

FOOD SCIENCE AND TECHNOLOGY SERIES



Handbook of Food Science and Technology 1

Food Alteration and Food Quality

Edited by

**Romain Jeantet, Thomas Croguennec
Pierre Schuck and Gérard Brulé**

ISTE

WILEY

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Jack Legrand & Gilles Trystram

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Contents

Introduction	ix
G�rard BRUL�	
Part 1. Water and Other Food Constituents	1
Chapter 1. Water	3
Pierre SCHUCK	
1.1. Structure and state of water	3
1.2. Properties of water.	7
1.2.1. Water activity (a_w)	8
1.2.2. Glass transition	19
1.2.3. Phase diagram	25
Chapter 2. Other Food Constituents	27
Thomas CROGUENNEC	
2.1. Carbohydrates	27
2.1.1. Structure of carbohydrates	28
2.1.2. Carbohydrates in solution	30
2.2. Proteins	36
2.2.1. Structure of proteins	36
2.2.2. Solubility of proteins.	38
2.3. Lipids	41
2.3.1. Composition of the lipid fraction.	41
2.3.2. Thermal properties of lipids.	47
2.4. Vitamins.	51

Part 2. Food Modifying Agents and Mechanisms	53
Chapter 3. Microbial Spoilage	55
Florence BARON and Michel GAUTIER	
3.1. Microbial profile of food	55
3.1.1. Origin of microorganisms	55
3.1.2. Factors influencing the growth of microorganisms	66
3.2. Food spoilage.	78
3.2.1. Changes in texture and structure	79
3.2.2. Changes in flavor	80
3.3. Sanitary risks	82
3.3.1. Foodborne disease outbreak.	82
3.3.2. Main pathogens and toxin producers	86
Chapter 4. Lipid Oxidation	99
Thomas CROGUENNEC	
4.1. Lipid substrates	100
4.2. Lipid oxidation mechanisms.	100
4.2.1. Lipid autoxidation	101
4.2.2. Lipid oxidation by singlet oxygen	106
4.3. Main compounds derived from lipid oxidation.	108
4.4. Factors affecting lipid oxidation	110
4.4.1. Oxygen content	111
4.4.2. Catalysts of lipid oxidation	112
4.4.3. Inhibitors of lipid oxidation.	116
4.4.4. Physical-chemical factors	119
4.5. Evaluation of susceptibility to oxidation and the level of oxidation	122
4.5.1. Measuring the consumption of oxidation substrates	124
4.5.2. Determination of the peroxide value.	124
4.5.3. Measurement of peroxide decomposition products	126
4.6. Control and prevention of lipid oxidation	126
4.6.1. Stabilization using physical means	128
4.6.2. Formulation	129
Chapter 5. Non-Enzymatic Browning	133
Thomas CROGUENNEC	
5.1. Substrates	134
5.2. Mechanism of non-enzymatic browning.	135
5.2.1. Condensation	136
5.2.2. Amadori or Heyns rearrangement	137

5.2.3. Degradation of ketosamines	138
5.2.4. Polymerization reactions	144
5.3. Factors influencing the Maillard reaction	145
5.3.1. Substrates	145
5.3.2. Physical-chemical conditions	147
5.3.3. Presence of activators and inhibitors	149
5.4. Consequences of non-enzymatic browning	149
5.4.1. Sensory consequences	150
5.4.2. Functional consequences	150
5.4.3. Nutritional consequences	151
5.5. Evaluation of non-enzymatic browning	152
5.6. Control and prevention of non-enzymatic browning	153
5.6.1. Removal of substrates	154
5.6.2. Physical-chemical factors	155
5.6.3. Formulation (addition of inhibitors)	155

Chapter 6. Enzymatic Browning 159

Thomas CROGUENNEC

6.1. Substrates and browning enzymes	160
6.1.1. Phenolic substrates	160
6.1.2. Browning enzymes	165
6.2. Mechanism of enzymatic browning	167
6.2.1. Formation of quinones	167
6.2.2. Reactions with quinones	167
6.3. Factors influencing enzymatic browning	169
6.3.1. Substrates	169
6.3.2. Physical-chemical conditions and presence of natural inhibitors	170
6.4. Consequences of enzymatic browning	171
6.5. Evaluation of enzymatic browning	174
6.6. Control and prevention of enzymatic browning	175
6.6.1. Denaturation or inhibition of polyphenol oxidase	175
6.6.2. Modification or removal of oxidation substrates	177
6.6.3. Control of reaction products	179

Chapter 7. Molecular Dynamics in Food Matrices 183

Thomas CROGUENNEC and Pierre SCHUCK

7.1. Water migration and changes in food quality	184
7.1.1. Water migration	184
7.1.2. Equilibration with the atmosphere	185
7.1.3. Equilibration in heterogeneous foods	186
7.1.4. Equilibration after a phase and/or structure change	186

7.2. Control and prevention	190
7.2.1. Thermodynamic factors	190
7.2.2. Kinetic factors	191
Part 3. Quality Control and Assessment	195
Chapter 8. Food Safety Control	197
Florence BARON and Gérard BRULÉ	
8.1. EU Legislation	197
8.1.1. Directive 93/43/EEC of June 14 1993 on the hygiene of foodstuffs	197
8.1.2. Food safety regulations	197
8.2. Tools	198
8.2.1. Guide to good practice	198
8.2.2. HACCP	199
8.2.3. Food safety and quality assurance management	204
Chapter 9. Evaluation of the Physical-chemical Properties and Quality of Food	205
Florence BARON, Gérard BRULÉ and Michel GAUTIER	
9.1. Microbiological evaluation	206
9.1.1. Choice of microbiological assays	206
9.1.2. Methods	210
9.1.3. Limitations of microbiological evaluation	219
9.2. Biochemical and physicochemical analysis	220
9.2.1. Texture analysis by rheological methods	220
9.2.2. Color analysis	228
9.2.3. Analysis of food composition	230
Bibliography	235
List of Authors	245
Index	247

Introduction

The first concern of primitive people was to find food in their immediate environment to meet their physiological needs to survive; with no knowledge of either their requirements or the properties of food products, whether they were of plant or animal origin, these food choices were based on very empirical observations. The development of agriculture and livestock farming gradually gave people greater control in procuring food compared to the randomness of gathering, hunting and fishing. However, the supply of agricultural and livestock products has long been highly irregular for reasons of climate, diseases or simply the seasonal nature of certain products. Due to this irregularity, and in order to meet the needs of people located far from production areas, man has always been in search of ways to preserve food, thereby creating the possibility of varying the time and place of consumption of agricultural products.

I.1. Traditional preservation methods at the beginning of the agri-food industry

Without any knowledge of the deterioration process of raw materials, man observed that certain natural changes led to more stable products with tasty characteristics. This is how fermentation emerged as an effective method of preservation: products of lactic acid, acetic acid and alcoholic fermentation (cheese, bread, wine, beer, etc.) are consequently some of the oldest foods since the raw materials used to produce them all contain the elements necessary for fermentation to occur. The reduction in pH, the

presence of metabolites such as alcohol and lactic acid, the occupation of the medium by bacteria or yeast and the depletion of microbial growth factors all generate high resistance to the development of potentially pathogenic spoilage flora.

Other preservation methods have gradually been developed, based in particular on the use of salt, combined with dehydration in some cases, so as to reduce the availability of water. Water is a vector of all the elements involved in microbial growth and in chemical and biochemical reactions (solvent for metabolites and reaction products). The unavailability of water hinders microbial growth and most of the reactions. Water availability can be reduced by eliminating free water, by changing its state (ice) and by transfer or by immobilizing water through the addition of highly hydrophilic solutes such as salts and sugars. Salting has long been the most common preservation method for meat and fish in particular, and still forms the basis of the meat curing industry today. Salting is sometimes combined with dehydration or smoking: smoke constituents also contribute to good microbial and biochemical stability due to their bacteriostatic and antioxidant properties, and have a favorable impact on both color and flavor.

Stabilization through heat treatment appeared late compared to the methods just described, around the middle of the 19th Century. By destroying food spoilage agents (microorganisms and enzymes), heating food is an effective way of ensuring longer product life. The canning industry is based on this method.

By integrating the knowledge of the role of microorganisms into food spoilage and fermentation processes, work that was initiated by Pasteur, food production and stabilization gradually moved from an artisanal level to an industrial level. This transition was facilitated by the integration of technological progress, such as the development of pasteurization and sterilization tools, the introduction of refrigeration and freezing, drum-drying followed by spray-drying, freeze-drying, etc. This transition accelerated after World War II due to the large rural exodus that ensued. The concentration of the population in urban areas generated problems of supply of agricultural and food products, which had to be tackled by improving product stability and distribution in order to vary the time and place of consumption. This need to regulate supply and adapt it to demand was instrumental in the development of the agri-food industry.

1.2. From quantitative demand to qualitative demands

Agricultural production at the end of World War II was insufficient, both quantitatively and qualitatively, to satisfy the needs of people who had moved to urban areas: the most important aspects of food were availability and accessibility. Thus, one of the first objectives of the agricultural sector and the food industry was to meet the quantitative demand by increasing productivity and reducing production and processing costs. Progress in animal and plant genetics, developments in the agricultural sector, and changes in crop protection, forage and livestock management all resulted in a considerable increase in productivity within a few years, which in some cases was at the expense of quality.

Once the quantitative demand was satisfied, consumers became increasingly aware of the dangers and risks associated with poor-quality food. The number of reported food poisoning cases increased due to the obligation to conduct a more rigorous monitoring of food poisoning, the development of mass catering and the increase in immunocompromised people, some of whom live in institutions (geriatrics). A fear of shortages, which disappeared in industrialized countries, was replaced by food scares: this phenomenon was aggravated by a series of crises that adversely affected the food chain (bovine spongiform encephalopathy, genetically-modified organisms, *Listeria*, *Salmonella*, bird flu, dioxin, etc.). The consumer's desire to eliminate food risk meant that food hygiene and safety has become the most important quality requirement today.

A number of sociological changes (increase in female employment, organization of the working day, family breakdown and the development of leisure activities) have impacted eating habits and created new needs. In addition to the development of mass catering, there has also been a rise in single serving and food services. This in turn corresponds to a very high demand for food with a long shelf life, fast preparation, individual portion sizes and highly processed food (ready meals). Trying to combine quality service (proposing a high diversity of individual portions of tasty ready-to-eat food products with long shelf life) as well as a guaranteed hygiene with the control of physicochemical and thermodynamic stability and the preservation of the sensory qualities of food is a real challenge faced by the food industry today.

The health benefit of food is also a qualitative element that has started to dominate consumer expectations: progress in the area of nutrition and data from epidemiological studies now make it possible to better assess the effects of poor diet on health. The development of a number of physiological disorders and the so-called lifestyle diseases (e.g. obesity, heart disease, allergies, intestinal and colon cancer, and diabetes) is attributed to changes in eating habits and food. As a result, health-conscious consumers are very sensitive to any communication extolling the virtues and benefits of certain foods.

Over the years and throughout the many health crises that have hit the agricultural and food sectors, consumers have become aware that several foods were the result of a production system based on profit, with little concern for them and even less for the well-being of animals and the environment. They discovered that the various stages of the food chain from production to distribution were opaque and that local products were disappearing. The agricultural model established after World War II and the food chain that underpins our diet are currently facing growing opposition. For the consumer, food is not solely a product that generates pleasure and satisfies nutritional needs, but it is also a symbol of the choice of society that is being supported or opposed through the act of purchase. There has been a shift from a “goods-based economy” to a “services-based economy”. These changes in expectations among consumers impact the entire food chain, and the agricultural sector should no longer be merely a place where raw materials and services are created, but also a place of living areas, beautiful countryside and natural resources.

The expectations and demands of consumers regarding their food are increasingly complex and sometimes difficult to understand because imagination also plays an important role, which in some cases can lead to contradictory behavior.

I.3. Better identification of quality criteria

Quality can be expressed in terms of five components: safety, health, sensory, service and society (Figure I.1); we can define a number of criteria for each of the five components.

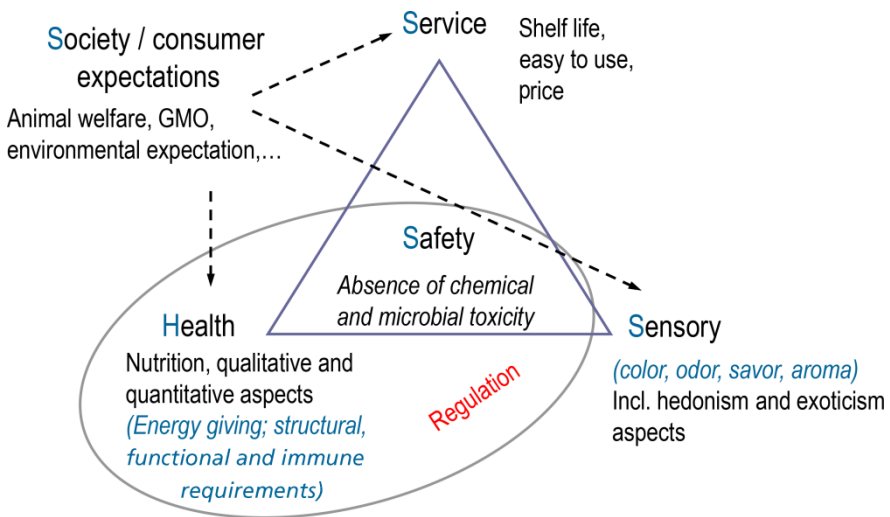


Figure I.1. Quality of food

I.4. Safety

Safety implies the absence of bacterial or viral pathogens, toxins or chemical residues. Pathogens can have several sources (e.g. raw materials and environment) and can be carried by different vectors (e.g. water, air and operators). The implementation of codes of good practice in production and processing, the application of health controls, the design of production and processing facilities, and ongoing progress are all elements that help reduce hygiene risks and limit cross contamination.

Toxins can be present in some raw materials as natural defense mechanisms against predators or microbial attack, or produced *in situ* by bacterial or fungal microorganisms that colonize the raw materials: this is the case, for example, with aflatoxins or patulin.

Chemical residues can originate from raw materials contaminated by the treatment of plants or crops (herbicides and fungicides) or animals (antibiotics, anabolic steroids and hormones), or can be generated by processing methods such as smoking (benzopyrenes) and salting (nitrites and nitrosamines). Products from traditional processes and technology are often considered a guarantee of quality, which can be true from a

sensory perspective, but is highly contestable from a health and safety perspective.

1.5. Health

The primary function of food is to satisfy the nutritional and physiological needs of the individual.

Energy requirements

Energy requirements vary not only depending on age and physiological condition, but also in relation to the amount of physical activity associated with muscular work during daily activities and sport. For adults, this can range from 8,000 to 15,000 kJ per day. Energy requirements are primarily fulfilled by the intake of fats and carbohydrates, which should constitute 30 and 55% of total energy, respectively, according to nutritional recommendations.

Structural and functional requirements

Our body needs certain amounts of organic and inorganic substances to build and repair bones and tissues as well as carry out certain biochemical reactions involved in metabolism.

In terms of minerals, macronutrient requirements (e.g. sodium, potassium, calcium, magnesium and phosphorous) are generally covered by diet. However, this is not always the case for some micronutrients (e.g. iron, iodine, selenium and fluoride).

Vitamin requirements can be met by a balanced diet that includes fruit, vegetables, and animal and vegetable fats.

Proteins supply amino acids, nine of which are essential since they are not synthesized by the body (methionine, lysine, tryptophan, threonine, phenylalanine, isoleucine, leucine, valine and histidine). Again, quantitative and qualitative needs change with age, physiological condition and activity. They are easily covered by an animal and vegetable protein-based diet.

Fats and carbohydrates, in addition to their energy function, play an important physiological role. Some fatty acids have reproductive, epidermal

and platelet functions. Two families of polyunsaturated fatty acids, *n*-6 (linoleic acid, 18:2) and *n*-3 (alpha-linolenic acid, 18:3), play an essential role. The recommended daily allowance, expressed as a percentage of energy from fat, is 60% monounsaturated fatty acids, 25% saturated fatty acids and 15% polyunsaturated fatty acids (linoleic acid and alpha-linolenic acid at a ratio of 5:1). Carbohydrates other than glucose play an important role as components of serum and tissue glycoproteins (galactose, mannose, fucose, sialic acid, etc.). Indigestible oligosaccharides are crucial in maintaining a balanced gut flora and limiting the probability of developing certain diseases.

Protection and defense requirements

The body is exposed to a certain amount of stress and attack. The immune system can therefore be affected by various diseases and treatments or during the process of aging, which results in greater susceptibility to viral attack and microbial infection. The immune status is maintained and even improved by the intake of micronutrients (copper and zinc) and vitamins (B₆) as well as the consumption of prebiotics and probiotics.

I.6. Satisfaction

Eating has and always should remain a pleasure through the sensations that food provides. Sensory stimuli, whether visual, olfactory (nasally or retronasally), gustatory (taste), tactile or auditory, contribute in varying degrees to the organoleptic or sensory quality of food. The integration of all these stimuli results in sensory perception and its hedonic expression. It can differ from one individual to another, given that the discriminating power of smell and taste depends on eating habits, and can vary for organic and physiological reasons. The perception of food can also be heavily influenced by the sociocultural context; for example, the imaginative aspect associated with food can have a considerable impact.

I.7. Service

Sociological changes (e.g. increase in female labor force participation, organization of working time and growing importance of leisure time) tend to limit the time spent by consumers in preparing meals. They have thus contributed to the development of non-domestic catering and food services, i.e. shelf-stable prepared and/or cooked products that are easy to use. The

development of freezing and thawing (using household microwaves, for example) has promoted the penetration of these ready-to-use foods into the market.

I.8. Society

The relationship between the consumer and food has evolved considerably. Consumers are increasingly aware of buying local, demanding more “naturalness” and “authenticity” and refusing any technical developments that might damage the quality of rural areas, animal well-being or the environment. Food, the link between consumers and their locality, conveys the values and choices of a society, of which consumers are increasingly aware.

PART 1

Water and Other Food Constituents

Water is the most abundant constituent of the majority of foods. It therefore plays a crucial role in the physicochemical characteristics and properties of the plant and animal foods we eat. These characteristics can be desired due to their contribution to food quality (the texture of fruit, vegetables and meat, which depends, among other things, on cell turgidity as well as on specific and complex interactions between water and other constituents). However, they can also contribute to food spoilage through biochemical and microbiological processes. As a result, several food preservation methods are based, at least partially, on lowering the water activity (a_w) or the water availability.

1.1. Structure and state of water

The water molecule, composed of two hydrogen atoms and one oxygen atom (H_2O), can exist, like many substances, in three different states: solid, liquid or gas.

In the liquid and vapor state, the water molecule is a polar monomer (see Figure 1.1).

In the solid state (i.e. ice), water molecules are linked by hydrogen bonds and form a crystalline polymer in which each monomer molecule is connected to four other molecules by hydrogen bonds. The distance between two oxygen atoms is 0.276 nm. At temperatures below -173°C , all hydrogen

Chapter written by Pierre SCHUCK.

atoms are involved in hydrogen bonds, whereas at 0°C only around 50% are involved, and at 100°C only a small percentage are involved.

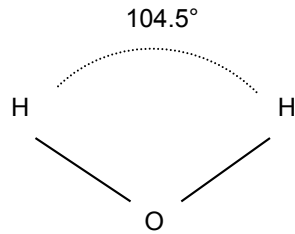


Figure 1.1. *Water molecule*

Certain water properties can be attributed to these intermolecular bonds, in particular the boiling point, melting point, latent heat of fusion, latent heat of vaporization, specific heat, surface tension and the dielectric constant. However, water in a liquid state behaves like a monomer in terms of viscosity and the diffusion coefficient (Tables 1.1 and 1.2).

Properties	Unit	Value
Molar mass	g mol^{-1}	18.01528
Melting point (at 101,325 Pa)	°C	0.00
Boiling point (at 101,325 Pa)	°C	100.00
Maximum density	kg m^{-3}	999.95
Temperature of maximum density	°C	4.00
Triple-point temperature	°C	0.01
Triple-point density (liquid)	kg m^{-3}	999.78
Triple-point density (gas)	$10^{-3} \text{ kg m}^{-3}$	4.88
Latent heat of sublimation at the triple point	10^3 J kg^{-1}	2800
Critical temperature	°C	373.99
Critical pressure	MPa	22.064
Critical density	kg m^{-3}	322
Specific volume at the critical point	$10^{-3} \text{ m}^3 \text{ kg}^{-1}$	3.11
Latent heat of freezing at 0°C	10^3 J kg^{-1}	335

Table 1.1 *Properties of water*

Different theoretical models have been proposed to explain the liquid and solid state behavior of water. Monomers as well as higher-energy molecules exist in a static equilibrium: each molecule is involved in one to four hydrogen bonds; the latter can form short-lived labile clusters.

Properties	Units	Frozen water		Liquid water						Water vapor	
Temperature	(°C)	−20	0	0	20	40	60	80	100	100	200
Density	kg m ^{−3}	919.3	916.8	999.8	998.2	992.2	983.2	971.8	958.4	0.589	0.452
Viscosity	10 ^{−6} Pa s	–	–	1,793	1,002	653	466	354	281	12.5	16.4
Surface tension	10 ^{−3} N m ^{−1}	–	–	75.64	72.75	69.60	66.24	62.67	58.91	–	–
Vapor pressure	(Pa)	103.4	611.3	611.3	2,338.8	7,381.4	19,932	47,373	101,325	–	–
Specific heat C _p	J.kg ^{−1} K ^{−1}	1,954	2,101	4,217	4,182	4,178	4,184	4,196	4,216	2,041	1,960
Energy of vaporization	10 ³ J kg ^{−1}	–	–	2,494	2,448	2,402	2,357	2,309	2,258	–	–
Thermal conductivity	W m ^{−1} °K ^{−1}	2.43	2.22	0.561	0.598	0.630	0.654	0.670	0.679	0.0248	0.0391
Diffusivity in the air	10 ^{−6} m ² s ^{−1}	–	–	21.8	24.6	27.5	30.5	33.7	–	–	–
Thermal diffusivity at 10 ⁵ Pa	10 ^{−6} m ² s ^{−1}	–	–	0.135	0.143	0.151	0.161	0.164	0.170	19.6	32.6

Table 1.2. *Properties of water*

The structure of water is largely influenced by its organic and inorganic environment:

– electrolytes such as Na⁺, K⁺ and Cl[−] are highly hydrated in solution, and lower the number of hydrogen bonds between water molecules, whereas hydrocarbon chains and non-polar groups of protein side-chains tend to increase it;

- substances in solution (carbohydrates and amino acids), which can themselves form hydrogen bonds, can modify the bonds between water molecules depending on their geometric compatibility with the existing network, e.g. urea has a strong effect whereas ammonia has none;
- substances with several different functional groups (e.g. amino acids, proteins, fatty acids and carbohydrates) affect the structure of water.

The effect of the environment is very pronounced in highly concentrated solutions and weakly hydrated systems. Water can form crystalline hydrates (clathrates) above 0°C with some gases (e.g. freon and propane). The formation of these hydrates can be used in the concentration or demineralization of aqueous solutions. Table 1.3 shows the different binding energies of water.

Type of bond	Binding energy (kJ mol ⁻¹)
Covalent bond	460
Hydrogen bond (liquid water)	< 20
Hydrogen bond (frozen water)	23
Hydration of polar group (monolayer)	4–6
Hydration of polar group (multilayer)	1–3
Van der Waals bond	0.5
Water retained by capillary forces	0.3

Table 1.3. *Binding energy of water [FAI 03]*

Water also affects properties such as the structure, diffusion and reactivity of substances in solution. For example, the role of water in the structure and functional properties of macromolecular compounds such as proteins is known [LEM 02]. The main functions of water in food are shown in Table 1.4.

Role of water	Example
Solvent	Molecule dissolution
Reaction medium	Enzymatic reactions
Mobility of reagents	Maillard reactions
Reagent	Lipid hydrolysis
Anti-oxidant	Hydrogen bond with peroxides blocking the oxidation of lipids, for low a_w
Pro-oxidant	– Swelling of proteins and accessibility of oxidizable sites – Sources of free radicals in irradiated foods
Structural role	– Formation of hydrogen bonds between texturing molecules – Acts on lipid/protein bonds in bakery products – Acts on the conformation and interactions between gelling agents and proteins

Table 1.4. Functions of water in food [DUC 76, FAI 03]

1.2. Properties of water

Among the physical and physicochemical properties of water, some greatly influence phase transitions as well as mass and heat transfers. Examples include specific heat, latent heat of fusion, latent heat of vaporization, thermal conductivity and viscosity. They determine the design and control of heat treatment (sterilization, cooking, etc.), concentration, drying or freezing processes. Others relate to the solvent properties of water: dielectric constant, surface tension or dipole moment. Water is, in fact, the dilution medium for many chemical species that can diffuse and react with each other. In addition to this, water can diffuse and participate in various reactions, such as hydrolysis. The introduction of different chemical species in solution or colloidal suspension in water also creates the so-called colligative properties, which depend on the number of molecules present. This is the case, for example, with lowering the freezing point and surface tension, increasing the boiling point and viscosity, and establishing osmotic pressure gradients through semi-permeable membranes.

The fact that the water present in food is more or less in interaction with the other constituents gives rise to concepts of “free water” and “bound water”. Various observations show that the so-called bound water can itself be bound to varying degrees and the state of water is just as important for the stability of a foodstuff as the total water content. This concept of “bound water” is also underpinned by the knowledge of the dipolar nature of water and its possible interactions with different chemical groups of other constituents.

In order to determine to what extent the concept of “bound water” is a physical reality, two categories of properties can be used to characterize this potential binding state: molecular mobility and thermodynamic properties. Of these, water activity (a_w) has attracted the most attention in food science and technology. The ability of water to freeze has also long been considered an indication of the binding state of water. These concepts are now combined with dynamic data (glass transition – T_g) to establish the state diagram [SIM 02]. These three concepts (a_w , T_g and the phase diagram) are explained hereafter.

1.2.1. Water activity (a_w)

1.2.1.1. Definition

The a_w of a food product is characterized by the ratio of the partial vapor pressure in a food product (P_p) and the saturated vapor pressure (P_w) at the same temperature θ :

$$a_w = \frac{P_p}{P_w} \quad [1.1]$$

The a_w is, therefore, a non-dimensional ratio and is thus a relative measure compared to a standard (pure water). Consequently, the a_w of pure water is equal to 1, with every other product having an a_w value below 1. The decrease in water activity can be explained by the fact that the solubilized chemical components partially mobilize the water and, therefore, decrease its capacity to vaporize; they also alter the chemical reactivity of water, which is exactly proportional to a_w .

The a_w of a food product should not be confused with its relative humidity (H_R). The H_R is the ratio of the partial vapor pressure in air (P_a) and the saturated vapor pressure (P_{sv}) at the same temperature θ :

$$H_R = \frac{P_a}{P_{sv}} \times 100 \quad [1.2]$$

At equilibrium, $P_p = P_a$ and $P_w = P_{sv}$ for a given food product and at a given temperature θ . The a_w or the H_R at equilibrium (H_{RE}) of a food product is the H_R of an atmosphere in equilibrium with the product. In other words, the a_w of a solution or a food is equal to the relative partial pressure of water vapor of the solution or food in a confined atmosphere at equilibrium. Therefore, H_R at equilibrium and a_w are proportional physical quantities linked by the following equation:

$$H_{RE} = a_w \times 100 \quad [1.3]$$

Some authors believe that such a thermodynamic equilibrium is never reached in the case of food. The a_w values obtained from measurements on food should therefore not be considered as absolute.

The a_w of a food product accounts for the availability of water as a solvent or a reagent. As a result, a_w is crucial in estimating the stability of food during processing and storage. The relative rates of change in food as a function of a_w are clearly illustrated in Figure 1.2. For low a_w values (< 0.1), the risk of lipid oxidation is very high. This negative correlation with a_w in this range can be explained by the fact that a monolayer of water molecules around the lipid fraction constitutes a resistance to oxygen transfer and, thus, a protective shell against lipid oxidation. For a_w values between 0.3 and 0.8, the reaction rates linked to non-enzymatic browning (maximum of 0.6–0.7 a_w), non-enzymatic hydrolysis and enzymatic activity gradually increase with a_w . There is very little microorganism growth when a_w is less than 0.6. Efstathiou *et al.* [EFS 02] estimated an optimum stability range for a_w values of between 0.2 and 0.3.

During freezing, the a_w of ice is calculated by using the vapor pressure value of subcooled water at the corresponding temperature as a reference [BLO 02]. It is equal to 1 at 0°C and decreases with temperature (see Table 1.5).

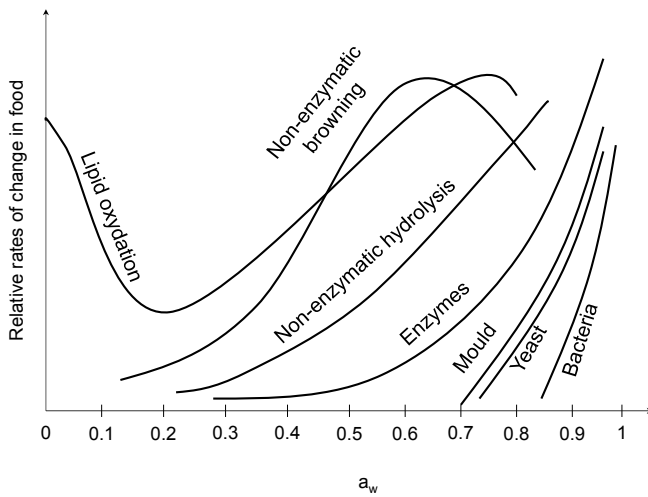


Figure 1.2. Relative rate of change in food as a function of a_w

Temperature (°C)	Vapor pressure of pure liquid water (Pa)	Vapor pressure of ice (Pa)	$a_w = \frac{P_{\text{ice}}}{P_{\text{liquid water}}}$
0	610.48	610.48	1.000
-5	421.70	401.70	0.953
-10	286.51	259.98	0.907
-15	191.45	165.45	0.864
-20	125.72	103.46	0.823
-25	80.93	63.46	0.784
-30	51.06	38.13	0.747
-40	18.93	12.93	0.683
-50	6.40	4.00	0.625

Table 1.5. Vapor pressure of water and ice measured at different temperatures

As it can be assumed that the cryo-concentrated phase and the ice phase are in equilibrium, a_w is the same in both phases: it depends, therefore, on the temperature of the frozen product regardless of its nature. The a_w value is

not a very significant criterion for stabilization (even at temperatures below 0°C). If a product preserves better at a lower storage temperature, and therefore has a weak a_w , the shelf-life variability of various frozen products clearly shows that this parameter cannot be considered as the sole index of stability. Chemical stability is related more to the mobility of solutes and the diffusion of oxygen than the availability of water. Another interesting aspect about the a_w of a product is its link with the initial freezing temperature. At this temperature, there is an equilibrium between the a_w and the temperature of ice. This parameter should be considered in the development of complex products that combine a frozen phase with another low-humidity and, therefore, ice-free phase, such as ice cream in a wafer; the difference in their a_w is the cause of water migration, which is inevitable without a barrier between the two phases.

1.2.1.2. Method

The methods for determining the a_w of food products involve placing the product in equilibrium with the atmosphere of a microchamber and then measuring the manometric or hygrometric characteristics of the air in equilibrium with the product [LAB 68, LAB 76, GAL 81].

a_w	Ideal molality	NaCl	CaCl ₂	Sucrose	Glycerol
0.995	0.280	0.150	0.101	0.272	0.277
0.990	0.566	0.300	0.215	0.534	0.554
0.980	1.13	0.607	0.418	1.03	1.11
0.960	2.31	1.20	0.87	1.92	2.21
0.940	3.54	1.77	1.08	2.72	3.32
0.920	4.83	2.31	1.34	3.48	4.44
0.900	6.17	2.83	1.58	4.11	5.57
0.850	9.80	4.03	2.12	5.98	8.47
0.800	13.90	5.15	2.58		11.50
0.750	18.90		3.00		14.80
0.700	23.8		3.40		18.3
0.650	30.0		3.80		22.0

Table 1.6. Molality (number of moles of solute per kg of solvent) of various solutes and corresponding a_w values at 25°C

	10°C	20°C	25°C	30°C	40°C	50°C	60°C	70°C	80°C
LiBr	0.07	0.07	0.06	0.06	0.06	0.05	0.05	0.05	0.05
NaOH		0.07	0.07	0.07	0.07	0.06	0.05		
KOH	0.12	0.09	0.08	0.07	0.06	0.06	0.05	0.05	
LiCl	0.13	0.11	0.11	0.11	0.11	0.10	0.10	0.10	0.10
LiI	0.21	0.19	0.18	0.17	0.15	0.12			
CH ₃ COOK	0.23	0.23	0.22	0.22					
CaCl ₂		0.33	0.30	0.22	0.19	0.17			
MgCl ₂	0.34	0.33	0.33	0.32	0.32	0.31	0.29	0.28	0.26
NaI	0.42	0.39	0.38	0.36	0.33	0.29	0.26	0.24	0.23
K ₂ CO ₃	0.43	0.43	0.43	0.43		0.41	0.39	0.37	0.35
Mg(NO ₃) ₂	0.57	0.54	0.53	0.51	0.48	0.45			
SrCl ₂		0.73	0.71	0.69		0.57	0.52	0.46	0.41
NaNO ₃	0.78	0.75	0.74	0.73	0.71	0.69	0.67	0.65	0.63
NaCl	0.76	0.75	0.75	0.75	0.75	0.75	0.74	0.74	0.74
KCl	0.87	0.85	0.84	0.84	0.82	0.81	0.79	0.78	0.77
BaCl ₂	0.92	0.91	0.90	0.90	0.89	0.88	0.87	0.86	0.85
K ₂ SO ₄	0.98	0.98	0.97	0.97	0.96	0.96	0.96	0.96	0.96
K ₂ Cr ₂ O ₇	0.98	0.98	0.98	0.98					

Table 1.7. Saturated salt solutions used to determine sorption curves

According to Dumoulin *et al.* [DUM 04], there are several methods for determining water activity, such as directly measuring the vapor pressure of water using a manometer or measuring the dew-point temperature or relative humidity of the air in equilibrium with the product using polyamide fiber or electrical hygrometers, for example.

Table 1.6 shows the ideal molality (number of moles of solute per kg of solvent) corresponding to a_w with experimental values for solutions of sodium chloride, calcium chloride, sucrose and glycerol. In reality, most solutes reduce a_w by more than would be expected theoretically. There are different reasons for this behavior: strong associations between water and solute molecules, almost complete dissociation of electrolytes and forces acting on the water structure.

Table 1.7 shows the a_w values of saturated salt solutions as a function of temperature: a_w is equal to 1 and 0 for pure water and saturated P_2O_5 , respectively. The a_w values generally have an accuracy of ± 0.02 [BIM 02].

1.2.1.3. Sorption isotherm

In addition to measuring a_w , it is also possible to establish a relationship between a_w and the water content of a product (kg of water per kg of dry matter) at a given temperature θ . This relationship is represented by a sorption isotherm (adsorption or desorption) in the shape of a sigmoidal curve (Figure 1.3). Sorption isotherms reflect the adsorption capacity of water as well as the water retention capacity of the product. This information is very important for the food technology sector.

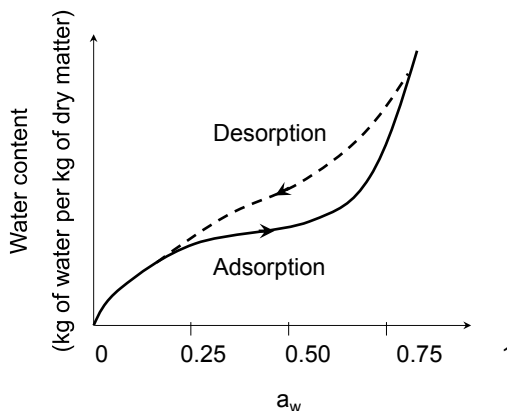


Figure 1.3. Water adsorption and desorption isotherm

Measuring these curves generally involves placing a product sample in an atmosphere of known H_R (Table 1.7) until equilibrium has been reached and then measuring the water content of the sample by weight. Depending on whether a high or low moisture product is used, a desorption or an adsorption isotherm is obtained, respectively. These are not superimposable. The gap between the two curves is referred to as hysteresis: for a product with the same water content, equilibrium is obtained during desorption, at each point, at a lower a_w value than during adsorption. Hysteresis occurs mainly in the intermediate regions of the isotherms where water is only “weakly bound”. It is linked to water condensation in the pores of the product. It also corresponds to the relationship, defined by the Kelvin equation, between the partial vapor pressure and the contact angle (which depends on the surface tension) or the pore diameter. The liquid–solid contact angle is greater when a liquid wets a dry surface (adsorption) than when it evaporates from a wet surface (desorption). The supersaturation phenomenon of sugars in solution may also partly explain the hysteresis observed in fruit and vegetables. Water activity drops rapidly during dehydration because sugars do not crystallize but form a supersaturated solution. In contrast, sugars only dissolve above a certain water content during rewetting.

Sorption isotherms vary with temperature (Figure 1.4). In theory, a_w does not depend on the temperature but solely on the composition of the solution. In reality, for most products and at a constant water content, a_w increases with temperature, but with a reverse reaction for products high in fat or soluble sugars [BIM 02].

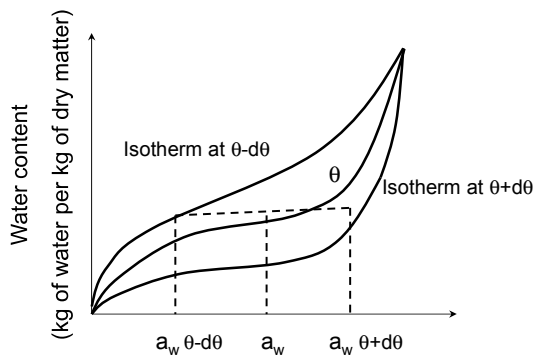


Figure 1.4. Influence of temperature on the water adsorption isotherm

Sorption isotherms also vary from one food to another (Figure 1.5). They are the result of the behavior of various chemical constituents of food with water. Proteins and starches retain more water in the lower region of the isotherms than fats and crystalline substances (e.g. sugar). Dried fruit high in sugar is particularly hygroscopic, but only above a certain H_R .

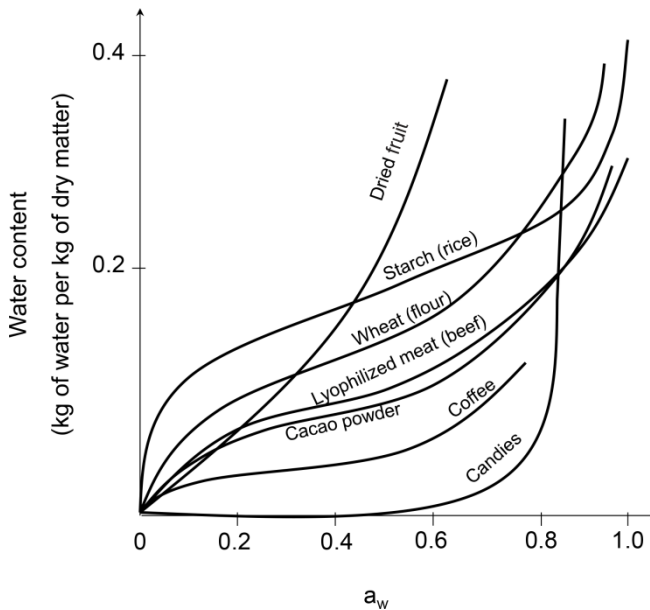


Figure 1.5. Water adsorption isotherm of various foods

The physical states (i.e. amorphous, intermediate or crystalline) of the matrix influence water retention. This physical state largely depends on the type of technological process, and the manner in which these operations are carried out can cause variations in the isotherms of dried products. Particle size also influences water retention.

Using the theoretical sorption isotherms given in Figure 1.6, it is possible to determine the ideal water content for the optimum preservation of a given powder. For an a_w of 0.2, the water content would be 4% for milk powder (maximum regulatory requirement), between 2 and 3% for whey powders and 6% for caseinate powders (maximum regulatory requirement).

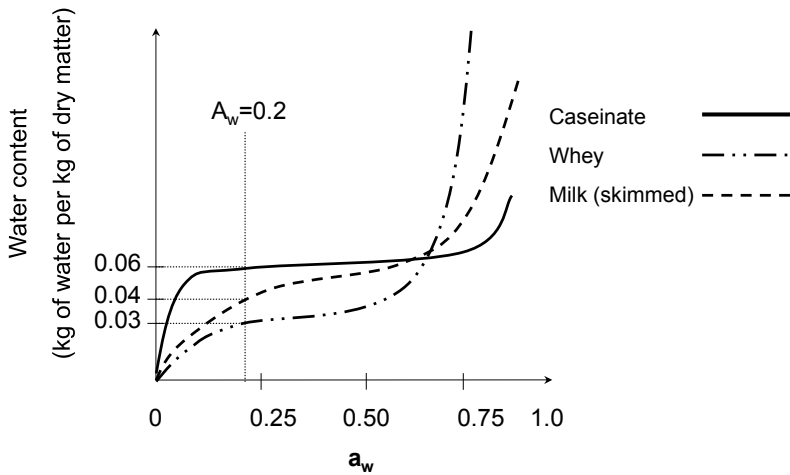


Figure 1.6. *Theoretical water adsorption isotherm for milk powders*

Sigmoidal sorption curves can be divided into three sections:

- The first section (a_w from 0 to 0.2) corresponds to the sorption of a monolayer of water molecules with strong hydrogen bonds (between 4 and 60 KJ mol⁻¹). These water molecules are bound to the polar groups of certain compounds, mainly NH₃⁺ and COO⁻ groups of proteins and OH groups of starches; this section also includes water of crystallization of salts and sugars (e.g. lactose). These water molecules are, therefore, very strongly bound, which means they are quite difficult to remove by dehydration and cannot undergo freezing.

- The second section (a_w from 0.2 to 0.6), the linear portion of the curve, corresponds to the additional water layers or multilayers with weaker hydrogen bonds (between 1 and 3 KJ mol⁻¹), representing more mobile water molecules with increasing a_w .

- The third section (a_w above 0.6) represents the condensed water in the pores of the food (energy 0.3 KJ mol⁻¹): this water allows the dissolution of soluble elements and can serve as a support to biological agents such as enzymes and microorganisms (Figure 1.2). This section is almost asymptotic and therefore very difficult to model.

However, some researchers believe that beyond the second section of the isotherm and despite an a_w as low as 0.2 to 0.3, water molecules have the

same properties apart from a few exceptions (ability to evaporate in particular). There would, therefore, be no fundamental difference between weakly bound water and unbound water (free water), where the property of water is very close to that of pure water, and its availability, as a solvent or reagent, would increase continuously with a_w . It is likely that weakly bound water and free water are able to rapidly interchange.

Many attempts have been made to establish mathematical models of sorption isotherms based on theoretical considerations and experimental observations. Despite this, and given the complexity of the phenomena, none of these models offers the ideal solution that covers the entire sorption field.

The most commonly used model is the Brunauer–Emmett–Teller model (BET; [BRU 38]) with two parameters, which is of particular significance in the study of curves corresponding to an a_w below 0.5. It is based on the following equation:

$$\frac{a_w}{M(1 - a_w)} = \frac{1}{M_1 C} + \frac{a_w (C - 1)}{M_1 C} \quad [1.4]$$

where M and M_1 are the values for the water content of the product and the monolayer, respectively (g per 100 g of dry matter), and C is the BET constant defined by:

$$C = e^{\left[\frac{H_m - H_n}{RT} \right]} \quad [1.5]$$

where H_m is the heat of sorption of the monolayer (J mol^{-1}), H_n is the heat of adsorption of the second and higher layers (J mol^{-1}), R is the ideal gas constant ($8.314 \text{ J mol}^{-1} \text{ K}^{-1}$) and T is the absolute temperature (K).

Using equation [1.4], M_1 and C can be calculated, with M and a_w determined experimentally. Plotting $\frac{a_w}{M(1 - a_w)}$ (y-axis) as a function of a_w (x-axis) results in a straight line (Figure 1.7), giving values for the intersection and slope of $\frac{1}{M_1 C}$ and $\frac{(C - 1)}{M_1 C}$, respectively. The advantage of this model is that it is possible to calculate M_1 and $(H_m - H_n)$.

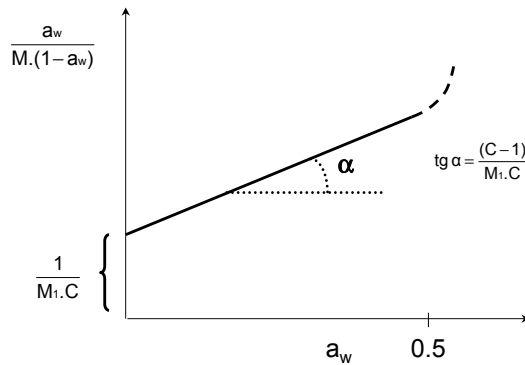


Figure 1.7. Representation of the Brunauer–Emmett–Teller equation

Another commonly used model is the Guggenheim–Anderson–de Boer model (GAB; [VAN 81]) with three parameters. It is often considered an extension of the BET model and can represent sorption curves up to an a_w of 0.85:

$$M = \frac{M_1 C K a_w}{[1 - (K a_w)][1 - (K a_w) + (C K a_w)]} \quad [1.6]$$

where M and M_1 are the water content values of the product and the monolayer, respectively (g per 100 g of dry matter), and C and K are the temperature-dependent parameters such as:

$$\begin{cases} C = C_0 e^{\frac{\Delta H_c}{RT}} \\ K = K_0 e^{\frac{\Delta H_k}{RT}} \end{cases} \quad [1.7]$$

where C_0 and K_0 are constant, and ΔH_c and ΔH_k are defined by:

$$\begin{cases} \Delta H_c = H_m - H_n \\ \Delta H_k = L - H_n \end{cases} \quad [1.8]$$

where H_m and H_n are, respectively, the heat of sorption values of the monolayer and multilayer of water (J mol^{-1}), and L is the heat of condensation of pure water (J mol^{-1}).

More recently, Pisecki [PIS 97] proposed an empirical model to directly estimate the a_w values of skimmed milk powder:

$$a_w = e^{\left[-\frac{b}{M^a} \right]} \quad [1.9]$$

where M is the water content of the product (g per 100 g of dry matter) and a and b are two adjusted coefficients ($a = 2.0544$ and $b = 54.387$ for desorption, and $a = 1.7764$ and $b = 24.8439$ for adsorption). This type of model, even though not explicative, still manages to closely match adsorption and desorption isotherms.

1.2.2. Glass transition

1.2.2.1. Principle

Glass transition has long been recognized for its technological importance for mineral and organic substances as well as food products. The concept, originally developed and used by physical chemists in the field of polymer science, characterizes the mobility of water in amorphous products (i.e. non-crystallized). A distinction can be made between:

- products in a glassy state, that are relatively hard, and present weak water mobility;
- products in a flaccid state (rubbery and sticky), having a higher water mobility and that are, therefore, less stable in terms of storage.

The gradual shift from one state to another is called “glass transition”: it occurs when there is a variation in temperature or water content. According to Genin and René [GEN 95], cooling a pure liquid can, in most cases, result in the formation of a crystalline solid. In theory, this change of state happens for a given product at a fixed temperature, known as the crystallization temperature (T_c). However, no change of state is observed during cooling even when the T_c has been exceeded. There are two possible outcomes if the temperature is lowered further:

- crystallization occurs, but at a temperature below T_c ;
- the liquid state remains until solid-like behavior occurs (no change of state in a thermodynamic sense) at a given temperature known as the “glass transition temperature (T_g)”.

This frozen liquid is called a glass or an amorphous solid. In terms of energy, it is a metastable state; a small amount of energy can switch it to a more stable state, which can be a liquid or a crystalline state.

The glass state can be achieved in two ways:

- when cooling is fast enough to avoid the appearance of ice crystals;
- when the dynamic viscosity affects the rate of crystal growth by lowering the diffusivity (dynamic viscosity and diffusivity vary inversely).

In the amorphous solid state, the molecules are not ordered and the system is said to be in a glassy state: the material has a high internal viscosity. During reheating above T_g , the system passes from a glassy state to a viscoelastic state where molecular mobility is higher [BHA 96]. This change can also be obtained at a constant temperature by increasing the water content: this is referred to as the plasticizing effect. Generally, the higher the water content is, the lower the T_g . This influence of the water content on T_g was observed for many products: amorphous lactose, skimmed milk powder, etc. [JOU 94a, LEM 90]. Water availability in a food matrix therefore depends on many different factors: water content, solute composition, hygroscopicity, viscosity and T_g (Figure 1.8).

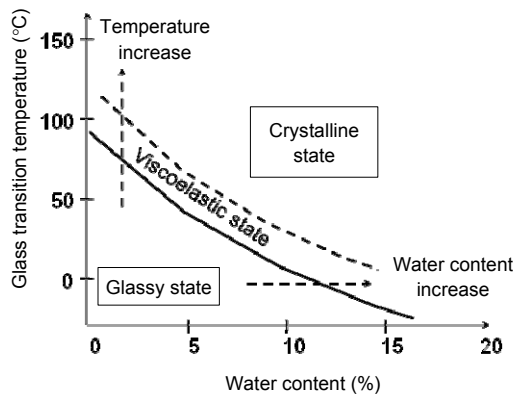


Figure 1.8. Representation of the glass transition temperature as a function of water content [ROO 97, ROO 02]

Thus, a change in temperature and water content around the T_g value will significantly alter the mechanical properties of the material [JOU 94b,

ROO 97]. Even though glass transition has been used to predict the stability of frozen or dried products, the complexity and variety of products make the interpretation difficult. Sugars, proteins and fat are affected by glass transition [ROO 91a, ROO 91b]. Figure 1.9 summarizes the relation between amorphous and crystalline structures.

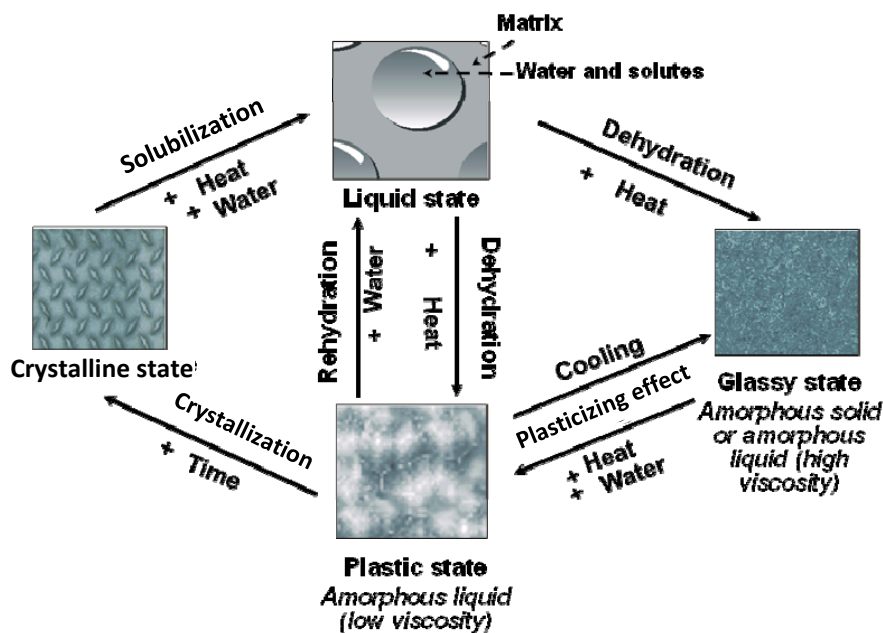


Figure 1.9. Representation of the relation between amorphous and crystalline structures [ROO 97, ROO 02]

The nature and range of molecular movements as well as the kinetics of the changes depend on the product temperature (T) in relation to T_g ($T - T_g$). For temperatures below T_g , only local, non-cooperative movements of small amplitude are possible (rotation movements and bond vibrations). Conversely, cooperative molecular movements of a higher amplitude occur at temperatures above T_g . Molecular mobility is enhanced in this case (translational movements), thus encouraging several reactions [LEM 01].

Table 1.8 gives some T_g values for dried ingredients. These values vary significantly from one ingredient to another (lactose/casein) and within the

same constituent category (lactose/galactose). For example, monosaccharides generally have a lower T_g than disaccharides.

Ingredients	T_g (°C)
Glucose	+36
Galactose	+30
Fructose	+10
Sorbitol	-2
Lactulose	-2
Maltose	+43
Sucrose	+67
Lactose	+97
Skim milk	+92
Whole milk	+92
Hydrolyzed milk	+49
Casein	+144
Sodium caseinate	+130
Maltodextrin DE 6	+168
Maltodextrin DE 33	+130
Maltodextrin DE 47	+103

Table 1.8. Glass transition temperature (T_g) of various dried food ingredients

Table 1.9 gives some examples of foods where sugars can affect the glassy state by causing visible modifications of food systems.

Food	Glassy state created by:	Types of sugars	Problems and solutions
Ice cream	Freezing	Sucrose–lactose	Grains (formation of lactose crystals, promoted by storage between -17 and -23°C). Avoid an excessive level of lactose in the formulae (lactose/water below 10% (w/w)).
Skim milk powder	Dehydration	Lactose (representing 50% of dry matter)	Sticking resulting in aggregates. Should be stored in waterproof packaging below the T_g in order to maintain stability. Possibility of instantizing.
Sweets	Concentration by boiling–cooling	Sucrose–glucose–invert sugar	Sedimentation – formation of grains (crystals) – sticky surface. Avoid a high hygroscopic sugar content and storing at overly high temperatures and/or relative humidity levels.
Lyophilisate	Dehydration	Different types	Partial liquefaction. Caking may occur during freeze-drying and slow it down. This also makes rehydration more difficult. Avoid overly high temperatures and relative humidity levels.

Table 1.9. *Sugars in the glassy state in foods: instability and approaches to overcome this*

1.2.2.2. Measurement and calculation

T_g can be measured in various ways; for example, in rheological measurements, T_g is defined as the temperature at which viscosity decreases. However, these measurements are often difficult in the agri-food sector because the glass phase is generally involved in a solid phase, rendering obsolete the notion of viscosity. Another method is to measure the dielectric

constant of the medium, which changes significantly during glass transition. In practice, T_g is measured by following the change in the specific heat flux as a function of temperature, generally by differential scanning calorimetry (Figure 1.10). Using this analysis, three successive thermal events can be identified during the heating of a pure substance in the amorphous state [SEN 95]:

- at T_g , a change in specific heat (endothermic), denoted by ΔC_p ;
- at the crystallization temperature, an exothermic phase transition;
- at the melting temperature, an endothermic phase transition.

