sugars: refractometry (must), densitometry Reducing sugars: Lane and Eynon method (wine). Individual sugars (inc. glycoside units): enzymic/UV-Vis spectrophotometric methods; HPLC ² ; NMR, MS, ICPAES.	QA/QC; determination of authenticity; structure determination.
Polyhydric alcohols	GC, HPLC, MS, NMR, ICPAES.	QA/QC; determination of authenticity.
Salts	Individual cations: colorimetry, atomic spectroscopy ³ , electrochemical methods ⁴ , CZE, ion chromatography, ICPMS, ICPAES Individual anions: colorimetry, CZE, ion chromatography, electrochemical methods.	QA/QC; determination of authenticity.
Ammonia, aminoacids and proteins	Total nitrogen content: Dumas and Kjeldahl methods (includes N of peptides and proteins). Yeast assimilable nitrogen (YAN): colorimetry, enzymic/colorimetric methods. Individual amino acids: colorimetry, fluorimetry, enzymic/colorimetric methods, HPLC, CZE, gel electrophoresis (proteins).	QA/QC; determination of authenticity; structure or molecular weight determination.

(Continued)

Table 17.11 *Continued*

Components	Methods of determination/ identification*	Contexts and comments
Phenolic substances	Total phenolic content ⁵ : redox titration, colorimetry, UV spectrophotometry; indirectly from chemometric/statistical methods Individual phenols: GC ⁶ , HPLC ⁶ , CZE ⁶ , NMR, MS.	QA/QC; correlation with antioxidant or radical scavenging ability; determination of authenticity. Structure determination.
Carbon dioxide	Foam stability, foam height, foamability	QA/QC

*Often performed on fractions isolated, for example, using column chromatography, preparative HPLC, GPC, CCC, solvent extraction, SFE or SPE.

QA = (routine) quality assurance.

¹Includes ion chromatography.

²Includes ion exclusion chromatography.

³Includes ICP-AES.

⁴Includes the use of ion selective electrodes and other sensors.

⁵Includes anthocyanins and other pigments, and volatile phenols.

⁶Especially with MS detection.

Note: Although most methods use electronic instruments, some non-instrumental (chemical or physical) methods have been included in this table for completeness. These will not be further discussed in any detail.

Electrochemical sensors or ‘electronic tongues’ (multisensor systems) have been used to determine numerous important must and wine parameters, including total acidity and pH. Moreno i Codinachs *et al.* (2008) used such a device to discriminate between must and wine samples, as well as to measure various parameters. Also, rapid indirect determinations of total acidity may be carried out using FTIR and chemometric/statistical analysis, usually a combination of PCA, PLSR and other modelling, pattern recognition or classification techniques. Such a method has used a combination of total acidity and pH data, as well as total soluble solids (a measure of the sugar content) data, with FTIR data to predict these important parameters for South African wine musts (Swanepoel *et al.*, 2007).

Individual must or wine acids, such as citric, fumaric, gluconic, malic or succinic acids, can be determined by enzymic methods (utilizing enzyme reactions and UV-visible spectrophotometry or colorimetry), often using dedicated kits from suppliers such as Boehringer Mannheim, Fluka, Megazyme or Sigma. These methods are generally precise and convenient, and they require no specialist equipment, other than a spectrometer or colorimeter. However, if several acids need to be analysed simultaneously, then electrophoretic or chromatographic methods are more suitable.

Tartaric acid is the major acid of wine and most musts. The reference method of the OIV is a manual colorimetric procedure based on that of Rebelein: the tartaric acid is separated by ion exchange chromatography

before development of colour at 500 nm by reaction with vanadic acid (OIV, 2007). Recently, a flow injection analysis (FIA) version of the Rebelein method, using an inline dialysis unit for dilution and elimination of matrix effects, was developed for simultaneous determination of tartaric acid and potassium in red wine (Oliveira *et al.*, 2010).

CZE and HPLC are the two major methods for the determination of a range of individual organic and/or inorganic acids in must and wine. CZE is more rapid than HPLC and is of similar accuracy and precision. Table 17.12 lists details of selected CZE methods, along with comments and references. Generally, standard CE equipment is used (e.g. Mato *et al.*, 2005; Buglass and Caven-Quantrill 2011d) using running buffers (sometimes with additives such as CaCl_2) of pH ~ 7 and with direct UV detection (Mato *et al.*, 2007) or with indirect UV detection in the presence of an added background absorbing electrolyte such as 3,5-dinitrobenzoic acid (Peres *et al.*, 2009). Also, pre-analysis derivatisation has been used to improve UV detection sensitivity (Santalad *et al.*, 2007). Often an electro-osmotic flow modifier in the mobile phase is used to improve resolution, but sometimes the internal capillary walls are coated with modifiers such as polyacrylamide or hexadimethrine bromide (HDB) (Saavedra and Barbas, 2003; Bianchi *et al.*, 2005).

On a miniature scale, 22 acids in Slovakian wines were determined using a miniaturised ('lab on a chip') zone electrophoresis system made of poly(methylmethacrylate) (PMMA) with channels for injection, separation and waste, and with two integrated platinum conductivity detectors (Masár *et al.*, 2005).

Many HPLC methods, with a variety of stationary phases, mobile phases and detectors, have been used in the analysis of must and wine acids, including additives such as bisulphite, sorbic acid and benzoic acid – see Table 17.13 and Buglass and Caven-Quantrill (2011b) and Mato *et al.* (2005) for reviews. At one time ion exchange and ion exclusion modes were most popular, because of significant decomposition of the bonded alkyl stationary phase that occurred when RP-HPLC was used with (necessary) low pH mobile phases. More recently, acid-resistant reversed phases, such as Phenomenex Aqua, have allowed consistently good chromatography using wholly or mainly aqueous acidic mobile phases (Buglass and Lee, 2003, and references therein).

17.6.3 Sugars

The fermentable sugar content of must and the residual sugar content of wine both have a direct bearing on wine sensory quality, and indeed generally have to conform to official regulations. Table 17.14 illustrates the major carbohydrates in grape must and wine, including those present as the glycosides of aroma compounds and polyphenols. Total sugars in grapes or must are estimated using a refractometer or by specific gravity

Table 17.12 Selected CZE methods for the determination of individual acids in must and wine

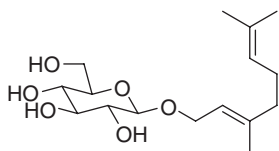
Reference	Running buffer solution (background electrolyte)	Special background electrolyte	Detection method	Other details, and comments
Saavedra and Barbas (2003)	200 mM phosphate buffer (pH 7.5)	None	UV (214 nm)	Polyacrylamide-coated capillary used. Eight organic acids plus nitrate and sulphite determined in Rioja wines.
Mardones <i>et al.</i> (2005)	Bis-tris ¹ with added CaCl ₂ or LiCl (pH 7.5)	<i>p</i> -Aminobenzoic acid	UV (260 nm)	C ₁₄ H ₂₉ (CH ₃) ₃ N ⁺ Br ⁻ (TTAB) used as electro-osmotic flow (EOF) modifier. Shikimic acid analysis to distinguish between wines of Cabernet Sauvignon, Merlot and Carmenère.
Mato <i>et al.</i> (2007)	7.5 mM NaH ₂ PO ₄ and 2.5 mM Na ₂ HPO ₄ with 0.24 mM CaCl ₂ (pH 6.4)	None	UV (185 nm)	2.5 mM C ₁₄ H ₂₉ (CH ₃) ₃ N ⁺ OH ⁻ (TTAOH) used as EOF modifier. Six organic acids analysed in 6 min.
Peres <i>et al.</i> (2009)	0.2 mM CTAB (pH 3.6)	10 mM 3,5-Dinitrobenzoic acid (3,5-DNB)	UV (254 nm)	Six acids analysed in 26 Brazilian wines.

¹ Bis[2-hydroxyethyl]imino-tris[hydroxymethyl]methane.

Table 17.13 Examples of HPLC methods for the analysis of must and wine acids

Reference	HPLC mode/stationary phase	Mobile phase	Detection	Comments
Masson (2000)	Ion exchange/Dionex As11	NaOH gradient elution in CH ₃ OH:C ₂ H ₅ OH:water (1.3:1.3:7.4)	Supressed conductivity	Organic acid anions and inorganic anions in grape must.
Buglass and Lee (2003)	Reversed phase/Waters Resolve C18 and chiral ligand exchange/Daiceal	1 mM or 0.5 mM aqueous H ₂ SO ₄ and 2 mM CuSO ₄ (respectively)	UV at 230 nm and 254 nm (respectively)	Sequential determination of malic acid and both D- and L-lactic acids in wine using column switch. Monitor of MLF.
Soyer <i>et al.</i> (2003)	Ion exclusion/Bio Rad Aminex PPX-87	5 mM aqueous H ₂ SO ₄	UV at 214 nm	Organic acids in Turkish grapes and must.
Pérez-Ruiz <i>et al.</i> (2004)	Reversed phase/Beckman Coulter Ultrasphere C18	5 mM aqueous H ₂ SO ₄	Photochemically induced chemiluminescence	Post-column redox reaction between acids in wine and UO ₂ ²⁺ /Fe ³⁺ and detection of Fe ²⁺ by chemiluminescent reaction with luminol system gave increased sensitivity.
Kritsunankul <i>et al.</i> (2009)	Reversed phase/Thermo Fisher Aquasil C18	Aqueous KH ₂ PO ₄ (pH = 2.5): MeCN 9.9:0.1	UV at 210 nm	Sample pretreatment: pre-column online flow injection dialysis (FID) of wines

Table 17.14 Main carbohydrates in grape juice and wine¹

Carbohydrate	Context	Comments
D-Glucose } D-Fructose }	As free sugars in grape juice and as residual sugar in wine	Glucose is more effectively fermented by wine yeasts: sweet wines tend to have excess of fructose
	(Glucose) as glucosides of aroma compounds such as terpenols and phenolic compounds, including anthocyanins (see Fig. 17.2)	An aroma compound glucoside: geranyl- <i>O</i> - β -D-glycoside (odourless)
		
D-galactose	As free sugar in grape juice (~0.1 g/l) As glycoside unit of flavonoid phenols	Poly(galacturonic acid), partially esterified by methanol, forms the backbone of grape pectic substances
L-Arabinose	As side chains or part of side chains in grape pectic substances	
L-Rhamnose	As glycoside unit of aroma terpenols and flavonoid phenols Forms part of rhamnogalacturonan region of grape pectic substances	

¹ Certain other sugars or derivatives, such as D-apiiose, L-fucose, 2-*O*-methyl-L-fucose, 2-keto-3-deoxy-D-mannooctulosonic acid, 3-deoxy-D-lyxo-heptulosaric acid, 3-*C*-carboxy-5-deoxy-L-xylose and 2-*O*-methyl-D-xylose are found in pectic substances.

measurement using a hydrometer or pycnometer. Strictly, these methods give the total soluble solids, but are sufficiently accurate for many purposes.

Total reducing sugars in wines or diluted musts of less than 2.2°Brix (1°Baume or ~1.1008 specific gravity or ~1.5% w:v sugar) are often determined by the Lane and Eynon method, which involves titration of the boiling sample with Fehlings solution until a distinct red colour of Cu₂O persists (Jackson and Schuster, 1981).

Individual free sugars, such as D-glucose, D-fructose and several others, can be determined conveniently by enzymic/spectrophotometric methods using specific kits (see Section 17.8). Typically, D-glucose, for example, in a diluted sample is oxidised by glucose oxidase to gluconic acid, which after

an incubation time and adjustment of pH, is reacted with *o*-dianisidine to give a red coloration (540nm). Such methods have been used to analyse the sugars (e.g. D-glucose) released from wine after hydrolysis of glucoside aroma precursors (Arévalo-Villena *et al.*, 2006).

Sugars and polyols (e.g. sorbitol) are frequently determined by ion exchange or ion exclusion modes of HPLC. These often employ a cation exchange column (typically a cross-linked sulphonated polystyrene-divinylbenzene particulate material, such as Alltech Prevail, Bio-Rad Aminex, Phenomenex Rezex, Supelco Supelcogel or Waters Sugar-Pak), a slightly acidic mobile phase and with UV, refractive index or evaporative light scattering detection (ELSD). A recent example is provided by Salinas *et al.* (2012), where glycosidic aroma precursors in red Bobal grape juice (after removal of free glucose and pigments) were estimated by determining the glycosyl glucose released after acid hydrolysis.

Dionex (2009) have introduced anionic ion exchange methods for sugar analysis using 16mM aqueous NaOH as mobile phase and a CarboPac PA column (a macroporous vinylbenzyl chloride/divinylbenzene substrates agglomerated with a quaternary ammonium functionalised microbead latex), with highly selective pulsed amperometric detection. Polyols (including glycerol) are less acidic than sugars and require 0.6M aqueous NaOH mobile phase and a CarboPac MA column (Dionex, 2009).

Glycerol, the major alcoholic fermentation byproduct, is a contributor to viscosity mouthfeel (smoothness). Its concentration in wine (Table 17.10) depends on winemaking processes, including yeast strain. Heat shock-treated yeast cells tend to produce wines with higher glycerol levels (Petropoulos *et al.*, 2010).

17.6.4 Salts

Dissolved ionic substances – salts of organic and inorganic acids (Table 17.10) – are important as they provide must and wine with buffer capacity and confer the wine with a mild minerality or saltiness of taste, which is more noticeable in white wines. Also, high concentrations of Fe^{3+} or Cu^{2+} , in the presence of phosphate, or sulphite, proteins and phenols can lead to serious wine defects such as ‘casse’ (cloudiness or coloured deposits). Total dissolved salts, only about 10% of total dry extract, can be estimated by ‘ashing’. Determination of ash and total dry extract are outlined in Fig. 17.22.

Although colorimetric methods (Amerine and Ough, 1980a) and ion chromatography can be used, individual cations and anions, as well as non-metallic elements, in must or wine are nowadays often determined directly by atomic spectroscopy in all its main forms (along with a number of variants): flame photometry (an emission technique), flame atomic absorption spectroscopy (FAAS), graphite furnace atomic absorption spectroscopy (GFAAS), inductively coupled plasma-mass spectrometry (ICPMS) and

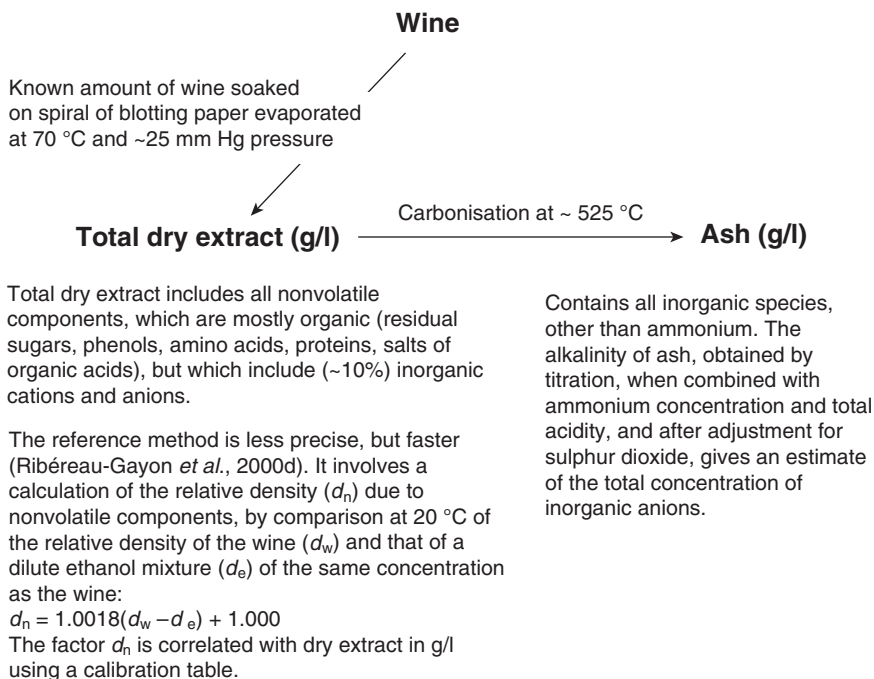


Fig. 17.22 Summary of total dry extract and ash determination. Total dry extract relates to 'body' mouthfeel; ash relates to minerality of taste.

inductively coupled plasma-atomic emission spectroscopy (ICPAES), although some emphasis has been placed on the determination of toxic heavy metals, which (with the exception of Cu and Fe) do not affect sensory quality.

Some examples are given in Table 17.15, where it can be seen many of these techniques require pre-concentration (focusing) and/or digestion-type sample pretreatment or the use of chemical matrix modifiers to enhance sensitivity. Background correction is generally present and flow injection, GC and HPLC techniques are often used to deliver the sample. Ion exchange or similar pretreatment of the sample into fractions can be used to determine the speciation of metals in wine samples (Karadjova *et al.*, 2002; Pohl and Prusisz, 2009).

HPLC-ICPAES can be used to estimate more difficult non-metals (such as B, C, P, S and Se, as organic or inorganic components) in wine (Paredes *et al.*, 2006) and GC-AED has been used to analyse sulphur compounds in wine (Campillo *et al.*, 2009).

Electrochemical methods are also widely used in the analysis of metal and nonmetal ions in grape must and wine (Table 17.16). They

Table 17.15 Selected examples of the determination of metals in grape must and wine by atomic spectroscopic techniques

Reference	Metals determined/ sample	Technique used	Sample pretreatment	Special features and general comments
Ajtony <i>et al.</i> (2008)	As, Cd, Cu and Pb in wine	GFAAS with transversely heated graphite atomiser (THGA)	None or with digestion in conc. $\text{HNO}_3/\text{H}_2\text{O}_2$	Chemical modifiers $\text{Pd}(\text{NO}_3)_2$ and $\text{Mg}(\text{NO}_3)_2$ used. Pyrolysis temperature: 600°C and atomisation temperature: 2200°C found in range $20\text{--}640\text{ }\mu\text{g/l}$. Limit of detection (LoD) for Cu = $1.2\text{ }\mu\text{g/l}$.
dos Santos <i>et al.</i> (2009)	Fe and Mn in wine	FAAS	Photo-oxidation with UV light/ H_2O_2	LoD for Cu = $30\text{ }\mu\text{g/l}$; for Mn = $22\text{ }\mu\text{g/l}$. Fe found in range $1.58\text{--}2.77\text{ mg/l}$ and Mn in range $1.30\text{--}1.91\text{ mg/l}$.
Schiavo <i>et al.</i> (2008)	Cd, Cu and Pb in must and wine	Thermospray flame furnace atomic absorption spectroscopy (TS-FF-AAS), a furnace AAS variant	None	Heated nickel tube/ceramic capillary used to increase residence time of atomic cloud in atomiser, thus increasing sensitivity. 0.14 M HNO_3 used as sample carrier.
Álvarez <i>et al.</i> (2007)	Wide range of metals in Montilla-Moriles fino wines (Spain)	ICP-AES	Digestion in conc. $\text{HNO}_3/\text{H}_2\text{O}_2$	Mineral profile used in chemometric determination of wine origin, using Spearman non-parametric sample correlation method. Correlations found allowed discrimination between these wines and similar wines from other areas.
Pohl and Prusisz (2009)	Fe in wine	FAAS	Sample fractionation using tandem XAD-7HP and Dowex-x8-200 columns. Digestion in conc. $\text{HNO}_3/\text{H}_2\text{O}_2$	Big majority of Fe is present as hydrated ions, or cationic complexed with hydroxyacids or amino acids, rather than as polyphenol-bound Fe or in neutral/anionic complexes.

