

Edited by **Olusola Lamikanra**

FRESH-CUT FRUITS AND VEGETABLES

Science, Technology, and Market



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Preface

Fresh-cut fruits and vegetables are a relatively new and rapidly developing segment of the fresh produce industry. Fresh-cut products have been freshly cut, washed, packaged, and maintained with refrigeration. They are in a raw state and even though minimally processed, they remain in a fresh state, ready to eat or cook. The International Fresh-cut Produce Association (IFPA) defines fresh-cut products as fruits or vegetables that have been trimmed and/or peeled and/or cut into 100% usable product that is bagged or prepackaged to offer consumers high nutrition, convenience, and flavor while still maintaining its freshness. Industry estimates in the U.S. indicate that fresh-cut sales of approximately \$11 billion in 2000 account for over 10% of the total fresh fruit and vegetable market, with food service sales making up 60% of the total. Sales are projected to increase by 10–15% annually for the next five years.

High levels of quality accompanied by superior safety are essential for sustained industry growth and fresh-cut produce consumption. Fresh-cut fruit and vegetable products differ from traditional, intact fruit and vegetables in terms of their physiology, handling and storage requirements. The disruption of tissue and cell integrity that result from fresh-cut processing decreases produce product shelf life. Consequently, fresh-cut products require very special attention because of the magnitude of enzymatic and respiratory factors as well as microbiological concerns that impact on safety.

Knowledge of the nature of fresh-cut fruits and vegetables as they relate to pre- and post-harvest handling, processing, packaging and storage are essential for ensuring their wholesomeness and nutritional value, and for developing the most effective procedures and innovative technologies for maintaining their quality to meet increasing consumer demand. Attention to the market and economic factors will also ensure the ability of the industry to consistently deliver value to consumers, develop and implement new technologies and reward all participants in the supply chain.

This book is a comprehensive interdisciplinary reference source for the emerging fresh-cut fruits and vegetable industry. It focuses on the unique biochemical, physiological, microbiological, and quality changes in fresh-cut processing and storage and on the distinct equipment and packaging requirements, production economics and marketing considerations for fresh-cut products. Based on the extensive research in this area during the past 10 years, this reference is the first to cover the complete spectrum of science, technology and marketing issues related to this field, including production, processing, physiology, biochemistry, microbiology, safety, engineering, sensory, biotechnology, and economics. It will be particularly useful for senior undergraduate and graduate students, food scientists, plant physiologists, microbiologists, chemists, biochemists, chemical engineers, nutritionists, agricultural economists, and molecular biologists.

I am grateful to each of the authors for their participation, promptness and cooperation as well as many others for their contributions, advice and encouragement in the development of this book.

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1 Fresh-cut Produce: Tracks and Trends

Edith H. Garrett

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INTRODUCTION

Fresh-cut produce has been one of the hottest commodities in grocery stores over the past 10 years. The industry soared to over \$10 billion in U.S. retail and food-service sales in 1999, and there are no signs of the trend slowing down (IFPA, 2000). In fact, sales for cut and packaged fruit are just getting off the ground, and new commodities such as cut tomatoes are emerging to answer the consumer's desire for more convenience in their daily lives.

What is driving this fresh-cut growth? Where did the industry come from, and what are the market influences affecting the continued growth of the industry? Where does the processor get ideas for new products, and what track did the processors

take to build success? This chapter will cover the history, current trends and issues affecting the fresh-cut produce industry.

SIZE OF THE INDUSTRY

According to the Produce Marketing Association (PMA), the size of the fresh produce industry was \$76 billion in sales for 1999, including foodservice and retail sales (PMA, 2000; Kaufman et al., 2000). Fresh produce has always been popular with consumers because of the wonderful flavors, the natural nutritious quality and freshness. In fact, the United States Department of Agriculture (USDA) reports that produce consumption in the U.S. rose from 284 pounds per capita in 1990 to 319 pounds per capita in 1998 (Kaufman et al., 2000).

FRESH-CUT PRODUCE

All these same attributes, along with added convenience, continue to drive sales for unique fresh-cut commodities. The International Fresh-cut Produce Association (IFPA) defines fresh-cut produce as “any fruit or vegetable or combination thereof that has been physically altered from its original form, but remains in a fresh state” (IFPA and PMA, 1999, p. 5).

IFPA estimates the U.S. fresh-cut produce market at approximately \$10–12 billion in sales in 2000, with foodservice sales making up about 60% of the total (IFPA, 2000). Packaged salads have been rising stars in the grocery store for the past decade, and, with cut fruits and vegetables included, this category is estimated by IFPA to continue to grow in sales in the U.S. retail market at 10–15% a year for the next five years. The category in U.S. foodservice sales is difficult to measure but is estimated by IFPA to grow 3–5% a year for the next five years.

ORGANIC PRODUCE

Organically grown fruits and vegetables are another segment of the fresh produce industry that have experienced strong growth in the 1990s. This category includes both whole commodities and fresh-cut products. Making up an estimated \$4 billion in sales in 2000 (PMA, 2000), the organic produce industry is projected to have an increase of 7% annually in sales in the next three years. Again, the consumer is looking for healthy, flavorful alternatives for their diets, and organic fresh-cut produce meets these criteria. As the availability of organic produce increases, production costs are reduced, making this an affordable product to serve in restaurants and sell in conventional grocery stores. Fresh-cut organic salads are now readily available in the marketplace.

IMPORTED PRODUCE

Consumption of imported commodities has grown in the past decade, and consumers now enjoy year-round availability of many produce items in the U.S. and Europe. Importation is necessitated by the fact that fruits and vegetables are not grown in any one locale every month. The market for imported produce continues to grow in

many parts of the world. The latest USDA reports show that U.S. imports of fresh fruits and vegetables accounted for \$4.1 billion in sales in 1997, a 105% increase over 1987's total of \$2 billion (Kaufman et al., 2000).

IMPROVEMENTS IN OPERATIONS

Since the 1940s, produce companies have devised unique ways to cut and package produce for sale. Initially, some used bathtubs to wash produce, while others used the spin dry cycle on washing machines for the drying step. Ice was used in water baths to chill produce, and rudimentary packaging provided little more than protection from contamination during distribution. The industry built much of their own equipment as production increased in the 1970s from the growth in foodservice sales, but real innovation coincided with an increase in the number of restaurants in the 1980s.

IMPROVED ORGANIZATION OF INDUSTRY

Many technological advances occurred in the 1980s and 1990s as the industry became organized via their own trade association, the IFPA. Suppliers joined the trade association and participated in a growing annual equipment trade show to sell equipment and network with processors. This new forum for technology exchange helped propel the industry forward and enhance the quality and safety of fresh-cut produce.

Industry research revealed many new steps for shelf life improvement and convinced the industry to focus on refrigeration as the most critical step in the production process. The mantra became "the earlier the chilling step, the better the finished product." In other developments, major equipment innovations that improved fresh-cut production standards included the closed flume water bath, advanced cutters for a variety of cut sizes, advanced drying machines, the automatic packaging machine, automatic sanitation equipment and electronic monitoring equipment.

Each technological advancement increased production speed but caused new bottlenecks. Thus, there has been increased movement toward greater automation and electronic control by the industry. Today, the design of fresh-cut operations centers on food safety and sanitation, excellent refrigeration, higher production speeds through automation, quality enhancement and product traceability.

FOODSERVICE DEMANDS

In the mid 1970s, restaurants saw a great opportunity to save on labor costs by switching to convenient fresh-cut produce. Meeting the growing demands of McDonald's and other fast-food chains, growers and processors built the shredded lettuce and chopped onion business into a formidable niche within the fresh produce industry (Lawn and Krummert, 1995).

In the mid 1980s, there was tremendous growth in restaurants in North America. Salad bars became the latest craze with consumers. Soon, fresh fruits and vegetables took the place of canned produce on salad bars across America. Consistently an industry innovator, McDonald's Corporation decided it wanted to eliminate salad

bars in its stores to reduce food safety risks to consumers. The company asked its suppliers for a fresh salad to be made and packed in 5-lb. bags that would be repackaged in single-serve trays for sale within its stores.

Mixing commodities together under hermetically sealed packaging was not a common practice at the time, but the success of the McDonald's salad motivated other restaurant chains to provide similar products. This was also a time when women began working outside the home in large numbers, and two-income families feeling a time crunch began looking for more convenience in their lives. Cut and packaged produce fit those needs perfectly, but the fresh-cut industry at that time could not provide consistent quality and sufficient shelf life for the retail marketplace. However, these obstacles were soon to be overcome.

IMPROVEMENT OF QUALITY CHARACTERISTICS

Even though fresh-cut produce had been sold at retail since the 1940s, it was not completely successful, because the quality was unpredictable and the shelf life limited. Initially, processors used cast-offs, blemished product or second-quality commodities for the cut produce. In addition, refrigeration was poor throughout distribution, and appropriate packaging had not been developed. As the demand for better products with longer shelf life grew from foodservice customers, the industry's efforts were concentrated on quality improvements.

One thing the processors knew — their leading challenge was to stop the produce from turning brown after it was cut. Product appearance was the primary focus for quality measurement at the time, and processors found that refrigeration alone was not going to control discoloration and other visible defects. Instead, they had to start with healthier raw products, gentler handling procedures during processing and better packaging. Today, processors are concentrating on the importance of enhanced flavor development to provide even better ready-to-eat products.

Growers began supplying first quality commodities for processors, and new equipment processes were introduced such as air drying and gentle water baths. Some processors experimented with chemical washes or edible films to prevent browning, but low rates of improvement did not justify the additional costs. Improved packaging became the next step in the quest to address these quality challenges.

NEW PACKAGING TECHNOLOGY

In the 1940s, during the early days of fresh-cut produce, packaging consisted of cellophane wrappers over cardboard trays for products like coleslaw or salads (Holderfield, 1946). Cellophane, styrene and other plastics were used to wrap cauliflower heads in the mid 1950s in California produce fields to reduce shipping weights and prolong shelf life. In the early 1960s, lettuce growers began wrapping head lettuce. Both products are still popular in today's retail markets (Anderson, 2000).

The next step for lettuce growers was to trim and core the iceberg heads before packing them in plastic bags for shipment to the East Coast. This practice is still carried out today, and growers are even packing cleaned and cored lettuce in large bins for shipment to processors around the country.

In the mid 1980s, the fresh-cut industry was small and fragmented in the U.S., and packaging suppliers did not focus research efforts on developing films specifically for use with cut produce. European companies, however, were consolidating and developing equipment and packaging systems to move their industry forward.

New packaging was not as easy to find in the U.S. in the 1980s, because polyethylene film was the only breathable film on the market that could preserve produce and hold up to the rough handling conditions. Initially, processors used bags that were designed for other foods such as turkey and other meats. The advent of automatic packaging machines in the late 1980s spurred the development of new and innovative packaging that solved quality problems and helped launch fresh-cuts into mainstream marketing and distribution channels.

With the advent of automated packaging machines for fresh-cut produce in the late 1980s, the plastics industry jumped into action to design materials for fresh-cut produce. Film companies looked for new polymers and manufacturing processes to create breathable films that could run on the automatic machinery. Companies like Mobile, Exxon and Amoco provided new polymers from petroleum products and entered the market to better understand the needs of the industry. Automatic machines and these new films combined to allow processors to launch smaller, branded bags for the new fresh-cut products in the early 1990s.

In 1995, the Flexible Packaging Association (FPA) reported in their annual survey of packaging converters that for the first time, produce had overtaken medical packaging as the number one product for their production facilities (FPA, 1995). Estimated at \$90 million in U.S. sales (Packaging Strategies, 1999), packaging for produce would be the number one product for the next five years, respondents reported in the 2000 survey (FPA, 2000).

SHELF LIFE IMPROVEMENT

Beyond the revolutionary impact on the plastics industry, the processors have also influenced fruit and vegetable growers to focus on the burgeoning fresh-cut market. Instead of second quality, misshapen commodities or blemished fruits and vegetables, processors ask for first quality and negotiate contracts for the best quality raw products they can procure. Today's trends include growers competing for processor contracts by committing whole fields to processors, seed companies developing new varieties to suit the needs of processors and equipment suppliers engineering innovative tools to reduce harvesting damage to the produce.

Other engineering feats positively impacting the fresh-cut industry today include advanced air-drying techniques to reduce damage to the cut produce, vastly improved refrigeration in the processing plants, retail outlets' increased attention to refrigeration and sanitation and application of HACCP and other food safety systems. Clearly, the industry's commitment to develop researchers and supplier partners who collaborate to solve quality and shelf life challenges has resulted in better quality, longer shelf life and steady sales growth today.

Today, salads and most vegetables have a 12–14 day shelf life, while fruits are more perishable and have a shorter shelf life of 8–10 days if held at temperatures between 33°F (1°C) and 41°F (5°C) (IFPA and PMA, 1999). Consumers now enjoy fresh-cut

salads, fruits and vegetables on a year-round basis, and the industry is committed to developing better products to continue delivering reliable quality for their customers.

MARKET PRESSURES

In North America, the fresh-cut business is comprised of two general categories of processors. National companies are represented by large grower/shipper/processor operations, frequently including multiple processing plants in several regional locations, with a main office located in California's agricultural areas. These grower-based companies are able to focus on a specific commodity such as baby carrots, packaged salads, broccoli or onions. Their facilities are designed for efficiency in the production of large quantities of a few commodities, and they specialize in selling to retail and/or foodservice chains.

A second category is made of medium- to small-sized regional processors that grew out of produce distribution companies in metropolitan areas. These companies are frequently family-owned single-facility operations that have evolved in a regional market and are usually designed for flexibility to serve the needs of retail or foodservice distributors. Their customer base may order small amounts of a variety of commodities to sell to many grocery or restaurant outlets within a defined region, or they may be large distributors for chains that are buying from several regional fresh-cut operators in different parts of the country. These processors often operate short production runs of numerous products during the course of a day.

CONSOLIDATION

The fresh-cut industry has not escaped the influence of recent corporate consolidation trends. Foodservice and retail buyers are combining at a rapid rate around the world, forcing processors to consolidate (Kaufman et al., 2000). Bigger companies want to buy from bigger suppliers, and this trend pushes down to the basic level of growers and other suppliers. This domino effect is resulting in the creation of larger processors who sell specific commodity lines to large customers, thus forming partnerships that make for tough competition. National operators who are looking for distribution rights, regional locations and volume consolidation are buying regional operations. In some cases, regional companies are combining to form larger companies to supply the growing foodservice chains.

Nelson (1999) identified 10 innovative options that processors are taking to remain competitive in the consolidating marketplace:

1. Joining the trend and selling out to a larger corporation
2. Concentrating on one commodity such as carrots or onions and becoming specialized in all aspects of that commodity, from growing through brand marketing (for example, Grimmway Farms' baby carrots)
3. Forming a strategic alliance with a larger company to process a branded product (for example, Verdelli Farms processing Mann broccoli)
4. Creating a cooperative buying or marketing group to reap the savings realized by other larger corporations

5. Specializing in processing under a private label for store-branded foods
6. Co-branding with a non-produce company that wants to have its brand associated with the successful fresh-cut product line (for example, Weight Watcher's salads)
7. Choosing a marketing niche for product line focus (for example, organic produce)
8. Developing or utilizing proprietary technology to set their products apart from others
9. Creating new market segments (for example, sliced tomatoes)
10. Specializing in the difficult or unusual (for example, hand-carved vegetables for luxury hotels and restaurants)

LABOR

Another pressure felt universally by the fresh-cut industry is a general labor shortage. Company owners continue to plan strategies to find new sources of reliable hourly labor, but they are rapidly investing their resources toward automation to reduce their reliance on hourly employees. In developed economies, immigrants make up the vast majority of the manual labor needed in fresh-cut operations. If immigration is impeded for any reason, the shortage increases. In addition, a variety of languages and cultures in one operation can result in barriers to effective training. These limitations continue to especially plague smaller operators in the metropolitan areas.

CUSTOMER DEMANDS

Aside from the enormous upheaval in the wake of customer consolidation, the fresh-cut industry continues to be influenced by the distribution characteristics, product development demands and purchasing specifications set by retail and foodservice corporations. These customers demand that their suppliers drive costs out of the system by requiring the use of internet technology for electronic data transfer and communication, productivity improvements, food safety audits, approved supplier programs and other system-wide streamlining.

The safety of produce continues to capture the attention of purchasing agents in the foodservice and retail sectors. The latest trend in North America is toward requirements from retailers for third-party food safety audits of growers (Hilton, 1999; Wright, 1999). Fresh-cut processors have complied with these types of audits for many years from foodservice customers, but this is new for fruit and vegetable growers.

As consolidation blurs the boundaries of foodservice and retail companies, exemplified by the recent purchase of PYA/Monarch, a large U.S. foodservice distributor, by Ahold, the sixth largest global retailer (Reuters, 2000), food safety and other standards may also blur between the two industries. A retail industry bellwether to watch in the consolidation game is the discount retailer, Wal-Mart, as they continue to set new standards. Global food chains and their suppliers struggle to keep up with formidable competitors like Ahold and Wal-Mart.

OTHER

Internet technology growth and increasing government regulation round out the list of major pressures for fresh-cut manufacturers around the world. Food safety regulation has been impacting the food industry around the world for the past five years and promises to continue to remain in the spotlight. Perhaps one consolation in today's global market is that many countries are working together to create food safety standards that will affect this industry on an even and fair basis. With food importation and exportation on the rise, it makes sense that new regulations should be harmonized around the world to level the playing field within the global marketplace.

FOOD SAFETY REGULATORY STATUS

The risk of developing foodborne illness from fresh produce is not precisely known at this time, because the outbreaks associated with fruits and vegetables have been sporadic and incompletely reported. There is even some debate of whether the incidence of foodborne illness associated with produce is on the rise or only tracked and reported more efficiently (Harris et al., 2000). Also, there are no definitive intervention strategies that assure the elimination of pathogens from fresh produce. Therefore, the industry must focus on the prevention of contamination of fresh produce with human pathogens to assure that these products are safe and wholesome for human consumption (Gorny and Zagory, 2002).

In the past five years, media stories featuring produce have not been very positive, and the result of this negative attention has been increased regulatory oversight of the produce industry. In the U.S. and Canada, guidance or regulations have been developed for the safe and hygienic production, harvesting, packing, processing and transporting of produce.

Likewise, in Europe, Australia and other countries, new standards or regulations are addressing contamination issues linked to produce. The international standards-forming body, Codex Alimentarius, hopes to have a document for hygienic procedures in the harvesting and packing of fresh fruits and vegetables ready in the next several years. There are currently two annexes to this draft standard, one covering sprouts and one covering fresh-cut produce (Codex, 2000). This particular initiative will apply to all countries in the World Health Organization and the Food & Agriculture Organization to further harmonize the global marketplace.

The food industry has received broad coverage in the news in the last five years due to many issues such as biotechnology, foodborne illness outbreaks and product recalls. But, according to the International Food Information Council Foundation (IFCF), the tide may be changing to a more positive image for food, and produce in particular, in the media.

IFCF reports that the number of food news stories increased from 810 to 1260 in May–July 1999, a 38% rise as compared to the same time frame in 1998. Twenty-nine percent of all the coverage measured focused on general wellness and health-boosting aspects of food, and these benefits outweighed negatives 57% vs. 43%. The previous year, the negatives outweighed the benefits, 54% vs. 45%. They also

noted that scientific researchers and experts were the most frequently quoted sources in food news reporting, which adds credibility to the stories (IFIC, 2000).

Food safety issues are very important, and the industry needs to institute updated sanitation practices, but the produce industry has a very positive message for the consumer, because most fruits and vegetables are low in fat and high in fiber and nutrients. A balanced, science-based approach is appropriate for media coverage of produce.

SUMMARY

The value of fresh-cut produce lies in the primary characteristics of freshness and convenience. Food safety, nutrition and sensory quality are required while providing extended shelf life and freshness. Fresh-cut produce is a safe, wholesome food when produced under Good Agricultural Practices (GAPs), Good Manufacturing Practices (GMPs) and sanitation procedures. Today's food marketplace is alive with new products and changing trends, and fresh-cut produce remains at the top of the list of products meeting the needs of today's busy consumers. This publication is providing the industry an up-to-date summary of the current science and marketing trends to assure that we continue to earn the trust and confidence of consumers everywhere.

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2 Quality Parameters of Fresh-cut Fruit and Vegetable Products

Adel A. Kader

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Quality of fresh-cut fruit and vegetable products is a combination of attributes, properties, or characteristics that determine their value to the consumer. Quality parameters include appearance, texture, flavor, and nutritive value. The relative importance of each quality parameter depends upon the commodity or the product and whether it is eaten fresh (with or without flavor modifiers, such as dressings and dips) or cooked. Consumers judge quality of fresh-cut fruits and vegetables on the basis of appearance and

freshness (“best if used by” date) at the time of purchase. However, subsequent purchases depend upon the consumer’s satisfaction in terms of textural and flavor (eating) quality of the product. Consumers are also interested in the nutritional quality and safety of fresh-cut products.

Quality of the intact fruit or vegetable depends upon the cultivar, preharvest cultural practices and climatic conditions, maturity at harvest, and harvesting method. Handling procedures, conditions, and time between harvest and preparation as a fresh-cut product also have major impacts on quality of intact fruits and vegetables and, consequently, quality of the fresh-cut products. Additional factors that influence quality of fresh-cut fruits and vegetables include method of preparation (sharpness of the cutting tools, size and surface area of the cut pieces, washing, and removal of surface moisture) and subsequent handling conditions (packaging, speed of cooling, maintaining optimum ranges of temperature and relative humidity, expedited marketing, and proper sanitation procedures). An effective quality assurance program must take into consideration all the factors that affect quality of the intact fruits or vegetables and their fresh-cut products.

QUALITY PARAMETERS

APPEARANCE (VISUAL) QUALITY FACTORS

These may include size, shape, color, gloss, and freedom from defects and decay. Defects can originate before harvest as a result of damage by insects, diseases, birds, and hail; chemical injuries; and various blemishes (such as scars, scabs, russetting, rind staining). Postharvest defects may be morphological, physical, physiological, or pathological. Morphological defects include sprouting of potatoes, onions, and garlic; rooting of onions; elongation and curvature of asparagus; seed germination inside fruits such as lemons, tomatoes, and peppers; presence of seed stems in cabbage and lettuce; doubles in cherries; and floret opening in broccoli. Physical defects include shriveling and wilting of all commodities; internal drying of some fruits; mechanical damage such as punctures, cuts and deep scratches, splits and crushing, skin abrasions and scuffing, deformation (compression), and bruising; and growth cracks (radial, concentric). Temperature-related disorders (freezing, chilling, sunburn, sunscald), puffiness of tomatoes, blossom-end rot tomatoes, tipburn of lettuce, internal breakdown of stone fruits, water core of apples, and black heart of potatoes are examples of physiological defects.

Examples of defects that do not influence postharvest life potential of fresh produce include healed frost damage, scars, and scabs; well-healed insect stings; irregular shape; and suboptimal color uniformity and intensity. Most other defects (listed above) reduce postharvest life potential of fresh fruits and vegetables.

Tissue browning, which can be a major defect of fresh-cut fruits and vegetables, depends upon the concentration of phenolic compounds, the activity of polyphenol oxidase (PPO), and the concentration of antioxidants in the tissue. Wound-induced loss of cellular compartmentation between the phenolic compounds (mainly in the vacuole) and PPO (in the cytoplasm) results in tissue browning at a rate that increases with temperature and water stress.

TEXTURAL (FEEL) QUALITY FACTORS

These include firmness, crispness, juiciness, mealiness, and toughness depending on the commodity. Textural quality of fruits and vegetables is not only important for their eating and cooking quality but also for their shipping ability. Soft fruits cannot be shipped long distances without extensive losses due to physical injuries. This has necessitated harvesting fruits at less than ideal maturity from the flavor quality standpoint in many cases, such as the melons sold during the winter months in the U.S. markets.

Tissue softening and associated loss of integrity and leakage of juice from some fresh-cut products can be the primary cause of poor quality and unmarketability. Increasing calcium concentration in the tissue can slow down its softening rate. Also, initial firmness, temperature, and vibration influence the rate of softening and juice leakage from fresh-cut fruits.

FLAVOR (EATING) QUALITY FACTORS

These include sweetness, sourness (acidity), astringency, bitterness, aroma, and off-flavors. Flavor quality involves perception of the tastes and aromas of many compounds. Objective analytical determination of critical components must be coupled with subjective evaluations by a taste panel to yield useful and meaningful information about flavor quality of fresh fruits and vegetables. This approach can be used to define a minimum level of acceptability. To find out consumer preferences of flavor of a given commodity, large-scale testing by a representative sample of the consumers is required.

Flavor quality of most fruits is influenced by their contents of sugars (sweetness), organic acids (acidity), phenolic compounds (astringency), and odor-active volatiles (aroma). More information is needed about the optimum concentration ranges of these constituents to assure good overall flavor (based on sensory evaluation) of each kind of fruit (to satisfy the majority of consumers). Also, future research and development efforts on objective quality evaluation methods must include nondestructive segregation of fruits on the basis of their contents of sugars, acids, phenolics, and or odor-active volatiles. In many cases, consumers are willing to pay a higher price for fruits with good flavor, and there is a growing trend of high-quality-based stores that serve this clientele.

NUTRITIONAL QUALITY FACTORS

Fresh fruits and vegetables play a significant role in human nutrition, especially as sources of vitamins (vitamin C, vitamin A, vitamin B₆, thiamine, niacin), minerals, and dietary fiber. Other constituents that may lower the risk of cancer, heart disease, and other diseases include flavonoids, carotenoids, polyphenols, and other phytonutrients. Postharvest losses in nutritional quality, particularly vitamin C content, can be substantial and are enhanced by physical damage, extended storage duration, high temperatures, low relative humidity, and chilling injury of chilling-sensitive commodities.

Nutritional value varies greatly among commodities and cultivars of each commodity. By using plant breeding and biotechnology approaches, it is possible to develop genotypes with enhanced nutritional quality and improved flavor quality to encourage consumers to eat more fruits and vegetables (at least five servings per day).

This can have a major positive impact on human health and should be given high priority in research and extension programs worldwide.

PREHARVEST FACTORS INFLUENCING QUALITY

GENOTYPES AND ROOTSTOCKS

Within each commodity, there is a range of genotypic variation in composition, quality, and postharvest life potential. Plant breeders have been successful in selecting carrot and tomato cultivars with much higher carotenoids and vitamin A content, sweet corn cultivars that maintain their sweetness longer after harvest, cantaloupe cultivars with higher sugar content and firmer flesh, and pineapple cultivars with higher contents of ascorbic acid, carotenoids, and sugars. These are just a few examples of what has been accomplished in improving quality of fruits and vegetables by genetic manipulations. However, in some cases, commercial cultivars, selected for their ability to withstand the rigors of marketing and distribution, tend to lack sufficient quality, particularly flavor.

Rootstocks used in fruit production vary in their water and nutrient uptake abilities and in resistance to pests and diseases. Thus, rootstocks can influence fruit composition and some quality attributes as well as yield, in many cases.

There are many opportunities in using biotechnology to maintain postharvest quality and safety of fresh-cut products. However, the priority goals should be to reduce browning potential and softening rate, to attain and maintain good flavor and nutritional quality to meet consumer demands, and to introduce resistance to physiological disorders and/or decay-causing pathogens to reduce the use of chemicals.

A cost/benefit analysis (including consumer acceptance issues) should be used to determine priorities for genetic improvement programs. For example, increasing the consumption of certain commodities and/or cultivars that are already high in nutritive value may be more effective and less expensive than breeding for higher contents of nutrients.

CLIMATIC FACTORS

Climatic factors, especially temperature and light intensity, have a strong influence on composition and nutritional quality of fruits and vegetables. Consequently, the location and season in which plants are grown can determine their ascorbic acid, carotene, riboflavin, thiamine, and flavonoids content. In general, the lower the light intensity, the lower the ascorbic acid content of plant tissues. Temperature influences uptake and metabolism of mineral nutrients by plants because transpiration increases with higher temperatures. Rainfall affects the water supply to the plant, which may influence composition of the harvested plant part and its susceptibility to mechanical damage during subsequent harvesting and handling operations.

CULTURAL PRACTICES

Soil type, the rootstock used for fruit trees, mulching, irrigation, and fertilization influence the water and nutrient supply to the plant, which can affect the nutritional quality of the harvested plant part. The effect of fertilizers on the vitamin content of

plants is less important than the effects of genotype and climatic conditions, but their influence on mineral content is more significant. For example, sulfur and selenium uptake influence the concentrations of organosulfur compounds in *Allium* and *Brassica* species. High calcium content in fruits has been related to longer postharvest life as a result of reduced rates of respiration and ethylene production, delayed ripening, increased firmness, and reduced incidence of physiological disorders and decay. In contrast, high nitrogen content is often associated with shorter postharvest life due to increased susceptibility to mechanical damage, physiological disorders, and decay. Increasing the nitrogen and/or phosphorus supply to citrus trees results in somewhat lower acidity and ascorbic acid content in citrus fruits, while increased potassium fertilization increases their acidity and ascorbic acid content.

There are numerous physiological disorders associated with mineral deficiencies. For example, bitter pit of apples; blossom-end rot of tomatoes, peppers, and watermelons; cork spot in apples and pears; and red blotch of lemons are associated with calcium deficiency in these fruits. Boron deficiency results in corking of apples, apricots, and pears; lumpy rind of citrus fruits; malformation of stone fruits; and cracking of apricots. Poor color of stone fruits may be related to iron and/or zinc deficiencies. Excess sodium and/or chloride (due to salinity) results in reduced fruit size and higher soluble solids content.

Severe water stress results in increased sunburn of fruits, irregular ripening of pears, and tough and leathery texture of peaches. Moderate water stress reduces fruit size and increases contents of soluble solids, acidity, and ascorbic acid. On the other hand, excess water supply to the plants results in cracking of fruits (such as cherries, prunes, and tomatoes), excessive turgidity leading to increased susceptibility to physical damage, reduced firmness, delayed maturity, and reduced soluble solids content.

Cultural practices such as pruning and thinning determine the crop load and fruit size, which can influence composition of fruit. The use of pesticides and growth regulators does not directly influence fruit composition but may indirectly affect it due to delayed or accelerated fruit maturity.

MATURITY AND RIPENING

MATURITY

Maturation is the stage of development leading to the attainment of physiological or horticultural maturity. Physiological maturity is the stage of development when a plant or plant part will continue ontogeny even if detached. Horticultural maturity is the stage of development when a plant or plant part possesses the prerequisites for utilization by consumers for a particular purpose.

Maturity at harvest is the most important factor that determines storage life and final fruit quality. Immature fruits are more subject to shriveling and mechanical damage and are of inferior quality when ripe. Overripe fruits are likely to become soft and mealy with insipid flavor soon after harvest. Any fruit picked either too early or too late in its season is more susceptible to physiological disorders and has a shorter storage life than fruit picked at the proper maturity.

All fruits and mature-fruit vegetables, with a few exceptions (such as European pears, avocados, and bananas), reach their best eating quality when allowed to ripen on the tree or plant. However, some fruits are usually picked mature but unripe so that they can withstand the postharvest handling system when shipped long distance. Most currently used maturity indices are based on a compromise between those indices that would ensure the best eating quality to the consumer and those that provide the needed flexibility in marketing.

For most non-fruit- and immature-fruit-vegetables (e.g., cucumbers, summer squash, sweet corn, green beans, and sweet peas), the optimum eating quality is reached before full maturity. In these vegetables, the problem frequently is delayed harvest, which results in lower quality at harvest and faster deterioration after harvest.

RIPENING

Ripening is the composite of the processes that occur from the latter stages of growth and development through the early stages of senescence and that results in characteristic aesthetic and/or food quality, as evidenced by changes in composition, color, texture, or other sensory attributes.

Fruits can be divided into two groups: fruits that are not capable of continuing their ripening process once removed from the plant and fruits that can be harvested mature and ripened off the plant. The following are examples from each group:

- Group one includes berries (such as blackberry, raspberry, strawberry), cherry, citrus (grapefruit, lemon, lime, orange, mandarin, and tangerine), grape, lychee, muskmelons, pineapple, pomegranate, tamarillo, and watermelon.
- Group two includes apple, pear, quince, persimmon, apricot, nectarine, peach, plum, kiwifruit, avocado, banana, mango, papaya, cherimoya, sapodilla, sapote, guava, passion fruit, and tomato.

Fruits of the first group, with the exception of some types of muskmelons, produce very small quantities of ethylene and do not respond to ethylene treatment except in terms of degreening (removal of chlorophyll); these should be picked when fully ripe to ensure good flavor quality. Fruits in group two produce much larger quantities of ethylene in association with their ripening, and exposure to ethylene treatment (100 ppm for 1 to 2 days at 20°C) will result in faster and more uniform ripening. Once fruits are ripened, they require more careful handling to minimize bruising. Fruits in group two must be ripened, at least partially, before cutting to assure better flavor quality in the fresh-cut products.

POSTHARVEST FACTORS INFLUENCING QUALITY

PHYSICAL DAMAGE DURING HARVESTING AND HANDLING

Harvesting method can determine the extent of variability in maturity and physical injuries and, consequently, influence composition and quality of fruits and vegetables. Mechanical injuries (bruising, surface abrasions, cuts, etc.) can accelerate loss of water

and vitamin C and increase susceptibility to decay-causing pathogens. The incidence and severity of such injuries are influenced by the method of harvest (hand vs. mechanical) and management of the harvesting and handling operations.

Physical damage before, during, and after cutting is a major contributor to tissue browning, juice leakage, and faster deterioration of the fresh-cut products.

TEMPERATURE AND RELATIVE HUMIDITY MANAGEMENT

Keeping intact and fresh-cut fruits and vegetables within their optimal ranges of temperature and relative humidity is the most important factor in maintaining their quality and minimizing postharvest losses. Above the freezing point (for non-chilling-sensitive commodities) and above the minimum safe temperature (for chilling-sensitive commodities), every 10°C increase in temperature accelerates deterioration and the rate of loss in nutritional quality by two- to threefold. Delays between harvesting and cooling or processing can result in quantitative losses (due to water loss and decay) and qualitative losses (losses in flavor and nutritional quality). The extent of these losses depends upon the commodity's condition at harvest and its temperature, which can be several degrees higher than ambient temperatures, especially when exposed to direct sunlight.

The distribution chain rarely has the facilities to store each commodity under ideal conditions and requires handlers to make compromises as to the choices of temperature and relative humidity. These choices can lead to physiological stress and loss of shelf life and quality. The weakest two links in the postharvest handling cold chain of fresh fruits and vegetables are the retail and home handling systems.

SUPPLEMENTAL TREATMENTS APPLIED TO THE COMMODITY

These include curing of "root" vegetables, cleaning, sorting to eliminate defects, sorting by maturity/ripeness stage, sizing, waxing, treating with fungicides for decay control, heat treating for decay and/or insect control, fumigating for insect control, irradiating for preventing sprouting or insect disinfestation, and exposing fruits to ethylene for faster and more uniform ripening. In most cases, these treatments are useful in maintaining quality and extending postharvest life of the produce. However, there is a need to determine the maximum storage period that can be used for each commodity between harvest and preparation as a fresh-cut product. Generally, the longer the storage duration of the intact commodity between harvest and cutting, the shorter the post-cutting life of the products.