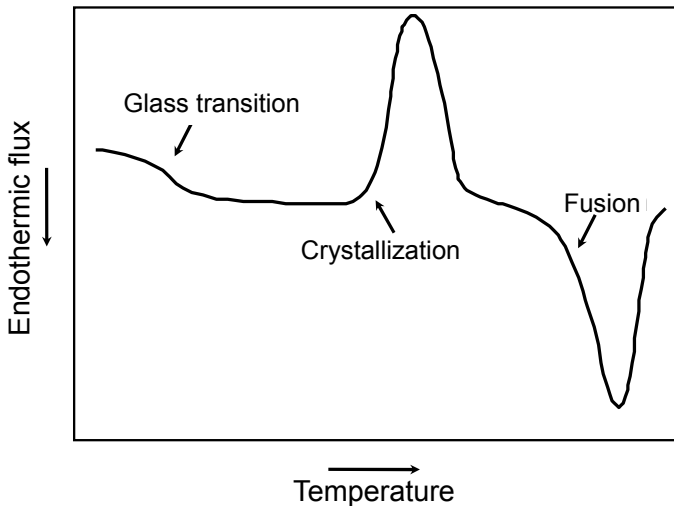


Figure 1.10. *Change in specific heat flux as a function of temperature by differential scanning calorimetry. Transition of a sugar from the glassy state to the crystalline state*

It is also possible to estimate a T_g value by using equations that link the glass transition temperature of a mixture to its composition and to the glass transition temperatures of its pure constituents. The Gordon and Taylor equation is as follows:

$$T_g = \frac{\sum_i w_i \cdot T_{gi}}{\sum_i w_i} \quad [1.10]$$

where W_i is the mass fraction of constituent i in solution (%) and T_{gi} is the glass transition temperature of the pure compound i (°C). In general, this equation slightly overestimates the T_g .

A more accurate T_g can be obtained using the Couchman–Karasz equation, modified and extended to ternary solutions of proteins, carbohydrates and water:

$$T_g = \frac{\sum_i W_i \Delta C_{pi} \cdot T_{gi}}{\sum_i W_i \Delta C_{pi}} \quad [1.11]$$

where T_{gi} is the glass transition temperature (°C) of constituent i at zero a_w , ΔC_{pi} is the change in specific heat of constituent i at T_{gi} ($\text{J kg}^{-1} \text{°C}^{-1}$) and W_i is its mass fraction (%). The T_{gi} values, referred to as “dry T_g ”, are obtained by extrapolating the T_g and ΔC_p values measured at different a_w levels. In this model, water is one of the constituents measured, with T_g and ΔC_p values equal to -139°C and $1.94 \text{ kJ kg}^{-1} \text{°C}^{-1}$, respectively. The reliability of the calculation of the overall T_g directly depends on the consideration of all the product constituents (water, proteins, amino acids, carbohydrates, minerals, etc.), subject to the availability of their thermodynamic characteristics, T_{gi} and ΔC_{pi} . This is a limiting factor in using equation [1.11].

1.2.3. Phase diagram

The phase diagram is a widely used tool in the agri-food sector. It is possible, for example, in the production of whole milk powder to relate temperature (θ) and dry matter concentration in the phase transitions for water and lactose, as shown in Figure 1.11:

- the freezing curve (a) starting from 0°C for water and gradually falling to around -6°C at around 50% dry matter, then extrapolated to 80% dry matter;
- the lactose solubility curve (b), adapted to whole milk;
- the glass transition curve (c) for lactose in milk according to Roos [ROO 97], using the Gordon and Taylor equation.

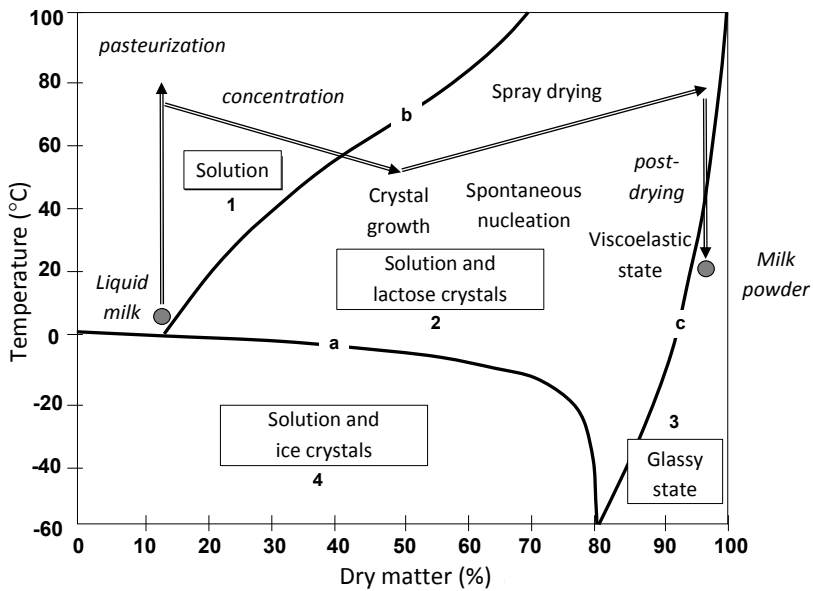


Figure 1.11. Phase diagram of whole milk [VUA 02]

The three curves define the main phase changes of lactose and water in four different areas:

- lactose solution;
- lactose solution with lactose crystals;
- solid amorphous lactose;
- lactose solution with ice crystals.

If we take into account the kinetic dimension of mutarotation and lactose crystallization, this dynamic phase diagram is particularly useful for describing the different processing stages (e.g. pasteurization, concentration, homogenization, spray drying, fluidized bed drying and cooling; Figure 1.11).

Other Food Constituents

2.1. Carbohydrates

Carbohydrates are abundant in nature. They can be defined as polyhydroxy aldehydes or polyhydroxy ketones, or compounds thereof. From a biochemical point of view, a distinction is made, on the one hand, between monosaccharides, or carbohydrate monomers such as ribose, glucose, fructose or galactose, and, on the other hand, oligosaccharides and polysaccharides, which are the condensation products of these monomers. Oligosaccharides contain a limited number of monomers (fewer than 10), which may be identical (as with maltose, a dimer of D-glucose) or different (as with both lactose, a dimer of D-galactose and D-glucose, and sucrose, a dimer of D-glucose and D-fructose). Polysaccharides are polymers that may consist of a very large number of identical monomers, in the case of starch, glycogen and cellulose, or different monomers, in the case of alginates, carrageenan, xanthan gum, etc. Carbohydrates play an important role in our diet. They play:

- A nutritional role by providing energy (16.7 kJ g^{-1}). In a normal diet, carbohydrates account for 55% of energy intake. In addition, some non-digestible polysaccharides are classified as dietary fibers; they have a role to play in intestinal transit.

- An organoleptic role by contributing to the food texture, color (non-enzymatic browning) and flavor. Sweetness is one of the four basic tastes. It is one of the characteristics of non-macromolecular carbohydrates. The sweet taste varies in intensity depending on the carbohydrate. For measuring

Chapter written by Thomas CROGUENNEC.

this intensity, the reference is sucrose, which is assigned a sweetening power of 1 (Table 2.1).

– A technological role as a preservative by their capacity to reduce water availability, but also as a coloring agent in baking (browning) and coating (sugar icing).

Carbohydrate	Relative sweetening power
Sucrose	1.0 (Standard)
Fructose	1.3
Xylitol	1.0
Maltitol	0.8
Glucose	0.7
Mannitol	0.7
Sorbitol	0.5
Maltose	0.5
Isomaltol	0.5
Lactitol	0.4
Lactose	0.3

Table 2.1. *Sweetening power of some sugars*

2.1.1. Structure of carbohydrates

Monosaccharides have a limited number of carbon atoms (usually five or six), a reducing group (aldehyde or ketone) and hydroxyl groups whose configuration is responsible for the optical properties of sugars (deviation of the plane of polarization of light). In the Fischer projection, carbons are numbered in ascending order from the aldehyde group for aldoses or the

primary alcohol group next to the ketone group for ketones (Figure 2.1). Moreover, it is the position of the hydroxyl group at the asymmetric carbon furthest from the reducing group, which determines whether it belongs to the D series (hydroxyl group on the right) or the L series (hydroxyl group on the left).

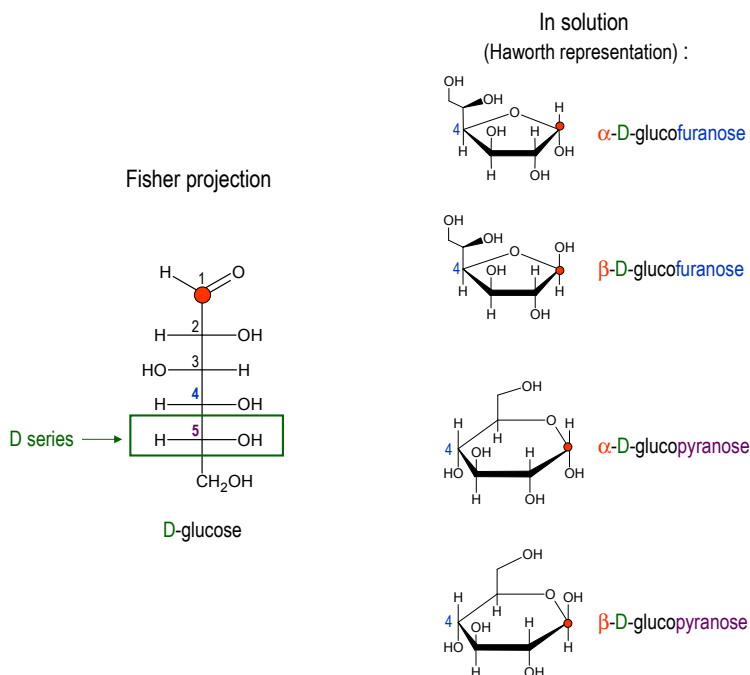


Figure 2.1. Chemical structure of D-glucose

In solution, the carbonyl group of monosaccharides is the site of an intramolecular nucleophilic attack by hydroxyl groups of carbons 4 or 5 (aldoses) or carbons 5 or 6 (ketones). This results in the formation of five-membered cyclic hemiacetals (furanose) or six-membered cyclic hemiacetals (pyranose). During cyclization, the carbon atom of the carbonyl group becomes asymmetric (anomeric carbon). Depending on the configuration of the substituents carried by the anomeric carbon, two isomers α or β (anomers) are obtained (Figure 2.1). Thus, in a monosaccharide solution at

equilibrium, cyclic molecules coexist as a five-membered ring (furanose) or six-membered ring system (pyranose) in α or β form and linear chains. In general, furanose anomers are preferred from a kinetic point of view, whereas pyranose anomers are preferred from a thermodynamic point of view; the latter are more abundant at equilibrium. Only a very small fraction of molecules are in linear configuration.

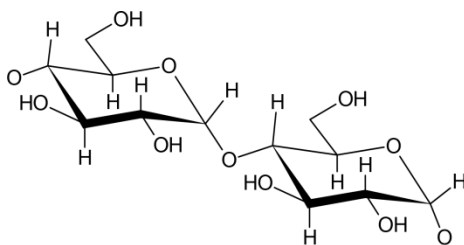
For oligosaccharides or polysaccharides, the various constituent monosaccharides are linked by osidic bond. This bond results from the condensation of the hydroxyl group from the anomeric carbon of one monosaccharide and a hydroxyl group from another monosaccharide or the condensation of the hydroxyl group from the anomeric carbon of two monosaccharides (in the case of certain disaccharides such as sucrose). The formation of the osidic bond traps the configuration of the anomeric carbon involved in the bond and removes the reducing group of the monosaccharide. Thus, lactose, which is a dimer of D-galactose and D-glucose (β -D-galactopyranosyl (1-4) D-glucopyranose [α,β]), has a single reducing group carried by a D-glucose residue. The configuration of the anomeric carbon of D-glucose defines the α or β nature of lactose (Figure 2.1). In contrast, sucrose is a dimer of D-glucose and D-fructose linked together by their reducing group (α -D-glucopyranosyl (1-2) β -D-fructofuranose). Thus, sucrose has no reducing power and cannot participate in non-enzymatic browning unless it is hydrolyzed.

In polysaccharides, the reducing group of the different monosaccharides is engaged in the osidic bond; they contain a maximum of one reducing group at the end of their hydrocarbon chain. For example, starches are made up of linear chains of α -D-glucopyranose linked by α (1-4) bonds in the case of amylose (Figure 2.2), on which there are branched chains linked by α (1-6) bonds in the case of amylopectin. Amylose and amylopectin are hydrolyzed by α -amylases, which exist in animals, plants and microorganisms. Cellulose results from the condensation of β -D-glucopyranose by β (1-4) bonds. Cellulose cannot be hydrolyzed in humans who do not exhibit cellulase activity (Figure 2.2).

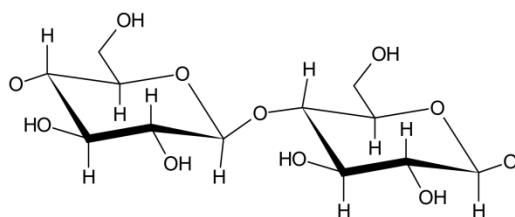
2.1.2. Carbohydrates in solution

In this section, we will only cover the most common simple sugars in the agri-food industry, which are mainly monosaccharides (glucose and

fructose) and disaccharides (lactose, sucrose and maltose). Sugars in solution are the site of complex interactions with surrounding molecules (water, other carbohydrates, proteins, etc.). They can have various conformations and configurations depending on their nature, concentration, temperature and pH.



Amylose: chain of α -D-glucopyranoses linked by $\alpha(1-4)$ bonds



Cellulose : chain of β -D-glucopyranoses linked by $\beta(1-4)$ bonds

Figure 2.2. Chemical structure of amylose and cellulose

2.1.2.1. Mutarotation

The anomeric carbon configuration of the reducing sugars is not stable. It can change quite quickly from the α to the β configuration, which is known as mutarotation. Polarimetry can then be used to follow mutarotation since each anomer has a particular ability to change the optical rotatory power. For example, α lactose has a specific rotatory power of $+89.4^\circ$, which is different from that of β lactose at $+35^\circ$. However, in solution, each form undergoes mutarotation and the specific rotatory power of the lactose

solution at equilibrium tends to be around $+55.4^\circ$ at 20°C . Thus, a lactose solution at equilibrium at 20°C contains 37.3% α lactose and 62.7% β lactose regardless of the initial composition of the mixture. The distribution of anomers at equilibrium varies from one sugar to another. At equilibrium and at 20°C , the proportions of D-glucose in α and β configuration are 36.2 and 63.8%, respectively. In the case of D-lactose and D-glucose, measuring the proportions of each anomer by polarimetry is relatively easy because the pyranose rings are the only cyclic forms in solution. For D-fructose, equilibria are more complex due to the additional presence of the furanose rings, making it more difficult to determine the proportion of each anomer. At equilibrium at 20°C , D-fructose has the following composition: 3% α -pyranose, 69.6% β -pyranose, 5.7% α -furanose and 21.1% β -furanose.

The rate of mutarotation of reducing sugars increases with temperature, and equilibrium can be reached faster. At equilibrium, the temperature also influences the proportions of different anomers. In the case of lactose, the proportion of the α anomer increases with temperature to the detriment of the β anomer. Moreover, hemiacetals are relatively stable in dilute acid; the rate of mutarotation is relatively constant between pH 2 and 7, but increases sharply either side of this pH range. However, the change in pH does not affect the distribution of anomers at equilibrium. The rate of mutarotation and the proportions at equilibrium of the different anomers also depend on the sugar concentration, ionic strength and viscosity of the medium.

The mutarotation requires the sugar chain to open. In solution, the proportion of linear chain is very small. It is the reactive form of the sugar molecule, especially for non-enzymatic browning reactions resulting from a nucleophilic attack of amine groups on the carbon of the reducing group.

2.1.2.2. *Solubility of sugars*

When there is no difference in chemical potential between sugar in solution (liquid phase) and sugar in crystal form (solid phase), the two phases are in equilibrium; the concentration of sugar in solution at equilibrium is therefore defined as the solubility limit (maximum solubility or saturation). The solubility limit of sugars varies depending on their nature and temperature (Figure 2.3).

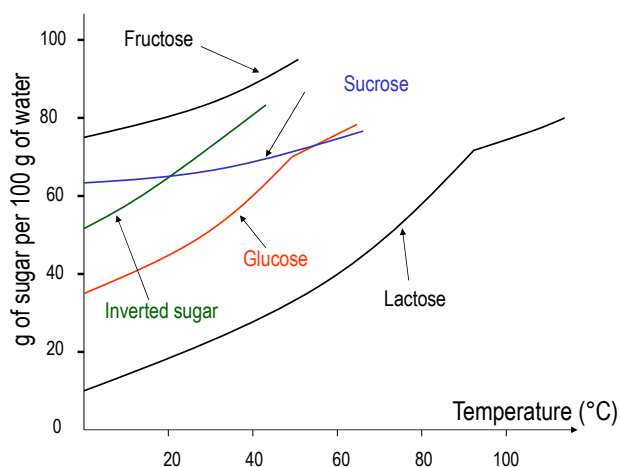


Figure 2.3. *Solubility of some sugars*

D-fructose has a higher solubility limit (80% at 20°C), much higher than that of lactose, which is less soluble (18% at 20°C). The solubility limit of sugars generally increases with temperature. Discontinuities in the solubility limit curves of reducing sugars result from differences in the solubility limit of the α and β anomers and their specific change according to temperature. For example, α lactose is less soluble than β lactose below 93.5°C, but becomes more soluble above this temperature. Thus, at a temperature below 93.5°C, the solubility limit of lactose depends on the solubility limit of α lactose, whereas above 93.5°C it depends on the solubility limit of β lactose.

When the sugar concentration is below the solubility limit, the solution is unsaturated; when it exceeds the solubility limit, the solution is supersaturated. Sugar crystals added to an unsaturated solution dissolve until the solubility limit is reached. However, if added in a supersaturated sugar solution, sugar crystals grow until the concentration of sugar in solution reaches the solubility limit. In a saturated solution at equilibrium, the addition of sugar crystals does not result in further dissolution and crystallization.

2.1.2.3. Crystallization

Depending on the conditions, crystallization may occur in supersaturated sugar solutions, the control of which is crucial in food products since it

impacts the texture. Schematically, sugar crystallization involves two stages: a nucleation stage in which nuclei (seed crystals) are formed and a crystal growth stage. Crystallization is an exothermic reaction.

In a supersaturated solution, molecules are in constant collision and form reversible aggregates (embryos). From a thermodynamic perspective, only embryos that can surmount an energy barrier (ΔG_c), associated with a critical size (r_c), can grow to form a new phase; they are called nuclei. Embryos below the critical size will dissociate (Figure 2.4).

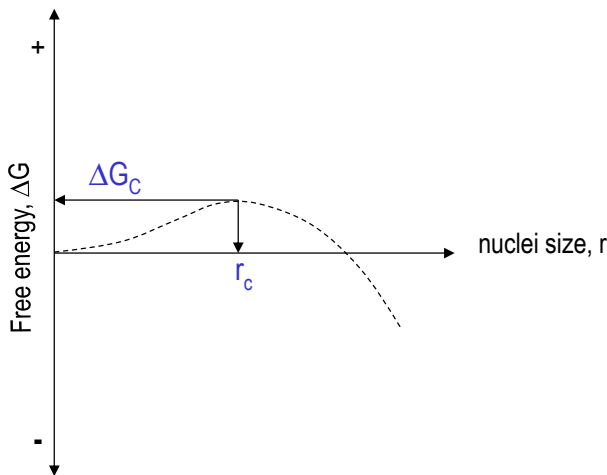


Figure 2.4. Variation in free energy depending on the size of the nuclei

This energy barrier explains why it is easy to concentrate a sugar solution beyond saturation without crystallization occurring. The more supersaturation increases (by concentrating a sugar solution or cooling), the more likely it is that embryos will reach the critical size and nuclei will form. In other words, spontaneous nucleation in a supersaturated sugar solution will only occur above a critical supersaturation concentration. The concentration range between the solubility limit (saturation) and the critical supersaturation concentration is defined as the metastable zone. In this zone, sugar crystallization only occurs if seed crystals are added (fine sugar crystals). The range of the metastable zone varies depending on the

temperature, agitation of the medium or the presence of impurities. The labile zone is defined as the concentration range within which nucleation spontaneously occurs (above the critical supersaturation concentration; Figure 2.5).

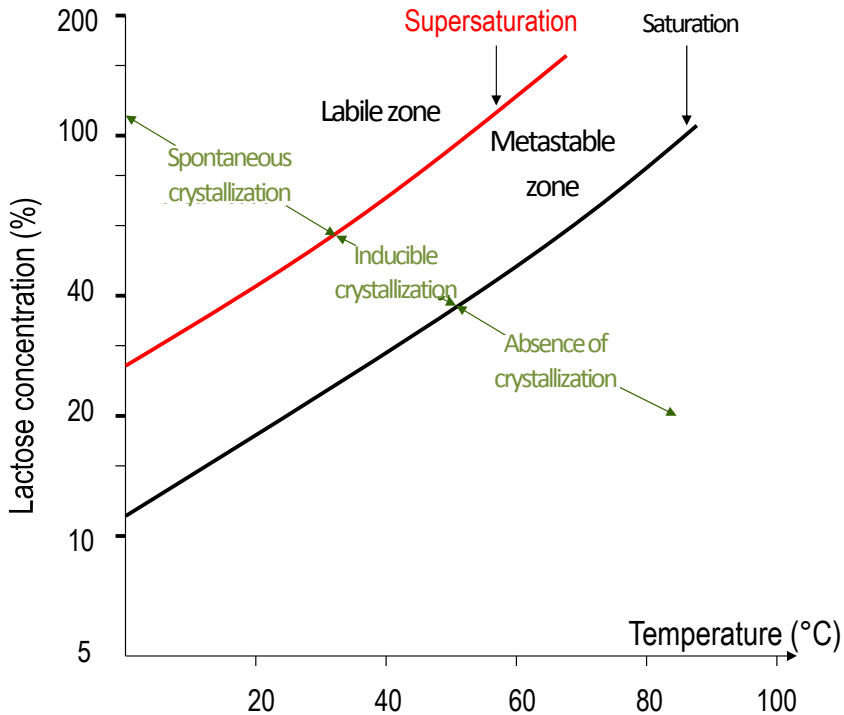


Figure 2.5. Lactose saturation and supersaturation curves as a function of temperature

Crystal growth occurs when sugar molecules are incorporated into the surface of the sugar crystal. They have to diffuse from the solution to the boundary layer surrounding the crystal. The rate of incorporation of new sugar molecules to growing crystals depends on many factors, the most important of which are supersaturation, the crystal interface area and the viscosity of the medium. However, a rapid concentration of the sugar solution far beyond supersaturation (by freezing or evaporation) can prevent crystallization by considerably limiting the molecular mobility necessary for nucleation and seed crystal growth.

2.2. Proteins

Proteins are linear chains of amino acids whose sequence is determined by the genetic code. They differ in their amino acid sequence and three-dimensional structure (globular proteins, fibrous proteins and supramolecular organization), as well as their function. Some are storage proteins while others have different biological functions within organisms (e.g. enzymes). The main dietary functions of proteins are:

- nutritional, by providing essential amino acids and biologically-active peptides;
- sensory, by contributing to food color (non-enzymatic browning), texture (e.g. water retention capacity, gelling, foaming and emulsifying properties), flavor (bitter or sweet amino acids and peptides) or aroma (vector of aromatic molecules or aroma precursors).

2.2.1. Structure of proteins

Proteins have several levels of organization:

- The primary structure corresponds to the sequence of amino acid residues constituting the protein. Traditionally, the numbering of amino acids starts at the N-terminus and finishes at the C-terminus. Acid hydrolysis of proteins generally results in 20 amino acids (building units of proteins), which differ in the nature of the side chain carried by the α -amino carbon; some are non-polar while others are polar, neutral or charged. The isoelectric point of the protein depends on the charge of the side chains of the constituent amino acids, which is defined as the pH at which the same number of positive and negative charges exist on the protein. There are 19 different primary amino acids and one secondary cyclic amino acid called proline. Apart from glycine, which has no asymmetric carbon, all amino acids are naturally in the L configuration. The different amino acids in a protein are linked together by a covalent bond, known as a peptide bond, to form an unbranched polymer called a polypeptide chain. The formation of peptide bonds results from the elimination of a H_2O molecule between the α -carboxylic group of an amino acid and the α -amino group of the next amino acid in the primary sequence. The six atoms in the peptide bond are in the same plane (pseudo-double bond) and the α carbons are in

trans-configuration (Figure 2.6). The free rotation of the peptide bond requires a consistent energy input.

– The secondary structure of a protein corresponds to the spatial arrangement of the polypeptide chain. Since the peptide bond is planar, the polypeptide chain only has two degrees of freedom per amino acid residue around the bonds involving the α carbon. Thus, the polypeptide chain can only fold by rotations around these bonds. Theoretically, bonds involving the α carbon have complete freedom of rotation. In reality, the side chains are too bulky to allow for a complete rotation. As a result, the possible conformations for the polypeptide chain are limited. The only repetitive secondary structures found in proteins are β sheets and α helices (Figure 2.6). The β sheet structure is characterized by a repetitive network of hydrogen bonds between two relatively distant parts of the polypeptide chain, while α helices are stabilized by hydrogen bonds involving groups close to each other in the primary sequence. In addition, proteins have many regions of non-repetitive, but ordered, structure: this is the case for turns.

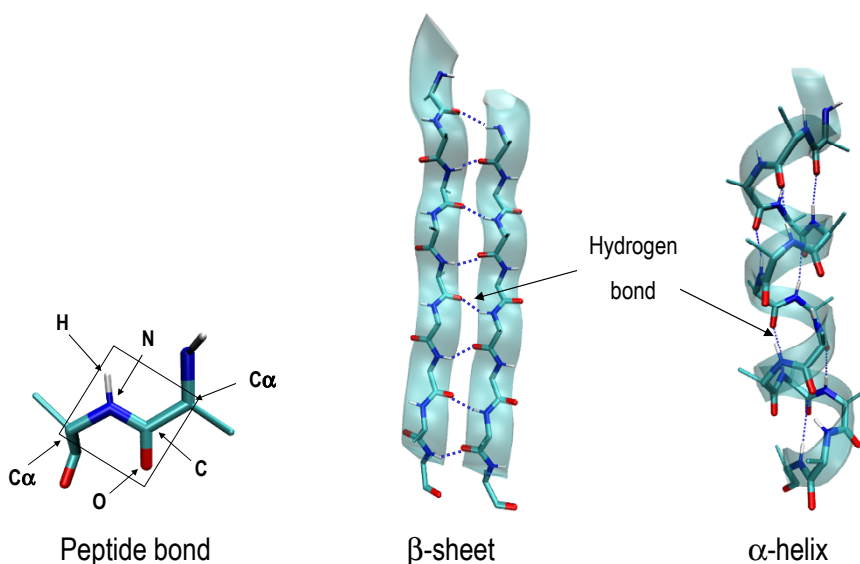


Figure 2.6. Representation of a peptide bond and secondary structures of proteins

– The tertiary structure corresponds to the three-dimensional organization of the polypeptide chain containing regions of well-defined secondary structures (e.g. α helices, β sheets and turns) or less well-defined structures (e.g. random coil). In aqueous solution, the driving forces for adopting the tertiary structure are hydrophobic: hydrophobic groups withdraw from the aqueous environment, which reduces the free energy of the protein. Interactions between protein fragments by hydrogen bonds, electrostatic bonds (ion–ion, ion–dipole, dipole–dipole), salt bridges or disulphide bridges stabilize the final structure. They are promoted by the conformation imposed by the hydrophobic associations and decrease the conformational entropy of proteins. They improve the stability of the native protein structure and make it more resistant to unfolding or denaturation.

– The quaternary structure of proteins results from the association of two or more polypeptide chains, also called “sub-units”. These sub-units can be identical or different, and their arrangement is not necessarily symmetrical. The forces or bonds that stabilize the protein quaternary structure are the same as those that stabilize the protein tertiary structures.

In solution, a protein is seen as a dynamic entity that constantly adopts a large variety of almost similar structures. The native structure of protein is defined as having the lowest free energy or the highest probability.

2.2.2. Solubility of proteins

Solubility is often a prerequisite for the performance of proteins in food systems. In order to have a technological (e.g. gelling, emulsifying and foaming properties) or biological functionality, a protein may need to change its structure so that it can interact with other components in the system. For the biological functions of proteins (transport or protection role), the mere presence of other molecules (ligands) in the system will allow interaction. However, external energy input (heating, shaking, etc.) is often required to activate the gelling, emulsifying and foaming properties of proteins, which involves a number of interactions between proteins, water molecules, fat and gas.

The solubility of a protein in a given medium is the thermodynamic manifestation of the interaction balance between protein molecules, as well as protein molecules and solvent molecules. A protein can be seen as a

polyelectrolyte surrounded by a double layer of water molecules and counter-ions. The double layer is characterized by a potential gradient due to the presence of charged groups on the protein surface (Figure 2.7).

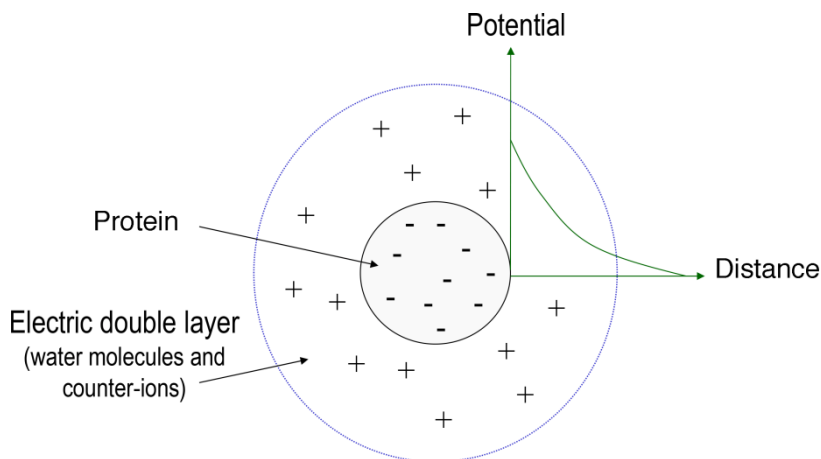


Figure 2.7. *Schematic representation of a protein in aqueous solution (pH above the isoelectric point of the protein)*

The hydration and charges carried by the amino acid side chains on the surface of the protein constitute repulsive interactions, acting against protein–protein associations. Conversely, hydrophobic amino acids on the surface of the protein constitute attractive interactions and favor protein–protein associations. In aqueous solution, these amino acids naturally withdraw from the surface of the protein; however, in proteins with greater than 30% hydrophobic amino acids, they cannot be completely embedded in the core of the protein structure due to steric requirements; therefore, some of them are exposed on the protein surface. For such proteins, solubility decreases if the attractive interactions counterbalance or are greater than the repulsive interactions.

The dielectric constant, ionic strength, pH and temperature all affect the solubility of proteins by changing their charge, hydration and the quantity of hydrophobic amino acids exposed on the protein surface.

– Generally, proteins have a minimum solubility at their isoelectric point (Figure 2.8). A change in pH toward the isoelectric point of the protein reduces its solubility mainly by lowering its surface charge and hydration,

but can also alter the conformation of the protein. Some globular proteins maintain a high solubility level regardless of the pH value.

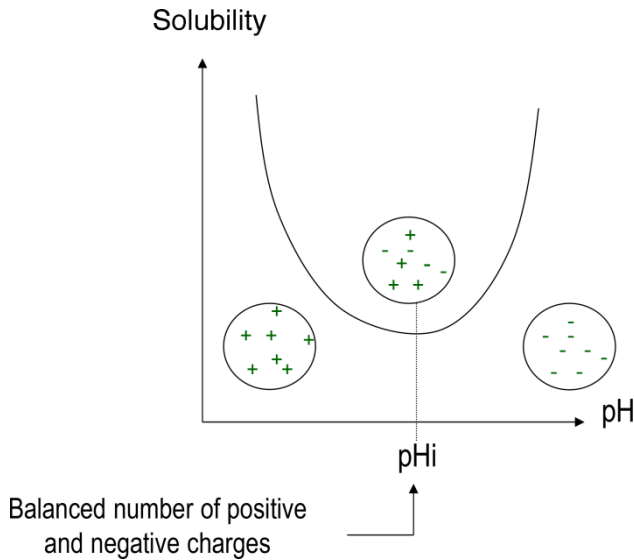


Figure 2.8. *Effect of pH on protein solubility*

– The effect of ionic strength on the solubility of proteins can be divided into two phases (Figure 2.9). For low salt concentrations, an increase in ionic strength enhances the surface charge of the protein, thereby increasing its surface potential and hydration: protein solubility increases. This effect is known as the salting-in effect. For higher values of ionic strength, the surface charges of the protein are shielded and protein hydration decreases: protein solubility decreases. This effect is known as the salting-out effect.

– A moderate increase in temperature, up to around 40°C, improves the solubility of proteins. Higher temperatures (>60–70°C) cause denaturation and exposure of hydrophobic residues originally buried inside the core of the protein structure. Therefore, protein solubility decreases sharply by increasing the attractive interactions between proteins.

– Modifying the dielectric constant of the medium (e.g. the presence of alcohol) decreases protein hydration and changes its conformation by weakening the hydrophobic associations, which are the protein stabilizing properties. The result is a reduction in solubility.

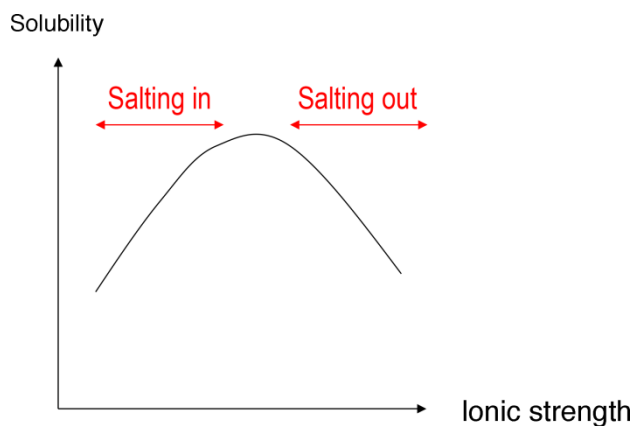


Figure 2.9. *Effect of ionic strength on protein solubility*

2.3. Lipids

More than 50% of lipids are consumed as “hidden” lipids in milk, eggs, meat, certain fruits like olives or avocados, hazelnuts, walnuts, etc. The rest are “visible”, and are found in processed foods such as butter, lard, fish oils, vegetable oils and their transformation products such as margarines, emulsified sauces, or lipid fractions with a controlled melting point suitable for industrial use (pastries, biscuits, etc.). Lipids, whether “hidden” or “visible”, play an important role in the diet and the agri-food industry. They have:

- a nutritional role by providing energy (37.7 kJ g^{-1}), essential fatty acids (e.g. linoleic acid and α -linolenic acid), fat-soluble vitamins (e.g. vitamins A, D, E and K), phytosterols and antioxidants;
- a sensory role by contributing to the food texture and flavor as carriers of aroma or aromatic precursors;
- a technological role as a heat transfer fluid (frying) or as a surface treatment agent (processing aid).

2.3.1. Composition of the lipid fraction

Lipids include a large variety of naturally occurring molecules that share a common property: they are insoluble in water and soluble in organic

solvents (ether, acetone, hexane, benzene, etc.). They can be divided into two categories:

- “saponifiable” lipids comprising neutral lipids (acylglycerols), phospholipids, waxes (esters made of fatty acids and long-chain alcohols such as those found in spermaceti and beeswax, etc.) and cutins (condensation polymers of long-chain hydroxy acids found on the surface of plant leaves);

- “non-saponifiable” lipids comprising hydrocarbons, pigments, sterols and fat soluble vitamins.

2.3.1.1. *Neutral lipids*

Biologically, neutral lipids are storage lipids, storing energy in living matter. Quantitatively, neutral lipids are predominantly composed of triacylglycerols. They are composed of a glycerol molecule where each of the three hydroxyl groups is esterified by a fatty acid molecule (Figure 2.10).

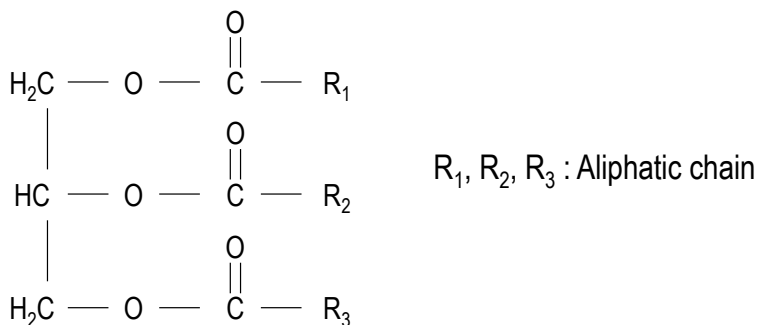


Figure 2.10. *Chemical structure of triacylglycerols*

Fatty acids consist of a carboxylic group and an aliphatic chain that can vary in length from 4 to 30 carbon atoms (usually 12–24 carbon atoms). The aliphatic chain is typically unsubstituted and has an even number of carbon atoms resulting from the condensation of acetate units during biosynthesis. The aliphatic chain can be saturated and linear or can possess one or more double bonds usually separated by a methylene group. As outlined later (Chapter 4), the methylene group plays an important role in the initiation of

lipid oxidation. Double bonds are generally in *cis*-conformation creating kinks in the aliphatic chains.

Exceptionally, fatty acids can contain conjugated double bonds, a double bond in *trans*-conformation, an uneven number of carbons, a methyl or a hydroxyl group: certain fatty acids of this type can be found in the milk fat and are produced in the rumen of cows by bacterial flora.

In the nomenclature of fatty acids, carbon acids are numbered from 1 to *n* from the carboxyl group to the methyl end. However, it is possible to follow the order of condensation of acetate units in the biosynthesis of fatty acid to identify carbon atoms on the aliphatic chain (Figure 2.11). Carbon in the 12th position on a chain of 18 carbon atoms is labeled *n*-6 (in this example: $18 - 6 = 12$).

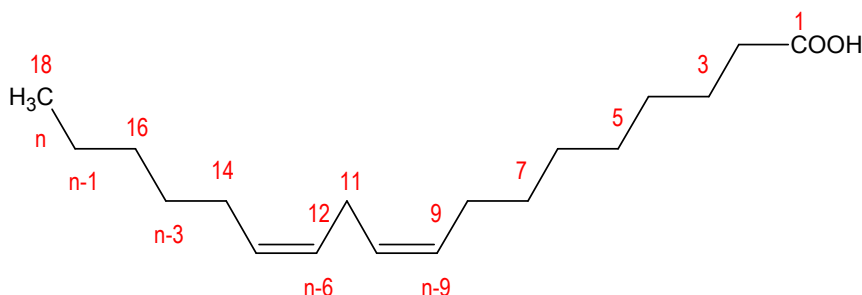
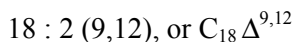
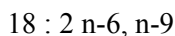


Figure 2.11. Structure and nomenclature of linoleic acid

The number of double bonds, together with the number corresponding to the position of the first carbon atom in the double bond (shown in brackets), gives the full name of the fatty acid. According to this nomenclature, linoleic acid, which is a chain of 18 carbon atoms with two double bonds (Δ) between carbons 9 and 10 and between carbons 12 and 13, is written as follows:



The double bonds can also be identified from the methyl end. In this case, linoleic acid would be written as:



It belongs to the $\omega 6$ family because the first double bond, starting from the methyl end, is located on the 6th carbon. α -linolenic acid belongs to the $\omega 3$ family.

Table 2.2 presents the common and symbolic names of the main natural fatty acids.

Common name	Symbolic name	Common name	Symbolic name
Butyric	4:0	Palmitoleic	16:1 (9)
Caproic	6:0	Oleic	18 : 1 (9)
Caprylic	8:0	Erucic	22:1 (13)
Capric	10:0	Nervonic	24:1 (15)
Lauric	12:0	Linoleic	18:2 (9, 15)
Myristic	14:0	α -Linolenic	18:3 (9, 12, 15)
Palmitic	16:0	γ -Linolenic	18:3 (6, 9, 12)
Stearic	18:0	Arachidonic	20:4 (5, 8, 11, 14)
Arachidic	20:0	Eicosapentaenoic (EPA)	20:5 (5, 8, 11, 14, 17)
Behenic	22:0	Docosahexaenoic (DHA)	22:6 (4, 7, 10, 13, 16, 19)
Lignoceric	24:0		

Table 2.2. *Common and symbolic names of the main natural fatty acids*

Of these fatty acids, linoleic acid and α -linolenic acid are essential for humans. They cannot be synthesized in the human body and therefore need to be included in the diet. The reason for this is that desaturases

(specific enzymes), allowing double bonds to be located beyond carbon 9, do not exist in human cells.

Given the diversity of fatty acids and the three esterification sites on the glycerol molecule, there are thousands of different triacylglycerols. In nature, esterification of glycerol with fatty acids results from biological processes and does not reflect a random distribution. The position of fatty acids on the glycerol molecule determines their intestinal absorption. While fatty acids in position 2 are absorbed as monoacylglycerols, fatty acids on either side (positions 1 and 3) of the triacylglycerol, released by pancreatic lipases, cross the intestinal barrier or are excreted as fatty acid salts.

Diacylglycerols and monoacylglycerols are usually found in small quantities in natural oils and fats. They are usually products of triacylglycerol degradation by lipases or simple chemical hydrolysis. They are widely used as emulsifying agents in food applications.

2.3.1.2. Phospholipids

Phospholipids are amphiphilic molecules consisting of two families of compounds: phosphoglycerides and sphingolipids. The first group derives from phosphatidic acid, which is a diacylglycerol whose hydroxyl group in position 3 is esterified by phosphoric acid (Figure 2.12). The second acid group of phosphoric acid is esterified by a nitrogen derivative (choline, ethanolamine or an amino acid like serine), inositol or another glycerol molecule.

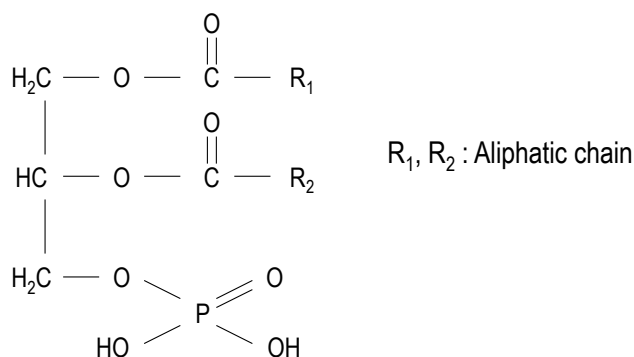


Figure 2.12. Chemical formula of phosphatidic acid

Sphingolipids are composed of one sphingosine molecule (amino alcohol with 18 carbon atoms having one double bond, two hydroxyl groups in positions 1 and 3, and an amino group in position 2) attached to which are a fatty acid in position 2 by an amide bond and phosphorylcholine or phosphorylethanolamine (sphingophospholipids or sphingomyelins) or carbohydrate residues (sphingoglycolipids) in position 1.

Phospholipids are important compounds of biological membranes. Due to their surface-active property, phospholipids are used as emulsifiers in the food industry. Their structure (high proportion of unsaturated fatty acids) and location (fixed at the interface directly in contact with the aqueous phase) make them particularly important for oxidation reactions and lipase action.

2.3.1.3. “Unsaponifiable” lipids

Generally, less than 10% of the lipid fraction is unsaponifiable. Molecules in this fraction have a very different chemical origin but have the common property of being resistant to alkaline treatment. According to their chemical origin, they can be classified into isoprene lipids, eicosanoid lipids and hydrocarbons. Isoprene lipids contain:

- isoprene hydrocarbons from the polymerization of isoprene units;
- sterols including cholesterol and phytosterols as well as sterol derivatives, some of which include bile salts, vitamin D and alkaloids;
- carotenoids with carotenes, xanthophylls, vitamin A;
- isoprenoid quinones including vitamins E and K.

Eicosanoid lipids derive from arachidonic acid including:

- prostaglandins;
- prostacyclins;
- thromboxanes;
- leukotrienes.

Hydrocarbons are naturally present in very low concentrations in most living organisms. They can also be produced during the processing or storage of lipids as a result of lipid oxidation.

2.3.2. Thermal properties of lipids

The thermal properties of lipids depend on the chain length and the unsaturation of fatty acid residues as well as the structure of triacylglycerols. These parameters determine the melting and crystallization properties of lipids, which are important in terms of the sensory perception of food.

2.3.2.1. Melting properties

Traditionally, oils and fats have been differentiated according to their melting properties. Oils are lipids that are liquid at room temperature (25°C): this is the case for most vegetable oils (soybean, sunflower, rapeseed, peanut, olive, etc.) and fish oils. Fats are solid at room temperature (25°C), such as goose fat, pork fat (lard) and beef fat (tallow); however, some vegetable fats are wrongly referred to as “oils” (e.g. palm oil).

The melting properties of triacylglycerols depend primarily on the nature of their constituent fatty acids. Triacylglycerols composed of saturated fatty acids can easily aggregate. They form compact crystalline structures held in place by van der Waals bonds. These bonds have very little energy but are sufficient to keep long-chain fatty acids associated with one another at room temperature. Thus, lipid fractions rich in saturated fatty acids are solid at room temperature and require energy input (heat) to become liquid. The presence of short-chain fatty acids reduces the number of bonds that may form between fatty acids, which tends to lower their melting point. Similarly, the presence of unsaturated fatty acids in triacylglycerols creates bends in the aliphatic chain, which interfere with the formation of compact crystalline structures. They hinder the alignment of triacylglycerols making crystal formation more difficult and thereby lowering their melting point. Lipid fractions rich in short-chain and/or unsaturated fatty acids are liquid at room temperature.

Furthermore, natural lipids contain a large variety of triacylglycerols. As a result, they never have a specific melting point but rather a melting range, which can be identified on the basis of the melting curve (Figure 2.13). The slope of the curve depends on the composition of triacylglycerols. At room temperature, triacylglycerols with a high melting point exist as several solid crystals suspended in liquid triacylglycerols. The presence of solid crystals gives solid fats their plasticity behavior. A lipid composed of only one triacylglycerol is either solid or liquid.

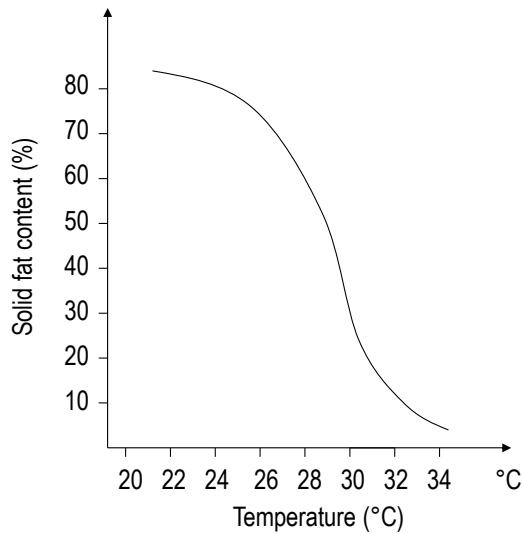


Figure 2.13. Representation of the melting curve of a fat

The melting of fat crystals is an endothermic reaction. When eating, the energy required to melt fat crystals comes from the mouth, giving a slight impression of freshness. The faster the crystal melting rate is, the stronger the impression will be. This is the case for cocoa butter triacylglycerols, whose melting points are limited to a very narrow temperature range corresponding to a very steep melting curve at approximately 30°C. These melting properties are responsible for the brittle texture of chocolate at room temperature and the fact that it melts in the mouth. Moreover, the flavors trapped in the fat are released during the phase transition, which adds to the pleasure of eating chocolate.

2.3.2.2. Crystallization behavior

Controlling the crystallization properties of lipids has two major implications for industry: first, the desired sensory properties can be achieved for many food products and, second, specific lipid fractions can be isolated from natural animal or plant sources containing lipids with varying chemical and physical properties.

In addition to the percentage of solid fat, the organization of aliphatic fatty acid chains, i.e. polymorphism, and crystal size, determine the melting

and rheological properties of lipids. Polymorphism occurs when the same set of molecules is able to organize itself in different ways by changing the operating conditions during crystallization. In fat crystals, the organization of triacylglycerols can be transversal or longitudinal:

– The transversal organization of fatty acid chains occurs in crystalline subcells. The three most common subcells are α , β' and β in the order of increasing stability, compactness and melting point (Table 2.3). They correspond to the hexagonal, orthorhombic perpendicular ($O\perp$) and triclinic parallel ($T//$) organizations (Figure 2.14).

	Crystalline forms		
	α	β'	β
Trilaurin	15	35	46
Trimyristin	33	46	57
Tripalmitin	45	56	65
Tristearin	54	65	71

Table 2.3. *Melting point (°C) of crystalline forms of some triglycerides*

– The longitudinal organization of triacylglycerols results from their molecular stacking. The thickness of the layers depends on the number of aliphatic chains stacked in each layer. They are generally type 2L or 3L (with 2 or 3 chain lengths) (Figure 2.14). The longitudinal organization of triacylglycerols depends primarily on the symmetry of the triacylglycerols. Triacylglycerols with a stacking “fault” (fatty acids with one or more double bonds or different chain lengths) tend to form 3L structures, with segregation of the defective chain. Triacylglycerols with fatty acids of similar chain length and the same number of double bonds mainly give crystals with a 2L organization.

The polymorphism of triacylglycerols is governed by thermodynamic and kinetic factors. It is monotropic for the most stable varieties, which means that the crystalline transitions are irreversible and always occur from the least stable crystals (α and β') to the most thermodynamically-stable crystal (β) (Figure 2.15). Moreover, cooling or heating kinetics determine the type of polymorph obtained. In general, rapid cooling produces less stable

crystalline varieties, while slow cooling results in more stable crystalline states.

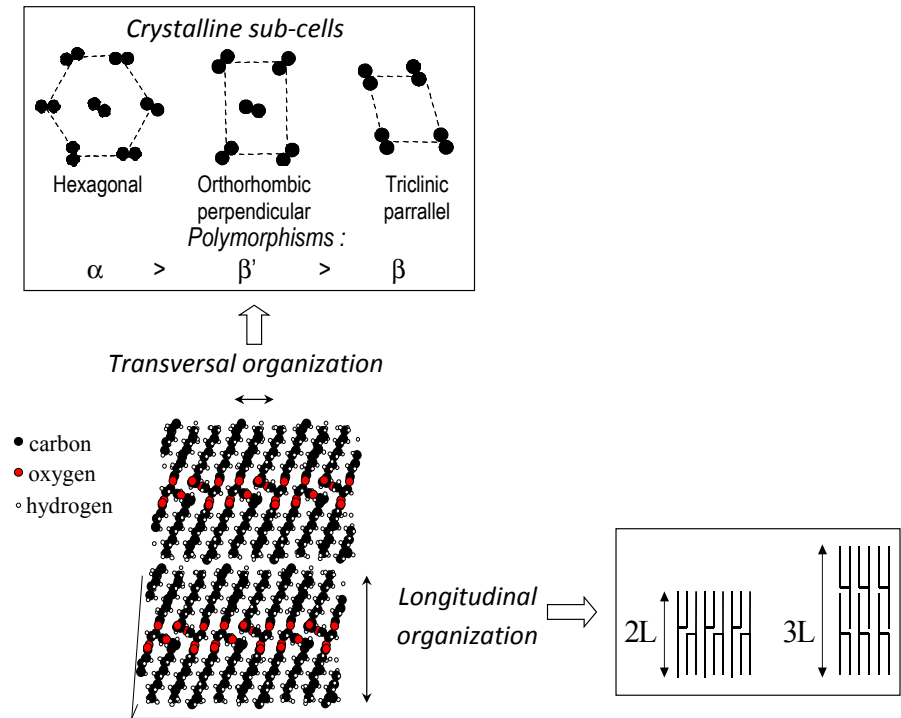


Figure 2.14. Structure of triacylglycerol molecules in longitudinal organization and with lateral stacking (crystal of β -trilaurin)

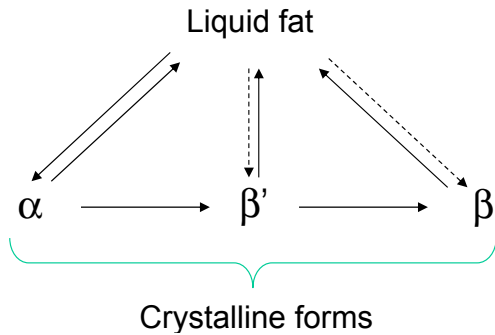


Figure 2.15. Crystallization and growth of fat crystals

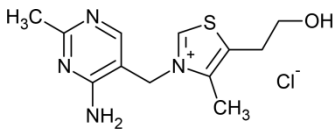
In chocolate-making, the tempering operation converts the crystallization of cocoa butter into the most stable crystalline form (β), which prevents gloss and texture defects during storage. The β crystal, together with the proportion of solid fat, is responsible for the brittle properties of dark chocolate: loud clean crack when broken by the fingers and adequate bite resistance. For other applications (e.g. pastries and ice creams), we have to stabilize the β' crystal, which promotes aeration and improves the smooth, melting sensation in the mouth. The β' crystals can be stabilized by adding certain triacylglycerols (1-palmitoyl, 2-stearoyl, 3-palmitoyl glycerol: PSP; 1-palmitoyl, 2-stearoyl, 3-stearoyl glycerol: PSS), which delay or prevent the conversion into the β crystals.

As with sugars, the crystallization process for lipids can be divided into two stages: a nucleation stage and a crystal growth stage. Nucleation can only occur by overcooling because an energy barrier exists for the formation of a solid phase; the activation energy to induce nucleation inversely decreases with overcooling. With slight overcooling, crystal growth is dominant; whereas substantial overcooling favors nucleation. Thus, when overcooling increases, the average crystal size decreases through an increase in the number of nuclei. The crystal size obtained has a major impact on the texture of lipids. Therefore, by controlling the crystal size, it is possible to standardize the spreadability of butter despite the fact that the fat composition varies during the lactation period.

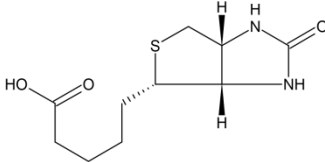
2.4. Vitamins

Vitamins are small molecules essential for various basic metabolic activities in humans, and a deficiency results in specific syndromes, which have often led to their discovery. Even though vitamins are needed in only very small quantities, humans are unable to synthesize sufficient amounts of them, and therefore they need to be incorporated into the diet.