Table 17.16 Electrochemical methods for the analysis of inorganic ions in must and wine

Method	Examples of analytes	General comments, special features and examples of specific applications
Ion selective electrodes (ISE) ¹	Ca ²⁺ , Cu ²⁺ , H ⁺ , K ⁺ , NH ₄ ⁺ (also Cl ⁻ , F ⁻ and other anions)	The pH ('glassy') electrode is the most widely used. Ammonium electrode is sometimes used to determine NH ₃ content of Kjeldahl digest in the analysis of total N content.
Potentiometric stripping analysis (PSA) ¹ , including derivative potentiometric stripping (dPSA) and adsorptive stripping chronopotentiometry (AdSCP)	Metal cations and some anions such as SO ₃ ²⁻	Working electrode is glassy carbon, plated with Hg film. Hg ²⁺ is often used as stripping oxidant and matrix modifiers like HCl or CaCl ₂ are often used. Cu in Marsala wine by dPSA (Dugo <i>et al.</i> , 2005). Ni(II) in Marsala wine by AdSCP on dimethylglyoxime complex of Ni(II) (Dugo <i>et al.</i> , 2004b).
Voltammetry ² including anodic/cathodic stripping voltammetry (ASV/CSV), square wave anodic stripping voltammetry (SWASV) differential pulse anodic stripping voltammetry (DPASV) and adsorptive stripping voltammetry (AdSV) and polarography ³	Metal cations, but often used to determine total acidity or anionic species such as SO ₃ ²⁻	Working electrode is often gold, platinum, silver or Hg plated glassy carbon, but chemically modified electrodes (CMEs) ⁴ are also used. Cyclic voltammetry is a popular variant. Fate of Cu and Zn in fermenting must by ASV (Esparza <i>et al.</i> , 2007). Speciation of Cu in white wine by polarographic DPASV (Wiese and Schwedt, 1997).

¹ Potentiometric methods.

² Potentiodynamic methods.

³ Uses a polarisable dropping mercury electrode as working electrode.

⁴ E.g. glassy polymeric carbon electrode with a poly(caffeic acid) thin film (da Silva *et al.*, 2008).

Note: The electrochemical methods listed can also be used to determine oxidisable organic components (like ascorbate, glucose and phenols).

are sometimes used in conjunction with other methods (especially atomic spectroscopy), although electrochemical methods per se are generally rapid, sensitive, accurate, precise, inexpensive and require little or no sample pre-treatment. On the other hand, some methods, especially voltammetric methods, can suffer from the presence of organic compounds in the sample, and others use toxic Hg or Hg(II).

17.6.5 Ammonia, amino acids and proteins

Apart from a contribution to saltiness, nitrogen compounds probably have only slight direct influences on sensory quality, but indirectly they are very important. Ammonia (as ammonium salts) and amino acids are required for the sound fermentation that produces a well-balanced wine, but too much nitrogen nutrient in the must is known to result in wines lacking in aroma (Ribéreau-Gayon *et al.*, 2000c).

Proteins retard release of aroma compounds in general (Tsachaki *et al.*, 2009) and enhance the foaming properties of sparkling wines (Marchal *et al.*, 2001), as well as forming complexes with polyphenols. Some, such as thaumatin-like proteins and chitinases, are also implicated in the formation of hazes or in the development of ‘casse’ (see above). The various organic and inorganic forms of nitrogen compounds in must and wine are described in Fig. 17.23, along with the major methods of determination.

17.6.6 Phenolic substances

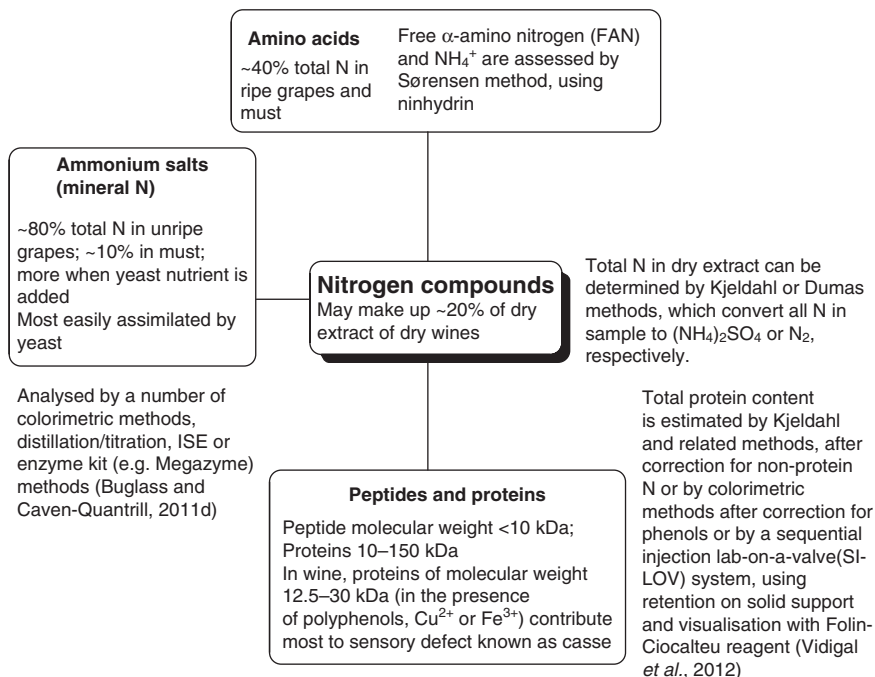
Phenolic substances (often loosely called tannins) are essential for the perception of bitterness of taste and astringency of mouthfeel when drinking wine. Without these the wine would appear flat and insipid, but in excess it would be unpleasant. Polyphenol levels in red wine depend on a number of growing factors and processing methods, the latter of which can be manipulated: for example, hot press techniques tend to give wine richer in phenols such as stilbenes (Fig. 17.24) (Leblanc *et al.*, 2008), whereas short skin contact times and whole bunch fermentations give less tannic wines.

Indirectly, phenolic compounds modify the impact of aroma compounds, probably by selective retention via complex formation. Non-volatile red wine fractions added to Sauvignon Blanc wine suppressed characteristic white wine fruity notes, especially those of 3-mercaptohexyl acetate (an important odourant of Sauvignon Blanc wine), in favour of red wine aroma notes (Sáenz-Navajas *et al.*, 2010).

Figure 17.24 gives a summary of non-anthocyanin phenolic substances found in must and/or wine, along with selected comparative bitterness and astringency impact.

Total phenolic content is normally determined by the colorimetric Folin-Ciocalteu method or by titration using the Löwenthal method (Buglass and Caven-Quantrill, 2011d), but also can be estimated by measurement of

Methods for the analysis of amino acids in must and wine have been reviewed (Callejón *et al.*, 2010; Buglass and Caven-Quantrill, 2011b, c, d). Individual amino acids can be analysed by ion exchange chromatography, derivatisation using ninhydrin and UV-Vis detection (amino acid analyser), or by RPHPLC on dansyl derivatives (Berlanga *et al.*, 2006) or with a binary mobile phase and automated pre-column derivatisation using *o*-phthalaldehyde/*N*-acetyl-L-cysteine reagent and UV-Vis detection (Kelly *et al.*, 2010)



Proteins in must and wine are analysed by SDS-PAGE isolation, followed (after removal of polyphenols) by solvent gradient RPLCMS, especially using ESI and Q-TOF MSⁿ detection. Proteins can be separated by lyophilising dialysed wine (Wigand *et al.*, 2009) or by adding $(\text{NH}_4)_2\text{SO}_4$ and redissolving in Tris-HCl buffer and 15% glycerol + 1.5% SDS (Marangon *et al.*, 2009)

Fig. 17.23 Major nitrogen compounds of must and wine, and main methods of analysis. *N* aroma compounds are covered in Section 17.3.

absorbance of a diluted sample at 280 nm (Ribéreau-Gayon *et al.*, 2000b). It can be determined indirectly by use of an array of voltammetric sensors (an ‘electronic tongue’) combined with chemometric/statistical processing to interpret the electrochemical signals and extract sensible data from them (Cetó *et al.*, 2012).

Instrumental methods for the analysis of individual flavonoid phenols in plant materials (de Rijke *et al.*, 2006) and (along with other families of phenols) in alcoholic beverages and their raw materials (Buglass and Caven-Quantrill, 2011a) have been reviewed. Also, Harbertson and Spayd

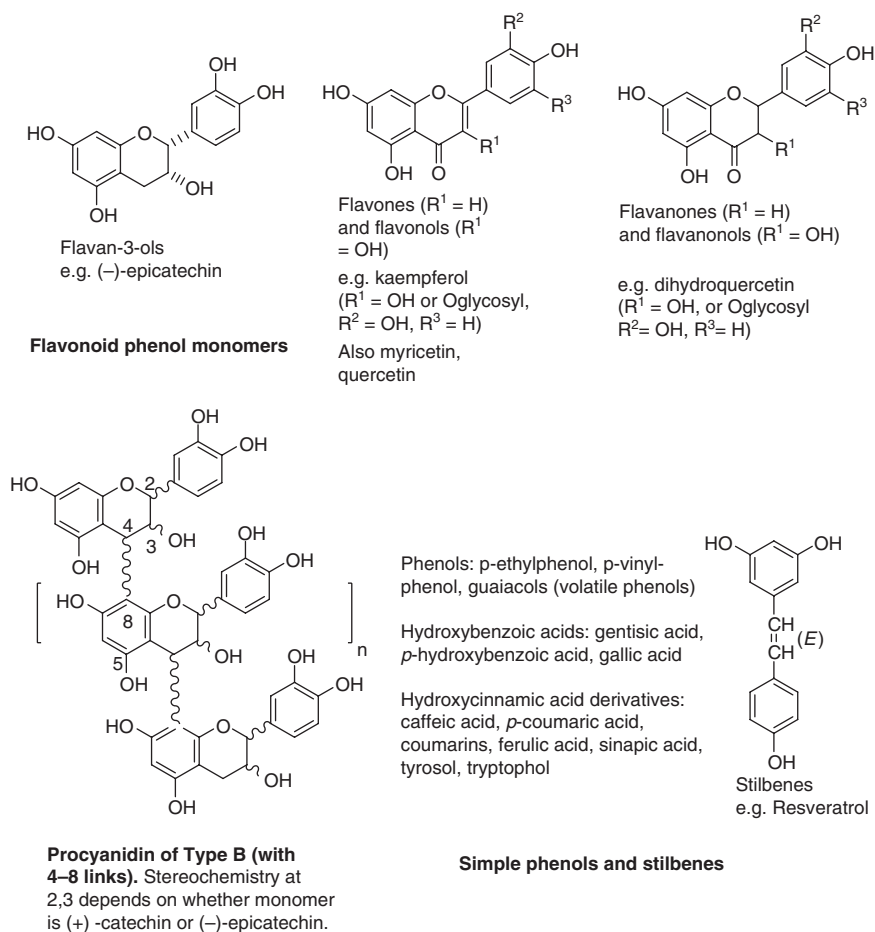


Fig. 17.24 Polyphenols in grapes and wine. Many of these are involved in copigmentation and reactions with anthocyanins and derivatives during fermentation/maturation. Other polyphenols, such as ellagatannin and pentagalloylglucose ('hydrolysable tannins') and some simple phenols, such as gallic acid, eugenol and sinapic acid, are derived wholly or partially from maturation in wood (especially oak). Bitterness: flavan-3-ol monomers are more intensely and more persistently bitter than procyanidins; type A procyanidin dimers (with 4–6 links) are more bitter than those of type B. Astringency: Higher for procyanidin dimers than monomers (but of same duration); higher for type B procyanidin dimer of catechin than other type A or B dimers.

(2006) have reviewed methods for the determination of phenolic substances in the winery. More specifically, NMR, MS and computational methods for the analysis of flavonoid phenols have been reviewed by Lin and Harnly (2007) and March and Brodbelt (2008).

Many of the methods described for the analysis of anthocyanins (Section 17.2, Table 17.6) can be applied to phenolic compounds in general. Non-anthocyanin phenols can be fractionated from anthocyanins using column chromatography, SPE, CCC or other techniques (Buglass and Quantrill, 2011b). Moreover, several families of polyphenols can be determined simultaneously by LC-MS and CZE in particular (Buglass and Caven-Quantrill, 2011b,d; Ballus *et al.*, 2012). MS (especially tandem MS or MSⁿ) and NMR (especially two-dimensional techniques), in particular, have much application in the determination of structure. In LC-MS, ESI of flavonoid phenols in the positive ion mode is often of low efficiency compared to the negative ion mode, but fragmentations from the protonated phenol are more distinctive. Efficiency can be increased by addition of Co(II), Mg²⁺ (or other cations) and/or chelating agents such as 2,2'-bipyridine. See Fig. 17.25 for examples of fragmentation modes and other aspects. The most common type of tandem MS used appears to be product ion scan using collision-induced decomposition in QqQ, Q-TOF or QIT systems.

As in the case of anthocyanins, 2D NMR techniques have been used extensively (often coupled to HPLC and in conjunction with MS) in the determination of must and wine phenolic compounds (Table 17.7). Additionally, 1D NMR has been used for polyphenol profiling of must after removal of carbohydrates by SPE (Savage *et al.*, 2011); for relating various components (including polyphenols) of wine to climatic conditions (Lee *et al.*, 2009); and in relating acids, glycerol, polyphenol content to production process, using PCA (Todasca *et al.*, 2011).

17.7 Future trends

Trends in instrumental analysis of must and wine sensory qualities that have been witnessed in the past decade or so are deemed to be reasonable indicators of future trends. These include the following:

- Application of increasingly sensitive and selective GC, LC and CZE techniques will give higher resolution, lower limits of detection and greater structure determination powers, especially when using a mass selective detector. Two-dimensional GC with spectral deconvolution, optimisation of the LC method to maximise the effectiveness of the MS detector and use of tandem MS systems are likely to play major roles here.
- Combination of instrumental methods with organoleptic techniques will continue to develop, with perhaps some emphasis on GC-O methods,

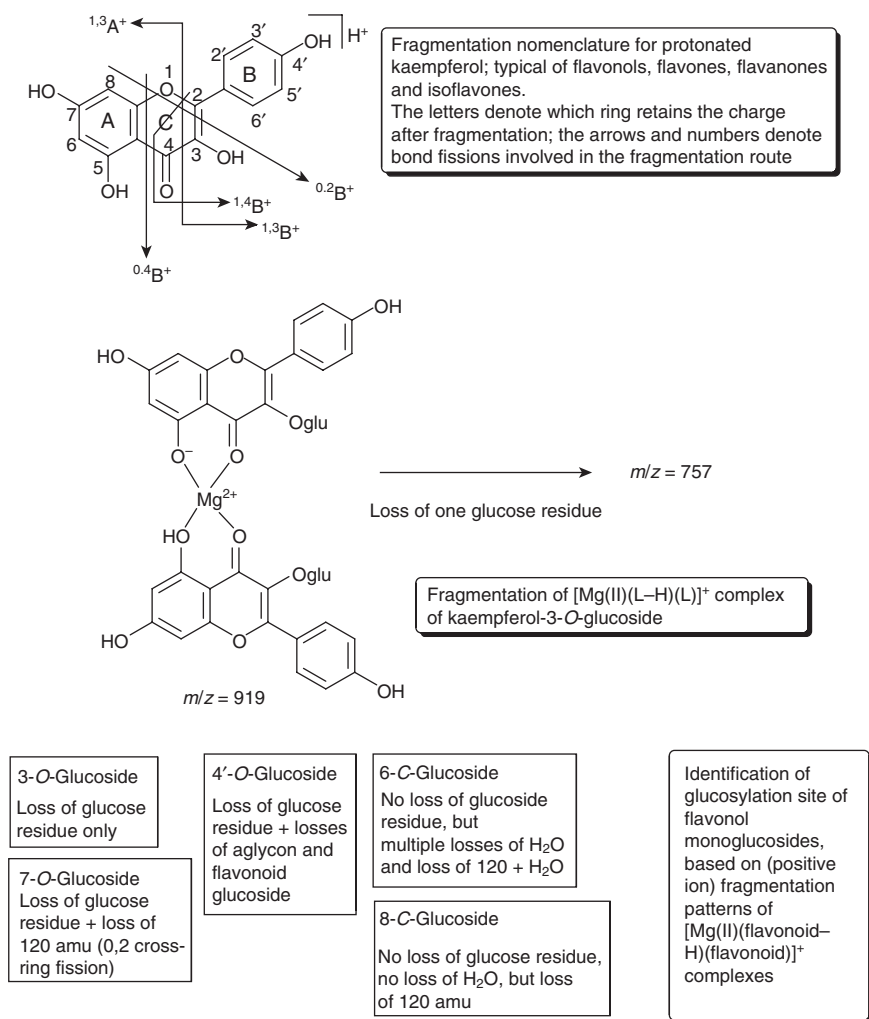


Fig. 17.25 Aspects of fragmentation observed in positive ion mass spectra of flavonoid phenols (March and Brodbelt, 2008).

interaction between odourants, the perception and role of low impact odourants and the influence of the matrix on odour perception.