SUPPLEMENTAL TREATMENTS INVOLVING MANIPULATION OF THE ENVIRONMENT

Responses to atmospheric modification vary greatly among plant species, organ type and developmental stage, and duration and temperature of exposure. Maintaining the optimal ranges of oxygen, carbon dioxide, and ethylene concentrations around the commodity extends its postharvest life by about 50–100% relative to air control. In general, low O₂ atmospheres reduce deterioration and losses of ascorbic acid in fresh produce. Elevated CO₂ atmospheres up to 10% also reduce ascorbic acid losses,

but higher CO₂ concentrations can accelerate these losses. On the other hand, CO₂-enriched atmospheres can be beneficial in delaying browning and microbial growth on some fresh-cut fruits and vegetables.

Exposure to ethylene can be detrimental to the quality of most vegetables and should be avoided by separating ethylene-producing commodities from ethylene-sensitive commodities, by using ethylene scrubbers, and/or by introducing fresh, ethylene-free air into storage rooms. Treating the fruits and vegetables or their fresh-cut products with 0.5–1 ppm 1-methylcyclopropene for about six hours protects them against ethylene action.

FLAVOR VS. APPEARANCE LIFE OF FRESH-CUT FRUIT PRODUCTS

Even under optimum preparation and handling conditions, postcutting life based on flavor is shorter than that based on appearance. More research is needed to identify the reasons for the flavor loss and possible treatments to slow it down and to restore the ability of the fruit tissue to produce the desirable esters and other aroma compounds.

Use of calcium chloride or calcium lactate in combination with ascorbic acid and cysteine as a processing aid (two-minute dip) has been shown to be effective in firmness retention and in delaying browning of fresh-cut fruits. Ethylene scrubbing and modified atmosphere packaging (to maintain 2–5% O₂ and 8–12% CO₂) can be useful supplements to good temperature management in maintaining quality of fresh-cut fruit products. Additional research is needed to optimize preparation and subsequent handling procedures for maintaining quality and safety of each fruit product.

QUALITY ASSURANCE PROGRAMS

An effective quality assurance system throughout the handling steps between harvest and retail display is required to provide a consistent good-quality supply of fresh-cut fruits and vegetables to the consumers and to protect the reputation of a given marketing label. Quality assurance starts in the field with the selection of the proper time to harvest for maximum quality. Careful harvesting is essential to minimize physical injuries and maintain quality. Each subsequent step after harvest has the potential to either maintain or reduce quality; few postharvest procedures can improve the quality of individual units of the commodity.

Exposure of a commodity to temperatures, relative humidities, and/or concentrations of oxygen, carbon dioxide, and ethylene outside its optimum ranges will accelerate loss of all quality attributes. The loss of flavor and nutritional quality of fresh intact or cut fruits and vegetables occurs at a faster rate than the loss of textural and appearance qualities. Thus, quality assurance programs should be based on all quality attributes, not only on appearance factors as is often the case.

Following is a list of handling steps and associated quality assurance functions:

1. Training workers on proper maturity and quality selection, careful handling, and produce protection from sun exposure during harvesting operations
2. Checking product maturity, quality, and temperature upon arrival at the processing plant

3. Implementing an effective sanitation program to reduce microbial load
4. Checking packaging materials and shipping containers to ensure they meet specifications
5. Training workers on proper processing and packaging operations
6. Inspecting a random sample of the packed product to ensure that it meets grade specification
7. Monitoring product temperature to assure completion of the cooling process before shipment
8. Inspecting all transport vehicles before loading for functionality and cleanliness
9. Training workers on proper loading and placement of temperature-recording devices in each load
10. Keeping records of all shipments as part of the “trace-back” system
11. Checking product quality upon receipt and moving it quickly to the appropriate storage area
12. Shipping product from distribution center to retail markets without delay and on a first-in/first-out basis unless its condition necessitates a different order

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3 Overview of the European Fresh-cut Produce Industry

Patrick Varoquaux and Jérôme Mazollier

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INTRODUCTION

HISTORY OF FRESH-CUT FRUITS AND VEGETABLES IN EUROPE

When research into optimal processing of fresh-cut produce began in France about 20 years ago, the per capita consumption of fruits and vegetables had steadily declined since 1971 due to the development of catering and the integration of women in the task force (Scandella and Leteinturier, 1989). As a consequence, the time devoted to meal preparation was reduced accordingly. Moreover, fruits and vegetables are short-lived commodities hardly compatible with one shopping trip a week. As shown in Figure 3.1, the reduction in butterhead lettuce consumption exceeded 25% from 1971 to 1982. It is noteworthy that easy-to-use vegetables such as tomato and endive tips (witloof) did not follow this trend.

This trend alarmed nutritionists and supervisors of supermarket fresh fruit and vegetable departments. During a visit to the United States in the 1970s, Claude Chertier, fruits and vegetables buyer with Monoprix (French supermarket chain), noticed the salad bar in fast-food restaurants and supermarkets and decided to adapt

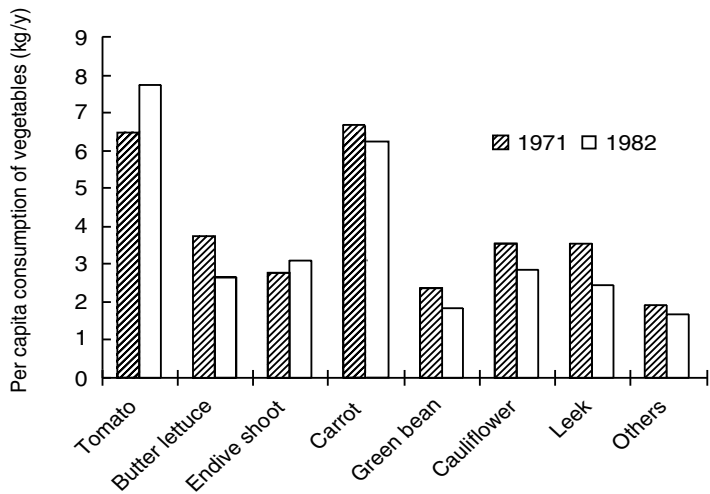


FIGURE 3.1 Per capita consumption of vegetables in France in 1971 and 1982 (Scandella and Leteinturier, 1989).

the concept of “ready-to-eat” salads to the French market. Claude Chertier got in touch with INRA (National Agronomic Research Institute) to develop his idea (1980).

Shredded celeriac and carrot, along with shredded iceberg lettuce, were already available in Northern Europe (1970), but these unpacked products (sometimes just overwrapped with stretchable PVC), mostly designed for catering, were not adapted to the French market, because their organoleptic and hygienic quality was poor, their shelf life was limited to two to three days, and iceberg lettuce was not popular in France. At this time, some French processors were already manufacturing precut fresh vegetable mixes for soups.

Claude Chertier wanted the new range of products to be recognized as fresh, safe, and user-friendly. The technical specifications were that the salads (200–300 grams) should be packed in order to facilitate supermarket distribution and to prevent microbial cross-contamination, the products should be distributed at room temperature (around 20°C), the shelf life should reach seven days plus an additional two days in the consumer’s possession, the salad composition should be adjusted to the taste of French consumers, and processing should not include any additives.

The proposed ingredients were broad-leaved endive (*Cichorium intybus* L. cv *latifolia*), curly endive (*Cichorium intybus* L.), red Italian chicories such as variegated-leaved chicory (i.e., *chioggia* cv), sugar loaf, lamb’s lettuce (*Valerianella locusta* L.), and some lettuces such as romaine and butterlettuce (*Lactuca sativa* L.) for the mixed salads. In order to offer consumers an acceptable range of salad, Claude Chertier also asked for packed shredded carrot (*Daucus carota* L.) and celeriac (*Apium graveolens* L.) plus shredded red and white cabbages (*Brassica oleracea* L.).

From 1981 to 1983, INRA therefore studied their first plant model, broad-leaved endive. The experiments on the effect of unit operations on physiological disorders, bacterial spoilage, and discoloration of the leaves resulted in a realistic process. Obviously, a shelf life of nine days was not attainable at 20°C but was possible at 4–6°C. In 1983, the procedure for each operation units of processing was established, and two processors invested in rudimentary processing equipment. At this time, the equipment was selected from other processing methods such as canning and freezing and was not well adapted to the fresh-cut industry. In 1984, a Swiss equipment manufacturer started to produce specific machines for the new fresh-cut industry. The production of “ready-to-use” fresh salads in France amounted to only 1400 metric tons in 1984, but their success was immediate since the production reached 8000 metric tons in 1985. These new products were rapidly known as “quatrième gamme” or “fourth range” in commercial terminology. Fruits and vegetables are fresh in the first range, canned in the second, frozen in the third, and fresh-cut or minimally processed in the fourth.

In 1985, CTIFL (Fruit and Vegetable Professional Technical Center) and other organizations such as ADRIA (Association for Agro-food Research and Development) Normandy, Pasteur Institute (Lyon), and different CRITT (Regional Center for Technology Transfer) were also involved in the development of the fresh-cut industry and provided processors with technical assistance. As a consequence, INRA focused its activity on a more theoretical approach to the field of the physiology and microbiology of “fresh-cut” plant tissues. Since the new produce was thought to be potentially hazardous, INRA undertook extensive research into the microbial hazards associated with prepacked plant tissues.

At the same time, the fresh-cut industry's approach spread throughout northern Europe, and a survey (Anonymous, 1986) concerning minimally processed vegetables counted eight processing units in Holland, four in Belgium, 11 in Germany, at least two large units in England, five in Switzerland (plus numerous small units around the cities), and 19 in France. The concept of ready-to-eat salad was not as successful in southern Europe. There, shelf life ranged from four to six days in the chill chain (from 2–4°C). At the same time, most European food-processing machinery developed specific processing lines fitted with American, Japanese, and European equipment. Bottled gas companies and film manufacturers proposed new gas mixtures and films designed to optimize actively and passively modified atmospheres.

DEVELOPMENT AND STATISTICS

After this development period, around 1990, there were up to 70 producers in France. Most manufacturers operated under poor hygienic conditions, and the chill chain was not respected either by transporters or by distributors. The visual quality of most fresh-cut produce at the end of their shelf life was poor. These factors inhibited industry growth (Figure 3.2). Fresh-cut processing was, nevertheless, responsible for a dramatic increase in the consumption of lamb's lettuce that had been steadily declining. This salad, which is grown on sandy soil, is difficult to wash. Presently, the production of fresh-cut lettuce is increasing (10–20% a year) in all European countries. In 1999, the annual tonnage production of fresh-cut leaf lettuce was, respectively, 45,000 in the UK, 39,000 in France, 21,000 in Italy, 20,000 in Germany, 10,000 in Spain and Netherlands, and 8,000 in Benelux.

In order to stop the decline and restore hygienic processing and distribution, CTIFL and DGCCRF (a French governmental organization similar to the American FDA) published a guideline for the fresh-cut produce industry. This guideline was turned into a regulation in 1988 (Anonymous, 1988) and was modified in 1993 (Anonymous, 1993), and was then modified again in 1996 (Anonymous, 1996). Its enforcement resulted in a rapid improvement in the quality and in a dramatic decline

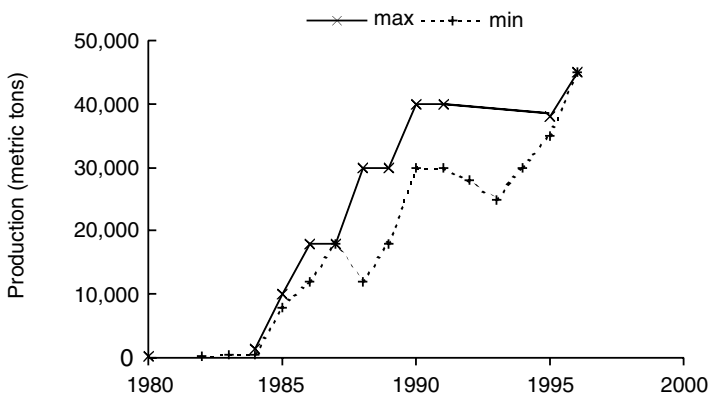


FIGURE 3.2 High and low estimates of fresh-cut produce production in France (Sabino, 1990).

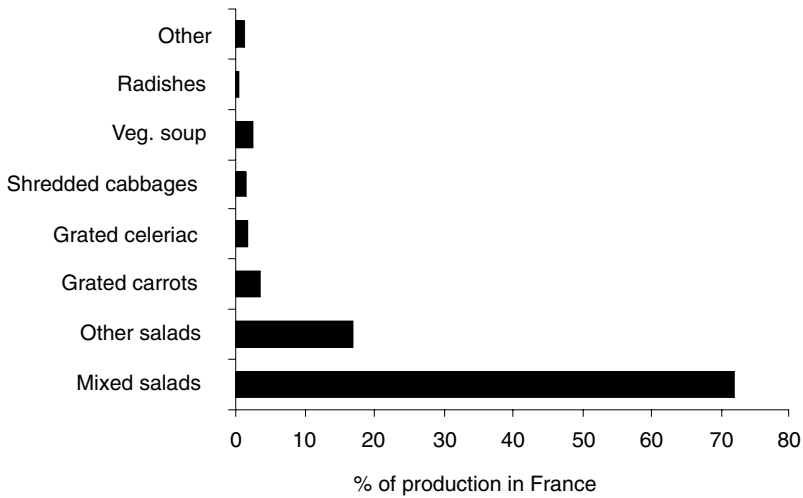


FIGURE 3.3 Percent of the different fresh-cut vegetables in 1986 (Scandella and Leteinturier, 1989).

in the number of processing companies. In 1998, four companies were responsible for 80% of the fresh-cut production. This trend was identical in all European countries.

In spite of an attempt to diversify the range of commodities proposed to the consumer (more details in the conclusion), fresh-cut green salads still account for about 85% of the overall production, as they did in 1986 (Figure 3.3).

GENERAL PROCESSING CONDITIONS

Processors apply HACCP principles as described in *Codex Alimentarius* (annex to CAC/RCP 1–1969, Rev. 3–1997) and in the code of hygienic practices for refrigerated packaged foods with extended shelf life (Alinorm 99/13, pp. 41–57) for all existing product types and for new product designs.

The guidelines for fresh-cut processing adapted by the French Administration are aimed at reducing biological, physical, and chemical hazards associated with this new type of produce. It proposes conditions under which raw materials are grown, as well as processing and distribution guidelines. In this review, details concerning recommendations and legislation that are specific to fresh-cut processing are presented.

FORWARD-ONLY MOVEMENT

This requires that there should be no “crossing over” in the processing line between the raw material and clean products.

The examples in Figure 3.4 show that the forward-only principle does not impose a linear processing, but it tolerates no crossing over (product line or waste disposal).

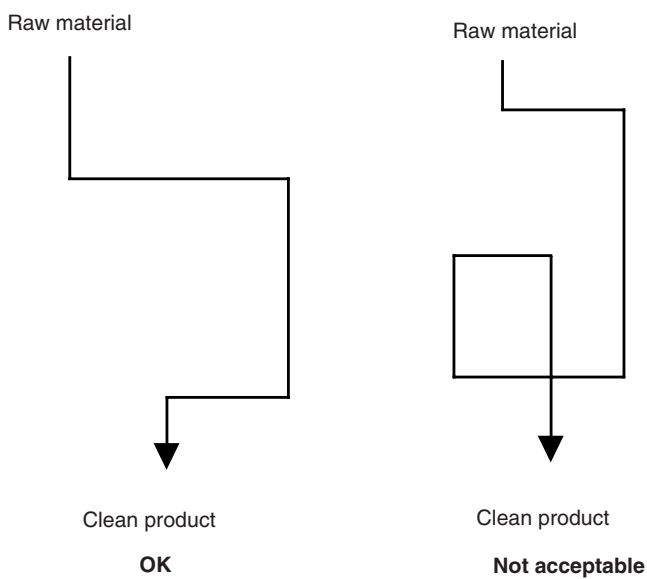


FIGURE 3.4 Principle of the forward-only movement.

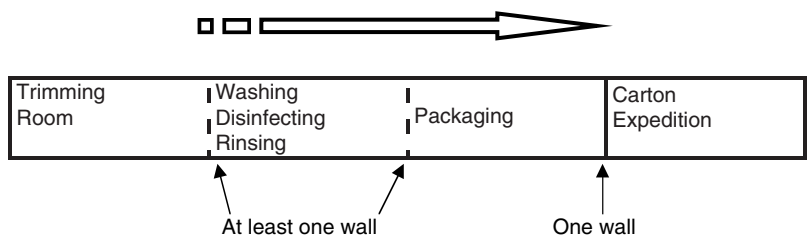


FIGURE 3.5 Segmentation of the processing line.

SEPARATION OF THE TRIMMING ROOM, THE WASHING ROOM,
AND THE PACKING ROOM

In order to prevent cross-contamination, the different processing rooms must be delimited by walls in order to progressively increase cleanliness from the trimming room to the packaging section (Figure 3.5).

TEMPERATURE CONTROL

Units are designed and equipped in such a way that the temperatures inside the different rooms are in accordance with the requirements summarized in Figure 3.6. According to French regulation, fresh-packed products must be immediately stored at 4°C and maintained at 0–4°C until delivered to consumers.

Airflow ← Positive pressure				
Ambient T	12°C	→ 4°C		4°C
Raw materials	Washing		Packing	Carton Expedition
	Trimming Prewashing	Disinfecting Rinsing Draining		

FIGURE 3.6 Temperature gradient and airflow in the processing unit.

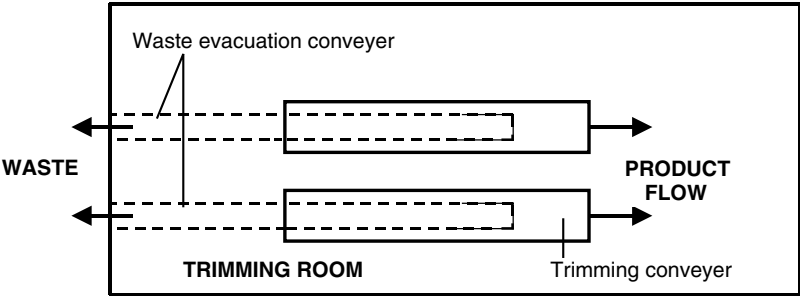


FIGURE 3.7 Waste disposal.

The following are therefore recommended:

- limit exposure to temperatures above 10°C
- refrigerate the product at 0–2°C before packing in order to be at the right temperature during the operation
- maintain this temperature during storage

The temperature gradient and flow of products run countercurrently. Temperature in the trimming and disinfecting rooms must not exceed 12°C and must not exceed 4°C in the packing room and warehouse.

AIRFLOW

Ventilation systems are designed to maintain the required temperature and prevent both condensation and circulation of dust. The air current must flow from the packing to the trimming room (Figure 3.6).

WASTES

Waste materials are evacuated from the facility to avoid any cross-contamination (Figure 3.7).

Inside the premises, equipment and machinery used for nonedible material and waste must be clearly identified and never used for edible products. Moreover, they should be easy to wash and sanitize.

Outside the premises, any reusable receptacle for nonedible material and waste should be waterproof and easy to wash and sanitize.

CLEANING EQUIPMENT, MATERIAL, AND UTENSILS

Washing should be performed by any method or combination of methods involving mechanical action (scrubbing, brushing, water jet spraying) or chemical cleaning (acidic or alkali detergent). The washing must include the removal of objectionable matter of any sort. A detergent or a disinfecting detergent should be applied so as to permit the elimination of dust and bacterial biofilms.

Efficient rinsing with potable water should eliminate the detached particles and detergent residues.

SANITATION

After washing the premises, the machines must be submitted to an efficient disinfecting, either by using steam or chemicals.

HYGIENIC PROCEDURE FOR OPERATORS

Personnel should know the hygienic procedure (International Code of Practice, General Principles of Food Hygiene) and wear protective clothing and footwear specific to the area.

CHLORINATING

Use of chlorine, associated with hygienic processing, permits a significant improvement in the microbiological quality of the product. According to French Regulations, chlorine disinfection must be followed by rinsing with potable water (less than 0.5 ppm active chlorine).

There are different forms of chlorine in water solution. A part of dissolved chlorine combines immediately with organic matters (combined chlorine). The remaining part is the “free” chlorine. Concentration of free chlorine, which averages 80% of total chlorine, may be assessed using a specific electrode (which also permits automatic regulation of chlorine content) or a spectrophotometric method with DPD (N,N-diethyl phenylene-1,4 diamine) as a reagent. Considering the instability of the chlorine solution, frequent determinations are required.

In most disinfecting equipment, there is a very large dispersion in transit time of the vegetable chunks. The recommended mean duration of disinfection is 2 minutes. pH is an important factor for chlorine efficiency. The pH of the disinfecting solution should range between 6.5–8. Microbial load (aerobic mesophilic bacteria) changes during processing are shown in Figure 3.8.

DISTRIBUTION CONDITIONS: CHILL CHAIN AND SELL-BY-DATE

In order to maintain produce quality until the time of purchase, fresh-cut manufacturers must stamp the “best before date” on the bag. Determination of the shelf life is the processor’s responsibility. The shelf life of the product must be established using scientific data, taking into account the chill chain temperature.

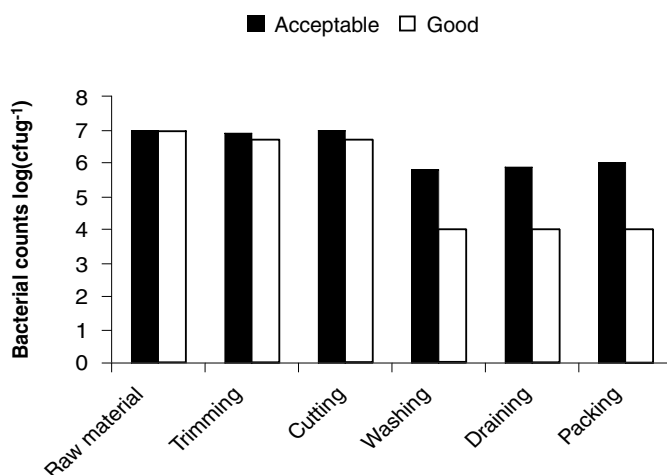


FIGURE 3.8 Microbial count (cfu·g⁻¹) during fresh-cut processing of green salads (Scandella and Leteinturier, 1989).

In order to simulate a realistic distribution of fresh-cut commodities, the temperature profile is two-thirds of the shelf life duration at prescribed temperature (4°C) and the remaining one-third at 8°C. The following are the microbial limits for fresh-cut commodities in France (Anonymous, 1993): *Listeria monocytogenes* and *Salmonella* should not be present in the final product (five samples of 25 g), but only 100 cfu·g⁻¹ *Listeria* is tolerated at consumption.

Escherichia coli tests are done to ensure that contamination is within the following limits: for five samples of 25 g, no count should exceed 100 cfu·g⁻¹, and three out of five should be below 10 cfu·g⁻¹. These conditions are similar to those recommended by the International Commission on Microbiological Specifications for Foods (ICMSF, 1986, 1988). The Good Manufacturing Practice Guide also recommends that aerobic mesophilic flora be lower than 5·10⁶ cfu·g⁻¹ with three out of five counts below 5·10⁵ cfu·g⁻¹ in bags after processing. This recommendation is not enforceable and cannot be attained for some commodities such as aromatic herbs (parsley, tarragon, chives, sweet basil, and coriander leaves, which are processed in France) and sprouted seeds.

UNIT OPERATIONS

RAW MATERIALS

It is obvious that the quality of the raw material is one of the most essential factors determining the quality of the final product. Green salads should be, as far as possible, cultivated in open fields. Broad-leafed and curly endives must be etiolated in the field in order to increase the processing output using either a rubber band or a plastic bell. This operation should be carried out carefully so as to avoid overstressing etiolated plant tissues. For hygienic reasons, no manure or fertilizer of animal origin should be used.

Numerous research projects in many countries, including the United States, Australia, and France are assessing the suitability of salad cultivars for minimally processing from the processor's point of view.

The main criteria in assessing this suitability of cultivars to fresh-cut processing are as follows:

1. Processing yield—for example, the output of butter lettuce ranges from 25–30% and reaches 50% for broad-leaved endive (Scandella and Leteinturier, 1989)
2. Low sensitivity to physiological disorders and microbial diseases
3. Mechanical resistance of the tissue
4. Resistance to elevated CO₂ concentration (Varoquaux et al., 1996) and/or low oxygen
5. High sugar contents because sugar depletion may be responsible for energy stress (Forney and Austin, 1988)
6. Low respiration rate (Varoquaux et al., 1996)
7. Special requirements—for example, all leaves of butter lettuces must be released when coring, because this salad is not cut thereafter in the process (Scandella and Leteinturier, 1989)

HARVESTING

- Most of the raw material for fresh-cut processing is cultivated under contracts that specify the cultivars and cultivation techniques (including acreage, sowing time, pesticide and fertilizer applications, and harvest conditions).
- It is required that the salads be harvested in the morning because of the cooler temperature, but the sugar content of the leaves is higher late in the afternoon.
- It is well known that produce should be precooled to 1°C as soon as possible after harvesting in order to extend the potential shelf life. One of the conditions required for processors to achieve the quality distinction called “Label Rouge” is vacuum cooling of the salads at 1–2°C within four hours after harvest.
- Most salads, except lamb's lettuce, which is more resistant, should be processed within two days. Radicchio can be stored for up to two months.

QUALITY ASSESSMENT

The first operation on receipt of the raw materials is quality control, which is necessary to achieve a standard product quality. The main criteria are the appearance of the salads, including overall freshness, the absence of insects, physiological and microbial diseases, presence of necrotic tissue, and compliance with regulations on pesticide residues and nitrate content. With some salads stored, as variegated-leafed Italian chicory, for example, the absence of pathogenic bacteria such as *Listeria monocytogenes* is checked. All quality assessments are noted on an input grid to comply with “tracing” requirements.

TRIMMING

The required proportions of the ingredients in salad mixes are achieved during trimming. The trimming table is supplied with the final percentage of each salad, taking into account their respective processing output. All unwanted parts of the plant, including most of the outer green leaves and core area, are removed manually. This operation causes injury that could be minimized by using very sharp knives (Bolin and Huxsoll, 1991). This is much easier said than done. In fact, classical stainless steel used to manufacture blades is rather soft, and intensively used knives should be sharpened very often (every hour or so). Carbon steel used for scalpel blades is brittle, may be dangerous for operators, and releases iron ions that may be involved in brown discoloration. Ceramic blades are also breakable and are very expensive. Trimming may be partly mechanized, at least for broad-leaved and curly endives. A new automatic trimmer was developed by a French company (Soleco SA). This patented machine (U.S. patent 5,421,250; 1995) improves the yield of raw material from 5 to 10 points and reduces the manpower cost by a factor of two. The use of the trimmer is, however, limited to broad-leaved and curly chicory. Mechanization of the trimming of butter-head lettuce is more complex. Wounding of plant tissue results in leakage of enzymes and their substrates that are normally in different cell compartments. The destruction of cell microstructures leads to biochemical spoilage such as texture breakdown, off-flavor, and browning (Varoquaux and Wiley, 1996).

One of the most conclusive examples of the effect of wounding on firmness was observed on kiwifruit after slicing. The slices lose about 50% of their initial firmness within two days at 10°C. It appears as if this phenomenon was due to the release of enzymes with pectinolytic and proteolytic activities by injured cells (Varoquaux et al., 1990).

It is well known that bruising or cutting plant tissues with browning capability will result in a brown discoloration. Because most green salads contain polyphenoloxidases and phenolic substrates, mainly chlorogenic acid, caffeoyl tartaric ester, and caffeoyl shikimic ester (Goupy et al., 1990, 1994), browning of the cut surface is a major problem for minimal processing. As previously mentioned, browning can be reduced by using very sharp blades and chill storage, but another extremely important factor is the interval between slicing and washing. This was demonstrated with apple slices.

In Figure 3.9 the reflectance absorbance difference at 440 nm of apple slices for two cultivars is reported as a function of time after slicing. The slices cut in air were dipped into water 30 seconds after slicing. Browning of these slices appeared as a peak in absorbance in the 400–440 nm region of the spectrum. Surprisingly, the slices cut in water did not visually turn brown for a few hours when stored at 8°C under air. Browning did not affect internal tissue, because no discoloration was observed when the slices were cut again.

It is most likely that the prevention of browning in slices cut in water is due to the instant washing out of cell sap liberated by cutting. In slices cut in air and rapidly dipped into water, the exudate immediately diffused into inner tissue layers prior to washing. The longer the interval between cutting and washing, the browner the slices turned during storage. A similar phenomenon occurs with cut salad leaves.

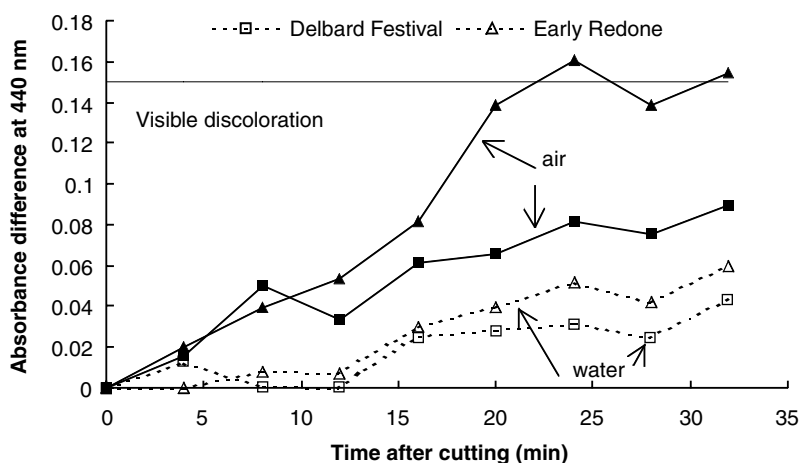


FIGURE 3.9 Absorbance of apple slices cut in air (open symbols) and cut under water (closed symbols) as a function of time for two cultivars (Kuczinski et al., 1993).

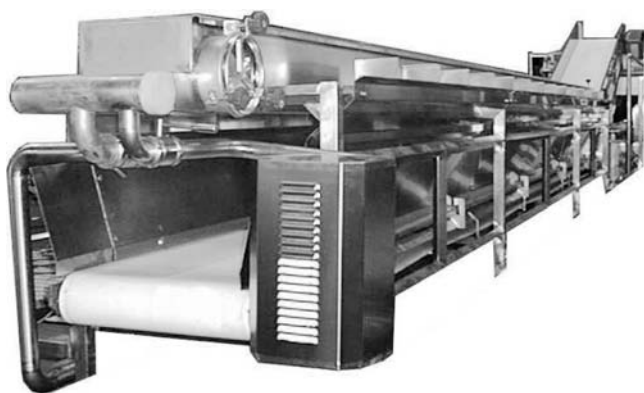


FIGURE 3.10 Trimming table fitted with water conveyor (upper part) to convey the trimmed product. (Photo courtesy of Turatti.)

It seems unrealistic to trim the salads manually under water. To reduce discoloration, new trimming tables were fitted with a hydraulic flow (upper part) to convey the trimmed parts and a belt conveyor (lower part) to evacuate the wastes, as shown in Figure 3.10.

SLICING AND SHREDDING

Chicory leaves are cut into 2–3 cm pieces using rotating blades (perpendicular to the flow) or disk knives (parallel to the flow). This process also causes injury to plant tissue that could be minimized by using very sharp blades sharpened once or twice a day. When trimming, salad leaves must be washed immediately after cutting;

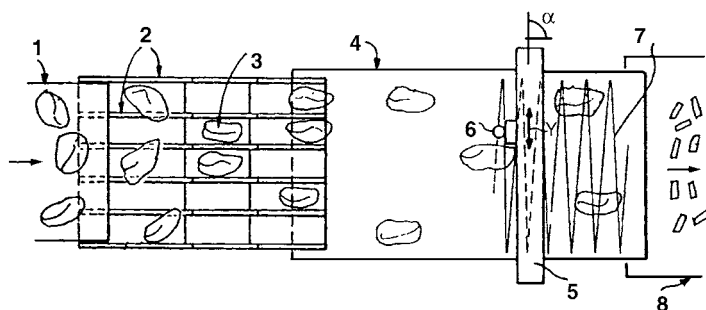


FIGURE 3.11 Sketch of a water jet cutter for fresh-cut commodities (Béguin et al., 1995).

any delay in prewashing will enhance browning. Washing the leaves after cutting is essential to prevent browning in the same way as trimming. In most processing lines, the product immediately drops into a washing tank after shredding. Since the cutting should take place under water, one of the approaches tested in France was water jet cutting (Béguin et al., 1995). Because the internal liquid of injured cells is removed by the water flow, browning is markedly reduced compared to any commercial cutting techniques. The principle of this machine is shown in Figure 3.11. The leaves (3) are conveyed (1) to a multi-U-shaped grooved belt (2) designed to position the main nervure of the leaves parallel to the direction of flow and to limit the thickness of the products to two or three layers on the stainless steel grill conveyor (4). The leaves (or other plant tissues) are cut by the transversal and alternative displacement of a water jet (6) on a fixed rail (5). The water jet pressure ranges between 50–100 MPa depending on the product to be sliced. The average width (cm) of the chunks is $P/2v$, where P is the period of the water jet cross-head (min^{-1}), and v is the conveyor velocity in $\text{cm}\cdot\text{min}^{-1}$ (7). The cut products are dropped into the washing (8).

PREWASHING

When the salad leaves are cut, they fall into the prewasher that washes away exudates and saps that would otherwise rapidly pollute the disinfecting tank.

WASHING WITH CHLORINATED WATER

The maximum active chlorine allowable in the disinfecting tank was set at 120 ppm by law in France in 1988, and the 1992 guidelines, which are still valid, proposed reducing it to 80 ppm. In current processing, the minimum chlorine concentration should not drop below 50 ppm. Chlorine may be hypochlorite or chlorine gas. The latter is more complex to handle but is slightly more efficient due to a noticeable decrease in pH of the disinfecting solution. Conversely, addition of hypochlorite increases the pH, resulting in a bigger dissociation of hypochlorite and, thus, in a decrease in disinfecting efficiency. In some processing units, the chlorine concentration is monitored and adjusted continuously, while in others, it is measured and readjusted every hour or so. Agitation in the disinfecting tank is insured either by tangential air bubbling or water jets or mechanically by rotating arms (Figure 3.12). It should be

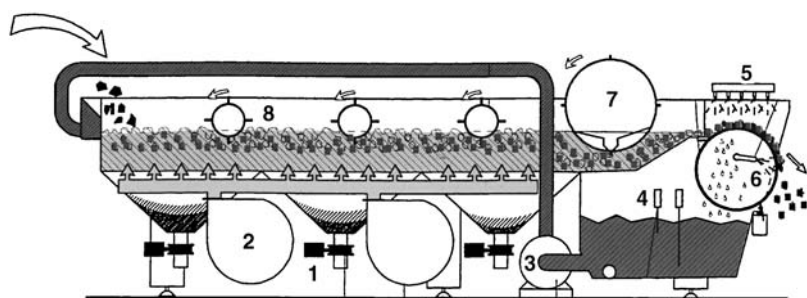


FIGURE 3.12 Conventional washer: (1) sediment discharge valves, (2) ventilator with blowing system, (3) pumps and hydraulic manifold, (4) level gauges, (5) cold water nozzles, (6) drum outlet filter, (7) insect removal drum, and (8) flow adjusting drum. (Courtesy of Turatti.)

noted that chlorine is not actually authorized but only tolerated by French regulations for disinfecting minimally processed products. Its use is, however, banned in some European countries such as Belgium, Germany, and Holland. The current trend is to eliminate chlorine from the disinfection process. In the washing equipment described below, a minimal chlorine concentration of 8 ppm was established as an efficient safeguard against possible contamination by pathogenic bacteria.