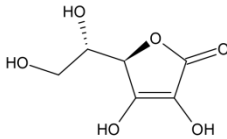
From a chemical point of view, vitamins have very diverse structures resulting in differences in terms of solubility in water and organic solvents. Thus, there are water-soluble vitamins such as vitamins B and C (Figure 2.16) and fat-soluble vitamins such as vitamins A, D, E and K (Figure 2.17). Each vitamin has a specific role and, as a result, they cannot be substituted for each other.



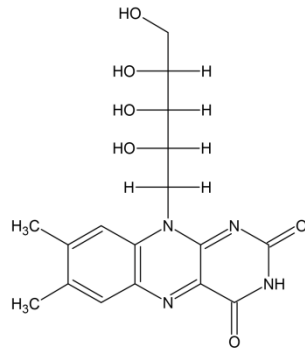
Vitamin B₁ : Thiamin



Vitamin B₇ : Biotin

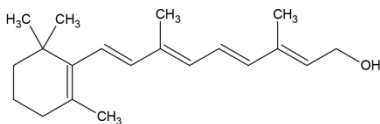


Vitamin C : Ascorbic acid

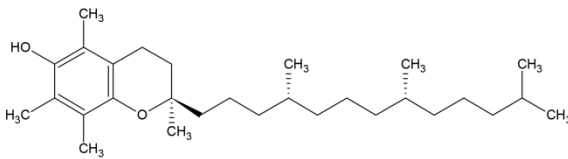


Vitamin B₂ : Riboflavin

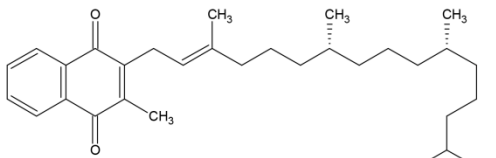
Figure 2.16. Water-soluble vitamins



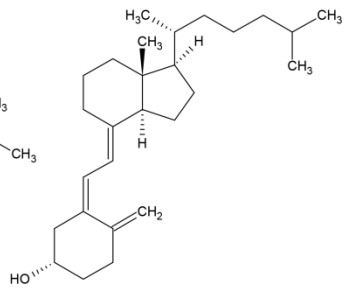
Vitamin A : Retinol



Vitamin E : α-tocopherol



Vitamin K₁ : Phylloquinone



Vitamin D₃ : Tocopherol

Figure 2.17. Fat-soluble vitamins

PART 2

Food Modifying Agents and Mechanisms

Microbial Spoilage

The contamination of food usually occurs on the surface; however, foods of animal origin can be contaminated from within. In most cases, food contamination occurs during the production process.

3.1. Microbial profile of food

3.1.1. *Origin of microorganisms*

3.1.1.1. *Endogenous origin*

The animal organism, like the human organism, carries a large number of microorganisms not only on its surface but also internally on mucous membranes and in the gastrointestinal tract. In fact, all internal surfaces of the animal organism that are in contact with their surrounding environment carry microorganisms and/or are contaminated by them. Thus, microorganisms are present in the entire digestive tract from the oro-pharynx to the anus, as well as on the mucosa of the female genital tract. It is often said that the human, or animal, organism contains more microorganisms than cells. The flora of the digestive tract contains 10^{11} to 10^{12} microorganisms per gram of feces. The dominant flora, at 10^8 microorganisms per gram, includes *Bifidobacterium*, *Peptostreptococcus* and *Bacteroides*, *Eubacterium*; the sub-dominant flora (10^5 microorganisms per gram) comprises *Lactobacillus*

Chapter written by Florence BARON and Michel GAUTIER.

and *Clostridium* and the fluctuating flora (10^5 microorganisms per gram) consists of *Staphylococcus* and *Streptococcus* [STE 80].

The main role of these microorganisms is to occupy space so as to prevent pathogenic microorganisms from invading. They are called saprophytes or commensals, depending on their relationship with the host. We will only refer to healthy animals here, since sick animals are generally expelled from the food chain by the veterinary services. In healthy human or animal organisms, pathogenic microorganisms are usually present, either temporarily or permanently, in relatively limited quantities compared to other so-called harmless microorganisms. They can be implanted in micro-ecological niches and, without actively multiplying, can co-exist with other microorganisms or simply pass into the digestive tract with food. Thus, humans can occasionally host pathogenic bacteria such as *Listeria monocytogenes*, *Salmonella*, *Staphylococcus aureus*, or *Clostridium* (which may survive and remain in the digestive tract) as well as viruses.

Staphylococcus aureus is a special case, since this bacterium's ecological niche is the sebaceous glands of hair follicles and it can therefore be hosted by human or animal skin. This bacterium is, therefore, often found in the oro-pharyngeal area of humans and animals.

These pathogenic microorganisms are generally confined to the mucosa of the digestive tract and, under proper slaughtering conditions, should not, in theory, contaminate the meat. It can happen, however, that under certain conditions they may migrate from the digestive tract or the mucosa to the muscle. This route of infection is called "bacteremia during slaughtering".

The other main routes of endogenous infection are linked to bleeding wounds or delayed evisceration.

Bacteremia during slaughtering [GIL 98]

The passage of microorganisms from the digestive tract to the muscle can occur after digestion. Microorganisms can pass through the intestinal wall into the lymph and blood system. They are usually stopped by the lymph nodes and the liver, which form the mononuclear phagocyte system.

When an animal is slaughtered in a state of stress or fatigue, catecholamines are produced, which paralyse this system, causing

microorganisms that were confined in the lymph nodes and the liver to be released into the blood. These microorganisms can subsequently multiply in the blood, the rate of which depends on the carcass cooling rate [FOU 85].

Microorganisms that contaminate the carcass in this way are mostly eliminated during bleeding, but some can attach to the blood capillaries and multiply. If pig feed was contaminated with *Salmonella*, it could be found inside ham, which proves that microorganisms can pass from the digestive tract to muscle.

Sticking point

The sticking point is the place in the lower part of an animal's neck where the knife is thrust in slaughtering. Strictly speaking, this does not constitute endogenous contamination, since in this case microorganisms originate externally and spread throughout the organism during bleeding. However, it results in contamination of the muscles, with the same consequences as previously mentioned.

During bleeding, microorganisms on poorly cleaned knives or animal hair (fecal soiling around the neck of cattle) can be carried along by the blood flow when the carotid arteries are severed by the operator. These infections can prove extremely dangerous because they potentially involve a large number of pathogenic microorganisms.

Delayed evisceration

Evisceration is considered delayed when it takes place more than 45 minutes after slaughtering, even if it is rare to find microorganisms in the muscles of animals after such a short period of time. Digestive tissues, weakened after the death of the animal, facilitate easy transit of microorganisms from the digestive tract to the muscle tissue. The dangers arising from delayed evisceration are amplified by temperature and the length of time between slaughter and evisceration of the animal.

“Faisandage” (hanging of game birds) involves storing a slaughtered animal that has not been eviscerated for a certain period of time. This practice is still used today, and changes the organoleptic qualities of the meat because of the passage of microorganisms to the muscle.

Other cases of endogenous contamination

Eggs [THA 94, BOA 94]

The content of eggs from healthy hens is usually sterile except in the case of transovarial transmission, which was shown in the case of *Salmonella* Enteritidis. This particular transmission assumes that *Salmonella* bacteria pass into the hen's bloodstream and spread to various organs, in particular the ovaries, with or without pathological manifestation. This type of transmission has been demonstrated by various studies, but transmission by fecal contamination of the shell, followed by penetration of *Salmonella* through the pores of the shell, is the most common. If egg yolks are not cooked at high temperatures, this could result in severe salmonellosis for the consumer.

Milk

Milk can also be contaminated endogenously by bacterial colonies in the udder. When such a colonization occurs, it causes inflammation of the udder known as mastitis, resulting in the excretion of large numbers of bacteria.

Staphylococcus mastitis is the most common. In fact, *S. aureus* is often present on the skin of the cow's udder and can therefore contaminate the teat during milking. There are also forms of mastitis caused by *Listeria monocytogenes* and *Salmonella*, which are less common but potentially extremely dangerous for the consumer.

3.1.1.2. *Exogenous contamination*

Raw food is often contaminated at its surface after production, and processing significantly alters the microbiological flora of the food. Some processes limit the development of microbial flora (freezing, heat treatments), while others create favorable conditions for the growth of certain useful flora (fermentation). Sources of food contamination are extremely diverse. They include the following:

- humans and animals;
- soil, water, air;
- waste and by-products;
- ingredients;

- surfaces and equipment;
- premises.

Humans and animals

Humans can contaminate food either through poor hygienic practices (for example, an operator picking up contaminated fruit from the ground and thereby introducing bacteria into the production chain) or by carrying pathogenic microorganisms.

In general, people suffering from infectious illnesses excrete large amounts of pathogenic microorganisms. Since such illnesses are often debilitating, these people are rarely in contact with food during production. However, it can happen that some pathogeneses, such as infected cuts, are not debilitating enough to prevent this risk. It is, therefore, recommended to limit the work of people who have wounds on their hands in the food production line. Operators working in the food industry are in fact more likely than most to injure themselves: they often use sharp instruments in humid conditions and handle raw materials that carry pathogenic microorganisms. For example, a wound infected with *S. aureus* can host more than 10^9 bacteria. Moreover, humans can be asymptomatic carriers (healthy carriers) of pathogenic microorganisms without developing an infectious disease: there are several pathogenic microorganisms (*L. monocytogenes*, *Salmonella*, *Clostridium perfringens*, etc.) and viruses (hepatitis A) in the human digestive tract. These microorganisms can simply pass through the digestive tract or remain there in equilibrium with the local flora. If humans do not respect basic hygiene principles, they may contaminate their hands through feces by not washing them and consequently contaminate equipment or utensils used in the preparation of food, or even the food itself. Humans can also host pathogenic microorganisms on their skin. Through handling or sneezing, humans can contaminate food with *S. aureus*.

Animals can also be vectors of several pathogenic microorganisms through their uncontrolled presence in establishments. The most common examples are rodents, birds and insects.

Through their excrement, rodents can contaminate stocks of raw materials and food during and after preparation. The fecal flora of rodents can harbor many pathogenic microorganisms such as *Salmonella*. For this

reason, every approved European Union (EU) establishment must have a contingency plan to deal with pests, which is monitored by the veterinary services.

The digestive flora of birds, which is very different to that of mammals, can harbor large numbers of microorganisms that are pathogenic to humans but harmless to birds. This is the case, for example, with *Campylobacter* and *Salmonella*, which are often found in bird droppings.

Insects, which are much more difficult to eradicate than rodents and birds, are also responsible for a considerable amount of contamination. In fact, they can spread several microorganisms on food through contact with their legs, after having landed on decaying matter or excrement. It is therefore necessary to store waste as far away as possible from production lines or have systems in place to confine it. Finally, it is not uncommon in some small companies to find pet cats or dogs who, through dirty hair and fur, can also be the vectors of pathogenic microorganisms.

Soil

The amount of microorganisms in soil varies greatly depending on the richness of organic matter. Up to 10^{10} microorganisms per gram can be found in fertile humus.

Microorganisms are mainly present in the surface layer, which is rich in decaying animal and plant matter. However, soil is not a favorable environment for the survival of microorganisms since the physicochemical conditions (temperature, surface water content) can vary considerably and quite rapidly. As a result, the soil flora contains microorganisms that are relatively resistant to changes in environmental conditions. There are more Gram-positive than Gram-negative bacteria, since the cell walls of Gram-positive bacteria are rich in peptidoglycans, making them more resistant.

The soil also contains microorganisms responsible for food fermentation, such as those involved in the production of bread (yeasts), cheeses (lactic bacteria) and fermented beverages, which may thus contaminate the raw materials. Pathogenic bacteria that are not very resistant to environmental media can, however, be transmitted by soil. Hence, soil can contain pathogenic bacteria found in the digestive tract and carried by animal excrement.

Water (Table 3.1)

Water is used in the food industry for food preparation, technological operations and cleaning. The quality level of water depends on its intended use. Water intended for cleaning premises may be of lower microbiological quality than water used in food preparation.

The level of contamination by microorganisms in water depends on its source. If it comes from a water distribution system, contamination depends on the state of the system (pipe maintenance, microbial colonization and the microbial content of the water drawn), the type of wastewater treatment and meteorological conditions. Heavy rain can wash soil into rivers, which can rapidly and significantly increase the microbial content.

Microorganisms	Tap water wells	Bottled water	Mineral water (bottled)
Viable colony count 22°C	< 100/l	< 100/l	< 100/l
Viable colony count 37°C	< 10/ml	< 20/ml	< 20/ml
Coliforms 37°C	0/100 ml (95%)	0/100 ml (95%)	0/250 ml
Coliforms 44°C	0/100 ml	0/100 ml	0/250 ml
Fecal streptococci	0/100 ml	0/100 ml	0/250 ml
ASR streptococci	≤1/20 ml	≤ 1/20 ml	0/250 ml
<i>Salmonella</i>	0/5l	0/5 l	absent
<i>S. aureus</i>	0/100 ml	0/100 ml	absent
<i>P. aeruginosa</i>	–	0/100 ml	0/250 ml
Enterovirus	0 pfu/10 l	0 pfu/10 l	absent
Bacteriophages	0/50 ml	0/50 ml	–

Table 3.1. Microbiological criteria of water for human consumption

Water used in the food industry can also come from the wells drawing from groundwater or catchment sources. In this case, it is necessary to filter the water in order to remove particles of mineral and organic matter. Several stages are sometimes necessary in the filtration process, depending on the degree of contamination of the water. These filtrations do not, however, remove microorganisms, which should be destroyed by ultraviolet radiation; the damage caused to the DNA of the microorganisms kills them. Tangential microfiltration, heat sterilization or the addition of bactericidal chemicals (chlorine, ozone, etc.) also eliminate microorganisms.

Relatively few bacterial species are capable of growing in water, mainly because its temperature is often below 20°C. However, the more organic contaminants in water, the greater the level of microbial growth, in particular for microorganisms capable of growing at these low temperatures (psychotropic microorganisms): this explains why *Pseudomonas* bacteria are often found in containers of water. Some microorganisms are also able to use mineral matter as a source of nutrition and energy production.

In some cases, technological processes that use water may be responsible for contaminating it. One example is the scalding tanks used to pluck poultry in slaughterhouses. It only takes one poultry flock contaminated by a pathogen (*Salmonella*, for example) to contaminate the water and consequently the other flocks that are dipped into the same tank. To avoid such cross-contamination, spray systems are a good alternative.

In general, therefore, not many pathogenic microorganisms are found in water, unless it has been in contact with a source of contamination. This is the case, for example, with river water contaminated upstream by a farm, which is used to irrigate crops of fruit and vegetables despite being illegal. Contaminated river water is discharged into the sea together with its pathogenic agents. Depending on their resistance to salinity, they can survive for different lengths of time in sea water and be concentrated by bivalves. Thus, viruses (hepatitis A, norovirus) can be found in shellfish.

Air

Microorganisms in the air are usually carried by solid particles from the soil, vegetables or humans and animals (pieces of skin or hair). Therefore, contamination of the air depends on its movement (winds, air currents) and

the density of contamination sources. Microbial levels are high in cattle slaughter halls, for instance.

Microorganisms in the air are usually not highly pathogenic, as in the case of soil and water, and correspond to microbial species that are rather resistant to changes in environmental conditions (Gram-positive bacteria, bacterial and fungal spores). The amount of fungal spores carried by air can vary seasonally: if no precautions are taken, they can contaminate food during processing depending on their susceptibility. For example, more attention is paid to the quality of the air in a ham slicing and packaging room than in a factory preparing biscuits or pastas. Some factories, therefore, require air filtration systems to eliminate microorganisms, resulting in the use of clean rooms, that is, places where incoming air is pre-filtered and maintained in a pressurized state to avoid the entry of outside air through openings used by operators. These clean rooms are usually equipped with airlocks allowing staff to change clothes and wash their hands. The rooms are designed in such a way that particles can be removed, for example, in the cured meat section; slicing and packaging machines are located under extractor hoods in clean rooms in order to create aseptic working conditions. The same facilities can be found in companies that manufacture packaged heat-treated beverages (pasteurized milk, ultra high temperature (UHT) milk, soft drinks and sodas packed in plastic bottles). The containers are usually sterilized by a chemical disinfectant (hydrogen peroxide) and then rinsed with sterile water, while packaging is done in a chamber where the air is sterilized by filtration.

Organic waste

Food companies have to manage several types of organic waste, ranging from animal bones and offal to vegetable peelings. They endeavor to minimize the spread of microbial contamination. Many kitchens purchase peeled and washed vegetables so as to avoid the entry of contaminated waste into their premises. To limit food contamination through waste, it should be prepared according to the principle of “forward flow”, which means that under no circumstances should a product from a clean area in the production line pass through a contaminated area beforehand. This basic principle, despite its importance, is unfortunately not respected in the agri-food sector, especially when faced with seasonal production (over production during the Christmas season, for example). Moreover, the addition of facilities and production lines in companies is not always the best solution. As needs

develop, there is a tendency to build additional premises without ensuring good overall functionality or the proper implementation of the “forward flow” principle.

Ingredients

Some ingredients can be a source of microbiological contamination if they are added at the end of a process without being sterilized or subsequently cooked. This is the case, for example, with spices that are added at the end of preparation. In fact, it is not uncommon to find a contamination of between 10^8 and 10^9 microorganisms per gram in pepper and some unsterilized spices. This also applies to herbs, dried or fresh (e.g. parsley or chives), which are added at the end of preparation. In the latter case, it is imperative that the cold chain is respected.

Surfaces and materials

Poorly maintained surfaces can accumulate organic matter in cracks and openings during food preparation and result in the spread of microorganisms. Bacterial biofilms containing different species and genera of microorganisms can form on the surface over time (Figure 3.1).

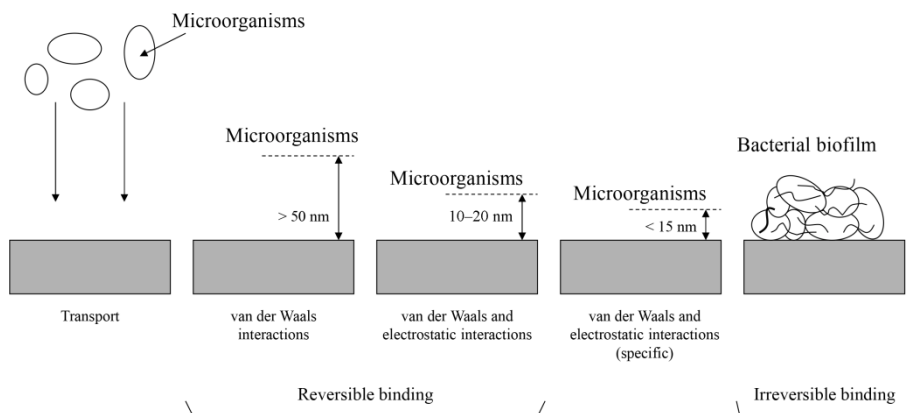


Figure 3.1. Formation of a biofilm (according to [FLE 73])

A biofilm is a microbial community in which cells adhere to each other on a surface. These cells are frequently embedded in a fibrous matrix of

extracellular polymeric substances and form a mucous layer protecting it from the environment. Biofilms allow microorganisms to survive; in fact, the resistance of biofilms to disinfectants is greater than that of cells in suspension: *L. monocytogenes* in a biofilm on glass resists 20 min of benzalkonium chloride compared to 30 s in suspension. This increased resistance is due to the low diffusion of disinfectant in the mucous layer, to the detoxification of substances in certain cases (bacterial enzymes) and the physiological state of the cells: slow-growing microorganisms have less permeable membranes, and the cells in a biofilm are often in a state of nutritional stress causing them to use different metabolic routes. Biofilms therefore pose a serious challenge to cleaning and disinfection operations in the food industry [COS 85]. They are also contamination carriers, since pieces of biofilm may break off due to mechanical action (the flow of a liquid food, for example) and contaminate the food, allowing the cells to multiply again. Manufacturers should therefore try to limit the formation of biofilm in their facilities by:

- using smooth, nonporous and wear-resistant surfaces;
 - quickly replacing worn, scratched or damaged surfaces;
 - using equipment that conforms with hygienic design guidelines (cleanability of machines);
 - managing water (restricting its use and optimizing its bacteriological quality);
 - regularly cleaning (avoiding the accumulation of organic matter) prior to disinfection, which should be effective (choice of disinfectants);
 - using hot detergents and combining them with a mechanical operation.
- The choice of materials used for equipment and work surfaces in the food industry depends on their capacity and resistance to cleaning and disinfection as well as technological constraints. This choice can be difficult because a material rarely has all the necessary qualities. For example, in butcher shops, flexible materials such as plastic or wood are better than stainless steel for cutting meat because it is easier to cut on them without blunting the knives. However, these materials wear very quickly, and any slits or openings made from continuous cutting render surfaces unsuitable in terms of good hygiene. It is therefore necessary to replace or smooth (by scrapping wood) these surfaces regularly. This is in fact one of the few cases where wood can be used in the food industry.

Cleaning and disinfecting surfaces considerably damages them; for example, stainless steel corrodes, plastic warps and splits. It is therefore necessary to have a suitable cleaning and disinfection method for each piece of equipment or workspace. The European “biocide” directive was introduced to impose an obligation on equipment manufacturers to draw up specific cleaning and disinfection procedures for each material. However, the development of technological processes makes this an increasingly complex task.

Premises

The principle of “forward flow” requires that premises should be specific and adapted to each food process. Designing functional premises must, therefore, be done in consultation with architects and food hygiene specialists.

However, even if premises are well designed, an increase in activity will result in non-functional premises after a certain amount of time. Thought should be given to the hypothetical future development of the company on its original site.

3.1.2. Factors influencing the growth of microorganisms

Every food has a microbial profile of its own. This profile depends on the flora of the food and the origin of contamination as well as physicochemical factors intrinsic to food such as structure, composition, pH, a_w , redox potential, the presence of natural antimicrobial compounds and environmental factors such as temperature.

3.1.2.1. Food structure

Raw food is often protected from attack by microorganisms by means of structures that are not easily degradable (fruit skin, nut shells, bran, collagen muscle tissue, egg shells, etc.). Only microbial species with appropriate enzymes (cellulase, pectinase, protease, etc.) can attack these structures. As a result, raw food prior to harvest is often only contaminated on the surface. However, as soon as the processing stage begins, this protection ceases and the food becomes more vulnerable to the spread of microorganisms. One

example is minced meat, which is a much more suitable substrate than a whole piece of meat for microbial growth.

3.1.2.2. Food composition

The nutrient composition of food also influences the growth of the most suitable species of bacteria (Figure 3.2): microorganisms containing proteases thrive in protein-rich foods, while microorganisms that metabolize sugars during fermentation thrive in sugar-rich foods. Thus, good slaughtering conditions (non-stressed animals that still have glycogen) allow lactic bacteria to develop in meat due to the presence of residual sugars. Through slight acidification, these bacteria inhibit the growth of spoilage bacteria containing proteases, such as *Clostridium* and *Pseudomonas*. In contrast, when an animal is slaughtered under stress and consequently has less residual glycogen, these spoilage bacteria may develop and lead to a faster deterioration of the meat.

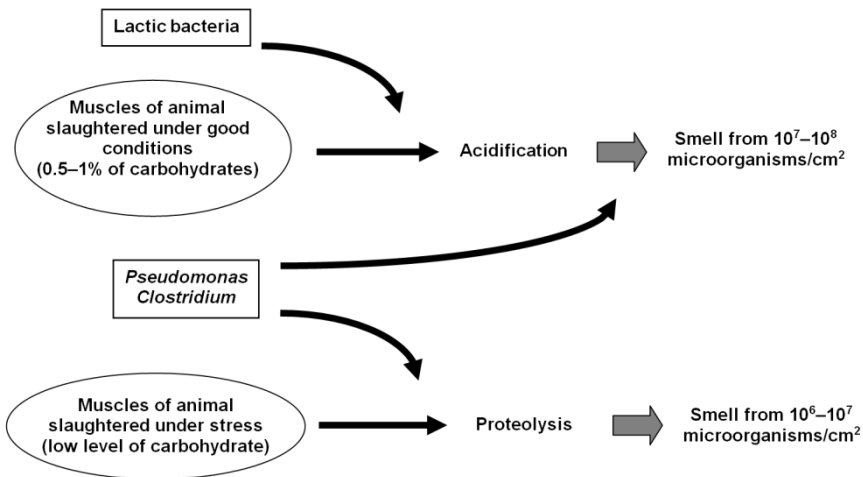


Figure 3.2. Influence of food composition on bacterial growth: example of muscle

3.1.2.3. Water activity [TRO 80, SPE 83]

Water activity (a_w ; see Chapter 1) is an essential factor for the growth of microorganisms. Water is vital for hydrolysis reactions allowing the

production of energy and various metabolites, as well as for the transfer of nutrients and the excretion of metabolites. When a bacterium is in an environment without water, the cell undergoes plasmolysis. This loss of water severely limits the bacterial growth and can, in some cases, result in cell death.

The amount of water available for microorganisms differs significantly depending on the food (Figure 3.3). Microorganisms vary in their capacity to grow and survive at low a_w [STR 70, CHI 82, BEU 83]. Some bacteria produce molecules called osmoprotectants (ectoine produced by *Brevibacterium linens*) that can keep water inside the cytoplasm. In general, yeasts and fungi can grow up to an a_w level of 0.6.

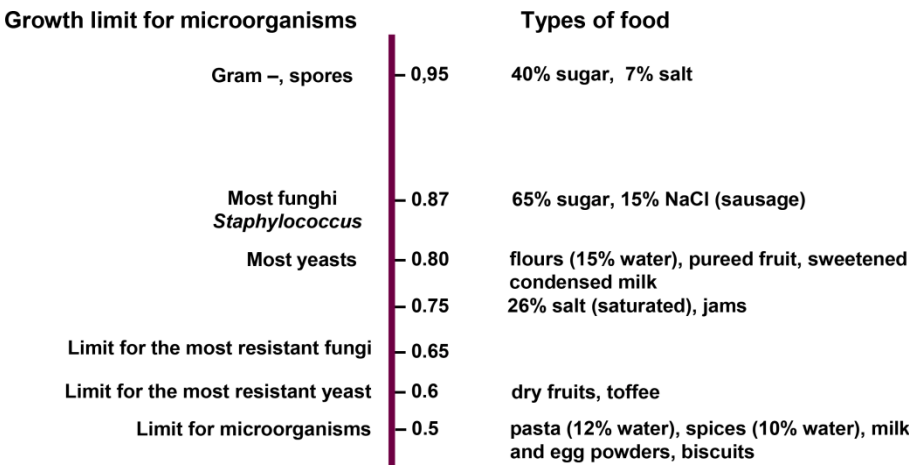


Figure 3.3. a_w values of certain foods and limits for microorganisms

3.1.2.4. pH

Most microorganisms develop around neutral pH, which corresponds to the pH of bacterial cytoplasm and is optimal for bacterial enzyme activities. When the microorganism comes into contact with a strongly acidic or basic environment, it regulates its intercellular pH to adapt to the change in environmental conditions. Substantial acidification or alkalinization of the

medium can considerably slow down bacterial growth and even cause cell death when the necessary enzymes are inhibited. Similarly, certain enzymes produced by bacteria and excreted in the environmental medium to allow the degradation of macromolecules and their assimilation can no longer remain active if the pH diverges from neutrality. Some molecules (weak acids, for example) produced by other microorganisms may, upon entering the cell, significantly alter the internal pH.

Microorganisms have established response mechanisms to deal with stress, enabling them to survive in certain pH conditions. With *Salmonella typhimurium*, there is a two-step process corresponding to two systems that are activated at different levels of acidity: tolerance response (between pH 4.5 and 6) and resistance response to more acidic pH. At pH values between 6 and 4.5, systems are synthesized, which maintain the internal pH above 5. Several decarboxylases contribute to this homeostasis: lysine decarboxylase (CadA) with the lysine transporter cadaverine (CadB). CadA decarboxylates intercellular lysine to cadaverine, an amine that consumes a proton through the process of protonation. Cadaverine is then excreted by antiport with lysine. This mechanism can maintain the internal pH through deacidification of the cytoplasm by mobilizing the systems appropriate to the amino acids in the cellular environment (lysine, ornithine, arginine decarboxylase; [FOS 91]). However, it does not provide sufficient protection for more acidic pH (below 4.5). It is believed that approximately 50 proteins can tolerate pH in this region. Three regulatory proteins (RpoS, Fur and PhoP) control the expression of different sets of acid shock proteins. These acid shock proteins act by protecting or repairing macromolecules [FOS 95].

As with a_w , bacteria can be quite heterogeneous with respect to changes in pH (Figure 3.4). In fact, some bacteria are able to grow at very acidic pH. This is the case, for example, for acetic acid bacteria that produce vinegar or lactic acid bacteria that produce lactic acid. This property is used in the food industry for the manufacture of several fermented foods (yoghurts, cheese, butter, bread, wine, etc.). Cheese-making involves decreasing the pH through the action of lactic acid bacteria and decreasing the a_w of milk by separating the whey from curd that is then salted: cheese is therefore less conducive to bacterial growth than milk.

Another effect of pH is to induce the complexation of certain nutrients in the culture medium or in the food. For example, some ions are chelated at acidic or basic pH.

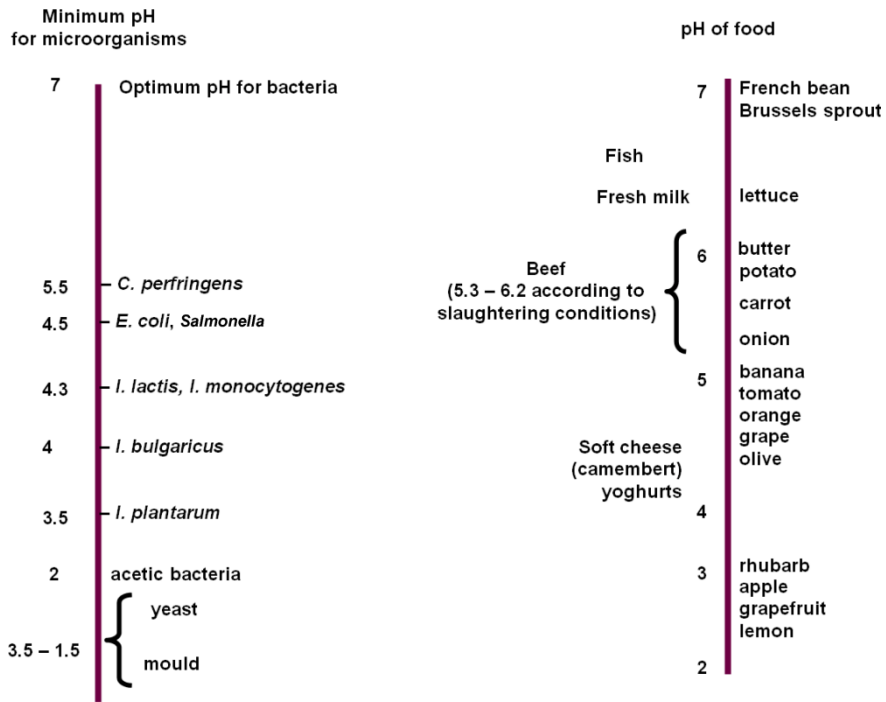


Figure 3.4. Minimum pH of microorganisms

3.1.2.5. Temperature

A distinction is usually made between the effect of temperature on the growth of microorganisms and the effect of temperature on their survival.

Effect of temperature on growth

Microorganisms can be classified according to their growth at different temperatures: psychrophiles grow at low temperatures, mesophiles at moderate temperatures and thermophiles at high temperatures.

However, this classification is arbitrary since a continuum exists in the bacterial world with regard to microorganisms’ capacity to grow at different temperatures. Some microorganisms are able to grow over a wide temperature range and can be both mesophilic and thermophilic or psychrophilic and mesophilic.

– *Psychrotrophic and psychrophilic microorganisms*

Psychrophilic microorganisms are especially suited to the cold and multiply at moderately low temperatures, since their optimum growth is at around 15°C.

Psychrotrophic microorganisms, which are better known, are able to adapt and grow at temperatures close to 0°C, but their optimum range is between 25 and 30°C, close to that of mesophiles. They have a slow metabolism and tend not to compete with other germs when the temperature increases.

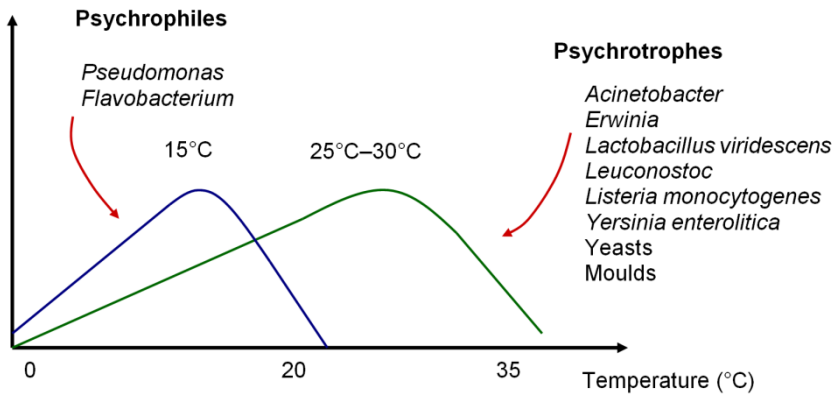


Figure 3.5. *Growth of psychrophilic and psychrotrophic microorganisms as a function of temperature*

Psychrotrophes are dominant in all chilled foods (meat, fish, milk, vegetables, etc.). The main genera are *Pseudomonas*, *Alcaligenes*, *Erwinia*, *Corynebacterium*, *Flavobacterium*, *Lactobacillus* and *Streptomyces*. Yeast and fungi are also mostly psychrotrophic.

Their slow growth rate allows them to contaminate food in 1–3 weeks, with a generation time of about 24 h at 0°C. Their metabolism remains very active at these temperatures, mainly because of the production of hydrolases (lipases or proteases), which poses technological problems (protein coagulation, color modification) as well as sensory problems (rancidity, unpleasant flavor and taste).

– *Mesophilic microorganisms*

Mesophilic microorganisms multiply at temperatures ranging from 20 to 45°C with an optimum at 37°C (Figure 3.6), at which point their growth rate peaks.

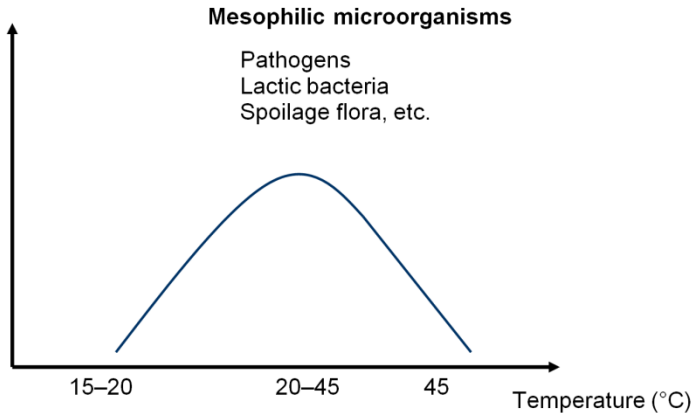


Figure 3.6. Growth of mesophilic microorganisms as a function of temperature

They are found in foods stored at room temperature or in refrigerated foods when the cold chain is broken. The main bacterial genera and species belong to the mesophilic group, including common species and species pathogenic to humans and animals; most are natural saprophytes.

– *Thermophilic microorganisms*

These micro organisms are able to multiply at temperatures between 45°C and 65°C with an optimum at 55°C (Figure 3.7). Thermophilic microorganisms are found in water, air and soil. They mainly exist in food in the form of the bacterial genera *Bacillus* and *Clostridium* and as the fungi *Cladosporium* or *Thermidium* [HOC 76].

Among the thermophilic microorganisms are bacteria called thermotrophs, which are mesophilic microorganisms capable of growth at high temperatures. An example is lactic bacteria, such as *Streptococcus thermophilus* or *Lactobacillus bulgaricus*, which can actively multiply at 45°C, as well as a fecal bacterium called *Streptococcus faecalis* that can multiply at up to 50°C.

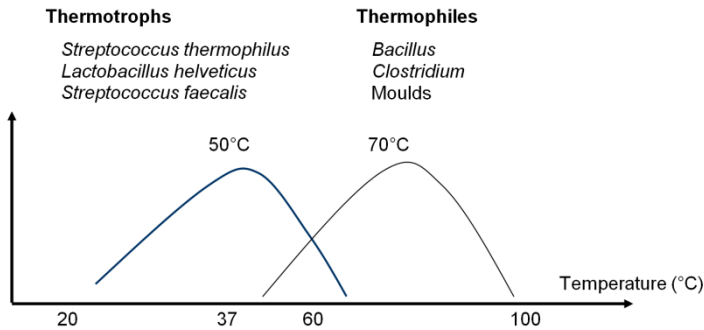


Figure 3.7. Growth of thermophilic microorganisms as a function of temperature

Temperature influences the speed of chemical and biochemical reactions. In living systems, the overall effect of a temperature variation means a change in the growth rate and the generation rate. It also has a differential effect on metabolic pathways and causes changes in cell size, and the secretion of toxins, pigments or polysaccharides.

Studies of *in vitro* enzyme kinetics show little difference in the oxidation rate between sugars and psychrophilic or mesophilic microorganisms. However, there are differences in terms of the enzyme thermostability of thermophilic and mesophilic microorganisms. The malonate dehydrogenase of thermophilic bacilli, for example, is stable for 120 min at 65°C while that of *Bacillus subtilis* is very quickly inactivated at this temperature.

Membrane proteins, ribosomes and flagella are also more thermostable among thermophiles. In contrast, some psychrophilic enzymes are more thermolabile than those in the other two groups and are inactivated at around 30–35°C.

The difference in thermosensitivity among certain cellular elements does not explain the entire behavior of microorganisms as a function of temperature. The sustainability of growth requires the sustainability of membrane transfers, and thus the integrity, structure and functionality of the cytoplasmic membrane.

This area has been widely explored, and many studies have reported significant differences in the average chain length of fatty acids in membrane

lipids as well as the changes in chain length in response to temperature variations. In psychrophiles, there is a large proportion of unsaturated fatty acids in membrane lipids, which ensures a low melting point, allowing membrane transfers to take place at low temperatures, as well as a high thermostability of the membrane. The percentage of saturated fatty acids is higher among mesophiles and thermophiles, which determines the physical properties of the membranes and the related biological properties.

Effect of temperature on survival

When considering the effect of temperature on the resistance of microorganisms, it is remarkable what high temperatures they can endure (Figure 3.8). Bacterial spores are among the most resistant, as well as certain fungal spores that can survive treatments lasting several hours at temperatures above 100°C (see Volume 2).

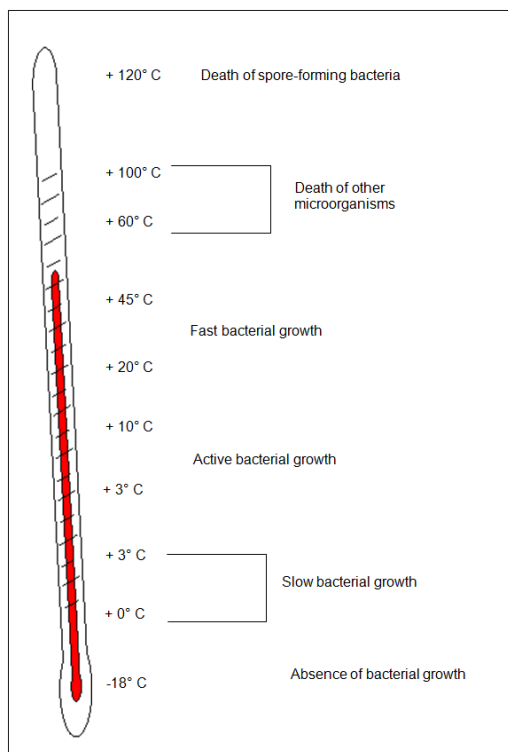


Figure 3.8. *Effect of temperature on the survival of microorganisms*

Heat treatment can fragment DNA, degrade RNA, induce a loss of lipopolysaccharides and alter the permeability of the membrane. However, proteins suffer the most damage as they can be denatured at relatively low temperatures (42°C). When microorganisms undergo sublethal heat treatment, the result is an induction of a set of specific proteins called “heat shock proteins” [LIN 88].

In many microorganisms, sublethal heat treatment is accompanied by an increase in thermotolerance. This capacity to withstand temperatures that are normally lethal can last for quite a long time. It depends on the treatment conditions (temperature and duration), the composition of the medium and the physiological state of the cells and varies from one species to the next. Generally, the induction of thermotolerance is related to the concomitant synthesis of heat shock proteins. These proteins protect cellular proteins from the lethal effects of stress by attaching themselves to the cellular proteins to prevent coagulation. By protecting the cells against heat shock, these proteins are consequently responsible for increasing the heat resistance.

Heat resistance is not necessarily exclusive to thermophiles. It applies, for example, to mesophilic fungi (*Byssoschlamys*, *Aspergillus*, etc.) and mesophilic or psychrotrophic spore-forming bacteria (*Bacillus cereus*, *B. subtilis*, etc.).

On another level, most microorganisms are more resistant to freezing when the process is rapidly carried out. This is because of the formation of intercellular microcrystals that do not alter the cells as much as large crystals formed during a slow freezing process (see Volume 2). Whatever the freezing method, cell structures suffer deformation, damage and destruction, which causes cell death at a rate of 1 Log. In general, Gram-positive bacteria are more resistant than Gram-negative bacteria.

3.1.2.6. Redox potential

Molecular oxygen (O_2) can form radicals that are very reactive to other molecules: superoxide (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^\bullet$). They can cause changes in DNA, RNA and proteins [FAR 91].

It appears that the most toxic molecule is superoxide, which can oxidize several compounds (thiols, ascorbate, catecholamines) and change

the oxidation state of iron, thereby impairing the functioning of cytochromes. Hydrogen peroxide reacts with many molecules, but in particular with iron and copper to generate hydroxyl radicals.

Bacteria may be divided into different categories depending on their ability to defend against these oxidants (Figure 3.9).

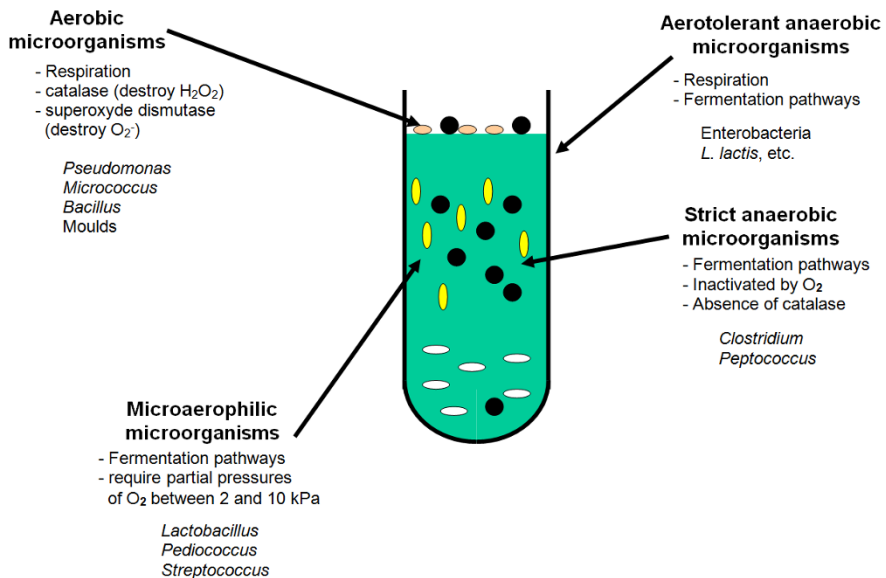


Figure 3.9. Influence of the redox potential on growth

Aerobic microorganisms

These microorganisms can only grow in the presence of oxygen. They possess enzymes like catalase and superoxide dismutase that can destroy the oxidizing compounds. Respiration is the metabolic process that enables the production of several ATP molecules. These microorganisms can grow on the surface of foods and generally multiply very quickly. They include, for example, bacteria of the genus *Pseudomonas* that grow on cold meat carcasses, or of the genera *Micrococcus* and *Bacillus*. Fungi are strictly aerobic with few exceptions (which can be found in the rumen, for example).

Anaerobic microorganisms

These bacteria not only have respiratory chains that allow them to grow in the presence of oxygen but also have fermentation pathways that allow them to grow in the absence of oxygen. This category includes a large number of microorganisms used in the fermentation process (lactic bacteria) as well as species comprising the spoilage flora of food.

Strict anaerobic microorganisms

These microorganisms do not usually have enzymes capable of deactivating oxidizing compounds and therefore use fermentation pathways to multiply. They are sensitive to oxygen and consequently can only grow in strictly anaerobic conditions (*Clostridium botulinum*).

Aerotolerant anaerobic microorganisms

These microorganisms use the necessary fermentation pathways due to their sensitivity to oxygen but can still withstand oxygen partial pressures (*C. perfringens*). They cause more foodborne infections than strict anaerobes.

Microaerophilic microorganisms

These microorganisms require partial pressures of oxygen of between 2 and 10 kPa, but can be impaired beyond this amount.

3.1.2.7. Antimicrobial compounds

These are molecules that have bacteriostatic and/or bactericidal properties (see Volume 2). The range of chemical compositions and heterogeneous actions is extensive. They can be of endogenous origin (lysozymes in eggs and milk, essential oils in spices or antioxidant gossypol in cottonseed), products of fermentation or added to food to preserve it (additives).

In general, Gram-positive bacteria are sensitive to many molecules such as citrate, nisin, butylated hydroxyanisole (BHA; Figure 4.16) or butylated hydroxytoluene (BHT; Figure 4.16), as well as molecules known for their antifungal activity, such as sorbates or benzoates. Gram-negative bacteria are

more resistant than Gram-positive bacteria, but are still susceptible to a broad spectrum of additives as well as SO₂.

Lactic and acetic acids, largely produced during fermentation, can be used as additives with substantial antibacterial activity; propionic acid and pimarinic acid are more antifungal.

3.1.2.8. Interaction phenomena

Not only do each of these physicochemical parameters (pH, a_w , temperature and additives) have a specific influence, but they also interact and it is necessary to consider their combined action with regard to the growth of microorganisms. These interactions can be positive or negative depending on the target microorganism, the food itself and the permissible levels of food additives.

During their growth, microorganisms alter the composition of food and certain physicochemical parameters: their metabolic activity lowers the amount of some nutrients and increases the amount of residual compounds, acting either as growth factors or as antimicrobial substances. The result is a modification of the overall flora through synergism and antagonism.

All these processes result in either the production of fermented products or the deterioration of the product. The antagonistic phenomena include the action of lactic bacteria in relation to the normal and psychrotrophic flora. The inhibitory effects are attributed to the drop in pH and the production of hydrogen peroxide and bacteriocins.

Synergies or even dependencies are also common. Examples can be found in lactic bacteria in starter cultures, either on their own or in conjunction with yeast.

3.2. Food spoilage

Spoilage due to microorganism activity lowers the sensory and health qualities of food. It involves a change in the food structure, color and taste. In some cases, what constitutes spoilage in certain foods is, in fact, desired in other foods, and is based on the same mechanisms. This applies to

fermented foods: in the production of cheese, milk fats and proteins undergo degradation, giving the desired texture and flavor.

Microorganisms grow on the surface or within the food. The impact of food degradation varies therefore depending on the case:

– *Spoilage by the growth of microorganisms on the surface*

When the surface of food is moist enough to allow microorganism growth, a biofilm, also called slime, forms after a certain amount of time depending on the environmental parameters (temperature, pH, etc.). If the microorganisms produce enzymes capable of degrading proteins on the surface of the food, unpleasant odors develop due to the catabolism of amino acids. However, some bacteria primarily responsible for acidifying the medium (lactic bacteria) are less harmful.

In some cases, slime can be removed by mechanical action: this process is sometimes used by butchers to treat the surface of carcasses. Fungal growth on the surface of food is usually irreversible. In fact, it is almost impossible to remove mycelium filaments from the surface, which is distinguished by a persistent residual color.

– *Spoilage by the growth of microorganisms on the inside*

This type of spoilage substantially alters the structure and texture of food. It results in the production of gas and causes severe irreversible damage (tearing) to the structure. It depends on the microbial species and, consequently, its metabolism (production of proteases capable of degrading proteins, production of gas resulting from different metabolic pathways).

3.2.1. Changes in texture and structure

3.2.1.1. Degradation of proteins

Food proteins can be degraded by a series of enzymes produced by microorganisms. Proteases break down proteins into smaller fragments called peptides, which can themselves be degraded into smaller peptides or free amino acids by peptidases. Peptidases are often specific because they

recognize particular amino acid sequences: aminopeptidases cleave from the amino-terminal, carboxypeptidases from the carboxy terminal, and endopeptidases cleave specific peptide bonds within the polypeptide chain. Protein degradation leads to a deterioration of the food structure. This phenomenon is evident in the ripening of soft cheese like camembert: the chalky structure composed mainly of milk caseins, and fat liquefies over time through the action of natural milk proteases (plasmin), rennet and microbial proteases.

3.2.1.2. Production of gas

Some microorganisms produce gas as they grow in food, thereby causing the food itself or the packaging to swell. The gases produced are carbon dioxide (CO_2), hydrogen (H_2), hydrogen sulfide (H_2S) and ammonia (NH_3). Numerous metabolic pathways are responsible for the production of these gases, and they vary according to the microbial species and the substrate on which they grow: carbon dioxide and ammonia may result from the decarboxylation and deamination of amino acids. Swelling may occur in packaging (spoiled preserves) or in the food itself (low-grade Emmental cheese due to an overproduction of gas (CO_2 , H_2) linked to the presence of *Clostridium tyrobutyricum*).

The production of gas by microorganisms may be desired in the production of fermented foods. This is the case for *Propionibacterium freudenreichii*, responsible for the formation of eyes during the ripening of Emmental, but also *Saccharomyces cerevisiae*, which produces CO_2 in the manufacture of bread, beer and other fermented beverages.

3.2.1.3. Production of polysaccharides

The polysaccharides produced by microorganisms during their growth can also cause food spoilage. They generally form a capsule that protects the bacterium from the environmental medium, bacteriophages or macrophages. These polysaccharides also play a role in the adhesion of microorganisms to surfaces and in the formation of biofilms (bacterial slime). They give the food a viscous appearance, which is highly undesirable. An example is the contamination of certain fermented beverages (cider or wine) by

leuconostocs giving the product an oily consistency. However, this ropy aspect can be desired in the production of certain yoghurts: strains of lactobacilli or streptococci with these characteristics are used in the acidification of milk.

3.2.2. *Changes in flavor*

Flavor is a combination of taste and smell that humans perceive when eating food. The growth of microorganisms can substantially modify the characteristics of food by producing molecules that have a direct effect on the flavor or an indirect effect by recombining with other molecules. They tend to be organic acids and compounds resulting from the catabolism of lipids and amino acids.

Many acids are produced by fermentation as microorganisms grow (lactic acid, butyric acid, propionic acid, acetic acid). Apart from the fact that they acidify the medium, these molecules also have their own smell: this is the case, for example, with propionic acid produced by propionic bacteria in Emmental.

Proteolysis generates new aromatic compounds through the formation of peptides and amino acids: this is the case for bitter peptides produced by certain fungi during cheese ripening. However, it is mainly the molecules resulting from the catabolism of amino acids that are involved in the development of new flavors: these include gases like ammonia resulting from the deamination of amino acids, hydrogen sulfide resulting from the catabolism of sulfur amino acids, and many other molecules like cadaverine and putrescine resulting from the decarboxylation of amino acids. Such mechanisms are desired during the production of fermented foods. In fact, much of the flavor of cheese is owing to the degradation of proteins.

Lipolysis also has a substantial impact on flavor; it involves the degradation of fats in food. The enzymes involved in this mechanism are less common among microbes than proteases. Lipolytic microorganisms include certain bacteria and many yeasts and fungi.

3.3. Sanitary risks

3.3.1. Foodborne disease outbreak

A foodborne disease outbreak (FBDO) is defined as an incident in which two or more persons experience a similar illness resulting from the ingestion of a common food. All foodborne outbreaks must be declared to a government health authority, either to the Department of Health and Social Affairs or to the Department of Veterinary Services in the case of France (OJ, June 1988). This declaration is obligatory for any physician who ascertains the existence of one case as well as for the main occupant, head of family or person in charge of premises where cases of the illness arise. Mandatory reporting of FBOOs allows medical and veterinary public health inspectors to conduct an epidemiological study aimed at identifying the foods responsible and contributing factors in order to take specific measures to prevent recurrences.

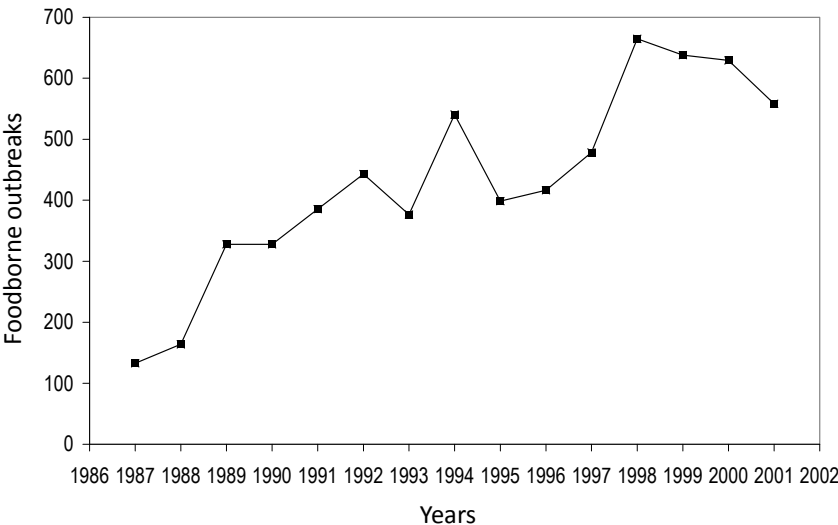


Figure 3.10. Foodborne outbreaks (all etiologies combined) reported in France between 1987 and 2001 [HAE 02]

In France, the production and analysis of data are carried out by the Institute for Public Health after pooling the information from the Department

of Health and Social Affairs and the Department of Veterinary Services and excluding any duplicate reports.