- Sample preparation, especially sorption methods, will further aid chromatographic or spectroscopic analysis, by removal of interferences and by increasing overall sensitivity thus, sample volumes required to carry out a fit for purpose analysis will likely decrease.
- Rapid indirect methods, such as FTIR, NMR and atomic spectroscopy, along with electrochemical methods (including miniaturised electronic

Table 17.17 Sources of information and advice

Source	Website (http://www or http://)	Description	Features
Agilent Technologies	agilent.com	Instrument and accessories company (chemical analysis, life sciences, diagnostics and genomics, electronic measurement) For academics and industrialists in wine industry and related industries	Applications library, solutions & technical support, training News, links, meetings, publications
American Society of Enology and Viticulture (ASEV)	asev.org/		
Association of Official Analytical Chemists (AOAC International)	aoac.org/	Dedicated to analytical chemistry and standardisation of methods	Standards and methods, publications
Gerstel	gerstel.com	Technically advanced sample preparation and sample introduction accessories for GC, GC/MS, LC, and LC/MS with a high level of automation Based in Europe, but major international oenological and viticultural site	Applications, customer training, technical support Scientific areas: publications, international methods of analysis
Organisation Internationale de la Vigne et du Vin (OIV)	oiv.int/		
Royal Society of Chemistry (RSC)	rsc.org/	For chemists, but with good analytical chemistry content	News, links, meetings, publications, RSS feed, AMC technical reports
Dionex (Thermo Fisher)	dionex.com	Deals with all aspects of ion chromatography (subsidiary of Thermo Fisher Scientific) Company specialising in enzymes, accessories and enzyme kits	Applications library: data sheets, manuals, technical notes, News/events Analytical applications (e.g. advanced wine testing kits), training videos
Megazyme	megazyme.com	Chromatographic equipment and accessories company	Technical support, applications
Phenomenex	phenomenex.com	Chromatographic equipment and accessories company	Learning centres, literature, applications
Supelco (Sigma-Aldrich)	sigmaaldrich.com	Research & development and adoption of innovative practices for grape and wine producers	R&D, industry support and education, commercial services, information services
The Australian Wine Research Institute	awri.com.au		
Thermo Fisher Scientific Waters	thermofisher.com waters.com	Instrument and accessories company Chromatographic instrument (esp. LC and LCMS) and accessories company	Brands, literature Resources: webcasts and videos LC and MS journal publications

noses and tongues), coupled with chemometric/statistical analysis will continue to give information correlating components or profiles with geographical, climatic, production and other factors that relate to sensory characteristics.

- Miniaturisation and automation will continue to be applied to many methods of analysis in order to provide portability (e.g. for analysis in the field or in distant parts of the winery) and rapid sample throughput (e.g. for production monitoring).

17.8 Sources of further information and advice

Good sources of information can be found on the websites of wine organisations (such as the OIV), scientific societies (such as the AOAC, ASEV, ASC and RSC) and instrument or accessory manufacturers. Often, methods of analysis and other publications can be obtained (downloaded as pdfs) through these sources, and sometimes advice can be obtained, although in some cases the reader may need to be a member. Most sites are also useful for news and links. Names of selected organisations, their website addresses and brief features are given in Table 17.17. The authors apologise for ‘omissions’ – the reader is advised to look at websites relevant to his/her own work.

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Instrumental assessment of the sensory quality of beer

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Abstract: Experiencing beer involves multiple senses. Color, clarity (the lack of turbidity) and foam (volume, duration and cling) are perceived visually. The temperature, carbon dioxide bite and astringency are tactile (chemesthetic) sensations. Various tastes are perceived in the mouth. Odors are perceived, either orthonasally or retronasally, by a huge number of receptor types located in the olfactory epithelium. Instrumental assessments corresponding to some of these, particularly appearance factors, relate well to human perceptions. For others, the nature of human and instrumental responses is quite different. It is interesting to consider which beer sensory perceptions have good instrumental analogs and which leave something to be desired.

Key words: chemesthetic sensations, instrumental measurements, olfaction, taste, visual perceptions.

18.1 Introduction

18.1.1 Sensory perceptions

Human senses largely follow the psychophysical response curve (Fig. 18.1) (Stevens, 1957). This shows the strength of a stimulus as the abscissa and the intensity of a sensation as the ordinate. At a low level of stimulus, there is no response and changes in stimulus strength in this range are not perceived. At some level (the detection threshold) the stimulus can just be perceived. At a slightly higher level the stimulus can be recognized (the recognition threshold). Above this level the perceived response increases essentially linearly with increasing stimulus strength, although the response slopes vary with the type of stimulus. At some level of stimulus, saturation is reached and higher levels of stimulus cannot be distinguished.

Most flavor thresholds of the compounds that have been determined in beer are actually difference thresholds, where substances have been added to a beer to determine the point at which a difference can be detected.

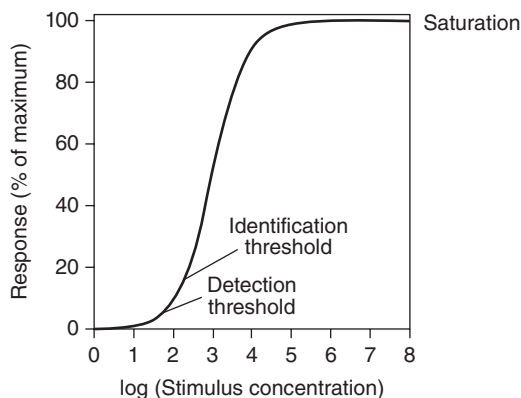


Fig. 18.1 Psychophysical response curve showing response as a function of stimulus intensity.

Frequently this has been done using the ASTM E679 Forced-Choice Ascending Concentration Series Method of Limits (American Society for Testing and Materials, 1979). In this approach, a series of triangle tests, each with two controls and one with the added substance in randomized order, are presented to a panelist. The concentration of the added substance increases with subsequent triangles in geometrically increasing steps (often by a factor of two). Panelists are required to identify one sample in each triangle as being different. Some low concentrations of an added substance may be correctly identified by chance; in this case it is likely that the panelist will miss picking the samples with the added substance at some higher concentration. At some concentration a panelist usually begins to correctly identify the samples with the added substance and also picks the additions correctly at all higher levels. A correct pick at a lower concentration with a miss at a higher level is disregarded. An individual's threshold for a compound is determined as the geometric average of the lowest concentration correctly indicated and the next lower sample. The panel's best estimated threshold is then computed as the geometric average of the individual thresholds. It is quite normal for individual thresholds for a compound to range over two orders of magnitude (Lawless and Heymann, 1998). Some individuals are typically quite sensitive for a compound, others average and a few quite insensitive. True anosmia (the complete inability to detect a compound) does occur, but rarely. A person who is quite sensitive for one compound is likely to be insensitive for another (there are no super-tasters). The patterns are quite different for different individuals. As a result, sensory testing intended to reflect the general population must be done with a panel of at least modest size rather than by one or a few 'expert' tasters.

Because flavor thresholds can easily range over at least ten orders of magnitude (Meilgaard, 1975), 99% chemical purity is no assurance of sensory purity. To determine thresholds properly it is necessary to determine a threshold, purify a compound in some manner (by activated carbon adsorption, recrystallization, distillation, preparative chromatography, etc.), again determine the threshold, purify by a different method, etc. until a constant threshold is achieved (Meilgaard, 1974).

For some purposes the concept of flavor units (FU) is useful. The number of flavor units of a compound present in a sample is:

$$\text{FU} = \frac{\text{concentration of the compound present}}{\text{flavor threshold of the compound}} \quad [18.1]$$

So 1.0 flavor unit of a compound should just be perceptible (equal to the detection threshold). With addition thresholds, it is presumed that a sample containing the concentration of a compound equal to the addition threshold would just be discernible for most people.

The strength of above threshold perceptions can be gauged by a sensory test format such as magnitude estimation (Lawless and Heymann, 1998). In this approach a panelist assigns a number to the perceived intensity of a stimulus. Often this intensity is expressed relative to a reference sample with a specified numeric value for a rated character. Such anchoring of the scale is a way to produce more similar scale usage and less variability among panelists.

With a technique called descriptive analysis (Lawless and Heymann, 1998), during discussion a panel agrees to use a modest number of descriptive terms to describe a set of samples. Samples are then rated for the intensity of each of these attributes.

Upon prolonged exposure to a stimulus, people tend to begin ‘tuning it out’. This phenomenon is called ‘adaptation’ (Lawless and Heymann, 1998). Most people will notice a strong odor upon entering a room, but if they stay there for a while the sensation fades. However, if they leave the room for a bit and then return, the odor is once again noticeable. As a result, care must be taken with a sensory panel not to fatigue panelists by overexposing them to stimuli.

Human beings are rather imprecise instruments (Lawless and Heymann, 1998). There is a high degree of variability among individuals because of inherent differences in sensitivity to particular compounds. And there is considerable variation within individuals; this is influenced by illness, allergy, recent food consumption and emotional state.

18.1.2 Instrumental measurements

Instruments, like humans, do not produce distinguishable responses below some stimulus level; see Fig. 18.2. The level of stimulus that produces a barely significant response is called the critical limit (L_c). This is defined in

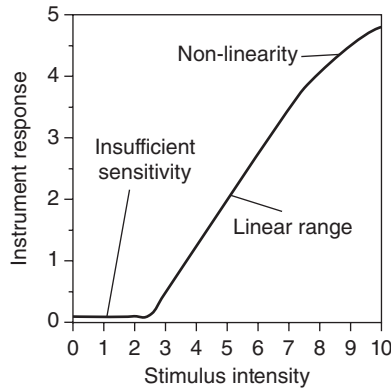


Fig. 18.2 Hypothetical instrumental response curve showing regions of non-significant response, linear increase and non-linearity.

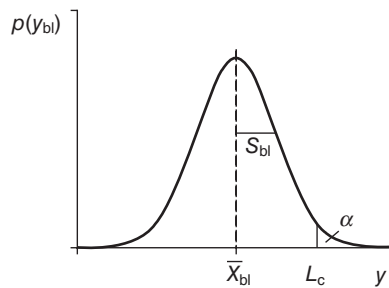


Fig. 18.3 The normally distributed probabilities of response magnitudes for observations of a blank. The critical limit (L_c) is greater than the mean blank result by a multiple of the standard deviation of repeated blank measurements.

terms of multiple measurements of a blank (Currie, 1999). A signal must exceed the mean level of blank responses ($\bar{y}_{bl}$) by a multiple (k_c) of the standard deviation of the blank responses (s_{bl}) to be deemed present (see Fig. 18.3):

$$L_c = \bar{y}_{bl} + k_c s_{bl} \quad [18.2]$$

For normally distributed data and a 95% significance level ($P \leq 0.05$), $k_c = 2.13$, and the likelihood of a false positive (α risk) is 1 in 20 (Currie, 1999).

However, a result less than L_c does not indicate that a stimulus is absent (see Fig. 18.4). For that, a signal must be less than the detection limit (L_d), see Fig. 18.5.

$$L_d = \bar{y}_{bl} + k_d s_{bl} \quad [18.3]$$

For normally distributed data and a 95% significance level ($P \leq 0.05$), $k_d = 4.26$ and the likelihood of a false negative (β risk) is 1 in 20 (Currie, 1999).

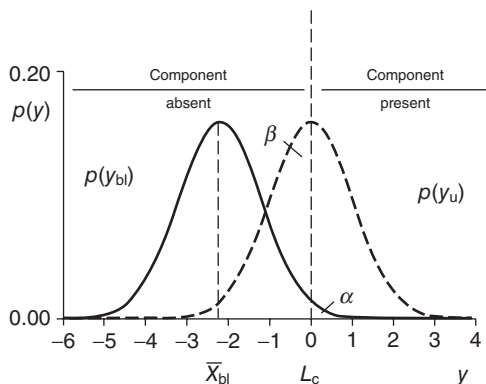


Fig. 18.4 A result less than the critical limit has only a 50% probability that a compound is absent (assuming normally distributed data).

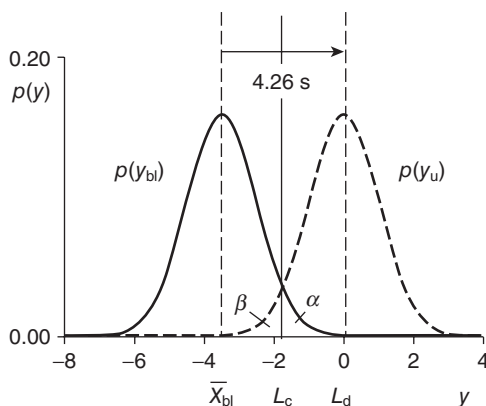


Fig. 18.5 For 95% probability that a compound is absent, a result must be less than the limit of detection (L_d), which is defined as greater than the mean of replicate blank observations plus a higher multiple (4.26 for normally distributed data) times the blank standard deviation.

Instruments often have response ranges where the relationship between stimulus and response is essentially linear (Fig. 18.2). Instruments typically do not become saturated at high stimulus levels, but often reach a region where the response is non-linear.

The American Society of Brewing Chemists (ASBC), the Institute of Brewing (now the Institute of Brewing and Distilling) and the European Brewing Congress (EBC) have for many years collaboratively tested analytical methods for substances or properties of beer and brewing materials. Methods that have been judged to perform satisfactorily by the ASBC have been published in *Methods of Analysis* (American Society of Brewing

Chemists, 2009). Some methods have been tested by both ASBC and EBC and accepted as 'International Methods'.

18.1.3 Univariate and multivariate phenomena

A univariate relationship is one where a single response is related to a single compound. An example would be an absorbance measurement at a wavelength where the analyte is the only absorbing substance. Then the relationship is simply between the concentration of the analyte and the absorbance. Another example would be the strength of a sensory response caused by a single compound in a sample. If glucose were the only sweet substance in a beer, then there would be a univariate relationship between glucose concentration and sweetness.

In a bivariate relationship, two variables jointly influence a response. Ethyl hexanoate and ethyl octanoate, both apple-flavored esters, jointly produce the apple estery flavor note in beer (Meilgaard, 1982).

In multivariate relationships, three or more variables jointly influence a response. Some of these may have a positive effect while others have a negative influence. Positive effects can be simple additivity, where the effects of individual substances are simply added together (e.g. where 0.5FU of one substance and 0.5FU of similarly flavored compound would be perceptible), less than perfect additivity (where, for example 0.6FU of one compound plus 0.6FU of another would be needed to be perceived) or true synergy, where, for example, 0.4FU of one compound + 0.5FU of another would be perceptible. Negative effects are evidenced as inhibition or masking, where the presence of one compound diminishes the response to another. Multivariate responses can occur when multiple compounds jointly impact one response, when a single compound impacts multiple responses, or when multiple compounds impact multiple responses.

18.2 Human and instrumental perceptions of beer appearance

Perceptions of beer appearance include color, foam and turbidity. All of these can be observed instrumentally with physical (optical) measurements, and typically these agree well with human perceptions.

18.2.1 Color

For many years beer color was thought of as the degree of a brown color, ranging from pale yellow through various shades of brown to nearly black. This was a reasonable assumption until the development of 'red' beers; these are not actually red but have a somewhat orange hue. Color observations were originally made with a visual comparator in which a sample was

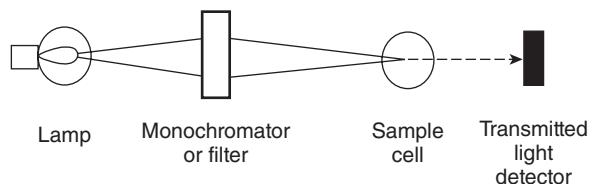


Fig. 18.6 The optical path for either a photometer or spectrophotometer; the former uses a filter and latter a monochromator to select the wavelength or optical band used.

placed in a tube, compared with reference standards, and expressed as degrees Lovibond. This approach was largely superseded by the use of photometers (colorimeters), see Fig. 18.6, with bandpass filters in the 420–450 nm portion of the visible light spectrum (Beer-10B. Color by Photometric Method, in American Society of Brewing Chemists, 2009). This approach in turn was largely supplanted by the use of a spectrophotometer at a single wavelength of 430 nm ('Beer-10A. Spectrophotometric Color Method' in American Society of Brewing Chemists, 2009). More recently, tristimulus instruments or measurements that represent more of the visible spectrum have been employed (Cornell *et al.*, 2002). In both human and instrumental perceptions of color, the strength of the absorbance of a portion of the visible range is observed. In the case of humans, the wavelength range is determined by the concentrations of absorbing compounds present (essentially all the color is derived from malt) while with instruments, the wavelength range is more precisely defined by a monochromator or filter. The correspondence between human and instrumental perception is very good with non-turbid samples. When turbidity is present, it appears to an instrument as if the absorbance is greater than it actually is, because some of the light that does not reach the detector is actually scattered rather than absorbed. A haze-corrected color measurement can be made with an instrument that determines both scattered light and absorbance. Instrumental observations of beer color are considerably more precise (repeatable) than human observations.

18.2.2 Turbidity

In the absence of scattered light, a beer appears clear or 'bright', although it has some color. Scattered light causes a liquid to appear hazy (turbid). Light scattering results from the presence of suspended particles of colloidal or larger size. It is influenced by the size and shape of suspended particles, the wavelength of light used and the angle at which scattering is observed relative to the incident beam (Siebert, 2009). The optical properties of the suspending liquid can also play a role.

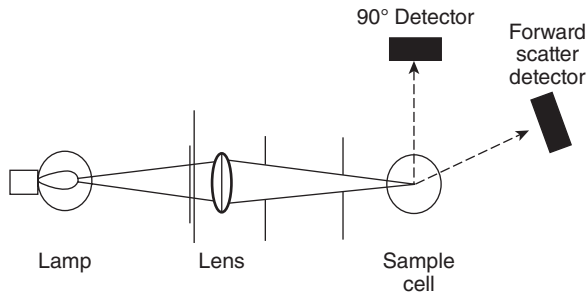


Fig. 18.7 The optical path (seen from above) in a turbidimeter. Often there is a bandpass filter in the optical path.

Turbidimeters (Fig. 18.7) typically observe light scattering either at a narrow angle to the incident light beam (usually in the 11–25° degree range) or at 90°. The latter instruments are also called nephelometers.

Haze has long been assessed visually using various arrangements, including the shadow box (Gales, 2000), or simply by holding a clear vessel containing beer to view against a light. Recent investigations using omnidirectional lighting have indicated good agreement between the haze visual threshold determined against a black non-reflective background and a turbidimeter employing 90° scattering with white light (essentially the entire visible range). Thresholds measured in this way fell in the range 0.21 to 2.2 nephelometric turbidity units (NTU) (Carrasco and Siebert, 1999; Fleet and Siebert, 2005); this is equivalent to 0.053–0.548 EBC turbidity units or 3.67–38.3 nephelos units. Reduced illumination intensity led to lower thresholds (greater detection sensitivity) up to a point (Fleet and Siebert, 2005). Using a light-colored background rather than the black one resulted in a huge increase in thresholds, corresponding to greatly decreased sensitivity (Fleet and Siebert, 2006). This appears to have been due to the difficulty in seeing scattered white light against a light colored background.

Visual perception of haze above the detection threshold using magnitude estimation showed a region of good agreement with a 90° turbidimeter, but diverged at higher particle concentrations (Carrasco and Siebert, 1999); see Fig. 18.8. Humans reached saturation, but the instrumental indication actually increased exponentially, possibly due to multiple scattering.