In previous work, American researchers stated that there was a significant relationship between the initial bacterial load and the spoilage of shredded iceberg lettuce (Bolin et al., 1977). Nguyen-The and Carlin (2000) reported a clear relationship between the number of aerobic mesophilic bacteria at the end of shelf life and spoilage on fresh-cut broad-leaved endive packed in sealed polypropylene film, but microbial pollution may be the consequence of the decay as stated by Carlin et al. (1989). Varoquaux and Wiley (1996) claimed that injury stress at processing and physiological disorders induced by detrimental packaging conditions along with temperature abuse were the main causes of the premature decay of fresh-cut produce.

The growth rate of aerobic mesophilic bacteria in highly disinfected salad is higher than that in control samples washed in tap water (less than 0.5 ppm free chlorine). After a 2 log reduction in bacterial count, due to an efficient sanitation, the bacteria population was identical to the untreated sample after only four days at 10°C (Carlin et al., 1996). The growth of *Listeria monocytogenes* under the same conditions is dramatically enhanced compared to that of the untreated control (Figure 3.13). Elimination of the saprophytic flora may favor the development of unwanted bacteria such as *Listeria monocytogenes*, which grows faster in highly disinfected samples.

It was postulated that a 2 log reduction in microbial count after disinfection was not necessary to ensure product quality. That is why alternative milder sanitation processes were developed (see Conclusion).

The last step of the washing operation is a rinsing with tap water containing less than 0.5 ppm active chlorine. This unit operation is necessary only when chlorine at a concentration higher than 1 ppm is used. The cold water (1–3°C) must be continuously renewed in order to avoid chlorine accumulation from the disinfecting section. This rinsing water can be recycled to the upstream washer after filtration and chlorinating.

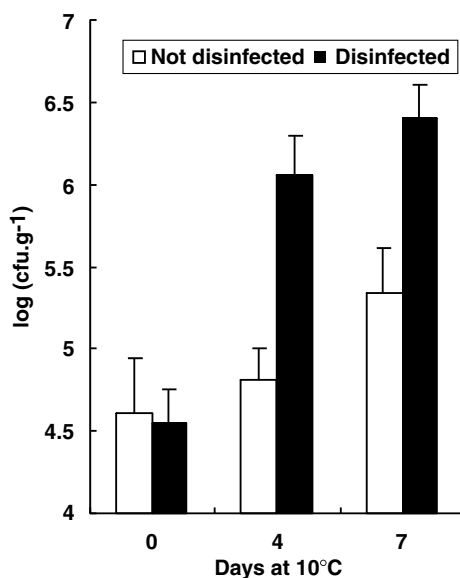


FIGURE 3.13 *Listeria monocytogenes* growth on inoculated broad-leaved leaves after drastic (empty bars) or light (closed bars) chlorine disinfecting (Carlin et al., 1996).

The new washer, illustrated in Figure 3.14, is based on the succession of torrential and laminar flows. The salad is elevated to the feeding hopper (3) filled with chlorinated water (5–8 ppm of active chlorine). The chlorine concentration is regulated with a specific electrode, and the recycled solution is filtered. The product is swept along in the overflowing water (11). The bottom of the first section (1) is equipped with bumps (10) which, combined with the optimal slope α of the disinfecting section, results in a succession of laminar and torrential flows. The surface of the commodity is washed in a permanently renewed turbulent chlorine solution. This process also eliminates aphids. At the end of the first section, the product is separated from the chlorine solution onto a perforated conveyor (5). The water is returned (7) to the water buffer tank (not shown) and partially recycled after adjustment of the chlorine concentration. The salad falls into a tank (9) filled with drinkable water (chlorine concentration lower than 0.5 ppm). The rinsing section (13) is also fitted with bumps (12) designed to turn over the leaves and to expose both sides to a UVc tube (24), which prevents any microbial cross-contamination in the rinsing section. The leaves are separated from water onto a second sieve (15). The water is recycled (14) and (17) either to the first section or to the upstream prewashing. The product is collected into crates (19) or sent directly to the drying system (spin dryer or tunnel).

DRAINING

Excessive free water in packs results in rapid bacterial spoilage mainly at the leaf-film interface (Herner and Krahn, 1973). Draining should result in about 1% residual moisture compared to the unprocessed salad. Two methods are presently used for this

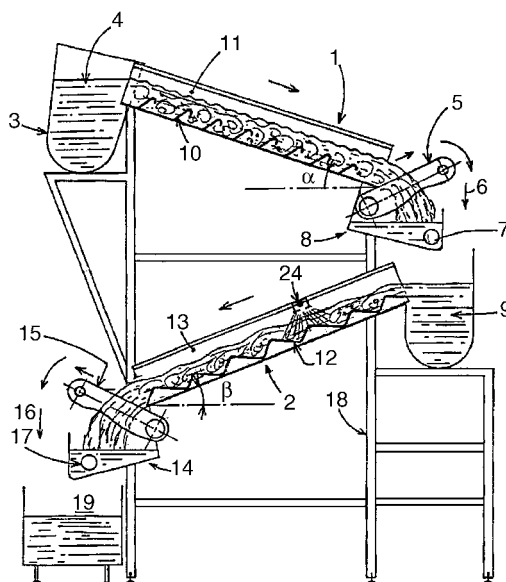


FIGURE 3.14 New washer based on a succession of laminar and torrential flows, including a regulation of chlorine concentration and a rinsing section (Béguin and Varoquaux, 1996).

operation: a spin dryer and an air tunnel. Drastic centrifugation results in bruising, so the process was improved using special centrifuges to achieve optimal draining. The centrifugation cycle begins with a soft loading of the fragile leaves followed by a smooth acceleration and a careful discharge of the drained products (Figure 3.15).

Air tunnel drying is a new technique developed in Italy that is currently used in several processing plants in Europe and in the United States (Figure 3.16).

The drying tunnel is composed of “cascade” vibrating tables to transport the product and a battery of air drying units. The product progression is countercurrent with both air temperature and dryness. The dryer is microprocessor piloted to optimize its efficiency. In order to limit cross-contamination by airborne microorganisms, the airflow is filtered and disinfected with a UV tube (250–280 nm).

WEIGHING AND PACKING

The packing room must be clean and refrigerated at 1–2°C and must be separated from the washing section. Packing is performed around a vertical tube at the top of which is the associative weighing machine, an example of which is shown in Figure 3.17.

Salad bits (or any other products) are poured into the infed funnel (or a vibrating cone) designed to distribute the vegetable chunks evenly into feed buckets, which release them into weighing buckets. The successive bucket system permits a continuous operation of the machine provided the level sensor is not activated. The weight of plant tissues in all the buckets is transmitted to a computer that calculates the best combination to optimize the required weight. Both mean weight and acceptable standard deviation are entered into the computer.

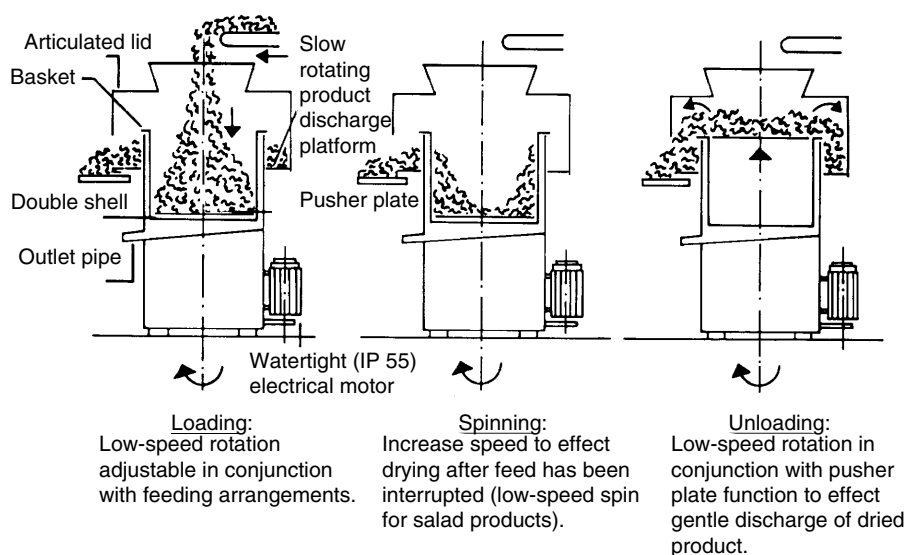


FIGURE 3.15 Semiautomatic spin dryer optimizing drying conditions for fresh-cut salads. (Courtesy of Rousselet Centrifugation.)

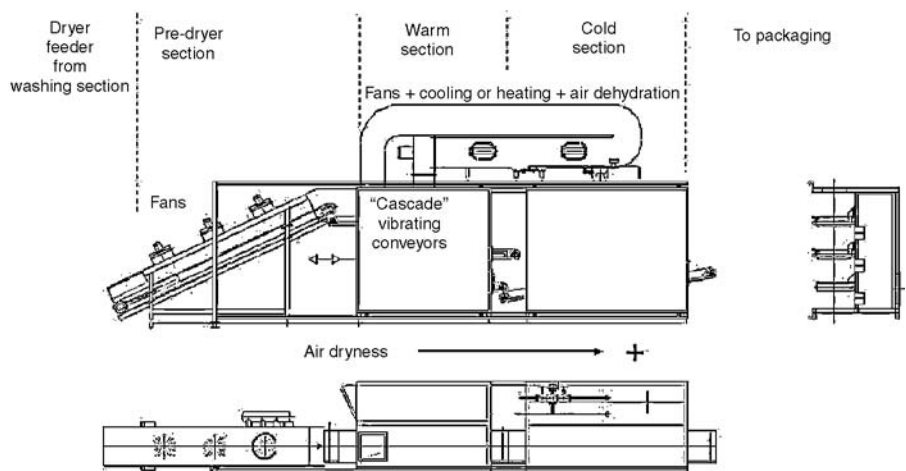


FIGURE 3.16 Air dryer. (Courtesy of Turatti.)

Vegetable chunks are collected in the collating funnel that feeds the packaging machine. The first part of the packaging machine is the conforming tube that drives the packaging film around the cylindrical tube at an optimal angle. The longitudinal sealing is performed, and the sleeve is driven by two conveying belts. The progression of the sleeve is guided by a photo cell that reads printed marks on the film. At the end of the tube, the lower part of the sleeve is sealed. Vegetable chunks are then

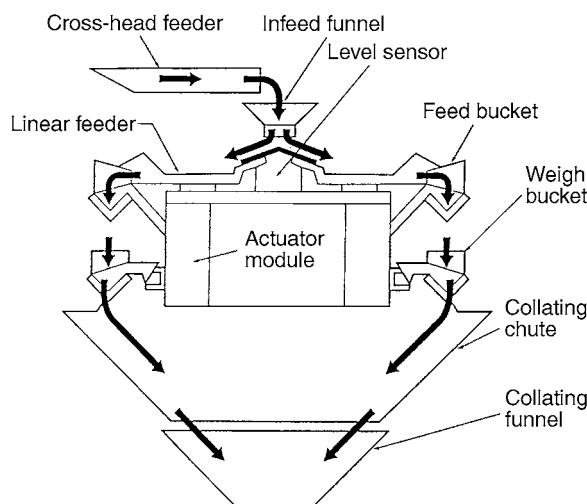


FIGURE 3.17 Associative weigher. (Courtesy of Yamato.)

released from the weighing buckets. The filled bag may be flushed with nitrogen, carbon dioxide or a binary gas mixture (N_2 and CO_2) to actively modify the atmosphere. The gas injection nozzle is withdrawn, and the upper side of the bag is sealed.

Most minimally processed vegetables in France are packed in bags of polypropylene that are 25–40 μm thick. In England and Ireland, a wider range of vegetables including spinach, broccoli, and cauliflower florets is processed. These highly respiring commodities must be packed with microperforated films more permeable to gases than oriented polypropylene. The permeability of oriented polypropylene is about $2 \cdot 10^{-16}$ and $7.2 \cdot 10^{-16}$ $\text{mole} \cdot \text{m}^{-1} \cdot \text{sec}^{-1} \cdot \text{Pa}$ for O_2 and CO_2 , respectively. In Europe, professionals use permeance to quantify gas transmission rate instead of permeability. Permeance is the permeability multiplied by the thickness of the film and is expressed in ml of gas per square meter, per day, and per atmosphere or Bar ($\text{ml} \cdot \text{m}^{-2} \cdot \text{day}^{-1} \cdot \text{atm}^{-1}$).

Oriented polyethylene (OPP) was preferred to polyethylene mostly for its brightness, crispness, and suitability for machine packing. The permeance of this film is suitable for packaging fresh-cut broad-leaved endive and lettuce provided that the distribution temperature does not exceed 10°C . This film generates an equilibrated modified atmosphere (steady state) within the bag that prevents necrosis of salad leaves. Pectinolytic bacteria *Pseudomonas fluorescens* and *Pseudomonas viridiflava* are responsible for soft rot on stored vegetables (Lund, 1983). They are also involved in the decay of shredded endive mixes (Nguyen-The and Prunier, 1989), but these bacteria are present in both spoiled and sound packs.

As shown in Table 3.1, broad-leaved leaves inoculated with a heavily concentrated *Pseudomonas marginalis* suspension do not develop necrosis when stored in CO_2 -enriched atmospheres. It is noteworthy that the leaves injected with a filtered culture medium of this bacterium develop the same symptoms. It has been postulated that the beneficial effect of CO_2 on soft rot induced by *P. marginalis* is due to the

TABLE 3.1
Effect of Controlled Atmosphere on the Development
of Soft Rot on Inoculated Broad-Leaved Leaves. (From
Nguyen-The and Carlin, 1988.)

Controlled Atmosphere		Inoculum		
% CO ₂	% O ₂	H ₂ O (Control)	<i>Ps. m</i>	Filtrate Medium <i>Ps. m</i>
40	10	0	0	0
20	10	0	0	(+)
0	22	0	++	++

Ps. m.: *Pseudomonas marginalis*; 0: no spoilage; (+): no browning, slight soft rot;
 ++: browning soft rot.

acidification of cell medium by CO₂ dissolution (Siriphanich and Kader, 1986), which in turn, inhibits the enzymatic activity of a pectate lyase produced by this bacterium (Nguyen-The and Carlin, 1988).

Salads that are highly sensitive to oxidation (such as those including butterhead and iceberg lettuces) are flushed with nitrogen so that residual oxygen within the packs ranges from 1–3%. Because the atmospheric composition at steady state does not depend on the initial gas mixture within the pack (Fishman et al., 1995), the active modified atmosphere will only accelerate the establishment of a protective atmosphere. Some processors introduce CO₂ into the pack to obtain 5–10% of CO₂ after sealing.

Shredded carrots and cabbages deteriorate rapidly when stored with excessively high (over 30 kPa) CO₂ and low O₂ partial pressures (Carlin et al., 1990a). Barry-Ryan et al. (2000) demonstrated that the spoilage of these products was triggered by the depletion of O₂ more than by the rise in CO₂. These detrimental atmospheric conditions result in a physiological disorder and exudation by the carrot tissue. This sap provides microorganisms, including lactic acid bacteria, with a good growth substrate (Carlin et al., 1990a). The spoilage is markedly reduced using a more permeable film than regular OPP. Microperforated films with a permeance of 15,000–20,000 ml O₂ (or CO₂, because these films are not selective to gas diffusion) markedly reduced spoilage of shredded carrot compared to 35 μ m OPP (Carlin et al., 1990b). A too permeable packing film results in a high respiration rate and a rapid decrease in sugar content of the carrot tissue.

Special products requiring an O₂-deprived atmosphere, such as sliced apples or prepeeled fresh potatoes, may be packed in high barrier films using a nitrogen (or other protective gases or gas mixtures) compensated vacuum.

The sell-by date must be stamped on the bag immediately after packaging. The packs are placed in cartons and stored in 1–4°C cold room according to the first-in, first-out principle. The expedition platform must also be fitted with a cold lock chamber. The chill chain must be respected and controlled up to the store's refrigerated cabinet.

CONCLUSION

NEW PRODUCTS

About 10 years ago, it was thought that diversification would boost the sales of fresh-cut commodities. The development of fresh-cut fruit salad was one of the first ideas.

Fresh-cut Fruits

In the first attempt to manufacture these products, the only additives or preservatives used were ascorbic or erythorbic acids. The shelf life of these fruit salads in juice was short (five days), and on the sell-by date, they were highly contaminated by yeast (10^7 – 10^8 cfu·g⁻¹). Production was stopped after one year or so. More recently, Belgian and Dutch processors have manufactured fresh fruit salads with syrup stabilized with sorbate (to prevent yeast growth), ascorbic acid (as an antibrowning agent), and calcium chloride (to reduce texture breakdown). Of these chemicals, only sorbate is controversial. The stabilization of fresh fruit salads packed without any liquid (syrup or fruit juice) requires only a few ppm of sorbate, because only the first layers of damaged cells must be protected (Varoquaux and Varoquaux, 1990). Dry fruit chunks must be packed in almost airtight containers and under anoxia in order to prevent browning. Nitrous oxide (N₂O) and carbon dioxide (CO₂) are more efficient than N₂. When the fruit chunks are immersed, the sorbate concentration must be much higher, from 350 ppm (sorbic acid) when distributed between 0 and 4°C to 1000 ppm when stored at ambient temperature (20°C). The organoleptic qualities (flavor, firmness, and appearance) are better protected in the “dry” version than in the immersed one.

Vegetable Mixes

Stir fry and other preparations to be served with meat are increasingly popular in Europe. These products, already developed in the United States, are being adapted to the European market.

Niche Products

Mushrooms, including truffles, and local salad mixes, such as “mesclun” (a Mediterranean salad mix), are examples of available niche products.

NOVEL PROCESSING TECHNIQUES

Automatic Trimming

The major fresh-cut French companies have developed automatic trimming machines. In one such machine, the stump of a broad-leaved plant is seized by a claw that spins, thus flattening the salads by centrifugal force. The green extremity of the outer leaves is torn off by static knives placed at an optimal distance and angle from the rotation axis. This machine has been considerably improved and is in operation

at three processing plants in France and England. In another machine (no longer in use), the knives rotated around the salads. A study has also been conducted in which the salad is trimmed with a water jet guided by computerized image analysis. The project did not result in an operable automatic trimmer.

Chlorine-Free Fresh-cut Commodities

As previously mentioned, the use of chlorine for disinfecting in the food industry is controversial and forbidden for organic food. The efficiency of ozone, organic acids, peracetic acid and hydrogen peroxide, the glucose oxidase lactoperoxidase system, air ionization, and other techniques is under investigation. Gamma irradiation and accelerated electrons are effective on shredded root vegetables but result in a dramatic texture breakdown when applied to green salads.

Modified Atmosphere Packaging (MAP)

Optimization of actively modified atmosphere and film permeance is being studied to make MAP more effective. This includes the use of high oxygen concentrations and noble gases.

Prevention of Temperature Abuse

Time-temperature indicators (TTI) have been used on cartons in France to detect temperature abuse during transport and distribution. The detrimental effect of temperature abuse may also be buffered by the packaging of fresh-cut bags or punnets in polystyrene boxes instead of cartons. This technique has been used in France, but its financial and environmental costs were high because the isothermal container could not be recycled.

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4 Safety Aspects of Fresh-cut Fruits and Vegetables

William C. Hurst

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FOOD SAFETY QUESTION

As food safety continues to be a worldwide public health issue, epidemiological studies have shown a significant increase in the number of produce-related foodborne illnesses over the past three decades. The Centers for Disease Control and Prevention (CDC) have reported that the mean number of outbreaks associated with fruits and vegetables more than doubled from 1973 to 1987 (4.3 per year) and again from 1988 to 1991 (9.75 per year). *Salmonella* spp. were the most common etiological agents linked to these outbreaks. During 1995 alone, major outbreak investigation linked infections with *Salmonella* serotype Stanley to alfalfa sprouts, *Salmonella* Hartford to unpasteurized orange juice, *Shigella* spp. to lettuce and green onions, *Escherichia coli* O157:H7 to lettuce, and hepatitis A virus to tomatoes (Tauxe, 1997). More recently in the United States, The Center for Science in the Public Interest ranked fresh produce the fourth highest cause of all food illness since 1990 (Figure 4.1), behind seafood, eggs, and beef. This data excluded bagged salads, fruit salad, or other processed produce. Sprouts and lettuce were the most frequent culprits. In a related study, labeled “multi-ingredient foods,” 14 outbreaks over the 10-year period (1990–2000) were attributed to bagged salads as well as salad bars and processed items not devoted exclusively to produce items (Scruton, 2000). The importance of fresh produce as a vehicle for pathogen transmission and those specific pathogens epidemiologically implicated in fresh produce-related diseases have been extensively reviewed and documented (Doyle, 1990; Nguyen-The and Carlin, 1994; Beuchat, 1996; Beuchat and Ryu, 1997; Tauxe et al. 1997; DeRoeve, 1999; Gillian et al., 1999).

Media attention to fresh and fresh-cut produce has heightened consumer awareness of produce safety. In 1998, a Fresh Trends survey, conducted by *The Packer* magazine, found that 60% of consumers are more concerned today than they were a year ago about *Salmonella* and other bacteria in produce. In response to consumer concerns, major retailers (e.g., Albertson’s and Safeway) and foodservice restaurants

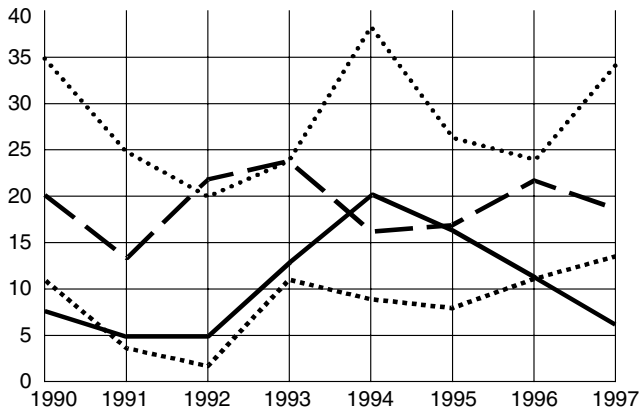


FIGURE 4.1 Cases of foodborne illnesses related to seafood (circles), eggs (solid broken lines), beef (solid line), and produce (squares) for 1990–1997. (Data reprinted with permission from the Center for Science in the Public Interest. Data for 1998–2000 are not yet complete.)

(e.g., Taco Bell and Burger King) have begun programs requiring their suppliers (growers) to have independent third-party inspections of their farms to certify that fruits and vegetables are being grown, harvested, and handled using good agricultural management practices (Hodge, 1998; Beers, 1999). Processors of fresh-cut produce have long understood their responsibility for providing a microbiologically safe, high-quality product to the consumer. They have always taken a proactive attitude toward safety. Within two years of establishing an industry association of processors and suppliers in 1987, a sanitation task force was commissioned to develop model sanitary guidelines for the membership. The culmination of this work was a publication entitled “Recommended Sanitary Guidelines for the Produce Processing Industry” that was released in 1992. This document set forth basic sanitary Good Manufacturing Practices (GMPs) and standardized processing procedures to ensure consistent quality and improved a processor’s credibility with local, state, and federal food inspectors for ensuring a safe product to the consumer (Hurst, 1992).

Despite the many technological and educational advances within the fresh-cut industry in its short history, the challenge remains how to best ensure product safety. The purpose of this chapter is to review some control measures for safety and provide a preventative strategy that has proven most advantageous for minimizing food safety hazards.

FRESH-CUT RISK FACTORS

Food hazards may be regarded as microbiological, chemical, or physical in nature. However, because microbiological safety is the major issue of concern in the fresh-cut industry, it will be the focus of this chapter. Many factors may be involved in the epidemiology of produce-associated disease. Risks for fresh-cut can be divided into two categories. One category concerns the factors or conditions contaminating fresh produce with indigenous pathogens during cultivation or at harvest. These have

been addressed by Hedberg et al. (1994) and Tauxe et al. (1997). They include poor agronomic practices, use of contaminated water for crop irrigation or mixing chemical sprays, application of improperly composted manure as fertilizer, and lack of training among field workers on good personal hygiene. Poor sanitary control during postharvest handling activities is another mechanism for pathogen contamination to fresh produce. DeRoever (1999) reported that two large cases of salmonellosis occurred in 1991 and 1993 due to fresh tomato consumption. The suspect tomatoes were epidemiologically linked to a single tomato packinghouse. Although the exact contaminating point was never pinpointed, possible sources included improperly cleaned bins, buckets, and trucks used for transporting tomatoes from the field to the packinghouse, cross-contamination of tomato dump tank water, poor personal hygiene among the employees, and/or improperly cleaned equipment that routinely went a full season without being disassembled for cleaning.

A second category of microbiological risk is the cutting or slicing operation in the fresh-cut plant. Internal tissue of fresh produce is normally protected from microbiological invasion by waxy outer skins and peels. However, cutting circumvents this physical barrier, allowing juices to leak from inner tissues onto the surfaces of fruits and vegetables. These juices contain nutrients for accelerating microbiological growth. Together with an increase in exposed surface area, large microbiological populations, including potentially higher human pathogen levels, develop on cut produce items (Brackett, 1987; Garg et al., 1990). Key microbiological risks of fresh-cut produce have been determined (Hurst, 1995; Fain, 1996; Zagory and Hurst, 1996) and include the following: there is no kill step (such as cooking) in the process to eliminate potential human pathogens; several pathogens (e.g., *Listeria monocytogenes* and *Aeromonas hydrophila*) are psychotrophic and can grow at temperatures used to store fresh-cut products; the longer shelf life (10–14 days) that is now common, due to good temperature control and sophisticated packaging, may provide sufficient time for pathogen growth; modified atmospheres suppress the growth of spoilage organisms, but certain pathogens (*Listeria monocytogenes*) survive and may actually thrive under these conditions; and unlike traditionally processed (canned and frozen) fruits and vegetables, fresh-cut produce is consumed raw.

CONTROL POINTS

Contamination of human pathogens on fresh produce may occur at any stage during its production, harvesting, handling, processing, storage, or distribution to the consumer. Growers, packers, and shippers of fresh produce have recently been provided a guide on how to minimize microbiological safety hazards during agricultural operations. This document (CFSAN, 1998) sets forth Good Agricultural Practices (GAPs) for producers to implement in their farm facilities. In addition, it focuses on three educational messages: to increase awareness of the common microbiological hazards in fruit and vegetable production, to stress prevention of contamination over corrective actions once contamination has occurred, and to establish a format for developing a system of accountability of sanitary practices at all levels of the

agricultural and packinghouse environment. The GAPs document identifies where and how to control microbiological risks between the field and the fresh-cut processing plant.

While GAPs address field sanitation practices, Good Manufacturing Practices (GMPs) provide the framework for minimizing product contamination in the processing plant. FDA mandates GMPs for all food handling establishments. Fresh-cut processors must have a comprehensive sanitation program, encompassing the facility, equipment, and personnel, as part of their quality assurance system. GMPs are the first line of defense in controlling pathogen buildup in the product or environment of a fresh-cut processing plant.

SAMPLING

To verify GMP control, food processors have traditionally used end-product testing to evaluate microbiological quality and safety of their foods. Microbiological testing is performed on a given number of samples collected from a production run, or lot, of product. When employing a sampling scheme to test for the presence of microorganisms, samples must be drawn in such a manner that the microbiological quality determined by the results is an accurate representation of the microbiological character of the lot. However, as demonstrated in Figure 4.2, microorganisms are not randomly distributed throughout the food. Instead, cells aggregate to form a contagious distribution (Jarvis, 1989), a type of nonhomogenous distribution. Several types

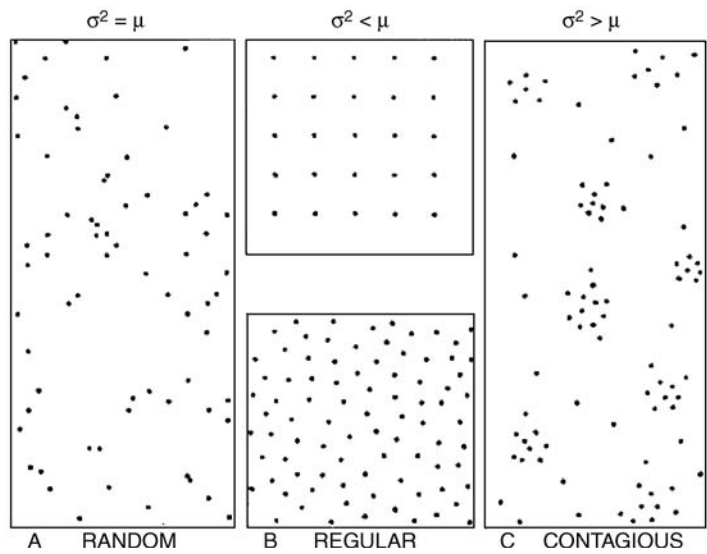


FIGURE 4.2 Possible types of spatial distribution of microorganisms in food. σ^2 , variance; μ , mean. (Reprinted with permission from Jarvis, 1989. *Statistical Aspects of the Microbiological Analysis of Foods*, Volume 21. Elsevier Science Publishers.)

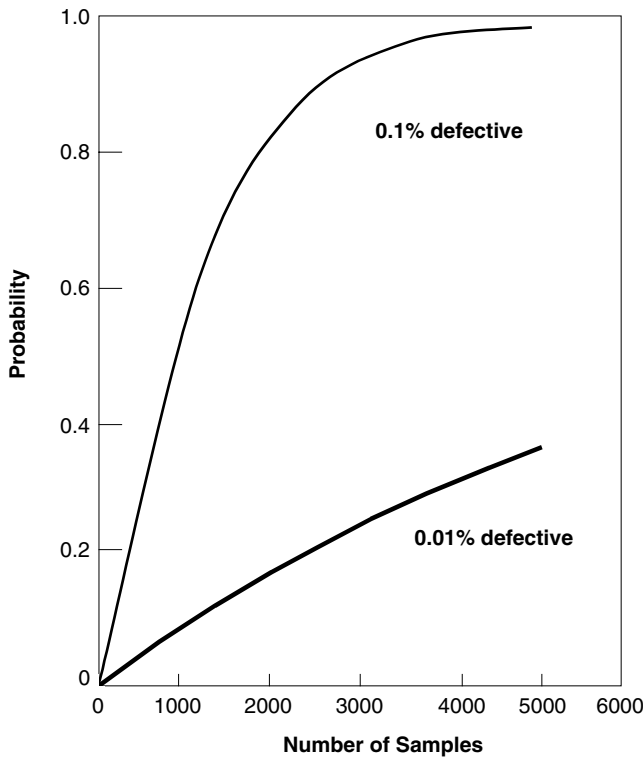


FIGURE 4.3 Probability of detecting a defective sample (positive for pathogens) based on the % defectives in a lot. (Reprinted with permission from Toledo, 2000.)

of probability distributions can be utilized to deal with nonrandom data. The probability distribution used to describe the statistically based attribute sampling plans for the microbiological examination of foods is the binomial (ICMSF, 1986). The problem of using attribute sampling (based on the binomial distribution) in looking for pathogens in fresh-cut products is that when the defective level p (e.g., pathogen) is very low, a very large number of fresh-cut samples n would have to be tested to give a high probability that one defective unit would be found. Figure 4.3 illustrates the futility of this procedure. It is a graph of the probability of detecting at least one defective sample (pathogen positive) for various sample sizes when the level of the defective units is 0.1% and 0.01%. The lower curve, which represents the probability of detection when there is a 0.01% level of defective units, does not even approach 0.5 probability at a sample size of 5000 units! On the other hand, a probability approaching one is shown when the sample size approaches 5000 at a 0.1% level of defective units. As the number of samples tested increases, so does our confidence in the results, but, so too does the cost of sampling. Thus, if close to 100% inspection must be employed to detect low levels of pathogens in a fresh-cut product, clearly, sampling is an inadequate means of assuring safety in products leaving the processing plant. Because sampling

techniques never provide a good enough answer for safe microbiological control, we must look to other approaches.

THE HACCP APPROACH

As indicated above, monitoring a finished food product is no guarantee of safety because unsafe samples may escape detection. What is needed is a more focused approach toward controlling food safety. Such a program is the Hazard Analysis Critical Control Point (HACCP) concept. HACCP is a structured approach to the identification, assessment of risk (likelihood of occurrence and severity), and control of hazards associated with a food production process or practice. HACCP addresses the root causes of food safety problems in production, storage, transportation, etc., and is preventative (FDA, 1994). It aims to identify possible problems before they occur and establish control measures at stages in production that are critical to product safety. One of the purposes of HACCP is to design safety into the process, thereby reducing the need for extensive microbiological testing of in-line samples and finished product (Silliker, 1995).

Design and implementation of a HACCP system involves following seven basic principles or steps (Stevenson and Bernard, 1999).

- *Step 1:* Conduct a hazard analysis. Flow diagram the steps of a process to determine where significant hazards exist and what control measures should be instituted.
- *Step 2:* Determine Critical Control Points (CCPs) required to control the identified hazards. CCPs are any steps where hazards can be prevented, eliminated, or reduced to acceptable levels.
- *Step 3:* Establish Critical Limits (CLs). These are specifications (target values and tolerances) that must be met to insure that CCPs are under control.
- *Step 4:* Establish procedures to monitor CCPs. These are used to adjust the process to maintain CCP control.
- *Step 5:* Establish corrective actions to be taken when monitoring indicates a deviation from an established critical limit.
- *Step 6:* Establish verification procedures for determining if the HACCP system is working correctly.
- *Step 7:* Establish effective record-keeping procedures that document the HACCP system.

HACCP for the fresh-cut industry must be built around a series of preservative factors (hurdles) to control pathogen growth, because there is no definitive kill step in the processing operation. Hurdle technology uses a combination of suboptimal growth conditions in which each factor alone is insufficient to prevent the growth of pathogens, but when combined in additive fashion give effective control (Gorris and Tauscher, 1999). Fresh-cut hurdles include purchasing produce from certified grower/packers, implementing comprehensive plant sanitation programs, using numerous antimicrobial agents (Beuchat, 1998) in the wash water, using modified atmosphere packaging techniques (Gorris and Tauscher, 1999), and following consistent low temperature management.

WHY USE HACCP?

Perhaps the foremost reason for implementing HACCP into a fresh-cut operation is to be ready for impending government regulations. HACCP has become a mandatory program for several food industries in recent years. FDA placed the seafood industry under HACCP at the end of 1997 (FDA, 1997), and the USDA required all meat and poultry establishments to be under HACCP by January 25, 2000 (FSIS, 1996). Most recently, FDA issued a possible fresh juice HACCP mandate, which is still under review (FDA, 1998). FDA has revised its 1999 Food Code, announcing that all retail businesses that handle, distribute, or process food shall implement a food safety plan based on the HACCP concept (FDA, 1999). The message is clear—all food processors will have HACCP in their future (Stier and Blumenthal, 1995). HACCP undoubtedly will be the food safety system of the future for regulatory use (Corlett, 1998).

HACCP is a proven, cost-effective method of maximizing food safety, because it focuses on hazard control at its source. It offers systematic control by covering all aspects of production and handling from raw materials to consumer preparation. HACCP builds customer confidence that food safety is being effectively managed at a fresh-cut operation. Because of its stringent controls, HACCP will bring about improvements in product quality. It demonstrates where to target resources to reduce risks. HACCP implementation will reduce losses from recalled or reworked product. HACCP complements total quality management because it offers continuous problem prevention.

WHAT HACCP IS NOT

Although HACCP implementation may lead to product quality improvement, it should be a distinctly separate program, not incorporated with quality control in a fresh-cut operation. HACCP is a food safety system and should focus solely on safety issues (Scott, 1993). For example, HACCP would be designed to prevent *E. coli* O157:H7 contamination in fresh-cut lettuce but would not guarantee the absence of brown leaves in a 2-lb bag (Hurst, 1995). In the early days of HACCP development, too many areas were designated as “critical,” causing the overlap of safety and quality points. The result was frustration and overwork among personnel who were responsible for monitoring and documenting these areas. Mixing safety and quality aspects in the same plan caused a dilution of the really critical areas and failures of many HACCP plans.