A total of 559 FBOOs, all causes combined, were reported in France in 2001 [HAE 02]. Since 1998, the number of FBDOs reported has decreased by 15% (–103 outbreaks).

Among FBOOs where the agent was confirmed, *Salmonella* was the most frequently isolated agent (64% of foodborne outbreaks; Table 3.2) and Enteritidis was the predominant serotype (52% of *Salmonella* outbreaks). The number of foodborne outbreaks caused by other confirmed pathogens has remained stable since 1987.

The hospitalization rate was 10% in 2001. Three deaths, all secondary to *Salmonella* infections, were reported among elderly people.

A recrudescence of FBDOs, all etiologies combined, is observed each year during the summer period (June to September). FBDOs with a suspected viral origin occur more frequently during the winter period (October–March).

In 2001, 61% of FBDOs originated in catering establishments (where the food was eaten) compared to 35% at home. The proportion of *Salmonella* FBDOs is still slightly higher at home than in catering establishments.

The most common foods responsible for *Salmonella* outbreaks are eggs and preparations containing raw or undercooked eggs (57%; Table 3.3). Eggs from packing centers have been accountable for 44% of cases and eggs from homesteads for 42% of cases. Milk products and dishes requiring handling are frequently held responsible for *S. aureus* outbreaks and dishes with sauces for *C. perfringens* outbreaks.

The factors that contribute to the emergence of FBDOs include the contamination of equipment, the contamination of raw materials, noncompliance with regulation temperatures and errors in the manufacturing process (Table 3.4).

Causative agent	Outbreaks reported to the DDASS ¹ and DDSV ²							
	Outbreaks		Cases		Hospitalized		Dead	
	N	% ^{3,4}	N	%	N	%	N	%
Confirmed agents								
<i>Salmonella</i>	174	64	17	57.7	272	15.8	3	0.2
Enteritidis	90	51.7	993	57.5	149	15	2	0.2
Typhimurium	30	17.2	308	17.8	80	26		
Hadar	1	0.6	2	0.1	2	100		
Other serotypes ⁵	5	8.6	131	7.6	9	6.9	1	0.8
Unknown serotypes	38	21.8	292	16.9	32	10.9		
<i>Clostridium perfringens</i>	8	2.9	208	6.9	1	0.5		
<i>Staphylococcus aureus</i>	43	15.8	620	20.7	131	22.3		
<i>Bacillus cereus</i>	8	2.9	139	4.6	9	6.5		
Histamine	8	2.9	22	0.7	2	9.1		
<i>Shigella</i>	3	1.1	8	0.3	2	25		
Other pathogens	28	10.3	270	9	20	7.4		
Total confirmed agents	272	48.6	2,993	44.4	437	14.6	3	0.1
Total suspected agents	189	33.8	2,647	39.3	177	6.7	0	
Total unknown agents	98	17.5	1,102	16.3	65	5.8	0	
Total foodborne outbreaks	559	100	6,742	100	679	10.1	3	0.04

1 DDASS: Department of Health and Social Affairs in France

2 DDSV: Department of Veterinary Services in France

3 For different agents: % compared to total known agents

4 For *Salmonella* serotypes: % compared to total *Salmonella*

5 Mandatory reporting of foodborne outbreaks: Anatum (1), Arizonae (2), Bredeney (1), Dublin (1), Mbandaka (1), Newport (7), Virchow (5)

Campylobacter (8 outbreaks = 121 cases), botulism (4 outbreaks = 16 cases), *Dinophysis* (1 outbreak = 2 cases), distomatosis (1 outbreak = 3 cases), *Shigella* (3 outbreaks = 8 cases), calcivirus (1 outbreak = 19 cases), toxoplasmosis (1 outbreak = 5 cases) *Vibrio parahaemolytica* (11 outbreaks = 100 cases), *Yersinia* (1 outbreak = 4 cases).

Table 3.2. Number of outbreaks and cases per confirmed or suspected etiological agent. Foodborne disease outbreaks and outbreaks of salmonellosis and shigellosis reported to the National Centre for Scientific Research in France (CNRS) in 2001. [HAE 02]

Foods	<i>Salmonella</i>				<i>Clostridium perfringens</i>	<i>Staphylococcus aureus</i>	Other agents	Unknown agents	Total
	Enteritidis	Typhimurium	Other serotypes	Unknown serotypes					
Milk and milk products	1	5	7	1	0	17	7	3	41
Eggs and egg preparations ¹	61	17	4	25	0	5	2	5	119
Meat	0	2	0	3	11	12	6	3	37
Charcuterie products	10	2	1	2	4	12	4	3	38
Poultry	1	0	3	7	6	2	4	4	27
Fish and crustaceans	0	0	0	3	1	3	24	3	34
Molluscs	2	0	0	1	0	0	21	0	24
Other foods ²	2	0	0	1	13	28	17	10	71
Drinking water	0	0	0	0	0	1	1	5	7
Foods not found	13	4	0	11	12	30	29	62	161
Total	90	30	15	54	47	110	115	98	559

1 Products containing eggs: chocolate mousse, cakes and pastries, mayonnaise, etc.

2 Non-animal or mixed foods.

Table 3.3. Identified or suspected agents and responsible or suspected foods. Foodborne disease outbreaks reported in France in 2001 [HAE 02]

Factors	% ¹
Contaminated raw materials	50
Environmental contamination:	55
– staff	2
– equipment	59
Error in the preparation process	46
Significant delay between preparation and consumption	35
Non-compliance with regulation temperatures:	49
– hot chain	19
– cold chain	43

1 Total > 100%, as there are several possible factors for a single foodborne outbreaks.

Table 3.4. *Factors contributing to the occurrence of FBDOs in 2001 [HAE 02]*

3.3.2. Main pathogens and toxin producers

3.3.2.1. Toxin-producing microorganisms

Clostridium botulinum [DOO83]

This Gram-positive bacterium is spore-forming and strictly anaerobic. It does not have any particular growth properties: it is a mesophilic bacterium with an optimum pH of 7. Like all spore-forming bacteria, *C. botulinum* is present in environments not conducive to bacterial growth and is ubiquitous in nature: it can be found in soil and water, from where it can contaminate all foods (vegetables, milk, eggs, meat, etc.). However, it can only grow in anaerobic conditions and at room temperature (> 15°C). As a result, this bacterium is found primarily in poorly sterilized preserves, ill-prepared cured foods and large pieces of meat where anaerobic conditions may exist

(smoked ham, etc.). The foods responsible for food poisoning by *C. botulinum* are mainly preserves and smoked hams prepared at home. The illness is caused by a heat-labile neurotoxin that is produced by the bacterium during its multiplication in the food. Symptoms appear between 2 and 24 h after ingestion, depending on the amount of toxin ingested. They include ocular paralysis accompanied by a dryness of the mouth, followed by speech and swallowing difficulties, constipation and urinary retention, and finally respiratory paralysis and reduced consciousness, which may lead to the death of the individual.

Prevention involves:

- avoiding, as much as possible, stressing animals prior to slaughter in order to avoid the release of microorganisms into the blood by the mononuclear phagocyte system;
- rapidly cooling carcasses after slaughter to avoid the combination of favorable growth temperatures and a depletion of oxygen;
- storing partially preserved products in cold conditions;
- salting foods properly;
- cleaning raw materials properly by removing all traces of soil;
- adhering to sterilization guidelines, with one of the objectives being to obtain a 12 log reduction of the pathogens (see Volume 2).
- destroying the toxin by heating in the case of a suspected contaminated foodstuff (15–30 min at 80°C).

Clostridium perfringens

This bacterium is very similar to *C. botulinum*. It is a Gram-positive bacterium, spore-forming, ubiquitous in nature and strictly anaerobic. However, it has the particular trait of growing at higher temperatures: its optimum growth temperature is 45°C. As a result, food poisoning by *C. perfringens*, capable of multiplying rapidly during the slow cooling of food, is more frequent than by *C. botulinum* [FAC 98]. Sauce dishes that are incorrectly cooled are of particular concern, since the cooking temperature ($\leq 100^\circ\text{C}$) does not destroy the spores, which can germinate around 50°C as the food cools and actively multiply.

The illness is caused by a toxin produced by *C. perfringens* in the digestive tract, which is not a suitable medium for its growth but facilitates its germination. A population of at least 10^8 bacteria per gram must develop before symptoms manifest themselves; the incubation period is 12 h. The toxin attaches itself to enterocytes in the digestive tract and induces a sodium and chloride secretion, which results in a flow of water into the intestine causing severe diarrhea that lasts 24–48 h.

Prevention of this illness involves:

- limiting endogenous contamination of meat at slaughter;
- avoiding the multiplication of bacteria by keeping the food at a temperature above 63°C or below 3°C (in this case, cooling to 10°C should take no more than 2 h).

***Staphylococcus aureus* [LEL 03]**

Staphylococcus is a Gram-positive bacterium whose main characteristic is that it can grow in foods with a relatively low a_w (up to 0.83). Its optimum growth temperature is 37°C and optimum pH is 7. Like all Gram-positive bacteria, it is a resistant bacterium in the environmental medium.

Foods responsible for *S. aureus* food poisoning are primarily those handled by humans, produced from raw milk (mainly soft cheeses) or dried foods (milk powder). The illness is caused by several toxins produced by the bacteria. Enterotoxin A, which is the most common, requires at least 10^6 bacteria per gram of food to be pathological. *S. aureus* toxins are generally resistant to heat and digestive enzymes. They enter the blood and act on the nerve centers in the brain responsible for controlling vomiting. Symptoms appear 1–6 h after ingestion and manifest themselves as violent vomiting, diarrhea and severe abdominal pain without fever. The illness spontaneously regresses after 2–3 days, but is followed by a long period of fatigue that can last from one to several weeks.

Prevention involves avoiding:

- the presence of bacteria in milk by controlling mastitis (monitoring livestock and milk quality);
- the contamination of food that is handled during production (wearing gloves and masks, removing staff members suffering from hand wounds);

– bacterial growth in food by deterring any break in the cold chain and rapidly cooling the food.

***Bacillus cereus* [NGU 03, DRO 01, CAR 98]**

This Gram-positive bacterium is facultatively anaerobic, spore-forming and capable of growing between 5 and 50°C (optimum between 30 and 37°C). *Bacillus cereus* is a large producer of enzymes with high phospholipase activity. Although it is generally not considered a psychrotrophic species, several reports have been made over the last 10 years of strains developing in cold conditions. Like all spore-forming bacteria, it is ubiquitous in nature. Offending foods are often rice, especially rice sold in Asian restaurants. In this type of preparation, the rice is steamed, which does not destroy the spores, and can be reheated several times a day, allowing the bacteria to germinate and multiply. Other foods, such as meat, precooked meals or pastries are also held responsible for *B. cereus* food poisoning. Milk, skimmed milk powder and pasteurized milk are sometimes altered by this bacterium (formation of clumps). Since this bacterium is not very competitive, it is particularly problematic in products containing few microorganisms (pasteurized milk). Given its ability to form spores, it is well adapted to heat treatment processes. The dose generally tolerated in the dairy sector is 10^3 spores per ml of milk. Foods responsible for causing *B. cereus* infections contain at least 10^5 bacteria per gram.

B. cereus usually causes a mild form of poisoning owing to the two types of toxins: emetic toxins (emetogenic) and enterotoxins (diarrheal toxins).

Emetic toxins act on nerve centers that control vomiting. They are preformed, that is they are in the food at the time of consumption. They are stable at 126°C for 90 min, at 4°C for 2 months, between pH 2 and 11, and even in the presence of trypsin and pepsin.

Enterotoxins are necrotizing and lethal for cells; they are produced during the exponential growth phase. The toxin works by stimulating the adenylate cyclase–cyclic AMP system, causing an accumulation of liquid in the intestine. It is more cytotoxic, unstable between 4 and 25°C, and completely inactivated when heated to 56°C for 5 min. It is sensitive to trypsin and pronase.

There are two different forms of the illness. One form is emetic syndrome, which has a relatively short incubation period (0.5–6 h) and results in nausea and vomiting, occasionally accompanied by abdominal pain and diarrhea. Symptoms persist for between 6 and 24 h. The other form is diarrheal syndrome, which has a longer incubation period (6–15 h) and results in profuse watery diarrhea and abdominal pain. Vomiting is very rare and nausea is experienced occasionally. The syndromes subside within 24 h; however, in immunocompromised patients complications may arise.

Prevention is approximately the same as for *C. perfringens*.

3.3.2.2. *Fungi responsible for the production of mycotoxins*

Like bacterial spores, fungal spores are extremely resistant in the environmental medium and are ubiquitous in nature. However, their germination requires specific conditions. Not all fungi are responsible for the production of mycotoxins, which are products of their metabolism and may vary depending on the substrate and environmental conditions (pH, temperature, humidity and presence or absence of oxygen). *Penicillium roqueforti*, which is used in the production of Roquefort, can produce mycotoxins when it grows on foods other than fermented dairy products.

Offending foods are those stored in the wrong conditions, allowing fungus to grow on their surface. In Europe, this happens primarily with plant foods such as corn and wheat. In these cases, fungus can develop during storage, but also in the field before harvest. Mycotoxins can also be found in poorly stored soybean meal and can contaminate the blood system of cows as well as their milk. Dried fruits that are stored in poor conditions may also contain mycotoxins.

In general, fruits and vegetables with visible fungal growth on the surface are rarely used, except in cases where such growth is removed during processing. It is thus possible to find large quantities of mycotoxins in apple juice produced from moldy apples.

The mode of action of mycotoxins is varied. Some are teratogenic, neurotoxic and/or carcinogenic. Toxicity varies depending on the toxin and the daily dose absorbed. Toxins pass from food into the bloodstream via the digestive mucous membrane and attack various organs. Aflatoxin is known to cause hepatic parenchymal necrosis, leading to cancer if ingested

regularly. Patulin (produced by *Penicillium expansum* and *Aspergillus clavus*) can be found on moldy apples and causes damage to the lungs, kidneys, spleen and neurons.

Prevention involves avoiding excessive storage periods in the wrong conditions (heat, humidity, aerobic conditions). To improve food preservation, certain fungicides like propionic or sorbic acid can be used. In order to avoid the accumulation of mycotoxins in livestock, it is recommended to vary their diet.

3.3.2.3. Other pathogenic microorganisms

Salmonella [COL 02]

Salmonella are mesophilic, Gram-negative bacteria. They are generally considered poor competitors in complex ecosystems, but are nevertheless relatively resistant in the environmental medium.

The primary ecological niche for *Salmonella* is in the digestive tract of humans and animals, in particular poultry. Intraovarian contamination may occur where the egg yolk is contaminated after its production in the oviduct. Given that the yolk is an ideal medium for the growth of *Salmonella*, the bacteria can continue to grow until the egg is sold, and if moderately cooked (fried egg, boiled egg or omelet) can lead to acute salmonellosis (10^9 to 10^{10} *Salmonella* per gram).

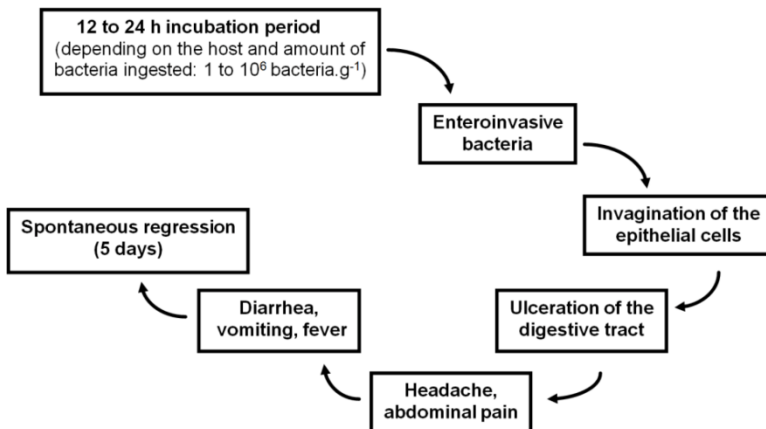


Figure 3.11. Pathogenicity of *Salmonella* Enteritidis

The incubation period is 12–24 h depending on the host and the amount of bacteria ingested. *Salmonella* bind to epithelial cells in the digestive tract where they are able to invaginate. By producing toxins, they cause ulceration of the digestive tract resulting in severe diarrhea, abdominal pain and vomiting. These symptoms are accompanied by fever and dizziness. The illness can last for several days. It usually regresses spontaneously after 5 days.

Salmonella populations are generally limited in raw materials. It is therefore important to limit their development by meticulously respecting the cold chain. Their presence in eggs can be avoided by closely monitoring livestock and slaughtering the flock in the case of contaminated laying hens.

Since humans can carry this bacterium, general hygiene rules should be respected.

Listeria monocytogenes [FLA 00, GOU 01]

This Gram-positive bacterium, despite not being spore-forming, is considerably resistant in the environmental medium. It can therefore be found in soil and consequently, in all foods. In humans and animals, it can travel into the digestive tract and implant itself in small quantities. Thus, humans can occasionally be healthy carriers of this bacterium. Furthermore, this bacterium is psychrotrophic and is able to form biofilms.

Infection if ≥ 100 bacteria.g⁻¹
Incubation period: 2 to 70 days

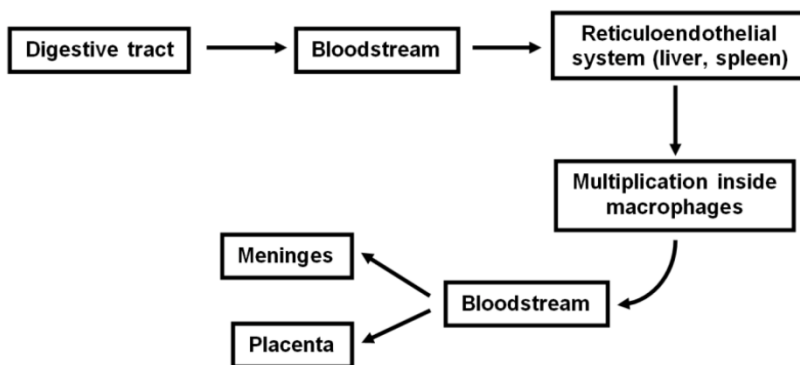


Figure 3.12. Pathogenicity of *Listeria*

Strains of serotype 4 are known to be the most virulent. The bacterium passes from the digestive tract into the bloodstream where it is able to colonize and, after multiplying, lyse macrophages. It is then dispersed through the blood, reaching certain organs where it multiplies (placenta, meninges, etc.).

The incubation period varies from a few days to several weeks and can result in many forms of the illness. In pregnant women, it can cause abortion in late pregnancy. In adults, the neuromeningeal form has a 26% mortality rate and the septicemic form, which generally develops in immunosuppressed individuals, has a 68% mortality rate. Finally, in babies, this bacterium can cause septicemia or meningitis. In general, *Listeria* has a 25% to 40% mortality rate. However, prevalence is low given that it is uncommon to encounter this bacterium in food.

Listeria can be found in many raw materials, such, as meat, milk or fish (Table 3.5). It can multiply in refrigerated foods with long shelf lives, as well as in some soft cheeses where the relatively high a_w allows bacterial growth. Prevention involves adjusting the shelf life of products at risk and carrying out effective stabilization, cleaning and disinfection treatments.

Year	Number of cases	Foods responsible
1975–1976	167	N.I.
1992	279	Pork tongue
1993	39	Rillettes ¹
1995	33	Soft cheeses
1997	14	Pont-Evêque and Livarot ²
1999	10	Rillettes ¹
1999–2000	32	Pork tongue in jelly

1 Coarse pâté.

2 Soft raw-milk cheeses.

Approximately 300–400 sporadic cases per annum in France with 25 to 40% mortality rate.

Table 3.5. Some examples of *Listeria* outbreaks

Escherichia coli O157: H 7 [VER 01]

The natural habitat of this Gram-negative bacterium is in the digestive tract of humans and animals. As it is not very resistant in the environmental medium, it is found primarily in meat and products of animal origin, and rarely in vegetables. In general, it is not present in sufficient quantities in raw materials to cause infection, which would require prior multiplication.

The incubation period is 3–9 days and results in severe watery diarrhea and abdominal cramps. This diarrhea can eventually become hemorrhagic and in some cases lead to complications such as hemolytic-uremic syndrome.

It is possible to avoid contaminating meat by applying good manufacturing practices when slaughtering cattle, and in particular during evisceration. Another way to prevent contamination is to increase the cooking intensity (temperature or time) of meat so as to kill bacteria. The latter method has been implemented by fast-food chains in the USA.

Campylobacter

This Gram-negative bacterium is mainly found in the digestive tract of animals, especially poultry. It is often found in raw milk.

There has been no recent research on this bacterium in France. However, the number of cases of campylobacteriosis is estimated to be at least equivalent to that of salmonellosis. The infective dose is relatively low at only 500 bacteria.

The illness starts after an incubation period of 2–7 days and is characterized by diarrhea that can be very severe, even purulent and bloody in certain cases, as well as very high fever and, occasionally, vomiting. A period of fatigue follows, which may last for several weeks and, in some cases (0.1%), a complication known as Guillain Barré syndrome may arise. Campylobacteriosis is responsible for a high infant mortality rate in developing countries.

3.3.2.4. *Viruses*

In France, the impact of viruses in foodborne infections is not well known. However, they figure among the leading causes of foodborne illnesses in the United States.

Poliovirus (enterovirus)

This virus is carried by water when it is contaminated with human excrement.

In industrialized countries, the effects of this virus have virtually disappeared due to widespread vaccination and the implementation of general hygiene measures since the beginning of the 20th Century (drinking water and sewerage schemes). However, this illness can be contracted even today in developing countries where this level of hygiene has not been reached.

Hepatitis A virus

This virus is relatively common in France, since it is quite often found in shellfish. However, contamination can occur from human to human (fecal–oral cycle), and more commonly via water contaminated by human feces. The incubation period is between 10 and 50 days, depending on the dose ingested and the individual, but the virus can be present in the feces 14 days before the illness manifests itself.

The illness is characterized by a first phase called the preicteric phase (4–7 days before the illness itself) with fever and gastrointestinal disorders. The icteric phase corresponds to the presence of pigments and bile salts in the urine and blood (jaundice) and severe fatigue. The recuperation period can be quite long for some individuals (several months to a year). The hepatitis A virus is relatively fragile, since it can be destroyed by heat (60°C for 1 h or 100°C for 5 min).

Rotavirus (reovirus) and Norwalk virus (parvovirus)

These viruses cause acute gastroenteritis. The incubation period is 1–3 days and the symptoms include diarrhea, heavy vomiting and fever with abdominal cramps and headaches. The viruses are present in the environment, especially in contaminated water and shellfish. When an individual is sick, he or she excretes large quantities of the virus in their feces (10^{10} viral particles per ml), and as a result foods handled by humans can often be contaminated in this way.

3.3.2.5. Prions [SAV 00, HIR 00]

Prions are responsible for transmissible spongiform encephalopathies (TSE), also called Creutzfeldt-Jakob disease.

There are four forms of prion disease:

- hereditary;
- iatrogenic;
- sporadic (around one case in a million inhabitants);
- transmissible by food.

In the last form, the prion passes from the digestive tract wall into the bloodstream. It is then captured by lymph nodes, the spleen and the thymus. It travels along the central nervous system to the brain causing a proliferation of glial cells that prevents neuronal regeneration. The cerebral tissue becomes increasingly atrophied resulting in death after about 9 months. In humans, the incubation period is very long lasting several decades.

So-called mad-cow disease began in 1986 in Great Britain; in the following decade, 165,000 animals were slaughtered or died of this disease. It also significantly impacted Switzerland, and to a lesser degree several European countries like France. There are currently 125 human cases. The first cases observed were in Great Britain about 10 years after the epidemic stuck among cattle. The disease usually affects young cattle.

The infectious agent is a glycoprotein, whose modified conformation is resistant to proteases. Unmodified glycoprotein is naturally found on the surface of neurons and is regularly renewed, like all cell proteins, through a turnover process. The modified glycoprotein resistant to proteases, and no longer degraded by them, accumulates in the cell causing neuronal death.

Most scientists share the assumption that the modified prion protein is the infectious agent responsible for this disease. However, other scientists believe that the disease is due to a prion associated with another infectious agent, such as a bacterium or a virus, or that the prion is simply a receptor for a conventional agent. Several measures have been taken to eradicate this disease:

- destruction of contaminated carcasses;
- ban on the use of meat and bone meal in cattle feed;
- ban on the consumption of potentially dangerous tissue (ileum, spinal ganglion, brain, spinal cord, retina, inner ear);
- detection of prion protein in carcasses at the slaughterhouse;
- slaughter of an entire herd when an animal is contaminated.

Lipid Oxidation

Lipids play a vital role in our diet. They have a nutritional function by providing energy, essential fatty acids, fat-soluble vitamins and hormone precursors, as well as a sensory function by contributing to the texture and palatability of food.

The main problem with lipids is that they oxidize easily. It is one of the primary causes of food spoilage. The lipid oxidation reaction is most commonly initiated between polyunsaturated lipids and oxygen. Several decomposition and polymerization reactions occur simultaneously, causing the formation of a complex mixture of reaction products: aldehydes, ketones, alcohols, hydrocarbons and polymers responsible for the physical-chemical and sensory properties of oxidized lipids. In most cases, the production of compounds that emit unpleasant tastes or odors, commonly referred to as rancidity, is undesirable. However, there are exceptions. The products of lipid oxidation also include desirable flavors created during the cooking of meat or the ripening of certain cheeses. At the same time, lipid oxidation can reduce nutritional quality, modify the texture and color of foods, and produce compounds that are harmful to human health.

The chemical reactions that occur during lipid oxidation are complex. This is due to the large number of substrates involved in oxidation, the diversity of oxidation mechanisms, the number of pro-oxidant and/or antioxidant agents that modulate reaction kinetics and, finally, the multitude of decomposition and polymerization reactions involved.

Chapter written by Thomas CROGUENNEC.

4.1. Lipid substrates

Free fatty acids or esterified fatty acids in the form of glycerides are susceptible to oxidation. Free fatty acids oxidize faster than triglycerides proving the importance of lipase action in the oxidation process. However, it is essentially the degree of lipid unsaturation that determines the susceptibility of fatty acids to oxidation. Lipid oxidation is either triggered by the addition of a “singlet” oxygen molecule to a double bond in the aliphatic chain of a fatty acid, or by the removal of a hydrogen atom from the aliphatic chain. In the latter case, the susceptibility of lipids to oxidation depends on the lability of hydrogen atoms in the aliphatic chain. The carbon–hydrogen bond energy in saturated aliphatic systems is approximately 400 kJ per mol. The energy of the carbon–hydrogen bond adjacent to a double bond (i.e. oleic acid) is lower, at around 340 kJ per mol, and lower again in a methylene group (i.e. linoleic acid), at around 300 kJ per mol. However, whether it is in a saturated or unsaturated aliphatic chain, the spontaneous removal of a hydrogen atom is difficult and unlikely. It is facilitated by high temperatures, light, the presence of certain polyvalent metal ions and the presence of a pre-existing radical. The relative oxidation rates at 100°C of a fatty acid with a chain length of 18 carbon atoms would be:

$$18:3 \text{ (relative oxidation rate: 3,000)} > 18:2 \text{ (1,000)} > 18:1 \text{ (100)} \gg 18:0. \quad [4.1]$$

Thus, oils rich in polyunsaturated fatty acids such as fish oils are very susceptible to oxidation. However, the oxidation rates are modulated by environmental conditions such as the oxygen content, pH, the water content of the product or the presence of antioxidants.

4.2. Lipid oxidation mechanisms

Lipid oxidation cannot occur spontaneously in the presence of molecular oxygen and fatty acid molecules in their ground state. Molecular oxygen in its ground state (“triplet state”), symbolized by $^3\text{O}_2$ or simply O_2 , is paramagnetic and contains two unpaired electrons, making it a diradical. In this form, oxygen cannot react with fatty acid molecules that are generally in a “singlet” state, since it is a spin-forbidden process. It can only react with molecules with unpaired electrons, i.e. free radicals.

Oxygen in its “singlet” state, denoted as $^1\text{O}_2$, in which the two highest energy electrons are paired, is the first excited state of the oxygen molecule; it has excess energy (92 kJ mol^{-1}) compared to “triplet” oxygen. In its singlet state, oxygen can react with fatty acid molecules in a singlet state.

Thus, to initiate the lipid oxidation reaction, fatty acids or oxygen needs to be activated. The activation of a fatty acid molecule to a free radical is known as the mechanism of lipid autoxidation. Lipid oxidation by singlet oxygen requires the prior activation of molecular oxygen, which is the second mechanism resulting in lipid oxidation.

4.2.1. Lipid autoxidation

Lipid autoxidation is an autocatalytic radical chain reaction. A sequence involving an initiation stage corresponding to the activation of the fatty acid molecule, a propagation phase and termination reactions was proposed to describe lipid autoxidation (Figure 4.1).

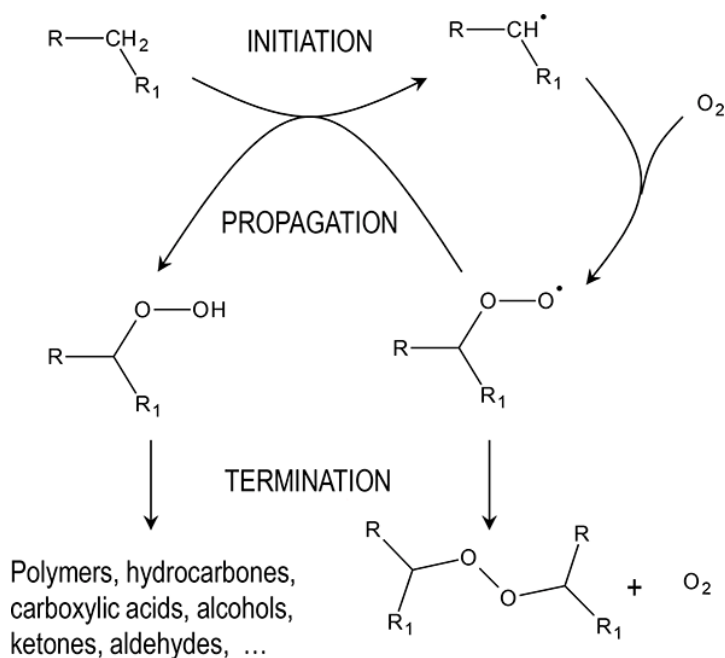


Figure 4.1. General outline of lipid autoxidation

Once lipid oxidation has been initiated by the removal of a hydrogen atom from a fatty acid chain, the three groups of reactions take place simultaneously.

4.2.1.1. Initiation

Reaction initiation involves the formation of a free radical by the removal of a hydrogen atom from a fatty acid chain that is usually unsaturated:



Lipid oxidation is very slow initially, owing to the low initiation rate. The removal of a hydrogen atom is in fact unlikely owing to the high activation energy of the reaction. However, it is facilitated by:

- heating (thermolysis);
- light (photolysis);
- ionizing radiation;
- the presence of polyvalent metal ions, free or bound to organic molecules;
- certain enzymes (lipoxygenase).

When the hydrogen atom has been removed from the α position of a double bond, the unpaired electron of the radical structure is stabilized by resonance. In the case of oleic acid, the radical is formed in position n-7 or n-10. Due to the delocalization of the unpaired electron by resonance, four fatty acid-free radicals (positional isomers) are obtained (Figure 4.2).

Fatty acid radicals in positions n-7 and n-10 are slightly more abundant than fatty acid radicals in positions n-8 and n-9. However, the relative proportion of each fatty acid radical varies with temperature. A temperature increase tends to homogenize radical populations. Due to the possibility of double bond isomerization during the delocalization of electrons by resonance in the radical state, oleic acid can be a source of eight allylic hydroperoxides: four of them with a double bond in *cis* conformation and the other four in *trans* conformation.

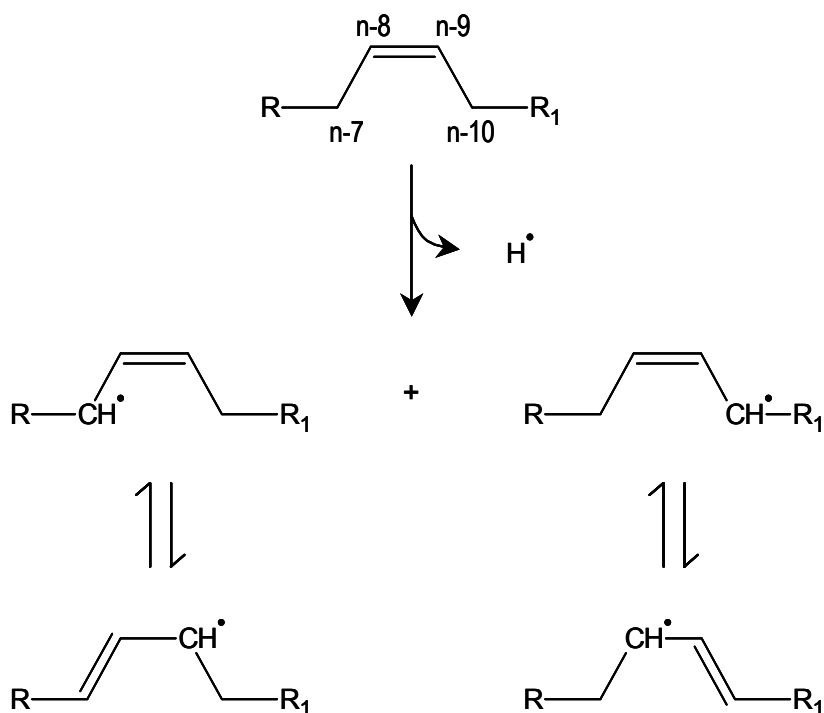


Figure 4.2. Autoxidation mechanism of monounsaturated fatty acids

In polyunsaturated fatty acid chains, the removal of the hydrogen atom generally occurs in the methylene group included in the *cis*, *cis*-1, 4 pentadiene moiety; for linoleic acid, the radical structure is formed in position n-7 (Figure 4.3).

Conjugated double bonds bearing a radical in position n-5 or n-9 are favored from an energy point of view because they stabilize the radical form. They are responsible for 98% of the peroxides formed from linoleic acid. The conjugated linoleic acid radicals can also change the conformation of their double bonds before reaction with oxygen: initially in *cis*, *trans* conformation, the conjugated double bonds can change to *trans*, *trans* conformation.

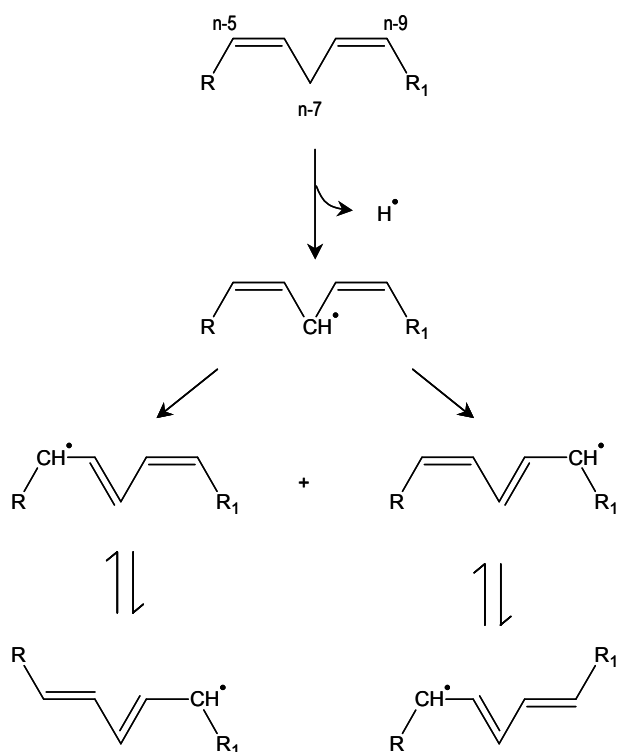
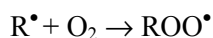


Figure 4.3. Autoxidation mechanism of polyunsaturated fatty acids

4.2.1.2. Propagation

These fatty acid radicals react with triplet oxygen $^3\text{O}_2$ dissolved in the lipid phase or atmospheric phase after diffusion. The reaction of a fatty acid radical with an oxygen molecule is very fast when oxygen is freely available, with a rate constant of $3 \times 10^8 \text{ mol}^{-1} \text{ l s}^{-1}$. The reaction leads to the formation of a peroxy radical ($\text{ROO}^\bullet$). Its structure is stabilized by a hydrogen atom coming from another fatty acid ($\text{R}' - \text{H}$). This newly-formed fatty acid radical ($\text{R}'^\bullet$) can continue the reaction based on the same principle, which constitutes the propagation phase, described as follows:



Propagation is possible without external energy input because the redox potential of hydroperoxides ($\text{ROO}^\bullet/\text{ROOH} \sim 1 \text{ V}$) is greater than that of fatty acids ($\text{R}^\bullet/\text{R}'\text{H} \sim 0.6 \text{ V}$); propagation is autocatalytic. The rate constant of the propagation reaction is $10 \text{ mol}^{-1} \text{ l s}^{-1}$. Therefore, if oxygen is freely available, the vast majority of radicals are in the peroxy form.

In the propagation phase, a single fatty acid free radical can initiate the formation of several hydroperoxide molecules (1,000 or more per minute). The quantity of hydroperoxides generated corresponds to the quantity of oxygen consumed during the oxidation of fatty acids. The rate of formation of hydroperoxides accelerates over time (Figure 4.4).

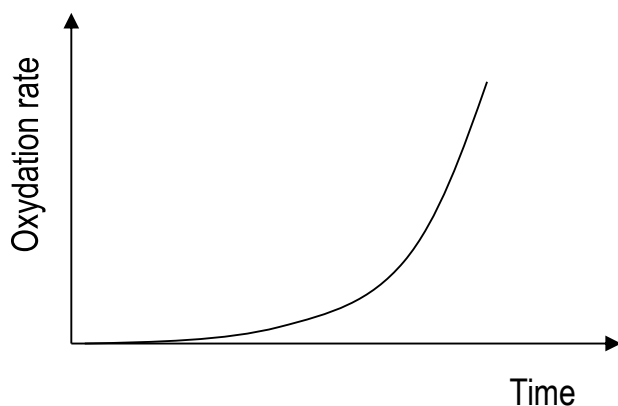
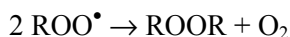
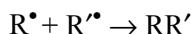
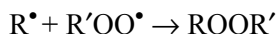


Figure 4.4. Schematic representation of the kinetics of lipid oxidation

4.2.1.3. Termination

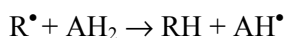
When the concentration of free radicals becomes high enough, they can combine to terminate the autooxidation reaction:



[4.4]

The last of these reactions predominates when the partial pressure of oxygen is high. The activation enthalpy of the termination reactions is low, but these reactions are limited by the collision probability of radicals. In oils heated to a high temperature, these reactions occur rapidly because hydroperoxides decompose spontaneously above 160°C and increase the concentration of free radicals.

Some molecules with a lower redox potential than fatty acid radicals and peroxy radicals can also terminate the autoxidation reaction. This is the case with antioxidants (AH_2) like tocopherols, ascorbic acid, BHA (butylated hydroxyanisole) or BHT (butylated hydroxytoluene). By reacting with free radicals, these molecules interfere with the propagation process. They therefore inhibit or delay lipid oxidation.



The termination rate constant of the propagation process in lipid oxidation by α -tocopherol (vitamin E) is $8 \times 10^4 \text{ mol}^{-1} \text{ l s}^{-1}$, or around 10^4 times higher than the rate constant of the propagation reaction. Consequently, low concentrations of α -tocopherol are sufficient for effective antioxidant activity.

4.2.2. Lipid oxidation by singlet oxygen

The activation of molecular oxygen to singlet oxygen occurs either by the loss of an electron from the superoxide anion $O_2^{\bullet-}$ obtained by the interaction of triplet oxygen with a metal, or by the photooxidation of triplet oxygen in the presence of a photosensitizer. The most common naturally occurring photosensitizers are chlorophyll, pheophytin, metalloporphyrin and riboflavin. Singlet oxygen is 1,000–1,500 times more reactive toward double bonds than triplet oxygen. As a result, edible oils rich in unsaturated fatty acids and stored in clear bottles should be exempt from chlorophyll residue.

The mechanism of lipid oxidation by singlet oxygen differs considerably from the radical pathway previously described. Singlet oxygen reacts

directly with unsaturated carbons in double bonds by addition reaction, inducing a relocation and a change in conformation of the double bond from *cis* to *trans*. Hydroperoxides are formed without the intermediate formation of radicals (Figure 4.5).

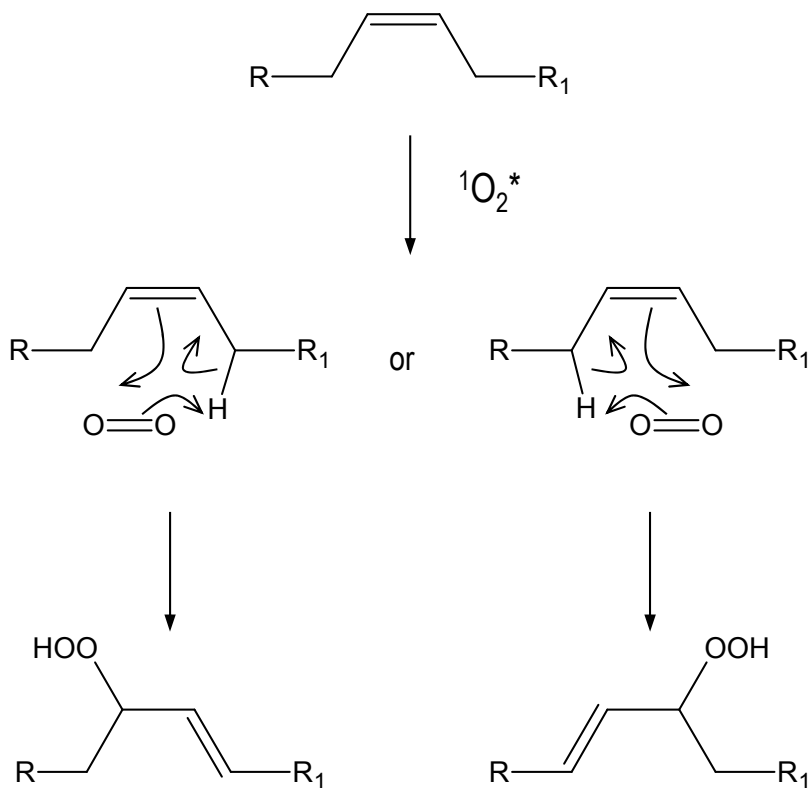


Figure 4.5. Oxidation mechanism of unsaturated fatty acids by singlet oxygen

With oleic acid, the attack of singlet oxygen generates two allylic hydroperoxides. Oleic acid hydroperoxides have a double bond in *trans* configuration (Figure 4.5). In comparison, the autooxidation of oleic acid produces a mixture of four allylic hydroperoxide isomers. Each can have a double bond in either *cis* or *trans* configuration.

4.3. Main compounds derived from lipid oxidation

There are several different derivatives of lipid oxidation such as alcohols, aldehydes, ketones, carboxylic acids, esters, hydrocarbons, and polymers as well as heterocyclic derivatives like lactones and alkylfurans. Some are volatile while others are not, but they all derive from the decomposition of hydroperoxides accumulated over the initial phase of lipid oxidation. Sensory perception is not affected by the accumulation of hydroperoxides. However, some products resulting from the decomposition of hydroperoxides have very low sensory perception limits. Many of these compounds are responsible for the rancid flavor of oxidized products. Hundreds of volatile compounds derived from lipid degradation are found in cooked meat.

Hydroperoxides accumulate at the beginning of the lipid oxidation reaction. They are relatively stable, but in the presence of metal ions or at a high temperature, they decompose rapidly into alkoyl and hydroxyl radicals:



Alkoyl radicals are highly reactive. They acquire a stable molecular structure, either by capturing a hydrogen atom from the first molecule they encounter or by decomposition. They form a wide range of volatile or non-volatile compounds.

In removing a hydrogen atom from a new fatty acid molecule, alkoyl radicals participate in the propagation of lipid oxidation. Meanwhile, alkoyl radicals are stabilized as alcohol (hydroxy acid or hydroxy ester):



Where steric conditions permit, γ - or δ -hydroxy acids or γ - or δ -hydroxy esters can undergo cyclization and form lactones (Figure 4.6), the olfactory characteristics of which depend on the *R* or *S* configuration.

When alkoyl radicals decompose they can create aldehydes or ketones by cleavage of the aliphatic chain (Figure 4.7). Cleavage of the aliphatic chain also leads to the formation of hydrocarbons. These compounds are themselves likely to participate in other chain reactions such as Maillard reactions, Diels-Alder cyclizations and polymerization reactions.

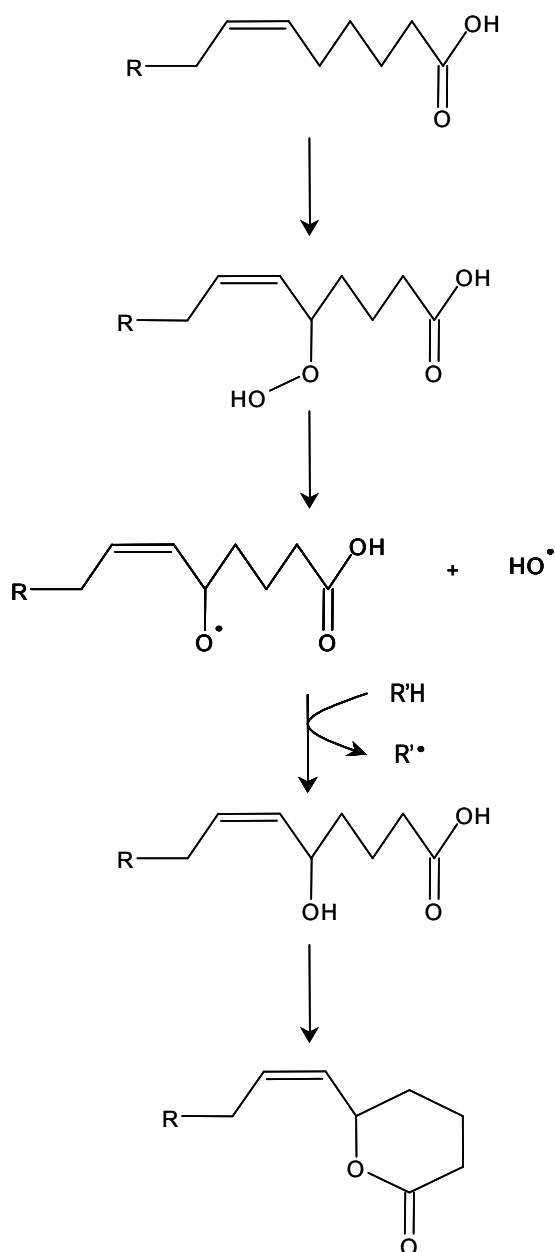


Figure 4.6. Oxidation of lipids and formation of lactones

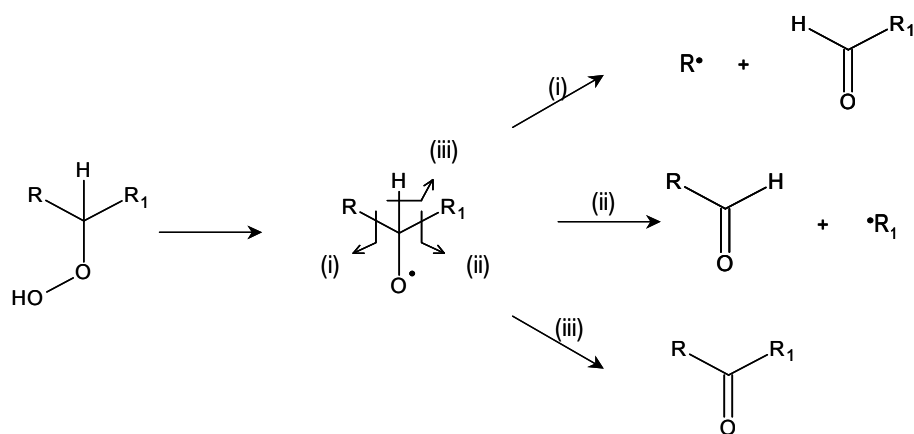


Figure 4.7. Formation of aldehydes and ketones by hydroperoxide decomposition. (i)–(iii) Different ways of stabilizing the alkoyl radical

Many products of lipid oxidation are small molecules richer in oxygen atoms than their precursors, which gives them surfactant properties and makes them more soluble in the aqueous phase. The distribution of lipid decomposition products between the aqueous and the lipid phase affects the sensory perception of foods because the flavor components are perceived more strongly in the aqueous than in the lipid phase. For this reason, in the refining of edible oils, lipid oxidation derivatives are removed during the washing stages.

4.4. Factors affecting lipid oxidation

Lipid oxidation depends on the nature of the fat (solid, liquid, whether or not it is emulsified), a number of physical-chemical parameters such as the oxygen content of the medium, temperature, a_w , pH, and the presence and activity of pro-oxidant or antioxidant agents. In foods, all these elements are in unstable equilibrium. Changes in the physical-chemical conditions of the foods during their handling, processing or storage can bring about rapid changes in lipid oxidation, and consequently in the sensory, nutritional or functional quality of foods.

4.4.1. Oxygen content

As previously indicated, the presence of oxygen is vital for lipid oxidation. The following equations represent the simplified expression of the lipid oxidation rate:

General expression:

$$v = -\frac{d[O_2]}{dt} = \frac{d[ROOH]}{dt} = \frac{A [RH] [O_2]}{[O_2] + k[RH]} \quad [4.8]$$

For a high oxygen content, the above general expression simplifies as:

$$v = A [RH] \quad [4.9]$$

For a low oxygen content, it simplifies as:

$$v = \frac{A[O_2]}{k} \quad [4.10]$$

where v is the rate of lipid oxidation; $[O_2]$ and $[RH]$ represent concentrations of oxygen and lipids, respectively; and A and k are constants obtained from the combination of the rate constants for the initiation, propagation and termination stages of lipid oxidation.

When the oxygen content is high, the rate of lipid oxidation becomes independent of the oxygen content (equation [4.9]), whereas the rate of lipid oxidation is directly proportional to the oxygen content when the latter is low (equation [4.10]). Thus, the representation of the rate of lipid oxidation as a function of the oxygen content of the medium consists of two linear parts for low and high levels of oxygen (Figure 4.8).

The lipid oxidation rates obtained at high and low levels of oxygen depend on the nature of lipids in the food and the physicochemical composition of the food.

Figure 4.8 shows that in order to significantly reduce lipid oxidation, it is necessary to lower the oxygen content of the medium to a level where the rate of lipid oxidation follows a first-order reaction with regard to the

oxygen concentration. This can be achieved by vacuum or nitrogen packaging or by using oxygen scavengers.

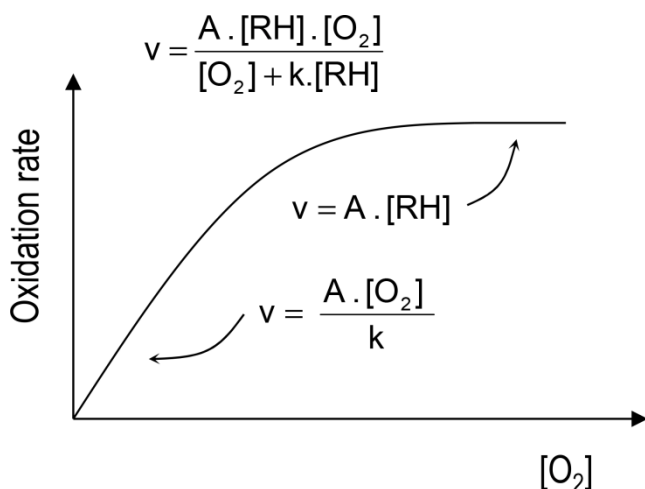


Figure 4.8. *Influence of the oxygen content on the rate of lipid oxidation*

Furthermore, oxygen is naturally more soluble in the lipid phase than in the aqueous phase (around three times more at 25°C). Thus, at low oxygen levels, the rate-limiting step in lipid oxidation (slope of the initial phase) is the diffusion of oxygen in the aqueous phase. The aqueous phase therefore acts as a protection barrier against lipid oxidation. Under these conditions, raising the temperature by reducing the solubility of oxygen in the aqueous phase slows down the rate of lipid oxidation. Conversely, the porosity of food or the agitation speed, which increases the surface/volume ratio, favors the diffusion of oxygen and lipid oxidation.

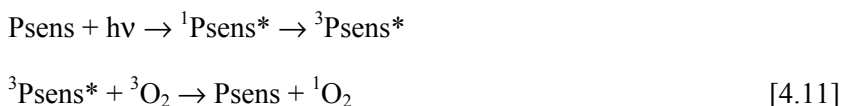
4.4.2. Catalysts of lipid oxidation

Due to the number of processes that food undergoes, it is exposed to several initiators of lipid oxidation or oxidation catalysts, with the most significant being exposure to light, transition metals or certain enzymes. Except those generated by light, free radicals in foods are usually generated in the aqueous phase or at the aqueous–lipid interface.

4.4.2.1. Electromagnetic radiation

Exposure of food to light enhances the flavor of oxidized lipids. Its effect depends on the wavelength of the incident electromagnetic radiation, the intensity and exposure time as well as the penetration capacity of the radiation in food.

Ultraviolet radiation can induce the loss of a hydrogen atom from the aliphatic chain of an unsaturated fatty acid due to the energy of the light itself. However, it is mainly through the intermediary of photosensitizers that photooxidation of lipids occurs. Natural photosensitizers like chlorophyll, porphyrins or riboflavin can transform triplet oxygen ($^3\text{O}_2$) into highly reactive singlet oxygen ($^1\text{O}_2$) upon the absorption of visible light. In this mechanism, the electromagnetic energy conveyed by the visible radiation excites the photosensitizer (Psens) first into a singlet state ($^1\text{Psens}^*$) and subsequently into a triplet state ($^3\text{Psens}^*$), which is compatible with molecular oxygen in its ground state (triplet state). The energy acquired by the photosensitizer is therefore restored and transferred to triplet oxygen, which is converted to singlet oxygen:



In turn, singlet oxygen reacts with the double bonds of a singlet state acceptor, in most cases lipids (RH), which results in the formation of hydroperoxides (ROOH), as indicated in Figure 4.7.