Descriptive analysis was applied to samples containing particles of three different sizes at suprathreshold concentrations, each suspended in three solution colors. The results indicated panelists responded to only two fundamental properties, scattering intensity and homogeneity (Carrasco and Siebert, 1999). The largest particle size used, 10.3 µm diameter, or about the size of a yeast cell, appeared homogeneous at both low and high concentrations, but at intermediate levels appeared 'lumpy'. It appears that moderate

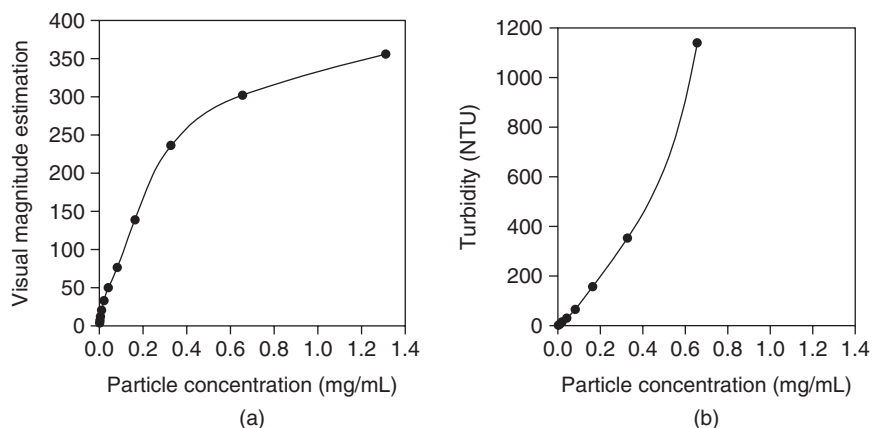


Fig. 18.8 Suspensions of $2.6\mu\text{m}$ diameter spherical particles in clear liquid at suprathreshold concentrations were assessed by (a) visual magnitude estimation: results are geometric average results from 18 panelists, and (b) turbidimeter observations. Data from Carrasco and Siebert (1999).

concentrations of relatively large particles are randomly concentrated and dispersed at different locations in a sample.

18.2.3 Foam

A number of aspects of beer foam have been described including beer foam height, foam volume, collapse time (or rate) and the amount of foam cling (or ‘lacing’) on a glass. Numerous foam assays have been devised to measure these in various ways (Bamforth, 1985; Evans and Sheehan, 2002).

There are two main approaches to foam measurement, pouring from a container (bottle or can) or foaming up degassed beer with CO_2 . Pour test results are affected by package geometry and the CO_2 level of the package. If N_2 is used in addition to CO_2 , this can have a very significant effect on foam bubble size (smaller) and foam duration (longer). Tests in which the beer is removed from the package by piercing through a bottle crown or can bottom rather than by pouring minimize the package effect. And tests where the beer is gently removed from the package and decarbonated before foaming up in some apparatus (e.g. the Rudin method) take away both the effects of the package and the carbonation level. As a result, such approaches should be most informative about the inherent foaming ability of a particular beer. The pour test, on the other hand, is closest to actual consumer practice, but only informative as to the behavior with the particular type of package tested.

The Nibem method foams beer into a glass-like beaker after piercing the package. The collapse of the foam is monitored by an instrument that

observes the time taken for a conductivity probe that senses the top of the foam column to sink by 10, 20 or 30 mm (Wackerbauer and Greif, 1980).

In the Rudin method (Rudin, 1957), beer is removed from the package with minimal foaming, allowed to degas spontaneously overnight in a covered beaker, and a measured volume of beer is placed in the apparatus. CO₂ at regulated pressure and flow rate is admitted through a sintered glass frit to foam up the beer to a certain height. The time taken for drainage of a fixed volume of beer from the foam is observed with a stopwatch.

Pour tests typically involve either pouring by hand or with an apparatus that tilts a package to pour the beer into a glass or glass-like beaker. The time taken for the foam to collapse is measured with a stopwatch. There is inherent difficulty in carrying out pour tests reproducibly.

With tests that assess foam cling, the foam, once generated, is allowed to collapse completely and the amount of foam adhering to the vessel walls is measured in some manner (by comparison with standard photographs or optically). The fact that many foam methods have been described in the literature likely attests to the fact that none is completely satisfactory. One of the difficulties is that the foam level observed at the side of a vessel is different from that in the center.

18.3 Human and instrumental perceptions of beer flavor

Flavor includes perceptions made in at least three ways. The main senses involved are taste, olfaction and chemesthesis.

18.3.1 Olfaction

Olfaction occurs either during inhalation of gas from above a beer, which draws odorant substances past the olfactory epithelium (called orthonasal olfaction) or during exhalation of gas after having ingested beer (retronasal olfaction). In the latter case gas from the lungs or stomach passes by the olfactory epithelium from the back of the nose. There are hundreds of different olfactory receptor types (Hasin-Brumshtein *et al.*, 2009), each of which responds to different degrees to individual chemicals. It has been estimated that humans are capable of recognizing thousands of different compounds (Zozulya *et al.*, 2001). The gas being sensed with any real food or beverage contains multiple compounds. The brain receives many responses in parallel and combines these to produce a pattern that, through experience, we may learn to identify as belonging to a beer, a particular beer brand or a beer style.

Because olfaction responds to large numbers of compounds, two general instrumental approaches have been employed. One of these is the use of gas chromatography (GC), which is suitable because odorants must, by definition, be volatile. GC separates compounds in the time dimension for

separate detection, often by a rather non-specific detector (e.g. flame ionization). This approach converts a multivariate phenomenon into a sequential univariate one (intensity as a function of time), where the size (height or area) of a GC peak (when a peak is due to a single compound) indicates the concentration of a compound and its sensory intensity if it is present above its flavor threshold. The second approach is to use multiple different sensors in parallel that are exposed to the headspace gas above a sample. Because the approach of multiple sensors operating in parallel is conceptually similar to the way olfaction operates, this approach has been dubbed the 'electronic nose' or 'e-nose'.

Olfaction is difficult to represent instrumentally because commonly employed detectors respond very differently to odorant chemicals than the olfactory epithelium. The flame ionization detector (FID) has widely been used in GC studies of organic compounds because it is sensitive, stable and responds to most organic compounds. With an FID, compounds exiting the GC column are passed through a hydrogen–air flame. As they are burned, ions are generated and the ion current between two electrodes is sensed. Many of the compounds causing large peaks in an FID chromatogram of a sample such as beer have no effect on flavor because their flavor thresholds are much higher than the concentrations at which they occur in beer. Often the compounds actually responsible for flavor produce small peaks that are obscured in the chromatogram by larger ones.

Some GC studies have been carried out using a mass spectrometer (MS) as the detector. When MS with selected ion monitoring is employed, the detector is in effect tuned to respond to a particular compound or compound class. This presupposes that the flavor compounds of interest and their mass spectra are known, which is often not the case.

Limitations of GC are the need for adequate separation and sufficient detection sensitivity (Siebert, 2011). GC-GC, where a peak from one column is switched to a dissimilar column to be further separated, can improve resolution. A detector that is highly specific for an element (e.g. nitrogen or sulfur) can measure compounds of interest without physically separating them. This is also the case with simultaneous observation of multiple different signals (like full spectrum MS); this provides data that can be de-convoluted to measure compounds whose peaks underlie other compounds.

An approach that produces a response similar to human sensory perception is gas chromatography-olfactometry (GCO), where the compound separation is carried out by GC but the detection is done with a human nose (Murakami *et al.*, 2003). Strictly speaking, however, this is not entirely an instrumental method.

In an e-nose, a detector array is exposed to headspace gas from a sample (see Fig. 18.9). Several types of detector arrays have been employed (Deisingh *et al.*, 2004). These include metal oxide semiconductor field-effect transistors (MOSFET), conducting polymers, quartz crystal microbalances,

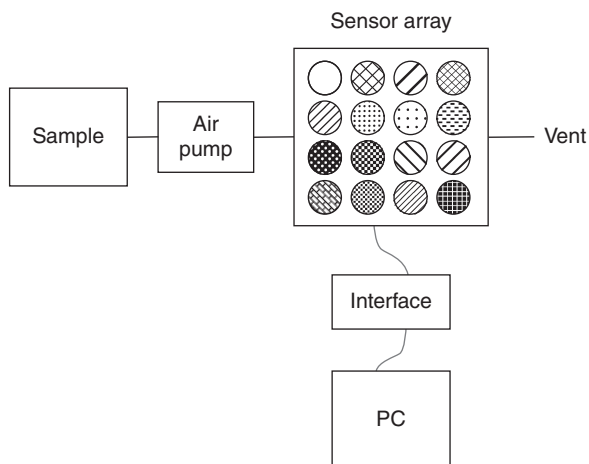


Fig. 18.9 In an electronic nose, the headspace above a liquid or solid is sampled and passed by a sensor array in which each sensor responds somewhat differently. The response data is acquired by a computer and processed using pattern recognition or in some cases a calibration to measure an analyte.

and surface acoustic wave (SAW) sensors. In each case different sensors in the array are modified (for example by different coatings) in order to produce somewhat different responses to a stimulus. E-noses have many fewer types of sensor than the olfactory epithelium and in many cases do not have adequate sensitivity to measure compounds at or below their flavor thresholds. Of necessity e-noses employ computers and multivariate analysis (Siebert, 2001) to process the data. Commonly used approaches are principal components analysis (PCA) and pattern recognition techniques for characterizing samples by raw material cultivar (Bailey *et al.*, 1995; Tomlinson *et al.*, 1995; Weber and Poling, 1997), beer type or brand (Tomlinson *et al.*, 1995), or to detect off-flavors or stale flavors (McKellar *et al.*, 2002; Weber and Poling, 1997). In some cases e-noses have been used to detect or measure particular compounds (Bailey *et al.*, 1995; Weber and Poling, 1997).

Fairly often the multivariate procedure used with the e-nose has been an artificial neural network (ANN) either to classify samples in some way or to predict a compound concentration or flavor sensation (Weber and Poling, 1997). This approach can model non-linear relationships using multiple nodes and layers to feed in inputs in different ways. One of the problems with ANN is that the analyst has no idea how it has operated (which individual sensors were used and in what manner) to produce a result. That severely limits what can be learned. Seasholtz and Kowalski (1993) suggested one should apply the principle of Occam's razor to the use of multivariate procedures: that no more complicated method should be used than

is needed to solve a problem. They recommend the use of ANN with multiple layers and nodes as the method of last resort.

18.3.2 Taste

The five tastes (bitter, salty, sour, sweet and umami) are well characterized (Lawless and Heymann, 1998). Different types of taste buds in the tongue perceive the tastes. Beers usually have noticeable bitterness resulting from the isoalpa acids derived from the hop alpha acids during kettle boiling. Isoalpa acids have a characteristic sharp (or clean) bitterness. Some beers have minor salty or sweet notes. Beers appear to lack umami (savory) flavor. Most beers have limited sourness, but lambics and gueuzes (Belgian sour styles) have a lot of this character.

The bitter aspect of beers is reasonably well measured by UV spectrophotometry after extracting isoalpa acids (and some closely related hop-derived compounds) from acidified beer with isooctane ('Beer-23A. Bitterness Units (BU)' in American Society of Brewing Chemists, 2009); this is an International Method, (where 1 BU is approximately equal to 1 mg/L isoalpa acids). The sweet character in some beers is either from residual amounts of simple sugars remaining after fermentation or, in some cases, from 'priming' sugar added to the beer shortly before packaging, mainly for in bottle 'conditioning' (generating CO₂ by the action of yeast). Sweetness in beer is closely associated with the levels of monosaccharides and disaccharides; these can be measured by HPLC using a refractive index detector ('Wort-14B. Fermentable Saccharides by HPLC' in American Society of Brewing Chemists, 2009). Individual sugars differ in their perceived sweetness (fructose is noticeably sweeter than either glucose or sucrose). Saltiness is associated with the sodium ion (Chandrashekar *et al.*, 2006); its concentration in beer can be determined by atomic absorption spectroscopy ('Beer-36. Sodium by Atomic Absorption Spectroscopy' in American Society of Brewing Chemists, 2009). Sourness is largely a function of pH, with lower pHs perceived as more sour. Low pH in beer is closely related to the concentrations of relatively strongly ionized organic acids, particularly acetic and lactic acids; beer total acidity can be determined either by titration using a pH meter ('Beer-8A. Total Acidity by Potentiometric Titration' in American Society of Brewing Chemists, 2009) or by indicator titration ('Beer-8B. Total Acidity by Titration of Diluted Beer with Phenolphthalein as Indicator' in American Society of Brewing Chemists, 2009).

An equivalent approach for taste to the e-nose is the 'electronic tongue' (Deisingh *et al.*, 2004). This is an array of sensors that are immersed in a liquid (see Fig. 18.10). Because taste is conceptually simpler than olfaction (in terms of the number of characterized qualities), one approach to the electronic tongue is to construct the device to produce responses equivalent to each of the five tastes. In practice, however, electronic tongues have

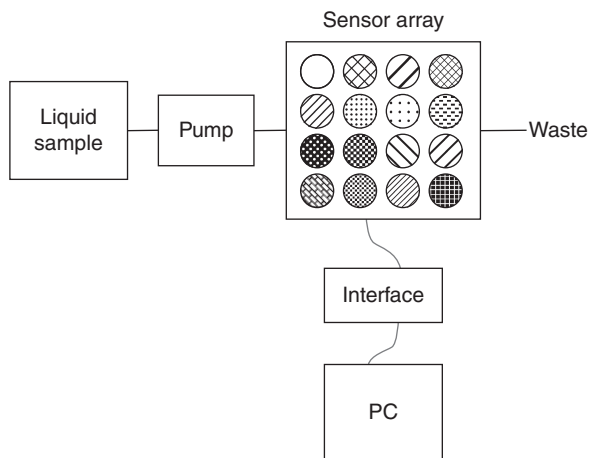


Fig. 18.10 In an electronic tongue, a liquid is sampled and passed by a sensor array in which each sensor responds somewhat differently. The response data is acquired by a computer and processed using pattern recognition or a calibration to measure an analyte.

usually been designed on similar conceptual lines to the e-nose. That is, arrays with considerably more than five sensors are used and multivariate analysis is used to map to the five tastes. The measurement principles used have included electrochemical (potentiometric, voltammetric, amperometric, impedimetric and conductimetric), optical, mass, and enzymatic sensors (also called biosensors) (Escuder-Gilabert and Peris, 2010). In beer, electronic tongues have been applied to relate to taste panel responses (Rudnitskaya *et al.*, 2009), to predict bitterness and alcoholic strength (Arrieta *et al.*, 2010), and to relate to basic beer parameters (Polshin *et al.*, 2010).

18.3.3 Chemesthetic sensations

Chemesthetic perceptions are actually tactile or pain sensations produced by the trigeminal nerve, which wraps around the throat and extends into the mouth. Among the impressions sensed in this way are hot and cold temperature, mouthfeel, CO₂ bite and astringency. Beer is normally served cool or even chilled, and this is perceived by chemesthesia. Smoothness is a general mouthfeel sense, probably resulting from a number of compounds acting in concert. Some beers (particularly very dark ales or lagers) produce an astringent sensation. Saliva contains proline-rich proteins (PRPs), which normally provide lubrication of oral surfaces. When polyphenols are ingested, they cross-link the PRPs, precipitating them and removing the lubrication they provide (Bajec and Pickering, 2008); this results in the astringent sensation. Acids intensify the PRP–polyphenol interaction and

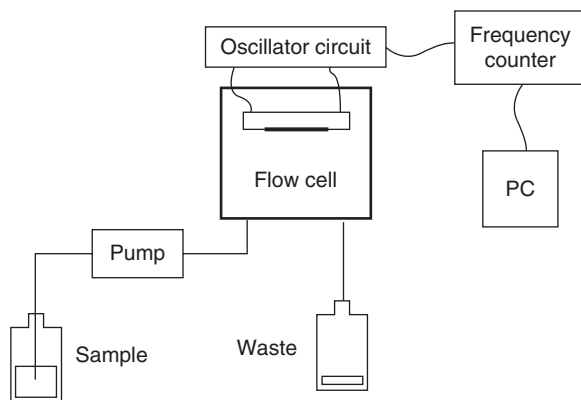


Fig. 18.11 Membrane sensor: when a liquid sample is passed by a quartz crystal coated with a lipid, non-polar compounds in the liquid adsorb onto the lipid. This changes the resonant frequency of the crystal in proportion to the mass adsorbed.

enhance astringency (Siebert and Chassy, 2003). So acids contribute to both sour taste and astringency. It is known that acids alone in water also produce astringency. This was recently shown to be due to the intensification of interaction of polyphenols already present in saliva with PRPs (Siebert *et al.*, 2011).

Some instruments designed to be equivalent to humans for chemesthetic sensations have been developed. Mainly these have employed lipid-coated quartz crystal microbalances (see Fig. 18.11). This approach has been used to assess body, smoothness and astringency in beer (Kaneda *et al.*, 2005). When a liquid is passed by the sensor, non-polar materials are adsorbed onto the lipid, increasing the mass of the crystal, which in turn changes the resonant frequency in proportion to the adsorbed mass.

18.4 Overall perception of beer flavor

When humans experience a product such as beer, the multiple flavor sensations detected by olfaction, taste and chemesthesis are combined in the brain in a manner akin to PCA. Only the three or four most prominent features are seized upon unless a conscious effort is made to steer attention to particular individual characteristics (Laing and Jinks, 1996).

One major difference between analytical measurements and sensory perceptions is that the former are often univariate, while the latter are frequently multivariate. Analytical measurements often originate in the size of a chromatographic peak, which is presumed to represent the concentration of a single compound. In spectrophotometry an absorbance at a particular wavelength is often taken to result from a single compound or class

of closely related compounds, either directly or after use of a reagent to develop a color (chromophore). Visual perceptions and instrumental observations of visual phenomena often perceive essentially the same phenomena and relate well even when the phenomenon actually is caused by multiple factors. For example, the color of beer is the combined effect of all the substances that absorb light in a portion of the visible range. Beer haze mainly results from interactions between proline-rich proteins and polyphenols that are affected by the beer pH and alcohol content. Beer foam is largely a result of proteins (different from those involved in haze) that interact with hop-derived bitter compounds and is also influenced by pH and ethanol content, among other factors. However, since haze and foam are directly measured, their multivariate nature is not apparent.

Studies of various beer properties are frequently made by carrying out sensory observations and chemical (or physical) analysis on the same set of beers. Univariate or multivariate regression is then typically applied to produce relations where the chemical analysis results are presumed to be independent variables and the sensory results are presumed to be dependent variables. In other words, researchers frequently assume that the analytical variables cause the sensory results. In this situation the actual independent variables are the raw materials and the manner of producing the beers. Both the sensory and analytical observations are actually dependent variables. As a result, any apparent relationships cannot demonstrate cause and effect but rather only associations. This may still be useful information as it can guide further experimentation to prove or disprove causation. To prove that a compound is flavor-active, it is necessary to show that the substance is present in beer at or above its flavor threshold or that, together with similarly flavored substance(s), two or more substances are jointly perceptible at the concentrations at which they occur in beer (Siebert, 1988).