HACCP is not a substitute for the FDA’s Sanitary GMPs (Good Manufacturing Practices). In fact, effective sanitation must be a prerequisite for successful implementation of a plant HACCP program. Sanitary procedures may, however, become incorporated as a control tool in HACCP plans to prevent a hazard from becoming a reality. For example, scarred cutting boards on a lettuce trim line may have shown to be a niche for microbiological pathogens based on equipment audits, if careful sanitation does not exert control over this area.

Also, HACCP plans, unlike GMPs, are not designed to cover all areas of sanitary control in a food operation. Instead, they narrowly focus on specific areas where hazards might be introduced. All GMP requirements are equal from a regulatory perspective. So, dust is filth under GMPs, and its presence on equipment is a violation.

However, under HACCP, control of dust is a sanitary step but is not *critical*, because its presence is unlikely a safety hazard.

ORIGIN OF HACCP

The HACCP concept was originally conceived by the Pillsbury Company in collaboration with the U.S. Army Natick Research and Development Laboratories and National Aeronautics and Space Administration to develop safe food for astronauts in the early 1960s. Pillsbury found that the only way to give 100% assurance that space foods were free of microbiological pathogens was to test all products. Finished product testing was impractical, because most of the product was used up during the destructive nature of the test procedures. A better method of food safety assurance was obviously needed, and this is how HACCP was born (Stevenson, 1993).

PREREQUISITES OF HACCP

As HACCP is integrated into food industry management systems, it becomes evident that HACCP cannot exist as a stand-alone program. Sperber et al. (1998) point out that HACCP cannot be successfully applied in a vacuum, but rather, it must be supported by a strong foundation of prerequisite programs. While not a formal part of HACCP, prerequisite programs must be developed and implemented in a food processing operation before attempting to put a HACCP plan in place.

Prerequisite programs are written, implemented procedures that address operational conditions and provide the documentation to help an operation run more smoothly to maintain a comprehensive food-safety assurance program. Fresh-cut processors should develop written prerequisite programs for the following operations: raw material receipt and storage; wash water quality; equipment maintenance; production controls for grading, washing, cutting, drying, and packaging; temperature and microbiological controls; chemical control; sanitary control for the facility, equipment, and employees; product coding and traceability; recall procedure control; and finished product storage and distribution control.

GOOD AGRICULTURAL PRACTICES (GAPs)

In 1997, President Clinton announced his Food Safety Initiative amid public and media pressure to improve safety in the U.S. food supply. An immediate result was that the FDA in conjunction with the USDA published a user's manual for the fresh produce industry entitled "A Guide to Minimize Microbiological Food Safety Hazards for Fresh Fruits and Vegetables." This document, which is not regulatory, identifies potential sources of microbiological contamination for fruits and vegetables during production and handling at the farm level and provides suggestions on "good agricultural practices" to minimize these hazards (CFSAN, 1998). Specifically, it addresses potential contamination from water sources, fertilizer use (manure or compost), worker health and hygiene, and field and packingshed sanitation, and calls for the development of trace-back procedures for fresh produce.

STANDARD OPERATING PROCEDURES (SOPs)

The heart of any prerequisite program are the Standard Operating Procedures (SOPs). SOPs are written references used to describe a specific sequence of events necessary to perform a task (Harris and Blackwell, 1999). In other words, they are step-by-step instructions that outline how an operation is to be carried out. SOPs must be written for both safety and nonsafety operational tasks. For example, a detailed procedure should be written for monitoring and maintaining correct disinfectant levels of fresh-cut wash water to insure product safety. Likewise, a specific SOP for checking product temperature should be developed that gives instruction on where and how often to perform the task to maintain product quality and shelf life. SOPs are used to assure that critical processing steps are accomplished and can also be used to train employees.

GOOD MANUFACTURING PRACTICES (GMPs)

GMPs are the minimum sanitary and processing requirements necessary to insure the production of wholesome food (Harris and Blackwell, 1999). FDA requirements for GMPs are listed in Title 21, Part 110 of the Code of Federal Regulations. GMPs are written for the following plant areas: building and facilities, equipment and utensils, employee practices, pest control, production and process controls, and warehousing practices. GMPs are broadly written, general in nature, and not intended to be plant specific. GMPs can be used to explain tasks that are part of many jobs (e.g., GMPs are written for personal hygiene and dress regardless of job title, management, production, quality assurance, maintenance, etc.).

GMPs differ from HACCP in a number of ways. First, they are not designed to control specific hazards; second, they do not provide methods for monitoring hazards; and third, they do not require specific record-keeping procedures. GMPs are not used to establish deviation limits and do not describe corrective action requirements.

SANITATION STANDARD OPERATING PROCEDURES (SSOPs)

Sanitation SOPs focus more narrowly on specific procedures that allow a fresh-cut processing plant to achieve sanitary process control in its daily operation. SSOPs can be categorized as two types. SSOPs refer to the sanitary procedures used prior to the start of production (preoperational sanitation). OSOPs (Operational Sanitation Operating Procedures) refer to sanitary actions taken during production to prevent product contamination or adulteration (Stevenson and Bernard, 1999). Preoperational sanitary procedures are written references that describe the cleaning of equipment, utensils, the processing line, and the facility area. Specific instructions must include a description of equipment disassembly, use of approved chemicals according to label directions, cleaning techniques, reassembly of equipment, and proper use of approved sanitizers.

Operational SOPs are sanitary practices that must be performed and documented daily to validate that fresh-cut product safety was maintained during production. There are five key elements to writing an OSOP. They include a written action plan identifying the task, the frequency of the task, the person responsible for the task, the person responsible for verifying the activity, and corrective actions taken if the expected outcome is not met. By establishing effective prerequisite programs prior to designing and implementing HACCP, the number of critical control points (CCPs) intended in a plan for fresh-cut plants may be reduced. HACCP becomes more “user friendly” and manageable because resources can be concentrated on the hazards associated with the product, not the processing environment. If SSOPs and/or OSOPs are included as part of the HACCP plan, they must lend themselves to all aspects of a CCP, including established critical limits and monitoring, corrective action, record keeping, and verification procedures.

COMPREHENSIVE PLANT SANITATION

GMPs serve as the basis for food plant sanitation programs that are mandated by the FDA. Comprehensive plant sanitation must address three areas: facility environment, processing equipment, and all employees (Zagory and Hurst, 1996). A designated person (sanitarian) who has satisfactorily completed a certified food sanitation program should be in charge of writing formal plans and procedures that are to be collected into a sanitation manual. The components of this manual should include the elements listed below.

GENERAL PRINCIPLES

1. Specific plant areas (e.g., employee restrooms, break rooms, waste areas, processing and warehouse room floors) must be cleaned and sanitized daily based on written procedures. Idle equipment, packaging supplies, and pallets should be removed from the processing area prior to cleanup. Storage and structural facilities, coolers, and other plant areas should be cleaned and sanitized on a frequent basis that is best determined by visual and microbiological auditing. Date, time, agent used, person doing the task, and person verifying the activity must be documented.
2. All processing equipment must be cleaned and sanitized based on written, documented, and verified procedures. The sanitarian would be responsible by assembling all SSOPs, OSOPs, and SOPs for GMPs into a sanitation manual.
3. Outside property, such as the exterior building walls, grounds, and landscaping should be included in the sanitation program.
4. A daily sanitation log of preoperational and operational sanitary conditions must be kept on file. Production personnel should be trained to report any unsanitary conditions to their supervisor immediately.
5. A container identification program must be initiated. Containers for product and those for waste must be clearly differentiated and never exchanged.

Employee training should focus on the correct sorting, handling, and storage of all finished, in-process, and waste fruit and vegetable products.

6. The sanitarian should design and implement continuous education programs on sanitary practices for new and existing employees.

FACILITY (ENVIRONMENTAL) SANITATION

The environment of a fresh-cut processing facility can be an ideal source of microbiological contamination. High humidity and numerous structural niches encourage microbiological buildup, which is often overlooked during regular equipment cleaning and sanitizing (Gabis and Faust, 1988). Of greatest concern is the presence of the environmental pathogen *Listeria monocytogenes*. Fresh-cut produce facilities can serve as a breeding ground for *L. monocytogenes* because they are cold and wet, and this microorganism is found on incoming fresh vegetables from the field. To preclude it from gaining a foothold in the cold storage or processing rooms, an environmental sanitation program must be established (Ryser and Marth, 1991; NACMCF, 1991). Information on where to clean and what to use can be found in the master sanitation schedule of Table 4.1. Dry-cleaning methods should replace wet cleaning in the finished product cooler, packaging-material and chemical-storage areas, and employee locker areas of the plant. Cleaning materials, such as dried, compressed air, and tools, such as brushes, brooms, and vacuum cleaners, can be used to remove dirt, dust, and other debris likely to attract rodents.

Routine monitoring for the possible presence of *L. monocytogenes* should be done at its commonly known niches in the plant. Generally, these are nonproduct contact surfaces such as wet floors, drains, cleaning aids, condensation off structural components, condensation catch pans under refrigeration units, etc. Verification for *L. monocytogenes* presence can be achieved by microbiological testing of air, water, and ice supplies used in the facility. These data are useful in determining sites where buildup and movement of airborne and other microorganisms occur throughout the facility.

EQUIPMENT SANITATION

Processing equipment should be designed to avoid microbiological growth niches (inaccessible areas that trap product residue and water). Specific directions should be written for disassembling each piece of equipment. Most importantly, sanitation crews must be trained to follow these procedures during cleaning and sanitizing. Some equipment manufacturers provide written instructions and/or videotapes for cleaning and sanitizing equipment (Shapton and Shapton, 1991). Detailed cleaning procedures should be documented for each functional piece of equipment as part of the total sanitation program. A preoperational sanitation schedule is given in Table 4.2 for an Urschel CC carrot shredder.

Operational SOPs generally focus on employee hygiene, product handling, unsanitary conditions that may arise during production, and a mid-shift cleanup. Table 4.3 gives an SOP example for hand washing/sanitizing.

TABLE 4.1
Processing Plant Environmental Sanitation Master Schedule

Area	Sanitation Method	Tool	Cleaning Materials	Frequency
Walls	Foam, brush, rinse	Soft nylon brush	Chlorine-quat-based cleaner	Once/month
Ceiling	Foam, brush, rinse	Nylon brush, high-pressure machine	Chlorine-quat-based cleaner	Once/month
Floors	Wash, rinse	Hard bristle broom, hose	Chlorine-quat-based cleaner	Daily
Doors	Foam, scrub	Scouring pad, cloth	Chlorine-quat-based cleaner	Once/quarter
Plastic curtains	Scrub, rinse	Scouring pad	Chlorine-quat-based cleaner	Once/quarter
Overhead pipes, electrical conduits, structural beams	Foam, brush	Brush, bucket, high water pressure machine	Chlorine-quat-based cleaner	Once/month
Hoist	Wipe, clean	Cleaning pad	Water, light soap	Once/quarter
Overhead light fixtures	Wipe, clean	Cleaning pad	Water, light soap	Once/quarter
Refrigeration coils	Rinse, sanitizer	High-pressure hose	Water, sanitizer	Once/quarter
Chillers	Scouring	Scouring pad	Acid cleaner	As needed/audit
Air distribution filter	Soak	Plastic bins	Chlorine-based soap	Once/quarter
Drains, trench	Clean, flood	Soft nylon brush, 50 gallon container	Chlorine-based soap quaternary ammonium sanitizer	Daily
Grids	Brush	Nylon brush, high-pressure machine	Chlorine-based soap	Once/week
Waste, dumpster areas	Foam, brush, rinse	Nylon brush, high-pressure foam machine	Heavy-duty chlorine-based cleaner	Daily
Employee break rooms/bathrooms	Wash, rinse	Nylon brush, washcloths	Chlorine-based soap	Daily
Maintenance areas	Scrub, rinse	Nylon brush	Degreasing agent	Once/month

Source: Reprinted with permission from Zagory and Hurst, 1996, *Food Safety Guidelines for the Fresh-cut Produce Industry*, IFPA.

TABLE 4.2
Sanitation Standard Operating Procedure (SSOP)
for an Urschel Machine

Date_____

Fresh-cut, Inc.

Standard Operating Procedure

Pre-Op SSOP #8

Urschel CC Carrot Shredder

Page 1 of 2

Revision #_____

Frequency: Nightly

Safety Precautions:

1. Always wear goggles or a full-face shield whenever handling, cleaning, and/or sanitizing products.
2. Ensure the equipment is locked at a zero mechanical state prior to beginning work or cleaning. Unplug any electrical service cords.
3. Follow chemical label instructions. Do not mix chemicals without appropriate supervisor authorization.
4. Wear goggles when using compressed air.
5. Wear a wet suit (rain slicker), rubber boots, a plastic hard-hat, and chemical resistant gloves.
6. Place plastic bags over electrical motors, electrical boxes, connections, etc. Remove the bags after the work is completed.

Required Chemicals:

Type	Formulation
FCC-2 (Chlorinated Alkaline Cleaner)	1 liter FCC-2 to 10 gallons water
Special Acid Cleaner	1 part acid to 5–10 parts water
Bio-Guard (Quaternary Ammonia)	200 ppm

Night Procedure:

1. Remove wheel-knife cover guards and wheel-knife assemblies and place in cleaning receptacle.
2. Remove and replace any knives requiring sharpening.
3. Remove accumulated produce residues using warm water.
4. Apply cleaner to interior and exterior surfaces. Rinse thoroughly.
5. Sanitize interior and exterior surfaces.
6. Remove accumulated residue, soap, rinse and sanitize interior and exterior framework of shredder.
7. Reassemble all equipment.
8. Inspect for cleanliness.

Biweekly Procedure:

1. Remove wheel-knife cover guards and wheel-knife assemblies and place in cleaning receptacle.
2. Remove and replace knives requiring sharpening.
3. Remove accumulated produce residues using warm water.
4. Apply a thin coat of acid to the interior above-mentioned parts and the interior of the shredder to remove film build-up; allow applied acid to stand for 8–10 minutes.
5. Rinse all parts thoroughly.

TABLE 4.2
Sanitation Standard Operating Procedure (SSOP)
for an Urschel Machine (Continued)

- 6. Sanitize interior and exterior parts of the shredder, including framework.
- 7. Re-assemble all equipment.
- 8. Inspect for cleanliness.

Note: Brushes or any other cleaning utensil used to help clean the Urschel CC Carrot Shredder must be identified for this use and stored separately from brushes or any other cleaning utensils used to clean the floors, bathrooms, etc.

Responsibility: _____
Approval: _____

Source: Reprinted with Permission from Zagory and Hurst, 1996, *Food Safety Guidelines for the Fresh-cut Produce Industry*, IFPA.

TABLE 4.3
Operational Standard Operating Procedure for Hand Washing

Date _____	Fresh-cut, Inc.	Page 1 of 1
_____	Standard Operating Procedure	
	Operational SOP #3	Revision # _____

Employee Hand Washing

Purpose: To ensure proper hand washing and sanitizing to reduce and control the possibility of cross-contamination of product.

Responsible Individual: Line Supervisor

Procedure

- 1. All employees shall use anti-microbial soap each time hands are washed.
- 2. Use warmest water possible when washing hands.
- 3. Wash by rubbing hands vigorously together for 20 seconds during each wash. (Do not forget underneath fingernails and between fingers.)
- 4. Wash hands at each of the following times:
 - a. before starting shift each day
 - b. after each break or when returning to work area
 - c. after handling any non-food item.
- 5. After thorough rinsing, dip hands 8–10 seconds in an appropriate sanitizing solution before returning to workstation.

Corrective Action: Add sanitizer as needed, replenish fresh sanitizing solution every 4 hours.

Verification: Quality Control Technician test sanitizer each hour.

SANITATION AUDIT

Visual Inspection

Prior to production start-up, line supervisors and the plant sanitarian should visually inspect processing equipment to insure that proper sanitation has been completed (Linjacki, 1996). Special attention should be given to observing any grooves, crevices, or inaccessible niches where produce debris has become embedded. A sanitation checklist or log should be used for each production line. Seriously frayed or cracked belts, excess grease accumulation, or loose nuts, hinges, or bolts should be reported to maintenance personnel for correction before the line starts up. Production should begin only after a pre-op inspection has been completed with satisfactory results.

Microbiological Monitoring

A microbiological survey can be used to evaluate the efficiency of sanitation procedures, ascertain the microbiological loads on equipment in relation to difficulty of equipment cleaning (based on design) and frequency of cleaning, identify areas of contamination throughout the plant that contribute to reduced product shelf life and possible pathogen contamination, and verify the HACCP plan (Plusquellec and LeVeau, 1995). Critical areas to test are those that contact produce on a consistent basis. Microbiological surveys can identify hard-to-reach sites in the plant or on processing equipment, which serve as niches for microbiological growth. Whenever an area is found to be unsanitary (based on high microbiological counts), the same area should be rechecked to determine if the changes instituted by the sanitation crew were effective. Data obtained over time from the survey will enable a processor to develop a baseline for the frequency of sampling. These areas should be tested randomly.

To augment control of sanitary monitoring, many fresh-cut plants have begun using ATP-bioluminescence. This technology provides virtually instantaneous results of the cleanliness of a surface based on the relative light units (RLUs), which equate to the general level of organic residue found (Flickinger, 1997). With ATP kits, processors can make on-the-spot decisions as to whether a piece of equipment or area has been properly cleaned so appropriate corrective actions can be taken before start-up. It is important to recognize that while the ATP data relates the degree of organic matter present, it is not adequate in judging the microbiological safety of the surface (Kornacki, 1999).

Tools to Use

Several microbiological techniques can be used to determine contamination on equipment surfaces and, therefore, validate the effectiveness of sanitation. These can be divided into three categories: traditional, modified, and rapid, real-time assays. Some representatives of each group are listed in Table 4.4. Giese (1995) has published a more complete listing of rapid microbiological test kits and instruments.

TABLE 4.4
Microbiological Procedures for Verifying Sanitation
in HACCP Program

Name	Method	General Procedure	Advantages	Disadvantages
RODAC (Replicate Organism Direct Agar Contact)	Traditional	Pre-poured raised agar (nonselective). Surface is pressed against equipment surface, and plates are incubated 48 hours at 32°C (89.6°F) to grow colonies.	FDA/USDA approved. Can vary agar medium. Prepared plates.	Labor intensive to prepare. Can use only on flat, lightly contaminated surfaces.
Swab contact	Traditional	Brush sterile, moistened swab over defined area and add sterile water/broth. Prepare appropriate dilutions and add to petri plate. Overlay with sterile agar and incubate 48 hours at 32°C (89.6°F) to grow colonies.	FDA/USDA approved. Can use for heavily contaminated areas. Suitable for hard-to-reach areas that are irregular, rough and seamed.	Labor intensive to prepare.
Petrifilm	Modified	Activated thin film of agar pressed against equipment surface. After rejoining to grid, Petrifilm is incubated 24 hours at 32°C (89.6°F) to grow colonies.	Eliminates agar-poured plates. Flexible for use on irregular surfaces. Convenient. AOAC approved for some foods.	Moderate cost.
Redigel	Modified	Presterilized nutrients in a tube are poured into a petri dish coated with calcium causing a hardened surface to form. Then use like RODAC plates.	Eliminates poured agar plates. Tube does not need heating before adding to petri plates. AOAC approved.	Moderate cost. Not flexible.
Lightning Biolumine- scence System	Rapid, real-time	Uses ATP bioluminescence preparation to determine gross contamination on equipment surfaces within 1–2 minutes.	Portable. No reagent preparation because all chemicals are contained within swabbing unit in ampoules.	Initial investment in luminometer is expensive. Measures total ATP. Cannot detect difference between microbial and nonmicrobial ATP.

(continued)

TABLE 4.4
Microbiological Procedures for Verifying Sanitation
in HACCP Program (Continued)

Name	Method	General Procedure	Advantages	Disadvantages
Lumac Hygiene Monitoring Kit	Rapid, real-time	Uses ATP bioluminescence preparation to determine gross contamination on equipment surfaces within 1–2 minutes.	Portable. Can determine total ATP or microbial ATP on surfaces. Because microbial ATP can be measured, serves as better indicator of microbial numbers on surface.	Initial investment in luminometer is expensive. Involves more reagent mixing which increases risk of operator error.

Source: Reprinted with permission from Zagory and Hurst, 1996, *Food Safety Guidelines for the Fresh-cut Produce Industry*, IFPA.

PEST CONTROL

A pest-control program is essential to good plant sanitation and must be maintained by a *certified* pest-control operator (Katsuyama, 1993).

1. Sanitation personnel should maintain an up-to-date inspection of all bait stations, mechanical traps, and glue-stations and should advise pest-control specialists of areas within the plant where pest problems are occurring. Bait stations should not be used inside the processing facility. Bait traps may be used outdoors.
2. All exterior windows, doors, and building surfaces should be inspected periodically to reduce the risk of pests entering the facility.
3. Written records should be maintained by sanitation personnel that include all of the following: bait station, mechanical trap and glue-station locations and activity, insect-electrocuting system inspection and cleaning, facility inspections, and pesticide applications.
4. If an outside pest-control company is hired to manage the program, the in-house quality assurance department should periodically evaluate the effectiveness of the pest-control program.

EMPLOYEE HYGIENE PRACTICES

The importance of employee personal hygiene to insure food safety has been the subject of several reviews including those by Troller (1993), Zagory and Hurst (1996) and Marriott (1999). In terms of safety, employees are one of the greatest potential sources of human pathogen transfer to fresh-cut produce. This is because they must physically handle the product during its preparation. *Salmonella* (Pether and

Gilbert, 1971), *Shigella* (Davis et al., 1988), and *Listeria* spp. (Kerr et al., 1993) have all demonstrated the ability to remain viable long enough on food workers' hands to contaminate the product.

An effective hand-washing regimen is implicit in good hygiene. The debate of whether or not to use gloves as a physical barrier to pathogen transfer between the food handler and food, as well as the effectiveness of hand sanitizers, remains unclear. The 1999 Food Code requires glove compliance by food workers to prevent pathogen transfer to ready-to-eat foods. However, skin can become heavily contaminated in the warm, moist (due to perspiration) environment under gloves. If the glove is breached, by sharp fingernails or jewelry, then massive contamination through leakage will be transferred to the product (Troller, 1993). While medical literature and regulatory agencies advocate glove protection, Fendler et al. (1998) reported that the most effective hand-washing system was when bare hands were washed and sanitized hourly. This procedure gave significantly higher hand sanitization levels than when gloves were employed. To minimize skin contamination, Taylor (2000) suggested using a protective, antiseptic lotion on hands under gloves. Miller et al. (1994) found that instant hand sanitizers resulted in a significant increase in bacterial numbers on hands, while Taylor (2000) reported them useful when washing was not possible, but, they did not have a lasting effect.

Regardless of the method used, employee training in sanitation must begin at the time of employment. Workers should be given a copy of the company's rules for hygienic practices and dress code when initially hired. Furthermore, management must be responsible for developing education programs to instill sanitary awareness among employees on a continuing basis.

DEVELOPING AN HACCP PROGRAM

HACCP design, implementation, and maintenance is not easy. It requires strong support from top management. A commitment of human and monetary resources is needed to make the system work. When Pillsbury first decided to implement HACCP, the CEO publicly stated that all raises, promotions, and evaluations would be based on developing and implementing HACCP to insure safe food production (Stier and Blumenthal, 1995). Now that was a strong statement of support! Employees ultimately determine the success or failure of HACCP. Therefore, training programs are essential to develop a positive attitude about food safety and to help empower personnel to maintain the HACCP program. Because prerequisite programs build the foundation of HACCP, these must be reviewed, and their soundness must be verified before starting HACCP. Implementing HACCP takes time. Experience has shown that installation and implementation takes between six months and two years.

ASSEMBLE THE HACCP TEAM

Once management agrees to the HACCP concept, an HACCP coordinator should be appointed to lead, coordinate, and build the HACCP team. The team leader may require an education in HACCP. In addition to leadership and coordination skills, this person should be creative, have good communication and listening skills, and

have an overall knowledge of the plant operation and its products. A qualified team leader is crucial to initiating the HACCP program and insuring it will be implemented and maintained in the correct fashion.

Because HACCP is a systems approach to safety, the team must have multitalented personnel from both management and production involved. They should have a real working knowledge of the operation and products. Fresh-cut produce teams should consist of managers, quality assurance/sanitation personnel, production operations, engineering/maintenance, purchasing/procurement, marketing, and labor (on-line personnel). The leader must instruct the team on HACCP and what the role of the team will be.

HACCP TRAINING

Because the level of technical expertise needed by the HACCP team will obviously be greater than that needed for the rest of the employees, this training is best carried out in two phases (Mayes, 1994).

- *Phase I:* Training for managers, supervisors, and HACCP team members should focus on hazard analysis and risk assessment issues, drawing flow-charts, determining critical control points in the process, and collecting data on control charts. This training consists of seminars, workshops, and even one-on-one instruction of how to design, implement, and maintain HACCP procedures.
- *Phase II:* Training for front-line production, maintenance, and sanitation will most often involve changing attitudes concerning handling activities, creating employee awareness, and paying attention to detail, as it relates to safety. Line employees must be taught the difference between quality concerns and safety concerns. Most importantly, employees need to understand that the HACCP might change their specific job activity and why this change is needed.

In staff training, keep in mind the ethnic and cultural differences of employees. English is not the primary language for a significant number of fresh-cut produce workers. Often out of fear of being incompetent or of losing one's job, an employee will indicate an understanding of policies or procedures when this might not be the case. Bridge the communication barrier and assure comprehension by providing training and operating manuals in the native language or languages of your employees (Rosete, 1998).

DEFINE THE PRODUCT

HACCP must be custom-designed for each fresh-cut product in a given plant operation. Although generic models can serve as useful tools to demonstrate how to create an HACCP plan, these models are not always applicable for every product, processing line, or specific plant facility. It is important to realize that HACCP is not a turnkey system. Each HACCP plan will be product, processing line, and plant specific.

TABLE 4.5
Product Description, Use, and Distribution

PRODUCT DESCRIPTION	
Common Name:	Shredded Lettuce; prepared from refrigerated iceberg lettuce; trimmed, cored and cut; washed in a solution of potable water and chlorine
Type of Package:	Packed in food grade plastic bags, 8 oz. to 10 lb. units
Length of shelf life, at what temperature?:	Optimum shelf life of 14 days if refrigerated at 34° to 38°F (1.1° to 3.3°C)
Labeling Instructions:	Bag and/or box contains “processed on” or “use by” date
Where will it be sold?:	Foodservice operations and retail markets
Intended use and consumer:	For use in salads and sandwiches for foodservice customers; prepackaged units for in-house use by consumer
Special Distribution Control:	Product distributed under refrigeration, stored in refrigeration at 34° to 38°F (1.1° to 3.3°C)
<i>Source:</i> Reprinted with permission from IFPA Technical Committee, 2000, <i>HACCP for the Fresh-cut Produce Industry</i> , IFPA.	

Before HACCP design begins, several preliminary tasks must be accomplished. Once the HACCP team has been assembled and trained, each product must be described, and its intended use must be defined. This consists of detailing its form, size, packaging and storage requirements, method of handling, and intended customer. Table 4.5 shows a sample product description, distribution, intended use, and customer.

DEVELOP A FLOW DIAGRAM

A flow process should be constructed for each product to detail how the product is created from raw material production, through processing and packaging, to distribution and consumer use. Its purpose is to identify specific areas where hazards could occur. Although a complete flow diagram takes into consideration the entire production process from farm to table, the HACCP plan for a processor usually involves only those parts of the process where control can be exercised (see Figure 4.4). Fresh-cut processors use vendor verification programs to document the safety of their raw ingredients before processing and retail/wholesale audits to verify safe handling after leaving the production plant. Once completed, the flow diagram should be verified with on-line supervisors for completeness and accuracy. Often, it must be reformatted or revised using descriptive words that are more easily understood by all members of the HACCP team. The flow diagrams should be reviewed on a quarterly basis. A number of computerized software programs (e.g., doHACCPZ version 2.3, and HACCP Documentation Software, version 2) have made updating flow processing changes to an operation quick and easy.

Process Flow Diagram

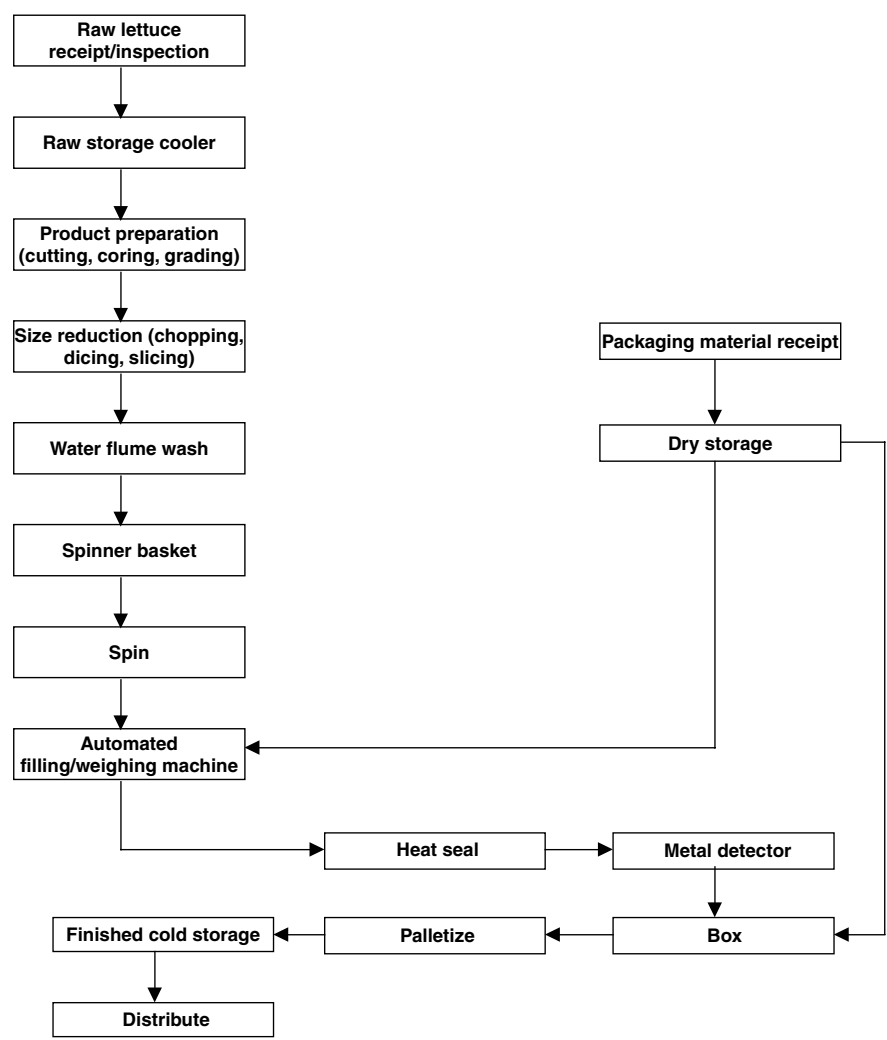


FIGURE 4.4 Example of a fresh-cut lettuce operation.

PERFORM A HAZARD ANALYSIS (PRINCIPLE 1)

The first step in formal HACCP design is to conduct a hazard analysis (Principle 1) of the process. The purpose of hazard analysis is to list all potential hazards that are significant enough to likely cause illness or injury if not effectively controlled. An extensive listing of possible hazards of a microbiological, chemical, or physical nature that might be imparted to food has been reviewed by Rhodehamel (1992).

Hazard analysis is a two-step procedure. First, the HACCP team must identify the potential of all hazards occurring at each step in the flow diagram. Once identified, it is important to consider how the hazard may become incorporated into a fresh-cut product. Brainstorming and cause-and-effect analysis are two quality improvement techniques that can be used to assess sources of hazard contamination (Rao et al., 1996). Brainstorming is a technique used to generate, through divergent thinking, a large number of creative possibilities to the cause of a problem. A cause-and-effect analysis can provide additional structure to brainstorming by grouping the convergence of ideas generated into key causes that will be addressed by further action.

Using a cause-and-effect diagram, the relationship between a given effect or problem and all identified causes of that effect can be narrowly focused. The effect or problem (e.g., microbiological contamination) is represented by a horizontal arrow or spine, and principal causes are identified by arrows entering the spine. Principal causes of any effect are inherently found in six major sources of variability, namely, machines, materials, methods, measurement, personnel, and the environment, all of which are part of any process. Each principal arrow can have secondary arrows representing sub-causes. Figure 4.5 illustrates part of a cause-and-effect diagram highlighting the environment as a possible source of *Listeria* contamination to fresh-cut lettuce. Once a comprehensive cause-and-effect diagram has been constructed and all possible causes of a problem verified, appropriate measures must be instituted to control the hazard.

Step two of the hazard analyses is an evaluation of each identified hazard based on its risk and severity. Risk is an estimate of the likely occurrence of a hazard, while severity is the seriousness of the hazard. For example, *Listeria monocytogenes* and *Clostridium botulinum* are potential microbiological hazards in the fresh-cut industry.

Epidemiological data and scientific research, however, have shown *L. monocytogenes* to be a greater risk in fresh-cut products, even though *C. botulinum* would be considered the more severe hazard based on mortality rates. Thus, routine screening of the processing environment for *L. monocytogenes* would be warranted to keep it out of fresh-cut products.

After all potential hazards have been listed, and control measures have been agreed upon, the HACCP team must face the challenge of determining the “significance” of each identified hazard based on risk assessment methodology. Traditionally, hazard analysis has been evaluated using qualitative risk assessment procedures that are subjective and do not adequately validate product safety (WHO, 1995; Buchanan, 1995). One method of gaining quantitative information is through the use of predictive microbiology, where bacterial growth responses are summarized under different environments to form mathematical equations (McMeekin et al., 1993). In recent years, several authors (Notermans et al., 1995a; Miles and Ross, 1999) have published research demonstrating “quantitative microbiological risk assessment,” where predictive models have been integrated into HACCP plans to provide a more objective way to quantify and rank microbiological hazards based on risk assessment. Because present predictive microbiology instruments, microbiological challenge testing (MCT), and storage testing (ST) are slow and expensive, Panisello and Quantick (1998) have developed computerized predictive microbiology software to assist in microbiological risk assessment in the HACCP system.