4.4.2.2. Transition metals

Transition metals (iron, copper, nickel, cobalt, etc.), whether free or complexed (hemoglobin), are powerful catalysts of lipid oxidation. They are usually found in the aqueous phase and catalyze lipid oxidation from the aqueous phase or at the lipid interface. In the latter case, the nature of the interface is important. When the emulsified fat interface is negatively charged, the rate of lipid oxidation by metals is faster than in the case of positively-charged interfaces. This is due to the electrostatic interactions between positively-charged metals and the negative charges of the interface; this results in an increase in the interfacial concentration of catalytic agents.

Transition metals are involved either through the decomposition of peroxides via the Fenton reaction or the removal of a hydrogen atom from the aliphatic chain of a fatty acid. In both cases, there is a formation of free radicals, and, at the same time, an oxidation or reduction of the metal ion. The reduced form of the metal ion is often the most reactive.

During the decomposition of hydroperoxides into alkoyl radicals, the metal ion is oxidized (Fenton reaction); it moves from its lowest valence state (M^{n+}) to its highest valence state ($M^{(n+1)+}$). This reaction is usually very fast. The regeneration of the reduced form (M^{n+}), which is much slower, can be obtained through the formation of a radical from a fatty acid chain. Thus, transition metals can participate in several redox cycles (Figure 4.9).

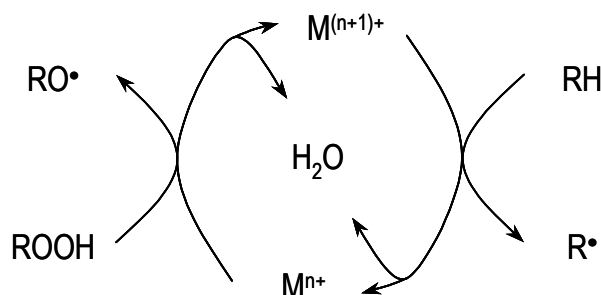
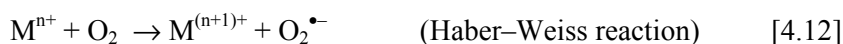
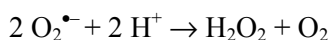


Figure 4.9. Redox cycle of metal agents in lipid oxidation

Typically, the metal ions in the reduced form, M^{n+} , are ferrous iron (Fe^{2+}) or copper (I) (Cu^+); the metal ions in the oxidized form, $M^{(n+1)+}$, are ferric iron (Fe^{3+}) and copper (II) (Cu^{2+}).

In addition, transition metals can initiate lipid oxidation by a reaction with oxygen and the formation of a superoxide anion ($O_2^{\bullet-}$). The latter forms hydrogen peroxide (H_2O_2) (Haber–Weiss reaction), which decomposes according to the Fenton principle into highly reactive hydroxy radicals ($OH^{\bullet}$):





Lipid oxidation in the muscle tissue of beef, chicken or fish can occur via hemoglobin in a similar mechanism. Hemoglobin possesses a porphyrin core and an iron ion bound to the heme by coordination bonds. In fresh tissue, almost all heme iron is ferrous iron: myoglobin or oxymyoglobin when an oxygen molecule is bound to ferrous iron. The oxidation of oxymyoglobin into metmyoglobin causes the release of oxygen in the form of a superoxide anion, which forms hydrogen peroxide. The latter further reacts with ferrous iron according to the Fenton reaction.

Transition metals can also participate in the formation of singlet oxygen after the loss of an electron by superoxide anions. As already mentioned, singlet oxygen is highly reactive toward lipid double bonds.



Based on these different mechanisms, trace metals may promote lipid oxidation by redox chain reactions. However, food naturally contains metal ions, which inevitably exposes it to oxidation.

4.4.2.3. Enzymatic catalysts

Several enzymes can catalyze lipid oxidation but the most active are lipoxygenases. These lipid oxidation catalysts are very common in plants such as legumes (soya bean), grains (wheat, barley, corn), tubers or leaves as well as some animal tissue. The substrate of lipoxygenases is a *cis*, *cis*-1, 4 pentadiene double bond system found in polyunsaturated fatty acids like linoleic acid, linolenic acid or arachidonic acid.

Lipoxygenases catalyze the formation of hydroperoxides via a process similar to that of lipid autoxidation. One feature of enzymatic oxidation is that the hydroperoxides formed are site-specific as well as stereo- and enantiospecific. For example, soya lipoxygenase LOX1 oxidizes linoleic acid to give 13(S)-hydroperoxy *cis*, *trans*-9, 11 octadecadienoic (Figure 4.10). Due to their optical activity, some hydroperoxides are markers for enzymatic oxidation.

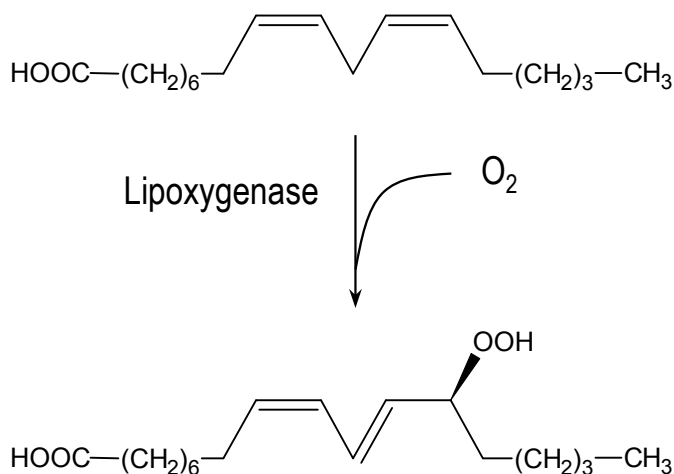


Figure 4.10. Oxidation of linoleic acid by soya lipoxygenase LOX1

To initiate the oxidation of lipids and to maintain their activity, lipoxygenases require the presence of hydroperoxides. In fact, lipoxygenases are metalloproteins with a non-heme iron, which is in the ferrous state in the inactive enzyme. Activation of the enzyme occurs by oxidation of ferrous iron to ferric iron coupled with the decomposition of a hydroperoxide to a radical alkoyl. Once active, the enzyme catalyses the removal of a hydrogen atom specifically located on the fatty acid chain. The iron redox cycle is similar to that described in Figure 4.9.

Although often unwanted, the oxidation of unsaturated lipids by lipoxygenase is sometimes desired. This is the case in bread-making, where hydroperoxides released by the enzyme contribute to the mechanical properties of the dough. In addition, oxidation reactions destroy pigments as well as some substances naturally present in the flour. This is favorable to the sensory qualities of the bread.

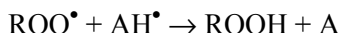
4.4.3. Inhibitors of lipid oxidation

Some molecules naturally present in foods have an antioxidant effect on lipids either by limiting the propagation of free radicals or by controlling the activity of oxidation catalysts. Thus, they delay or slow down the onset of coloration and/or flavor development due to lipid oxidation.

4.4.3.1. Antiradical action of antioxidants

Some antioxidants are radical scavengers. They act by transferring a hydrogen atom to the radical species. Antioxidants generally react with peroxide radicals as they are the most common radicals in lipid oxidation. This hydrogen exchange significantly delays the propagation of lipid oxidation.

The effectiveness of an antioxidant is due to the presence on its structure of a low energy bond involving a hydrogen atom. The lower the bond energy, the more likely it is that the hydrogen atom rapidly transfers to a lipid radical. The effectiveness of an antioxidant also depends on its ability to reduce the energy of its radical structure to prevent it turning into an oxidation catalyst. Thus, radical antioxidants ($AH^\bullet$) are often stabilized by a resonance delocalization of the unpaired electrons. They can transmit a second hydrogen atom to another lipid radical and adopt an even more stable non-radical molecular structure (A) or react with each other to form a stable dimer ($HA-AH$):



The most common chemical structures involved in scavenging radicals are the hydroxyl groups of phenol derivatives, thiol group of cysteine or amino groups of uric acid, certain alkaloids or proteins. Of these, phenol derivatives, the general formula of which is given in Figure 4.11, are the most effective.

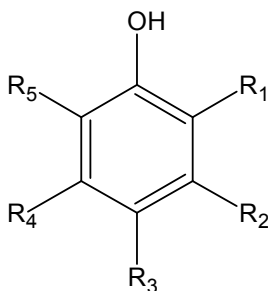


Figure 4.11. Chemical formula of phenol derivatives

They rapidly transfer one or two hydrogen atoms and form a stable phenol derivative, radical or non-radical (Figure 4.12). Ortho and para forms are the most effective derivatives because they provide relatively stable radicals due to the favorable electron delocalization by resonance. The larger the substituent group is, the more stable the antioxidant radical; however, the antioxidant action decreases in this case.

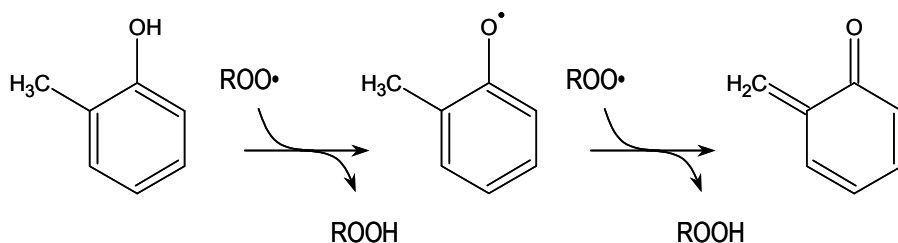
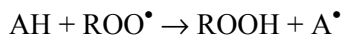


Figure 4.12. *Mechanism of action of antioxidants*

The protection of foods provided by antioxidants is temporary, since they are consumed until complete disappearance by the scavenging of radicals. The disappearance kinetics of antioxidants is directly linked to the unsaturation of the fat. Highly unsaturated lipids quickly consume antioxidants. Conversely, in lipids low in unsaturated chains, the consumption of antioxidants is much slower. As long as the antioxidants are present in the system, the oxidation process is limited to the initiation phase. As soon as the antioxidants are fully consumed, oxidation accelerates rapidly.

Sometimes, there is a synergy between the antioxidant and a compound with no direct antioxidant properties:



The BH compound must be less reactive than the antioxidant with regard to $\text{ROO}^\bullet$, and the free radical $\text{B}^\bullet$ should be more stable than $\text{A}^\bullet$. In this case, the antioxidant AH is recycled.

4.4.3.2. Anticatalytic action of antioxidants

Other antioxidants act through indirect mechanisms by inactivating the lipid oxidation catalyst. Some molecules such as carotenoids interfere with the photooxidation mechanism of lipids by singlet oxygen. They compete with unsaturated lipids for the addition reaction of singlet oxygen or they inactivate photosensitizers in the excited state by absorbing their excess energy. During the inactivation of photosensitizers, carotenoids go through an excited state. Unlike photosensitizers, carotenoids in the excited state are able to return to their lowest energy state by dissipating excess energy into the environmental medium without the formation of singlet oxygen.

The pro-oxidant activity of transition metals is controlled, at least partially, when included in complexes. Several molecules are able to complex metal ions, but not all have the same ability to inhibit oxidant activity. Some can even promote lipid oxidation by increasing the solubility of metal ions or by altering their redox potential.

However, usually transition metals are complexed with proteins (transferrin, ferritin, carnosine), peptides, amino acids (histidine), organic salts (citrate, phytate) or inorganic salts (polyphosphates) in an oxidized form incapable of generating superoxide anions or decomposing hydroperoxides. Some enzymes also play a vital role in inhibiting lipid oxidation. Ferroxidase catalyzes the oxidation of highly reactive ferrous iron to less reactive ferric iron. Superoxide dismutase, together with catalase and glutathione peroxidase, converts the superoxide anion into water, thereby reducing the production of highly reactive hydroxyl radicals. Phenolic compounds are able to indirectly inhibit the activity of lipoxygenase by reducing the iron in the active site of the enzyme to ferrous iron.

4.4.4. Physical-chemical factors

4.4.4.1. Water activity (a_w)

Water activity and the physical state of water strongly influence the oxidative stability of a food. The maximum stability of lipids with regard to oxidation is reached at an a_w range of between 0.2 and 0.4. The relative rate of lipid oxidation increases sharply either side of this range, for a_w values of

between 0.2 and 0 or 0.4 and 0.7. The rate of lipid oxidation slows down or decreases above an a_w of 0.7 (Figure 1.2).

The influence of a_w is complex because it involves several mechanisms. Water can increase the rate of lipid oxidation by increasing the mobility of the reactants. It can also slow oxidation down by delaying the decomposition of hydroperoxides and diluting the oxidation catalysts.

Starting from a low a_w , an increase in the water content results in the formation of a monomolecular hydration layer around the solute molecules. It protects lipids from direct contact with oxygen. It also protects hydroperoxides from decomposition and lowers the catalytic activity of metal agents by solvating them. The a_w values responsible for the monomolecular hydration layer correspond to the optimum stability of lipids with regard to oxidation. When the water content is greater than that required for the formation of a hydration monolayer around the solute molecules, the water facilitates the diffusion of molecules, which increases the rate of oxidation. At a_w values above 0.7, the rate of lipid oxidation slows down or decreases through the dilution of oxidation catalysts.

4.4.4.2. pH

pH is involved in the oxidation mechanism of lipids, mainly by modifying the solubility and activity of catalysts and oxidation inhibitors.

Regarding oxidation catalysts, metal agents are not very soluble at alkaline pH where they precipitate as hydroxides. Lowering the pH increases their solubility and may induce release from complexation: in the case of milk, maximum lipid oxidation occurs at pH 3.8. Lipoyxygenase activity depends on pH. Some are more active at acidic pH and others at alkaline pH, but the majority have a maximum activity level around neutral pH (pH 6.0–8.0).

The activity of antioxidants is generally linked to their solubility, but not exclusively. Polyphenols are more active at alkaline pH, where their solubility is the highest. Lowering the hydroxyl bond energy while increasing pH facilitates the transfer of a hydrogen atom to lipid radicals. Conversely, cepharanthine, which belongs to the alkaloid family, is more soluble at acidic pH but its antioxidant activity is optimum at basic pH due to the much more effective transfer of the hydrogen atom carried by the

asymmetric carbon. Therefore, antioxidant solubility is not the only criterion for assessing antioxidant activity (Figure 4.13).

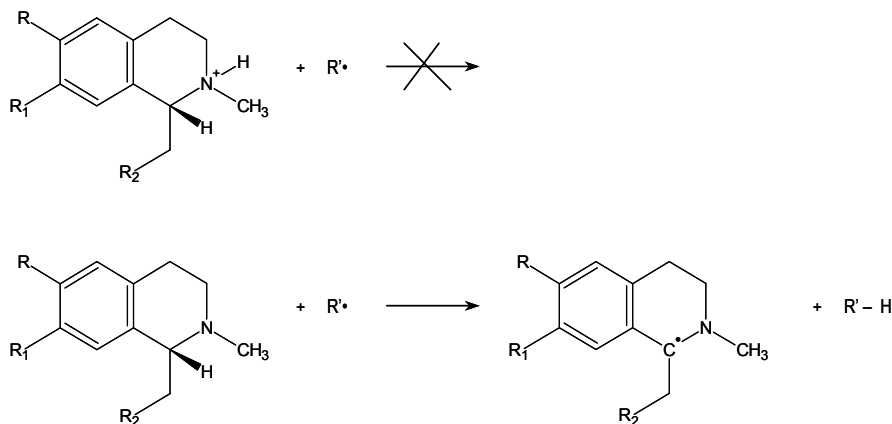


Figure 4.13. *Effect of pH on the antioxidant activity of cepharanthine*

The accessibility of fat to catalysts or oxidation inhibitors also influences the rate of lipid oxidation. In emulsion, when fat is protected by negatively-charged surfactant molecules, lipid oxidation catalyzed by metal agents is greatly accelerated by the drop in pH because of the increasing solubility of metal ions. Electrostatic interactions between the negatively-charged surfactants and the positively-charged metals allow their concentration at the interface. In contrast, no variation in the rate of lipid oxidation as a function of pH is observed when the interface is composed of positively-charged surfactants. For protein interfaces, the rate of lipid oxidation is minimal at the pI (isoelectric point) of proteins. In fact, proteins are likely to form a thicker viscoelastic interfacial film close to their pI, which slows down the penetration and diffusion of oxidation catalysts towards fatty acids.

4.4.4.3. Temperature

The effect of temperature on lipid oxidation is also complex and depends on the oxygen concentration in the medium. When oxygen is not limited, the rate of lipid oxidation is generally governed by the Arrhenius law and increases with temperature. However, the relative contribution of various

oxidation initiation mechanisms varies depending on temperature. For example, when temperature is increased, non-enzymatic initiation processes contribute more compared to enzymatic initiation processes. In cooked products, lipid oxidation reactions are almost exclusively catalyzed non-enzymatically, since most enzymatic systems are destroyed. Thus, blanching of vegetables (or fruits) delays the development of undesirable colors and flavors compared to simply refrigerating or freezing them. Blanching is a brief heating process used to inactivate enzymes that catalyze lipid oxidation, such as lipoxygenase. However, overly intense heat treatments can cause protein denaturation and the release of complexed metal ions, thereby promoting lipid oxidation.

When the oxygen concentration is limited, the rate of lipid oxidation increases as the temperature drops due to the increased solubility of oxygen in the aqueous phase. So as the temperature drops, crystallization of the lipid fraction with a higher melting point excludes oxygen from crystallized zones. Oxygen is therefore concentrated with the most unsaturated lipid fraction, which facilitates the propagation of lipid oxidation.

4.5. Evaluation of susceptibility to oxidation and the level of oxidation

Lipid oxidation is accompanied by the appearance and disappearance of many compounds at varying rates depending on the physical-chemical conditions of the food. However it can be summarized as shown in Figure 4.14, which gives the oxygen consumption as well as levels of lipid peroxides, polymers or aldehydes as a function of time.

There is a lag phase during which oxidation occurs slowly, followed by a sharp acceleration in lipid oxidation with an accumulation of peroxides that are subsequently decomposed into small volatile molecules or condensed as polymers. The onset of rancidity usually occurs at the end of the lag phase.

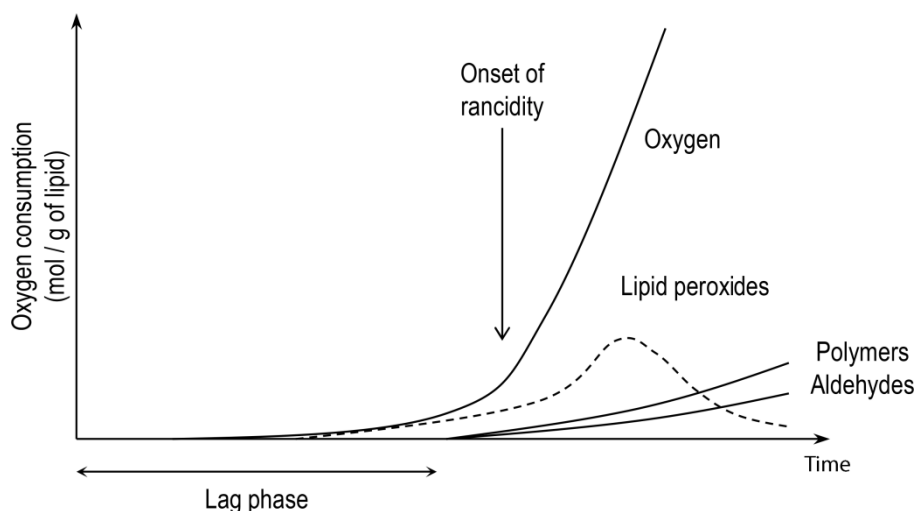


Figure 4.14. *Kinetics of appearance and disappearance of compounds during the lipid oxidation reaction*

Determining the level of lipid oxidation or the susceptibility of a food to oxidation is not always easy, since the correlations between the concentration of oxidation indicators and the sensory deterioration or shelf-life of a product are not always satisfactory. The methods developed are based on measuring selected products from lipid oxidation, which estimates the level of oxidation at any given time, or on measuring the length of the lag phase. The length of the phase corresponds to the resistance or susceptibility of a food to oxidation. It is usually estimated based on the time needed to reach a sudden increase in the oxidation rate or for a rancid flavor to develop. Although tests on oxidation resistance performed in conditions as close as possible to food storage conditions are the most informative in terms of food stability, they are nevertheless unsuitable from a practical point of view. Thus, the susceptibility of a food to oxidation is measured by accelerated oxidation tests under standardized conditions (active oxygen method (AOM); Rancimat; oxygen radical absorbance capacity (ORAC)). Heating is often used to accelerate lipid oxidation. These methods have certain disadvantages since the oxidation rate depends on the oxygen concentration, the solubility of which decreases with increasing temperature. Furthermore, the degradation (phenolic compounds) or volatilization (BHA, BHT) of antioxidants that contribute to food stability with respect to

oxidation and polymerization, cyclization or cleavage reactions become significant at high temperatures.

The methods used to assess the level of oxidation and susceptibility to oxidation can be categorized into three groups.

4.5.1. *Measuring the consumption of oxidation substrates*

The kinetics of oxygen consumption gives an indication on the length of the lag phase. Basically, either the increase in the weight of fat after the fixation of oxygen or the drop in pressure resulting from oxygen consumption is measured. The results always need to be interpreted with caution since, in complex mixtures, oxygen is involved in several reactions and not only in oxidizing lipids. Moreover, the production of volatile compounds through peroxide decomposition can distort measurements.

It is also possible to measure a residual oxidation substrate. This substrate is usually a fatty acid such as linoleic acid or linolenic acid, which is measured by gas chromatography.

4.5.2. *Determination of the peroxide value*

Determination the peroxide value is done via a simple test and is therefore widely used. Peroxides are measured by iodometry, polarography or colorimetry after reaction with ferrous thiocyanate or by gas chromatography after heat shock. The peroxide value is expressed in mg of active oxygen per kg of fat.

In the Swift test or AOM, a lipid sample is continuously aerated or oxygenated at 97.8°C throughout the experiment. The determination of peroxides or the latency time after which incipient rancidity can be detected by smell is used to measure the susceptibility of lipids to oxidation. This method is used to analyze oils or fats but is not suitable for analyzing foods. It can be used in production control but provides no information on the expected shelf life.

The ORAC method measures, through the decrease in fluorescein fluorescence, the peroxidation of a food caused by a stable water-soluble radical initiator, AAPH [2, 2' azobis (2-amidinopropane) hydrochloride].

Fluorescein reacts more slowly with fatty acid radicals than most natural antioxidants, allowing the stability of a food with respect to radical oxidation to be measured. It is expressed by the integrated area under the curve, representing the fluorescence intensity at 515 nm in response to excitation at 493 nm as a function of time (Figure 4.15).

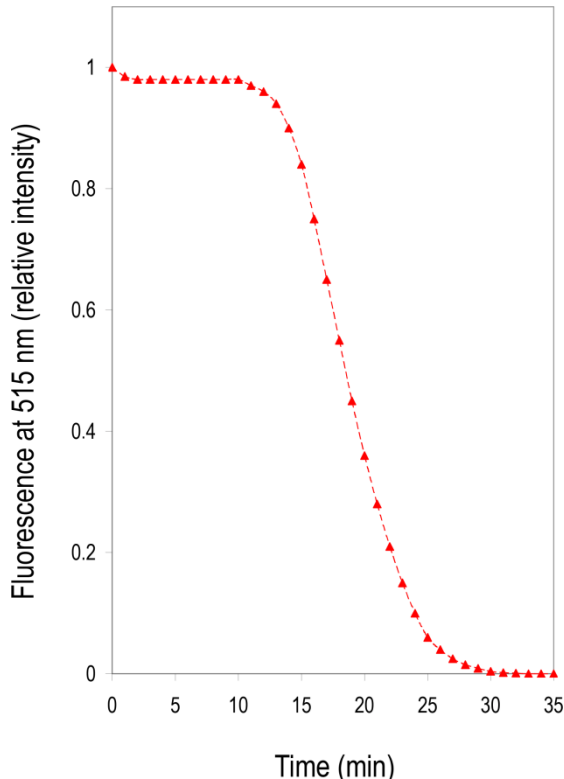


Figure 4.15. *Determination of the oxidative stability of a food product using the ORAC method*

Electron paramagnetic resonance (EPR) spectroscopy can also be used to determine the susceptibility of fat to oxidation. This analysis is based on measuring radicals formed during the initial stages of oxidation. Free radicals are highly reactive species with very short lifetimes. EPR requires the use of radical scavengers such as *N-tert*-butyl phenylnitron (PBN). These species react with free radicals to form much more stable radicals

whose lifetimes are compatible with the EPR analysis time. Unlike accelerated oxidation tests, EPR analyses can be carried out at lower temperatures, around 60°C.

Measuring conjugated dienes by using ultraviolet spectrophotometry provides information on the oxidation state of lipids. The formation of hydroperoxides from polyunsaturated fatty acids like linoleic acid or linolenic acid is accompanied by a double bond migration, which leads to the formation of a conjugated diene that absorbs at 233 nm.

4.5.3. Measurement of peroxide decomposition products

Several methods exist to analyze peroxide degradation products. Their presence reflects an advanced stage of oxidation.

The analysis of volatile compounds by gas chromatography can be well correlated with the development of a rancid flavor. Furthermore, this method can provide useful information on the origin of volatile compounds.

The thiobarbituric acid (TBA) test is a measure of a colored complex resulting from the reaction of certain aldehydes, in particular malonaldehyde, with TBA. The colored complex absorbs at 523 nm. Malonaldehyde comes mainly from the oxidation of polyunsaturated lipids. The TBA test may overestimate the oxidation state, since other colored compounds can interfere with the measurement at 523 nm.

The Rancimat method is an automated instrumental test that measures the conductivity of low-molecular-weight acid, for example formic acid produced during the autoxidation of an aerated or oxygenated fat at a temperature above 100°C. This method requires a greater oxidation level than other methods to achieve measurable values.

4.6. Control and prevention of lipid oxidation

As already mentioned, lipid oxidation in foods depends on the composition, concentration and activity of reaction substrates, as well as the balance of pro-oxidant and antioxidant agents. It is responsible for a loss of nutritional value (decrease in the content of polyunsaturated fatty acids

and/or antioxidants), as well as a modification of the sensory (formation of molecules responsible for undesirable flavors, formation of substrates for non-enzymatic browning reactions) and functional (loss of protein solubility) properties of foods. Furthermore, the lipid oxidation reactions mostly involve free radicals or highly active forms of oxygen, which are involved in the degenerative aging process of the organism or in serious pathologies (especially certain types of cancer, atherosclerosis, cardiovascular diseases and diabetes).

Tackling lipid oxidation is a considerable challenge for food manufacturers. Several options are available to stabilize lipids against oxidation: the first would be to reduce the degree of fat unsaturation, either by chemical hydrogenation as in the case of margarines or by adjusting the diet of animals, or genetically by selecting plants with low desaturase activity. However, for nutritional reasons, reducing the degree of unsaturation of dietary fat is not always desirable.

In these cases, the stability of lipids with regard to oxidation should be achieved by modulating the natural antioxidant and pro-oxidant activity of foodstuffs. First, the endogenous antioxidant activity of foods should be kept to a maximum. To do this, it is necessary to minimize the loss of antioxidants during the technological stages, such as the refining of edible oils (tocopherols, carotenoids), select plants or plant ingredients that are naturally rich in antioxidant molecules, or even incorporate antioxidants into the animal's diet. At the same time, it is important to avoid exposing foods to oxidation catalysts (overly high temperatures or light, especially when foods contain photosensitizers). Other possible solutions include reducing the content of pro-oxidant agents, either by heat treatment to inactivate lipoxygenase (e.g. blanching vegetables), using separation techniques to reduce the chlorophyll content of edible oils, or limiting the amount of metals from water or ingredients in the food composition. It is also possible to genetically select vegetables and grains with low lipoxygenase activity. However, some pro-oxidant agents are of nutritional benefit in foods, for example iron, copper or riboflavin, or of sensory benefit, such as myoglobin or chlorophyll, which are widely used as natural colorings.

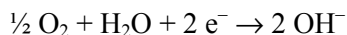
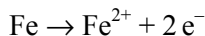
Thus, stabilizing lipids against oxidation requires either the use of particular physical methods to avoid contact between unsaturated fatty acids and oxygen or oxidation catalysts (vacuum or nitrogen storage,

oxygen scavengers, fat encapsulation), or the incorporation of antioxidant molecules.

4.6.1. *Stabilization using physical means*

The oxygen content has a major effect on the rate of lipid oxidation. Thus, oxidative stability can be improved by reducing the oxygen content of the medium by vacuum or nitrogen packaging foods in an oxygen-impermeable material or by glazing, as in the case of frozen fish. In addition, opaque packaging plays a role in protecting oxidizable foods from light-induced oxidation.

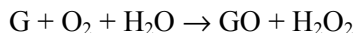
It is impossible using these techniques to remove all the oxygen molecules, since occluded and adsorbed oxygen on the product only desorbs slowly. This is especially true for lyophilized products, which are very porous and highly susceptible to oxidation, so an atmosphere containing even a residual amount of oxygen is not enough to stabilize them. By using oxygen scavengers that absorb residual oxygen after packaging, it is possible to minimize the spoilage of foods susceptible to oxidation. The most common oxygen scavengers operate on the principles of enzymatic oxidation (glucose oxidase coupled with catalase) or iron oxidation. The latter can trap oxygen in the form of iron hydroxide:



These oxygen scavengers come in sachets and are packed at the same time as the food. They are very effective and help ensure the almost complete absence of oxygen during the shelf life of the product. However, there is a potential risk of accidental ingestion of a large quantity of iron despite the warning label “Do not eat”.

Oxygen scavengers based on enzymatic oxidation use glucose oxidase to catalyze the oxidation of glucose (G) to gluconolactone in the presence of

oxygen. This gluconolactone, spontaneously formed, hydrolyses to gluconic acid (GO) and releases hydrogen peroxide. The latter, which is undesirable in foods, is removed by catalase:



In the oxidation reaction catalyzed by the glucose oxidase–catalase enzymatic system, a half mole of oxygen is removed by the oxidation of one mole of glucose. Enzymatic systems for stabilizing foods against oxidation are not suitable for foods with low a_w . They are also very susceptible to variations in pH and temperature. Enzymes can be incorporated as a separate sachet when the food is being packaged or attached to the packaging itself. Polypropylene and polyethylene are effective substrates for immobilizing enzymes.

An alternative to delaying the autoxidation of lipids is to encapsulate the fat phase in a carbohydrate matrix (maltodextrin, arabic gum) or a protein matrix (gelatine), which acts as a barrier against the transfer of oxygen but also oxidation catalysts. The most commonly used encapsulation method is the dehydration of a lipid emulsion where the aqueous phase contains the carbohydrate or protein encapsulation material. It is also possible to solubilize the lipid fraction in a solution containing encapsulating agents before dehydration. Fine lipid droplets spontaneously form because of the supersaturation that occurs with dehydration. Antioxidants (ascorbic acid, α -tocopherol) can also be encapsulated in liposomes that are used as emulsifiers to stabilize lipid dispersions. Thus, antioxidants are released in a controlled manner to where the oxidation reactions have occurred, i.e. the lipid–water interface. In addition, due to their encapsulation, they are protected from reactions with other food compounds.

4.6.2. Formulation

We have seen that molecules naturally present in foods can delay the onset of spoilage due to lipid oxidation. In some cases, these antioxidant molecules are insufficient in quantity to effectively protect the food since they are naturally present in only small amounts or are removed or destroyed

during processing. Thus, effective control of lipid oxidation can be achieved by inactivating free radicals or oxidation catalysts by adding antioxidants.

The most common antioxidants used in foods are chemically synthesized even though the general trend is to reduce the use of synthetic food additives in favor of natural additives. Their use is regulated by dose limits and food categories in which their use is authorized.

Synthetic antioxidants, BHA and BHT (Figure 4.16) are still used despite reservations by nutritionists. They are soluble in the lipid phase and heat resistant, but have the disadvantage of quickly evaporating, which makes it difficult to use them in dried foods. Propyl gallate (Figure 4.16) is water soluble but not very heat resistant and in the presence of iron produces dark salts; it should be used with a metal complexing agent like citric acid, for example.

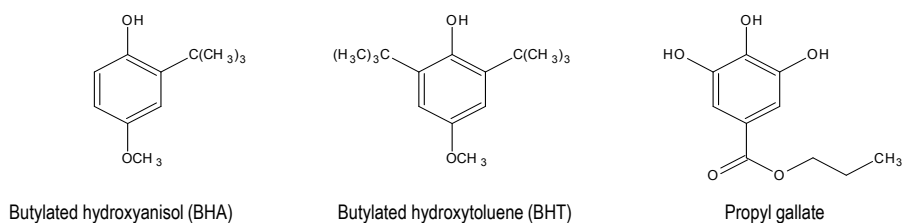


Figure 4.16. *Chemical formulas of certain antioxidants*

Natural antioxidants belong to different chemical families (phenolic compounds, tocopherols, ascorbic acid). Phenolic compounds are essentially phenolic acids, flavonoids or anthocyanins. They operate either by interrupting the propagation phase of oxidation or by inactivating oxidation catalysts. Tocopherols are heat-resistant lipid antioxidants, while ascorbic acid is soluble in the aqueous phase. In the presence of metal agents, ascorbic acid can have a pro-oxidant effect.

Limiting factors for the use of antioxidants in food matrices include their solubility, their interactions with other food components (proteins and phenolic compounds) or changes in color or flavor (herbal extracts) that they

may induce. Fat-soluble antioxidants are ineffective in blocking oxidation initiated in the aqueous phase. The antioxidant activity of carotenoids (which are fat soluble) is not very effective if singlet oxygen is generated by riboflavin (which is water soluble). In contrast, antioxidants soluble in the aqueous phase are effective at inhibiting oxidation reactions catalyzed by metal agents. However, in lipid emulsion they are unable to block the propagation of lipid oxidation, which occurs in the lipid phase or at interfaces. Interfacial antioxidants (α -tocopherol) are usually the most efficient.

Due to these specific features, a combination of antioxidants will, in many cases, be more effective against lipid oxidation due to synergy phenomena.

Non-Enzymatic Browning

Non-enzymatic browning involves a set of chemical reactions that take place during the preparation or storage of foods. It is responsible for the formation of brown compounds, which are volatile flavor molecules that affect the sensory quality of foods. Consequently, non-enzymatic browning is desired in the roasting of coffee and cocoa beans, in grilling, in bread and pastry products, in the manufacture of beer, balsamic vinegar, whiskey and even certain cheeses like hard cheeses. Non-enzymatic browning is undesirable when, if uncontrolled, it negatively affects the sensory characteristics of foods. It is responsible for the cooked flavor of some sterilized milks and fruit juices or the visual defects of dried products (milk powders). Moreover, the nutritional value of food may be modified by non-enzymatic browning. Some examples include the degradation of essential amino acids like lysine, reduced protein digestibility, the formation of potentially toxic compounds or mutagens, or a change in the antioxidant capacity of food. Thus, one of the challenges facing the food industry with regard to non-enzymatic browning is to achieve stability conditions and the desired sensory characteristics for final products while at the same time maintaining their nutritional value.

Non-enzymatic browning is often associated with the Maillard reaction. However, a number of other chemical reactions that do not fall within the definition of the Maillard reaction are involved in non-enzymatic browning. This is the case with the thermal degradation of sugars (caramelization), the oxidation of phenols or the chemical reactions involved in maderization. In this chapter, we will focus more specifically on the Maillard reaction, although other reactions will be referred to for comparison.

Chapter written by Thomas CROGUENNEC.

Defined for the first time by Louis Camille Maillard in 1912, the Maillard reaction comprises a wide range of complex reactions. It refers to all reactions initiated by the interaction of an amino group and a carbonyl group. This involves a whole host of reactions including dehydration, cyclization, retro-aldolization, rearrangement, isomerization as well as condensation resulting in volatile and aromatic molecules on the one hand and high-molecular-weight brown nitrogenous polymers known as melanoidines on the other hand. Several factors influence the rate of the various stages of the Maillard reaction, and therefore the nature and quality of the constituents responsible for the color and flavor profile of products during preparation or storage. They include the nature of the substrates, the time–temperature relationship used during heat treatment or storage, pH, water activity and the presence of activators or inhibitors.

5.1. Substrates

The compounds participating in the Maillard reaction must have a free carbonyl group or a non-protonated amino group. As a result, there are a large number of potential substrates in foods. They are naturally present or are generated during processing or storage, and are the source of many reaction products.

Traditionally, the carbonyl group is the reducing group of a sugar. The latter reacts when the carbohydrate chain conformation is linear. Thus, monosaccharides (e.g. ribose, glucose and fructose) as well as some reducing disaccharides (lactose, maltose) or reducing oligosaccharides participate in the Maillard reaction. Sucrose (a non-reducing disaccharide) does not participate in the Maillard reaction unless it is first hydrolyzed (to an inverted sugar). However, sucrose, like all reducing or non-reducing sugars, can be degraded at high temperatures in the absence of the amino group and participate in non-enzymatic browning (caramelization).

It should be kept in mind that some polyphenols, ascorbic acid, uronic acids and other carbonyl compounds (aldehydes or ketones) that may form during oxidative processes (e.g. lipid oxidation) have one or more carbonyl groups, and may also participate in the Maillard reaction.

In general, the amino group originates from free amino acids or the ϵ -amino group of lysine when the latter is in the primary sequence of a protein or a peptide. Under certain conditions, the side chain of arginine can

also participate in the Maillard reaction. Similarly, the N-terminal amino group of proteins has a reactivity that allows it to participate in the Maillard reaction, but in reality its contribution is negligible. Ammonium and certain primary or secondary amines also have chemical characteristics enabling them to attack the carbonyl group and initiate the Maillard reaction.

5.2. Mechanism of non-enzymatic browning

The Maillard reaction comprises a series of highly complex reactions. They can broadly be divided into four phases:

- 1) the condensation reaction of the amino group and the carbonyl group;
- 2) the Amadori and Heyns rearrangement;
- 3) the decomposition of ketosamines;
- 4) polymerization reactions.

Figure 5.1 summarizes these main stages.

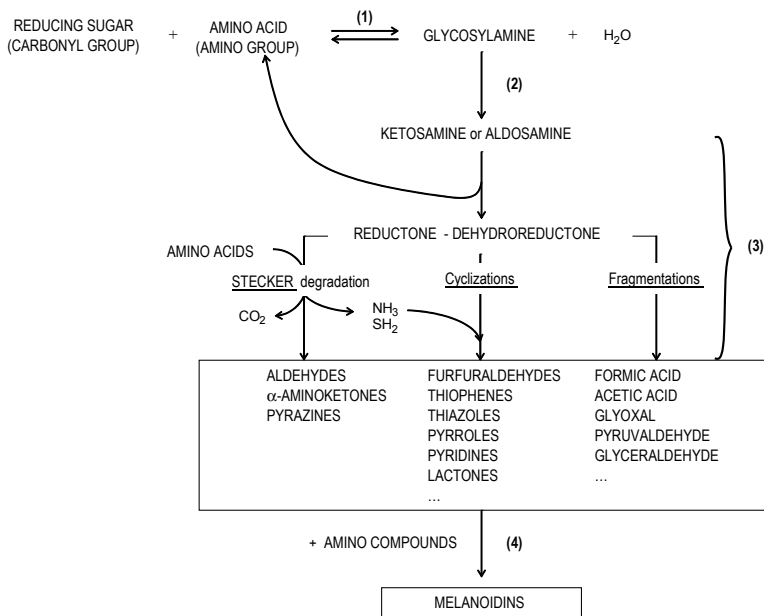
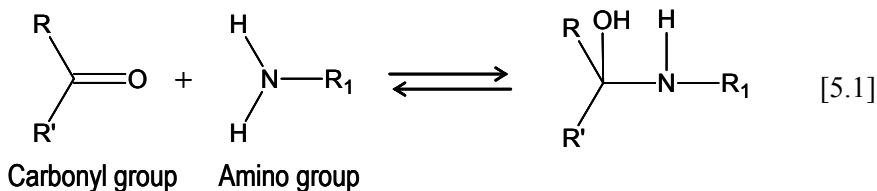


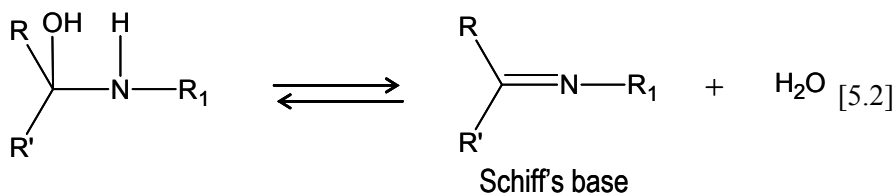
Figure 5.1. Reactions involved in non-enzymatic browning

5.2.1. Condensation

The Maillard reaction is initiated by the nucleophilic attack of a primary or secondary amino group of an amino acid or protein on the α -hydroxy carbonyl group of a reducing sugar (or more generally a nucleophilic attack of an amino group on a carbonyl group):



The nucleophilic attack is possible when the amino group has a lone pair of electrons, and consequently is favored at basic pH. The condensation product, highly unstable in aqueous solution, loses a water molecule resulting in the formation of a glycosylamine, also known as “Schiff’s base” (or more generally a carbonyl amine; equation [5.2]). Tertiary amines are unable to undergo this reaction.

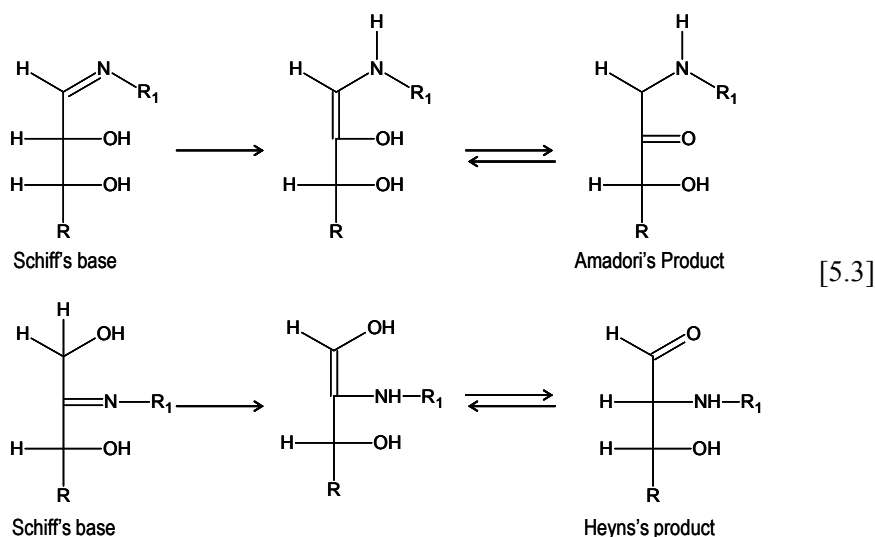


The reactions that result in glycosylamines are reversible. Considering the law of mass action, we can observe that the Maillard condensation is favored in a partially dehydrated medium. The quantity of water released during this reaction is negligible even in dried products. Furthermore, the sugar and the amino derivative can be fully regenerated in a highly acidic medium. Glycosylamines produced from amines and proteins are relatively stable, whereas those of amino acids are rapidly subjected to Amadori or Heyns rearrangement due to the catalytic action exerted by the carboxylic group of the amino acid.

The Maillard reaction is a non-enzymatic glycosylation that can be differentiated from enzymatic glycosylation insofar as both the reaction substrates and products are different. During enzymatic glycosylation, enzymes catalyze the binding of oligosaccharide residues to the side chain of certain asparagine or serine residues to form glycoproteins.

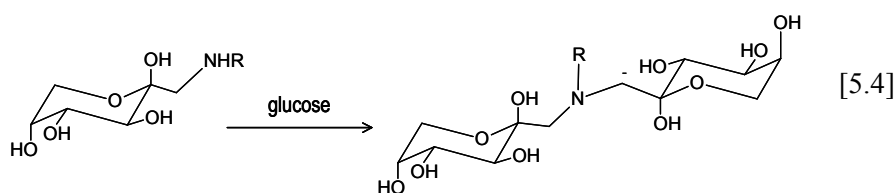
5.2.2. Amadori or Heyns rearrangement

Glycosylamines may undergo irreversible isomerization and yield more stable products: ketosamines if the initial carbohydrate substrate is an aldose (Amadori products), or aldosamines if the initial carbohydrate substrate is a ketose (Heyns product):

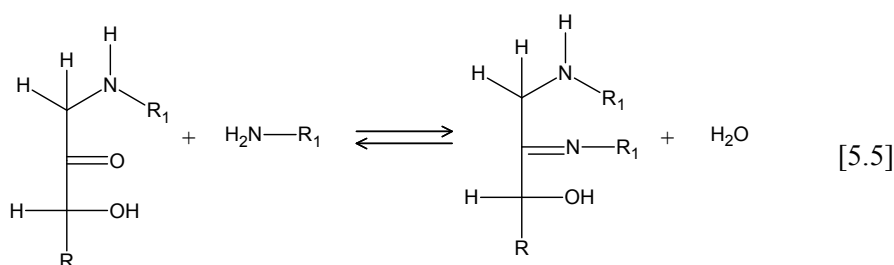


These reactions are catalyzed by the carboxyl group of amino acids. Aldosamines and ketosamines remain stable provided the heating conditions are not too severe. The main outcome at this stage is a decrease in the amount of available lysine; the color and flavor of products are only slightly affected.

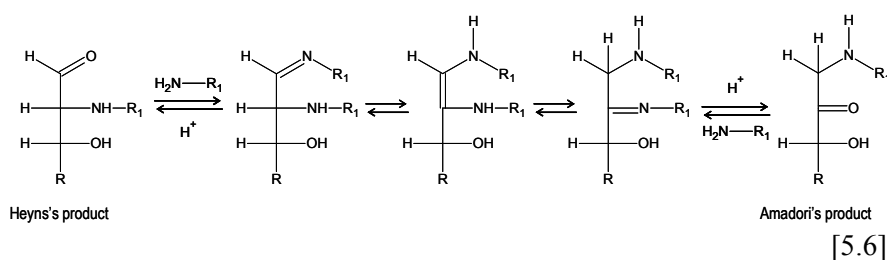
The Amadori or Heyns products can react, through their secondary amine group, with a second molecule of reducing sugar to give diglycosylated reaction products:



The carbonyl group of Amadori or Heyns products can also be the site of a new nucleophilic attack by an amine group from another amino acid or protein side chain:



In the presence of free amino acids, Heyns products are unstable. They undergo isomerization resulting in Amadori products. The mechanism is similar to the isomerization of glucose to fructose (Lobry de Bruyn–Alberda van Ekenstein rearrangement):



5.2.3. Degradation of ketosamines

Even though ketosamines are relatively stable, they can undergo a series of parallel and consecutive reactions, most of which are classical transformations such as enolization, dehydration, oxidation, cyclization, decarboxylation, retro-aldolization, etc. It is the diversity of reactions that

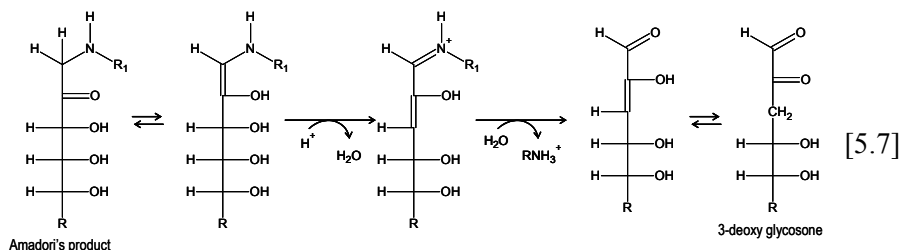
can occur simultaneously, which produces a complex reaction matrix. There are, however, three main reactions:

- 1) formation of highly reactive carbonyl compounds;
- 2) Strecker degradation;
- 3) fragmentation of carbohydrate units (retro-aldolization).

5.2.3.1. Formation of highly-reactive carbonyl compounds

Depending on pH conditions, ketosamines may undergo 1,2 or 2,3 enolization. After regeneration of the amine group, α -dicarbonylated compounds are formed, also known as deoxyglycosones or reductones (equation [5.7]). Due to their reactivity, they are highly reactive intermediates in the Maillard reaction.

In foods, the main decomposition route of ketosamines to reductones begins with enolization in position 1,2. Then a rearrangement, accompanied by a loss of a water molecule, results in a 2,3 double bond and the deamination of carbon 1. These reactions are favored under acidic conditions. The optimum pH of the dehydration reaction is around 5.5.



Heated in an acidic medium, dicarbonyl compounds can lose a water molecule and give an unsaturated dicarbonyl that, through cyclization, results in furfuraldehyde molecules, which include hydroxymethylfurfural (HMF). These are the main compounds formed with lactones. In pasteurized fruit juices, the 5-HMF content appears to reflect the intensity of the heat treatment. In the presence of primary amines, such as amino acids, the proportion of furfuraldehyde formed decreases in favor of nitrogen heterocycles like pyrroles (Figure 5.2).

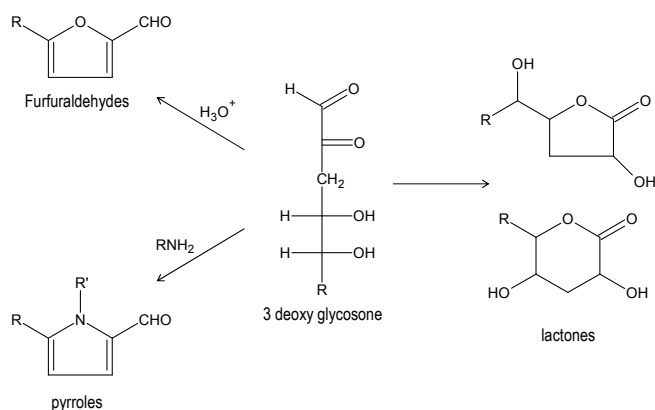
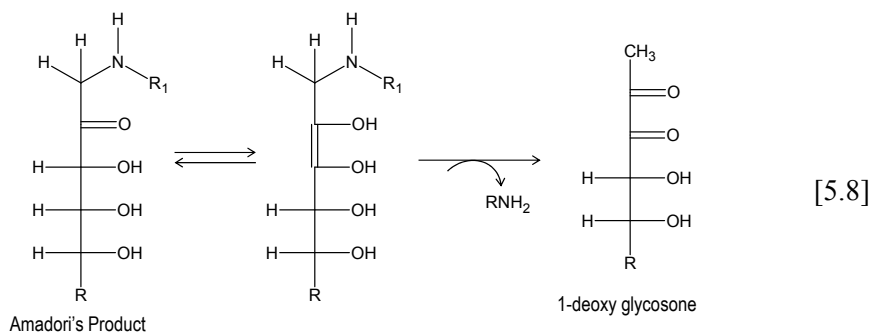


Figure 5.2. *Reactions of 3-deoxyglycosone*

Another degradation route of ketosamines starts with a 2,3 enolization and results in the formation of reductones (1-methyl-2, 3-dicarbonyl). Reaction is favored under basic conditions or when the amine bound to the carbohydrate is very basic. This degradation route can also occur to a limited extent when pH is close to neutral:



These reductones decompose via complex reactions in a large variety of mono- and dicarbonyl compounds: furanone, maltol, and isomaltol (Figure 5.3).

At this level, regardless of the degradation route taken, the regenerated amine has only played a catalytic role, and the free amino acid or the amine group on protein has not yet been degraded. Only reducing sugars have been converted into highly reactive reductones. Similar changes can occur during

the intensive heating of sugars, which occurs during caramelization. The compounds that are formed are potent precursors to nonenzymatic browning; however, by reacting with sulfur dioxide they produce sulfonates, which are relatively stable and not very reactive. This is probably what largely explains the inhibitory effect of sulfur dioxide on this type of browning.

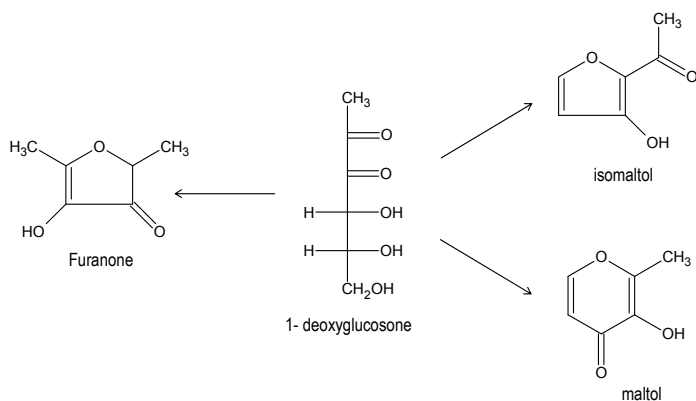
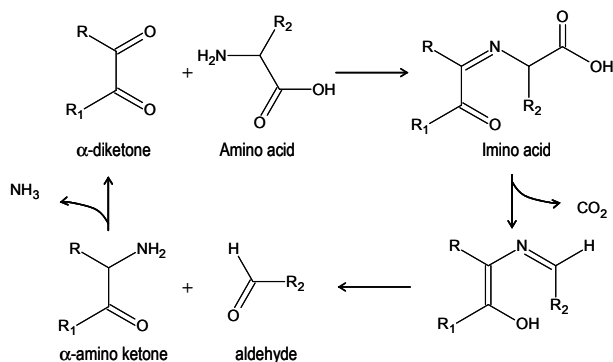


Figure 5.3. *Reactions of 1-deoxyglucosone*

5.2.3.2. Strecker degradation

Reductones formed during the decomposition of ketosamines react with the α -amino group of free amino acids through a nucleophilic attack on the carbonyl group to give imino acids that first decarboxylate and then hydrolyze resulting in aldehydes. At the same time, the dicarbonyl compound is converted to an α -amino ketone that regenerates the reductone by deamination:



[5.9]

Strecker degradation is an important source of aroma and flavor molecules. It involves a degradation of amino acids by releasing carbon dioxide, ammonia and an aldehyde of the corresponding amino acid. Depending on the nature of amino acids, various aldehydes with different aromatic properties are obtained. If the amino acid is cysteine, Strecker degradation leads to a production of mercaptoaldehyde that decomposes to acetaldehyde and hydrogen sulfide. The latter is the source of several strong-smelling sulfur compounds such as thiazoles, thiazolines, thiophenes, trithiolanes, etc. (Figure 5.4).