18.5 Future trends

Some evolutionary improvements in instrument response in terms of sensitivity and stability are likely to occur; this would lead to better precision and accuracy. However, since many improvements in this area have already occurred, remaining improvements will likely be small. One such area, the use of digital rather than analog electronics, has enabled more signal averaging and digital smoothing, which reduces noise.

Knowledge of the compounds responsible for particular flavors is certain to improve. Many measurements will be moved on-line or in-line. This gives faster response than grab sampling by laboratory personnel and provides continuous information. It also offers the possibility of on-line control for critical parameters such as alcohol, calories, original gravity and CO₂ content.

Instruments developed relatively recently will undoubtedly improve and mature. To some extent this will be so for the e-nose, but probably even more so for the electronic tongue and chemesthesis sensors.

There may be some developments in which sensory properties are predicted from observations of substances that cause a phenomenon. For example, haze at some time point could perhaps be predicted from the concentrations of haze-active (HA) proteins and HA polyphenols.

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Instrumental assessment of the sensory quality of juices

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Abstract: Sensory properties, mainly colour, aroma and taste, are major factors affecting quality perception and consumer's acceptance of fruit and vegetable juices. Colour and appearance are the initial quality attributes attracting us; nevertheless, the flavour (the overall combination of oral and nasal stimulation) may have the largest impact on acceptability and desire to consume it again. Due to the importance not only on the consumer's acceptability but also on other specific factors, the juice industry needs to control the organoleptic characteristics of their products. The sensory properties can be assessed by organoleptic analysis, but this is a subjective description. For this reason, the objective instrumental methods to measure them described in this chapter are sometimes preferred.

Key words: colour, instrumental assessment, juices, odour, sensory properties, taste.

19.1 Introduction

Fruit juices are defined by the EU legislation (Council of the European Union, 2002) as the fermentable but unfermented products obtained from fruit which is sound and ripe, fresh or preserved by chilling, of one or more kinds mixed together, having the characteristic colour, flavour and taste typical of the juice of the fruit from which it comes. Flavour, pulp and cells from the juice which are separated during processing may be restored to the same juice. In the case of citrus fruits, the fruit juice must come from the endocarp. Lime juice, however, may be obtained from the whole fruit, by suitable production processes whereby the proportion of constituents of the outer part of the fruit is reduced to a minimum. The juice may have been concentrated and later reconstituted with water suitable for the purpose of maintaining the essential composition and quality factors of the juice. The addition of sugars or acids can be permitted but must be endorsed in the individual standard. As stated in Codex Standard 247-2005 (Codex, 2005):

The fruit juices and fruit nectars shall have the characteristic colour, aroma and flavour of juice from the same kind of fruit from which it is made. The fruit shall retain no more water from washing, steaming or other preparatory operations than technologically (Codex, 2005)

Consumers' perception of juice quality is mainly related to the organoleptic or sensory properties of the product. Other characteristics like nutritional value, wholesomeness and safety cannot readily be determined by consumers, but if this information is given to the consumer, it will influence the acceptability of the product. Colour, aroma and flavour (the odour and taste sensations) are major factors affecting quality perception and consumer acceptance of fruit and vegetable juices. While colour and appearance may be the initial quality attributes that attract us to a fruit or vegetable, the flavour may have the largest impact on acceptability and desire to consume it again. The human tongue can distinguish only five distinct qualities of taste, sourness, sweetness and bitterness being the most important ones regarding the flavour of fruit juices. The human nose, on the other hand, can distinguish among a vast number of volatile compounds, even in minute quantities. Any changes in the compounds responsible for the sourness, sweetness, bitterness or odour of fruits and vegetables may result in changes in their flavour.

Currently, there is an increasing demand to develop methods for simulating the human perceptions (visual, odour or taste), by means of objective instruments. This is the case of electronic tongue, an instrument for measuring taste attributes.

19.2 Juice appearance

Colour is the first contact point of the consumer with foods. We first judge foods from their appearance (colour, texture, shape) and then from other sensory attributes such as aroma or taste. The prominent role of food colours in their acceptability is therefore unquestionable (Hutchings, 1994; Calvo *et al.*, 2001). In this sense, relationships between the colour and the perception of flavour, sweetness and other organoleptic characteristics related to the quality of citrus products have been reported (Huggart *et al.*, 1977; Tepper, 1993).

Colour can be visually assessed during the tasting of the product or by comparison (colour atlases, dictionaries), although a subjective description rather than an objective definition is achieved. In contrast to this, it is possible to define any colour objectively by using appropriate instruments (mainly, spectrophotometers, colorimeters and spectroradiometers) and following the CIE (International Commission on Illumination) recommendations, a series of standard conditions (illuminant, observer, etc.) and colour spaces (CIEXYZ, CIELUV, CIELAB) (CIE, 2004) aimed at standardizing the objective measurement and definition of colour. The implementation of

appropriate instrumental methodologies for the objective assessment of colour is an expanding field, since the instrumental measurement of colour presents several advantages to food industries:

- Affordability: a wide choice of instruments is affordable for any size industries.
- Simplicity: any person can be appropriately trained for their use in a short time.
- Rapidity: the colour measurements can be made in seconds.
- Non-destructiveness: little or no sample manipulation (filtration, homogenization).
- Automation: some instruments can carry out automatic readings at set time intervals.
- Versatility: many instruments can be easily adapted to carry out different sorts of measurements (reflection, transmission, scattering).
- Portability: portable instruments are particularly useful for field measurements.
- High volume of information obtained: both spectroscopic and colorimetric data.
- Easy interpretation of results: colour parameters easy to interpret across colour spaces.

Thus, the instrumental measurement of colour can be applied not only to monitor the sensory quality of the product, but also to obtain rapid information about the pigments accounting for them.

The juice industry needs to control the colour of their products, not only because of the clear relationship with their acceptability but also for other more specific purposes, such as the study of the influence of the amount of pulp, juice blends, composition, process, spoilage level, etc. on the colour parameters (Gullett *et al.*, 1972; MacDougall, 1983, 2002; Lee and Chen, 1998; Al-maiman and Ahmad, 2002; Meléndez-Martínez *et al.*, 2004; Kırca *et al.*, 2006; Fernández-Vázquez *et al.*, 2011; Turfan *et al.*, 2011).

19.2.1 Instruments for colour measurement

In the case of juices, especially orange juices, much attention was paid to the assessment of colour in the United States during the second half of the twentieth century as this attribute is one of the parameters considered for the commercial classification of the product in relation to its quality. The United States Department of Agriculture (USDA) assigned 40 points out of a scale of 100 points for colour in their commercial grading (Stewart, 1977; Lee, 2001), and a series of points within that scale for grapefruit juices (Huggart and Petrus, 1976). Also, coloured plastic tubes were developed as standards for the classification of orange juices, to be used under standardized observation conditions for the measurement. Instrumentally, the Citrus Colorimeter was widely used; until 1985 it was the only officially approved

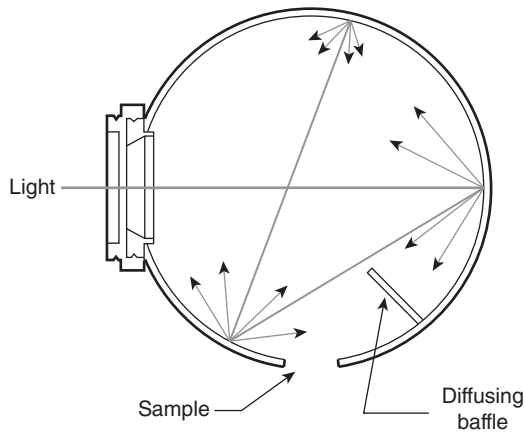


Fig. 19.1 Diffuse reflectance measurement scheme.

instrument to measure the colour of orange juices in the USA (Huggart *et al.*, 1977) and the producers of orange juices had to use it to measure the colour of the product to avoid the inherent subjectivity related to the nomenclature of colour.

In two interesting studies, different instruments, optical designs and sample presentations were evaluated. Of all the possibilities tested, many showed correlation coefficients over 0.97 relative to the official method and new formulas for the calculation of the colour number were proposed (Eagerman, 1978; Buslig and Wagner, 1985a). Some of the best correlations were obtained when using a diffuse reflectance sphere attached to the instrument, whose usefulness for the measurement of orange juice colour has also been reported by other authors (Fig. 19.1; Rummens, 1970; Buslig and Wagner, 1985b; Buslig, 1991, 1993; Meléndez-Martínez *et al.*, 2006).

As a result of the design and development of instruments with improved components, accuracy and reproducibility, a new generation of apparatus and their specific equations have been approved for the measurement of juice colour such as spectrophotometers and colorimeters (Buslig and Wagner, 1986, 1988; Buslig *et al.*, 1987; Buslig and Buslig, 1988; Buslig, 1989, 1991, 1992, 1993; Lee and Coates, 1999, 2002, 2003; Lee, 2000, 2001; Lee and Castle, 2001).

Spectroradiometers have been widely used: to detect the colour changes due to the effect of the dilution of orange juices (Figs 19.2 and 19.3; Meléndez-Martínez *et al.*, 2005) or the characterization of different kinds of these products (Meléndez-Martínez *et al.*, 2007a, 2008; Fernández-Vázquez *et al.*, 2011). Likewise, they have proved useful to estimate carotenoid contents (Meléndez-Martínez *et al.*, 2003, 2010, 2011) and to detect colour changes due to deterioration of the juices (Meléndez-Martínez *et al.*, 2009b, 2010).

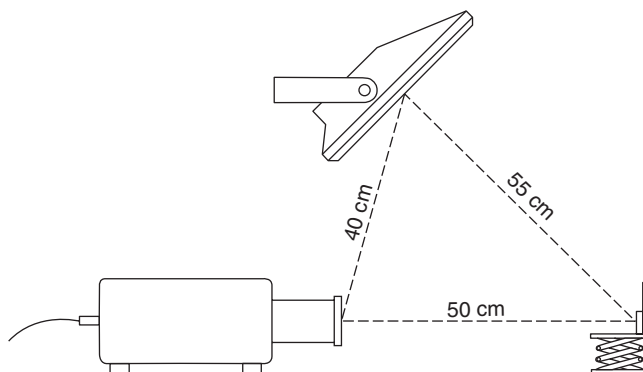


Fig. 19.2 Spectroradiometric measurement scheme.

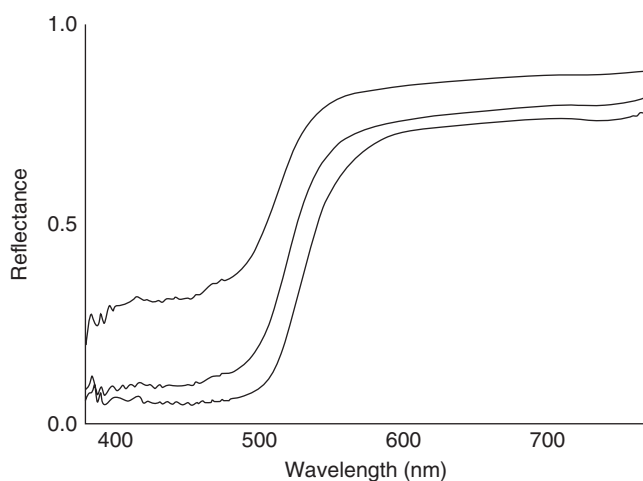


Fig. 19.3 Reflectance spectra of orange juices.

More recently, reports on the use of digital image analysis for the colour assessment of orange juices have appeared (Fig. 19.4; Fernández-Vázquez *et al.*, 2011). In principle, one of the main advantages of this methodology is that it allows for a wider area of the sample to be considered for the readings, as well as that every colour present in the image captured from the sample can be taken into account (pixels). Furthermore, other attributes related to the appearance of the product (texture, distribution of colour, homogeneity, etc.) apart from colour can be assessed.

Regarding the colour calculations, grape and orange juices (together with other liquid foods such as olive oil, vanilla milkshake, brandy, honey, vinegar and petunidin solutions at different pH values) were included in a



Fig. 19.4 Image for digital analysis.

study to determine differences in colour coordinates obtained from the use of different spectral features in the calculation by considering different sources of errors (truncation, abridgement or different bandwidths) (Montes *et al.*, 2004). It is interesting to know how much difference can be expected from those factors in order to avoid confusion between colour differences attributable to instruments and those attributable to actual colour changes.

19.2.2 Chemical and technological quality assessment by colour measurement

The orange, yellowish or reddish colours of oranges, watermelons, tomatoes, and other fruits and vegetables are mainly due to carotenoids, which are lipophilic compounds located in the pulp particles (Liu *et al.*, 2007; Meléndez-Martínez *et al.*, 2007b; Alquezar *et al.*, 2008). Other red-bluish colours (red grape, strawberry, raspberry, blood orange, black carrot, etc.) are due to anthocyanins, which are water-soluble pigments (Meléndez-Martínez *et al.*, 2007b; Veitch and Grayer, 2011). Both types of pigments have been reported to have antioxidant activity and are associated to several health benefits (Krinsky *et al.*, 2004; Weisel *et al.*, 2006; Spormann *et al.*, 2008; Khandare *et al.*, 2011). As a result of the industrial processing, storage and other factors changes in the composition take place and colour changes can occur, sometimes visually noticed (Meléndez-Martínez *et al.*, 2008, 2009a, 2009b; Borochov-Neori *et al.*, 2009; Sadilova *et al.*, 2009; Caminiti *et al.*, 2011; Turfan *et al.*, 2011).

Characterizing the chromatic features of pigments present in solutions like juices is of great importance, as stated in the case of 3-monoglucosidein

anthocyanins of grape juices (Heredia *et al.*, 1998). The applicability of instrumental colour measurements to estimate carotenoid levels deserves to be highlighted since some of them (lutein, zeaxanthin, lycopene, provitamin A carotenoids) have nutritional relevance, and hence their relevance for quality control purposes (Meléndez-Martínez *et al.*, 2003).

Concerning the effect of chemical changes in carotenoids on the colour of orange juices, Cortes *et al.* (2006) observed that the high intensity pulsed electric fields (HIPEF) treatments led to lower decreases in the carotenoid levels than pasteurization and that the changes in the pigment levels were accompanied by decreases in a^* and increases in b^* . Moreover, it was concluded that some of the resulting colour changes could be visually detectable by the consumers. The colour changes associated to the processing and storage of orange juices can lead to visual colour changes due to the conversion of 5,6-epoxycarotenoids to 5,8-furanoidcarotenoids (Meléndez-Martínez *et al.*, 2009a, 2009b).

Meléndez-Martínez *et al.* (2003) found statistically significant correlations between the levels of major orange juice carotenoids and b^* , C_{ab}^* and h_{ab} , proposing formulas for the estimation of carotenoid levels from colorimetric information. In a later study it was reported that the parameters best correlated with the total carotenoid content of ultrafrozen orange juices were b^* and C_{ab}^* , although the correlation with h_{ab} was very poor (Meléndez-Martínez *et al.*, 2007a).

Besides the chemical composition, changes in the colour of juices can be related with a series of factors including agronomic or processing such as heat treatments, preservative methods or storage time and conditions. In this sense, colour measurements have appeared very useful in following or detecting modifications in quality characteristics and chemical composition.

Regarding agronomic factors, the CIELAB colour coordinates (L^* , a^* , b^* , C_{ab}^* and h_{ab}) have been successfully applied to studying mandarin juices from a series of eight varieties; it was found that the Clemenules variety provided the darkest juice (Beltrán *et al.*, 2008). Other authors found that the juice of the Hernandina variety cultivated in organic farming had better sensory qualities, including colour, with higher contents of minerals and carotenoids (Beltrán-González *et al.*, 2008a). The colour measurements are also valuable tools in quality control for the evaluation of the maturation degree of the fruits or for the differentiation among varieties (Lee, 2000). In the case of grapes, the berry size influences the colour of the juices and wines obtained. This effect was evaluated in Shiraz grapevines growing in a warm climate (Walker *et al.*, 2005) and better colour density and higher anthocyanin concentration was found for the very smallest mass category (0.3–0.55 g) compared with larger berries (1.4–2.0 g).

The changes in colour due to the effect of the temperature of storage were evaluated in several fruit juices. Mandarin orange juice was packed in two different non-transparent cartons and kept at two storage conditions:

4°C and room temperature ($25 \pm 3^\circ\text{C}$). During the shelf-life the CIELAB colour parameters were determined and also the sensory colour of the juices was evaluated by a consumer panel (Beltrán-González *et al.*, 2008b; Beltrán *et al.*, 2009). Significant changes in colour were found when two types of freezing storage, normal (5 and 20°C) and accelerated (30, 40 and 50°C), were tested as influencing the sensory quality of orange juice made from a Brazilian concentrate (Petersen *et al.*, 1998), and mathematical models were developed to predict colour variation of concentrated and reconstituted prickly pear juices, as a function of the CIELAB parameters, when they were stored at different time and temperature conditions. An equation which predicts the sensory response as a function of the CIELAB parameters was also developed (Sáenz *et al.*, 1993).

The effect of light exposure during the storage was also evaluated by colour measurements in a highly pigmented pulp, from red coloured Star Ruby grapefruit juice stored in glass test tubes and kept in a refrigerated locker at 4.5°C for 8 weeks (Shim and Kim, 2002), which causes a slight loss of red character. In the case of pasteurized juices of Bearss Seedless lime stored in glass bottles under refrigeration and freezing the colour evaluation study resulted in a gradual increase of the browning index and ΔE (Ziena, 2000). Lee and Lee (1999) used the colour measurements to study the influence of the concentration technique (vacuum evaporation, freeze concentration and reverse osmosis) on the quality characteristics (colour, turbidity, heat stability and sensory preference) of pear juice.

Usually some processes, such as heat treatments, affect the colour of juices; hence, colour measurement can be used to evaluate modifications in quality characteristics and sensory properties. For example, the effect of thermal treatments has been assessed by using the Hunter colour scale values L^* , a^* and b^* . In tomato purée and strawberry juice treated with two types (normal and high pressure) of thermal processes (Rodrigo *et al.*, 2007). It was found that the L^*a^*/b^* parameter combination best described the colour changes and was useful to state the treatment which induced lowest colour modifications; in watermelon juice it was used to predict the lycopene and total carotenoid contents (Sharma *et al.*, 2008). Different heat treatments related to the quality of grape juices were studied based on colour evaluation. Grape juices processed at different temperatures and times, showed slight increase in L^* and b^* values and a decrease in coordinate a^* , having higher influence for the temperature than the time (Cabrera *et al.*, 2009). Other studies assessed the effect of heating in a microwave oven (Cinquanta *et al.* 2009; 2010) or pasteurization (Gabriel and Azanza, 2009) in orange juices. In the case of apple juice, which is very affected by enzymatic browning, different pasteurization processes were evaluated colorimetrically by comparing it with the untreated juice (Mehmood *et al.*, 2008; Sanchez-Vega *et al.*, 2009).