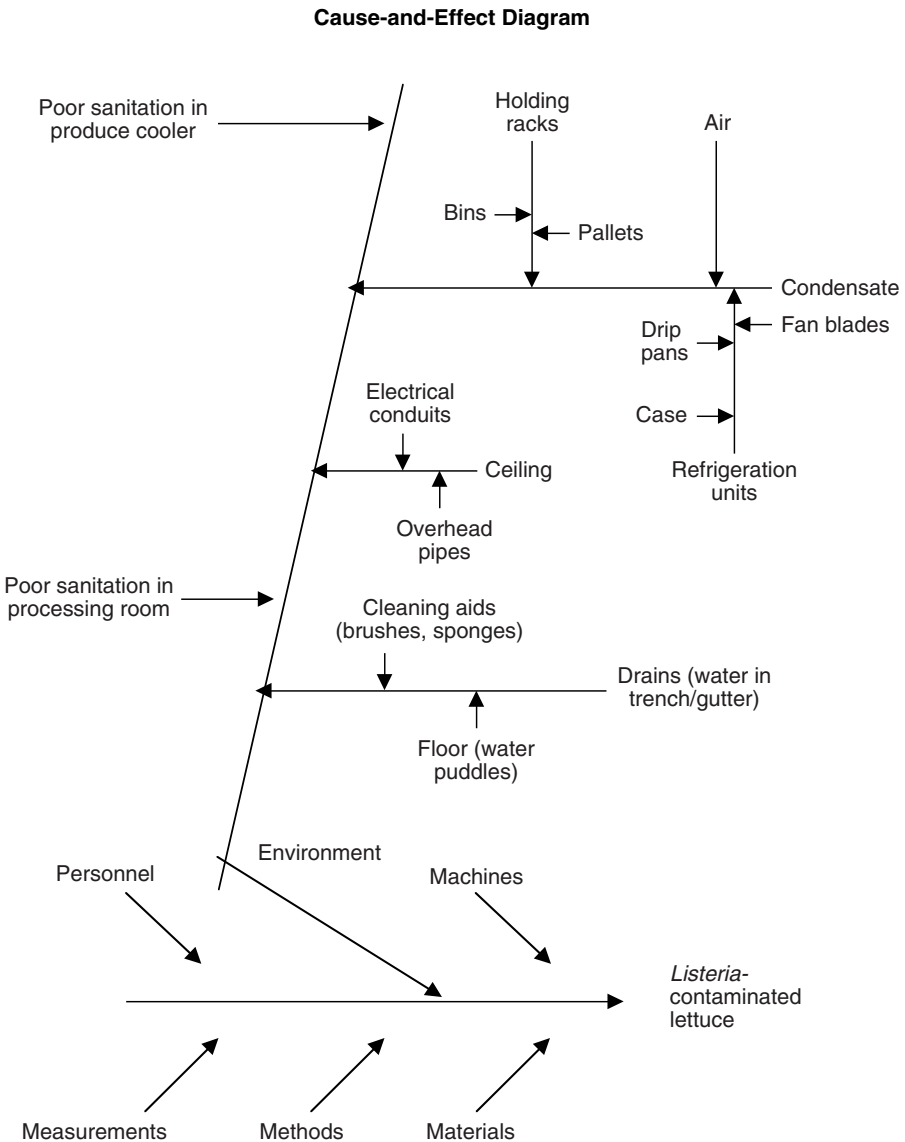


FIGURE 4.5 Part of a cause-and-effect diagram highlighting the environmental component as a source of *L. monocytogenes* contamination.

A thorough hazard analysis is essential to the design of an effective HACCP plan. If this is not done correctly and the significant hazards warranting control within the plan are not properly identified and evaluated, then HACCP will not be effective regardless of how well the plan is followed (Bernard, 1997). As shown in Table 4.6, when the HACCP team does a hazard analysis in the production of fresh-cut lettuce, microbiological pathogens would be the biological hazard associated

TABLE 4.6
Excerpt from Hazard Analysis Worksheet

HAZARD ANALYSIS PROCEDURE (Principle 1)					
Process Step	Biological Chemical Physical	Potential Hazard Controlled, Introduced, or Enhanced	Is Potential Hazard Significant? Yes/No	Justification for Determination of Hazard Significance	What Control Measures Can Be Applied to Reduce or Eliminate this Hazard?
Water flume wash	B	Pathogen controlled	Y	Water reuse; pathogen introduction into water through cross- contamination	Chlorine added to and monitored in wash water; water cold and has optimum pH for chlorine action

Source: Reprinted with Permission from IFPA Technical Committee, 2000, *HACCP for the Fresh-cut Produce Industry*, IFPA.

with the water flume wash step. Pathogen buildup would be significant, if not controlled, because of water reuse and cross-contamination over time. Proper chlorination is the control measure applied to reduce this hazard.

IDENTIFY CRITICAL CONTROL POINTS
(PRINCIPLE 2)

Once the significant hazards and control measures have been identified through hazard analysis, the HACCP team must determine the best place to control these hazards in the process. This process, called establishing the critical control points (CCPs), is the heart of the HACCP plan. A critical control point is a step at which control can be applied and is essential to prevent or eliminate a food safety hazard or reduce it to an acceptable level (NACMCF, 1998). For every significant hazard identified, one or more CCPs must be designated to control the hazard. The CCPs are the points in the process where HACCP control activities will occur. But, it may not be possible to fully prevent or eliminate a significant hazard, only minimize it to an acceptable level. For example, chlorination of flume water in the fresh-cut industry is practiced to minimize the introduction of pathogens into the water through cross-contamination of the produce items being washed. Using chlorinated water to rinse sliced chicory leaves for “ready-to-use” salads, Nguyen-The and Prunier (1989) found that, on the average, 10³ bacteria per milliliter were inoculated into the wash water. This is low compared to the numbers 10⁵ to 10⁶ cfu per gram found on the product, but it demonstrates that microorganisms can be rinsed off fresh-cut surfaces by washing. Although no human pathogens were reported, presumably, they may have been part of the microflora. It is known that chlorine efficacy as a sanitizer is

affected by certain physiochemical characteristics of water. Chlorine antimicrobial activity against both plant and human pathogens (Bartz and Eckert, 1987; Maier et al., 2000) decreases as the particulate matter in the water increases. Particulate matter may interfere by either chemically reacting with the disinfectant, thus neutralizing its action, or physically shielding the organism from the disinfectant. Clearly, the goal in chlorinating flume water is to maintain levels at ≥ 1 ppm free available chlorine (IFPA, 2000) and to keep any pathogens from reaching infectious levels in the water which may be imparted to fresh-cut products.

Many points in a flow diagram not identified as CCPs may be considered control points. A control point (CP) is any step at which biological, physical, or chemical factors can be controlled (NACMCF, 1998). Many types of CPs can exist in a fresh-cut operation. They include those that address quality control (color, flavor, texture), sanitary control (SSOPs, GMPs), and process control (filled weights, seal closures). Pinpointing the right CCPs is a most crucial and problematic aspect of an effective HACCP program (Demetrakakes, 1997). HACCP is about safety, not about quality. Perhaps the biggest mistake processors make is to define too many CCPs, some of which are really CPs, in their HACCP program. Quality and safety issues are confused for two reasons. Quality is vital to a product's well being in the marketplace, which can tempt processors to elevate it to the highest level of scrutiny. In addition, most processes have points where a breakdown could affect both quality and safety.

To keep HACCP programs plant-friendly and sustainable, Bernard (1997) recommends that the number of CCPs should be kept to a minimum and none should be redundant. Redundancy will add to the cost of record keeping. Experience has shown that HACCP plans that are unnecessarily cumbersome will likely be the ones that fail. A CCP should be limited to that point or those points at which control of the significant hazards can best be achieved. For example, a metal hazard can be controlled by ingredient sourcing, magnets in the water flume, screens, and a metal detector, all in one processing line. However, sourcing magnets and screens would not be considered CCPs (but instead upstream CPs). A possible metal hazard is best controlled by use of a metal detector and product rejection at the end of the packaging line (Lockwood et al., 1998). This is an example of where the CCP can be several process steps away from the point where a significant hazard may be introduced.

To assist in finding where the correct CCPs should be located in a unit operation, we can use a tool called the CCP Decision Tree. Several versions of this tool can be found in the literature (NACMCF, 1998; Mortimore and Wallace, 1994; Wedding, 1999a). The decision tree is a logical series of questions that are asked for each identified hazard at each process step. The answer to each question will follow a process of elimination and ultimately lead to a decision as to whether or not a CCP is required at that step. Using a CCP decision tree promotes structured thinking and insures a consistent approach of every hazard at each step. It also has the benefit of forcing and facilitating HACCP team discussion, teamwork, and study (Mortimore and Wallace, 1994). However, as pointed out by Wedding (1999a), this is not a perfect tool and is not a substitute for common sense and process knowledge, because complete reliance on the decision tree may lead to false conclusions. Application of how to use the CCP decision tree for metal contamination at the water flume is demonstrated in Figure 4.6. If the answer to Q1, as shown, is yes, then ask Q2. To

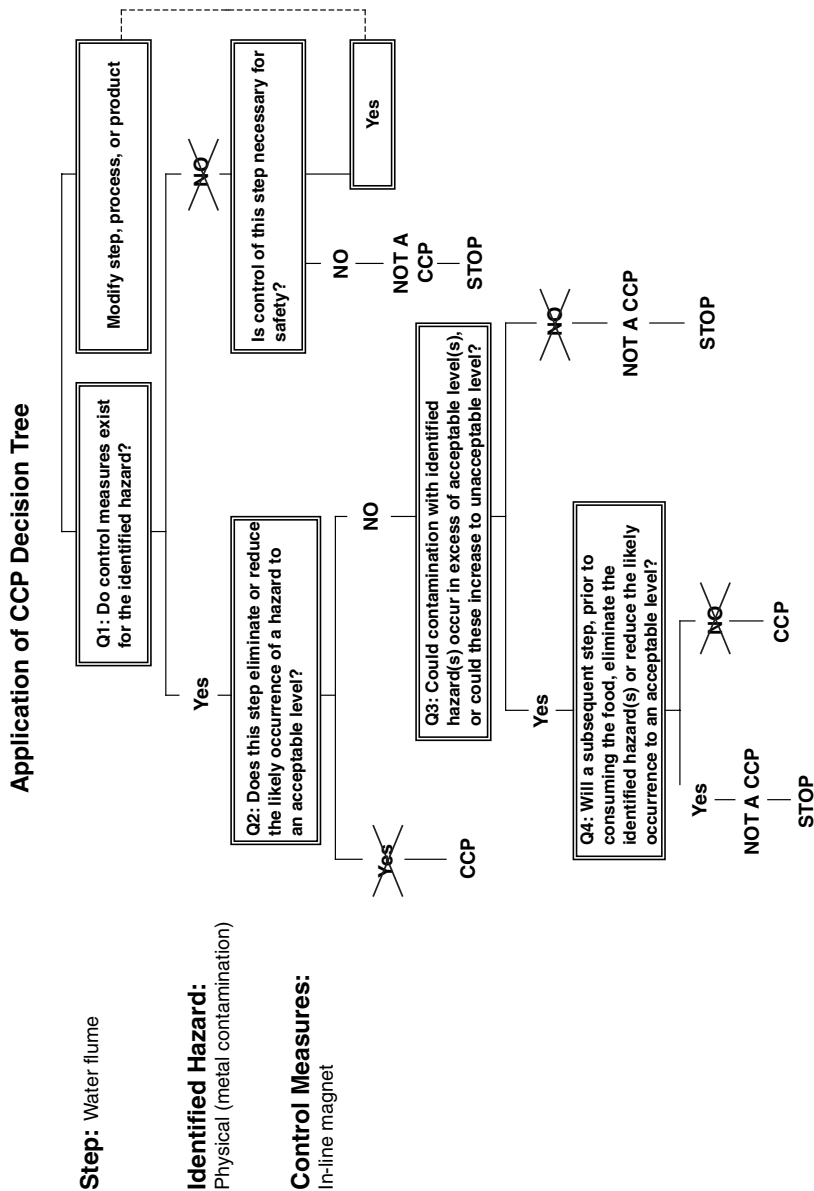


FIGURE 4.6 Using CCP decision tree to illustrate whether metal contamination at the water flume step should be a CP or a CCP.

answer this question, consider if this is the best step at which to control the hazard. If the answer is yes, then the step is a CCP; move to the next step with a significant hazard. If the answer is no, as shown, then ask Q3. This question refers to contamination occurring or increasing to unacceptable levels beyond this step. If the answer is no, then the step is not a CCP for that hazard. If the answer is yes, then ask Q4. If the answer is no, then the step is a CCP. If the answer is yes, then this step is not a CCP for the hazard. In this case, magnets are a CP, but not a CCP because metal contamination can occur anywhere in the processing line. The best location to designate a CCP for metal contamination in the product is at the end of the packaging line.

HACCP teams often ask, “How many CCPs do we need in our HACCP plan?” (Wedding, 1999a). Unfortunately, there is no simple, clear-cut answer to this question. It depends on plant layout and design, product being produced, ingredients used, equipment age and condition, processing methods employed, and, especially, the effectiveness of the prerequisite programs implemented. The situation often arises where a similar step may be designated a CCP in one facility but covered under a prerequisite program in another facility. Each processor must determine the best location to designate CCPs in his unique operation (IFPA, 2000).

SET CRITICAL LIMITS (PRINCIPLE 3)

Critical limits are the safety boundaries that must be established for each identified CCP in the hazard analysis step. A critical limit (CL) is a maximum and/or minimum value to which a biological, chemical, or physical parameter must be controlled at a CCP to prevent, eliminate, or reduce to an acceptable level the occurrence of a food safety hazard (NACMCF, 1998). It must be understood that CCPs do not implement the control of hazards; they are just affected by them. The parameters that actually control hazards are the critical limits. They are individual values, not averages, that signify whether the control measure at a CCP is “in” or “out” of control (Wedding, 1999b). Critical limits define the boundaries between safe and unsafe product. For the HACCP team to set correct critical limits, they must have in-depth knowledge of the potential hazards, full understanding of the factors involved in their prevention or control, and knowledge of the control mechanisms of the process (Mortimore and Wallace, 1994). Criteria or factors that make up critical limits can be grouped into several categories as demonstrated in Table 4.7.

Because critical limits must be measurable in real-time, microbiological limits are not suitable for controlling CCPs. Conventional testing takes days for results,

TABLE 4.7
Criteria Utilized in the Fresh-cut Industry to Set
Critical Limits

Chemical	Physical
Chlorine	Temperature
pH	Absence of foreign materials (metal, etc.)
Titrateable acidity	ORP (millivolts)

and even rapid screening methods can take several hours. Microbiological limits will not allow instant action to be taken when a CCP deviation occurs. Also, the probability of low pathogen levels, nonrandomly distributed through a batch, significantly increases the chance of failing to detect pathogens when statistical sampling is used (Moberg, 1992).

A recent exception to the general rule of microbiological ineffectiveness has been the development of ATP bioluminescence. Although truly a rapid testing method, i.e., results in minutes rather than hours, it is used by some fresh-cut processors to verify hygiene control at CCPs. But, this new technology has several limitations. ATP is nonspecific in distinguishing between eukaryotic and microbiological ATP (Griffiths, 1996), its light signal may be quenched or enhanced by cleaning/sanitizing residues (Velazquez and Feirtag, 1997), and the limit of its sensitivity is 1000 cells (Forsythe and Hayes, 1998), which is greater than the infectious dose for several food pathogens, including *E. coli* O157:H7, *Listeria monocytogenes*, and *Shigella* spp. (Doores, 1999). These limitations make ATP a better tool for monitoring hygienic compliance with prerequisite programs (e.g., SSOPs and GMPs) than serving as a critical limit parameter.

When a critical limit is violated, it signals that a potential hazard may be introduced at that CCP, thus, immediate control measures must be taken to bring the CCP back within its CL range. Exceeding the control limit indicates that one of the following situations has occurred (Moberg, 1992):

- evidence of the existence of a direct health hazard (e.g., detection of *Listeria monocytogenes* in a fresh-cut salad product)
- evidence that a direct health hazard could develop (e.g., no chlorine or other antimicrobial agent used to sanitize recirculated flume wash water)
- indications that a product was not produced under conditions assuring safety (e.g., metal detector not running during a production shift)
- indication that a raw material may affect the safety of the product (e.g., *E. coli* O157:H7 found in whole cabbage heads from supplier)

Each CCP must have one or more CLs set for each significant hazard, and these must be scientifically based (NACMCF, 1998). In many cases, the appropriate CL may not be readily apparent or available to HACCP team members. Wedding (1999b) has listed some sources to consult for his information. These include research articles, government documents, trade association guidelines, in-plant studies, university extension publications, and industry experts. If outside sources are used to establish CL, they should be documented and become part of the HACCP plan as shown in Table 4.8.

Because of the need for “real-time” monitoring and quick data feedback, chemical and physical measurements made at CLs also serve as an indirect measure of microbiological control at the CCPs. In these instances, correlation between chemical and physical parameters and microbiological parameters must be predetermined in order to set safe control limits. With this correlation, exceeding a chemical or physical limit would mean that the corresponding microbiological limit had been violated, and a potential health hazard may exist or develop (Moberg, 1992). Notermans et al. (1995b)

TABLE 4.8
Excerpt of HACCP Plan Summary Page for Fresh-cut Lettuce

Process Step	Biological Chemical Physical Hazard Description	Critical Limits	Monitoring Procedures/Frequency/Person Responsible	Corrective Actions/Person Responsible	Verification Procedures/Person Responsible	HACCP Records
Water flume wash	<i>L. monocytogenes</i>	Potable water containing ≥ 1 ppm free residual chlorine for 30 seconds; at pH ≤ 7.0	Prior to start of processing and each 30 minutes thereafter, QC personnel will monitor free chlorine using standardized test kit, and a calibrated pH meter will be used to monitor water pH three times per shift	QC personnel will adjust water chemistry to maintain pH and chlorine added to maintain CL; held product will be rewashed and CL deviations noted in a log	QC personnel will maintain chlorine monitoring logs, pH temperature monitoring logs, CCP deviation/corrective action logs, calibration logs for thermometer, pH test and chlorine test kit used, microbiological tests will be run on finished product at least once/year to validate pathogen absence, HACCP plan will be revalidated at least once/year	HACCP coordinator reviews all HACCP records weekly, HACCP coordinator will conduct calibration tests, plant manager reviews records daily, state food inspector/FDA audits, customer audits/internal/consultant audit once/year, all records kept at least one year, random sampling/testing product to ensure process verification
CCP 1B	<i>E. coli</i> O157:H7 <i>Salmonella</i> and <i>Shigella</i> spp. and other microbial pathogens					

Source: Reprinted with Permission from IFPA Technical Committee, 2000, HACCP for the Fresh-cut Produce Industry, IFPA.

proposed a method to quantify microbiological levels and their reduction at each CCP. This approach would require knowledge of the kind and number of potentially hazardous microorganisms in the raw food material and the use of computer models, storage tests, and microbiological challenge testing to predict microbiological survival and death rates at each stage of the production process.

Once CLs have been set for all CCPs, the task is to keep the parameter being measured in control within the established tolerances. This may or may not be an easy job depending on the kind of variation in the process. Establishing operating limits is a practical means to help prevent routine violation of the CLs (Wedding, 1999b). Operating limits are criteria that are more stringent than critical limits and are established at a level that would be reached before the critical limit is violated (Lockwood et al., 1998). Process adjustment should be taken when the operating limit is exceeded to avoid loss of control and the need to take corrective action at the critical limit.

ESTABLISH MONITORING PROCEDURES (PRINCIPLE 4)

Monitoring is one of the most important components of the HACCP plan, because it is what the HACCP team relies upon to maintain control at the CCPs. It documents that a process can operate consistently within the CL to control the identified hazards. By definition (NACMCF, 1998), monitoring a planned sequence of observations or measurements to assess whether a CCP is under control produces an accurate record for future use in verification. Monitoring serves three purposes: first, to track the operation of a process and enable the identification of trends toward a critical limit that may trigger process adjustment; second, to identify when and where there was a loss of control (a deviation occurred at the CCP) such that corrective action is needed; and third, to provide written documentation of the process control system (Lockwood et al., 1998).

The HACCP team will be responsible for designing the monitoring activities at each CCP. Procedures must identify what control measures will be monitored, how frequently monitoring should be performed, what procedures will be used (data collection methods and equipment), and who will perform the monitoring.

WHAT WILL BE MONITORED?

Monitoring usually means measuring a physical, chemical, or sensory parameter, or taking an observation of the product or process to determine compliance within a CL. Examples in the fresh-cut industry include the following:

- checking that a vendor's certificate for safety accompanies a lot of produce materials (required by some customers)
- measuring free chlorine levels in the flume wash water
- checking the metal detector at the end of the packaging line
- measuring packaged product temperatures at the end of the processing line (required by some customers)

WHEN WILL MONITORING BE PERFORMED?

To assume adequate control, the frequency of monitoring must be consistent with the needs of the operation in relation to the variation inherent in the control step (Bernard, 1997). Continuous monitoring at a CCP is always preferred, but sometimes, it may not be practical or available for use. When it is not possible to monitor a CL at the 100% level, or continuously, then it is necessary to establish a monitoring interval (e.g., discontinuous) that will be reliable enough to insure the hazard in question is under control. It is vital that the sampling procedures used to monitor CCPs be statistically valid. Data collection programs will be most accurate and of greatest benefit if established under a structured system of statistical process control. An example of a discontinuous monitoring system (e.g., measuring free residual chlorine at specified intervals) for the CCP designated at the water flume wash step of fresh-cut lettuce is described in Table 4.8.

HOW WILL MONITORING BE PERFORMED?

As noted earlier, physical and chemical measurements are the preferred monitoring methods, because testing can be done rapidly. The equipment chosen for CCP monitoring must have the degree of sensitivity to accurately control hazards. In fresh-cut processing, CCP monitoring equipment includes thermometers, pH meters, ORP meters, chlorine test kits and probes, metal detection units, etc. Daily calibration or standardization of this equipment is necessary to insure accuracy. Records should be maintained on the equipment calibration and must become part of the support documentation for the HACCP plan.

WHO WILL PERFORM THE MONITORING?

An underlying concept of HACCP is to promote safety awareness to production line employees. A good way to achieve this goal is to involve them in the monitoring activities. Monitoring by the personnel and equipment operators can be advantageous because they are continuously viewing the product and/or equipment and are in the best position to observe changes from the norm quickly. Also, there is the benefit of a broad base of understanding, commitment, and ownership to the HACCP program. The role of quality control personnel may be more appropriate in verification or “checking the checker” (Bernard, 1997).

According to Lockwood et al. (1998), those responsible for monitoring a control measure at a CCP must do the following:

- be trained in the CCP monitoring techniques
- fully understand the importance of CCP monitoring
- have ready access to the monitoring activity
- accurately report each monitoring activity
- immediately report critical-limit infractions so that immediate corrective actions (Principle 5) can be taken

Should any unusual occurrences and/or deviations from the CL occur at a CCP, the monitor is responsible for taking corrective action immediately. All process

adjustments must be reported on the corrective action form. All records and documents associated with CCP monitoring must be signed or initialed by the person doing the monitoring.

STATISTICAL MONITORING FOR PROCESS CONTROL

An underutilized tool for monitoring and verifying HACCP systems is the use of Statistical Process Control (SPC). Implementation of SPC techniques is the most effective way to achieve process control. What is process control? Hubbard (1996) defines it as a process functioning or operating within its optimum level of capability with only common cause (inherent) variation occurring among its manufactured products. The kind of variation and its capability of being controlled can only be determined by the application of applied statistics. Hurst (1996) presented a systematic guide for implementing statistical tools in the monitoring of quality of fresh-cut products that could also be applied to safety issues.

Process control is not a natural state. Most processes do not operate in a state of control. Furthermore, once in control, a process will not remain there. It is not known whether a process is “in” or “out” of control until it is measured using SPC techniques. SPC is a quality management system that uses graphical and statistical tools to analyze, control, and reduce variation within a process. The heart of SPC is the Statistical Control Chart, the specific tool for monitoring process control. Control chart theory is based upon the notion that the parameter being measured, when in statistical control, will vary normally (e.g., only common cause variation) about a central value (ICMSF, 1988). Control chart methodology is the only SPC tool that can distinguish between common-cause (inherent) and special-cause (unnatural) variation in a process. The control chart allows the highlighting of special-cause variation, if present, when monitoring a process. If the special-cause variation source can be found and eliminated in the process, then the process will exhibit only common-cause variation. Only when common-cause variation is present, is the process in a state of “statistical control.” What makes statistical control so important? The essence of statistical control is predictability. A process is predictable when it is in a state of statistical control, and it is unpredictable when it is not in a state of statistical control (Wheeler and Chambers, 1992). In addition to predictability, there are two other sound reasons for putting a process into statistical control (Clements, 1988). The process becomes stable (meaning it is operating normally with only the expected amount of variation), and it becomes capable (meaning the parameter being measured can meet the specification or boundaries within which it is supposed to operate). The most effective way to avoid a safety hazard is to have a process that is stable, capable, and predictable.

INTEGRATING SPC AND HACCP

Both SPC and HACCP focus on process control. The difference between the two is that HACCP focuses on safety issues, while SPC has addressed quality and production control issues. In terms of process control, however, HACCP has two inherent limitations. First, it has historically been implemented as a management tool for

product safety, not for production line process control (Giese, 1999). Second, the HACCP monitoring system takes an attribute approach to safety. The process at which some parameter, like temperature, is being monitored at the CCP (Critical Control Point) is either categorized as “safe” (within critical limit) or “unsafe” (exceeding its critical limit). Attribute charting is not a good warning tool for signaling an impending change in the process leading to a safety hazard in the product. Attribute charts do not detail the relative position of a parameter being monitored within its critical limit. Therefore, they are poor tools for anticipating process changes (Surak et al., 1998).

So, how can HACCP be made a more effective prevention tool for safety hazards in the production process? The key lies in integrating SPC techniques into the HACCP plan. The focus must be on the process not the product. Also, a new type of process control chart must be implemented. Specifically, variable control charts are used. Variable charts are better than attribute charts for monitoring and verifying process control at HACCP CCPs.

Variable charts should be used for quantifying and measuring process output and identifying if this output can remain within statistically defined control limits. More importantly, variable charts can signal the beginning of trouble in a process based on the pattern of points plotted so that action can be taken before a safety hazard has time to become incorporated into the product.

The integration of SPC into an HACCP plan will increase its effectiveness because both systems focus on prevention, both are process-related tools (if variable charts are used), both can be used to document and verify safe product to customers, and both meet regulatory requirements.

SPC MONITORING OF CCPs

Statistical control charting is ideally suited for HACCP monitoring of designated CCPs, because it provides an early warning signal of when to take corrective action before a CCP exceeds its CL. The application of SPC allows a fresh-cut processor to control his CCPs systematically, predictably, and most importantly, demonstrably (Grigg, 1998). To be able to predict future safety of a product to customers and have the statistically valid evidence (documented charts) to back up your statements is a powerful marketing tool.

It is important to note, however, that process control may not be HACCP control. If the common-cause variation of the parameter monitoring a CCP is too great, the process may exceed the critical limit. Thus, a process in statistical control may not be capable of producing a safe product. Likewise, the parameter monitoring a CCP may be within the CL but not in statistical control. In fact, any one of four scenarios may exist, as demonstrated in Figure 4.7.

There is a major difference in the manner of setting SPC limits vs. HACCP limits. SPC limits are set based on the standard deviation of the statistic plotted from the data when the state of the process is assumed to be in statistical control. In other words, the width of the SPC control limits are based on the common-cause variation exhibited by the process. In contrast, HACCP critical limits must be set based on valid research evidence that has demonstrated boundaries of safety for the statistic being plotted.

Applying SPC to Monitor HACCP CCPs

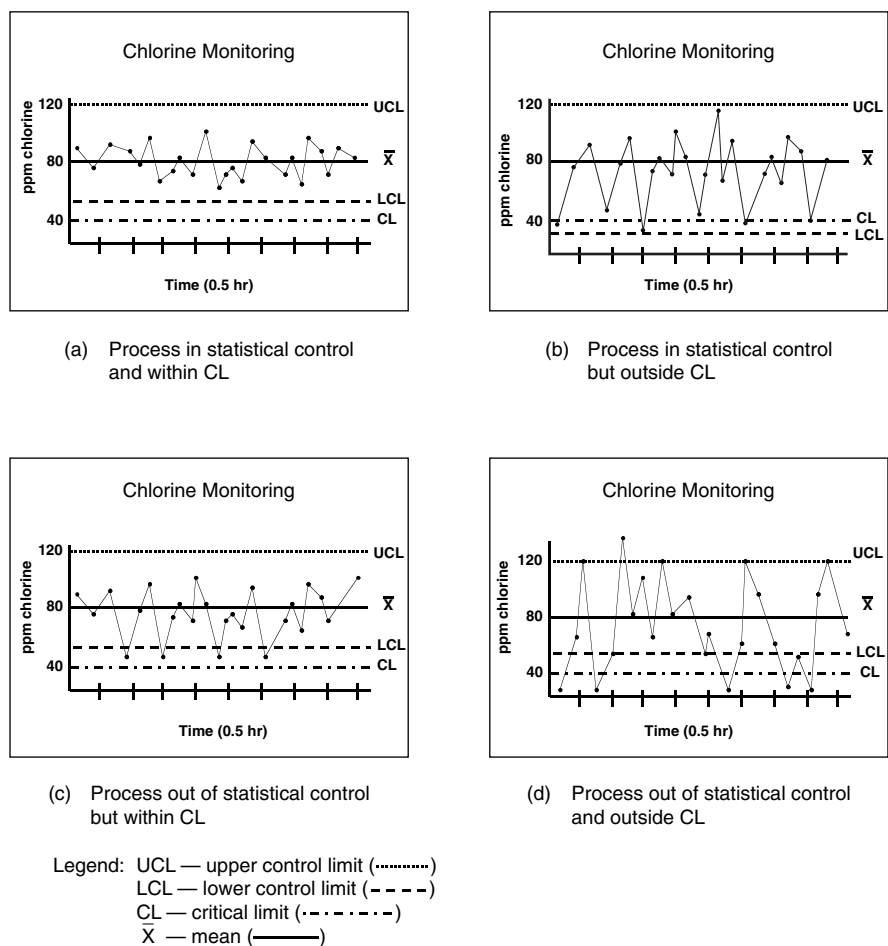


FIGURE 4.7 Chlorine monitoring of wash water using SPC and HACCP methodology.

SPC limits must have less variability than HACCP limits (Van Schothorst and Jongeneel, 1994).

It must be remembered that any statistical chart that relies on plotted data averages may obscure extreme values that could pose a health hazard (ICMSF, 1988). While plotted averages for a CCP may be within critical limits, individual values may be above or below the CL for safety. For this reason, it is recommended to first monitor CCPs using individual values plotted on individual/moving range charts (Surak et al., 1998) to be certain they can remain within their predetermined CLs. Once process stability has been achieved, then one can proceed to construct average/range charts. These are better indicators of any process shift that may occur for a CCP within the CL.

ESTABLISH CORRECTIVE ACTIONS (PRINCIPLE 5)

Although HACCP is designed as a preventative strategy to hazards entering the process, ideal conditions do not always prevail. There may be deviations from CLs, and corrective actions may be needed. By definition, a corrective action is taken when there is a CL deviation identified by monitoring a CCP (NACMCF, 1998). Tompkin (1992) pointed out that corrective action involves four activities:

1. Bring the process back into its CL through process adjustment
2. Determine and correct the cause of the deviation
3. Determine the disposition of the noncompliant product
4. Record the corrective action taken and the disposition of the noncompliant product

When CLs are violated at a CCP, *predetermined* (developed in advance for each CCP and included in HACCP plan) corrective action procedures must be instituted. The goal is to adjust the process on the spot to minimize the amount of noncompliant product and determine the safe disposition of the affected product (Lockwood et al., 1998).

The great diversity in fresh-cut products and the variation in equipment, type of processing, raw material quality, etc., require that specific corrective actions be developed for each CCP according to the parameters of the processing. Examples of corrective actions to be taken if the CL for chlorine in water fails can be found in Table 4.8.

Individuals who have a thorough understanding of the product, process, and HACCP plan should be assigned responsibility for writing and overseeing that the corrective actions are implemented (Bernard, 1997). At a minimum, the HACCP plan must specify what is done when a deviation occurs, who is responsible for taking corrective action, and what happens to the noncompliant product. Detailed accurate records must document all of these actions and procedures.

ESTABLISH VERIFICATION PROCEDURES (PRINCIPLE 6)

The purpose of the verification step is to confirm through documentation that the HACCP plan is followed as designed and implemented. The verification step provides assurance that the HACCP program is achieving the established objective of food safety (Prince, 1992) and that the plan will operate consistently on a day-to-day basis. Verification is defined as those activities, other than monitoring, that determine the validity of the HACCP plan and determine that the system is operating according to the plan (NACMCF, 1998).

An inherent aspect of verification is the initial validation of the HACCP plan. Before implementing HACCP, the HACCP team must review the plan to determine that it is accurate in all details. Validation, as defined by Stevenson and Gombas (1999), is that element of verification that focuses on collecting and evaluating scientific and technical information to determine if the HACCP plan will effectively control the hazards.

According to Hudak-Roos (1999), verification is a daily activity that should answer the following question: Are your activities in compliance with the written/implemented HACCP plan? In contrast, validation is a periodic function performed every six months to a year. It should answer the question: where is the scientific evidence that the HACCP plan has been properly designed? Simply put, validation is the proof that the intended result can be achieved. It is evidence of process capability (Sperber, 1999).

SPC VALIDATION/VERIFICATION OF A PROCESS

For each CCP in the HACCP plan, there will be a need to validate that, under normal operating conditions, the process can be maintained within its CL. In other words, is the process verifiable? An ideal way of assessing whether a process is capable of remaining within specified limits is to use statistical analysis. Statistical validation of a process to determine the probability (confidence) of its ability to stay within specified (critical) limits is known as establishing its process capability (Mortimore and Wallace, 1994). Remember, all processes should be validated before the HACCP plan is implemented. Conducting a process capability analysis accomplishes two goals (see Figure 4.8). First, can the process be validated so that it is capable of achieving the Critical Limits (CLs) that have been established? Second, can the process be verified so that it is capable of being controlled? Can it realistically remain within the CLs on a day-to-day basis? A process capability study validates whether or not CCP control is achievable and verifies whether or not the process can remain consistent. The statistical format for calculating process capabilities has been reviewed by Hubbard (1996) and is graphically demonstrated in Figure 4.8.

Each HACCP plan will include verification procedures for individual CCPs. The major verification activities include plant audits, calibration of instruments/equipment, targeted sampling and microbiological testing, and HACCP records review (Lockwood et al., 1998).

Audits are conducted by an unbiased person not responsible for performing monitoring activities. The objective is to compare actual practices with what is written in the HACCP plan. Audits can be performed by a member of the HACCP team, plant management, outside experts or consultants, regulatory agencies, and customers.

Calibration of instruments and equipment used to monitor CCPs is extremely important. If the monitoring devices are out of calibration, then results will not be accurate. Significant deviations might go unnoticed, creating a potential health hazard. If this happens, the CCP could be considered out of control because the last documented acceptable calibration. This situation must be considered when establishing monitoring frequency.

Verification may also include targeted sampling and microbiological testing. Vendor compliance can be checked by targeted sampling when receipt of material is a CCP and purchase specifications are relied on as control limits. Microbiological testing can be used as a verification tool to determine if the overall operation is under control.

All records connected with the above activities must be reviewed. Especially important are CCP records; deviation, corrective action, and disposition records; calibration records; and microbiological test results. Verification should be performed

Process Capability Analysis

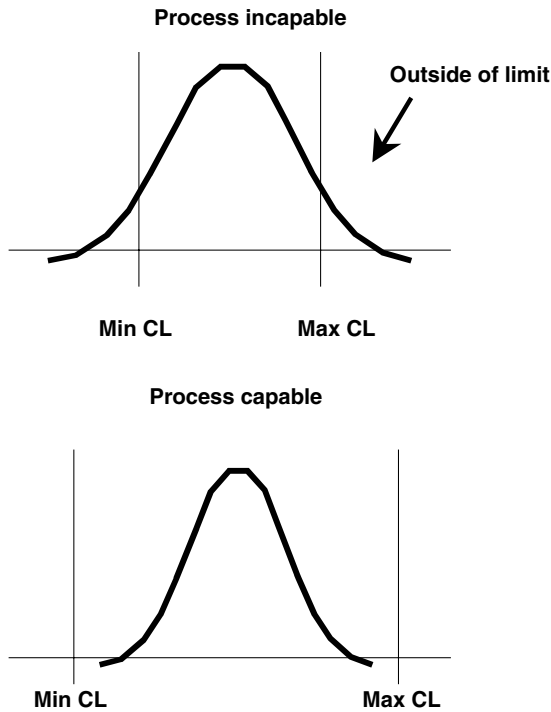


FIGURE 4.8 Graphic display of an incapable vs. capable process.

whenever there are indications that a process is unstable or out of control or whenever there is a change in product or processing equipment. HACCP plants should be revalidated on a periodic basis, even if no significant changes have occurred in the process. In this way, the plan will retain its support base (Bernard, 1997). Examples of verification activities for fresh-cut lettuce at the water flume wash step are found in Table 4.8.