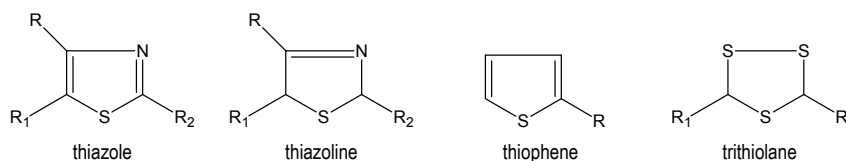
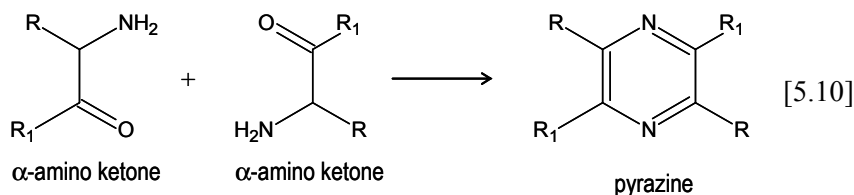


Figure 5.4. Main classes of sulfur heterocycles

The α -amino ketones resulting from the Strecker degradation produce pyrazines by condensation reactions:



Pyrazines are highly active aromatic substances found in roasted products as well as grilled meat and fish. In the latter, due to the presence of creatinine (existing only in animal products), pyrazines react with aldehydes to form heterocyclic amines belonging to the imidazoquinoline, imidazoquinoxaline or imidazopyridine family.

Some foods in their natural state contain highly reactive carbonyl compounds. Ascorbic acid, present in many types of fruit and vegetables, has a molecular structure in the oxidized state, which is similar to reductones. It is an important substrate for Strecker degradation in some fruit juices and fruit juice concentrates, which lose some of their vitamin C activity due to these reactions (Figure 5.5).

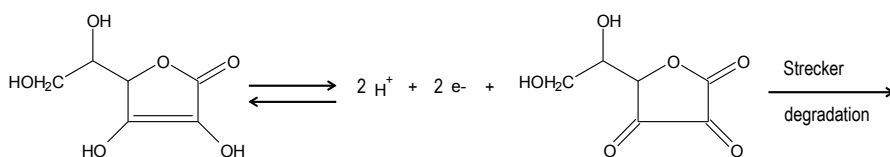
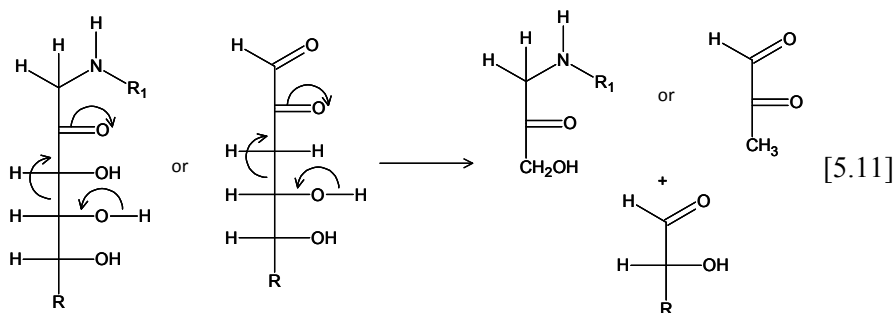


Figure 5.5. *Degradation of ascorbic acid*

Vitamin C can be degraded in the absence of nitrogen compounds at least in the first stages of degradation, resulting in furfural or furoic acid. Nitrogen compounds are subsequently involved in the formation of brown pigments. This reaction occurs in the presence or absence of oxygen, depending on the medium conditions (pH, presence of catalysts). Thus, the addition of ascorbic acid to fruit juices to prevent enzymatic browning and compensate for the loss of vitamin C has the disadvantage of promoting non-enzymatic browning. In addition to spoilage due to non-enzymatic browning, the release of carbon dioxide creates excess pressure in bottles of concentrated citrus fruit juice and fruit juice rich in vitamin C, which is sometimes associated with poor preservation.

5.2.3.3. *Fragmentation of ketosamines*

Parallel to enolization reactions, ketosamines or their α -dicarbonylated derivatives can undergo fragmentation reactions (retro-aldolization) resulting in small molecules of one to five carbon atoms (equation [5.11]): formaldehyde, formic acid, acetic acid, glyoxal, glyceraldehyde, and pyruvaldehyde as well as several α -hydroxyketones, α -hydroxyaldehydes, and α -diketones, etc. Fragmentation reactions are favored under neutral or alkaline conditions.

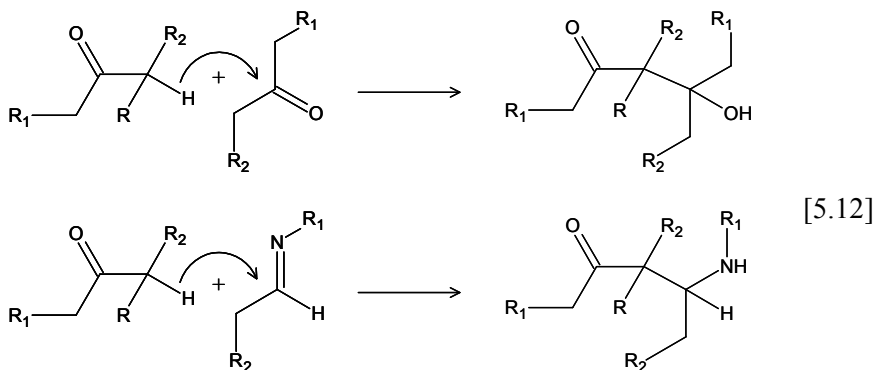


These molecules are highly reactive and likely to subsequently participate in various reactions, such as the degradation of amino acids according to Strecker, dehydration reactions, oxidation or condensation contributing to the formation of aromatic molecules and brown pigments. Some molecules such as glyoxal, methylglyoxal or diacetyl can participate in cross-linking (bridging), e.g. between proteins via lysine or arginine side chains.

5.2.4. Polymerization reactions

In the final stage, successive polymerization reactions involving highly reactive intermediates formed during the initial stages of the Maillard reaction lead to the formation of high-molecular-weight pigments called melanoidins. By their brown color, they are a visible indication of the Maillard reaction; the intensity of browning increases with the degree of polymerization. It is usually quantified by measuring the absorbance between 420 and 540 nm. In food, a large proportion of brown pigments is associated with proteins through the lysine and arginine side chain.

The nature of products formed and their formation process are still poorly understood. Basically, melanoidins are high-molecular-weight brown nitrogen compounds containing repeating groups: furan, pyrrole, indole, carbonyl, amine, amide, alcohol, ester, ether, etc. It appears that the polymerization of low-molecular-weight brown compounds occurs primarily through addition reactions, substitution and aldol condensation (equation [5.12]):



Polymerization reactions are accelerated by the presence of amine catalysts, such as amino acids. However, they are delayed by the use of sulfur dioxide or sulfites and sulfur amino acids. Even though the inhibition mechanism is not clear, it appears that the strong nucleophilic properties of sulfur compounds make carbonyl groups unavailable for the reactions responsible for browning. During the browning phase, many other reactions can take place at the same time as the reactions previously mentioned, such as cyclization, dehydration, oxidation or isomerization.

5.3. Factors influencing the Maillard reaction

Several physical or chemical factors affect the development of the Maillard reaction: the nature and quantity of the substrates, temperature and heating time, pH, water content and the presence of activators or inhibitors. By regulating the reaction rate, these factors affect the amount and nature of aromatic and colored molecules formed, and consequently the quality of the food product.

5.3.1. Substrates

The nature and quantity of substrates has a major effect on the Maillard reaction.

5.3.1.1. Nature of substrates

For reducing sugars, the sugar size is an important factor. Pentoses (ribose) react faster than hexoses (glucose, fructose, and mannose), which are in turn more reactive than disaccharides (lactose, and maltose). Sugars within the same group have different reactivities, which is the case in particular for aldoses like glucose and ketoses like fructose.

As already mentioned, molecules other than reducing sugars can participate in the Maillard reaction; this is particularly true for some lipid oxidation products, phenolic compounds or any other molecules with carbonyl groups. By interfering with the initial or final stages of the Maillard reaction, these molecules alter the balance of aromatic and colored compounds formed during food processing or preservation. The interaction between lipid oxidation products and molecules from the Maillard

reaction plays an important role in the development of flavor in meat during cooking.

Similarly, there is a considerable difference in reactivity among amino acids. Due to its strong basic character, lysine is the most reactive amino acid followed by arginine, especially in the early stages of the Maillard reaction. Even though cysteine is involved in the development of flavor in cooked meat, it hardly plays a role in browning due to the inhibitory action of its thiol group.

5.3.1.2. Quantity of substrates

In food systems, the amount of amino acids (lysine) and reducing sugars available for the Maillard reaction must be taken into account. They participate, in particular, in the rate of condensation (v) between the amino group (concentration $[A]$) and the carbonyl group (concentration $[C]$), which is expressed as follows:

$$v = k_{\theta} [A] [C] \quad [5.13]$$

where k_{θ} is the reaction rate constant.

In addition to the nature of reducing sugars, their reactivity depends on the quantity of molecules in a reactive form, i.e. with a straight chain configuration.

Furthermore, certain physical-chemical or biological conditions can promote the release of carbohydrate units that can participate in the Maillard reaction. In meat and fish, increasing the temperature promotes the release of ribose from inosine monophosphate and other ribonucleotides. It also promotes the inversion of sucrose during the manufacture of table (granulated) sugar from beet or during the preparation of jams. Storing potatoes at a temperature below 10°C promotes the accumulation of reducing sugar (glucose) from starch. This sugar is involved in the formation of brown pigments during frying, which is popular among consumers. For disaccharides and polysaccharides, the use of biological agents (enzymes, and microorganisms) can increase the amount of reducing sugar and promote the Maillard reaction (*Dulce de leche* or milk caramel, bread, beer, etc.).

The reactivity of proteins is mainly through the non-protonated amino group of the lysine side chain. Thus, foods containing proteins rich in lysine (milk) are more susceptible to the Maillard reaction. However, the availability of reactive amino acids depends on other factors like pH or the denaturation state of the protein, which modify their accessibility.

5.3.2. *Physical-chemical conditions*

5.3.2.1. *Temperature and heating time*

The rate of the Maillard reaction is strongly influenced by the temperature–time relationship applied to the product. The effect of temperature on the reaction rate constant can be expressed by the Arrhenius equation:

$$k_{\theta} = ae^{\frac{-E_a}{RT}} \quad [5.14]$$

where k_{θ} is the rate constant, a is the frequency factor, E_a is the reaction activation energy (J mol^{-1}), R is the perfect gas constant ($8.314 \text{ J mol}^{-1} \text{ K}^{-1}$) and T is absolute temperature (K).

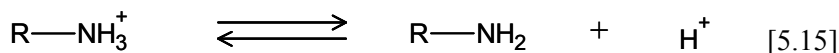
The activation energy of the different stages of enzymatic browning is relatively high. Increasing the temperature by 10°C multiplies the rate constant of the browning reactions (Q_{10} , Volume 2) by a factor ranging from approximately 2 to 8. Moreover, increasing the temperature increases the solubility of sugars, their mutarotation rate and the amount of molecules in reactive conformation. Browning is therefore strongly stimulated by an increase in temperature. In contrast, it slows down at low temperatures, but can still occur even below 0°C .

The flavor profile and the color intensity vary depending on the time and temperature applied to the food products. Those stored at room temperature can develop flavor defects linked to the Maillard reaction. When heating at an intermediate temperature (100°C), caramel or cooked flavors dominate, while grilled or roasted flavors take over at higher temperatures (150°C). This is due to the fact that pyrazines mostly form at high temperatures. As a result, for a given time–temperature relationship, there is a particular flavor profile that is hardly reproducible using another heating combination. At the

same time, the color intensity increases with exposure time at a given temperature.

5.3.2.2. *pH*

The effects of pH are complex, since each reaction involved in browning has its optimum pH, ranging between 6 and 9. This is due to the fact that only amino groups with a lone pair of electrons participate in the condensation reaction with carbonyl groups. Increasing the pH shifts the equilibrium as follows:



Furthermore, the hemiacetal structure of sugars is more stable in a dilute acid medium: at a pH above 7, the proportion of sugars in linear and reactive conformation increases sharply. This pH dependence implies that food products with a neutral pH are more susceptible to the Maillard reaction than those with a lower pH. At a pH below 3 or above 9, other nonenzymatic browning phenomena compete with Maillard browning.

Even if there is no clear limit, it is possible to classify the effect of pH on the different types of reactions involved in the Maillard reaction as follows: basic media accelerate addition reactions and retro-aldolization, while acidic media accelerate hydrolysis and dehydration reactions. Thus, the flavor profile and color intensity developed by the Maillard reaction differ depending on pH. The flavor of cooked meat is more intense in the pH range of 4 to 5.5; pyrazines are formed in large quantities at pH values above 5; the intensity of browning increases with pH.

During the Maillard reaction, the pH of the medium gradually decreases due to the formation of acids, in particular formic acid and acetic acid. Their formation creates a phenomenon of feedback inhibition of the Maillard reaction, since the development of browning is slowed down with the drop in pH.

5.3.2.3. *Water activity*

Water activity considerably changes the development of the Maillard reaction. The rate of browning reaches a maximum at an a_w of between

0.5 and 0.8. The rate of browning decreases sharply at either side of this range.

The decrease in the rate of browning at high water activities is due to the fact that water is a product of the carbonyl–amine condensation reaction. As a result, in line with the law of mass action, the initial browning reaction occurs more slowly in foods with higher a_w . Moreover, since the reaction is bimolecular, an increase in the water content tends to dilute the reactants and slow down the rate of browning.

The decrease in the browning rate at low water activities reflects a slowdown in the diffusion rate of molecules toward each other. Dried foods are stable with regard to browning, especially below the glass transition temperature where there is absence of molecular mobility. However, any moisture absorption that lowers the glass transition temperature or any increase in the storage temperature above the glass transition temperature of the product triggers the resumption of browning reactions.

5.3.3. Presence of activators and inhibitors

Apart from the catalytic role of amino groups, the Maillard reaction is accelerated by the presence of metal agents such as copper or iron, and when exposed to oxygen. These elements accelerate oxidation and the formation of highly active α -dicarbonylated compounds.

On the other hand, the elements that react with the carbonyl group of reducing sugars and highly reactive carbonyl intermediates are inhibitors of the Maillard reaction. This is the case with sulfites or sulfur dioxide as well as amino acids with a sulfur atom, such as cysteine. Sulfites react with carbonyl compounds to form sulfonates that have a low reactivity in the Maillard reaction. Cysteine acts through its nucleophilic sulfur group but also through its reducing property.

5.4. Consequences of non-enzymatic browning

The Maillard reaction results in the formation of molecules, which can have a positive or negative effect on the sensory, functional and

nutritional qualities of foods. These effects depend on the given food product.

5.4.1. Sensory consequences

The first perception of the sensory quality of food is visual. The formation of melanoidins responsible for browning during the Maillard reaction is therefore particularly important in foods like breads, pastries, meat, etc. However, it is undesired when it affects the appearance of powders such as protein ingredients. On an industrial level, controlling browning is not always easy since the structure and formation mechanisms of melanoidins are still poorly understood. The color of melanoidins changes from yellow-brown to reddish brown depending on the conditions of the medium, in particular pH and heating temperature.

The Maillard reaction is also an important and diversified source of flavors. They are the result of a multitude of reactants that can combine during technological treatments. In some cases, the formation of a class of aromatic molecules can positively affect the flavor of the product, whereas it may be perceived as a negative effect in other cases. The quantity of molecules produced must also be considered. Because of their weak detection threshold, Strecker aldehydes actively affect the flavor of a very wide variety of foods; they are found, for example in both potato products as well as cocoa. Like Strecker aldehydes, furans, furanones and pyranones are found in nearly all foods. They impart a strong, sweet and caramelized flavor. Pyrroles contribute a smoky or cooked flavor to cereal products. Oxazoles and thiazoles are heterocycles with a nitrogen atom and another heteroatom that can be either an oxygen or sulfur atom. They have a very low detection threshold. Oxazoles add a nutty taste and are found in many roasted or fried products such as cocoa, coffee, meat or potatoes. Thiazoles give off a sulfur aroma, such as in onions and meat. Pyrazines and pyridine derivatives are found more specifically in roasted products. Some develop a pleasant flavor, but on the whole they impart bitter or astringent flavors.

5.4.2. Functional consequences

During the Maillard reaction, the functionality of proteins can be strongly modified. The initial stages of the Maillard reaction tend to improve it.

Non-enzymatic glycosylation can increase the solubility of poorly soluble proteins like gluten. But the most significant results were seen in the improvement of the emulsifying properties of egg white proteins.

In the later stages of the Maillard reaction, there is a formation of highly reactive molecules that can form cross-links between proteins. They can participate in the viscoelastic properties of bread dough and the quality of foods after cooking. However, the accumulation of highly aggregated proteins tends to decrease their solubility and digestibility.

5.4.3. Nutritional consequences

The Maillard reaction may be responsible for reducing the nutritional quality of foods by degrading essential molecules or decreasing the digestibility of proteins. It can generate anti-nutritional factors and potentially toxic compounds that further reduce the nutritional value of the food and even affect its safety. However, it also results in the formation of molecules with a known benefit for human health.

In order to serve as a source of nutrients, amino acids must first be released by protein digestion and then absorbed through the intestinal wall. However, the participation of amino acids in browning can significantly alter their bio-availability either by lowering protein digestibility or reducing the intestinal absorption of modified amino acids. The fact that lysine, and to a lesser extent arginine, are involved in the nonenzymatic glycosylation of proteins means that the nutritional quality of proteins is reduced through a decrease in the availability of essential amino acids. Proteins whose conformation is changed after binding carbohydrate molecules can block the accessibility of peptide bonds to proteolytic enzymes or directly inhibit digestive enzymes. In some cases, Maillard products derived from lysine can be used as a source of lysine. The size, charge and basicity of the molecule influence its susceptibility to enzymatic hydrolysis, transport and use as a source of lysine. As the reaction develops, which depends on the intensity of the treatment, other molecules like essential amino acids and ascorbic acid may be destroyed.

As already mentioned, the Maillard reaction generates anti-nutritional, even potentially toxic compounds; these include melanoidins, carbonyl

compounds and heterocyclic amines among others. It has been argued that the development of the Maillard reaction in foods alters their allergenic response, and that melanoidins and certain non-assimilable precursors alter the mineral metabolism through their chelating ability. α -dicarbonylated molecules such as glyoxal, methylglyoxal and diacetyl are highly reactive and have been found in instant or decaffeinated coffees. They exhibit mutagenic activity. However, no correlation with carcinogenic activity has been shown. Heterocyclic amines (carbolines, imidazoquinolines, and imidazoquinoxalines) have been found in roasted meats and fried fish; they are potent mutagens. The type of cooking and temperature as well as the presence of other molecules modulate the quantity of heterocyclic amines formed and their mutagenic activity. The higher the temperature applied to the product, the greater the amount of heterocyclic amines. The presence of antioxidants, such as vitamin C, β -carotene or tocopherols, reduces the quantity of heterocyclic amines formed. Chlorophyll, flavones and flavonoids inhibit their mutagenic activity. More recently, acrylamide has been found in crisps, chips or crackers. Acrylamide is a neurotoxin causing cancer in rats and is “probably carcinogenic to humans” according to the International Agency for Research on Cancer.

Certain benefits can be attributed to Maillard reaction products, like the formation of antioxidant molecules. The antioxidant capacity of food is generally associated with the formation of brown pigments—melanoidins. It increases as the browning reaction progresses. Several mechanisms have been proposed to explain the antioxidant activity of melanoidins. They have aromatic structures that can easily transmit hydrogen atoms to radical structures and thus block the propagation reaction of lipid oxidation. They are also able to chelate heavy metal catalysts of lipid oxidation or trap singlet oxygen molecules. In addition, some Maillard reaction products display antibiotic activity by inhibiting the growth of pathogenic flora and spoilage flora found in food. Similarly, antigenic sites on food proteins can be selectively altered by modification with a reducing sugar.

5.5. Evaluation of non-enzymatic browning

The progress of the initial stages of the Maillard reaction can be estimated by quantifying the residual reducing sugars or the available lysine

by enzymatic or chemical methods. An alternative is to monitor the accumulation of their condensation products or Amadori products. In the latter case, determining furosine levels allows the quantification of the condensation product of a reducing sugar and the lysine side chain of a protein. Furosine is an artificial amino acid released during acid hydrolysis (6M HCl, 110°C, 24 h) of the Amadori product. Furosine is easily quantified by high-performance liquid chromatography (HPLC). The disadvantage of this method is that the conversion factor between furosine, which is quantified experimentally, and the actual quantity of the Amadori product is approximate: only 30–40% of Amadori products are converted to furosine.

In the more advanced stages, the decomposition products of ketosamines are most commonly separated and quantified by HPLC and/or gas chromatography (GC). Together, GC and mass spectrometry can separate and identify several volatile products that contribute to the flavor of foods resulting from the Maillard reaction. Despite the need for post-column derivatization, HPLC has an advantage over GC in the analysis of water-soluble, non-volatile compounds.

Finally, the browning intensity can be measured either by reflectance (solid foods) or absorbance after the extraction of pigments at room temperature, possibly with the assistance of a proteolytic enzyme. The measurement is carried out between 420 and 540 nm. In addition, some precursors of brown pigments are fluorescent compounds and can be used to monitor the development of the Maillard reaction.

Furthermore, storage tests can be carried out at a temperature of approximately 40°C and at a controlled a_w in order to study the susceptibility of dried products to browning. Such “accelerated” tests are more useful than excessive heat treatment as the latter does not lead to immediate browning, but rather the accumulation of reactive carbonyl compounds susceptible to polymerization during storage.

5.6. Control and prevention of non-enzymatic browning

In a given food product, some effects of the Maillard reaction may be desired (e.g. improved antioxidant capacity and/or flavor of the food) while

others are not (e.g. loss of nutritional value). There are relatively few ways of preventing non-enzymatic browning or influencing the balance of products formed. This is due, among others, to the lack of understanding of the structure of products formed during the Maillard reaction as well as the reaction mechanisms generating them.

5.6.1. Removal of substrates

Sugar can be removed by biological treatments as in the case of egg white ingredients. Due to the thermal sensitivity of liquid egg white, it is difficult to preserve the egg white from a microbial perspective. An alternative is the dry-heating of egg white powder (between 65 and 90°C for approximately 7–10 days), which destroys pathogenic flora like salmonella. However, these are ideal conditions for the development of nonenzymatic browning unless the glucose is first transformed into gluconic acid by enzymatic treatment (glucose oxidase) or eliminated by fermentation.

In potatoes, there is a balance between starch, the energy reserve component and reducing sugars that are used in cell reactions. Storage at temperatures below 10°C shifts the equilibrium toward the release of reducing sugars, which participate in the Maillard reaction during processing. However, storage at temperatures above 10°C (e.g. 20°C for two weeks) shifts the equilibrium toward the synthesis of starch at the expense of the reducing sugars.

Where possible, it is desirable to substitute reducing sugars during formulation. Otherwise, it is preferred to heat or pre-heat the various ingredients separately before final blending.

In addition, modifying the amino groups of proteins limits or prevents browning reactions. The reaction of proteins with transglutaminase transforms the amino group of the lysine side chain to an amide group, which is unable to participate in the Maillard reaction. The enzymatic hydrolysis of asparagine to aspartic acid using asparaginase drastically reduces the occurrence of acrylamide in products heated at high temperatures (French fries, for instance).

5.6.2. Physical-chemical factors

Due to the high activation energy of some browning reactions, foods should not be subjected to severe heat treatments and should be stored at moderate temperatures. In addition, temperature influences the nature of the products formed. With regard to heating, alternatives like microwave heating can limit the development of browning. Unlike traditional methods of heating, which induce surface dehydration and involve relatively high temperatures, this heating method maintains moderate temperatures and a more humid atmosphere around the product during generally shorter periods.

During the concentration and dehydration processes, temperature and residence time in the critical zone of a_w (0.5–0.8) should be as low as possible. However, the temperature used directly affects the residence time at these intermediate a_w . The most satisfactory conditions should be experimentally determined in each case. Moreover, concentrated or dried products should ideally be stored at a temperature below the glass transition temperature, away from moisture and heat.

Lowering the pH can in some cases slow down browning, but the product must of course be suitable for moderate acidification, i.e. without any other undesirable changes.

5.6.3. Formulation (addition of inhibitors)

The most effective inhibitors against non-enzymatic browning are sulfites. They compete in the reaction with carbonyl compounds (reducing sugars, aldehydes, ketones), Schiff's bases or unsaturated carbonyl compounds resulting in particularly stable sulfonates (Figure 5.6).

By binding to the most reactive non-enzymatic browning intermediates, sulfites lengthen the induction period and considerably delay the appearance of pigments. However, when free sulfites are depleted, browning resumes at its initial rate (Figure 5.7).

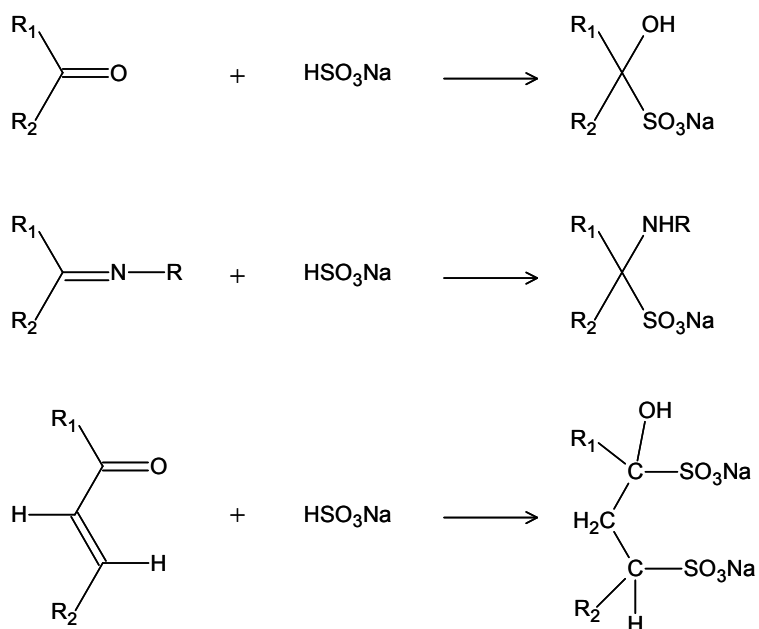


Figure 5.6. Inhibition mechanisms by sulfites

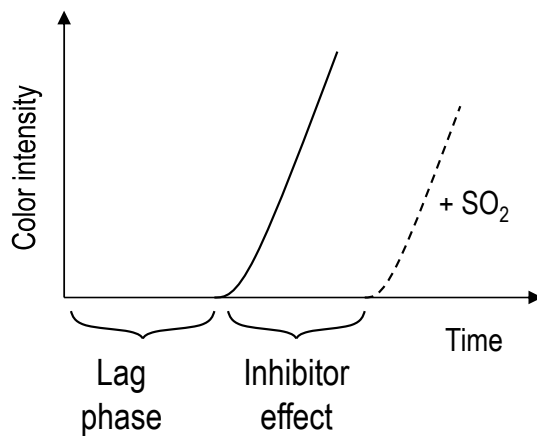


Figure 5.7. Kinetics of non-enzymatic browning: effect of inhibitors

By reacting with sulfur dioxide, nitrites lower the amount of available inhibitors: the inhibition period is shortened. The final color obtained is changed by sulfite, from a reddish brown to a yellow-brown color. The intervention of sulfites in the structure of melanoidins has yet to be explored.

Sulfites also inhibit enzymatic browning and the growth of microorganisms. As a result, they are widely used as antiseptics in grape must and wine, dried fruit and juice concentrates stored in barrels. Antiseptic action generally requires high doses, well above those needed to avoid browning. When it is solely a matter of avoiding browning (dehydrated mashed potatoes or citrus juice concentrates), relatively small amounts of sulfites are used.

Enzymatic Browning

“Enzymatic browning” is the transformation of phenolic compounds to colored polymers, changing from intermediate shades of pink, red or blue to mostly brown or black. This process is enzymatic in the initial stages and occurs in the presence of oxygen. The dark pigments that form at the end of this chain of reactions are generally known as melanins. Enzymatic browning occurs in plants rich in phenolic compounds. It is also responsible for the development of color in the skin, retina, hair and fur of mammals as well as the brown color of insect or crustacean cuticles.

Like non-enzymatic browning, enzymatic browning is undesirable when it negatively affects the sensory and nutritional properties of foods. It rarely occurs in foods of animal origin. However, it can arise during the storage of certain seafood (shrimp, crab and lobster). In contrast, it often occurs in plant products, especially during postharvest handling such as storage and processing procedures (e.g. trimming, peeling, cutting, juicing, dehydration and freezing). This phenomenon occurs in fruits and vegetables such as apples, pears, apricots, peaches, bananas, avocados, potatoes and mushrooms.

The formation of brown pigments is not, however, always undesirable. Some degree of browning is, in fact, desired in the production of dried fruit (dates, prunes and raisins), the preparation of cider, the fermentation of tea and the drying of tobacco and fermented cocoa and coffee beans.

Chapter written by Thomas CROGUENNEC.

6.1. Substrates and browning enzymes

6.1.1. Phenolic substrates

Many natural substrates (mono-, di- or polyphenols) are involved in enzymatic browning. The most significant are pyrocatechol derivatives, benzoic acid and cinnamic acid derivatives, flavonoids, tannins and lignins. Their reactivity depends on their structure and the origin of the enzymes that catalyze their oxidation; for example, meta-diphenols are weakly reactive.

The pyrocatechol nucleus found in 3,4-dihydroxyphenylalanine (DOPA) or 3,4-dihydroxyphenylethylamine (dopamine) is a powerful substrate for enzymatic browning (Figure 6.1).

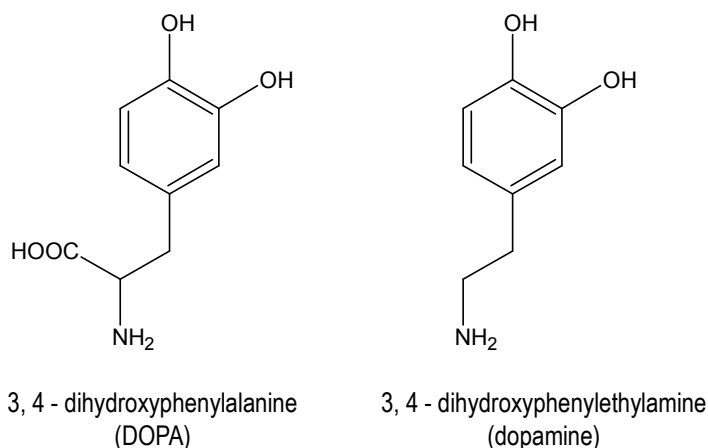


Figure 6.1. Chemical structures of DOPA and dopamine

DOPA formed from tyrosine is likely to be further oxidized to dopaquinone. Tyrosine and chlorogenic acid are the major browning substrates in potatoes and 3,4-dihydroxyphenylethylamine is the main browning substrate in bananas.

Benzoic acid derivatives (gallic acid and protocatechuic acid) and cinnamic acid derivatives (*p*-coumaric acid and caffeic acid) are actively involved in enzymatic browning (Figure 6.2).

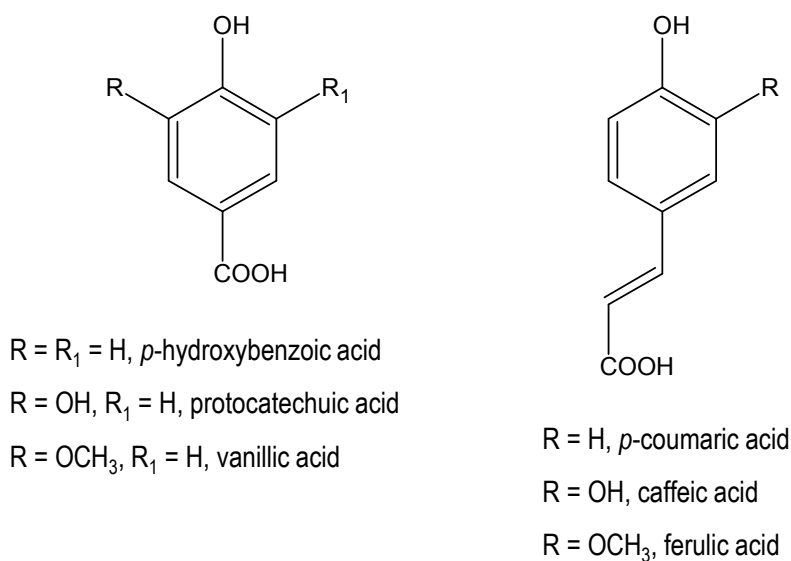


Figure 6.2. *Benzoic acid and cinnamic acid derivatives*

Chicoric acid (dicafeoyl tartaric acid) is a major constituent of chicory escarole. Chlorogenic acid (5'-cafeoylquinic) accounts for 60–70% of phenolic compounds isolated from carrots. In addition to its presence in apples and pears, it is also involved in the formation of blue–black pigments that may appear in potatoes during cooking in the presence of traces of iron. The formation of pigments can be avoided by lowering the pH (below 4, which is not always suitable because cooking at this acidity results the transformation of starch into dextrin), and using iron complexing agents such as citrate or ethylene diamine tetra-acetic acid (EDTA). Glucose derivatives of *p*-coumaric acid, caffeic acid and ferulic acid were observed in large quantities in tomatoes.

Flavonoids are also major substrates for enzymatic browning. They exhibit a 15-carbon basic skeleton ($C_6-C_3-C_6$) forming a benzopyran derivative substituted by a phenyl ring (Figure 6.3) and are present in all vascular plants, bacteria, fungi and lichens.

This large family is divided into several subclasses, which are differentiated by the position of the phenyl ring (B) and the oxidation state

of the benzopyran nucleus. Flavan-3-ols, flavonols, flavones, isoflavones, flavanones, isoflavanones and anthocyanidins are the main flavonoids responsible for enzymatic browning in vegetables (Figures 6.4 and 6.5). They are often present in plants in the form of glycosides.

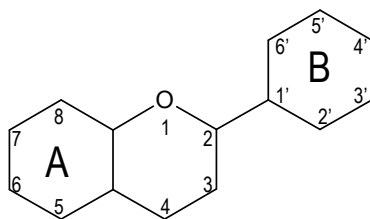


Figure 6.3. General structure of flavonoids

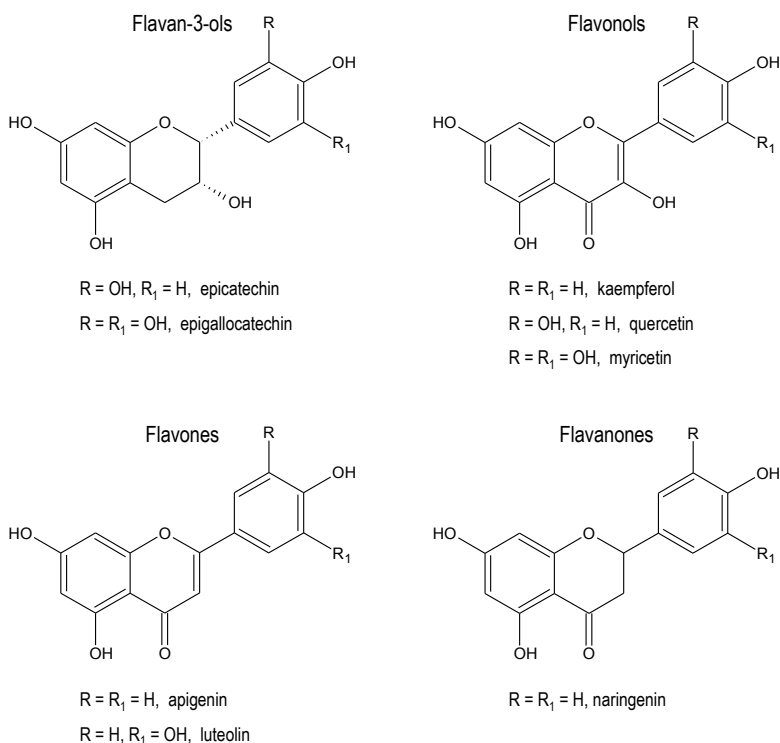
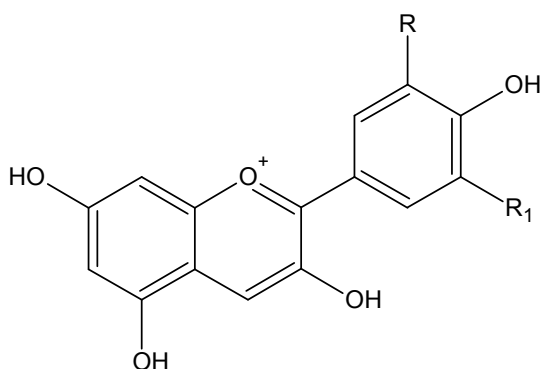


Figure 6.4. Chemical structure of flavonoids



R = OH, R₁ = H, cyanidin

R = R₁ = OH, delphinidin

Figure 6.5. *Chemical structure of anthocyanidins*

Flavan-3-ols such as catechins and their derivatives are some of the main plant polyphenols (grapes, tea, etc.), the quantity of which depends on ripeness. Catechins have two asymmetric carbon atoms in positions 2 and 3 resulting in four stereoisomers. They are not colored but undergo rapid change to yellow- and brown-colored compounds during enzymatic oxidation. During the fermentation process of tea leaves in the manufacture of black tea, approximately 75% of catechins undergo enzymatic oxidation and polymerization reactions. In apples, the most abundant flavan-3-ols are epicatechins, which are catechin isomers. The ratio between the amount of epicatechins and catechins is around 5:1.

Flavonols have a carbonyl group in position 4 and a hydroxyl group in position 3. In addition, the presence of a double bond between carbons C₂ and C₃ is responsible for the planar conformation of the benzopyran nucleus. They are abundant in onion layers in the form of quercetin glycosides (sugar units linked in positions 3 and/or 7 and/or 4'). Flavones such as luteolin and apigenin glycosides can be found in carrots.

Flavanones have a carbonyl group in position 4 and an asymmetric carbon atom in position 2. Among these, naringin, or naringenin glycoside (glucose and rhamnose disaccharide linked in position 7), is responsible for

the bitter taste of some grapefruits, especially before fully ripened. A compound of similar structure, hesperidin (5, 7, 3', 4' tetrahydroxy 4'-methoxyflavanone) is present in oranges.

Anthocyanidins are plant pigments that are red, purple or blue, which depend on their structure (Figure 6.5). They are usually in the form of glycosides linked in position 3, and less frequently in position 5 or 7.

Increasing the number of hydroxyl groups on the B ring results in a shift in the absorption maximum to longer wavelengths, causing the color of the molecule to change. It is also very sensitive to changes in pH, going from blue to red when the pH drops and vice versa. Vegetables are not a rich source of anthocyanidins apart from some exceptions (e.g. red cabbage). The color of fresh aubergines is due to delphinidin glycosides in the pericarp. Anthocyanidins in the form of cyanidin, delphinidin, malvidin, peonidin, pelargonidin or petunidin glycosides are major flavonoids in ripe fruit, both in terms of quality and quantity (grapes, blueberries, blackcurrants, cherries, strawberries, raspberries, figs and tropical fruit). They are also found in lesser amounts in apples, pears, bananas and oranges.

The tannins that contribute to the texture (cell wall structure) and flavor (astringency) of plant tissues are also enzymatic browning substrates. There are two groups of tannins: hydrolysable (or pyrogalllic) tannins, resulting from the esterification of five hydroxyl groups of glucose by various polyphenolic acids (gallic, digallic, ellagic and luteic), and condensed (or catechin) tannins, the chemical composition of which is very close to that of anthocyanidins. The main physical-chemical property of tannins is their ability to bind proteins and polysaccharides by hydrophobic interactions and/or hydrogen bonds and to precipitate them. This binding may explain why polyphenols can inhibit protein hydrolysis by proteases. The formation of complexes between tannins and proteins or polysaccharides often causes the appearance of cloudiness in beverages (wine and beer). In addition, interactions between tannins and salivary proteins and mucopolysaccharides of mucous secretions result in their precipitation with the loss of saliva lubricity. A sensation of dryness develops in the mouth, referred to as astringency. The blackening that sometimes occurs in chestnut purees is due to the formation of pigments formed by the reaction of pyrogalllic tannins with traces of iron.

Finally, lignins that are responsible for the rigidity of certain plant tissues are polyphenolic polymers that also participate in enzymatic browning.

6.1.2. Browning enzymes

The enzymes involved in browning reactions are polyphenol oxidase (PPO) and, to a lesser extent, peroxidase. PPO acts on phenols in the presence of oxygen, while peroxidase involves hydrogen peroxide. As the latter is only present in small amounts in cells since it is quickly detoxified, the involvement of peroxidase in browning is therefore limited.

PPO exists in many organisms in slightly different forms, which are called isoenzymes. It differs in its affinity for oxygen and phenolic substrates, its maximum reaction rate and its hydroxylation/oxidation activity ratio. Hydroxylation activity is not always present and when both activities are present, the hydroxylation/oxidation activity ratio can vary considerably. PPO specifically catalyzes the oxidation of diphenols in bananas, peaches, tea and tobacco; it also shows hydroxylation activity in apples, pears, potatoes and mushrooms. Both reactions consume oxygen. PPOs are metalloproteins containing copper, which is essential for the catalytic mechanism of the enzyme. Their optimum pH activity is generally between 5 and 7, and activity decreases rapidly as the pH drops. A distinction is made among PPOs between catechol oxidase and laccase.

Catechol oxidase, which is found in bacteria, mammals and plants, catalyze the hydroxylation of monophenols to *o*-diphenols (cresolase activity) and the oxidation of *o*-diphenols to *o*-quinone (catecholase activity). It contains two copper atoms at its catalytic site; each copper atom is coordinated by three histidines through the ϵ nitrogen of the imidazole ring. It appears that the catalytic cycle of catechol oxidase includes three forms of enzymes (E_1 , E_2 and E_3) depending on the oxidation state of the copper atoms and the presence of molecular oxygen: the reduced form (E_1), $\text{Cu}^+ - ^+\text{Cu}$, and oxidized forms (E_2), $\text{Cu}^{++} - \text{O}_2 - ^{++}\text{Cu}$, and (E_3), $\text{Cu}^{++} - \text{OH} - ^{++}\text{Cu}$ (Figure 6.6).

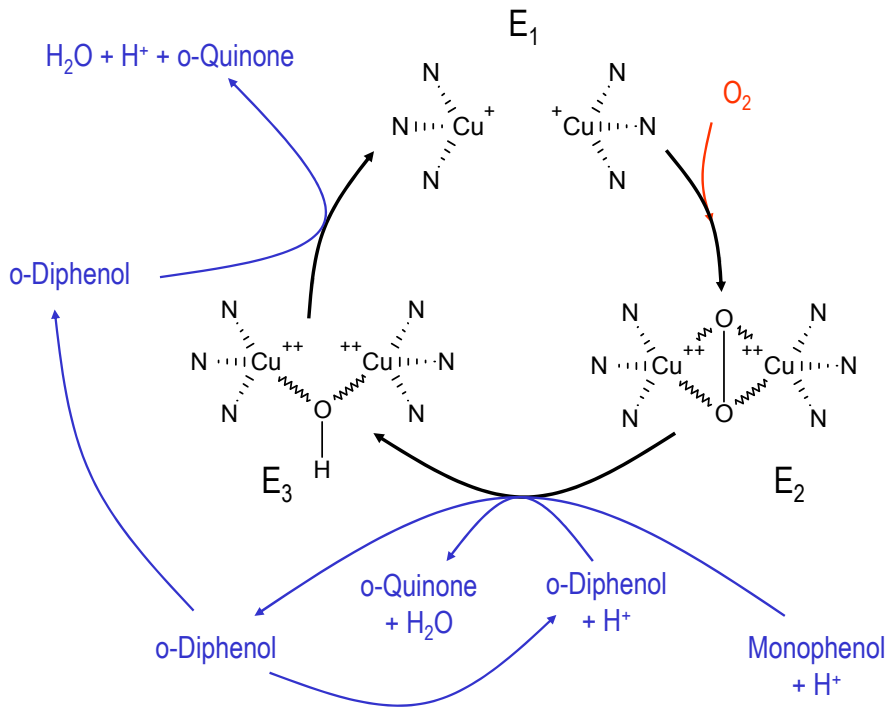


Figure 6.6. Change in the oxidation state of the active site of catechol oxidase during the oxidation of polyphenolic compounds

The reduced form (E₁) is unable to react with phenolic compounds but can be activated by the binding of molecular oxygen.

Laccases are present in higher plants, fungi and certain bacteria. Excreted by *Botrytis cinerea*, they have a constructive role in the manufacture of sweet wines. Laccases, less specific than catecholases, catalyze the oxidation of a large range of phenolic compounds: *p*-diphenols, *o*-diphenols, *m*-diphenols, *o*-triphenols (gallic acid) and various monophenols except tyrosine. Laccases contain four copper atoms. They are more stable in acidic medium than catechol oxidases, and are less sensitive to inhibitors like sulfites, but are less resistant to temperature increases.

6.2. Mechanism of enzymatic browning

6.2.1. Formation of quinones

PPO is responsible for the hydroxylation of monophenols to *o*-diphenols and the oxidation of *o*-diphenols and *p*-diphenols to quinones (Figure 6.7). Only the oxidized form of the enzyme, $\text{Cu}^{++} - \text{O}_2 - {}^{++}\text{Cu}$ (E_2), is able to react with monophenols and insert an oxygen atom in the *ortho* position of the phenolic compound. The oxidized forms of the enzyme (E_2 and E_3) can carry out the oxidation of *o*-diphenols to *o*-quinones and the simultaneous release of a water molecule.

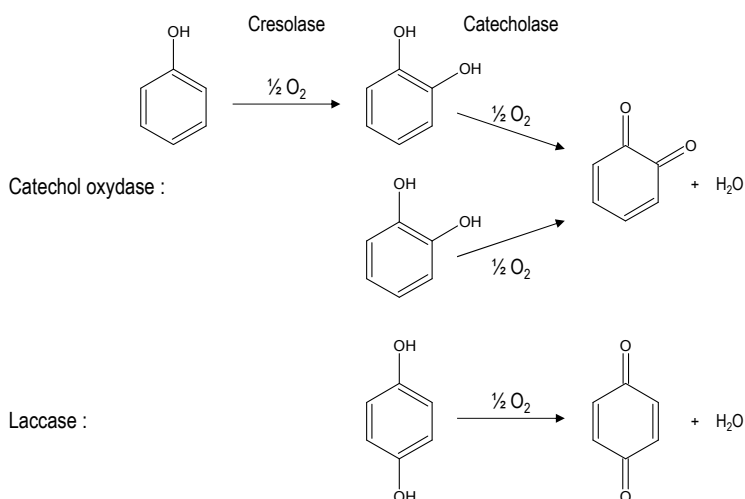


Figure 6.7. Oxidation of phenolic compounds during enzymatic browning

These reactions are associated with a change in color of the products. While phenolic compounds are mostly colorless, quinones are slightly colored, usually in shades of yellow, orange, pink, red or brown. The color of *o*-quinones depends mainly on pH and the phenolic compounds from which they originate.

6.2.2. Reactions with quinones

Quinones are very unstable compounds, both powerful and highly electrophilic oxidants, which form a wide variety of colored or colorless

products. Quinones are involved in either the oxidation reactions of reducing compounds, with the regeneration of the *o*-diphenolic compound, or the addition reactions of nucleophilic compounds leading to the formation of several addition products (Figure 6.8).

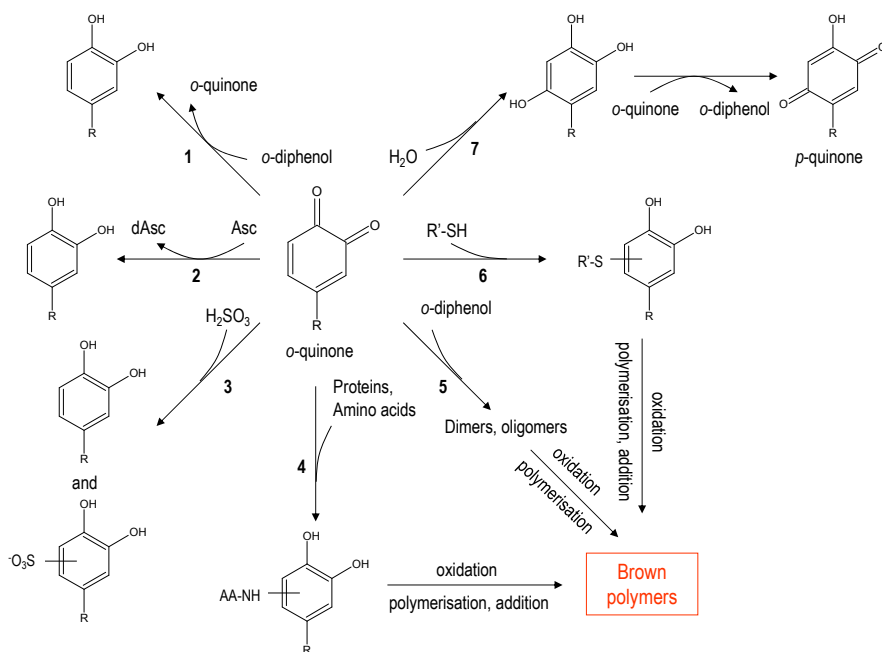


Figure 6.8. Chemical reactions of quinones

At this stage, the participation of PPO is less pronounced. *O*-quinones can carry out oxidation reactions coupled with *o*-diphenols (see 1, Figure 6.8). These reactions can be rapid, and depend on the respective redox potentials of the different present substrates (quinones and phenols). *O*-quinones also react with various polyphenols to form condensation products (dimers, oligomers and copolymers; see 5, Figure 6.8). The latter, having an *o*-diphenolic structure themselves, may, in turn, be subjected to enzymatic oxidation by PPO or non-enzymatic oxidation by *o*-quinones. The addition of water molecules to quinones gives trihydroxybenzene (see 7, Figure 6.8); these are then oxidized by excess quinones or PPO to form hydroxyquinones, which are ultimately the site of oxidative condensation resulting in colored polymers, the intensity of which changes with the degree

of condensation. Quinones can react with proteins (thiol and amino groups), free amino acids and amines (see 4 and 6, Figure 6.8). Due to their structure, some compounds formed act as competitive inhibitors of PPO. *O*-quinones can oxidize ascorbic acid (Asc) to dehydroascorbic acid (dAsc) by regenerating the corresponding *o*-diphenol (see 2, Figure 6.8). Sulfites are involved in reduction or addition reactions on *o*-quinones (see 3, Figure 6.8).

After many oxidation, addition and polymerization reactions, pigments that are called melanins are formed. The color (pink, red, blue, brown and black) and the color intensity of the pigments vary depending on the polyphenols involved and the environmental factors of the oxidation reaction (essentially temperature and pH).

6.3. Factors influencing enzymatic browning

The rate of onset and intensity of enzymatic browning in plant products depends on the nature and content of polyphenols, oxygen availability, enzyme activity, which itself depends on the physical-chemical environment (temperature, pH and water activity), as well as the presence of natural inhibitors. It is difficult to predict the rate of onset and the intensity of enzymatic browning since each phenolic compound has its own set of kinetic parameters (K_M , v_{max}) and produces pigments of a particular color.

6.3.1. Substrates

Enzymatic browning is mainly correlated to PPO activity and the phenolic compound content. Thus, organisms containing few PPOs and/or phenolic compounds are less susceptible to enzymatic browning. The phenolic compound content of plant products depends on several factors such as variety, ripeness and environmental conditions (light, temperature, nutrients and pesticides). The concentration of phenolic compounds is higher in unripe fruit and decreases rapidly during their ripening due to dilution. After harvesting, the concentration of phenolic compounds remains constant or decreases slightly. Furthermore, a wide qualitative and quantitative variation exists in the distribution of phenolic compounds within the same organism.

Even in fruit and vegetables susceptible to browning, there are practically no browning reactions as long as the tissue remains healthy and intact since the enzymes and substrates are not in contact; the enzymes are in the cell organelles (mitochondria, chloroplasts and microsomes), whereas the phenolic compounds are in the vacuole. Moreover, the contact between the compounds susceptible to oxidation and oxygen, which is the second enzymatic browning substrate, is reduced; dissolved oxygen is preferentially used by respiration enzymes that have more affinity for oxygen than enzymes responsible for browning.

However, cell relocation caused by different kinds of stress, whether it is mechanical (shock, cuts and wounds), pathological (attack by saprophytic or parasitic microorganisms) or physiological (cell deregulation of various kinds), is likely to induce browning of the plant tissue. The more plant organs are stressed, the greater the risk of browning. This is particularly the case when stressed areas are directly in contact with atmospheric oxygen. As a result, ready-to-use fresh precut fruit and vegetables (fourth-range products) are very susceptible to browning. Thus, handling these products involves the maintenance of cutting tools, the removal of damaged areas and packaging in a low-oxygen environment.

6.3.2. Physical-chemical conditions and presence of natural inhibitors

Temperature has a direct effect on enzymatic reactions in general and on the modification of color in plant products in particular. The optimum temperature for PPOs is around 25–35°C, which takes into account both an increase in the rate of catalysis and the inactivation of enzymes when increasing temperature. In addition, oxidation reactions and non-enzymatic condensation are favored with increasing temperature. Thus, the optimum temperature for enzymatic browning is between 35 and 40°C. A drop in temperature below these values results the reaction to slow down. However, cooling only reduces the reaction rate but does not fully inhibit it. Storing plant products at too low temperatures, below the “chilling injury” temperature, results in cell relocation. Under these conditions, more biochemical reactions occur, such as enzymatic browning, than if the storage temperature had been higher. Thus, the optimum storage temperature of plant products, in order to prevent the onset of browning, is the point at

which there is a maximum reduction in enzymatic activity without any damage to the structure of the plant tissues.

The optimum pH for enzymatic browning is between pH 4 and 7. It reflects the optimum pH of PPO activity as well as non-enzymatic addition and condensation reactions, oxidation reactions resulting browning and reactions inhibiting browning. Lowering the pH below these values reduces the enzymatic browning of plant products. Depending on the origin of the plant material, the optimum pH of PPO activity is generally between pH 5 and 7. However, PPO in apples maintains 40% of its maximum activity at pH 3. Controlling enzymatic browning by acidification alone is therefore difficult unless pH values are very low.

Lowering water activity slows down enzymatic activities by a reduction in the mobility of the substrates and reaction products, which in some cases have an inhibitory effect on the enzyme. However, in the case of plant products, lowering a_w is incompatible with the survival of the plant. The dehydration of tissues by lowering a_w results in physiological stress, with a far greater impact on enzymatic browning than the effects expected by reducing enzyme activity.

Among the naturally occurring inhibitors of enzymatic browning, ascorbic acid is undoubtedly the best known and the most common. Ascorbic acid delays the onset of browning by reducing *o*-quinones to their corresponding phenolic derivatives. It is also possible that ascorbic acid has a more direct effect on PPO by reducing copper at the catalytic site of the enzyme. In this oxidation state, PPO is unable to exhibit cresolase or catecholase activity.