The colorimetric parameters (reflectance spectra, CIELAB colour space and colour difference) were applied to evaluate non-enzymatic browning

due to thermal treatment of cashew apple juice related to variations in ascorbic acid, 5-hydroxymethylfurfural and sugar contents (Damasceno *et al.*, 2008), and the decay of the main carotenoids. It was found that ΔE^* is good predictor of both all-*trans*- β -cryptoxanthin and all-*trans*- β -carotene thermal degradation (Zepka *et al.*, 2009).

High-pressure treatment can also induce colour modifications. The effect of different treatments on the colour of several juices has been studied: in white grape juice during the storage (Daoudi *et al.*, 2002), ultra-high-pressure homogenization (UHPH) in fresh apple juice (Saldo *et al.*, 2009), continuous high-pressure carbon dioxide (HPCD) in orange juice (Kincal *et al.*, 2006), and ultra-high hydrostatic pressure (UHP) in single-strength natural tomato juice (Porretta *et al.*, 1995).

The effect of the pulsed electric field (PEF) process on the flavour and colour of orange juice has been investigated at different temperature and time storage conditions (Ayhan *et al.*, 2001, 2002), compared with thermal processing (Min *et al.*, 2003), or combined with thermosonication (TS) technology (TS/PEF) (Walkling-Ribeiro *et al.*, 2009). The colour characteristic during storage of PEF-processed and thermally processed tomato juices was assessed and significantly lower non-enzymatic browning and higher redness was found for the juice treated by PEF (Min and Zhang, 2003).

Irradiation (UV, gamma) is highly effective in inactivating microorganisms so it is used as decontamination method in food industry. The influence of ultraviolet (UV) irradiation on some quality attributes, the colour among them, was studied on fresh apple juice made from different varieties (Kiss and Farkas, 1972; Falguera *et al.*, 2011) on fresh strawberries and grapes (Kiss and Farkas, 1972) and on ready-to-use tamarind juice (Lee *et al.*, 2009).

Colour measurements have been very useful to assess the influence of other preservation methods on the quality of fruit juices: electrodialysis process on four tropical fruit juices (passion fruit, castilla mulberry, najanrilla and araza) (Vera *et al.*, 2003, 2007), ultrasonic treatments on orange juice (Gómez-López *et al.*, 2010), canning process on mango juice (Hansawasdi *et al.*, 2009), ozonation process on tomato juice (Tiwari *et al.*, 2009), clarification agents and methods on pomegranate juice (Vardin and Fenercioğlu, 2003), enzymatic mash treatments on apple juice (Mihalev *et al.*, 2004) and on date juice obtained from immature fruits (Kulkarni *et al.*, 2010), and packaging with oxygen scavenging films (OSF) on orange juice (Xu *et al.*, 2012).

19.2.3 Correlation between visual and instrumental assessments

The visual analysis of colour is part of the sensory analysis and can provide information about the perceptions and preferences of potential consumers, but describing the colour of the product in this way has an important subjective component. Some guidelines for these assessments concerning physical requirements, types of tests, panel selection, sample presentation and

data handling, among others, can be found elsewhere (Hutchings, 1994; MacDougall, 2002; Meléndez-Martínez *et al.*, 2004).

There are many reports about the correlation between instrumental and visual analysis of orange juices colour. Gullett *et al.* (1972) studied the influence of different pulp contents on colour by both approaches and concluded that the higher the size of the pulp particles the lower the regression coefficients obtained. Rummens (1970) performed a methodological study aimed at optimizing the instrumental measurement of orange-based drinks, and found that the use of a hollow sphere internally covered with MgO and with the sample in the centre (allowing a multiple scattering and reflection from all sides) correlated well with the corresponding colours in the Munsell Atlas. In some studies assessing the colour of turbid juices by measuring the reflection of a layer of sample thick enough to be considered opaque, good correlations with the visual analyses were obtained (Wenzel and Huggart, 1962; Edwards *et al.*, 1966). However, other reports indicate that for the detection of small colour differences in translucent samples, presentation of thin layers of the sample would be more appropriate (Little, 1964; Little and Mackinney, 1969; Calvo, 1993). The colour of orange juices diluted with distilled water to obtain eight levels of concentration (10, 25, 40, 50, 60, 75, 90 and 100% of juice) was assessed visually and by spectroradiometry, in both cases against white and black backgrounds. It was found that the use of a black background led clearly to better classifications of the samples as a function of their concentration, and also the colour parameters best correlated with the level of concentration of orange juice were different as a function of the background used (Meléndez-Martínez *et al.*, 2005; Stinco *et al.*, 2012). Plate VIII (between pages 242 and 243) shows the background effect in colour measurement. These observations were in agreement with those obtained on other food-stuffs reported by Huang *et al.* (1970a, 1970b).

The colour of orange juices from five cultivars was assessed instrumentally, by spectroradiometry and digital image analysis, and visually by a panel trained to assess the CIELAB colour space parameters, which was used to evaluate the relationships between the instrumental and the visual assessments. The results indicated that the hue h_{ab} was better correlated with the spectroradiometric measurements while lightness L^* was better correlated with the digital image analysis (Fernández-Vázquez *et al.*, 2011). The quantitative colour attribute chroma, was not well assessed by the panel and showed poor correlation with the instrumental measurements, which agreed well with other findings (Meléndez-Martínez *et al.*, 2004). Wei *et al.* (2012) quantified the food appearance and studied the relationship between colour appearance and sensory characteristics of orange juices by visual assessment carried out using a calibrated digital display. They applied the CIELAB colour difference formula (ΔE_{ab}^*) to determine the colour tolerance and proposed a new formula to predict the sourness and freshness of orange juices.

19.3 Juice aroma

Extracting and identifying the compounds related to flavour/off-flavour composition of foods has been a challenge for scientists since the beginning of the last century and it was not until gas chromatography (GC) was invented in the 1950s when most important aroma compounds were separated and identified (Reineccius, 2006). Nowadays, aromagrams or aroma profiles obtained by chromatographic methods or electronic noses (e-noses) can be valuable tools in assessing the sensory quality of juices (Plutowska and Wardencki, 2007).

Flavour compounds arise as secondary metabolites formed during metabolic pathways of carotenoids, fatty or amino acid precursors (carboxylic acids, alcohols, carbonyl compounds, lactones) or during ripening of fruit (terpenes esters, ethers) (Jiang and Song, 2010). An overview of flavour formation in fruits and vegetables can be found in Reineccius (2006) and an updated reference treatise on the flavours of fruits and vegetables has been edited by Hui (2010). The quality of flavour is influenced by genetic, pre-harvest, harvesting and post-harvesting factors, processing technologies and storage conditions (Dixon and Hewett, 2000; Averbeck and Schieberle, 2011). An overview of the processing effects on orange juice flavour has been published by Ruiz Perez-Cacho and Rouseff (2008). Fruit type can be identified by the volatile constituent of the juice while variety is related to the relative proportion. The main flavour compounds detected in fruit juices are shown in Table 19.1.

One of the problems in quality assessment is to identify which compounds contribute to the desirable aroma and off-flavour, their threshold concentration, potency and interaction with other compounds (Kader, 2008). In this sense, the traditional research methods of aroma have advanced from methods that provided information only about compounds present at high concentrations in juices to the current application of sophisticated extraction, separation and detection techniques that provide information about component concentration and identity at nanogram per litre (ng/L) levels (Ebeler, 2004). However, to elucidate the odour-active components in complex aroma profiles, a molecular sensory science approach is needed, based on aroma reconstitution assays (Greger and Schieberle, 2007). For example, in freshly squeezed orange juice more than 300 aroma compounds have been identified by gas chromatography–mass spectrometry (GC-MS) analytical methods, of which less than 25 appear to have significant odour activity at levels found in fresh orange juice, but the number and composition of aroma-active compounds required to reproduce the orange odour still has not been generally accepted. It must also be taken into account that the volatile composition changes with agricultural factors (variety, maturity, soil) and technological process (pasteurization, concentration) (Ruiz Perez-Cacho and Rouseff, 2008).

Table 19.1 Important or abundant flavour compounds in fruit juices

Juice	Odour compounds	CAS Number	References
Orange	Citral	5392-40-5	Robards and Antolovich (1995)
	Limonene	95327-98-3	
	Linalool α -Pinene	78-70-6	
	Ethyl butanoate	80-56-8	
	Acetaldehyde	105-54-4	
	Octanal	75-07-0 124-13-0	
Apricot	Benzaldehyde	100-52-7	Guichard and Souty (1988) Guichard (1988)
	Linalool	78-70-6	
Peach	Benzaldehyde	100-52-7	Riu-Aumatell <i>et al.</i> (2005)
	Methyl acetate	79-20-9	
	Ethyl acetate	141-78-6	
Pear	2,4-decadienoic,ethyl ester	3025-30-7	Kralj Cigic and Zupancic-Kralj (1999) Shiota (1990) Chervin <i>et al.</i> (2000)
	(<i>E,E</i>)- α -farnesene	502-61-4	
Apple	β -Damascenone	23696-85-7	
	Hexyl hexanoate	6378-65-0	
	Ethyl butanoate	105-54-4	
	Butyl hexanoate	626-82-4	
	Hexyl butanoate	2639-63-6	
	Ethyl-2-methyl butanoate	7452-79-1	
Blackcurrant	Methyl butanoate	623-42-7	Varming <i>et al.</i> (2004)
	Ethyl butanoate	105-54-4	
	Ethyl hexanoate	123-66-0	
	Cineole	8024-53-1	
	Linalool	78-70-6	
	4-Terpineol α -Damascenone	562-74-3	
	1-Octen-3-one	24720-09-0	
	2-Methoxy-3-	4312-99-6	
	Isopropylpyrazine	25773-40-4	
	4-Methoxy-2-methyl-2-butanethiol	94087-83-9	
Grapefruit	Ethyl butanoate	105-54-4	Buettner and Schieberle (1999)
	<i>p</i> -1-Menthene-8-thiol	71159-90-5	
	(<i>Z</i>)-3-Hexenal	6789-80-6	
	4,5-Epoxy-(<i>E</i>)-2-decenal	188590-62-7	
	4-Mercapto-4-	19872-52-7	
	methylpentane-2-one1-	2918-13-0	
	Heptene-3-one	182699-77-0	
	Wine lactone		

Table 19.1 *Continued*

Juice	Odour compounds	CAS Number	References
Grape	2,5-Dimethyl-4-hydroxy-3(2H)-furanone	3658-77-3 431-03-8	Baek <i>et al.</i> (1997) Kotseridis and Baumes (2000)
	2,3-Butanedione	105-54-4	
	Ethyl butanoate	7452-79-1	
	Ethyl 2-methylbutanoate	60-12-8	
	2-Phenylethanol-	551-93-9	
	Aminoacetophenone	3268-49-3	
	3-(Methylsulfanyl) propanal	557-48-2	
	(<i>E,Z</i>)-2,6-nonadienal	112-31-2	
Lemon	Decanal		
	Geranial	96680-15-8	Ikeda <i>et al.</i> (1962) Moshonas and Shaw (1972) Allegrone <i>et al.</i> (2006)
	Neral	96680-15-8	
	Ethyl acetate	141-78-6	
	2-Methyl-3-buten-2-ol	115-18-4	
	4-Methyl-2-pentanone	108-10-1	
	Limonene γ -terpinene	95327-98-3	
	Linalool	99-85-4	
	Terpinen-4-ol α -Terpineol	78-70-6	
	Carvone	562-74-3	
	Methanol	10482-56-1	
	Ethanol	99-49-0	
Pineapple	Perillaldehyde	67-56-1 64-17-5 2111-75-3	Tokitomo <i>et al.</i> (2005) Berger <i>et al.</i> (1985) Flath and Forrey (1970)
	4-Hidroxy-2,5-dimethyl-3(2H)-furanone	3658-77-3 97-62-1	
	Ethyl 2-methylpropanoate	7452-79-1	
	Ethyl 2-methylbutanoate	868-57-5	
	Methyl 2-methylbutanoate	19883-27-3	
	1-(<i>E,Z</i>)-3,5-undecatriene	23696-85-7	
	β -damascenone	81717-82-0	
	1-(<i>E,Z,Z</i>)-3,5,8-undecatetraene	104-50-7 698-76-0	
	γ -Octalactone, δ -Octalactone γ -Nonalactone	104-61-0	
Strawberry	(<i>Z</i>)-3-hexenal	6789-80-6	Schieberle and Hofmann (1997)
	4-Hydroxy-2,5-dimethyl-3(2H)-furanone	3658-77-3 623-42-7	
	Methyl butanoate	105-54-4	
	Ethyl butanoate	9011-14-7	
	Methyl 2-methylpropanoate	431-03-8	
	2,3-Butanedione		
Tomato	(<i>Z</i>)-3-Hexenal α -Ionone	6789-80-6	Buttery <i>et al.</i> (1987)
	Hexanal	127-41-3	
	(<i>Z</i>)-3-Hexenol	66-25-1	
	(<i>E</i>)-2-Hexenal	95123-47-0	
	3-Methylbutanol	73543-95-0	
	2-Isobutylthiazole	6423-06-9	
	6-Methyl-5-hepten-2-on	18640-74-9 110-93-0	

19.3.1 Chromatographic techniques

The isolation of low-concentration volatile compounds from a complex matrix containing sugars, complex carbohydrates, lipid proteins and water, which is the case of most juices, may be problematic. The chromatographic analysis of flavours and off-flavours in juices usually requires that the samples first be processed to remove as many interfering compounds as possible, assuming that discarding the irrelevant chemicals will not risk the loss of significant flavour factors. Sample preparation for food flavour analysis has been exhaustively reviewed by Wilkes *et al.* (2000). Although a number of flavour isolation methods are known, the most appropriate way to attain an optimum recovery of the flavour chemicals in a juice is the employment of more than one extraction technique.

According to the isolation and concentration techniques there are two groups of methods: those not involving concentration (direct sampling) and those involving concentration (concentration methods). Direct injection of juices on GC has been rarely applied to the fruit juices analysis due to its low sensitivity and the high temperatures used.

Static headspace (SHS)

Static headspace (SHS) is the simplest method for analysis of volatiles. It is often used in quality control of juices, when only major components have to be measured. It has many advantages (simple, gentle and automated) but one of its major drawbacks is low sensitivity for substances of intermediate and high boiling point. During the SHS analysis, equilibrium between the juice and the headspace above it is achieved, and a fraction of this headspace gas phase is withdrawn for GC analysis. The odorants present are assumed to render the so-called 'top note' to the juice and are expected to be related better to the profiles experienced by human subjects (Hinterholzer and Schieberle, 1998). However, owing to the comparatively low sensitivity, the amounts of volatiles detected by SHS method are quite limited. To improve the sensitivity of detection the addition of salts to the juice can be used, e.g. the addition of sodium chloride to pear juice was extremely effective for improving the sensitivity of detection of flavour components by SHS-GC (Tobitsuka *et al.*, 2009).

Solvent extraction (SE)

Solvent extraction (SE) is the most widely used method for aroma collection. This method provides a comprehensive extraction of volatiles and measures the composition of volatile compounds present in the juice. However, the goal of flavour analysis is to identify volatile compounds, which are perceived by the human nose in the air above a food, and the solvent extracts do not always resemble the aroma perceived by a human nose. However it has been reported to be representative to the odour quality evaluated by a sensory panel in blood and blond orange juices (Näf *et al.*, 1996; Selli and Kelebek, 2011). SE is a better sample preparation

technique for the determination of polar compounds such as acids or alcohols (Sánchez-Palomo *et al.*, 2009). This technique requires large amounts of high-purity solvents, is relatively tedious and time-consuming and causes serious environmental problems. Furthermore, extracts often have to be concentrated hundreds of times, and loss of analytes and artefact formation, due to elevated temperatures, during the concentration step are generally observed. An improvement of this technique which minimizes the main drawbacks of solvent extraction is liquid–liquid microextraction (LLME) which is faster and simple. It has been successfully applied to juices obtained from melons, peaches, grapes, strawberries and tomatoes (Aubert *et al.*, 2005).

Steam distillation (SD)

Steam distillation (SD) has been applied to flavour extraction in different juices for identification purposes (Hinterholzer and Schieberle, 1998). Methods involving heating the sample can modify the flavour composition quantitatively and qualitatively. Moreover, odour-active compounds in fruits often present glucosidic precursors which are converted to the free aroma compound during heating processes, or at low pH values. For that reason distillation under reduced pressure is of great interest in juice analysis. Derail *et al.* (1999) reported differences in key odorants of handmade juice of yellow-flesh peaches (*Prunus persica* L.) induced by the workup procedure (solvent extraction and high-vacuum distillation vs. simultaneous steam distillation/extraction).

Simultaneous extraction of steam distillates by solvents (SDE)

Simultaneous extraction of steam distillates by solvents (SDE) is among the most versatile methods used today in food flavour isolation. Although aroma extracts can be obtained very quickly and simply by this method, the elevated temperatures applied during distillation may lead to artefact formation as mentioned above. SDE has a higher extraction capacity and higher recovery for polar compounds. It has been applied to grape juice (Huxelrebe, a variety of half Muscat ancestry) (Caven-Quantrill and Buglass, 2006) and noni fruit (*Morinda citrifolia* L.) (Wei *et al.*, 2011).

Engel *et al.* (1999) designed a compact and versatile distillation unit for the fast and careful isolation of volatiles from complex food matrices called SAFE (solvent-assisted flavour evaporation) offering the possibility of quickly isolating food volatiles from different food matrices including juices. It has been applied to orange juice reconstituted from concentrate (Averbeck and Schieberle, 2011) and Kabosu (*Citrus sphaerocarpa* Hort. ex Tanaka) juice (Tomiyaama *et al.*, 2011).

Solid phase microextraction (SPME)

Solid phase microextraction (SPME) is a widely applied technique, being a solvent-free method which exploits the high adsorption power of a fused

silica fibre coated with a specific extraction phase, which is selected according to the type of matrix. Miller and Stuart (1999) compared the efficiency of SPME-sampled SHS with traditional SHS sampling for various juice samples. They concluded that the SPME method provided better sensitivity over the traditional SHS method and could also extract more compounds. Headspace solid phase microextraction (HS-SPME) has been used for the isolation of volatile compounds in commercial fruit juices of pear, apricot and peach (Riu-Aumatell *et al.*, 2005). The chemical profile of the collected volatiles depends upon the type, thickness and length of the fibre, as well as on the sampling time and temperature. Reviews dedicated to the application of SPME in the analysis of food taints and off-flavours and for quality control have been published (Jeleń, 2006; Nicolai *et al.*, 2006; Balasubramanian and Panigrahi, 2010).