ESTABLISH RECORD-KEEPING PROCEDURES (PRINCIPLE 7)

Accurate record keeping is an essential part of any successful HACCP program. Record keeping assures that there is written evidence of all HACCP activities that have occurred in the plant. HACCP records should be kept in a file separate from quality control records so that only product safety records are reviewed during HACCP audits. This master file should contain a record of the deliberations (meetings) of the HACCP team and documentation for all aspects of the plan. It should include all HACCP records that have been generated, including justification for the setting of critical limits, details on sampling and monitoring procedures, methods of analysis,

corrective actions taken, product dispositions, details on all prerequisite SOPs, examples of all forms, and procedures and reviews of the HACCP plan (Bernard, 1997). The FDA requires that HACCP records be kept on file for at least one year from the date of production for refrigerated foods (e.g., fresh-cut produce).

Although record keeping may appear to be a burden, there are some sound reasons for this activity, which benefit the processor. First, records provide documentation that CCPs comply within their CLs to insure product safety. Records are the only reference the processor has available for tracing his product once it is distributed in the marketplace. Records provide a monitoring tool so that process adjustments can be made before there is loss of process control. Records are the necessary data needed for regulatory compliance and HACCP auditing by regulator and customers. Records provide irrefutable evidence that proper procedures/processes were followed in strict accordance with HACCP requirements (Wedding and Stevenson, 1999).

Record keeping includes records that go beyond those that are manufactured on a day-to-day basis. NACMCF (1998) endorses the maintenance of four types of records:

1. Summary of the hazard analysis—including records on the HACCP team's deliberations on the rationale for determining hazards and control measures
2. The HACCP plan for each product—including records on the product description, distribution and end use, verified flow diagram, and all HACCP plan summaries addressing the seven required components
3. Support documentation—CCP records, CL records monitoring and corrective action records, and verification and validation records
4. Daily operational records—including records generated daily and which really control the HACCP process for each CCP (Specifically, these include monitoring, corrective action, and verification.)

Information to be found on various HACCP records can be quite diverse. For example, the FDA requires all monitoring records to have the following:

- title of form
- name/location of processor
- date/time of activity
- product identification (including product type, package size, processing lie, product code where appropriate)
- critical limits
- actual observations/measurements
- operator's signature or initials
- reviewer's signature or initials
- date of the review

An example of a fresh-cut HACCP form for monitoring chlorine/pH levels in tomato dump tank water is illustrated in Figure 4.9. Specific records to validate and verify CCP control at the water flume wash step of a fresh-cut lettuce operation are found in Table 4.8.

Chlorine/pH Daily Monitoring Log
S & W Tomato House
Donaldsonville, Georgia

Processing Line: Tomato grading line
Specific Location: Water in dump tank
Critical Limits: Chlorine 150–200 ppm/pH 6.5–7.5

Date	Time	Total Chlorine			pH		Operator Initials
		7:15 am	9:30 am	12:15 pm	7:15 am	12:15 pm	

Date Reviewed: _____ Verified: _____

FIGURE 4.9 Example of HACCP monitoring record.

SUMMARY

To meet the challenges of today's food safety issues, the FDA has increased its emphasis toward programs that are proactive and prevention oriented. The most comprehensive, science-based program to date for reducing pathogen contamination in fresh-cut products is HACCP. The HACCP approach focuses on controlling pathogens at their source rather than trying to detect them in finished products. Final product testing is futile, because by this time, pathogens have had the opportunity to cross-contaminate many plant areas and fresh-cut products.

The International Fresh-cut Produce Association (IFPA), which is the trade association for over 530 fresh-cut processors and suppliers in 25 countries, has been very active in providing food safety information to its membership. It has published food safety guidelines, designed HACCP models for implementation in fresh-cut operations, created a yearly technical seminar on current topics, and established a two- to three-day HACCP workshop for its members. Here, participants learn the mechanics of putting plant-specific HACCP programs together. According to a 1997 IFPA membership survey, 61% of the respondents had a written, implemented, verifiable HACCP program in place (DeRoeve, 1999).

Although useful in preventing and reducing the failure rate at CCPs, the HACCP program has an inherent weakness. It gives no advanced warning as to when a CCP has a high probability of exceeding its CL, thus going out of control. The HACCP program's reliability and effectiveness as a prevention tool can be greatly strengthened by the incorporation of statistical techniques. SPC can provide an objective, statistically valid means of predicting CCP control during monitoring and verification activities. Integration of SPC into an HACCP program can only help to validate and verify HACCP performance.

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5 Physiology of Fresh-cut Fruits and Vegetables

Peter M. A. Toivonen and Jennifer R. DeEll

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INTRODUCTION

There have been several reviews on the physiology of fresh-cut (minimally processed) fruits and vegetables (Rolle and Chism, 1987; Watada et al., 1990; Brecht, 1995; Watada et al., 1996). This chapter is devoted to the integration of the principles presented in these reviews and recently published information on fresh-cut fruits and vegetables.

The fundamental principle underlying quality of fresh-cut fruits and vegetables is that they are living tissues, and as a consequence, show physiological response

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to minimal processing procedures as well to post-processing handling and treatments and ultimately to the package environment in which they are enclosed. In addition, the intrinsic physiology and quality of the raw produce will have an influence on the response to minimal processing and packaging. Microbial growth is influenced by the physiology of the minimally processed product, and thus, must also be considered. Physiological processes leading to tissue senescence and deterioration can be minimized or modulated through the implementation of an integrated approach involving proper cultivar selection, pre-harvest management, pre- and post-processing treatments, and application of appropriate packaging that provides optimal atmospheres. All of these issues will be discussed in detail, and a summary on the consequences to quality will be presented.

PHYSIOLOGICAL EFFECTS OF CUTTING ON TISSUES

ETHYLENE PRODUCTION

Wounding of plant tissues has long been known to induce ethylene production, and the time line for the initiation of this response can range anywhere from a few minutes to an hour after wounding, with maximal rates being produced between 6–12 h (Abeles et al., 1992) (Figure 5.1). The potential effects of wound ethylene are dependent on the type and physiology of the tissue in question. Large increases in ethylene production, as a consequence of cutting, have been shown in kiwifruit (*Actinidia deliciosa* L.) (Watada et al., 1990; Agar et al., 1999), tomato (*Lycopersicon esculentum* Mill.) (Lee et al., 1970; Mencarelli et al., 1989; Abeles et al., 1992; Brecht, 1995; Artés et al., 1999), winter squash (*Cucurbita maxima* Duch.) (Abeles et al., 1992), papaya (Paull and Chen, 1997), and strawberry (*Fragaria x ananassa*

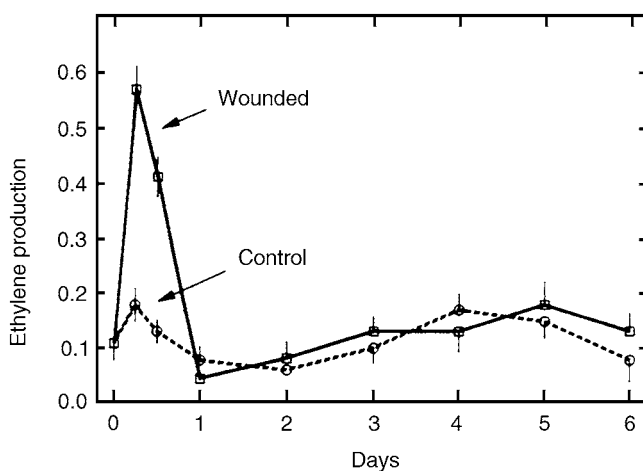


FIGURE 5.1 Effect of wounding on the rate of ethylene production [$\text{nl (g FW)}^{-1} \text{h}^{-1}$] from lettuce tissue. All lettuce tissues were incubated at 5°C . The vertical error bars represent SE of the mean. (From Ke and Saltveit 1989, used with permission from Munksgaard International Publishers.)

Duchesne) (Rosen and Kader, 1989). However, there are some products that respond differently to wounding. For example, pear (*Pyrus communis* L.) does not show an increase in ethylene production in response to cutting (Gorny et al., 2000). In another report, cut pear slices were found to have lower ethylene production as compared with whole fruit (Rosen and Kader, 1989).

A few contradictory results regarding the effects of wounding on ethylene production have been reported in bananas (*Musa* spp. AAA) and cantaloupe (*Cucumis melo* L., var. *Reticulatus*). Slicing of banana has been shown to increase ethylene production in one case (Abe et al., 1998), while no increase in ethylene production was observed in another (Watada et al., 1990). The contradictory results may be explained by the fact that maturity of the bananas was different in the two studies; in the first study, they were at the green tip stage, and in the second study, they were at a full yellow, post-climacteric stage at the time of cutting. Cutting of cantaloupe in one case (Hoffman and Yang, 1982) resulted in increased ethylene production, but in another study, resulted in a reduction of ethylene production (Luna-Guzmán et al., 1999). Again, the difference can be explained by the fact that wound-induced ethylene production is influenced by fruit maturity; in the first case, the fruits were cut in the pre-climacteric phase, whereas in the second case, the fruits were cut in the post-climacteric phase, when tissue capacity to produce ethylene has declined (Luna-Guzmán et al., 1999). Therefore, maturity of the product (especially for climacteric fruit) must be considered in understanding the effect of cutting on ethylene production.

Ethylene production is localized to tissue in the close vicinity of the wound or cutting injury. It has been shown that increase in ethylene production was limited to within a few millimeters of the cut surface in sliced bananas (Dominguez and Vendrell, 1993). The importance of secondary responses of tissues adjacent to the cut cells has been only partially documented (Rolle and Chism, 1987), but this may explain the localization of such phenomena as cut surface browning.

Storage temperature also has an effect on wound-induced ethylene production (Figure 5.2). It has been shown that storage of cantaloupe pieces at 0–2.5°C will almost completely suppress wound-induced ethylene as compared to higher storage temperatures (Madrid and Cantwell, 1993). Similar reductions in ethylene production for other cut fruits and vegetables would be expected at low post-cutting storage temperatures.

RESPIRATION

Wounding results in increases in respiration, but the initiation of this response is delayed compared to that found for wound-induced ethylene (Brecht, 1995). Increases in respiration in response to cutting may depend on the commodity under consideration, since bananas do not appear to show an increase but kiwifruit do (Figure 5.3). The increase in respiration has been assumed to be due to enhanced aerobic mitochondrial respiration. This assumption is supported by the fact that changes in mitochondrial structure and increases in their numbers and function have been shown to be induced by wounding (Asahi, 1978).

Respiration rate is associated with product shelf life potential, with high rates of respiration being correlated with short shelf life (Kader, 1987). Therefore, it has

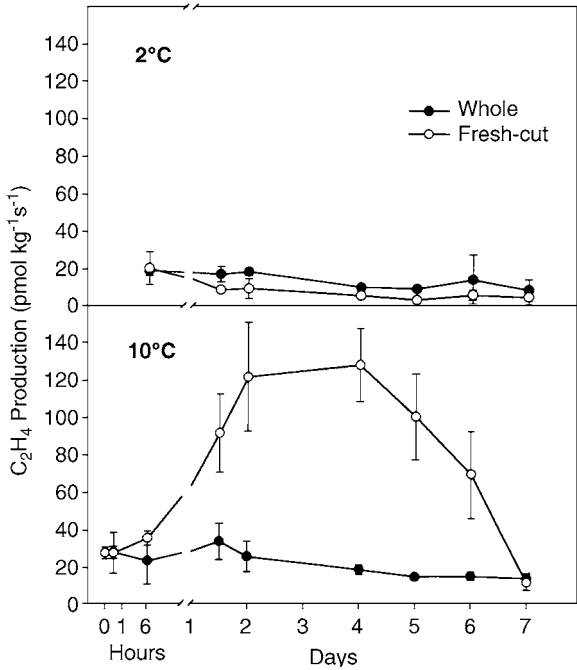


FIGURE 5.2 C_2H_4 production of whole and fresh-cut ‘Durinta’ tomato at 2 and 10°C during the first hours after slicing and up to seven days. Each point represents the mean of six whole fruits or six fresh-cut fruits. Vertical lines represent S.D.s. (Reprinted from Artés et al. 1999, *Postharvest Biology and Technology*, Vol. 17, pp. 153–162, with permission from Elsevier Science.)

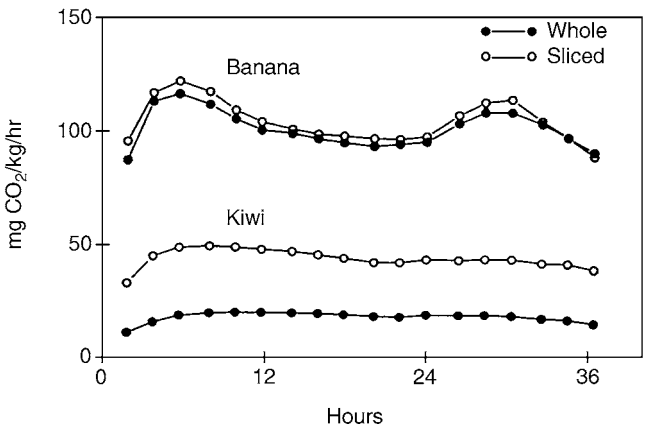


FIGURE 5.3 Respiration rates of sliced and whole banana and kiwifruit held at 20°C. Banana is sliced to 4-cm length sections and kiwifruit to 1-cm thick slices. (From Watada et al. 1990, used with permission from the Institute of Food Technologists.)

been assumed that increases in respiration brought about by cutting are expected to result in shorter shelf life (Rolle and Chism, 1987). A wide range of fresh-cut products shows significant increases in respiration, and generally, this effect is only seen when the cut product is stored at higher temperatures (Watada et al., 1996). There are some exceptions—pear and strawberry slices have been shown to respire at a greater rate than whole fruits at both 2.5 and 20°C (Rosen and Kader, 1989). Gorny et al. (2000) did not find an immediate effect of cutting on respiration of pear, but respiration rates in slices rose after six days at 10°C. The delayed increase in respiration in response to cutting may have been related to the growth of microorganisms on the cut slices, as has been suggested in other fresh-cut products (Varoquaux et al., 1996a).

Increases in respiration in response to cutting may be quite substantial in some cases. Slicing of mature green tomatoes results in increased respiration by up to 40% when stored at 8°C, as compared to intact product (Mencarelli et al., 1989). Many other fruits and vegetables show such large increases in respiration, mainly when stored at higher temperatures (Watada et al., 1996) (see Figures 5.4 and 5.5).

The basis for the rise in respiration may not always be completely explained by an enhancement in aerobic respiration. It has been demonstrated in cut potatoes that the rise in respiration after cutting or wounding is at least partially a result of α -oxidation of long-chain fatty acids (Martin and Stumpf, 1959; Laties, 1964;

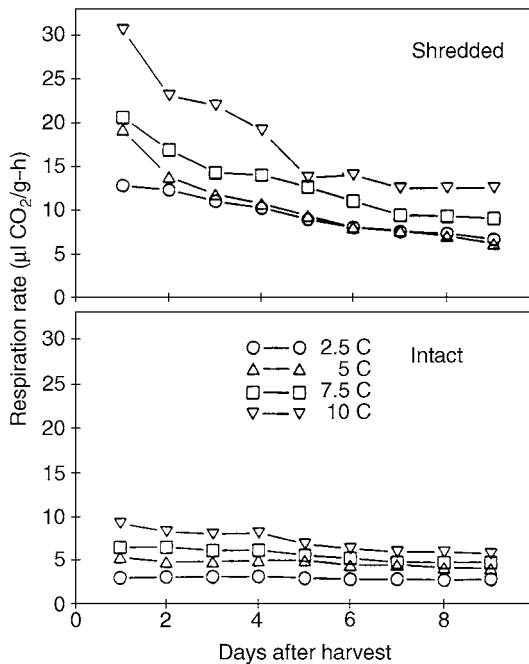


FIGURE 5.4 Respiration rates of intact and shredded cabbage stored at 2.5°C (36.5°F), 5°C (41°F), 7.5°C (45°F), and 10°C (50°F). The intact heads of cabbage had been harvested, cooled, and processed the same day. (From Cantwell 1992, used with permission from the University of California Board of Regents.)

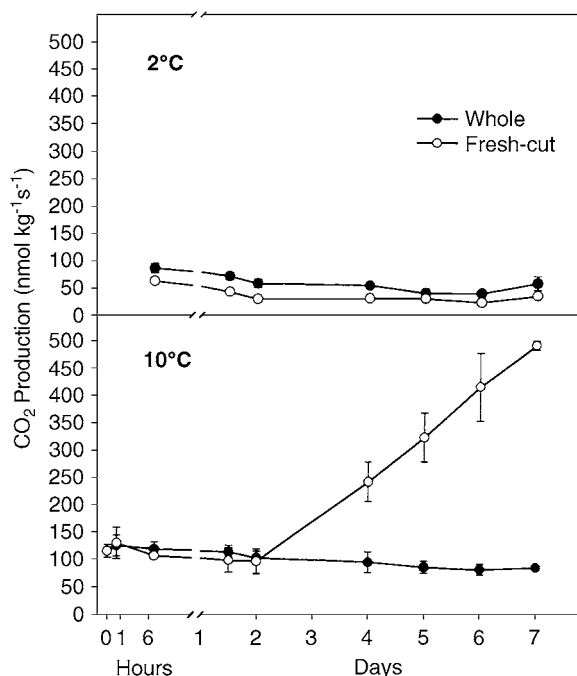


FIGURE 5.5 Respiration rate of whole and fresh-cut 'Durinta' tomato at 2 and 10°C during the first hours after slicing and up to seven days. Each point represents the mean of six whole fruits or six fresh-cut fruits. Vertical lines represent S.D.s. (Reprinted from Artés et al. 1999, *Postharvest Biology and Technology*, Vol. 17, pp. 153–162, with permission from Elsevier Science.)

Laties et al., 1972). Increases in O₂ consumption associated with α -oxidation coincide with membrane deteriorative processes, which will be described in a separate discussion on membrane deterioration.

Increases in respiration in response to cutting might be expected to be at least partially explained by removal of barriers (i.e., periderm or cuticle) to gas exchange in the tissues. However, it has been shown in apples that minimal processing does not contribute to increased respiration due to reduction of resistance to diffusion for O₂ (Lakakul et al., 1999). Therefore, the physical removal of gas diffusion barriers may not be an important factor in the physiological response to cutting.

Another aspect of respiration and minimal processing is that of susceptibility to anaerobic metabolism. When cut product is placed into modified atmosphere packaging, it is exposed to high CO₂ and/or low O₂, and the sensitivity of cut product to modified atmospheres may be quite different than for whole product. Cut lettuce tissues are less susceptible to developing fermentative metabolism than whole heads when exposed to high CO₂ (Mateos et al., 1993). In contrast, shredded carrots are more susceptible to developing anaerobic metabolism than whole carrots, and this may be associated with the tissues' requirement to supply the ATP requirements to ensure cell survival (Rolle and Chism, 1987).

The level of anaerobic metabolism is determined by the O₂ threshold for anaerobic metabolism induction, as well as the handling temperature of the fruit or vegetable product (Lakakul et al., 1999). The target atmosphere for real-life distribution must reflect a potential for exposure to non-ideal temperatures, because package O₂ atmospheres are generally designed to be low at ideal temperatures (e.g., 0°C). If the packages are exposed to higher temperatures during distribution, there is a risk of anaerobic metabolite accumulation. The risk of off-flavors in response to anaerobic metabolite accumulation is high in some fruit cultivars, whereas in others, the risk is much lower (Ke et al., 1991). In some commodities, such as iceberg lettuce, where a flavored dressing is used by the consumer of the product, some accumulations of anaerobic metabolites might be tolerated (Smyth et al., 1998).

MEMBRANE DETERIORATION

Membrane deterioration results in decompartmentation of cellular structure and organization and loss of normal cellular function. Many secondary events are a consequence of membrane deterioration, the most commonly recognized being tissue browning (Rolle and Chism, 1987; Brecht, 1995). Another is the development of off-odors (Brecht, 1995). Wounding of tissues can result in relatively rapid deterioration in membranes, and this has been associated with oxygen-free radical production in response to wounding (Thompson et al., 1987). In potato, wounding has been shown to rapidly cause membrane lipid breakdown (Galliard, 1970). Wounding has also been shown to result in enzymatic degradation of membrane components. Induction of lipid acyl hydrolase (Wardale and Galliard, 1977) and phospholipase D (Galliard, 1979) activities result in the production of free fatty acid from membrane lipids. These liberated fatty acids can disrupt the cellular function via direct lysis of organelles and through binding to and subsequent inactivation of proteins (Galliard, 1979). The free fatty acids are also subject to oxidation via either α -oxidation (Galliard and Matthews, 1976; Laties et al., 1972) or lipoxygenase activity (Galliard and Phillips, 1976). Wound respiration has been at least partially attributed to the α -oxidation of fatty acids in potato tissues (Laties and Hoelle, 1967). Ethylene production as a consequence of metabolism of free fatty acids by lipoxygenase has been demonstrated in tomato (Sheng et al., 2000), suggesting that the wound-induced membrane breakdown may be associated with wound-induced ethylene production. However, not all fruits and vegetables show a wound-induced membrane lipid breakdown, with carrot, avocado, and banana being notable examples (Theologis and Laties, 1980).

SECONDARY METABOLITE ACCUMULATION

Phenolic accumulation is one of the most studied phenomena in response to wounding. Wounding has two effects on phenolic metabolism (Rhodes and Woollorton, 1978). The first is the oxidation of endogenous phenolics as a consequence of cell membrane breakdown, allowing the mixing of the phenolics with oxidative enzyme systems, which are normally separated by membranes. The second is the stimulation of cells adjacent to the injury to produce more phenolics in an attempt to initiate repair processes (i.e., lignification). Phenolic accumulation is initiated via increased activities in phenylalanine

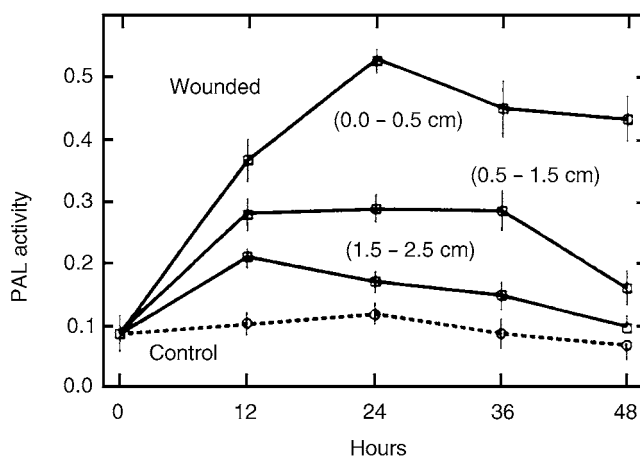


FIGURE 5.6 Changes in wound-induced PAL activity [$\mu\text{mol (g FW)}^{-1} \text{h}^{-1}$] as affected by distance from the wound. The numbers in the parentheses represent the distance from the wounded surface. The vertical error bars represent SE of the mean. (From Ke and Saltveit 1989, used with permission from Munksgaard International Publishers.)

ammonia lyase activity, and wounding has been clearly shown to increase the activity of this enzyme in iceberg lettuce (Figure 5.6). Chlorogenic acid can also accumulate in shredded carrots when stored in air (Babic et al., 1993). Isocoumarin levels increase in response to ethylene exposure, cutting, or bruising in carrots (Lafuente et al., 1996). This response is not seen in cut-and-peel ('baby') carrots though, likely due to the fact that most of the phenolic metabolism is localized in the peel tissue (Sarkar and Phan, 1979), which is removed in the cut-and-peel process. However, if carrots are bruised or exposed to ethylene prior to processing, then isocoumarin accumulations will occur, affecting quality of cut-and-peel product. Wounding increased phenolic acids and anthocyanins in midrib tissues of red pigmented lettuce; however, there was no significant effect in the green and red tissues (Ferrerres et al., 1997). Onion tissue, when disrupted, will enzymatically produce a bitter compound (either a triterpenoid or flavonoid) over time, and its accumulation can be controlled by acidification (Schwimmer, 1967). Precursors to a pink pigment accumulate in response to wound-induced alliinase activity (Shannon et al., 1967). These precursors react with free amino acids and carbonyls to develop a pink pigment in cut onion tissues.

Sulfur-containing compounds can also accumulate in response to wounding. Several unpleasant sulfur compounds can increase with time after cutting in cabbage tissues, such as methanethiol and dimethyl disulfide (Chin and Lindsay, 1993). The accumulation of such compounds is known to be associated with membrane deterioration. The loss of cellular compartmentation allows enzymes such as cysteine sulfoxide lyase to come into contact with various sulfur-containing substrates and oxidize them into these unpleasant sulfur volatiles (Dan et al., 1997). Allyl isothiocyanates can also accumulate in response to cutting in shredded cabbage (Yano et al., 1986).

Tissue disruption of green bell peppers results in the rapid production of six-carbon aldehydes and alcohols, which are a consequence of the oxidation of free

fatty acids that have been enzymatically (by lipoxygenase and hydroperoxide lyase) released from membranes (Wu and Liou, 1986). Some of these compounds are associated with off-flavors in peppers.

Accumulation of long-chain aliphatic compounds (fatty acids or alcohols) that comprise suberin polymers occurs in response to wounding in tomato fruit (*Lycopersicon esculentum* Mill.) and bean pods (*Phaseolus vulgaris* L.) (Dean and Kolattukudy, 1976). The production of suberin is an important part of the process leading to wound healing.

WATER LOSS

Water loss in fruits and vegetables is determined by many factors, probably the most important being the resistance of outer periderm or cuticle to transpirational movement of water vapor (Ben-Yehoshua, 1987). However, peeling and cutting result in reduction or elimination of the resistance by these barriers to transpiration. Two issues are important to water loss: reduction of tissue bulk, i.e., increase in surface area to volume ratio, and the removal of protective periderm tissues. Both mechanisms for resultant increases in water loss, are demonstrated by the fact that slicing of kiwifruit results in increased rates of water loss, and subsequent peel removal from the slices results in a further increase in weight loss (Figure 5.7). Increased rates of water loss result in greater susceptibility to wilting and/or shriveling. Slicing of pears resulted in high rates of moisture loss from cut surfaces, which was an important factor in quality loss (Gorny et al., 2000). Peeled ‘Majestic’ potatoes have a water loss of $3.3\text{--}3.9\text{ mg H}_2\text{O cm}^{-2}\text{ mbar wpd}^{-1}\text{ h}^{-1}$, while nonpeeled, cured potatoes have a moisture loss rate of $0.007\text{ mg H}_2\text{O cm}^{-2}\text{ mbar wpd}^{-1}\text{ h}^{-1}$ (Ben-Yehoshua, 1987).

The peeling method of carrots influences the water loss of subsequently processed fresh slices. Coarse abrasion peeling results in three times greater weight loss of

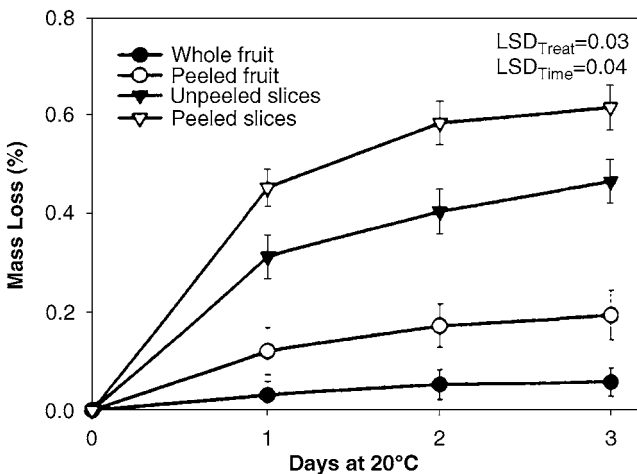


FIGURE 5.7 Effect of wounding on mass loss of whole kiwifruit, whole-peeled kiwifruit, peeled slices, and unpeeled slices stored at 20°C for three days. (From Agar et al. 1999, used with permission from the Institute of Food Technologists.)

packaged slices as compared to slices made from hand-peeled carrots (Barry-Ryan and O'Beirne, 2000). Slices made from carrots that had been abrasion-peeled using a fine peeling plate had intermediate levels of water loss. The slicing process can also influence the water loss in carrot slices, with machine-sliced product losing water 30% faster than manually razor-sliced product (Barry-Ryan and O'Beirne, 1998).

SUSCEPTIBILITY TO MICROBIOLOGICAL SPOILAGE

A wide variety of microorganisms have been found to be actively growing on packaged, minimally processed fruits and vegetables, and these include mesophilic bacteria, lactic acid bacteria, coliforms (some fecal in origin), and yeasts and molds (Nguyen-The and Carlin, 1994). Increases in microbial populations on minimally processed products are often associated with apparent increases in respiration rates with time in storage (Figure 5.8). Tissue decay is closely associated with microbial activity (aerobic and lactic bacteria). Spoilage of packaged bean sprouts was associated with the reduction of O_2 levels in the package, and the accumulation of acetate and lactate linked the spoilage with microbial activity (Varoquaux et al., 1996a). Many microorganisms

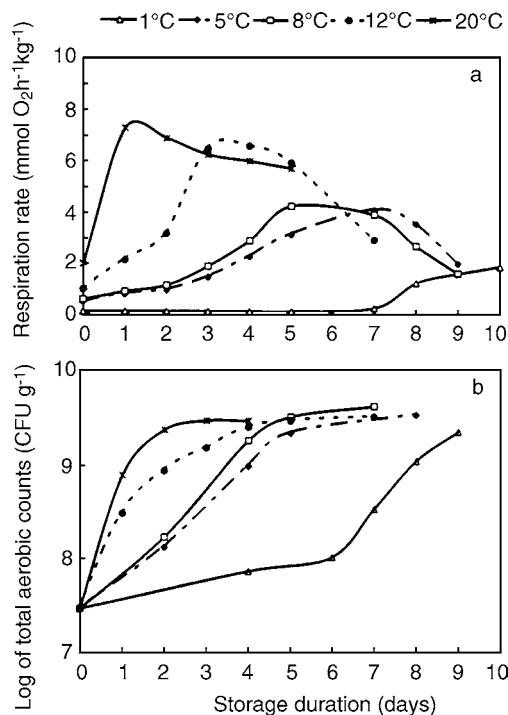


FIGURE 5.8 Changes in (a) oxygen uptake rates and (b) total aerobic counts during storage of bean sprouts at various temperatures. Results are expressed as $\text{mmol h}^{-1} \text{kg}^{-1}$ and cfu g^{-1} of fresh weight, respectively. (From Varoquaux et al. 1996a, Copyright Society of Chemical Industry. Reproduced with permission. Permission is granted by John Wiley & Sons Ltd. on behalf of the SCI.)

produce pectin degrading enzymes, which lead to tissue softening and breakdown. However, relatively high microbial counts can be found on what would be considered acceptable quality product (Watada et al., 1996). In some products, microbiological spoilage does not appear to be of great concern. For example, it has been reported that packaged minimally processed Chinese cabbage does not show any microbial spoilage even after 21 days of storage at 5°C (Kim and Klieber, 1997).

The largest populations of microorganisms are found within broken cells or in tissues adjacent to broken cells after packaged fresh-cut product has been stored (Watada et al., 1996). Presumably, the damaged tissue and broken cells provide nutrients and a protected environment for growth of most types of microflora. This is demonstrated by the fact that microbial growth is much greater on minimally processed product as compared with intact product. For example, cut lettuce has generally higher microbial count than intact heads (Priepke et al., 1976). In addition, higher populations of microbes have been associated with faster rates of tissue decay in minimally processed product (King and Bolin, 1989; King et al., 1991).

The tissue from which the minimally processed product is derived can determine the rate of growth of microbial population. Inner (younger) leaves of endive have smaller populations of microbes, which might be explained by the effect of protective outer leaves (Jacques and Morris, 1995). However, the growth of bacteria inoculated on these inner leaves is inhibited as compared with outer (older) leaves. This suggests that there is a physiological basis for the resistance of such tissue to bacterial growth.

FACTORS AFFECTING RESPONSE TO CUTTING

CULTIVAR

Romig (1995) discussed the importance of the appropriate selection and development of fruit and vegetable cultivars specifically for use in fresh-cut products. The effect of cultivar selection on the raw product initial physiology and quality was considered to have a subsequent impact on the acceptability of a packaged, fresh-cut fruit or vegetable product in the retail market. In a few cases, traits that could be improved through conventional breeding and/or genetic transformation have been selected for certain vegetables (Romig, 1995). However, the literature does not show any specific successes in this area. There have been numerous reports regarding identification of cultivar differences, and these will be dealt with in the following discussion.

Different cabbage cultivars will produce different levels of various sulfur volatiles in response to injury (Chin and Lindsay, 1993). Some cultivars produce more dimethyl disulfide and dimethyl trisulfide in response to cutting, and these compounds are considered unpleasant. This information would, therefore, suggest that cultivars that produce large amounts of these volatiles on cutting should not be used for fresh cabbage salads. Different cabbage cultivars also produce different levels of allyl isothiocyanates, and their accumulation accounts for reductions in rates of browning and ethylene production of the shredded product (Yano et al., 1986; Nagata, 1996).

There are no differences in wound-induced phenolic accumulation and browning of 'Baby' and 'Romaine' lettuces (Castañer et al., 1999). In butterhead lettuce, the cultivar 'Ritmo' produced higher CO₂ concentrations in 30 µm thick polypropylene

packages than 'Musette' or 'Nancy', and it was also more susceptible to tissue injury in the package in response to high CO₂, resulting in greater levels of tissue browning (Varoquaux et al., 1996b). Minimally processed 'Calmar' and 'Sea Green' iceberg lettuces were the least susceptible to browning out of eight tested, and 'Nerone' was the most susceptible after six days of storage at 5°C (Couture et al., 1993). There are also differences in wounding-induced accumulations of phenolics that are dependent on cultivar for shredded carrot (Babic et al., 1993).

No single apple cultivar tested in New York state showed an overall better suitability for use as packaged fresh slices. The numbered cultivar 'NY 674' developed the least browning of all 12 tested, however, it was the least firm of all the cultivars (Kim et al., 1993b). In contrast, 'Liberty' was the firmest cultivar, but it was susceptible to cut-surface browning. It was concluded that 'NY 674', 'Cortland', 'Golden Delicious', 'Empire', and 'Delicious' were considered to be acceptable for fresh slices, whereas 'Mutsu' and 'Rome' were poorly suited for this purpose. There were differences in respiration in slices made from the 12 cultivars, but these were not related to suitability for packaged, fresh slices (Kim et al., 1993b).

There is limited evidence that there are cultivar differences in rates of membrane deterioration in response to minimal processing. Membrane deterioration in shredded carrots in response to the shredding process is dependent on cultivar, with 'Caropak' showing greater rates of membrane deterioration than 'Apache' (Picchioni and Watada, 1998).