6.4. Consequences of enzymatic browning

Phenolic compounds are actively involved in the color and taste quality of foods (bitterness and astringency). Therefore, the oxidation of phenolic compounds to quinone and their polymerization during enzymatic browning result in changes to food sensory properties. As previously mentioned, the color becomes increasingly browner as the rate of polymerization of phenolic compounds increases.

In addition, some phenolic compounds have antifungal and antibacterial activities. Resveratrol (3, 5, 4' trihydroxystilbene) (Figure 6.9), a phytoalexin of the stilbene family, is synthesized in response to a defense signal against an attack on grapes by the *botrytis cinerea* fungus (botrytis bunch rot). It has a relatively broad spectrum of activity, which makes it a strong contender for use as a natural antifungal agent to control fungal growth during the storage of fruit and vegetables.

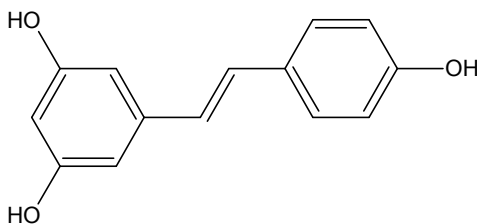


Figure 6.9. Chemical structure of *trans-resveratrol*

Despite the fact that this mechanism of action still remains poorly understood, it has been observed that polyphenols are more effective against Gram positive bacteria than Gram negative bacteria. Some degradation products of polyphenols such as quinones are more active than non-oxidized forms. Although it is difficult to generalize, it appears that changing the degree of oxidation of phenolic compounds generates a change in its antibacterial character.

Finally, phenolic compounds possess antioxidant properties with all the potential consequences for public health. These consequences manifest themselves through the capacity of phenolic compounds to scavenge or inhibit the formation of free radicals as well as chelate transition metals, primarily iron and copper, which are radical reaction catalysts. In many cases, the technological processes used in the preparation of food products are responsible for a reduction in the amount of natural antioxidants. This is due to the exposure of antioxidant molecules, in particular phenolic compounds, to high temperatures, atmospheric oxygen or light radiation. For example, the process of peeling, trimming or cutting fruit and vegetables is responsible for enzymatic browning and lowering the amount of natural antioxidants.

However, even though enzymatic oxidation is assumed to cause a progressive decrease in the amount of antioxidant molecules in food products, it appears that some phenolic compounds in an intermediate oxidation state may be better able to scavenge free radicals than the non-oxidized molecules. Thus, black tea possesses few phenolic compounds in the monomeric state due to successive oxidation and polymerization reactions occurring during the fermentation stage of tea leaves; yet, it has the same antioxidant capacity as green tea, rich in unoxidized and monomeric phenolic compounds. Short contact times between catechins and browning enzymes improve their capacity to interrupt the propagation of free radicals. Longer contact times result in the formation of brown compounds and a decrease in the antioxidant capacity is observed (Figure 6.10).

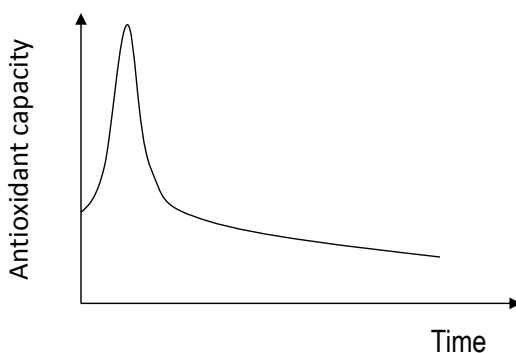


Figure 6.10. *Changes in the antioxidant capacity of foods containing phenolic compounds during enzymatic oxidation*

The increased antioxidant capacity of partially oxidized phenolic compounds is attributed to a better ability of the hydroxyl groups of the polymerized aromatic structure to give hydrogen atoms to free radicals and to stabilize the resulting unpaired electrons. However, the antioxidant activity of polyphenolic compounds decreases due to steric hindrances beyond a certain degree of molecular complexity.

6.5. Evaluation of enzymatic browning

Among the criteria for evaluating food quality, color plays a crucial role and color changes away from standard are detrimental. Thus, experimental methods based on the quantification of the color of food products are also used to evaluate enzymatic browning. Ultraviolet (UV)-visible absorbance spectroscopy, tristimulus colorimetry and reflectometry are most commonly used, but it is also possible to monitor the oxygen consumption or the indirect oxidation of certain compounds. These different methods are not interchangeable. Instead, they provide additional information on the enzymatic browning.

UV-visible absorbance spectroscopy uses absorbance measurements, usually at wavelengths of between 400 and 500 nm. These measurements are carried out in solution and require the prior extraction and purification of the compounds responsible for browning. As a result, the absorbance measurement takes into account only soluble pigments. However, during enzymatic browning, polymerization reactions lead to the insolubilization of some of the pigments. These are removed during the filtration or centrifugation stages prior to the absorbance measurement, and are therefore not quantified by this analytical technique. Moreover, depending on the type of pigment formed, which itself depends on the phenolic substrates used and the reaction conditions, the absorbance maximum varies. After oxidation, catechins form yellow pigments with maximum absorbance at 380 nm, chlorogenic acid produces yellow–orange pigments with maximum absorbance at 420 nm, while *o*-dihydroxyphenylalanine (DOPA) is the precursor of pink pigments with maximum absorbance at 480 nm. The absorbance value at a single wavelength does not always correlate with the visual appearance of browning.

Tristimulus colorimetry and reflectometry measure the light reflected by an object and can be directly applied to cut surfaces or pureed fruit or vegetables. Tristimulus colorimetry uses three filters (red, green and blue) based on the three retinal sensors (see section 9.2.2 in Chapter 9). Reflectometry uses multiple sensors to measure the spectral reflectance of an object for each wavelength. Reflectometry yields the same results as tristimulus colorimetry, but it also provides the spectral curve for the color in question.

6.6. Control and prevention of enzymatic browning

Postharvest changes are responsible for a considerable loss of fruit and vegetables worldwide; enzymatic browning is a major contributor to this loss. In winemaking, the incidence of PPO is particularly visible: the color changes to pronounced shades of brown, often with a deterioration in clarity and flavor, which thereby reduces the quality of the product. Thus, the control and prevention of enzymatic browning are vital. There are several methods for preventing enzymatic browning but only some are used in practice. They can be classified into three categories depending on whether they directly affect enzymes, substrates or the reaction products of enzymatic browning, although some may act on all three targets simultaneously. Their use depends on the type of food as well as nutritional, technical, sensory, regulatory, economical and even ethical considerations.

6.6.1. *Denaturation or inhibition of polyphenol oxidase*

The thermal stability of PPO, which depends on pH, is lower than that of other enzymes responsible for food spoilage. Thus, the thermal denaturation of PPO (bleaching) by steam, microwave treatment or immersion in hot water is an effective means of preventing the development of enzymatic browning in canned and frozen products. Brief exposure to temperatures of 70–90°C is generally sufficient to totally inactivate the enzyme. The destruction of enzymes follows a first-order law (the rate of inactivation is proportional to the residual activity of the enzyme). In many cases, it is necessary to ensure that the heat treatment is intense enough to destroy all the enzymes responsible for browning; otherwise, browning intensifies after cooking. The change in the selective permeability of cell membranes during cooking places phenolic substrates in contact with enzymes. Moreover, the complete inactivation of browning enzymes does not always imply a lack of color after cooking. For example, the gray color that appears during the cooking of mushrooms is due to secondary reactions affecting preformed quinones. Heat treatments that denature PPO are not always applied since they can adversely affect the flavor and texture of food products. It is, however, possible to implement conditions or compounds to reduce the binding of copper to the active site of the enzyme, or compounds

that interact at this active site and compete with enzymatic browning substrates.

Lowering the pH to values below or equal to 4 can be used to reduce the browning of fruit juices, sliced fruit, avocados and guacamole as long as the acidity is tolerated from a taste perspective. Citric acid (lemon juice), malic acid, fumaric acid, sorbic acid or inorganic acids (hydrochloric and phosphoric acids) are most commonly used. The drop in PPO activity is due to a reduction in copper coordination by the active site of the enzyme at low pH. In addition, the complexing activity of the citrate can remove copper from the active site and inactivate PPO. Sodium pyrophosphate and EDTA are also effective inhibitors of enzymatic browning due to their ability to complex copper.

Halides, including chlorides, exert an inhibitory effect on PPO. Thus, sodium and calcium chlorides are used to delay the enzymatic browning of fruit and vegetables intended for processing. Calcium chloride has the advantage of maintaining the firmness of the plant tissue through the interaction of calcium with pectin molecules. The inhibitory action of chlorides is strongly dependent on pH, and therefore, they are often used in addition to the acidification of the medium between pH 3.5 and 5. The inhibition by halides is linked to the electronegativity of the anion; fluoride is the most inhibitory halide. Halides form a complex with copper facilitating its release after the protonation of histidine residues at the active site of the enzyme.

4-Hexylresorcinol (Figure 6.11) or derivatives of benzoic acid and cinnamic acid, the structure of which is similar to the phenolic substrates of PPO, can be used to inhibit enzymatic browning.

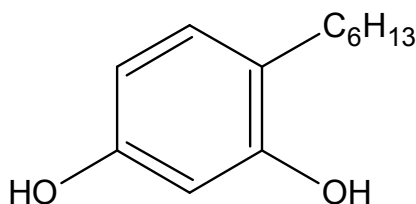


Figure 6.11. Chemical structure of 4-hexylresorcinol

These compounds act as competitive inhibitors of PPO. Diamino derivatives of coumarin and 4-hexylresorcinol effectively inhibit the appearance of brown spots on seafood like prawns. 4-Hexylresorcinol prevents the browning of cut fruit and fruit juices. It also inhibits the PPO of mushrooms, potatoes or avocados. The major advantage of this compound is its specificity toward PPO, which means that it can be used in low concentrations thereby limiting the effect on the taste, color and texture of the treated products.

Finally, since PPO does not seem to play a vital role in the organism, attempts to select species that are less susceptible to browning were considered through conventional techniques or genetic engineering. The latter rely on directed mutations in the active site of PPO (histidine), or the reverse insertion of gene coding for PPO in plant cells. During transcription, messenger ribonucleic acid derived from the reversely inserted gene hybridizes with that of the endogenous gene coding for PPO, thereby preventing its translation.

6.6.2. *Modification or removal of oxidation substrates*

Enzymatic browning can be inhibited by removing one or two substrates, oxygen or/and phenolic compounds from the reaction medium. The removal of oxygen is achieved by immersing the product in syrups or brines, or by coating. Low oxygen atmospheres are also a satisfactory method of slowing down the appearance of enzymatic browning. This can be achieved by inert or modified atmosphere packaging. When oxygen availability is the limiting factor of the reaction, its influence on browning is measured by K_M (Michaelis constant), which represents the dissolved oxygen concentration for which the reaction rate reaches half of its maximum value. Furthermore, the depletion of oxygen in the atmosphere is accompanied by physiological changes such as a slowdown in respiration, an inhibition in the production of ethylene and a delay in the ripening and senescence of tissues responsible for the release of enzymes and substrates for browning reactions. Respiration enzymes have greater affinity for oxygen than enzymes responsible for browning. This allows the inhibition of enzymatic browning by reducing the partial pressure of oxygen (modified atmosphere packaging of many plant foods) without the onset of anaerobic catabolism. These conditions, combined with chilled storage, are applied to inhibit the enzymatic browning

of ready-to-use fresh precut fruit and vegetables. However, once the product comes into contact with oxygen, browning quickly sets in again.

Phenolic substrates can be reduced or eliminated by selecting varieties that are naturally low in phenolic compounds or by using physical methods:

- ultrafiltration can be used to selectively remove polyphenols from fruit juices. By converting polyphenols to oligomers or polymers that are more easily eliminated by ultrafiltration, pretreatment with laccase further reduces the amount of polyphenols in juices. However, the action of laccase appears to increase the susceptibility of juices to browning during storage. It is likely that the formation of highly reactive *o*-quinones and their passage through the pores of the ultrafiltration membrane is the cause;

- cyclodextrins have been used to remove naringin, in particular, in order to reduce the bitterness of certain fruit juices. At the same time, treated juices were less susceptible to enzymatic browning. Cyclodextrins are cyclic oligosaccharides composed of six (α -cyclodextrin) or more (7: β -cyclodextrin; 8: γ -cyclodextrin) glucose units linked by α 1–4 bonds. Cyclodextrins have a conical structure with a hydrophilic external surface and a hydrophobic internal cavity. The latter allows the formation of inclusion complexes with polyphenols preventing the action of PPO;

- chitosan, derived from the chitin of crustaceans, has been used to reduce the browning of fruit juices. Although the principle of action of chitosan is not well known, it would appear that its positive charge allows it to establish electrostatic interactions with the solid particles on which PPOs are linked. The complex is then removed from the juice by filtration;

- carrageenan is also used to remove certain phenolic compounds. It apparently forms complexes with polyphenols but can also chelate copper ions of the active site of the enzyme via their sulphate group.

The use of physical methods to remove phenolic compounds is limited to liquid products. However, the reduction or removal of phenolic compounds is not always desirable since this has a direct effect on the sensory and nutritional quality of foods containing them, as well as on the inhibition of pathogens. One alternative is to irreversibly and specifically transform *o*-diphenols into methylated phenols using *o*-methyltransferase. The catecholase action (*o*-diphenol oxidase) of PPO is therefore inhibited. In fact, the cost of having *o*-methyltransferase limits its use in the prevention of enzymatic browning.

6.6.3. Control of reaction products

Several compounds are used to prevent the enzymatic browning of foods by interaction with reaction products. They essentially act as reducers of *o*-quinones, which are retrograded to their corresponding *o*-diphenols, and/or by producing mostly colorless nucleophilic addition products. Their effect is generally temporary because they are irreversibly consumed during the redox reactions in which they participate. The quantity of browning inhibitors depends on the abundance of phenolic compounds susceptible to enzymatic oxidation and on the length of time (shelf life) during which browning should be inhibited. Moreover, the non-specificity of anti-browning molecules can negatively impact the taste or color of food products due to their involvement in side reactions.

Among these molecules, the most effective are sulfites. They can reduce quinones or form nucleophilic addition compounds, which result in colorless products after polymerization. When sulfites are added after the onset of browning, a partial decoloration of the food product can be observed. However, coloration becomes increasingly resistant to their action as the degree of condensation of the phenolic compounds increases. Sulfites also inhibit the activity of PPO by binding to it and changing its structure. They are used to inhibit the formation of brown polymers in prawns, cut potatoes, mushrooms and apples. However, despite their multifunctional properties (antioxidant, antimicrobial and anti-browning), their use is increasingly regulated given their potential impact on human health (see Volume 2 [JEA 16]). They are tolerated up to a level of 50 mg per kg in ready-to-use fresh precut fruit and vegetables intended for cooking, and must be labeled accordingly. As a result, other alternatives to sulfites are being sought.

The most common substitutes for preventing the enzymatic browning of foods are ascorbic acid and its derivatives as well as its less expensive stereoisomer, erythorbic (isoascorbic) acid. They retrograde newly formed *o*-quinones to corresponding *o*-diphenols. The reaction continues as long as ascorbic acid, oxygen and the active enzyme remain in the medium. When all the ascorbic acid is oxidized to dehydroascorbic acid, the quinones begin to accumulate and browning occurs. In addition, ascorbic acid could reduce the copper in the active site of PPO and thus inhibit its activity. This class of inhibitors is rapidly inactivated by traces of metals like iron or copper, naturally present in foods. They are less effective than sulfites at inhibiting enzymatic browning due to lower stability and less penetration in the food

matrices. As a result, they are often used in combination with other preservatives such as citric acid or sorbic acid. The dehydroascorbic acid generated during these reactions may participate in non-enzymatic browning reactions (Strecker degradation; see section 5.2.3.2 in Chapter 5).

Compounds with thiol groups (cysteine, reduced-form glutathion) are potent inhibitors of enzymatic browning. Cysteine exerts its reducing activity by reducing o-quinone and forming colorless cysteine–quinone addition products. The latter act as competitive inhibitors to PPO but can undergo cross-oxidation with excess quinones. They are responsible for colored compounds, the color of which ranges from pink to red below pH 5 to brown and black above pH 5, suggesting a reduced efficiency of cysteine (Figure 6.12).

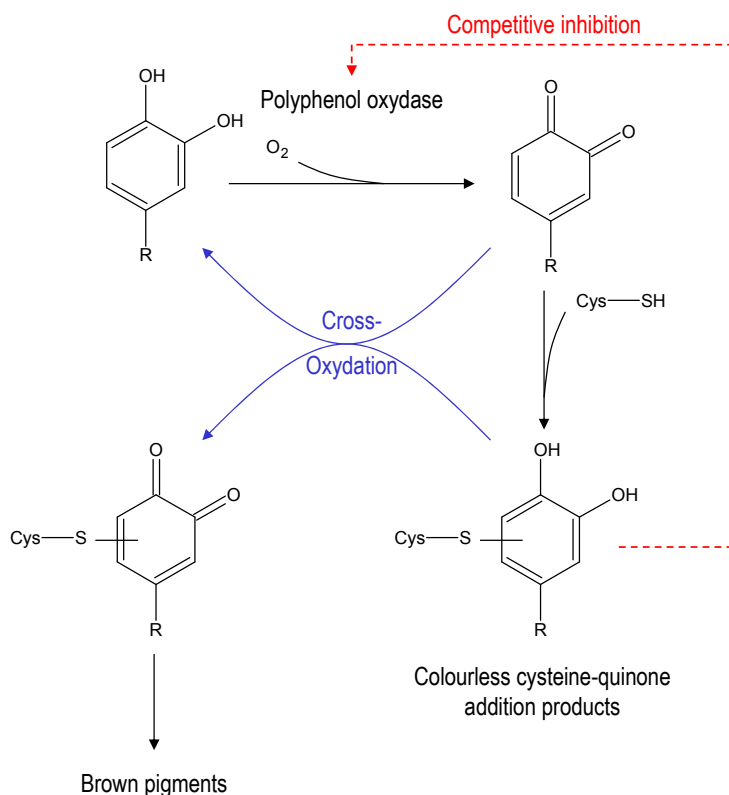


Figure 6.12. Effect of cysteine on the enzymatic oxidation of polyphenols

To effectively inhibit enzymatic browning, the quantity of cysteine must exceed that of the total phenolic compounds in the medium. Its effect has been demonstrated on pieces of avocado or banana. However, in many cases, the levels of cysteine used to inhibit enzymatic browning are unsuited to the sensory properties desired for final products. Reduced-form glutathione has the disadvantage of being too expensive for large-scale use in the food industry.

Molecular Dynamics in Food Matrices

Molecular migrations within the food matrix can considerably alter the sensory, microbiological and nutritional quality of food during its shelf life, resulting in softening, staling, sandiness, non-enzymatic browning and fungal growth. These migrations are governed by thermodynamic and kinetic parameters, which represent the quantity of molecules that are able to diffuse and the time required to achieve thermodynamic equilibrium.

Migrations occur not only between a food and its environment but also in “heterogeneous” foods as soon as a chemical potential gradient exists for a given molecule. These migrations continue under the influence of the gradient until the food system has reached thermodynamic equilibrium. The rate of molecular migration is proportional to the chemical potential gradient, which decreases continuously as the molecular diffusion progresses, and a diffusion resistance, which depends on the microstructure of food as well as the interactions between molecules in the medium. In most cases, molecular migration can be described according to the diffusion model established by Fick’s law (see Volume 2 [JEA 16]). However, in food matrices, diffusion phenomena can be accompanied by phase changes as well as structural changes within the food, which affect the migration rates. Thus, in the study of molecular migration, it is important to link molecular diffusion phenomena with phase and structural changes in the matrices.

Chapter written by Thomas CROGUENNEC and Pierre SCHUCK.

7.1. Water migration and changes in food quality

Water migration between foods within the same package, between parts of a “heterogeneous” food or exchanged with the surrounding atmosphere (Figure 7.1) are dynamic phenomena widely encountered in products such as pizzas, tarts, filled pastries, cereals with fruit, coated ice cream or ice-cream cones, etc. They greatly influence the consumer acceptability of food mainly in terms of sensory and microbiological qualities.

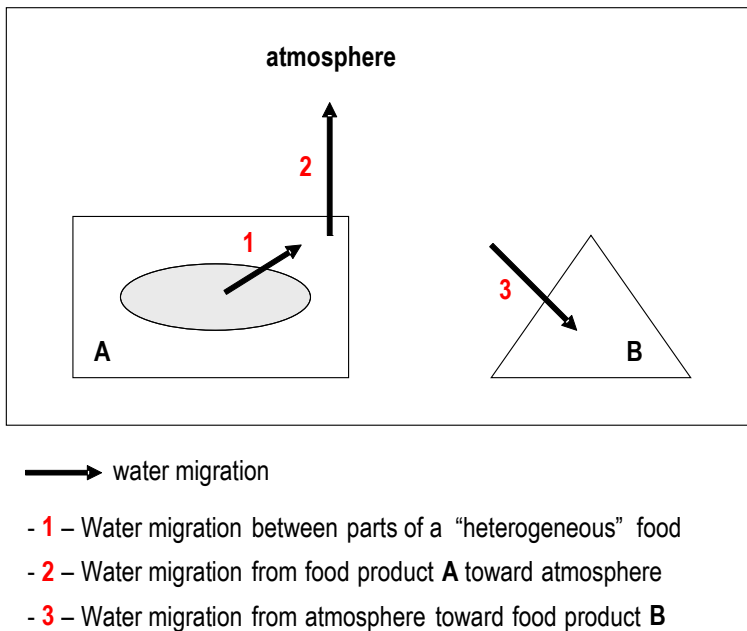


Figure 7.1. *Migration of water from food to the atmosphere or vice versa*

7.1.1. Water migration

The driving force behind water migration corresponds to the difference in water activity (a_w) between the various components or areas of the food, or the difference between the partial pressure of water vapor of the food and

that of the storage atmosphere. Water migrates from high to low a_w media. The transfer of water ceases once equilibrium has been reached, which corresponds not to an equal amount of water in each component or area but to an equal a_w . Thus, foods with both high and low water contents, sealed together in a package, will exchange water unless their a_w is the same. For some food systems, a_w at equilibrium is acceptable in terms of the physical, chemical or biological stability of each of the components or areas of the food; the shelf life of such systems is therefore not limited by water transfers. However, in systems where a_w at equilibrium is unsuitable for the stability of one or more components or constituents, the shelf life of the product is determined by the rate of water migration.

Water transfer is not independent of the transfer of other molecules in the food matrix. It can create local chemical potential gradients for other molecules triggering their migration. Furthermore, water migration may carry solutes by convection or, conversely, oppose their migration; this is the case with sugar- or salt-coated foods for which the direction of the water migration from the core of the product to the surface is opposite to that of the migration of salt or sugar molecules.

7.1.2. Equilibration with the atmosphere

Generally, products with low water activity may be subject to moisture absorption from the surrounding atmosphere when left in the open air. Biscuits, bread or cereals tend to lose their crunchiness. Powder ingredients are susceptible to clumping. In the case of milk, it is possible to obtain an instant powder with improved dissolution through lactose crystallization facilitated by controlled moisture absorption and an additional fluid bed drying step. Powder agglomeration (e.g. semolina) can be obtained in a similar way.

In contrast, fruit and vegetables are made up of cells saturated with water; they contribute to the firmness and freshness of the fruit and vegetables. Depending on the storage conditions (temperature and relative humidity), fruit and vegetables undergo dehydration at varying rates, losing moisture to the surrounding atmosphere. The dehydration rate depends on the exchange surface between the plant (saturation) and the surrounding atmosphere as

well as the difference in the partial pressure of water vapor between both media. The cutting of fruit and vegetables in fourth range products and the storage under dry atmosphere promote dehydration. Excessive dehydration causes not only wilting of the plant and loss in mass, but also exerts adverse physiological effects by stimulating the synthesis of ethylene, which accelerates senescence. The maximum permissible water loss without any decline in the sensory quality of the plant varies considerably. It is generally between 3 and 10%. Dried fruit (raisins) have a “soft” texture due to the sufficiently high water content. Under certain storage conditions (packaged in a dry atmosphere and/or with foods with low a_w), they may undergo a loss of water resulting in the hardening of the texture, which is unacceptable from a sensory point of view.

7.1.3. Equilibration in heterogeneous foods

In the production of biscuits, the water content decreases from the center to the surface of the biscuit once removed from the oven, which tends to equilibrate during storage. Water migration from the center to the surface of the biscuit causes parts that absorb water to expand and parts that release water to shrink. These movements create tensions, which the structure of the biscuit cannot tolerate unless sufficiently elastic; otherwise cracking may occur during storage. To avoid such cracking, it is possible to modify the cooling conditions after baking. Slow cooling enables the biscuits to absorb the mechanical stresses due to water transfer.

Pizzas, tarts and pastries often have a topping or filling, which has a higher a_w than the pastry or base. In such foods, water transfer is almost inevitable and generally results in softening of the pastry or base and loss in crispness. In addition, a local increase in the water content of heterogeneous foods with low a_w (e.g. dough) may lead to the growth of microorganisms if the a_w increases above their a_w growth limit. It can also promote chemical reactions such as lipid oxidation, non-enzymatic browning and enzymatic reactions.

7.1.4. Equilibration after a phase and/or structure change

Solid foods may be in a thermodynamically stable crystalline state or in an amorphous state, which is an unstable equilibrium that depends on

temperature and a_w . Depending on the glass transition temperature, T_g (see section 1.2.2), the amorphous state can be divided into a state of low water mobility (glassy, rigid, and brittle) and a state of higher water mobility (soft, rubbery, flexible, and pliable). The transition from the glassy state to the rubbery state involves a change in the thermodynamic properties, molecular mobility and mechanical properties of the matrix. This can cause physical, chemical or biological deterioration, thereby limiting the product's shelf life.

Some sugars may be responsible for physical or rheological damage in food products such as sweets, ice creams, milk powders, etc. They pose a problem in amorphous matrices because of the possibility of transition from a hygroscopic amorphous form to a non-hygroscopic crystalline form along with release of water molecules. Figure 7.2 shows that from an a_w of 0.4, amorphous lactose begins to crystallize causing a “break” in the isotherm.

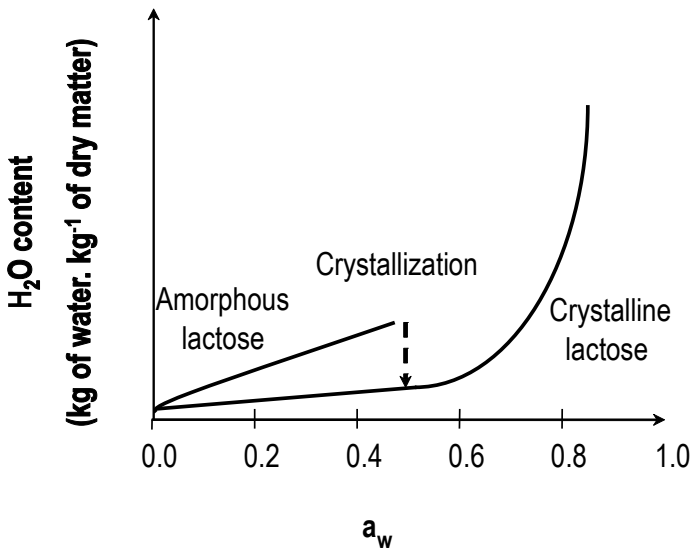


Figure 7.2. Sorption isotherm of crystalline and amorphous lactose

This phenomenon can occur at different rates depending on the difference between the temperature of the product and the glass transition temperature (Figure 7.3).

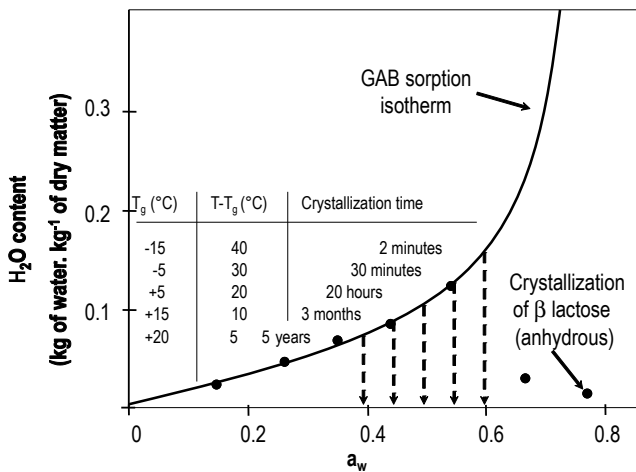


Figure 7.3. Crystallization time of amorphous lactose as a function of $T - T_g$ [ROO 02]

Amorphous lactose at 25°C can crystallize within approximately 5 years, 20 h or 2 min if the $T - T_g$ temperature difference is 5, 20 or 40°C, respectively. During the crystallization of sugar, the release of water increases the $T - T_g$ temperature difference for a given storage temperature, which in turn increases the rate of crystallization. Similarly, for a given a_w , the rate of crystallization increases as the temperature rises, thereby increasing the $T - T_g$ temperature difference. Figure 7.4 shows how, in the case of amorphous glucose at 25°C, this transition from an amorphous state to a crystalline state is affected by relative humidity (H_R). A minimum of 8.6% H_R is needed for glucose to crystallize in less than 400 days and at least 16.2% H_R for it to crystallize in a few days.

Released water can also bind to other constituents resulting in the formation of a poorly soluble sticky mass, which often results in caking or clumping. It occurs with coffee, orange juice powders and dried milk products when the storage conditions are not optimal. In addition, sugar crystallization can break down the structure of porous products. It can also cause the release and subsequent concentration of volatile compounds, fat, etc., initially encapsulated in the amorphous matrix (Figure 7.5). The decrease in viscosity that accompanies the transition from the glassy state to the rubbery state also accelerates bimolecular reactions such as lipid oxidation and non-enzymatic browning.

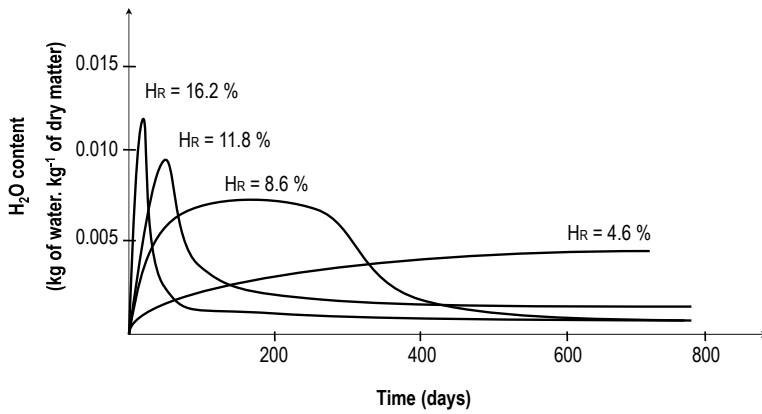


Figure 7.4. Crystallization of amorphous glucose to crystalline glucose as a function of relative humidity (H_R)

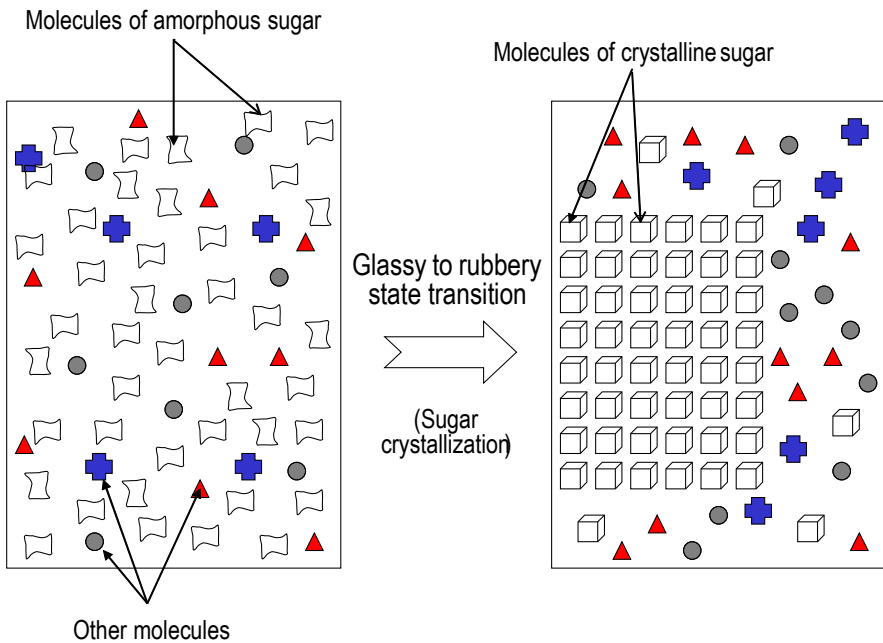


Figure 7.5. Concentration of compounds initially encapsulated in an amorphous matrix

During storage, bread gradually loses its freshness; it goes stale. Staling involves softening of the crust, which becomes more elastic, and hardening of the crumb. It is a complex phenomenon in which the reorganization of the starch chains and the migration of water play a major role. In the baking of bread, a starch network is formed consisting of amylopectin chains trapped in a continuous matrix of amylose chains. Bread is also dehydrated during baking, more in the crust than in the crumb. After cooling to room temperature, the molecules in the bread crust are in a rigid glassy state giving the “crunchy-crispy” character whereas those in the crumb are in a pliable rubbery state giving the softness of the crumb. During storage, a reorganization of the amylose and amylopectin chains to more stable semi-crystalline structures, known as starch retrogradation, occurs in the crumb. The rate of retrogradation depends on the $T-T_g$ temperature difference. Crystallization is accompanied by the release of water molecules that migrate from the crumb to the crust and soften it. Stale bread can be “refreshed” by reheating it, which breaks these reformed semi-crystalline structures.

7.2. Control and prevention

Water migration associated or not with a phase change may initiate irreversible damage to food quality. Combating such water migration is a major challenge because it can increase the shelf life of the product, a criteria valued by many consumers. Water migration can be controlled by acting on the thermodynamic and kinetic factors affecting it in heterogeneous foods, or with the surrounding atmosphere; limiting the a_w gradients and reducing the rate of water migration are the main issues.

7.2.1. Thermodynamic factors

One factor in controlling water migration is linked to thermodynamics; it involves reducing the a_w gradients between parts of the heterogeneous food or with the surrounding atmosphere. Food formulation is a means of limiting the differences in a_w values. In some cases, it involves increasing the a_w , whereas in other cases, the a_w needs to be decreased. Increasing a_w can be achieved by reducing the total solids, the soluble compounds or the fat content for a given solid. Among the many molecules that reduce the a_w of food, salts (mainly sodium chloride) and sugars (mainly sucrose) are most commonly used. However, these solutes can negatively affect the sensory

quality of foods. Variations in the solids and fat content often change food texture; added salts and sugars are responsible for increasing the perception of sweetness or saltiness in food. Beyond a certain level, flavor defects are unacceptable for the consumer. Moreover, the solubility limit of these molecules is quickly reached especially when a_w is low. Physical changes linked to crystallization phenomena may also create a limit in the consumer acceptability of products. The use of glycerol and propylene glycol as a_w depressants does not negatively affect texture. However, the potential toxicity of propylene glycol limits its use to maximum permissible levels. As mentioned in previous chapters, changes in a_w can promote chemical reactions such as non-enzymatic browning during storage. These measures to limit a_w gradients are only applicable if the a_w difference to be corrected does not exceed 0.1 within the intermediate a_w range (0.3–0.7).

In bread-making, emulsifiers such as monoglycerides are known to slow down staling. Monoglycerides are able to associate with amylose chains and limit the breakdown of starch grains. Through this property, they limit gelatinization during heating and consequently the number of molecules involved in retrogradation during the cooling and storage of the product. Moreover, monoglyceride–amylose complexes are less likely to undergo retrogradation.

7.2.2. Kinetic factors

Another factor in controlling water migration is related to kinetics. Water migration within heterogeneous foods is strongly dependent on the tortuosity of the diffusion path and the viscosity of the aqueous phase. The presence of crystals or fat hinders water migration. Thus, the greater the quantity (i.e. of finely dispersed crystals or fat), the slower the diffusion of water.

A local increase in the viscosity of the aqueous phase through the addition of hydrocolloids can also reduce the rate of water migration. In this respect, the T_g of food products is also very important in maintaining food quality during storage. Below the T_g , viscosity is very high and molecular migration is limited. During the drying of dairy products, water is eliminated so rapidly that lactose does not have sufficient time to crystallize; the product is in a glassy state (as amorphous glass, Figure 7.6).

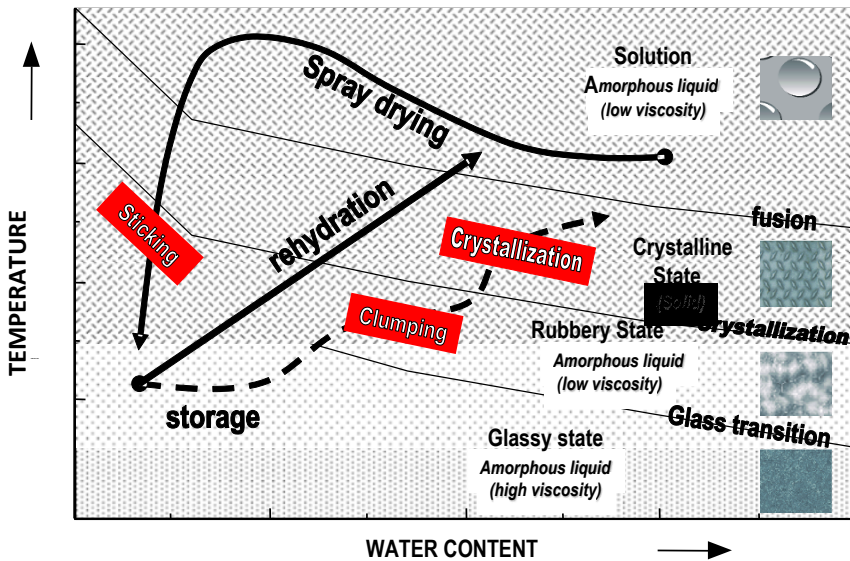


Figure 7.6. Phase changes in lactose and glass transition

During storage, it is necessary to maintain the product in an amorphous state in order to preserve its sensory and structural characteristics. The product should therefore be stored at a temperature below T_g to avoid lactose crystallization and water migration responsible for clumping. For example, skimmed milk powder at 4% moisture will be stable over time if its a_w remains close to 0.2 (at 25°C) and if it is stored below its T_g , which is approximately 50°C (Figure 7.7). If the water content increases to 7%, a_w increases to approximately 0.4 and T_g drops to 25°C, the powder will be more susceptible to physical and chemical changes (e.g. lactose crystallization and enzymatic browning) due to the higher a_w . In order to limit these changes, the powder should be stored below 25°C, which is more difficult and expensive.

Similarly, pasta can be stored for long periods when kept below T_g . After rehydrating durum wheat semolina, kneading the pasta dough under vacuum and shaping the fresh pasta, it is finally dried, usually between 70 and 90°C, to ensure the formation of a gluten network without inducing starch gelatinization, and then cooled below T_g .

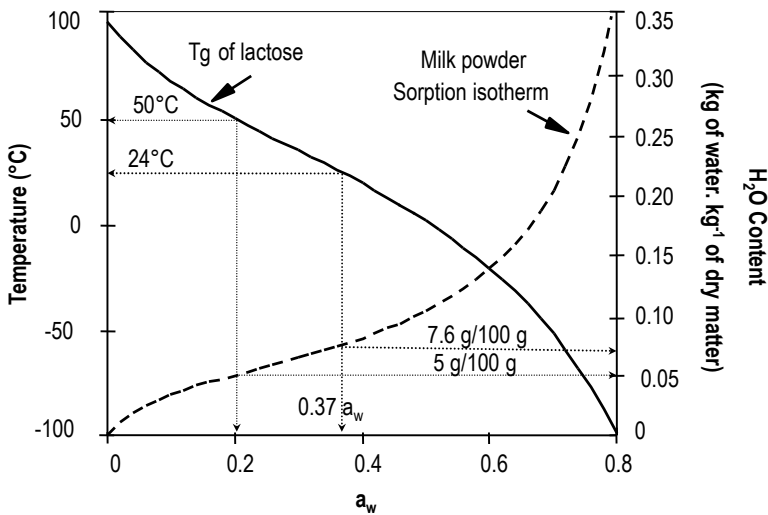


Figure 7.7. Change in the water content of skimmed milk powder and the T_g of amorphous lactose as a function of a_w

Another approach is to use “barrier” films to separate areas with different a_w . In heterogeneous foods, the “barrier” films used should be edible. Edible barrier films have the same properties as synthetic packaging but are generally less effective. The effectiveness of barrier films depends on several factors such as composition and thickness of the film, difference in a_w between the areas to be protected, temperature, etc. The chocolate layer that coats the inside of ice cream cones acts as a barrier against the transfer of water from the ice cream to the cone. The latter maintains its crispness during storage. Edible barrier films, generally made up of starch and fat, are used to separate the base from the toppings in frozen pizzas and tarts. The oil used as an anti-adhesive agent in baking wafers also acts as a barrier against moisture absorption during storage. The same applies to confectionary where edible barrier films cover the surface of sweets to avoid moisture gain and stickiness.

Packaging is used to restrict the exchange of water with the surrounding atmosphere. Such packaging may be highly impervious to water vapor to prevent during storage either moisture absorption and softening in products

with a low a_w (e.g. crisps), or to avoid dehydration in products with a higher a_w (e.g. cheeses coated in wax or wrapped in aluminum foil). In some cases, water vapor permeability is desired to avoid saturation of the packaging atmosphere. This is the case with fruit and vegetables packaged under modified atmosphere to delay ripening. If the relative humidity of the storage atmosphere of fruit and vegetables is too high, there is a risk of microbial growth on the plant surface. Packaging is also used to reduce volatile molecule exchanges between packaged food and the atmosphere and for hygiene reasons (protective role).

PART 3

Quality Control and Assessment

Food Safety Control

8.1. EU Legislation

8.1.1. *Directive 93/43/EEC of June 14 1993 on the hygiene of foodstuffs*

The manufacturer is responsible for the quality of products placed on the market and the “standard of hygiene throughout all stages of preparation, processing, manufacturing, packaging, storing, transportation, distribution, handling and offering for sale or supply to the consumer”.

This directive sets out “potential risk analysis, [...] risk assessment and management techniques to identify, control and monitor critical control points”. It is therefore compulsory for food business operators to identify all activities that may pose a risk to food safety and to implement adequate safety procedures (FAO-WHO, 1995). Inspections by competent authorities include a general assessment of the potential food safety hazards with particular attention to the critical control points (CCPs) identified by food businesses. Competent authorities are therefore responsible for both monitoring the fulfillment of regulatory objectives and evaluating the measures implemented by food business operators.

8.1.2. *Food safety regulations*

New hygiene rules were adopted in April 2004 by the European Parliament and the Council. They became applicable on January 1, 2006. They are provided for the following key acts:

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- Regulation (EC) No 178/2002 laying down the general principles and requirements of food law;
- Regulation (EC) 852/2004 on the hygiene of foodstuffs;
- Regulation (EC) 853/2004 laying down specific hygiene rules for food of animal origin;
- Regulation (EC) 854/2004 laying down specific rules for the organization of official controls on products of animal origin intended for human consumption;
- Regulation (EC) No 882/2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules.

Particularly, the new hygiene rules take into account the following principles:

- primary responsibility for food safety borne by the food business operator;
- food safety ensured throughout the food chain, starting from primary production;
- general implementation of procedures based on the hazard analysis and critical control points (HACCP) principles;
- application of basic common hygiene requirements, with further specifications for certain categories of food;
- registration or approval for certain food establishments;
- development of guides referring the good practices for hygiene or for the application of HACCP principles as a valuable instrument to aid food business operators at all levels of the food chain to comply with the new rules;
- flexibility provided for food produced in remote areas (high mountains, remote island) and for traditional production and methods.

8.2. Tools

8.2.1. Guide to good practice

Drawn up by food business operators in each agri-food sector (with other partners in the same sector and consumers), validated by government and

published in the Official Journal, guides to good hygiene practice describe the manufacturing process of a given product and propose measures to limit health risks. These recommendations can reduce the likelihood of certain hazards occurring, in particular those relating to handling by humans and cross-contamination. Guides to good practice take into account EU and national legislation.

8.2.2. HACCP [JOU 91, BUC 95, BRY 90]

HACCP is a preventive approach to hygiene problems. It helps to prioritize and guide the course of action needed to improve the health quality of products. It is a rational and systematic method carried out according to a logical working plan consisting of four steps:

- 1) identification of need(s);
- 2) data collection;
- 3) data analysis;
- 4) action.

HACCP is used to identify and assess the hazards associated with the different stages of the production process of a foodstuff and identify the necessary means to manage them. The concept of hazard corresponds to any biological (microorganism, toxin, etc.), chemical (additive, preservative, etc.) or physical factor (temperature, foreign body, etc.) that could pose an unacceptable risk to consumer health and safety or product quality. The concept of critical point implies any operation or procedure where a lack of control could result in an unacceptable risk to the microbiological health and safety of the product.

It is therefore a structured and progressive procedure, which is also multidisciplinary since all industry sectors are involved, participatory as it includes working groups, and specific to a company, production line, process or product.

It is a preventive method since operational measures are identified to avoid accidents, but it is also a critical, creative and evolving method as new requirements are constantly arising (pathogenic, equipment, and customer), to which it must adapt.

The HACCP method can be applied by following 11 steps (Table 8.1).

Step	Aims	Output
1	Assemble a HACCP team	Action plan
2	Describe the product	Product description
3	Identify intended use	
4	Describe the production process	Flow diagram
5	Confirm on-site the accuracy of the flow diagram	On-site, product flow
6	a) Analyze hazards (risk identification and assessment) b) Identify and evaluate preventive measures c) Establish preventive measures	List of hazards and preventive measures Operational procedures
7	Identify critical control points (CCPs)	List of CCPs
8	Establish critical limits for each CCP	Critical limits
9	a) Establish a monitoring procedure for each CCP b) Define corrective actions in the case of a deviation from the critical limit	Operational procedures Corrective actions, procedures
10	Establish verification procedures	Operational procedures
11	Ensure proper documentation	Documentation Records

Table 8.1. HACCP plan

8.2.2.1. Assembling a team

HACCP requires the formation of a team on the basis of an HACCP system; it should include the following members:

- the plant manager, who should coordinate actions and ensure their completion;
- production manager (process flow diagram);
- maintenance manager (state of equipment and consequences for product safety);
- quality control manager;

- laboratory manager;
- occasionally any other specialist from a particular area of expertise: purchasing manager, product manager, external expert, person responsible for cleaning and disinfection, logistics, transport, etc.

The team is responsible for the implementation and management of the procedure (transfer of responsibility). It has functional and non-hierarchical relationships, and should determine:

- the issue: production line, product, hazard, etc.;
- the objectives: e.g. improve the hygiene quality of the product.

8.2.2.2. Description of the product

A full description of the product must be given: raw materials, ingredients, formulation and composition, volume, shape, structure, texture, physical-chemical properties (pH, a_w , preservatives), storage, cooking and distribution temperatures, packaging, etc.

8.2.2.3. Intended use

The terms and instructions of normal and intended use (use-by date, cooking method and time, etc.) should be described.

8.2.2.4. Description of the production process

The production process should be described and presented in a detailed flow diagram that includes the nature and function of the process, equipment, materials and premises, characteristics (objectives, parameters, constraints), internal flow (material, equipment, personnel) and the environment.

8.2.2.5. Confirmation of the production process

The flow diagram must be verified on-site during production in order to confirm that it is in line with actual operations.

8.2.2.6. Hazard analysis

Hazard analysis consists of three main steps: identification of hazards and related causes, risk assessment and the establishment of preventive measures.

Firstly, all hazards must be identified (e.g. broken glass in a product, pathogens) and linked to situations and causes (raw material, ingredient, practice, procedure, etc.) that could increase the likelihood of their occurrence or allow them to increase to an unacceptable level. All malfunctions and faulty operations must also be accounted for.

Subsequently, there should be a risk assessment, i.e. evaluating the likelihood of risk at each stage of the process while taking into account three points: the frequency of occurrence of the cause, the severity of the hazard associated with this cause and the probability of non-detection of the cause.

Finally, preventive measures specific to each identified hazard should be implemented, i.e. actions to eliminate the hazard or reduce its risk of occurrence: e.g. changing a cooling tunnel, modifying a cleaning procedure, walling up an opening.

8.2.2.7. Identification of critical control points

A CCP is any step or procedure where a lack (or loss) of control creates an unacceptable risk. A CCP is generally any step to eliminate or reduce a hazard, to prevent the risk of reaching an unacceptable level, or steps during which the hazard may be introduced or increased without a further step to eliminate or reduce it.

The use of a decision tree for each raw material or ingredient and for each step of the process can help to determine the CCPs. CCPs should be listed in order of priority.

8.2.2.8. Establishing target values and tolerances for each CCP

Critical limits are established for each CCP, which are criteria indicating whether an operation is under control. With regard to microbiological hazards, the most common control criteria include temperature, refrigeration time, pH, a_w and frequency of cleaning and disinfection.

8.2.2.9. Monitoring procedures and corrective actions

The requirements for each CCP must be verified. The ideal situation is continuous monitoring that delivers real-time information, but this is often not possible. Monitoring is therefore mostly intermittent, and it is necessary to define the number and frequency of monitoring operations. These can include visual observations (cleaning), physical-chemical measures or

microbiological analyses. Monitoring should be described on the basis of operational procedures with a definition of responsibilities. The results should be recorded and interpreted.

Corrective actions must be established when the monitoring system indicates a loss of control for a CCP in order to regain control. It is also necessary to manage products affected by loss of control, change monitoring procedures where necessary, specify responsibility and draw up accurate records.

8.2.2.10. *Verification*

It is essential to verify the effectiveness of the system by analyzing deviations, corrective measures and customer complaints and validating values set as critical limits (e.g. pasteurization rate). Verification procedures should be described (mode, frequency, methods to implement).

8.2.2.11. *Documenting HACCP*

Evidence is essential in the validation of the HACCP system: food safety has no basis without reliable contractual evidence. A summary table combined with a production flow diagram is used to gather all HACCP analysis elements. This table takes into account the responsibility of the person in charge of monitoring and/or corrective action, and subsequent verification. Improvements and/or investments are based on these findings.

HACCP is a method that can be used to implement quality assurance. It takes into account the company's activities to prevent a specified hazard (or number of hazards). It can be applied to a product, process or particular project (hazard = consumer safety).

HACCP includes:

- identification elements and needs analysis (hazard analysis and associated risks);
- intervention elements (CCPs and monitoring);
- performance evaluation methods (verifications, system audits);
- reaction measures in response to deviations (corrective actions, HACCP meetings);
- a documentation system (records, operational procedures).

The HACCP system is therefore a set of rational and structured procedures that follow in a logical order to achieve a defined goal. If the company does not have a quality assurance system, the HACCP system constitutes the first key element to establish. If the company already operates a quality assurance system, the HACCP system can be adapted to specific quality plans.

8.2.3. Food safety and quality assurance management

Some companies engage in a process of quality assurance that is beyond the scope of quality control. The standard ISO 22000 is a Food Safety and Quality Assurance Management System. It is an international standard that specifies the requirements for HACCP in organizations involved in the production, processing, transport or distribution of food products. This type of certification is suited to businesses that require international recognition of their food safety management system. ISO 22000 is also for companies seeking to integrate their quality management system, for example ISO 9001, and their food safety management system.

The food safety standard combines generally recognized key elements to ensure food safety along the food chain, including:

- interactive communication;
- system management;
- control of food safety hazards through prerequisite programs and HACCP plans;
- continual improvement.

Evaluation of the Physical-chemical Properties and Quality of Food

The quality of raw materials and foods is defined by processing constraints, consumer expectations and regulatory requirements. Quality can be expressed on many different levels (Figure I.1):

- *hygienic quality*: raw materials and foods derived therefrom should be free of pathogenic organisms, toxins, chemical residues or undesirable components generated during processing.

- *nutritional quality*: the relative concentration and nature of various nutrients should not, if possible, deviate too far from nutritional recommendations. While it is unrealistic to hope to achieve an ideal balance in each food, this balance can, however, be reached in the overall diet.

- *technological quality*: raw materials and intermediate products intended for the preparation of food must have all the necessary qualities for processing, either in terms of technological feasibility or processing yield.

- *sensory quality*: sensory qualities influence palatability and the pleasure associated with consuming a product; they include color, texture, smell, flavor and aroma.

- *quality of service*: this includes service (storability, ready to use, etc.) and quality of use (packaging convenience, single portions, etc.).

These qualitative components can be understood and evaluated by biological (microbiological analysis), physical-chemical (color, texture and

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composition), biochemical (composition) and sensory (flavor and aroma) methods, which are not dealt with in this book.

9.1. Microbiological evaluation

Evaluating the microbiological quality of a food product involves two aspects:

- hygienic quality, which indicates the health risk to the consumer;
- commercial quality, which indicates the existence or risk of spoilage.

The ideal situation for manufacturers wanting to know the microbiological quality of the foods they produce would be to analyze all the manufactured food products and find and list all the pathogenic and spoilage microorganisms. However, the diversity of microorganisms, the number of necessary tests and the destructive effect of analyses make it necessary to select a few sufficiently informative analyses as an indicator of quality.

9.1.1. Choice of microbiological assays

It is important to search primarily for pathogenic microorganisms likely to be present in the food. The origin of the raw materials and the type of technological operations performed during production determine the microbial profile that is unique to the food. For example, it is more likely to find *Salmonella* in a chicken carcass than biscuits.

When choosing which pathogen to target, the manufacturer can refer to the microbiological criteria defined in a number of regulatory texts, in particular the Order of December 21 1979, and the memo of DGAL/SDHA/N2001-8090 of June 27 2001. A microbiological criterion indicates a maximum tolerable microbial population calculated by a defined method in a given food. It consists of the following elements:

- relevant product statement;
- statement of microorganism(s) identified;
- analytical methods for detection and quantification;
- appropriate numeric limits for the given product;

- sampling protocol;
- indication of where this criterion is applicable (e.g. before or after pasteurization).

A criterion introduced into a regulatory framework is known as a standard. It always involves compulsory action by the competent administrative authorities in the case of breach. This action may involve the process alone or both the process and the affected product. This is why a general distinction is made between an “imperative standard” involving an action by official services with regard to the products or batches concerned, and a “guideline standard” involving only corrective actions regarding the process.