Dynamic headspace (DHS)

Dynamic headspace (DHS) methodology, also called ‘purge and trap’, employs purge of the sample with an inert gas stream and concentration of volatiles by continuously flushing or purging headspace and trapping of the removed volatiles onto an adsorbent. The volatile components may be trapped by a cryogenic, tenax, charcoal or other suitable trapping system (Reineccius, 2006). Despite the methodological difficulties connected to headspace analysis in dynamic conditions, multiple application of this technique in the analysis of odour compounds related to quality of juices can be found in the literature. It has been used in blackcurrant juice (*Ribes nigrum* L.) (Varming *et al.*, 2004), orange juice (Bylaite and Meyer, 2006), guava juice (Yen and Lin, 1999), tomato juice (Sucan and Russell, 2002) and apple juice (Nikfardjam and Maier, 2011).

Chromatographic techniques are necessary for the qualitative and quantitative analysis of aroma compounds in fruit juices. GC has an excellent separating power and great sensitivity of detection. Some of the most difficult flavour studies need to use two-dimensional GC to improve sensitivity as in fresh lemon juice (Komura, 2006).

Flame ionization detector (FID) is a universal detector with a great number of applications in the analysis of fruit juices aroma. MS has a very high sensitivity (10–100 pg) and as an identification tool is unequalled by other instruments. GC-MS is being currently applied to the identification of flavour compounds in many juices (see Table 19.1).

The use of the nose as a detector, in so-called olfactometry is uniquely applied to aroma studies, with a huge number of applications in juices (more than 5000 publications in the last 20 years). Olfactometric detector together with other detectors (FID, MS) allows the identification and exact analysis of components essential for quality, odour-active as well as character impact compounds, responsible for the characterizing odour of a food sample.

An extensive review on gas chromatography–olfactometry (GC-O) application to food flavour analysis, including fruit juices, has been published by d'Acampora Zellner *et al.* (2008).

CG-O or GC-O/MS has been applied to the characterization of aroma volatiles in different juices: yellow passion fruit juice (*Passiflora edulis* Sims F. Flavicarpa degner) (Jordán *et al.*, 2002), Jincheng sweet orange juice (*Citrus sinensis* L. Osbeck) (Qiao *et al.*, 2008) and orange juice (Rega *et al.*, 2003). It has also been used to evaluate the impact of vanillin on the flavour of orange, grapefruit, tangerine, lemon and lime juices (Goodner *et al.*, 2000) and to characterize the aromatic profile from the thermal degradation of thiamin in model orange juice (Dreher *et al.*, 2003).

The off-flavour and volatile substances originated during the storage of fruit juice are an important indicators of fruit juice quality. GC-O has been used to identify the volatile compounds responsible for the strong metallic off-flavour formed by photo-oxidation in cloudy apple juice stored in glass bottles under fluorescent light (3000 lx, 8°C) (Hashizume *et al.*, 2007). In mango and pineapple juices, medicinal/antiseptic compound and 'cheese' off-aromas associated with 2-methylbutyric acid and 3-methylbutyric acid respectively were identified by GC-O/MS (Danyluk *et al.*, 2011).

High-performance liquid chromatography (HPLC) has also been used to evaluate flavour changes due to 2-furaldehyde formation in processed citrus juices, as an indication of quality deterioration. The method is based on the formation of the 2,4-dinitrophenylhydrazones of carbonyl compounds and subsequent reversed-phase separation of these derivatives (Coco *et al.*, 1994).

19.3.2 Non-chromatographic techniques

E-noses have been commercially available since 1993. Although expensive and needing trained personnel, e-nose instruments are valuable tools in quality control since they require simple or no sample work-up, automated measurement capability and easy, but mostly black box, pattern recognition and interpretation software (Reinhard *et al.*, 2008). An extensive review on the application and advances on electronic nose has been published by Wilson and Baietto (2009). It has been described as an emerging tool in the quality control of fruit juices to detect spoilage by microbial contamination in apple and orange juices (Karlshøj *et al.*, 2007; Cagnasso *et al.*, 2010; Valencia-Chamorro *et al.*, 2011) and, in particular, spoilage due to *Alicyclobacillus* spp. in peach, orange and apple juices (Gobbi *et al.*, 2010). Other applications are: classification of juices from different apple fruit cultivars (Marrazzo *et al.*, 2005), classification of different juices (blackcurrant, mango and orange juices) (Penza *et al.*, 2001; Mamat and Samad, 2010; Mamat *et al.*, 2011) shelf-life investigation and authenticity assessment, for characterization and classification of three different treatment methods of *Myrica*

rubra juice (Zhang *et al.*, 2010). An e-nose was able to distinguish between two orange juices, ideal and volatile stripped, but the differences were reduced when the volatile stripped orange juice was enriched with four commercial orange juice essences at concentrations up to 2% (Farnworth *et al.*, 2002). A portable e-nose for beverage quality assessment has also been reported (Lorwongtragool *et al.* 2011).

MS is generally used either to determine the identity of an unknown flavour or to act as a mass-selective GC detector. GC-MS without chromatographic separation has been proposed as a 'sensor' that can monitor changes in odour quality of juices (Goodner and Rouseff, 2001). It has been applied to the rapid screening of off-odours in apples (Farkas *et al.*, 2011), optimization of processing conditions in sweet potato juice (Tamaki *et al.*, 2007) and the preliminary characterization or classification of citrus juices (Reinhard *et al.*, 2008). An extensive review on gas sensor array technology combined with multivariate data processing methods for foodstuff analysis has been provided by Dymerski *et al.* (2011).

19.4 Juice taste

19.4.1 Sweetness and sourness

The main non-volatile contributors to fruit juices taste are sugars and acids. The soluble solid contents relate to both. Other components such as pectins, glycosidic materials and salts of metals, when present, may have some influence on this value. Generally, °Brix is used to indicate the amount of dissolved solids in a solution. The °Brix of a fruit juice is a key control parameter in juice processing. There is a direct relationship between °Brix value and the specific gravity of the solution (representing dissolved solids in the juice), for that reason it is more accurate to use a °Brix hydrometer which reads directly the percentage of sucrose. Optical refractometers provide a direct reading of % w/w sucrose (°Brix), which is used in the fruit juice industry to indicate the degree of concentration or 'folding' (Taylor, 2005). To obtain the true and accurate dissolved solids content in a fruit juice from a refractometric °Brix reading, the optical distortion of the reading due to the acid content must be corrected (Shachman, 2004). The minimum °Brix values for reconstituted fruit juices and purée are shown in Table 19.2.

For a more specific sugar analysis, various enzymatic and colorimetric methods may be used; however HPLC with various detection systems is the preferred option. Refractive index detection is usually used in the low parts per million (ppm) ranges and above, whereas electrochemical detection is used in the analysis of sugars in the low parts per billion (ppb) range. The addition of beet sugar or cane sugar to fruit juices can be determined by stable carbon isotope ratio (Guillou *et al.*, 1999).

Table 19.2 Minimum degree Brix values for reconstituted fruit juice and reconstituted fruit purée

Common name	Botanical name	Minimum degree Brix values
Apple (*)	<i>Malus domestica</i> Borkh.	11.2
Apricot (**)	<i>Prunus armeniaca</i> L.	11.2
Banana (**)	<i>Musa</i> sp.	21.0
Blackcurrant (*)	<i>Ribes nigrum</i> L.	11.6
Grape (*)	<i>Vitis vinifera</i> L. or hybrids thereof	15.9
	<i>Vitis labrusca</i> L. or hybrids thereof	
Grapefruit (*)	<i>Citrus</i> × <i>paradise</i> Macfad.	10.0
Guava (**)	<i>Psidium guajava</i> L.	9.5
Lemon (*)	<i>Citrus limon</i> (L.) Burm.f.	8.0
Mango (**)	<i>Mangifera indica</i> L.	15.0
Orange (*)	<i>Citrus sinensis</i> (L.) Osbeck	11.2
Passion fruit (*)	<i>Passiflora edulis</i> Sims	13.5
Peach (**)	<i>Prunus persica</i> (L.) Batsch var. <i>persica</i>	10.0
Pear (**)	<i>Pyrus communis</i> L.	11.9
Pineapple (*)	<i>Ananas comosus</i> (L.) Merr.	12.8
Raspberry (*)	<i>Rubus idaeus</i> L.	7.0
Sour Cherry (*)	<i>Prunus cerasus</i> L.	13.5
Strawberry (*)	<i>Fragaria</i> × <i>ananassa</i> Duch.	7.0
Mandarin (*)	<i>Citrus reticulata</i> Blanco	11.2

*Products which are produced as a juice – a minimum relative density is determined as such in relation to water at 20/20 °C.

**Products which are produced as a purée – only a minimum uncorrected Brix reading (without correction of acid) is determined.

Note: If a juice from concentrate is manufactured from a fruit not mentioned in the above list, the minimum Brix level of the reconstituted juice shall be the Brix level of the juice as extracted from the fruit used to make the concentrate.

Source: Commission Directive 2009/106/EC of 14 August 2009 amending Council Directive 2001/112/EC relating to fruit juices and certain similar products intended for human consumption OJ L 212, 15. 8. 2009, pp. 42–44.

Organic acids also play an important role in the sensory quality of fruit juices. Moreover, it has been reported that the perception of flavour intensity is modulated in the presence of fruit acids (Zampini *et al.*, 2008).

The main organic acids in some fruit juices are shown in Table 19.3. Each type of fruit has a distinct organic acid profile so the profile can be used to assess whether an expensive juice has been illegally adulterated with a cheaper juice. While malic acid is the predominant one in pome and stone fruits, citric acid is the most abundant in berries and tropical fruits, and tataric acid is only present in grape juices. Addition of exogenous citric or malic acids to fruit juices is a common adulteration practice. By conventional methods of analysis, an addition of, for example, citric acid to orange or pineapple juices is usually controlled by the determination of the ratio of citric to isocitric acid, which should remain in the range 80–130,

Table 19.3 Main tastant compounds in common fruit juices

Phytonutrient class	Typical component	Taste quality	Food source	Reference
<i>Phenolic compound</i>				
Flavanonoid Flavones	Naringin	Bitter	Grapefruit juice	Puri <i>et al.</i> (1996)
	Tangeretin	Bitter	Tangerine juice	Rouseff and Ting, (1979), Ting <i>et al.</i> (1979)
	Nobiletin	Bitter	Juice from concentrates Orange juice	Mouly <i>et al.</i> (1998) Rouseff and Ting (1979), Veldhuis <i>et al.</i> (1970)
Flavonols	Sinensetin	Bitter	Juices from concentrates Orange juice (Fresh) Juice from concentrates	Mouly <i>et al.</i> (1998) Sendra <i>et al.</i> (1988), Ting <i>et al.</i> (1979) Mouly <i>et al.</i> (1998), Pupin <i>et al.</i> (1998), Veldhuis <i>et al.</i> (1970)
	Quercetin	Bitter	Grapefruit juice Lemon juice Apple cider	
	Catechin polymers	Astringent		
<i>Triterpenes</i>				
Limonoid aglycones	Limonin	Bitter	Lemon juice Grapefruit juice Orange juices	Puri <i>et al.</i> (1996) Hsu <i>et al.</i> (1998)
	Nomilin	Bitter	Grapefruit juice	Hsu <i>et al.</i> (1998)
	Limonin Glucoside	Tasteless	Lemon juice Grapefruit juice	Hsu <i>et al.</i> (1998)

Organic acids

Citric acid	Acid	Apple juice	Ehling and Cole (2011), Friedrich (2001), Jeuring <i>et al.</i> (1979)
		Orange juice	Buettner and Schieberle (2001), Ehling and Cole (2011)
		Grapefruit juice	
		Cranberry juice	Ehling and Cole (2011)
		Lemon juice	
		Pineapple juice	
		Strawberry juice	
		Grape juice	
		Blackcurrant (concentrates)	
Isocitric acid		Apricot juice	Boccorh <i>et al.</i> (1998)
		Grapefruit juice	Kvasnicka <i>et al.</i> (2002)
		Pomegranate juice	Kvasnicka <i>et al.</i> (2002)
		Pineapple juice	Friedrich (2001)
		Apricot juice	
Malic acid		Red grape juice	Ehling and Cole (2011)
		Cranberry juice	Ehling and Cole (2011)
		Blackcurrant (concentrates)	Boccorh <i>et al.</i> (1998)
		Apple juice	Ehling and Cole (2011), Jeuring <i>et al.</i> (1979)
Tartaric acid		Pomegranate juice	Ehling and Cole (2011)
		White grape juice	Ehling and Cole (2011)
Quinic acid		Cranberry juice	Ehling and Cole (2011)
		Orange juice	Ehling and Cole (2011)
Ascorbic acid		Blackcurrant (concentrates)	Boccorh <i>et al.</i> (1998)
		Orange juice	Buettner and Schieberle (2001)

depending on the regional origin, and possibly by other factors, too. Because of this range of possible variation of the citric/isocitric ratio and the availability of cheaper isocitric acid, which can be added too, additional methods for detecting acid addition to fruit juices have been developed based on stable isotope MS (Guillou *et al.*, 1999; Rossmann, 2007).

To measure acidity in juices two common units of measurement are titratable acidity and pH. However, no direct relationship exists between them. Titratable acidity measurement is related to the total acid content in the juice and is determined by titration with a base.

Volumetric, electrochemical, enzymatic and chromatographic (paper, thin-layer, gas-liquid, or HPLC) methods are available for organic acid determination. However, some of these methods are not able to assay organic acids comprehensively; for example, the enzymatic methods are specific kits for individual organic acids (i.e. they detect only one of the acids present). HPLC (reverse phase or ion exchange) coupled to UV detection, allows analysing all organic acids in a sample to be analysed in one run. However owing to the high variability of this method, a more accurate approach based on liquid chromatography-tandem mass spectrometry (LC-MS/MS) has been proposed for authenticity testing of fruit juices (Ehling and Cole, 2011).

The °Brix/acid ratio of a fruit juice (the degrees Brix corrected for acid and temperature, divided by the total acid concentration by titration) is commonly used as a measurement of fruit juice flavour. This ratio of °Brix to acidity could be described as the 'sweetness to sourness' relationship in the taste of the juice. The higher °Brix values (in relation to the acid content of the juice), the sweeter taste of the juice. At the beginning of the ripening process the sugar/acid ratio is low, because of low sugar content and high acid content, which make the fruit taste sour. During the ripening process the fruit acids are degraded, the sugar content increases and the sugar/acid ratio achieves a higher value. Overripe fruits have very low levels of fruit acid and therefore lack characteristic flavour.

The fruit juice processing industry together with international government agricultural and food organizations established the standards of °Brix/acid ratio specifications for most of the world's known edible fruits. The values established are expressed as a °Brix/acid ratio range or a minimum (or maximum) limit.

A new index in which the °Brix reading is modified to account for the sweetness-reducing effect of the acids present has been proposed by Jordan *et al.* (2001). This index is based on the linear weighted difference between the °Brix and the total acid measured rather than their ratio. It is called BrimA for °Brix minus acid: $\text{BrimA} = \text{Brix} - k \times \text{total acid}$. They indicate that the value of k reflects the tongue's higher sensitivity to acid than to sugar. This seems to range from 2 to 10, depending on the specific acids and sugars in a given sample. The index allows smaller amounts of acid than sugar to make the same numerical change to BrimA and in the opposite direction.

19.4.2 Bitterness

Bitterness is also relevant in some fruit juices. A broad range of different chemical structures can be found in this group, so specific methods for each compound can be found in the literature. In orange juices, naringin, a flavanone neohesperidoside, and neohesperidin are very bitter, whereas hesperidin is tasteless. An HPLC method is recommended for the determination of naringin and neohesperidin in orange juice (AOAC, 2002).

Limonin, a triterpene, is responsible for the so-called delayed bitterness of citrus juices. A tasteless limonin precursor, is released when the fruit tissue is damaged, and is gradually converted to limonin, resulting in bitterness. Bitterness due to flavonoids and limonoids poses a major problem for the citrus industry (Drewnowski and Gomez-Carneros, 2000). A wide variety of patented techniques have been developed to remove or absorb excess naringin and limonin from citrus juices (Singh *et al.*, 2003). HPLC in reversed and normal-phase HPLC phase is the most commonly used method to quantify limonoids from citrus juices. In addition to these methods, a variety of techniques using fluorometry GC, radioimmunoassay, enzyme-linked immunoassay, nuclear magnetic resonance (NMR), electron ionization–mass spectrometry (EI/MS) and atmospheric pressure chemical ionization/MS (HPLC-APCI) have been reported (Manners, 2007).

19.4.3 The electronic tongue (e-tongue) in the taste assessment

Organic and inorganic chemical compounds responsible for taste are perceived by human taste receptors; in the brain, taste signals are transduced by nerves into electric signals. In this biological mechanism, taste quality perception is based on recognition of activated sensory nerve patterns by the brain and on the taste fingerprint of the product, i.e., the chemical composition of the product and their brain interpretation.

Like human receptors, the sensors of an electronic tongue (e-tongue) detect the chemical compounds and generate electric signals as potentiometric variations. Most of the e-tongue sensors have detection thresholds similar or even better than human receptors. Each sensor gives a different spectrum of reactions, offering complementary information. The combination of all the sensors results generates a unique fingerprint. In the case of the electronic instrument, the interpretation of the taste quality perception is achieved by the e-tongue's statistical software, which interprets the sensor data into taste patterns.

Thus, an e-tongue can be defined as an analytical instrument, which reproduces the sensory appreciation of the taste in an artificial way. Usually, this device consists of several components: (1) automatic sampler, (2) chemical sensors having different specificity, (3) instrumentation for acquiring the signal, and (4) software with the appropriate algorithms to process the signal and to obtain the desirable results, according to qualitative or quantitative analysis.

Although e-tongue systems are still being developed, their advantages are already clear. Among these strengths are the fast determination of compounds in foodstuff and the classification of samples with a direct measuring stage. It is intended that an e-tongue will be able to measure taste attributes such as sweetness, bitterness, salty, sourness or tasty. In general, the attributes measured by an e-tongue are very similar for each application area, unlike those measured by e-noses. The importance of the e-tongue in modern analytical chemistry is highlighted in the increasing number of publications that are currently investigating flow-based e-tongues. Escuder-Gilabert and Peris (2010) indicate the following principal characteristics and applications:

- The development of new measurement methods, the search for new chemosensitive materials and new techniques for the preparation of chemosensitive layers.
- The coupling of e-tongues with distributed expert systems for the advanced in-line monitoring of food production processes, especially alcoholic fermentations.
- The application as a detection scheme in flow-based analytical systems.

Nevertheless, these systems have a big disadvantage: a huge amount of previous measurements are required for the modelling calibration of learning step.