The accumulation of fermentation products in response to high CO₂ can be influenced by the cultivar of the fruit or vegetable under consideration. High CO₂ atmospheres in package atmospheres have been suggested as a good approach for preserving fresh-cut strawberry slice quality (Rosen and Kader, 1989). However, it has been found that there is a great variability in response of strawberry cultivars to high CO₂ (Watkins et al., 1999). In addition, Watkins et al. (1999) found that the different cultivars showed different degrees of firmness improvement under high CO₂ atmospheres. Similar differences in response of packaged raspberry cultivars to package CO₂ levels have been demonstrated (Toivonen et al., 2000).

There are large differences in shelf life potential for peach and nectarine slices made from different cultivars (Gorny et al., 1999). It is assumed that these differences relate to differences in ripening-related physiology of the cultivars.

Susceptibility to microbial spoilage can also be influenced by cultivar. The shelf life of different cultivars of packaged spinach was found to be dependent on the rainfall conditions during production (Johnson et al., 1989). The cultivars 'Seven R' and 'Grandstand' had the best shelf life under normal rainfall conditions, whereas 'Gladiator' and 'Melody' had the best shelf life under high rainfall conditions. The differences in shelf life were primarily associated with differences in susceptibility to microbial decay in the packaged product.

PREHARVEST CROP MANAGEMENT

Very little information has been published in respect to preharvest crop management on the postharvest physiology of fresh-cut fruits and vegetables. A series of reviews on preharvest factors and their effects on fruits and vegetables have been published

recently (Crisosto et al., 1997; Prange and DeEll, 1997; Weston and Barth, 1997), and some of the discussions in these reviews are pertinent here. Good pest and disease management may be the most important preharvest factor affecting the quality of fresh-cut fruit and vegetable products. Other issues that appear to be consistently important to fruit and vegetable quality are irrigation and calcium nutrition. Rates of irrigation and calcium nutrition have effects on postharvest decay and on tissue firmness. Excess irrigation results in development of tissues that are susceptible to bruising and injury (Prange and DeEll, 1997). In some cases, there are physiological responses to nitrogen and phosphorus fertilization (Prange and DeEll, 1997; Weston and Barth, 1997), however, these are often overshadowed by the effects of climatic variations (Prange and DeEll, 1997). Excess irrigation will also reduce the soluble solids content of fruit and vegetable tissues (Crisosto et al., 1997; Prange and DeEll, 1997; Weston and Barth, 1997), and this may have an effect on respiration rates (Blanchard et al., 1996).

The climatic conditions in which fruits and vegetables are produced can have a significant influence on fresh-cut product quality. Muskmelon that is shaded before harvest can have lower sucrose levels and higher acetaldehyde and ethanol levels, and this can lead to "water-soaked" flesh tissues. This particular problem has been shown to occur under dull, cool summer growing conditions (Nishizawa et al., 1998). Carrots of the same cultivar grown in different geographical regions will produce different levels of phenolics in response to shredding (Babic et al., 1993); however, the basis of these differences are not understood. Growing region also has an effect on shelf life of pear slices (Gorny et al., 2000) as related to effects on browning and softening, but again, these effects are not understood.

There is very little information on crop nutrition on the physiological response to cutting in minimally processed fruits and vegetables. Calcium is the best studied of all the crop nutrients in terms of postharvest quality (Fallahi et al., 1997). In general, preharvest calcium nutrition improves firmness retention and delays membrane deterioration and ripening in whole fruits. Recent work is showing that preharvest calcium applications can improve the firmness retention in green and colored peppers (Toivonen, unpublished data). Further work is required in this area, because improvements in nutrition may have tremendous impacts on the physiological response to minimal processing.

PHYSIOLOGICAL MATURITY

The physiological maturity of fruits or vegetables is known to impact the wounding response. This is especially true for climacteric fruits. Cantaloupes harvested early ($\frac{1}{4}$ slip) had better quality retention (decay/discoloration) than those harvested at late maturity (full slip) (Madrid and Cantwell, 1993). Pieces cut from the $\frac{1}{4}$ slip fruit produced less ethylene, had lower respiration rates, and stayed firmer than those cut from full slip fruits. Slices from partially colored or fully colored bell peppers retained better quality over 12 days in controlled atmospheres than slices from green bell peppers (López-Gálvez et al., 1997a). This is opposite to results found with whole peppers where fully ripe (red) fruit deteriorated much more quickly than immature (green) fruit (Lurie and Ben-Yehoshua, 1986). Immature carrots produce

greater amounts of isocoumarin, a compound responsible for bitterness, in response to ethylene than mature carrots (Lafuente et al., 1996). Minimally processed immature iceberg lettuce was less susceptible to browning than mature or overmature lettuce (Couture et al., 1993). Therefore, while most fruits and vegetables are better suited to minimal processing in less mature physiological stages, some products such as bell peppers may be most suitable at more advanced stages of maturity.

Papaya fruit at 55–80% skin yellowing were found to be the most suitable for minimal processing (Paull and Chen, 1997). Fruit with less than 55% skin yellowing showed greater increases in ethylene production and respiration in response to slicing and de-seeding, and the flesh of processed product was not soft enough to be acceptable for consumption. At the other end of the spectrum, papaya fruit which were full yellow at cutting showed little increase in ethylene or respiration, and the pieces were easily bruised and too soft to handle.

Pears with 44–58 N firmness were found to be optimal for quality of fresh slices (Gorny et al., 2000), and small fruit (122–135 g) were more susceptible to browning than large fruits (152 g). Peaches and nectarines at a firmness of 18–31 N were found to be optimal for packaged fresh slices (Gorny et al., 1999), however, not much information was given on the maturity characteristics at picking.

In areas where root crops can be left in the soil and harvested throughout the winter (e.g., Ireland), carrots that are harvested after the winter produce slices that break down faster than carrots that are harvested earlier in the fall (Barry-Ryan and O'Beirne, 2000). This is presumably due to the higher microbial loads on the carrots harvested after the winter. Similar findings were reported for shredded carrots by Babic et al. (1992). Therefore, root crops used for fresh-cut products should be harvested as soon as they mature.

SEVERITY OR DEGREE OF CUTTING-INDUCED INJURY

The severity of cutting can have a large influence on ethylene production and respiration rates (Figure 5.9). In bananas, it was found that as the angle of cut was increased, total cut area increased, and this resulted in parallel increases in respiration rates and ethylene production (Abe et al., 1998). These differences in respiration and ethylene production were related to shelf life of the sliced bananas. In another study, cut potatoes were found to have twice the respiration rate of whole-peeled potatoes that, in turn, have double the respiration of intact unpeeled potatoes (Gunes and Lee, 1997).

Susceptibility to anaerobic metabolism can also be affected by severe cutting procedures. For example, carrot shreds show a significant accumulation of ethanol and acetaldehyde even in 2% O₂, which is not considered to be at the anaerobic threshold (Kato-Noguchi and Watada, 1997a), suggesting the extensive injury to the tissues induces anaerobic metabolism in order to supply the ATP requirements for cell survival (Rolle and Chism, 1987).

Phenolic metabolism is also affected by the severity of the processing method. As tissue injury levels increase, so does phenylalanine ammonia lyase (PAL) activity in the tissue (Figure 5.10). This results in increased accumulations of phenolics in products such as lettuce (Ke and Saltveit, 1989). Severity of abrasion on the surface of peeled carrots affects the degree of lignification of the surface, and use of a sharp

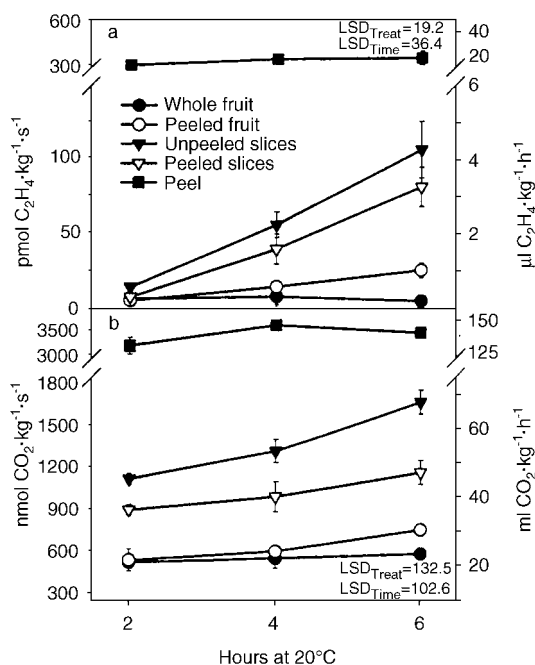


FIGURE 5.9 Effect of wounding on the C_2H_4 (a) and CO_2 (b) production rates of whole kiwifruit, whole-peeled fruit, peel, peeled, and unpeeled slices stored at 20°C for 6 h. (From Agar et al. 1999, used with permission from the Institute of Food Technologists.)

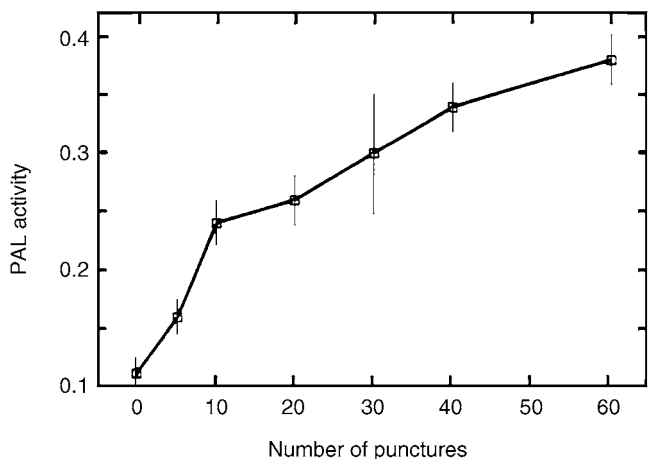


FIGURE 5.10 Phenylalanine ammonia lyase (PAL) activity [$\mu mol\ (g\ FW^{-1}\ h^{-1})$] as related to the degree of wounding. Wounding was done by uniformly puncturing an 8 cm² area of midrib tissue with a sterile 26-gauge hypodermic needle. All measurements were taken on the second day of incubation. The vertical error bars represent SE (standard error) of the mean. (From Ke and Saltveit 1989, used with permission from Munksgaard International Publishers.)

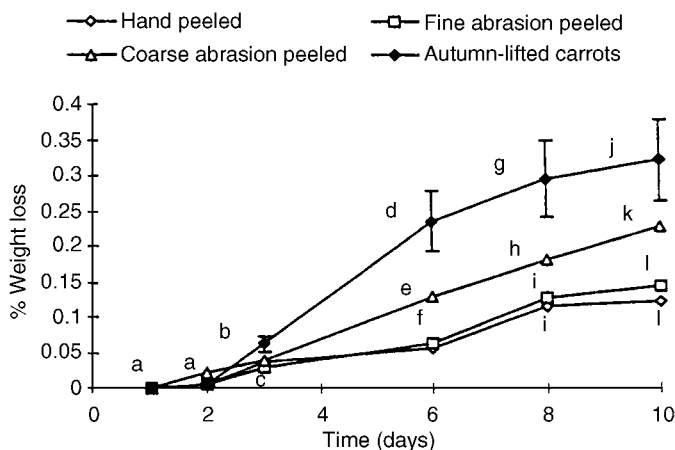


FIGURE 5.11 Effects of peeling method on weight loss of carrot disks during storage at 8°C. Values are means for four determinations, each done in duplicate, separated by Fisher's least significant difference ($P < 0.05$), denoted by different letters. (From Barry-Ryan and O'Beirne 2000, used with permission from Blackwell Science Ltd.)

blade completely prevents the lignification even at 35 days of post-process storage at 2°C (Bolin and Huxsoll, 1991). However, if much of the outer carrot tissue is removed with the peeling process, there is less problem with lignification, which is likely due to the fact that metabolic machinery to produce lignification is localized in the outer peel tissue (Sarkar and Phan, 1979).

The type of peeling or cutting process can also influence the degree of physiological response by tissues. Fine abrasion peeling results in lower weight loss of packaged slices made from the peeled carrots as compared with coarse abrasion peeling, which causes more tissue injury (Figure 5.11). In addition, the respiration rates of slices made from fine abrasion-peeled carrots were significantly lower than for slices made from coarse abrasion-peeled carrots. Carrots that were hand-peeled with a sharp blade (which causes the least amount of tissue damage) exhibited lower water loss, respiration, and microbial counts than slices made from either fine or coarse abrasion-peeled carrots. The severity of injury from the slicing procedure also has effects on water loss and microbial growth in sliced carrots (Barry-Ryan and O'Beirne, 1998). Machine slicing as opposed to manual slicing with a sharp razor blade results in more bacterial, yeast, and mold growth in packaged sliced carrots. Weight loss also increases by ~30% in the machine-sliced product as compared with the manually sliced product. This difference relates to the degree of tissue injury induced by the machine slicer (Barry-Ryan and O'Beirne, 1998). Hand-peeling with a sharp blade and lye peeling resulted in less surface browning than abrasion peeling in potatoes (Gunes and Lee, 1997). Enzymatic peeling to produce minimally processed orange (*Citrus sinensis*) segments resulted in half the weight loss than for segments made from manually peeled oranges, presumably due to the reduction of injury to the segments with enzymatic peeling (Pretel et al., 1998).

Manual peeling resulted in greater bacterial growth than enzymatic peeling for these orange segments.

PRE- AND POST-CUTTING TREATMENTS

One of the most studied post-cutting treatments is the use of calcium dips. CaCl_2 (1% w/v) has been shown to prevent softening of strawberry and pear slices, especially when combined with modified atmospheres (Rosen and Kader, 1989). CaCl_2 was also effective in slowing ripening and softening in sliced tomato when packaged slices were stored at 2°C, but not at 10°C (Artés et al., 1999). CaCl_2 at concentrations between 1 and 5% (w/v) suppressed wound-induced respiration in fresh-cut cantaloupe but did not have any effect on ethylene production (Luna-Guzmán et al., 1999; Luna-Guzmán and Barrett, 2000). CaCl_2 dips at a 0.05 M concentration delayed the catabolism of membrane phospholipids in cabbage leaf tissue (Chéour et al., 1992), delaying senescence in the tissues. CaCl_2 dips enhance the maintenance of membrane structure and function in shredded carrots (Picchioni and Watada, 1998). Calcium lactate dips can increase the shelf life of peach slices via effects on firmness retention (Gorny et al., 1999).

Organic acids such as citric acid have also been used to control physiological changes in fresh-cut tissues. Citric acid dips (i.e., low pH) will reduce peeling-induced surface lignification in minimally processed cut-and-peel carrots, likely due to the inactivation of the enzymes that are responsible for processes leading to lignification (Bolin and Huxsoll, 1991). Citric acid dips of 1 mM or higher concentration reduce the respiratory rate of shredded carrots by 50% or more (Kato-Noguchi and Watada, 1997b).

Ascorbic acid (vitamin C) is a reducing agent often used to prevent oxidation reactions such as browning; however, there may be effects on other physiological processes in the cut tissues. Ascorbic acid dips reduced the respiration of 'Fuji' apple slices stored in a 0% O_2 atmosphere (Gil et al., 1998). In air atmosphere, the ascorbic acid dips reduced ethylene production and increased the respiration of apples slices (Gil et al., 1998). Browning of slices was reduced in both air and 0% O_2 atmospheres.

The use of mild heat treatments has been found to have profound physiological effects on fresh-cut fruit and vegetable products. Heat shock treatments of 45°C for 120 s, 50°C for 60 s, or 55°C for 30 s reduced PAL activity that resulted in less wound-induced phenolic accumulation in iceberg lettuce (Loaiza-Velarde et al., 1997). Pre-cutting heat treatments (45°C) have been shown to reduce browning and enhance firmness retention of slices made from treated apples (Kim et al., 1993a). However, not all cultivars respond favorably to heat treatments—only 'Delicious' and 'Golden Delicious' showed a significant benefit for slices after eight days of storage at 2°C. In contrast, other cultivars (e.g., 'Liberty', 'Munroe', 'Rome', and 'RI Greening') showed increased browning in response to the heat treatments (Kim et al., 1993a). The mechanism of the response to heat treatments relates to their effect on physiological processes; heat treatment inhibits ethylene synthesis, tissue response to ethylene, and cell wall degradation associated with hydrolytic enzymes such as polygalacturonase and galactosidases (Lurie and Klein, 1990; Lurie, 1998).

While respiration is initially enhanced, it subsequently falls below the level found in nontreated whole products. These effects may explain why heat treatments inhibit cutting-induced changes in fruits and vegetables. Heat treatments up to 60°C for 1 min also improved CaCl_2 uptake and firmness retention in fresh-cut cantaloupe (Luna-Guzmán et al., 1999).

The postharvest storage method and/or duration can be considered postharvest treatments and have been, in a few cases, found to influence post-cutting physiology. Storage for 30 days will reduce isocoumarin accumulation in response to ethylene by severalfold in fresh-cut carrots, as compared to freshly harvested carrots (Lafuente et al., 1996). Freshly harvested pears and those stored in CA (2% O_2 , 98% N_2) produced better quality slices than those stored in air (Gorny et al., 2000).

Edible coatings offer several possible benefits to fresh-cut fruits and vegetables. Such coatings can provide a modified atmosphere for the cut pieces, and thus, can reduce water loss from cut surfaces. With the incorporation of additives or preservatives, they can control cut-surface browning and microbial growth on the damaged tissues (Baldwin et al., 1995). This technology is still emerging, but there have been several successful developments using an array of materials, including lipids, polysaccharides, and/or proteins as the base components in the coatings. Functional additives are expected to improve the benefits from using these coatings. Potential preservative additives that are being considered are benzoic acid, sodium benzoate, sorbic acid, potassium sorbate, and propionic acid (Baldwin et al., 1995). Potential antioxidant additives include ascorbic acid, citric acid, phosphoric acid, and other compounds.

A cellulose-based edible coating applied to cut apples and potatoes was most effective in controlling moisture loss when the formulation contained soy protein (Baldwin et al., 1996). The addition of ascorbic acid to the formulation delayed surface browning, while the addition of sodium benzoate or potassium sorbate helped to control microbial growth. Adjustment of pH to 2.5 resulted in optimal control of both browning and microbial growth (Baldwin et al., 1996). A wrap made from edible film composed of fruit puree and lipid material was found to be an effective approach to controlling moisture loss and surface browning in apple slices (McHugh and Senesi, 2000).

Low-dose ionizing irradiation is being investigated as an approach to sanitize fresh-cut fruit and vegetable products. The effects of such treatments on physiology and quality of fruit and vegetable tissue must be ascertained to determine the acceptability of this approach to product sanitation. Low-dose irradiation (0.19 kGy) significantly reduces microbial populations in packaged, cut iceberg lettuce and moderately increases respiration (Hagenmaier and Baker, 1997). Overall, the quality of the packaged cut lettuce was improved by the irradiation treatment. The use of 2 kGy of irradiation increased the respiration of shredded carrots but slowed the loss of sugars and inhibited the growth of aerobic mesophilic and lactic acid bacteria (Chervin and Boisseau, 1994). The quality of irradiated shredded carrots was much higher than those sanitized using conventional industry practice (i.e., chlorine washes). Hagenmaier and Baker (1998) found that a 0.5 kGy dose of gamma irradiation had only a minor effect on respiration but significantly reduced the microbial population in shredded carrots as compared with chlorine-washed shredded carrots. This effect was persistent over nine days of storage in modified atmosphere packaging (MAP).

Doses of up to 2.4 kGy reduced ethylene production and caused a transitory increase in respiration of apple slices (Gunes et al., 2000). Softening of the tissues resulted from doses above 0.5 kGy, however, it is not known whether the level of softening is commercially important.

ATMOSPHERIC COMPOSITION

The basic principle for the use of packaged, fresh-cut products is that modified atmospheres are theoretically expected to control physiological and quality changes in the product. Low O_2 and high CO_2 are known to inhibit ethylene action (Burg and Burg, 1967) (Figure 5.12). For some fruits or vegetables, O_2 is the main factor that controls respiration. As an example, respiration rates in packaged carrot cubes were dependent on the O_2 levels in the package but were not affected by the CO_2 levels (Sode and Kühn, 1998). The level of respiration suppression that a controlled atmosphere may cause is dependent on the product. Furthermore, the sensitivity of respiration to elevated CO_2 and reduced O_2 levels depends on the commodity in question. The respiration of cut carrots appears to be unaffected by CO_2 levels, as they increase well above 21%. On the other hand, cut lettuce in a relatively impermeable container appears to maintain a balance of 21% between CO_2 and O_2 (Priepke et al., 1976). Lettuce respiration slows as the package atmosphere becomes more modified, suggesting that cut lettuce is less susceptible to the development of anaerobic metabolism than is cut carrot. Celery behaves similarly to carrot, whereas radish, green onion, and endive behave similarly to lettuce. Similar differences are seen in fresh-cut fruits. It was shown that an atmosphere of 2% O_2 and 10% CO_2 significantly

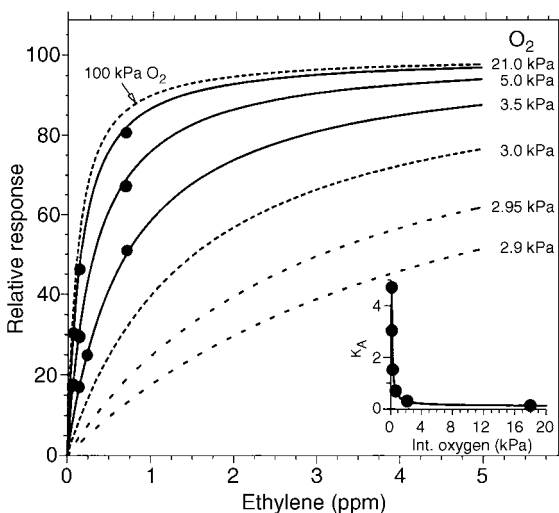


FIGURE 5.12 Effect of external oxygen levels on the response of pea seedlings to ethylene. Inset: the effect of internal O_2 on the concentration of ethylene required to give a response half that of the maximal response K_A . (Reprinted from Beaudry 1999, *Postharvest Biology and Technology*, Vol. 15, pp. 293–303, with permission from Elsevier Science.)

TABLE 5.1
Carbon Dioxide Production and Oxygen Uptake Rates of Fresh-cut Products Stored in Air and Controlled Atmospheres

Commodity	°C	Atmosphere	CO ₂ Production (ml kg ⁻¹ h ⁻¹)	O ₂ Uptake (ml kg ⁻¹ h ⁻¹)
Kiwifruit slices	0	Air	2.1	2.8
		1% O ₂ + 5% CO ₂	2.3	1.8
	5	Air	2.4	2.6
		1% O ₂ + 5% CO ₂	3.3	2.4
Peach slices	0	Air	3.0	3.0
		0.5% O ₂ + 10% CO ₂	3.1	1.0
	5	Air	5.5	4.6
		0.5% O ₂ + 10% CO ₂	3.9	1.1
Large muskmelon cubes	5	Air	4.0	4.0
		1% O ₂ + 10% CO ₂	2.3	0.8
	10	Air	9.6	10.6
		1% O ₂ + 10% CO ₂	5.4	2.2
Small muskmelon cubes	5	Air	2.7	3.8
		1% O ₂ + 10% CO ₂	5.5	2.1
	10	Air	5.2	7.0
		1% O ₂ + 10% CO ₂	6.5	3.2
Broccoli florets	0	Air	12.9	15.8
		0.5% O ₂ + 10% CO ₂	6.9	8.1
	5	Air	22.6	31.0
		0.5% O ₂ + 10% CO ₂	7.7	11.3
	10	Air	41.2	68.0
		0.5% O ₂ + 10% CO ₂	15.3	20.5

Source: Extracted from Watada et al. (1996), *Postharvest Biology and Technology*, 9: 115–125, used with permission from Elsevier Science.

reduced respiration and ethylene production in cut honeydew melons but had a much smaller effect on cut strawberries and peaches (Qi and Watada, 1997). Therefore, it must be recognized that controlled or modified atmospheres may not have consistent or predictable effects on CO₂ production and O₂ uptake in different types of tissues, and this lack of consistent response is further complicated by the effect of storage temperature (Table 5.1).

Due to the lack of predictability of response to atmospheres for the varying range of products, the potential effects of modified atmospheres on a specific fruit or vegetable product must, therefore, be considered on an individual basis. For example, an atmosphere containing 0.5% O₂ was found to be effective in preventing browning and softening in sliced pears, whereas an atmosphere containing 12% CO₂ prevented softening in strawberries (Rosen and Kader, 1989). In contrast, high CO₂ and low O₂ atmospheres both have detrimental effects on quality of shredded carrots—high CO₂ leads to enhancement of lactic bacteria growth, and low O₂ leads to quality deterioration (flavor and texture) (Barry-Ryan et al., 2000).

High CO₂ atmospheres can have apparently contradictory effects on bacterial and fungal growth, depending on the product. A high CO₂ atmosphere (30%) inhibited both phenylalanine ammonia lyase activity and accumulation of phenolics in shredded carrots, but lactic acid bacterial growth was rapid (Babic et al., 1993). The phenolic acids have been shown to be antibacterial in activity (Harding and Heale, 1980; Barber et al., 2000). This line of thinking is supported by the observation that lactic acid bacteria never grow in shredded carrots held in air atmosphere (Carlin et al., 1990). At the other end of the response spectrum is the well-known phenomena of the fungistatic effect of high CO₂ atmospheres (Wells and Uota, 1970). Microbial growth is inhibited by commercial low O₂, high CO₂ atmosphere packages of minimally processed iceberg lettuce (King et al., 1991). High CO₂ (10%) showed suppression of fungal and bacterial growth in fresh-cut honeydew, strawberries, and peaches (Qi and Watada, 1997). Low oxygen atmospheres (1 or 3%) had little effect on microbial growth in cut cantaloupe, but a combination of low O₂ (3%) and high CO₂ (7.5 or 15%) was effective in controlling microbial growth and decay in cut cantaloupe (Portela et al., 1997).

CO₂ can also have contrasting effects on quality retention in different products. High CO₂ (15%) improved firmness retention in cut cantaloupe (Madrid and Cantwell, 1993), whereas high CO₂ caused tissue softening and electrolyte leakage in cut peppers (López-Gálvez et al., 1997a). Negative effects of CO₂ on product quality may not be apparent until after product is removed to air atmospheres. High CO₂ (20%) increased extractable phenylalanine ammonia lyase (PAL) in cut lettuce tissue, although phenolic accumulations and tissue browning are not a problem while the lettuce remained in the package (Mateos et al., 1993). This is due to the low PAL activity *in situ*, because CO₂ readily dissolves in the tissue and thereby reduces cytoplasmic pH, which in turn, inhibits the activity of the enzyme. Once removed to air, the tissue pH rises and *in situ* PAL increases, providing substrates for browning reactions. This phenomenon was reported for butterhead lettuce in which browning has been associated with high package CO₂ levels, but the browning did not occur until the lettuce was transferred to air (Varoquaux et al., 1996b).

Low O₂ (0.25%) reduces respiration, ethylene production, weight loss, browning, and microbial counts on sliced zucchini (Izumi et al., 1996). Low O₂ has also been found to reduce browning in cut lettuce (López-Gálvez et al., 1997b; Smyth et al., 1998) and appears to reduce water loss in broccoli florets (Bastrash et al., 1993). Rapid development of low O₂ and high CO₂ is also key to controlling cut-surface browning, and actively modified atmosphere packaging is commercially used in lettuce and potato products (Gorny, 1997).

One of the greatest challenges is that modified atmosphere packages cannot provide acceptable O₂ and CO₂ concentrations for some commodities. Therefore, in many cases, the attainable atmospheres are quite deviant from those considered optimal (Beaudry, 1999). While recommendations have been made for optimal atmospheres in which to package most fruit and vegetable products, the reality is that not all commodities can be maintained in appropriate atmospheres using packaging films (Figures 5.13 and 5.14). For example, cut lettuce and lettuce salads are packaged in atmospheres that are very different than those recommended from prior research reports (Figure 5.13) and that appear to do relatively well in commercial practice

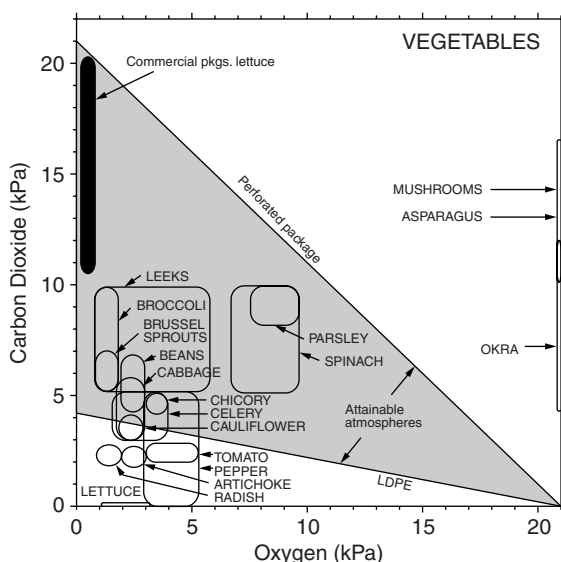


FIGURE 5.13 Recommended O_2 and CO_2 combinations for the storage of vegetables. The shaded area depicts atmospheres theoretically attainable by modified atmosphere packaging (MAP) using low-density polyethylene (LDPE, lower boundary) and perforated packages (upper, dashed line). The darkened area represents atmospheres observed in commercial modified atmosphere packages of mixed, lettuce-based salads. (Reprinted from Beaudry 1999, *Postharvest Biology and Technology*, Vol. 15, pp. 293–303, with permission from Elsevier Science.)

(López-Gálvez et al., 1997b). There are some risks associated with such packaging, but in practice, by using conservative shelf life expectations, these risks can be tolerated (Smyth et al., 1998).

CONSEQUENCES OF CUTTING-INDUCED INJURY ON QUALITY RETENTION

Wound-induced ethylene production is associated with an increased rate of ripening in papaya flesh (Paull and Chen, 1997) and softening in pear and strawberry slices (Rosen and Kader, 1989). This leads to the conclusion that ethylene removal would be beneficial to quality retention of packaged fresh-cut products. However, the benefit of ethylene removal in modified atmosphere packages has yet to be demonstrated to be of significant merit. Ethylene removal has been shown to reduce softening in kiwifruit and banana slices held in air atmospheres and yellowing of cut spinach, but ethylene removal was of no benefit in reducing yellowing of broccoli florets (Abe and Watada, 1991). It has been pointed out that most fresh-cut package atmospheres are highly modified and that high CO_2 and low O_2 would certainly inhibit, if not completely prevent, ethylene action on the fresh-cut tissue (Gorny, 1997). This is supported by the work of Howard et al. (1994) who showed that ethylene absorption had little, if any, effect on the quality of diced onions held in modified

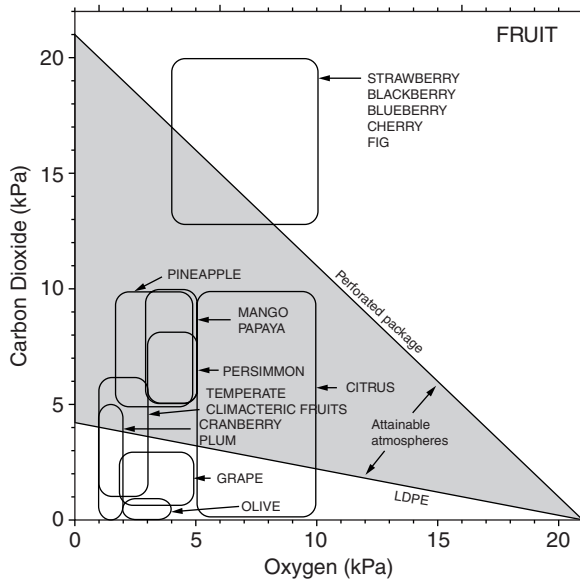


FIGURE 5.14 Recommended O_2 and CO_2 combinations for the storage of fruit. The shaded area depicts atmospheres theoretically attainable by modified atmosphere packaging by film permeation alone (low-density polyethylene, LDPE, lower boundary) and via perforations alone (upper, dashed line), or their combination (shaded area). (Reprinted from Beaudry 1999, *Post-harvest Biology and Technology*, Vol. 15, pp. 293–303, with permission from Elsevier Science.)

atmospheres (2.65% O_2 , 3.65% CO_2 at equilibrium). In fact, they found increased microbial growth in packages containing the ethylene adsorbent. This was likely due to the fact that sulfur volatiles were also removed from the package headspace by the adsorbent, and it is known that some sulfur volatiles produced by injured onion tissues have antimicrobial properties (Blanchard et al., 1996). No response or improvement in quality was found with use of ethylene adsorbents in packaged, minimally processed carrot sticks (Howard and Griffin, 1993). Another issue regarding ethylene activity is that wounding induces the production of allyl isothiocyanates in cabbage, and these compounds are strong antioxidants that can suppress both wound-induced ethylene production and browning (Nagata, 1996). Therefore, accumulation of secondary metabolites may impinge on other physiological responses to cutting, depending on the product in question, and thus, must be kept in mind.

Another aspect of wound-induced changes is the production of volatiles other than ethylene. Sulfur volatiles in onions are typically produced within hours of cutting, and their production may last for a few days (Toivonen, 1997a). If these volatiles are not removed from the tissue (i.e., with appropriate adsorbents), then physiological and biochemical changes can be initiated in response to their accumulation (Toivonen, 1997b) leading to declines in tissue quality. It is, therefore, very important to minimize the effects of the responses to cutting in the early stages in order to control quality changes. Also, it has been shown that production of some oxidation

products of free fatty acids as a consequence of wound-induced membrane deterioration can lead to off-flavors (Rolle and Chism, 1987).

Storage temperature after cutting will certainly influence the effect of wounding on product quality. It was determined that quality of packaged tomato slices was better maintained at 2°C than at 10°C. This was associated with the fact that ethylene and respiration production rates were increased by cutting at the higher storage temperature as opposed to the lower storage temperature (Artés et al., 1999), despite the fact that the atmospheres in the packages were less modified at the lower storage temperature. In another example, honeydew cubes were firmer and had reduced respiration and ethylene production in a 4% O₂ and 10% CO₂ atmosphere at 10°C, while there is no appreciable response seen at 5°C (Qi et al., 1999). This is likely due to the fact that the lower temperature is already controlling quality deterioration, and the modified atmosphere can provide little additional benefits under those conditions. However, it must be cautioned that the quality of the honeydew cubes were poorer (controlled atmosphere or not) at 10°C. Bacterial populations were lower in controlled atmosphere conditions than in air at both 5 and 10°C.