In addition to these criteria, the manufacturer may also look for pathogenic microorganisms that may be present in the food. Manufacturers have an obligation of results: they are responsible for the quality of the product placed on the market and, as a result, should ensure that the product is free of pathogens (even those not specified by existing standards). Similarly, manufacturers may search for microorganisms known to cause spoilage or microorganisms responsible for sensory quality (e.g. fermented foods).

However, for the reasons previously mentioned, an exhaustive search is not possible. Therefore, the strategy involves looking for the main pathogens that may be present in the food and, by detecting indicator microorganisms, assessing the potential of the food to host pathogenic or spoilage microorganisms.

9.1.1.1. *Indicator microorganisms*

Total mesophilic flora

The mesophilic flora, which is assessed on an agar medium rich in nutrients (Plate Count Agar (PCA)), includes chemoorganotrophic mesophilic microorganisms corresponding to bacteria present in most foods. The number of microorganisms after incubation at 30°C for 3 days gives an indication of the effectiveness of the safety operations in place, the quality of care during packaging and the level of hygiene in general. A high mesophilic flora may indicate that the spoilage process is well underway or that pathogens are likely to be present. Usually, this flora is not pathogenic since it consists of the natural flora of raw materials and the processing plant. However, it could comprise mostly pathogenic flora (e.g. *Listeria monocytogenes* in pasteurized charcuterie, contaminated during storage).

Counting total flora can lead to misinterpretation in the following cases:

- fermented products: they normally have high total flora values corresponding to the presence of microorganisms responsible for fermentation (10^9 microorganisms g^{-1}). Part of the lactic flora can grow on PCA, and although the resulting colonies are small, they are included in the total flora. To avoid this confusion, it is more common in the analysis of these products to make a count on MRS medium, which is more selective in terms of lactic acid bacteria;

- sterilized products: the absence of microorganisms on PCA is not indicative of the quality of the raw material (*Clostridium*);

- refrigerated products: cold storage produces a number of microbial profiles.

Enterobacteriaceae

This phylogenetic group includes bacteria that live in the digestive tract of mammals. However, some species can colonize other ecological niches or, simply due to their resistance, survive outside the gut. They are counted by culture on Violet Red Bile Glucose (VRBG) agar, which is a selective medium. The presence of enteric bacteria in food can result from contaminated raw materials where the code of good conduct has not been adhered to (e.g. contaminated meat during the improper evisceration of carcasses, poorly cleaned equipment, a break in the cold chain or improper cooling, etc.).

Unless there is a lack of hygiene during processing, the level of enteric bacteria found in foods is relatively low. Although the *Enterobacteriaceae* family contains pathogenic species, their presence is not necessarily correlated to a risk for the consumer.

Coliforms

Coliforms include lactose-negative, gas-producing enteric bacteria. The main species are *E. coli*, *Klebsiella spp.* and *Serratia sp.*. Coliforms live in the digestive tract of mammals, but some species can withstand variations in the environmental medium and consequently survive outside the gut. They are counted on a selective medium of Deoxycholate Lactose Agar incubated

at 30°C for 2 days. Their presence in foods leads to essentially the same conclusions as for enteric bacteria.

Fecal coliforms (also known as thermotolerant coliforms)

This group includes coliforms capable of growing at 44°C, i.e. essentially *E. coli*. This bacterium, which shows low survival in the environmental medium, indicates recent fecal contamination when present in food (contamination of raw milk by cow dung or contamination of egg products by droppings on the egg).

While some serotypes are pathogenic (*E. Coli* O 157: H7), the vast majority of strains are not dangerous to humans. As with coliforms, the level of contamination in food is rather low, and, when high, often indicates a break in the cold chain. While a moderate number of *E. Coli* cells are not pathogenic, a high number can cause intestinal disorders. The presence of *E. Coli* in food may be an indicator of the presence of other pathogens not covered by the analyses (e.g. virus).

Permissible levels in food are generally very low because they indicate questionable hygiene practices. The concept of faecal coliforms is, however, criticized as being inaccurate from a taxonomic point of view and because counting methods, varying from one laboratory to the next, often lead to misinterpretation.

Sulfite-reducing anaerobes (SRAs)

This group includes certain pathogens (*Clostridium perfringens*). The presence of spores in sterilized foods highlights a shortcoming in the sterilization procedure.

The concept of SRA is deemed somewhat vague because it encompasses benign microorganisms (*Bacillus*) as well as species highly pathogenic for humans (*Clostridium perfringens*).

Yeasts and moulds

These are spoilage organisms: they can be analyzed to assess the spoilage status of the product.

Lactic flora

In some products, the development of lactic bacteria is desired (yoghurt, cheese, meat, sausages and wine). In others, these bacteria are spoilage organisms (fruit juice). This flora is measured in fermented foods where the total aerobic mesophilic flora may be overly abundant, but does not imply a lack of hygiene.

Other flora

Psychotropic flora or spore-forming flora (mainly *Bacillus*) may be analyzed by the food producer depending on the product and upon consumer request.

9.1.1.2. *Pathogenic microorganisms*

Some pathogens may also be analyzed depending on the type of product or at the request of the consumer. This is the case, for example, with *Salmonella*, *Listeria*, *Staphylococcus*, but also *Campylobacter*, *Clostridium* or prions.

9.1.2. *Methods*

The reference method of counting microorganisms in foods is based on growth in liquid agar media. However, due to time-consuming experiments and the long delay in obtaining results (5 days to detect *Salmonella* and 3 days to determine total flora), alternative methods based on molecular biology have been developed.

The choice of technique depends on many factors, the main one being:

- the objective of analysis, which may be to count or simply detect;
- the urgency of the analysis;
- the cost and possibility of outsourcing analyses;
- the validity of the method with control services (AFNOR certified method, etc.).

All these methods may pose difficulties with regard to sampling and the recovery of microorganisms from given food matrices.

9.1.2.1. Conventional plate counting method

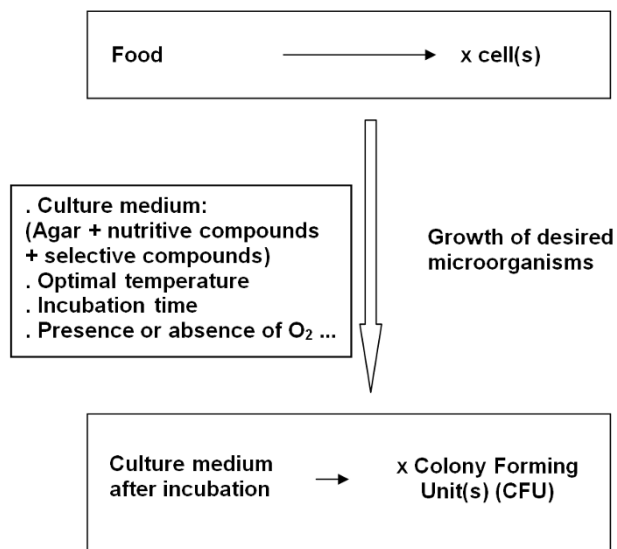


Figure 9.1. *Principle of standard agar plate counting*

Sampling

Reference must be made to the specific International Standard for the given product. If absent, a strategy must be agreed upon prior to analysis. In the case of self-monitoring, the onus is on the manufacturer to present a sampling plan.

Transport and storage before analysis

Samples must be transported to the laboratory under conditions that avoid any change in the number of microorganisms present. Storage temperatures depend on the products:

- dry or sterilized products: room temperature;
- fresh or chilled products: between 0 and 4°C;
- frozen or deep-frozen products: less than –18°C;
- pasteurized and similar products: between 0 and 4°C.

Test sample

The part used for analysis must be as representative as possible of the product. If the food is composed of several elements, each element must be taken in a similar proportion to that of the food.

During sampling, the microflora of the product must not be modified through the addition of foreign microorganisms. Sampling should be carried out aseptically in close proximity to an open flame or under a laminar flow hood. A typical sample should weigh 10 or 25 g ($\pm 2\%$) depending on the analysis.

Counting

The method involves grinding the food (usually 1:10) in a buffered medium in order to break it up and extract the microorganisms in the food matrix. The ground material is then diluted logarithmically in the same diluent in order to obtain increasingly lower concentrations of microorganisms. A portion of each dilution (generally 1 ml or 0.1 ml) is then mixed with a culture medium maintained in a supercooled state in the petri dish. This medium can be selective depending on the type of microorganism.

After solidification of the medium, the dishes are incubated under conditions that allow the optimum growth of desired microorganisms (temperature, gas atmosphere and duration). After a certain amount of time, each cell in the agar medium multiplies to form a colony visible to the naked eye. Counting the colonies gives the number of microorganisms present in the food before grinding. For accuracy reasons, counts are only valid if carried out on dishes containing between 30 and 300 colonies. The appearance of colonies (shape, size, color, etc.) can give an indication of the variety of microorganisms; however, two very different microorganisms may produce very similar colonies.

When counting microorganisms grown on selective media, manufacturers' instructions must be strictly followed. This method is very effective because it can be used to selectively count certain microbial groups in a complex flora down to 10 microorganisms per gram of food. It has, however, some drawbacks:

- it is a cumbersome method in terms of equipment (culture media, petri dishes and pipettes) and the number of experiments (labor cost);

- it takes several days to obtain a result;
- accuracy can be low – estimated at 0.5 log units;
- the number of microorganisms is sometimes underestimated because cells stressed by technological operations or the physical-chemical composition of the food can have difficulties growing in agar medium: they are known as viable but non-culturable (VBNC) forms;
- species that form cell aggregates or chains result in the formation of colonies by group, thereby greatly reducing the number of counted microorganisms.

9.1.2.2. *Alternative bacterial enumeration methods*

To overcome these drawbacks, a number of alternative methods have been developed. A distinction can be made between those that are based on the plate counting method and those based on totally different principles.

Petrifilm

Petrifilm consists of two permeable films containing a dehydrated nutrient agar medium that is rehydrated before use: rehydration occurs when the diluted sample is added. After incubation, the colonies are counted. This technique is advantageous to companies with basic analytical laboratories as it helps to reduce preparation time (culture media). However, it is costly and more difficult to count since colony growth is less than on conventional agar medium.

SPIRAL system [GIL 77]

This technique provides automated sample dilution and spreading on an agar medium. The diluted sample is drawn up by a syringe and spread by a stylus in a spiral direction on a rotating plate; dilution increases as the stylus moves from the center of the plate outward. The advantage of this technique is the accuracy of the count, the amount of time and labor saved and the ability to automate colony counting by a laser scanning system. However, the drawback is the relatively high cost of the device.

ATP method [STA 89]

This indirect counting technique is based on measuring one of the cellular components, adenosine triphosphate (ATP). It is present in all

microorganisms and rapidly degrades after cell death. The amount of ATP in a sample is proportional to the number of bacteria present. The first step of the assay involves cell lysis, allowing the release of ATP molecules into the medium; an enzyme (luciferase) and its substrate (luciferine) are added, which in the presence of ATP emit light, the intensity of which is proportional to the amount of ATP. It is not possible using the ATP method to selectively count microorganisms but it does give an indication of the amount of total flora. It is an ideal method for assessing in real time the level of cleanliness and disinfection of surfaces before resuming production. This technique is, however, not as accurate as conventional plate counting.

Real-time PCR

Real-time polymerase chain reaction (PCR), which is also known as quantitative PCR, can be used to rapidly detect microorganisms in food.

PCR is a rapid technique for amplifying a specific double-stranded deoxyribonucleic acid (DNA) sequence. In quantitative PCR, the amplification of DNA is characterized by an increase in fluorescence generated in a reaction tube and can be measured by optical density. An electrophoresis gel, as used in conventional PCR, is no longer necessary. This type of technique can therefore easily be used in company laboratories. The sensitivity of this technique is, depending on the food, between 10^2 and 10^4 microorganisms per gram. The ease of extraction of bacteria from food matrices is variable, e.g. recovering bacteria from a high-fat medium (cheese) is relatively difficult, whereas recovery from a high-cellulose medium (salad) is quite easy.

One of the disadvantages of this technique is the lack of discrimination between living and dead cells. A variant approach for specifically targeting living cells involves detecting only RNA present in the cells, by PCR. This technique is known as reverse transcriptase PCR (RT PCR). It is well suited to counting a specific species since only primers specific to the target species are selected. The task of counting a bacterial group containing several species is, however, much more complex. It is, in fact, very difficult to find a specific sequence in a group of different bacterial species (e.g. enteric bacteria or total flora).

9.1.2.3. Bacterial detection: conventional and rapid methods

Pathogenic microorganisms can be found in small numbers in food and are often accompanied by a much large number of other microorganisms that may be phylogenetically very close. In this case, selective enrichment allowing the growth of desired microorganisms is necessary (Figure 9.2). It may be preceded by non-selective pre-enrichment: colonies observed after culture in agar do not therefore represent the number of microorganisms initially present in the food. As a result, this is not referred to as counting but rather a search or detection and only their absence or presence can be concluded (requiring confirmation). Assays for these microorganisms generally require 25 g of product.

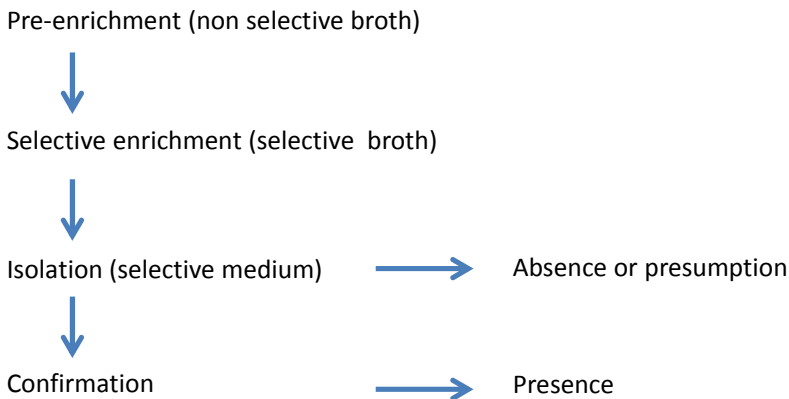


Figure 9.2. *Principle of the conventional detection method*

Conventional or rapid detection methods are based on the differences that may exist between families, genera and species of bacteria. These differences, from the largest to the smallest, correspond to morphological, biochemical, immunological and genetic characteristics.

Conventional criteria in microbiology are morphological (size, flagellum and colony shape) and biochemical (respiratory type, wall and metabolism). There are assays for carrying out a series of biochemical identification tests (API), which save time, lab space and material. Rapid techniques use genetic or immunological characteristics.

Thus, faster and more specific technology is being researched for each stage of conventional bacteriological analysis. Conventional analysis can be divided into three stages:

- enrichment;
- detection;
- analysis (visualization).

Enrichment

In this stage, the bacteria is isolated from its medium and placed in favorable conditions so as to be able to detect it. It includes a regeneration period in one or more liquid media, optionally selective, and lasts different lengths of time (at least 24 h).

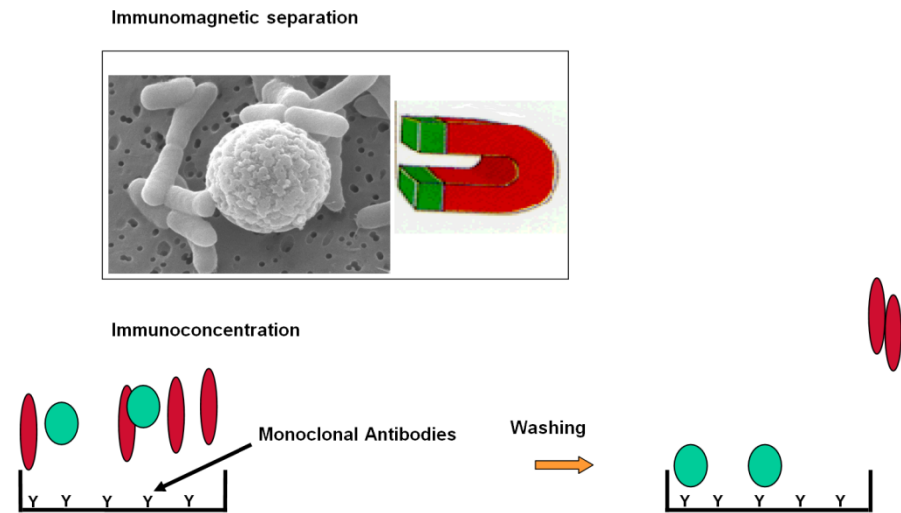


Figure 9.3. Principle of different rapid enrichment methods prior to detection

Immunomagnetic separation and immunoconcentration can significantly shorten this step (Figure 9.3). Using antibodies, specific bacteria can be enriched and/or extracted. Immunomagnetic separation involves using antibodies bound to magnetic beads which capture the bacteria using a magnet. Immunoconcentration uses antibodies attached to a support that can be washed in order to keep only a “bacteria concentrate”.

Detection

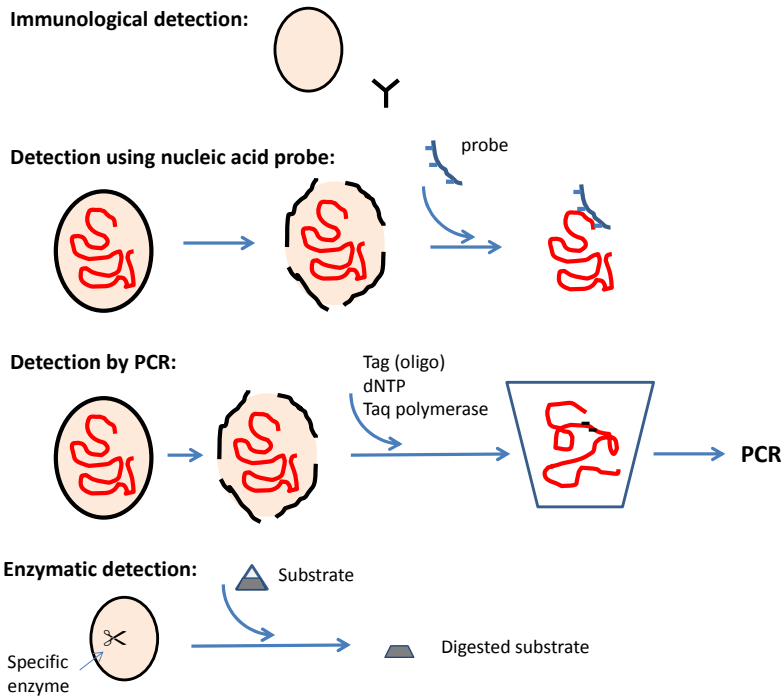


Figure 9.4. Principle of different rapid detection methods

There are different types of detection (Figure 9.4):

- immunological detection: it involves producing specific antibodies capable of binding to bacterial antigens to form antigen–antibody complexes, which can be visualized.

- detection using nucleic acid probes: these probes, based on the specific bacterial genome, can detect the presence of the genes in the bacterium.

- detection by PCR: by amplifying the DNA before starting detection, sensitivity can be increased. Copies are then confirmed by a visualization technique.

- enzymatic detection: based on knowledge of the enzymatic system of the bacteria and their specificity, the bacteria can be detected using a specific substrate.

Analysis and visualization

This last step involves interpreting the result of the detection.

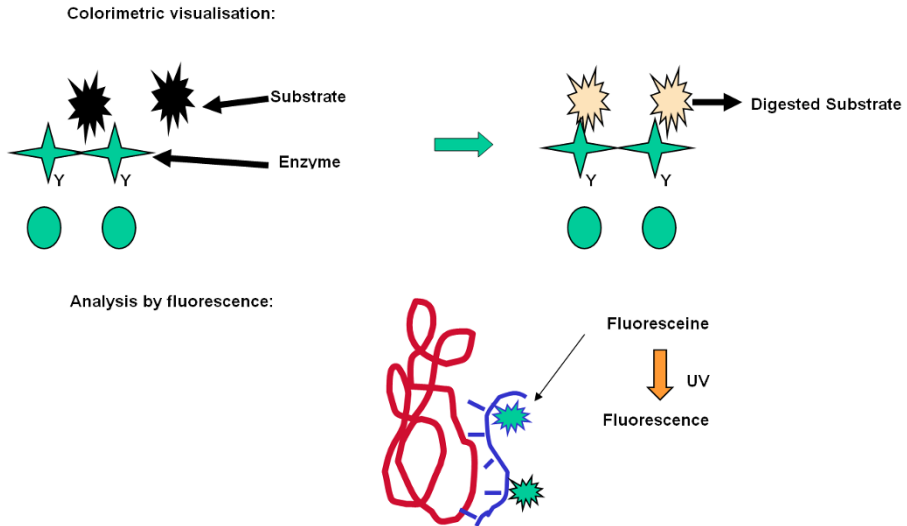


Figure 9.5. *Principle of different rapid visualization methods*

Many methods are possible (Figure 9.5):

- colorimetric visualization: by binding an enzyme (alkaline phosphatase or peroxidase) to specific antibodies or probes and then adding the corresponding substrate, this can cause a color change that can be detected and quantified by spectrophotometry.

- analysis by fluorescence: this is based on the addition of a fluorescent molecule (fluorescein) bound to a nucleic acid probe or antibody. Under ultraviolet (UV) light, the fluorescence can be detected.

Molecular biology techniques have a higher detection threshold than conventional counting or isolation techniques after plating on an agar medium. Immunological techniques are used to detect microorganisms if the sample contains at least 10^4 or 10^5 microorganisms per milliliter. PCR techniques are slightly more sensitive since the detection threshold is as low as 10^2 microorganisms per milliliter.

These techniques can be used to detect microorganisms after enrichment (the enrichment solution typically contains over 10^4 microorganisms per milliliter), but is not suitable for counting limits close to those of the agar medium (10 microorganisms per gram or 1 microorganism per milliliter). Certain foods contain PCR inhibitory molecules that are difficult to eliminate, and as already mentioned, the method does not distinguish between live or dead cells.

The choice between counting and/or detection techniques and between conventional and rapid methods therefore depends on various factors, which is summarized in Figure 9.6.

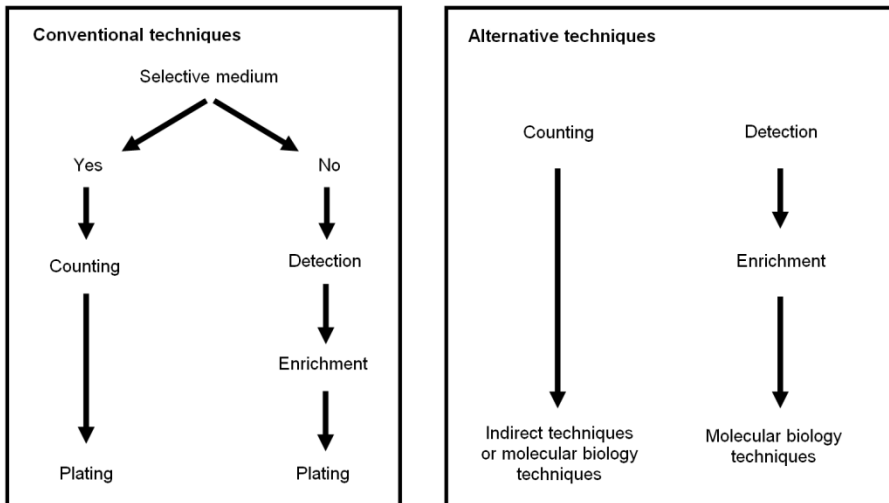


Figure 9.6. *Choice between conventional or alternative counting and detection techniques*

9.1.3. Limitations of microbiological evaluation

As previously mentioned, microbiological analysis can be time-consuming and expensive and generally involves destroying the food to be analyzed. For these reasons, the number of samples is limited. However, in order to highlight a specific problem in the production of a food, it is often necessary to analyze a large number of samples; thus, this is incompatible with the methods currently available. However, if an entire batch of product

or raw material were contaminated, microbiological analysis would easily detect this problem.

In conclusion, if the results of microbiological analysis indicate a high level of contamination in the sample, it is likely that the entire production is affected. However, good microbiological analysis does not necessarily mean that the microbiological quality of the batch is satisfactory.

9.2. Biochemical and physical-chemical analysis

9.2.1. Texture analysis by rheological methods

As outlined in section 9.1.3.1, the relationships between stress and strain or the strain rate can be defined based on Newton's law (see Volume 2 [JEA 1b]), which form the basis of rheology.

9.2.1.1. Definitions

Shear stress

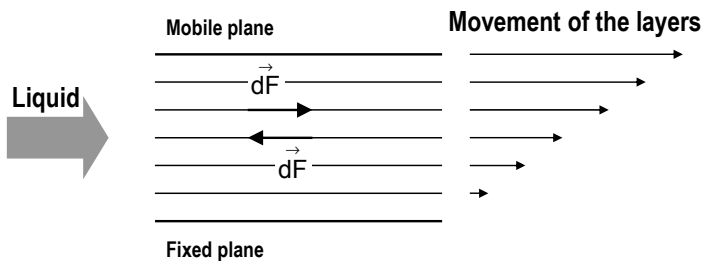


Figure 9.7. *Shear forces in the case of a shear plane*

When shear movement is applied to a liquid (the liquid is divided into a series of thin layers that slide against each other), shear force acts tangential to movement, dF , which tends to slow down or accelerate the adjacent layers in the case of a shear plane. The layers close to the moving plane move faster than those close to the fixed plane. The shear force, dF , applied to a surface area dA gives the shear stress τ :

$$\tau = \frac{dF}{dA} \quad [9.1]$$

Stress is the ratio of a force and a surface, and is expressed in N m^{-2} or Pascal.

Strain

In the case of shear, strain is the variation in displacement or deformation between adjacent layers. Take two molecules in the same plane and separate from each other by a distance dx at t_0 (Figure 9.8). After a given amount of time, dt , they reach points 1 and 2. Strain is the difference in displacement between the two molecules ($y_1 - y_2$) relative to the distance between them (dx). By convention, strain is written as γ , which is the ratio of two distances, and is therefore dimensionless. In the case of a solid gel compressed at low strains (below 10%), strain is the ratio of the compression distance to the initial height of the gel.

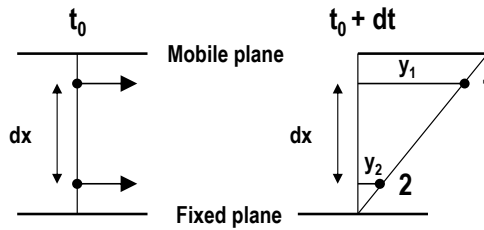


Figure 9.8. Strain

Strain rate

Denoted as $\dot{\gamma}$, this is the derivative of strain over time, which is measured in inverse seconds (s^{-1}). It is called strain (or shear) rate or velocity gradient because it represents the distribution of displacement rates in the fluid or material.

9.2.1.2. Rheological behavior

Liquid

A particular feature of liquids is that they flow irreversibly; this flow is characterized by the strain rate $\dot{\gamma}$. Any energy input, known as strain, is lost in viscous friction. A liquid is characterized by its viscosity η (volume 2), which is the ratio of stress to strain rate; viscosity is therefore expressed in

Pa·s. For a viscous liquid, the rate of change in strain over time is more important than the amount of strain applied. If strain is applied slowly, there is little resistance. The viscous behavior can be seen as a dashpot (Figure 9.9).

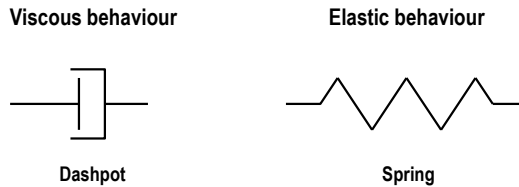


Figure 9.9. Graphical representation of viscous and elastic behavior

Figure 9.10 shows the main types of behavior of “time-independent non-Newtonian” fluids: viscosity is the tangent at the start of the curves $\frac{\tau}{\dot{\gamma}}$, and does not change with time at constant shear rate.

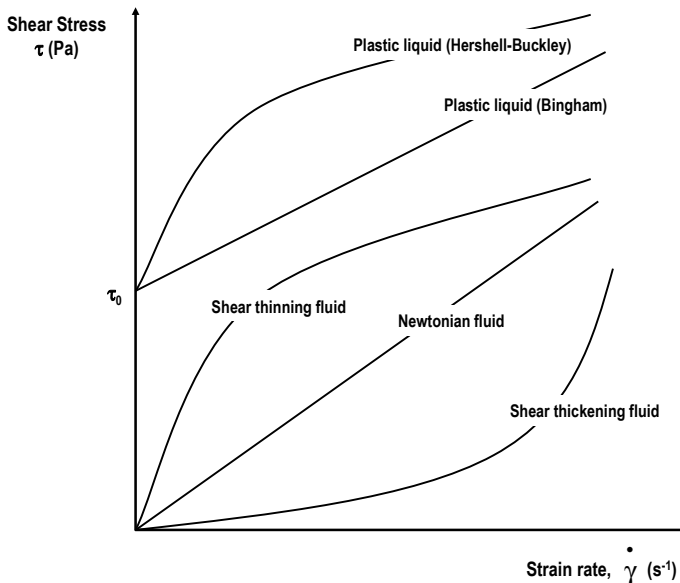


Figure 9.10. Main types of viscous fluid behavior

A distinction is made between:

– *Newtonian liquids* or linear viscous or pure viscous liquids, where viscosity is constant at different strain rates. This is the case with solutions containing low molecular weight solutes (water, solvents and sugar solutions) and diluted solutions of macromolecules (skimmed milk, clarified fruit juice). Viscosity always depends on the temperature and pressure applied to the product. Generally, the viscosity of Newtonian liquids decreases with temperature according to $\log(\eta) = f\left(\frac{1}{T}\right)$, where T is the absolute temperature (K). The viscosity of water at 20°C is 10^{-3} Pa·s.

– *Shear thinning or shear thickening fluids*, where viscosity drops or increases with the strain rate; it is therefore necessary to indicate the strain rate at which stress or viscosity is measured. Polymer solutions of increasing concentrations often display shear thinning behavior (protein concentrates, milk retentate) as well as liquids with weak intermolecular interactions: in this case, shear weakens these interactions (yoghurt). Shear thickening fluids are not as common and are limited to concentrated starches.

– *Plastic liquids*, where stress increases to a certain threshold (τ_0) before movement can be observed in the product. The value τ_0 can be obtained by extrusion of the contents through an opening in a container (e.g. tube). This behavior is common for solid emulsions or dispersions such as cream, butter and margarine, chocolate, ketchup or emulsified sauces (mayonnaise). The dispersed phase behaves like a solid, with weak interactions between the particles or droplets, but beyond this threshold, only flow of the continuous phase is observed. In the case of fat suspensions, the threshold is correlated to the melting properties of the fat. When the behavior beyond the threshold is linear, this is referred to as a Bingham liquid. When it is not linear, it is known as a Hershell–Buckely liquid.

Solid

In the case of solids, stress causes a constant strain at equilibrium. The energy supplied is stored and used to recover the original shape of the solid. It is said that the solid has a memory or that it has a preferred spatial configuration that is regained after stress. The parameter that characterizes a solid is its coefficient of elasticity or modulus of elasticity, i.e. the ratio of

stress to strain. The modulus of elasticity is expressed in Pascal. In the case of solids, stress is proportional to the penetration distance, regardless of the speed. Elastic behavior can be seen as a spring (Figure 9.9).

Viscoelastic

Most food products are viscoelastic, i.e. simultaneously liquid and solid: some of the energy is lost in viscous friction and some is stored.

9.2.1.3. *Measurement methods*

Liquid

Liquids are characterized by their flow. This flow can be induced in a capillary tube (capillary viscometer, by means of gravity or a pressure difference created by a pump). Flow time is inversely proportional to viscosity and proportional to the density of the fluid. The most common viscometers are cylindrical rotary systems with coaxial cylinders (Figure 9.11). These systems are well suited to Newtonian liquids. The liquid fills the space between the two cylinders, which is known as the (air) gap. The radii of the two cylinders are very close ($R_2/R_1 < 1.15$). The rotational movement produces a strain rate that is a function of the angular velocity of rotation and the two radii of the cylinders. The shear stress is measured in the product using a torsion bar (cross-section). The temperature control of the product is generally carried out on the outer cylinder. A cone- and plate-type geometry is also used (Figure 9.11). Two types of systems are used: those that apply controlled rotation and measure stress, and those that apply controlled force and measure rotational movement. The temperature control of the product is ensured by the plate on which the sample is placed.

Elastic or solid

The most common test is uniaxial compression, i.e. where the solid is compressed between two plates. The depth of compression and strain is known at any given moment (compression/original height). Stress is measured using a stress or force sensor. Tensile force can also be applied but the solid must be attached between two clamps. The ratio of stress to strain is used to evaluate the modulus of elasticity.

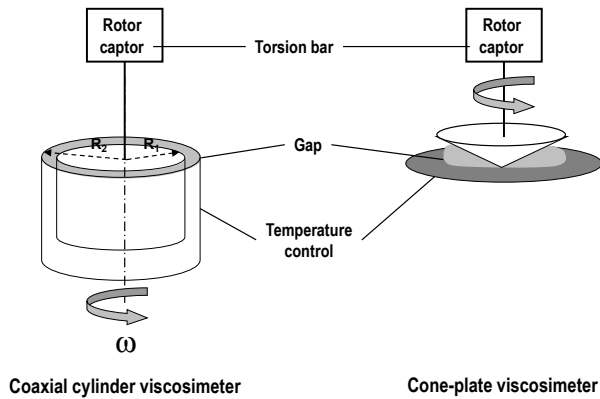


Figure 9.11. Coaxial cylinder and cone–plate viscometers

Viscoelastic

Three methods exist depending on whether one wants to control strain (stress relaxation) or stress (creep), or apply sinusoidal strain:

– *Harmonic shear (or dynamic method)*: a repetitive sinusoidal strain is applied and the resulting stress is measured, which should be a sinusoidal function of time at the same frequency. Figure 9.12 shows pure viscous, pure elastic solid and viscoelastic behavior.

In the case of elastic solid behavior, stress and strain are proportional at all times, and the phase angle (δ) between stress and strain is zero.

In the case of a pure viscous liquid, stress is proportional to the shear rate, phase shifted by $\pi/2$ with respect to strain, i.e. $\delta = \pi/2$ (the derivative of a cosine is a cosine phase shifted by $\pi/2$). The component of stress in phase with strain corresponds to the elastic or storage modulus G' , which is equal to the ratio of stress to strain and characterizes the solid properties of the product. The component of stress that is $\pi/2$ out of phase with strain corresponds to the viscous or loss modulus G'' , which characterizes the liquid properties of the product. The tangent of the phase angle characterizes liquid behavior:

$$\tan \delta = \frac{G''}{G'} \quad [9.2]$$

Viscoelastic products have a phase angle between 0 and $\pi/2$.

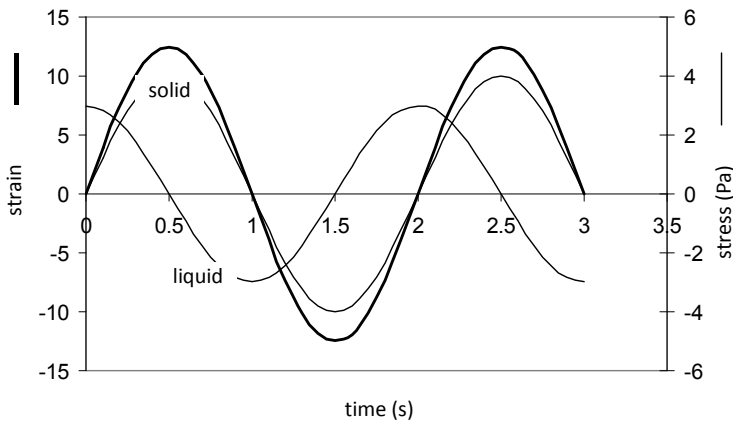


Figure 9.12. *Harmonic shear method*

– *Stress relaxation*: in this method, increasing low strain is applied and stress is observed over time. At time t , strain becomes constant and a resultant decrease in stress relaxation is determined. For a pure elastic solid, there is a constant stress at a constant strain with no stress relaxation (Figure 9.13). For a viscous liquid, the strain rate is zero at constant strain, and therefore stress is zero. With a pure viscous liquid, stress is instantly zero. Viscoelastic bodies display intermediate behavior. The proportion between the value at equilibrium and the value of maximum force is used to determine the elastic properties of the material. The whole relaxation curve can be modeled by different mathematical models; for instance, cheeses can be modeled by Peleg's equation.

– *Creep*: In creep, a low stress (that is non-destructive to the product) is applied for several minutes. Strain is observed; then stress becomes zero and strain is observed, i.e. how the product returns to its equilibrium position (compliance). For an elastic solid, it immediately returns to its original position (zero stress = zero strain). For a viscous liquid, there is a zero strain rate at zero stress, so the product keeps the position it has when the stress is removed. Viscoelastic products do not return completely to their original position and the position of equilibrium is reached at varying speeds (Figure 9.14).

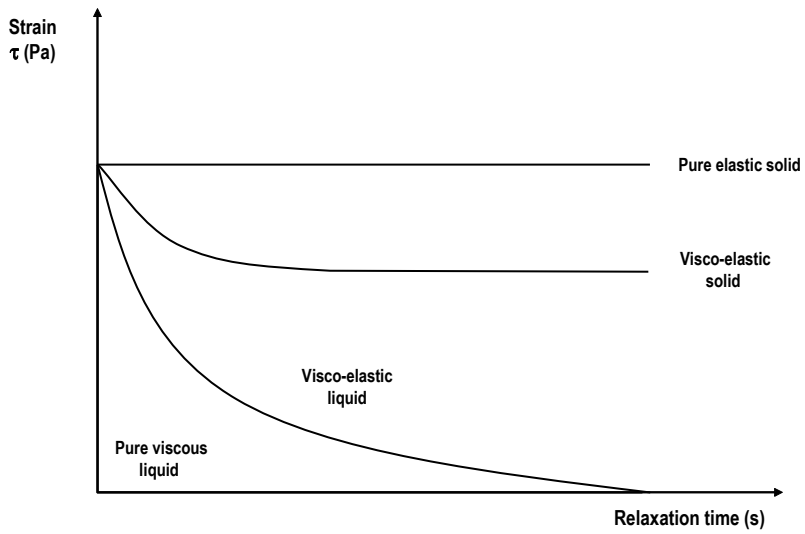


Figure 9.13. *Stress relaxation*

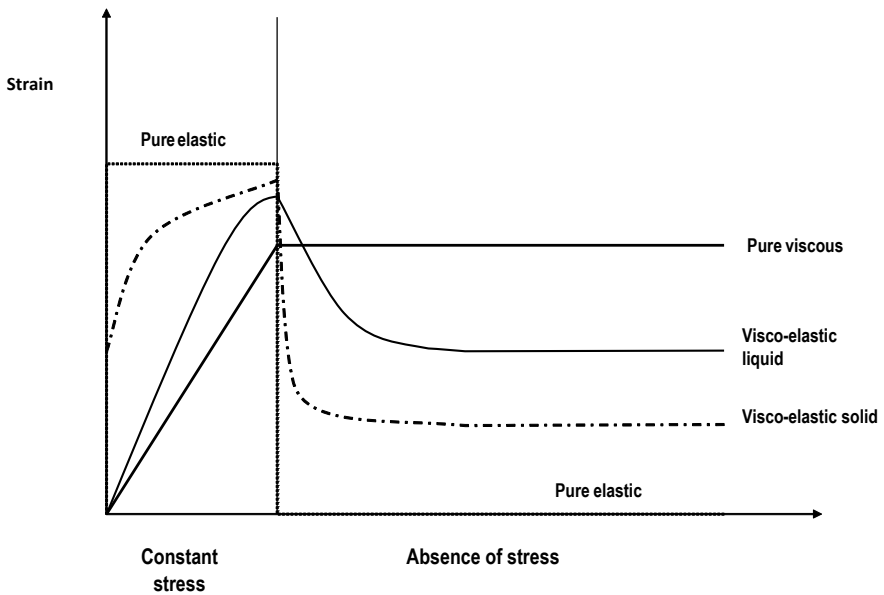


Figure 9.14. *Creep*

9.2.2. Color analysis

The color of a food has a significant impact on the purchasing decision and sensory perception of consumers. It is an objective criterion of quality as it varies with the level of ripeness of fruit and vegetables, and can be an indicator of the state of freshness or spoilage of a food (e.g. meat or fish).

The exact characterization of a color is quite complex. Several criteria must be taken into account to define it. The perceived color of an object depends on the lighting, the viewing angle with respect to the illumination angle, the background, geometric dimensions and the observer. Colors are classified based on three criteria: hue (color), saturation (intensity) and brightness (luminosity).

The two most common methods used to evaluate color are:

- the *XYZ* color space developed in 1931 by the International Commission on Illumination (CIE), which is based on the tristimulus values *XYZ*;

- the $L^* a^* b^*$ color space developed in 1976.

The concept of the tristimulus values is based on the theory that color perception depends on three factors. The human eye has receptors for the three primary colors (red, green and blue) and all colors are perceived as mixtures of these three colors. Three basic light stimuli [*X*, *Y*, *Z*] have been defined. The values of *X*, *Y* and *Z* ($\bar{x}$, $\bar{y}$, $\bar{z}$), as shown in Figure 9.15, can be used to define a color. The basic stimulus *Y* was chosen so that its value corresponds exactly to the visibility curve of the eye, therefore giving the physiological light intensity. Color is characterized based on the trichromatic coefficients *x*, *y* and *z*:

$$\begin{aligned} x &= \frac{\bar{x}}{\bar{x} + \bar{y} + \bar{z}} \\ y &= \frac{\bar{y}}{\bar{x} + \bar{y} + \bar{z}} \\ z &= \frac{\bar{z}}{\bar{x} + \bar{y} + \bar{z}} \end{aligned} \quad [9.3]$$

Two coefficients are enough to define color, given that their sum is equal to 1. Thus, $\bar{y}$ characterizes intensity and the coefficients x and y chromaticity.

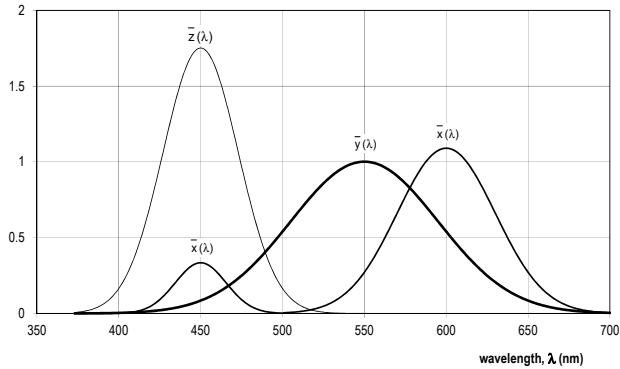


Figure 9.15. Spectrum of XYZ stimuli

In the chromaticity diagram in Figure 9.16, point A ($\bar{x} = 0.48$ and $\bar{y} = 0.30$) corresponds to the color of the analyzed object.

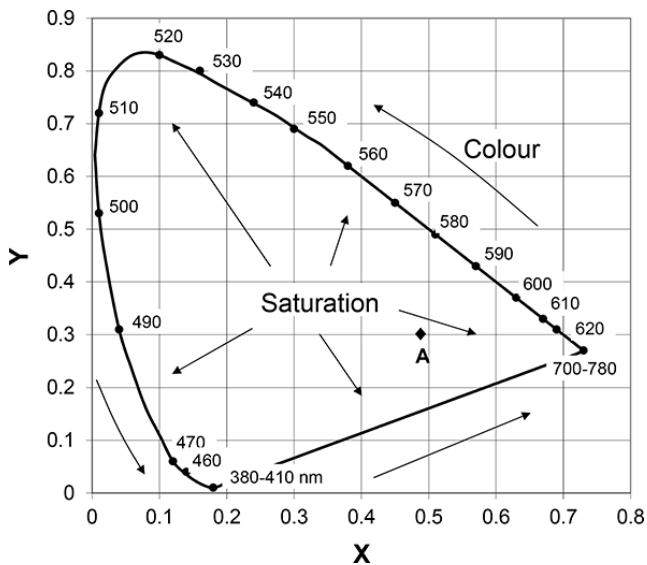


Figure 9.16. x, y chromaticity diagram

The $L^* a^* b^*$ color space (sphere) is widely used at present. In this space, L^* indicates lightness, while a^* and b^* are the chromaticity coordinates. Figure 9.17 shows a horizontal section of the sphere at value L^* . In this space, $+a^*$ goes toward red, $-a^*$ toward green, $+b^*$ toward yellow and $-b^*$ toward blue; saturation increases with increasing distance from the center.

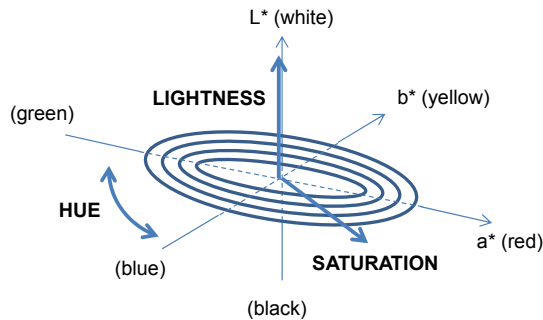


Figure 9.17. a^* and b^* chromaticity diagram

9.2.3. Analysis of food composition

Compositional analysis can be divided into four categories, as shown in Table 9.1. Not all methods and food analysis techniques are described in this book as they are described in detail elsewhere [LIN 81, POM 00].

Type of analysis	Constituents	Techniques
<i>Total</i>	Solid–water content	Drying
	Minerals	Determination of ash content
	Protein	Determination of nitrogen content, infrared spectroscopy
	Fat	Volumetric or gravimetric analysis, infrared spectroscopy
	Carbohydrates	Analysis of the reducing function, infrared spectroscopy
	Acids–bases	Titration

<i>Specific</i>	Carbohydrates–acids	Enzymatic assay
	Inorganic cations	Atomic absorption and emission
	Proteins	Immunochemical assay
<i>Separation</i>	Carbohydrates–acids–amino acids	Reverse phase liquid chromatography, ion exchange chromatography
	Proteins	Electrophoresis, reversed-phase liquid chromatography
	Lipids: triglycerides–fatty acids	Gas chromatography
<i>Structural</i>		Mass spectrometry Nuclear magnetic resonance

Table 9.1. *Analysis of food composition*

A total compositional analysis can be carried out using physical-chemical methods that are inexpensive and easy to implement. By drying in an oven, it is possible to determine the water and solid content. High-temperature treatment (ashing) is used to ascertain the mineral content. By chemical decomposition in highly concentrated sulphuric acid, the total nitrogen content (protein and non-protein) is converted to ammonium, which is then determined by titration as ammonia (Kjeldhal method) and converted to the equivalent protein using a coefficient depending on the origin of the protein. As lipids are in the form of emulsions in most liquid foods, it is sufficient to separate the phases by centrifugation and measure and weigh the supernatant. In the case of dried or low-moisture foods, the process involves solvent extraction and evaporation, and weighing of the extracted lipids. Carbohydrate analysis is often performed using chemical methods based on the reactivity of reducing functions. A quantitative analysis of the main constituents (lipids, proteins and carbohydrates) can be simultaneously carried out by infrared spectroscopy on both liquids and solids based on the specific absorption bands of the functional groups of the various constituents. The status of different biological processes such as fruit ripening, lactic or acetic fermentation or fish spoilage can be measured by titration of acids (lactic acid, acetic acid, tartaric acid and malic acid) or bases (endogenous amines).

Several biological and spectroscopic methods are currently available for specific quantitative analyses. Among the biological methods, there is a distinction between enzymatic and immunochemical methods. In the first case, enzymes are used, which react in a complex medium with their specific substrates. The most common reactions are those involving dehydrogenases and their cofactors (NAD^+/NADH , $\text{NADP}^+/\text{NADPH}$) whose spectral properties can be used to quantify the converted substrate by UV-visible spectroscopy. In the case of immunochemical methods, antibodies are used, which react with the specific antigen. These antibodies are extracted from the blood serum of animals such as rabbits that have been injected with the antigen: the antibody forms stereospecific complexes with the antigen of the product, which precipitate in some cases. A quantitative analysis can be carried out with antibodies that have been directly or indirectly labeled, making them detectable by spectroscopy (Enzyme-Linked Immunosorbent Assay (ELISA) method).

Another specific analytical method is the analysis of mineral cations by atomic absorption or atomic emission spectroscopy, based on the specific atomic energy levels of the elements.

When there are no specific analytical methods to quantify a constituent in a complex medium, it is necessary to separate the constituents by chromatographic or electrophoretic methods. Chromatographic techniques are widely used; they are based on the differential transfer of molecules in a mixture by a flow of liquid or gas known as the mobile phase. This phase carries the analyte over the stationary phase with surface-active properties that promote adsorption, affinity or exchange. Technological advances in recent years in the design of columns as well as detection systems have significantly improved resolution and sensitivity.

Gas chromatography is used to separate volatile constituents. It is mainly used to analyze volatile compounds responsible for aroma and smell in food but can also be used to analyze molecules such as carbohydrates and fatty acids. A “flame ionization detector” is generally used to detect gas eluates. There are, however, specific detectors to analyze organochlorine compounds or sulphur products. Quantitative analysis often requires the use of an internal standard since the exact volume of injected sample is not always ensured. Considerable progress has been made in the analysis of volatile substances by combining gas chromatography and mass spectrometry

(GC–MS) because mass spectral data can be used to unequivocally identify eluted molecules.

Liquid chromatography (LC) is used to separate a large range of low or high molecular weight molecules, whether hydrophilic or lipophilic. There are polar (carboxylic, sulphonic, amine/amino, etc.) and non-polar stationary phases (aliphatic groups); it is possible to vary the polarity of the mobile liquid phase by using solvent mixtures. The detection of eluates is usually carried out using spectrophotometric detectors (UV or visible), but for molecules with no absorption properties, refractometric methods can be used. Unlike gas chromatography, it is easy to control the injection volume and thus make a quantitative analysis, provided that the molar extinction coefficients are known at the detection wavelength. The two most common techniques are ion exchange chromatography and reversed phase chromatography:

- ion exchange chromatography uses matrices attached to which are acid or amino groups, which are, respectively, negatively or positively charged. It separates ionic molecules (proteins, peptides, amino acids and amines) based on their affinity to the ion exchange matrix. Elution conditions involve varying pH and/or the ionic strength of the mobile phase. Carbohydrates, although not charged, can interact with these supports and thus be separated and analyzed;

- reversed phase chromatography uses hydrophobic stationary phases that react with hydrophobic molecules if the mobile phase is sufficiently polar, which is the case with water. If the polarity of the solvent is reversed by the gradual introduction of an organic solvent to the liquid mobile phase, the hydrophobic interactions are weakened causing a gradual release of the sample molecules from the stationary phase. Reversed phase chromatography is used to separate proteins and peptides (all of which have hydrophobic side chains), vitamins, alkaloids, etc.

Electrophoretic methods are used to separate molecules according to their charge density and molecular crowding. The most common technique involves placing the sample onto a gel (starch or polyacrylamide) and placing it into an electric field that allows the migration of ionic molecules. This method is well suited to the separation of proteins. The difficulty lies in the detection and quantification of separated components; it is generally

necessary to use specific reagents, which stains the bands allowing them to be located and quantified. A variation to electrophoresis has been developed to further improve separation, which is known as isoelectric focusing. In this case, a pH gradient is created within the gel by the migration of ampholytes before or during the separation of proteins: the charge of the protein gradually decreases as it migrates within the gel, and when it reaches the pH region corresponding to its pI, migration ceases.

It is easy to use separation methods when the chromatographic or electrophoretic behavior of the sample molecules is known. However, in some cases, the elution volume or elution time is unknown or no suitable reference molecule is available. However, elution volume or elution time is not necessary proof of identification and it is often necessary to add structural analysis using mass spectrometry, which can be directly combined with chromatographic equipment (GC–MS, LC–MS) and/or nuclear magnetic resonance.

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List of Authors

Florence BARON
Agrocampus Ouest
Rennes
France

Gérard BRULÉ
Agrocampus Ouest
Rennes
France

Thomas CROGUENNEC
Agrocampus Ouest
Rennes
France

Michel GAUTIER
Agrocampus Ouest
Rennes
France

Romain JEANTET
Institut national de la recherche
agronomique (INRA)
Rennes
France

Pierre SCHUCK
Institut national de la recherche
agronomique (INRA)
Rennes
France

Index

A, B, C

absorbance, 144, 153, 174
adsorption, 13–17, 19, 232
amphiphilic, 45
antioxidant, 41, 77, 99, 100, 106,
116–133, 152, 153, 172, 173, 179
Arrhenius, 122, 147
ascorbic acid, 106, 129, 130, 134,
142, 143, 151,
169, 171, 179, 180
biofilm, 64, 65, 79, 80, 92
carotenoid, 46, 119, 127, 131
clostridium, 55, 56, 67, 72,
208, 210
crystallization, 16, 19, 24, 26,
33–35, 47–51, 122, 185,
188–192

D, E, F

desorption, 13, 14, 19
dry heating, 154
enzymatic browning, 167
flavonoid, 130, 152, 160–162, 164
fluorescence, 124, 125, 214, 218
freezing point, 7

G, H, L, M

glassy state, 19, 20, 23, 24, 187, 188,
190, 191
Gram
negative, 60, 75, 77, 91,
94, 172
positive, 60, 63, 75, 77,
78, 86–89, 92, 172
hysteresis, 14
linoleic acid, 41, 43, 44, 100, 103,
115, 116, 124, 126
linolenic acid, 115, 124, 126
lipid
composition, 47
oxidation, 99
Maillard reactions, 108
mesophilic, 70
molecular migration, 184, 191
mutarotation, 26, 31, 32, 147

N, P

non-enzymatic
browning, 133
pathogen, 62, 206
polyphenol oxidase, 165, 175–177

psychrophilic, 70

psychrotrophic, 71, 75, 78, 89, 92

R, S

reducing sugar, 31–33, 136,

137, 140, 145, 146, 149,

152–155

rheological properties, 49

saturation, 32, 34, 35, 185, 194, 228,

230

Strecker degradation, 139, 141–143,

180

sulfites, 145, 149, 155–157, 166, 169,

179

supersaturation, 14, 34, 35, 129

T, W

tannins, 160, 164

texture, 3, 27, 34, 36, 41, 48, 51, 78,

79, 99, 164, 175, 177, 186, 191,

201, 205, 220–227

thermophilic, 70

tocopherol, 106, 127, 130, 152

water

activity, 3, 8–19, 67, 68, 119, 120,

134, 148, 149, 169, 171, 184, 185

migration, 11, 184–192