Different types of devices have been developed to determine instrumentally the taste. Non-destructive techniques such as near infrared (NIR) spectroscopy can be of great interest as rapid analytical methods to quantify the chemical components of juices related to the taste characteristics. Cozzolino *et al.* (2011) underwent an interesting review on the applications of the NIR spectroscopy for the measurement of chemical parameters in both fruit and fruit juices. Compared with traditional methods, multivariate data analysis combined with NIR instrumental techniques gives a new and a better insight into complex problems by measuring a great number of chemical compounds at once, thus enabling the ‘fingerprinting’ of each sample.

In this sense, different authors have applied NIR spectral analysis to the study of juice quality. Wu *et al.* (2010) successfully applied Vis-NIR spectroscopy and partial least squares (PLS) regression models for the non-invasive determination of soluble solid contents and pH in grape juices, in relation to sweet and acid tastes, respectively. Different apple juices were studied with three different analytical techniques: HPLC, e-tongue multi-system based on potentiometric chemical sensors and Fourier transform infrared (FTIR) spectroscopy. E-tongue and attenuated total reflectance (ATR)-FTIR were demonstrated to be promising tools for the discrimination of apple juices and determination of organic acid content in apples (Rudnitskaya *et al.*, 2006).

The e-tongue and ATR-FTIR were evaluated as rapid techniques in tomato juice taste research (Beullens *et al.*, 2006). The most abundant sugars (glucose, fructose and sucrose) and organic acids (citric, malic, tartaric, fumaric and succinic acids) were measured by HPLC. The results were studied by means of supervised and unsupervised multivariate analysis techniques. The electronic tongue was able to classify different tomato juices. However, this device was not sensitive enough to accurately detect individual sugars. The combination of e-tongue and ATR-FTIR resulted to be a valuable technique to describe the sugar and acid profile of tomato juices.

Malmendal *et al.* (2011) have tested the potentiality of NMR spectroscopy as a predictive tool to measure sensory descriptors. In particular, they were able to correlate the NMR metabolomic fingerprints recorded for tomato juices to the sensory descriptors bitterness, sweetness, sourness, saltiness, tomato and metal taste, redness, and density, suggesting that NMR might be a very useful tool for the characterization of sensory features of tomatoes.

Recent developments in systems to measure the quality of fruits, including e-tongue and biosensors array, were reported by Nicolai *et al.* (2006). Optical techniques (NIR and ATR-FTIR spectroscopy) were discussed as regards the measurement of taste components of intact fruits and juices.

There is no doubt that the flow-based analytical systems may be used for the resolution of more complex analytical problems, increasing the number of analytes to be determined, and also the identification/classification of even more similar samples (Escuder-Gilabert and Peris, 2010). A planar e-tongue based on an integrated array of solid-state microelectrodes (portable e-tongue system) was checked in the recognition of different orange juices being stated the high ability of the system (Ciosek *et al.*, 2007). Another approach was proposed (Ciosek and Wróblewski, 2007), taking into account that partially selective sensors provide independent information due to the 'chemical orthogonality' of the sensor array data. It was found that an array based on a selective sensor has a higher recognition ability, for example in classifying orange juices in flow mode.

The usefulness of the flow-through analysis for the monitoring of food-stuff production is due to a reduced response time and the possibility of miniaturization (Ciosek *et al.*, 2006a; Ciosek and Wróblewski, 2008). Based on miniaturized solid-state electrode, a flow-through e-tongue was constructed and tested in the recognition of orange juices. The miniaturized e-tongue can be coupled with miniaturized pre-treatment systems. It can combine sensors working on the base of various principles in the same measurement, a so-called hybrid e-tongue. Dias *et al.* (2011) developed an all-solid-state potentiometric multi-sensor device, made of non-specific lipo/polymeric (PVC) membranes. This e-tongue was applied to the analysis of non-alcoholic beverages with different flavours and amounts of added fruit juices. By means of linear discriminant analysis (LDA, a

supervised learning technique), the soft drinks were classified according to their juice content.

Penza *et al.* (2001) designed and developed an array of four thin films WO₃ metal oxide sensors capable of discriminating several juices. Three different electrodes (Pt and Au electrodes and an electrode modified with poly 3,4-thylenedioxythiophene, PEDOT-conducting polymer) were tested (Martina *et al.*, 2007) to be used as non-specific amperometric sensors for blind analysis on two sets of juices. The first set consisted of four different fruit juices from the same manufacturer (orange, pear, peach and apricot). The second set of samples consisted of orange juices from three different brands. The PEDOT-modified electrode presents several advantages over Pt and Au electrodes. The modified electrode demonstrated the highest discriminating ability, and it was the only system capable of satisfactorily performing the most complex task attempted during the analysis: discriminating among juices from the same fruit but from different brands. Principal components analysis (PCA) and partial least squares discriminant analysis (PLS-DA) confirm the high discriminating capabilities of amperometric devices based on such electrodes when they are applied to matrices such as fruit juices.

An e-tongue that can analyse liquids, based on similar concept of previous e-nose with gas sensors, was designed by Winquist *et al.* (1997). The e-tongue, based on pulse voltammetry, was used to classify fruit drinks, demonstrating its ability to follow some ageing processes by oxidation. Good discrimination was obtained, especially for orange juices.

Bleibaum *et al.* (2002) used a series of nine apple juices, including mixtures of three apples, pear/apple juice fortified with vitamin C, and cider (an alcoholic beverage made from apple juice). Some samples were modified in sweetness and sourness with sucrose and citric acid, respectively, to determine how these products are differently perceived by (a) consumers, (b) a sensory panel, and (c) electronic instruments (e-nose and e-tongue). Results indicated that some sensors of e-tongue were better correlated with the sweetness, whereas others were better correlated to the flavour and fresh attributes of apples.

A combined electronic nose and tongue as a flavour sensing system was developed by Cole *et al.* (2011) to test orange juices, resulting in 100% discrimination using principal components analysis. The system comprises both an 'e-tongue' based on shear horizontal surface acoustic wave (SH-SAW) sensors analysing the liquid phase and an e-nose based on chemical field-effect transistor (chemFET) sensors analysing the gaseous phase.

The e-tongue based on potentiometric sensor arrays has been applied to the food analysis to distinguish simple tastes and to classify food samples. The sensory properties of common citrus juices (orange, grapefruit and tangerine) were analysed with e-nose and e-tongue to measure the possible changes of the juices related to modifications in the physico-chemical parameters (pH, °Brix, etc.). The effect of applying pulse electric field and

high hydrostatic pressure technologies was also highlighted. These electronic tools can be used as a potential detection to differentiate each treatment type. As indicated, these results provide promising principles for the elaboration of new methods which could be used in the area of quality control and assurance of novel foods (Hartayáni *et al.*, 2011).

Efficient sensoactive layers in taste sensors were applied for the quality assessment of commercial and freshly squeezed orange juices (Medeiros *et al.*, 2009). Sensor ability in classifying the juices (separating different types and monitoring the ageing process) was successfully tested. The sensor array was able to detect changes in citric acid concentration down to values as low as 2 ppm.

Zoltán *et al.* (2008) developed a study with the main objective of predicting the concentration of orange and apple juices based on data from the e-tongue with a multivariate statistical treatment. The e-tongue can differentiate the juices based on the taste, and also monitor the changes in taste and the stability of the juice components. The sensor was able to recognize the differences between apple juice samples with 5% concentration difference and orange juice samples with 10% concentration difference.

Bleibaum *et al.* (2002) compared the apple juice quality measured by a sensorial trained panellist and a liquid taste analyser instrument, as an e-tongue, and a sensor array coupled to a mass spectrometer, as an e-nose. Good results were obtained, demonstrating that the combination of e-nose and e-tongue can be used to predict the sensory characteristics of apple juices. Also, the relationships to the quality measured by consumers can be assessed in this way.

Voltammetric e-tongues have been applied to classify different juices (Ivarsson *et al.*, 2005): orange juices (Winqvist, 2008) and apple juices (Winqvist *et al.*, 2008) using a miniaturized device in the case of apple juices. The e-tongue systems prove to be suitable for apricot juices. Kantor *et al.* (2008) demonstrated that the device was a good tool for monitoring the effects of post-harvest techniques on the fruit ripening process. They achieved the classification of apricot varieties and obtained good correlations between e-tongue, chemical properties and the sensory analysis results, these being the instrumental measurements more sensitive to differences than the sensory analysis.

A multiparametric array containing ion sensitive field effect transistors (ISFET) was used as an e-tongue for grape juice analysis. The juices from four different grape varieties were well distinguished by this integrate multisensor and the application of multivariate PLS regression method to the data (Moreno i Codinachs *et al.*, 2008). The distinguishing abilities of e-tongues mean they can recognize complex liquids that are very close when assessed by human tasting (Legin *et al.*, 2000; Vlasov *et al.*, 2000, 2002).

The use of grape juice in masking bitter tastes has been compared with other sweeteners. The quantification of bitterness intensity and the effectiveness of bitterness suppression have been assessed using an e-tongue

based on potentiometric chemical sensors. The most effective taste masking was found to be produced by the grape juice (Legin *et al.*, 2009).

After validating the device by classifying different synthetic samples prepared with controlled variability of their composition, Gallardo *et al.* (2005) applied this e-tongue to classify orange-based drinks. They obtained good correlations with the natural juice content by means of PCA. With the objective of differentiating four non-alcoholic beverages with different added fruit juices contents, Peres *et al.* (2009) developed an all-solid state potentiometric multi-sensor device. A 100% overall correct classification was obtained by applying appropriate statistical analysis.

The combination of the e-tongue's chemical sensors with appropriate multivariate statistical methods means rapid, objective and flexible results are obtained. Some multivariate statistical techniques such as PCA, step-wise discriminant analysis (SDA) or artificial neural networks (ANN) have been applied in the treatment of data from e-tongue sensors. In the case of PCA the main applications are focused on reducing the number of variables and detecting structure in the relationships between variables, i.e., to classify variables, whilst SDA is used to determine which variables discriminate between two or more naturally occurring groups. ANN is a very sophisticated nonlinear technique capable of modelling extremely complex functions.

PCA and ANN statistical analyses were applied as pattern recognition tools by Legin *et al.* (1997) to an e-tongue based on the sensor array of non-specific solutions sensors. The device was capable of discriminating reliably between various sorts of the same types of juices as complex beverages, and of monitoring the process of ageing of juices. To analyse apple juices qualitatively an e-tongue based on the sensor array of ion-selective electrodes was developed together with a procedure used to reduce the number of sensors (Ciosek *et al.*, 2004b). This device was capable of reliable discriminations among apple juices even after reducing the number of sensors. PCA and ANN techniques reduced the amount of redundant and unsubstantial information, providing similar or even better discriminations of juice samples when the most suitable sensors were chosen.

Different strategies of data analysis for artificial tastes by a sensor array used as an e-tongue were further applied (Ciosek *et al.*, 2005; Ciosek and Wróblewski, 2006). ANNs and PLS-DA applied to raw data (as direct processing methods) and also applied to PCA outputs (as two-stages processing methods) demonstrated a considerable increase of the classification capability of different brands of orange juices from various manufacture lots. Zhou *et al.* (2010) also designed an e-tongue to identify samples of fruit juices applying learning vector quantization neural network (LVQ-NN). This e-tongue with universal pattern recognition (seven working electrodes and one reference electrode) could discriminate one category of all the foodstuffs directly, and had many advantages such as simpler training methods, shorter training time and higher efficiency. An e-tongue based on

the sensor array of polymeric membrane ion-selective combined with pattern recognition tools was applied to the analysis of orange juices and other foodstuffs. In this case, the device could discriminate among not only different brands but also among different days of manufacture. In this work, the importance of the calibration procedures and the use of more sophisticated data processing methods are highlighted (Ciosek *et al.*, 2004a, 2006b).

Winquist *et al.* (2002) described a voltammetric e-tongue based on flow injection analysis and applied it to classify different types of apple juices. The multivariate treatment of the data by PCA permitted good classifications to be obtained from an untrained sensor panel. Zoltán *et al.* (2008) developed a complete method for sensing different taste attributes in carrot juice by an e-tongue and a trained sensory panel. The e-tongue was able to distinguish the juices according to the sensorial data evaluated by multivariate statistics.

Two types of e-tongues (with 18 and 7 potentiometric sensors) were evaluated in the taste analysis of tomato juice. The concentration of sugars (glucose and fructose), organic acids (citric, malic and glutamic acids) and minerals (Na and K) were also determined by reference techniques. By means of multivariate statistical methods (PCA, canonical discriminant analysis and PLS regression) different tomato juices were classified according to similarity in taste profile and to quantitatively relate the taste compounds to the sensory panel scores. The system, consisting of seven sensors, was able to some extent to predict tomato juice taste, as scored by a trained sensory panel (Beullens *et al.*, 2008).

19.5 Juice flavour: instrument–sensory relations

Flavour is generally understood to be the overall experience from the combination of oral and nasal stimulation and is principally derived from a combination of the human senses of taste (gustation) and smell (olfaction). Juice flavour depends upon taste (balance between sweetness and sourness or acidity, and low or no astringency) and aroma (concentrations of odour-active volatile compounds) and is specific to the species and cultivar. Although taste and aroma are well integrated in their contribution to the overall flavour, attention has been traditionally focused on aroma, minimizing the influence of taste stimuli on flavour perception (Voilley and Etiévant, 2006).

Instrumental methods are used to predict the sensory quality of the juices, but this is finally evaluated by consumers. When sensory and instrumental data are correlated a better understanding of the role of physico-chemical parameters in the sensory quality of the juice is obtained. For that reason studies correlating sensory quality evaluated by panellist with physico-chemical parameters have been conducted on orange juice (Elston *et al.*, 2006), elderberry (*Sambucus nigra L.*) juice (Kaack *et al.*, 2005),

pomegranate, blueberries, blackberries and raspberry juices (Vázquez-Araújo *et al.*, 2010), cashew apple (*Anacardium occidentale* L.) juice (Garruti *et al.*, 2003), freshly squeezed pineapple juice (Li *et al.*, 2010), Bearss seedless lime (*Citrus latifolia* Tan) juice (Ziena, 2000), concentrated orange and passion fruit juices prepared by osmotic evaporation (Shaw *et al.*, 2001), fresh and processed mandarin juices (Pérez-López and Carbonell-Barrachina, 2006), apple juice (Komthong *et al.*, 2006) and mandarin juice var. Hernandina (Beltrán-González *et al.*, 2008a). Some research have pointed out that sensory evaluation remains the ultimate means to reliably assess juice quality (Nikfardjam and Maier, 2011).

It is possible, by means of simple measurements, to provide an objective analysis of attributes such as sweet and acid tastes in food products like juices. The soluble solids content (°Brix) was correlated with the sweetness results given by a trained sensory panel for apple juices. It was found that the panellists could predict a difference in taste when apple juice differed by more than 1°Brix (Harker *et al.*, 2002). Better results were found for the titratable acidity as the best predictor for the acid taste. The authors suggested that titratable acidity may be an important tool in predicting taste of apples.

Unlike the traditional research exposed above, flavoromic is a new approach based on an untargeted methodology aimed at linking the chemical composition with the sensory quality of foods. Instead of focusing on compounds already known to influence the flavour quality, it considers all (ideally) low molecular weight compounds as candidate chemical stimuli in flavour perception (unbiased). Its application to orange juice flavour offers the advantage of finding potential correlation between chemical compounds and an increased accuracy in flavour predictions as it includes inputs from more compounds (Charve *et al.*, 2011).

19.6 Conclusion

Colour, aroma and taste are the major sensory attributes related to the quality perception and consumers' acceptability of foods in general and juices in particular. The need to control such parameters in the context of quality control is therefore beyond doubt. Despite its inherent subjectivity, sensory analysis (carried out by trained panellists or potential consumers) remains essential for the industry for such purposes such as the development of new products, the enhancement of existing products, the assessment of preferences and so on. However, the instrumental assessment of sensory attributes offers a number of advantages that make them powerful analytical tools for the industry, one of the main ones being their objectivity. Currently, there are instrumental methods that mimic human abilities with regards to perception of appearance, taste and aroma. Indeed, the objective colour assessment of the colour of foods has been successfully applied in

the industry for several decades and many instruments are available for general or specific purposes that cater for the needs of companies of diverse sectors and size. In this regard, it can be stated that the objective measurement of colour can be readily carried out in most food companies due to the availability of affordable instrumentation and the simplicity of the measurements and the data interpretation. More recently, the development of e-noses and e-tongues opened new possibilities for the quality control of other sensory attributes in the industry. In this regard, the development of more simple and affordable instrumentation amenable to their use in food companies of different characteristics appears as an important research need.

On the other hand, it is important to consider that, apart from sensory appealing-foods consumers increasingly demand more wholesomeness in them, hence the recent boom in the functional food industry. Considering that some pigments (mainly carotenoids and phenolics) are thought to provide health benefits, the analysis (either instrumental or by panellists/consumers) of the colour of products that contain them can be harnessed to estimate (to a certain extent) some aspects of their nutritional value. This appears to be a novel interesting research field, the transfer of which not only to the industry but also to consumers (who could relate the colour of certain foods to potential health benefits) could have an important economic impact on the industry.

19.7 References

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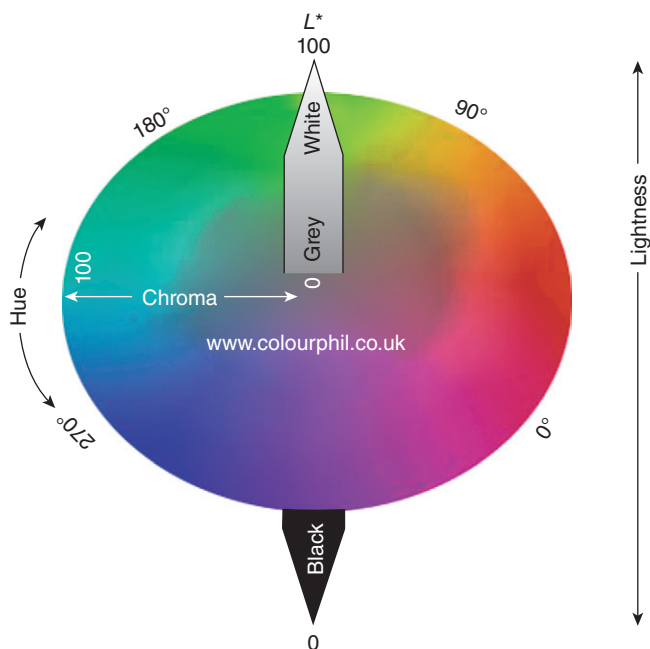


Plate VII (Chapter 16) LCH colour space (http://www.colourphil.co.uk/lab_lch_colour_space.html).



(a)



(b)

Plate VIII (Chapter 19) Background effect in colour measurement. Different orange juice dilutions over white (a) and black (b) backgrounds for visual and spectroradiometric analysis.

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