The prediction of respiration rate is a very important factor in the selection of the appropriate packaging film for fresh-cut products (Lee et al., 1997). A plethora of information on the CO₂ production by fresh-cut products exists (see Table 5.2), however, the oxygen level in the package is probably as important, if not more important, in the package selection. Avoidance of hypoxic or anaerobic conditions in the package is considered to be critical to ensuring quality of fresh-cut product (Lakakul et al., 1999). Modeling of the ideal film for a product is relatively simple if the ideal temperature is assumed for storage and handling (Lee et al., 1997), however, in the real world, most minimally processed products are exposed to non-ideal temperatures during distribution and retail (Talasila et al., 1995). Several research groups have proposed modeling approaches to deal with non-ideal temperature situations. Many sophisticated approaches to model temperature dependence of respiration to assist in MAP film design have been published (Makino et al., 1997). Probably the most manageable is one that establishes that most abusive situations can be tested using a single superoptimal temperature of 7°C (Jacxsens et al., 2000) and that gives a reasonable selection for films usable in the range between 2 and 10°C. The greatest issue in obtaining accurate respiration values is which approach is used to determine respiration rate at different atmospheres. There are two that are commonly practiced: use of flow-through respiration setups, which have been suggested to give the closest physiologically correct values (Lee et al., 1997) and a closed or static respiration approach, where respiration is assessed either in a semipermeable flexible film package (Lakakul et al., 1999) or in an impermeable chamber (Beveridge and Day, 1991; Gong and Corey, 1994; Jacxsens et al., 2000). The closed approach for respiratory behavior is likely a better simulation of a modified atmosphere package, because there is evidence that a flow-through system does not accurately duplicate actual quality changes in a package situation (Ben-Arie et al., 1991; Larsen and Watkins, 1995). Another aspect of the issue is that most package O₂ and CO₂ permeabilities change differentially with increases in temperature (Moyls et al., 1998; Lakakul et al., 1999). In some cases, CO₂ transmission increases faster than O₂ transmission (Lakakul et al., 1999), and in others, the O₂ transmission increases faster than CO₂ transmission (Moyls et al., 1998).

TABLE 5.2
Respiration Rates of Fresh-cut Fruits and Vegetables at 0–2.5°C
and 10°C

Commodity	Processed Form	Respiration (mg CO ₂ kg ⁻¹ h ⁻¹)	
		0–2.5°C	10°C
Green beans	cut	7.1 ^a	39.7
Zucchini	sliced	6.1 ^a	23.9
Cucumbers	sliced	1.7 ^a	4.9
Yellow summer squash	sliced	3.3 ^a	9.0
Bell pepper	sliced	3.6 ^a	7.1
Tomatoes	sliced	0.7 ^a	5.1
Kiwifruit	sliced	3.7 ^a	11.9
Banana, without peel	sliced	4.0 ^a	10.7
Strawberry	hull removed	3.1 ^a	11.3
	sliced	8.5–11.2 ^b	—
Green seedless grapes	without stems	1.0 ^a	2.9
Peach	sliced	3.1 ^a	9.5
Muskmelon (large type)	cubed	1.9 ^a	6.2
Muskmelon (small type)	cubed	1.4 ^a	5.0
Crenshaw melon	cubed	0.6 ^a	4.6
Honeydew melon	cubed	1.2 ^a	4.2
‘Anjou’ pears	sliced	1.7–3.8 ^a	6.4–7.5
‘Bartlett’ pears	sliced	2.8–4.9 ^a	9.9–11.8
‘Bosc’ pears	sliced	1.7–2.7 ^a	7.3–7.8
‘Red Anjou’ pears	sliced	1.7–4.4 ^a	6.1–8.1
‘Bintje’ potato	whole, pre-peeled	3.0 ^c	9.0
	half	4.0 ^c	12.0
	sliced	5.0 ^c	20.0
‘Van Gogh’ potato	whole, pre-peeled	4.0 ^c	10.0
	half	4.0 ^c	11.0
	sliced	6.0 ^c	20.0
Red beet	whole, pre-peeled	2.0 ^c	10.0
	cubed	5.0 ^c	14.0
	grated	6.0 ^c	20.0
Carrot, from muck soil	whole, pre-peeled	3.0 ^c	9.0
	sliced	6.0 ^c	17.0
	grated	5.0 ^c	20.0–25.0
Carrot, from sandy soil	whole, pre-peeled	4.0 ^c	11.0
	sliced	6.0 ^c	15.0–18.0
	grated	8.0 ^c	27.0
Onion	whole, peeled	4.0 ^c	11.0
	rings	7.0 ^c	20.0
	grated	6.0 ^c	12.0
Broccoli	florets	12.9 ^a	41.2
Chinese cabbage	half	5.0 ^c	9.0
	coarsely shredded	9.0 ^c	25.0
	finely shredded	12.0 ^c	30.0

(continued)

TABLE 5.2
Respiration Rates of Fresh-cut Fruits and Vegetables at 0–2.5°C
and 10°C (Continued)

Commodity	Processed Form	Respiration (mg CO ₂ kg ⁻¹ h ⁻¹)	
		0–2.5°C	10°C
'Apex' white cabbage	quartered	4.0 ^c	12.0
	coarsely shredded	9.0 ^c	25.0
	finely shredded	12.0 ^c	30.0
'Lennox' white cabbage	eighth sections	4.0 ^c	10.0
	coarsely shredded	8.0 ^c	22.0
	finely shredded	9.0 ^c	27.0
'Delicious' apple	sliced	2.4–3.5 ^c	—
'Golden Delicious' apple	sliced	2.9–5.1 ^c	—
'Fuji' apple	sliced	—	3.0–8.5
Iceberg lettuce	shredded	3.9 ^b	6.4

^a0°C, ^b2.5°C, or ^c2.0°C.

Source: Adapted from Rosen and Kader (1989), Kim et al. (1993b), Mattila et al. (1994), Watada et al. (1996), Gil et al. (1998), Gorny et al. (2000).

Therefore, the specific transmission properties at different temperatures of the film being considered must be ascertained to better predict the package atmosphere response at different handling temperatures.

The cutting process can induce several physiological responses that may interact in closed package systems. For instance, shredded cabbage may, depending on cultivar, produce significant levels of allyl isothiocyanates (Yano et al., 1986). The accumulation of these compounds has strong inhibitory effects on respiration, polyphenol oxidase activity, phenylalanine ammonia lyase activity, ethylene production, and ACC synthase (Nagata, 1996). Therefore, the totality of the physiological responses must be understood to accurately predict the quality responses of packaged minimally processed fruits and vegetables. In addition, many fresh-cut products are mixes of various commodities. In the case of cut lettuce salads, would mixes containing shredded cabbage have less browning than mixes containing no cabbage? This is one of the next generation of questions that must be studied in packaged fresh-cut products.

CONCLUSION

The physiological effects of wounding are important factors in determining quality and shelf life of most fresh-cut fruit and vegetable products. While respiration rates are generally thought to be important in determining shelf life, there is no clear evidence that respiration rate changes in response to cutting are directly associated with deteriorative processes that lead to the end of useful shelf life. This may be because other processes are more limiting to shelf life than respiration. However, respiration rates of various fresh-cut products are important in selecting packaging films. It is

surprising that respiration rates of various fresh-cut fruits and vegetable fall into three or four ranges (Table 5.2). Therefore, if similar package atmospheres were desired for all commodities, then only a few package film permeabilities would be required for commercial purposes. Also, the mixing of different commodities to produce salad mixes would be relatively straightforward, because most of the components might be expected to have similar film permeability requirements. However, if extreme packaging atmospheres are required, for example, those required to control browning in iceberg lettuce (Figure 5.13) (Smyth et al., 1998), then special permeability requirements need to be specified. Even with such cases, it is important to have information on the respiration rates of the product so that the appropriate film can be selected to avoid anaerobic package atmospheres.

The use of ethylene adsorbents to remove wound-induced ethylene may be redundant if atmospheres are modified (Gorny, 1997). Reduced O_2 (<5%) and elevated CO_2 (>5%) will significantly inhibit ethylene effects. Rapid establishment of modified atmospheres is especially beneficial in preventing the effects of softening and deterioration produced by ethylene effects (Gorny, 1997). It may be that suppression of ethylene action is one of the most important functions of modified atmosphere packaging in fresh-cut products, next to controlling water losses.

Wounding also results in a wide range of physiological effects on many aspects of quality, including secondary metabolite accumulations, microbial growth, and water loss. Appropriate cultivar selection, crop management, post-cutting treatments, and packaging can minimize these effects. It is the challenge of the commercial fresh-cut practitioner to integrate the physiological information that is available and can be applied to their product and to determine the information that cannot be applied directly. The challenge for researchers is to fill the existing gaps of knowledge so that critical information is available to the fresh-cut industry.

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6 Enzymatic Effects on Flavor and Texture of Fresh-cut Fruits and Vegetables

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INTRODUCTION

Enzymes and substrates are normally located in different cellular compartments, and their transfer is actively regulated. Cutting of produce removes the natural protection of the epidermis and destroys the internal compartmentation that keeps them separate. The first visually observed change at the cut surface of plant tissue is desiccation

of the first layer of broken cells and one to a few additional subtending layers of cells (Kolattukudy, 1984). Tissue disruption increases permeability and mixing of enzymes and substrates that are otherwise sequestered within vacuoles. The consequent increase in enzymatic activity may cause sensory deteriorations such as off-flavor, discoloration and loss of firmness. Tissues of fresh-cut fruits and vegetables, are thus, more perishable and senescence-prone, than the intact organs from which they are obtained. Rupture of membranes also triggers metabolic changes that encompass wound healing processes (Picchioni et al., 1994; Ramamurthy et al., 1992; Bernards et al., 1999). This chapter is a review of the occurrence, distribution, function and properties of some enzymes that could significantly affect the flavor and texture of fresh-cut produce. The main enzymes covered are lipoxygenase, peroxidase, polyphenol oxidase and the pectic enzymes. Other related enzymatic reactions and conditions that potentially affect their activity in cut fruits and vegetables are also discussed.

LIPOXYGENASE

OCCURRENCE AND FUNCTION

Lipoxygenase (linoleate: oxygen oxireductase—EC 1.13.11.12) (LOX) is present in most plant tissues and, in the presence of oxygen, catalyzes oxidation of polyunsaturated fatty acids (PUFA) containing a *cis*, *cis*-1,4-pentadiene structure. The generally accepted nomenclature for the most common LOX isozymes is as follows: LOX I, which exhibits alkaline pH for maximal activity and isoelectric point of about 5.7; LOX II, with optimum pH activity and isoelectric points around 6.5 and 6.25, respectively; and LOX III, with a broad optimum activity pH centered around pH 7 and isoelectric point of 6.15 (Siedow, 1991; Boyes et al., 1992). While all three LOX types could be present in plants such as legume seeds (Siedow, 1991), some LOX types are more dominant in others, such as LOX II in kiwifruit (Boyes et al., 1992), asparagus (Gavanthorn and Powers, 1989), cucumber (Feussner and Kindl, 1994; Wardale and Lambert, 1980), tomato (Bonnet and Crouzet, 1977) and apple (Kim and Grosch, 1979). LOX I converts linoleic acid preferentially to 13-hydroperoxide derivatives, LOX 2 forms 9-hydroperoxide compounds while LOX III yields a mixture of hydroperoxides. LOX types II and III, in the presence of fatty acids and dissolved oxygen, co-oxidize carotenoids (Lebedev et al., 1978; Canfield and Valenzuela, 1993; Ueno et al., 1982) and chlorophyll (Cheour et al., 1992; Zhuang et al., 1994, 1997). LOX II is activated by calcium, whereas LOX III is inhibited by calcium (Siedow, 1991). In apple flesh disks, fruits rich in calcium and/or phosphorous had lower LOX content (Marcelle, 1991). Kato et al. (1992) reported the appearance of new LOX (LOX IV, V and VI) in germinated soybean cotyledons. LOX V and VI preferentially produced 13(*S*)-hydroperoxy-9-*cis*, 11-*trans*-octadecadienoiic acid (13*S*-HPOD) as a reaction product of linoleic acid, whereas LOX IV produced both 13*S*-HPOD and 9(*S*)-hydroperoxy-10-*trans*, 12-*cis*-octadecadienoic acid. All three isozymes have pH optima of 6.5, no activity at pH 9.0 and preferred linolenic to linoleic acid as substrate.

Conjugated hydroperoxy acids (HPO) produced by LOX catalysis undergo metabolism by hydroperoxide lyase (HPO lyase). HPO lyase catalyzes the cleavage

of HPO to aldehydes, such as *cis*-3-noneal and hexanal from linoleic acid HPO and *cis*-3-, *cis*-6-nonadienal and *cis*-3-hexenal from linolenic acid HPO (Grun et al., 1996; Wardale and Galliard, 1975). Besides hydroperoxidation, formation of oxoacids and ketodienes are catalyzed by LOX (Vick and Zimmerman, 1980; Sanz et al., 1993). Hydroperoxide isomerase could also catalyze the isomerization of hydroperoxide as an intermediate reaction pathway (Vick and Zimmerman, 1976). HPO lyase is a membrane-based/bound enzyme present in small amounts in plant tissue. On the basis of substrate specificity, HPO lyase has been classified into three types: 9-HPO lyase, 13-HPO lyase and nonspecific HPO lyase (Perez et al., 1999a). The substrate specificity determines aroma composition of many plant products, despite the specific action of LOX. The formation of volatile aldehydes of chain lengths C6 and C9 is widespread in fruits and vegetables (Kim and Grosch, 1981; Gray et al., 1999; Perez et al., 1999a) and proceeds rapidly when plant cells are disrupted in the presence of oxygen. Siedow (1991) described two metabolic pathways for HPO (Figure 6.1). One involves the action of HPO lyase on the LOX product, 13-hydroperoxylinolenic acid, resulting in the formation of hexanal and 12-oxo-*cis*-9-dodecenoic acid. The latter is then isomerized into the more stable 12-oxo-*trans*-10-dodecenoic acid, also known as traumatin or as “wound hormone.” Traumatin is known to mimic some physiological effects associated with wounding in plant tissues, inducing cell division and subsequent callus formation. It can also be converted to the acid derivative by a nonenzymatic oxidation of the aldehyde moiety. Traumatinic acid also appears

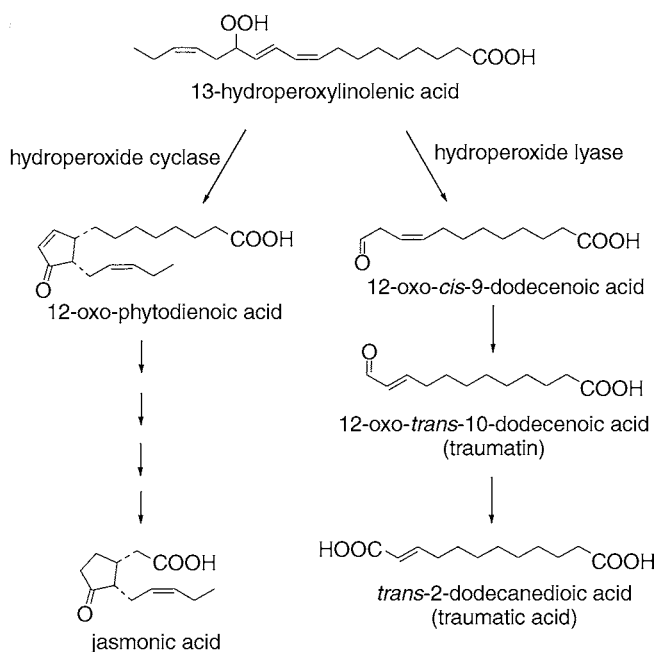


FIGURE 6.1 The “lipoxygenase pathway” for the biosynthesis of jasmonic acid, traumatin and traumatic acid from the lipoxygenase product, 13-hydroperoxylic acid (Siedow, 1991).

to be involved in plant responses to wounding. The second pathway is initiated by the enzyme hydroperoxide cyclase (HPC) that catalyzes the formation of 8-[2-(*cis*-2'-pentenyl)-3-oxo-*cis*-4-cyclopentenyl] octanoic acid, commonly referred to as 12-oxo-phytodienoic acid. This compound serves as the precursor for the formation of jasmonic acid that has also been suggested to be a promoter of plant senescence and to be involved with plant signal transduction.

EFFECT OF LIPOXYGENASE ON SENESCENCE AND RESISTANCE MECHANISM

An increase in LOX activity is a common feature in senescent plant tissues. The catalysis of *cis*, *cis*-1,4-pentadiene structures is related to the critical role of LOX in plant tissue senescence. Treatments believed to delay the onset of senescence, such as the addition of cytokinins or antioxidants reduce the level of endogenous LOX relative to untreated controls (Siedow, 1991). Inhibition of LOX delays ripening and softening in peaches (Wu et al., 1999) and kiwifruit (Chen et al., 1999). LOX activity has also been correlated with plant tissue development (McLeod and Poole, 1994; Tanteerataarm et al., 1989), as well as pathogen (Avdiushko et al., 1993a; Gardner, 1991; Ohta et al., 1990) and insect (Avdiushko et al., 1997; Duffey and Stout, 1996; Thaler et al., 1996) resistance mechanisms. The protective mechanism implicated is the further catabolism of oxidation products to jasmonic acid and methyl jasmonate, which are members of an intracellular signal transduction chain transferring signal to the nucleus and selectively activating gene expression (Gardner, 1991; Feussner and Kindl, 1994; Avdiushko et al., 1993a). An increase in LOX occurs concurrently with a decrease in linolenic and linoleic acids and total fatty acids in cucumber plants inoculated with *Colletotrichum lagenarium* or tobacco necrosis virus (Avdiushko et al., 1993b). The involvement of LOX in disease resistance of kiwifruit is evidenced by the elevated LOX activity in *Botrytis cinerea* inoculated fruits (McLeod and Poole, 1994). Linoleic acid and LOX products of linoleic acid also inhibit germination and growth of *Botrytis cinerea* in carrot slices (Hoffman and Heale, 1989). Gorst and Spiteller (1988) detected large quantities of LOX-unsaturated fatty acid oxidation compounds, 10-hydroxy-octadeca-8,12-dienoic acid and 10-hydroxyoctadeca-8-enoic acid, in aqueous homogenates of freshly picked strawberries. These compounds possess fungi toxic activity and appear to be involved in the self-defense mechanism of the plant.

LIPOXYGENASE MEDIATED AROMA AND FLAVOR COMPOUNDS

Aroma compounds in fresh-cut fruits and vegetables and the involvement of LOX in flavor biogenesis are reviewed in Chapter 12. Flavor production by LOX pathway is generally quiescent unless triggered by maceration or cell damage (Gardner, 1989). Free radical intermediates in the reaction damage biological membranes (Eskin et al., 1977), and a pool of free fatty acids results from lipid hydrolysis (Biale, 1975). In cauliflower florets (*Brassica oleracea* L., *Botrytis* group), LOX-mediated increases in the lipid phosphate-to-protein ratio occur via free radical intermediates (Voisine et al., 1991). In envelope membranes from spinach (*Spinacia oleracea* L.) chloroplasts, LOX catalyzes the rapid breakdown of fatty acid hydroperoxides (Blee and

Joyard, 1996). Fatty acids are quantitatively the major precursors of volatile compounds responsible for the aroma of plant products. Typical flavors generated by LOX were demonstrated by treating LOX extracts from green sea algae (*Enteromorpha intestinalis*) with linolenic acid (Kuo et al., 1996). Fresh apple-like, green, cucumber, mango and algal aromas were found in the volatile concentrates. The addition of LOX to green bean puree also caused flavor and aroma changes described as “unripe banana,” “grassy,” “straw” and “ammonia” (Williams et al., 1986). LOX contributed to sourness in the puree. Lactones that are important flavors of peaches and apricots are produced via LOX activity (Crouzet et al., 1990; Sevenants and Jennings, 1966). LOX pathway products from lettuce (*Lettuca sativa* L.) are primarily *cis*-3-hexenal, *cis*-3-hexenol and *cis*-3-hexenyl-acetate (Charon et al., 1996). In both green and red bell pepper homogenates, the addition of linoleic acid considerably increased the levels of hexenal and hexanol, whereas the levels of *cis*-3-hexenal and *trans*-2-hexenal are markedly enhanced by the addition of linolenic acid (Luning et al., 1995). In mushrooms (*Agaricus bisporus*), a clear relationship exists between the production of 1-octen-3-ol and LOX activity (Belinky et al., 1994).

LIPOXYGENASE AND PHENOLIC COMPOUNDS

Phenolic compounds inhibit LOX activity and have the potential to prevent LOX-mediated oxidation of carotenoids in vegetables (Oszmianski and Lee, 1990). Catechin and epicatechin have the highest inhibitory potency, and *p*-coumaric and ferulic acids exhibit the lowest inhibitory efficacy. In general, flavans show the highest inhibitory effect followed by flavonols and acidic phenolic compounds. Thus, the concentration of phenolics appears to have a high correlation with the prevention of carotene bleaching. The mechanism of LOX inhibition seems to be due to reduction of the free radical intermediates formed during lipoxygenation (Takahama, 1985).

LIPOXYGENASE IN FRUITS AND VEGETABLES

Apple

Unsaturated straight-chain ester volatiles appear to arise in apples only by the LOX pathway (Rowan et al., 1999). An investigation of the biosynthesis of *R*-octane and *R*-5-*cis* octene-1,3-diol, two naturally occurring antimicrobial agents in apples and pears after application of labeled LOX substrates and LOX-derived metabolites showed that almost all precursors applied were partly transformed into *R*-octane-1,3-diol (Beuerle and Schwab, 1999). Linolenic acid derivatives, still containing the 12,13-*cis* double bond and octanol derivatives oxy-functionalized at carbon 3 were the most efficient precursors of the 1,3-diol. The relationship between mineral content, ethylene biosynthesis and LOX activity in ‘Jonagold’ apple is one in which fruits rich in calcium and/or phosphorous have lower LOX activity, 1-aminocyclopropane-1-carboxylic acid (ACC) content and ethylene emission, while fruits rich in potassium and/or magnesium with relatively high K/Ca have a higher LOX activity, ACC level and ethylene emission. The fruit mineral content is an agent acting on LOX activity and the ethylene-forming system with a consequent effect on storage capacity (Marcelle, 1991).

Asparagus

Asparagus LOX utilizes only monolinolein and linoleic acid as substrates but exhibits low activity with di- or tri-linolein substrates. The enzyme has a pH activity optimum of 5.5–6.0 and is stable at pH 4.5–8.0 when stored at 2°C (Ganthavorn and Powers, 1989). Ganthavorn et al. (1991), based on the first-order kinetics nature of the heat inactivation, suggested that LOX isozymes in asparagus do not have different heat stabilities. However, Pizzocaro et al. (1988) observed a biphasic pattern of heat inactivation and a relatively heat stable LOX in asparagus compared to LOX extracted from beans, carrots and zucchini.

Broccoli

Temperature abuse during storage and handling of broccoli results in reduced polyunsaturated fatty acids (PUFAs) content and LOX activity and increases thiobarbituric acid-reactive substances compared to those stored at 2°C (Zhuang et al., 1994, 1997). Treatment of broccoli heads dipped in hot water (45°C for 14 minutes), however, results in better retention of chlorophyll and soluble protein contents when stored at 20°C. This treatment also lowers LOX and chlorophyllase activities (Kazami et al., 1991). Optimal pH of broccoli floret LOX occurs at 5.5–6.0 (Zhuang et al., 1994). Modified atmosphere packaging (MAP) storage (7.5% CO₂ and 11.2% O₂) of broccoli florets increases chlorophyll (Chl) and reduces LOX-induced degradation of C-18 PUFA (Zhuang et al., 1994). Changes in water-soluble LOX activity in MAP and nonpacked samples follow a trend similar to PUFA. LOX activity, C18 PUFA, C18 PUFA hydroperoxides, total fatty acids, thiobarbituric acid-reactive substances (TBA-RS) and Chl also increase in senescent broccoli flower buds during postharvest storage of broccoli florets at 5°C. Lack of packaging causes significant decreases in moisture, PUFA, LOX activity, Chl and soluble protein, and an increase in thiobarbituric acid-reactive substances (TBA-RS) (Zhuang et al., 1995).

Cucumber

Free and bound polysomes contribute to LOX synthesis, and the isozymes are located in different compartments of the plant cell. The cucumber fruit is often used as a model for extracting and locating organelles (Feussner and Kindl, 1994; Wardale and Lambert, 1980). In the determination of the intracellular location and organellar topology of LOX forms in lipid bodies, microsomes and cytosol, Feussner and Kindl (1994) demonstrated that LOX isozymes occur in different compartments of the plant cell. The catalytic properties of lipid-body and microsomal LOX in cucumber were also found to be different. Optimum pH for lipid-body LOX was 8.5, whereas that of the microsomal LOX was 5.5. Wardale and Lambert (1980) observed high LOX activity in intact cucumber protoplasts from both the peel and flesh tissue of cucumber fruit. Fewer intact vacuoles and decreased LOX activity occurred following osmotic rupture than from macerated tissue. The most active substrates for cucumber LOX are linoleic acid (100%), linolenic acid (77%) and arachidonic acid (23%) with optimum pH and temperature being 5.5 and 40°C, respectively. Calcium, EDTA, dithiothreitol, imidazole, cysteine and *p*-chloromercuribenzoic acid do not inhibit cucumber LOX activity.

Kiwifruit

Kiwifruit LOX contains at least one class II LOX. The enzyme is relatively heat stable and exhibits higher activity relative to other fruits (Boyes et al., 1992). The main fatty acids in ripening kiwifruit were palmitic (16:0), stearic (18:0), oleic (18:1), linoleic (18:2) and linolenic (18:3). LOX activity can be correlated with a decrease in firmness of fruits stored at 20°C. At 0°C, however, LOX is markedly inhibited to less than 30% of those in fruit stored at 20°C, and ripening is delayed (Chen et al., 1999). The pH profile of kiwifruit LOX is one in which two pH maxima (6.2 and 7.6–8.0) occur when linoleic acid is the substrate. Linolenic acid causes a shift in peak activities to 5.75 and 7.4–7.7. Maximum LOX activity of the fruit occurs at 36–46°C.

Melon

Cantaloupe melon (*Cucumis melo reticulatus*) shows no LOX activity in the middle-mesocarp (mesocarp) tissue of all maturities unlike in the hypodermal-mesocarp (hypodermis) (Lester, 1990). Linolenic and linoleic acid in hypodermis tissue and plasma membrane integrity also declined at 30 days post-anthesis. In the hypodermis tissue, LOX appears to play a major role in cantaloupe melon senescence by the production of peroxides from free fatty acids, which in turn, results in the perturbation of membranes. Enzymatic activity increases with ripening and senescence. LOX activity during cantaloupe melon cell division and enlargement periods of development is also higher than in the mature fruit. In hybrid honeydew (*Cucumis melo inodorus* Naud.), LOX activity occurred on both hypodermal- and middle-mesocarp tissues (Lester, 1998). The hypodermal-mesocarp tissues, however, show higher activity than the middle-mesocarp tissues in the mature fruit. Such differences in LOX activities could be attributed to differences in the development of the tissues and in tissue water deficit. Homogenizing the flesh of cantaloupe melon activates LOX and HPO lyase activity and alters the aroma profile appreciably (Wyllie et al., 1994). LOX-derived compounds such as *cis*-2-hexenol are produced concurrently with a substantial loss of esters. Temperature and pH optima for LOX in cantaloupe are 20°C and 7, respectively (Lester, 1990). Hybrid honeydew LOX has only one activity peak at pH 6.8 and is temperature dependent with higher activities at higher temperatures. Increase in LOX activity that coincides with lipid biophysical changes in hybrid honeydew plasma membrane also occurs with a resulting loss in membrane integrity (Lester, 1998).

Strawberry

LOX and HPO lyase in strawberry (*Fragaria X ananassa* Duch.) fruits are located mainly in the micosomal fraction (Perez et al., 1999a). Linolenic acid is the preferred substrate for strawberry LOX, forming 13- and 9-hydroperoxides in the proportion 70:30. HPO lyase in strawberry cleaves 13-hydroperoxides of linoleic acid (13% relative activity) and linolenic acid (100% relative activity) to form hexanal and 3-*cis*-hexenal, respectively. A detrimental effect of ozone treatment on strawberry aroma occurs with a reduced emission of volatile esters in ozonated fruits (Perez et al., 1999b).

Tomato

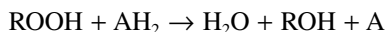
LOX-induced conversion of linolenoyl and alpha-linolenoyl fatty acyl groups to hexanal and hexenal (*cis*-3-hexenal and *trans*-2-hexenal), respectively, during the maceration of tomato fruits greatly contributes to the mix of volatile compounds that determines tomato flavor (Gray et al., 1999). Formation of the C6 aldehydes from C18 polyunsaturated fatty acids (PUFAs) proceeds by way of a sequential enzyme system involving LOX (that preferentially oxygenates at the 9-position) followed by a hydroperoxide cleavage system which is, however, specific for the 13-hydroperoxy position (Gallard and Matthew, 1977). LOX and HPO lyase activities determined in the microsomal fraction, a post-microsomal pellet (PMP) and cytosolic fraction of tomatoes indicated that the microsomal form of LOX had the greatest increase in activity, with fruit development between the mature-green and breaker stages (Riley et al., 1996). Activity declined as the fruit turned red, unlike HPO lyase, in which the microsomal fraction activity dominated in all stages of development. HPO lyase in tomato is insensitive to pH over the range 6–8 and does not show any significant change in activity with ripening. Soluble LOX activity also predominates in the PMP fraction of the fruit for all stages of development. For the subcellular fractions, LOX activity is higher at pH 6 than at pH 7 and pH 8 at all stages of development (Riley et al., 1996).

PEROXIDASE

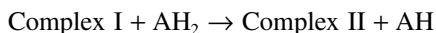
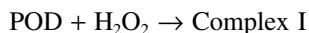
Peroxidases (POD; EC 1.11.1.7) are iron-porphyrin organic catalysts that are widely distributed in plants and seem to be normal components of most plant cells. Most flavor changes in raw and unblanched fruits and vegetables could be correlated to POD activity, and there is an empirical relationship between residual peroxidase activity, off-flavors and off-odors in foods (Burnette, 1977; Loaiza-Velarde et al., 1997; Cano et al., 1998). Changes that occur in POD activity in wounded and fresh-cut fruits and vegetables significantly contribute to their product quality (Svalheim and Robertsen, 1990; Loaiza-Velarde et al., 1997; Lamikanra and Watson, 2001).

PEROXIDASE-MEDIATED REACTIONS

Reactions catalyzed by POD enzymes are typically peroxidatic, oxidatic, catalatic and hydroxylation (Vamos-Vigyazo, 1981). The peroxidatic reaction is the most important. The mechanism of reaction essentially involves an oxidative action by way of an initial formation of a complex intermediate with a hydrogen acceptor. The transfer of hydrogen from a donor substrate results in a second complex intermediate before the regeneration of the POD enzyme and formation of a reaction product. The reaction can be summarized as follows:



ROOH can be HOOH or some other organic peroxide, AH₂ is the hydrogen donor in the reduced form, and A is the hydrogen donor in the oxidized form. The hydrogen donor complexes and two univalent oxidation intermediate steps occur:



Peroxidative reactions could take place by way of a number of reaction intermediates. Although the enzymatic action appears to be capable of proceeding by way of heterolytic pathways (Dixon and Webb, 1964), peroxidase activity could be catalyzed by free radicals and retarded by radical scavengers (Adak et al., 1997). POD enzymes may also enhance radical scavenging properties (Staelin et al., 1992; Wang, 1995). The optimum pH and temperature for peroxidase activity depend on the source of the enzyme. Optimum pH could range from 1.8–8.5 (Lee and Tu, 1995; Rodrigo et al., 1996; Otter and Polle, 1997). Temperature optimum range appears to be 25–60°C (Koshiba and Matsuyama, 1993; Campos et al., 1996), but there are cases reported in which peroxidase activity increased under freezing conditions (Scott, 1975; Cano et al., 1998).

OCCURRENCE AND STABILITY

Peroxidases occur in both soluble and bound forms in fruits and vegetables (Sanchez et al., 1993; Brzyska et al., 1992; DaSilva et al., 1990; Ingham et al., 1998). The POD isoelectric points that vary from 3.5–9.5 evidence the existence of both cationic and anionic forms of POD in plants (McLellan and Robinson, 1983). Bound POD are usually attached by ionic interactions to the cell wall in the intact cell (Giordani, 1977; Thompson et al., 1998), and possibly other organelles such as mitochondria and ribosomes (Hideg et al., 1991; Raa, 1973). Properties of bound POD differ from those of POD isolated from the soluble fraction of the cell (Penon et al., 1970). POD enzymes in vegetables usually have high stability to heat and, because of this, they are often used as the index of effective heat treatment in processed vegetables. Heat stability and regeneration properties of POD are often influenced by whether they are in the soluble or bound state. Ionically bound POD is more heat stable in mango and orange (Khan and Robinson, 1993; McLellan and Robinson, 1984) in contrast with the findings for apple POD (Moulding et al., 1987). In pear, however, both the soluble and ionically bound POD are heat labile with only approximately 2% of the original enzymatic activity remaining after 2 min at 80°C (Moulding et al., 1989). Heat inactivation of peroxidase is nonlinear. A large decrease in activity is observed during the initial stages of a given heat process, but the rate of inactivation then changes to a much slower process (Clemente, 1998; Powers et al., 1984). The biphasic nature of heat inactivation of POD is related to the presence of isozymes with differing heat stabilities in vegetables (Yamamoto et al., 1962). Regeneration of POD following heat inactivation of enzymes from Brussels sprouts and cabbage (McLellan and Robinson, 1981), apple (Moulding et al., 1987), kiwifruit (Prestamo, 1989) and mango (Khan and Robinson, 1993) occurs. POD was completely inactivated after 2–10 min heat treatment of cauliflower at 80–110°C (Bottcher, 1975; Vamos-Vigyazo, 1981), and it takes only 15 min to reduce its POD activity by 98% at 50°C (Lee et al., 1984). A heat-resistant

isozyme in cauliflower that is responsible for less than 5% of its POD activity, however, takes over 30 min to reduce activity to 50% at 50°C (Lee and Pennesi, 1984). Regeneration following heat inactivation of Brussels sprouts was not observed for isolated POD isoenzymes (McLellan and Robinson, 1987a). Arroyo et al. (1999) did not inactivate POD in lettuce, tomato, asparagus, spinach, cauliflower and onion with combined high-pressure and low-temperature treatments. Heat-shock treatments (ranging from 45°C for 120 sec to 55°C for 60 sec), however, caused a decrease of about 30% in POD activity from iceberg lettuce (Loaiza-Velarde et al., 1997).

The location and types of POD enzymes in plant tissues were described by Kvaratskhelia et al. (1997). Plant PODs are classified into two types (Welinder, 1992). Ascorbate peroxidase (APX; class I), which is from the plant chloroplast and cytosol, is distinguished from classical plant POD (class II) isozymes by significant differences in their primary structure. APX is an H_2O_2 -scavenging POD that uses ascorbate as an electron donor in plants and algae (Miyake and Asada, 1996; Asada, 1992). APX occurs in two isoforms with respect to its cellular localization. One isoform of APX is localized in chloroplasts and has been found in both a thylakoid (tAPX) form and a soluble form in the stroma (sAPX). tAPX scavenges the H_2O_2 that is photoproduced in the thylakoids. The other form of APX is localized in the cytosol, and its function seems to be the scavenging of H_2O_2 produced in the cytosol (Miyake and Asada, 1996). During plant development, APX activity is regulated by ascorbate content. Wounding, as in fresh-cut processing, generally leads to an increase in ascorbate biosynthesis and content (Diallinas et al., 1997; Oba et al., 1994). Classical, secretory plant POD that share 50–95% sequence homology within the superfamily of POD (class II) are assigned to class III (Welinder, 1992). Because guaiacol (2-methoxyphenol) is commonly used as a reducing substrate, these enzymes are also referred to as guaiacol-type POD. Most secretory plant POD are glycosylated (Johansson et al., 1992). Unlike guaiacol POD that is characterized by broad specificity with respect to electron donors and participates in many physiological processes, such as the biosynthesis of lignin and ethylene, ascorbate POD exhibits high sensitivity for ascorbate as the electron donor (Asada, 1992; Amako et al., 1994) and is specific in its physiological role in scavenging potentially harmful H_2O_2 (Dalton et al., 1998) and free radicals (Wang, 1995).

In the presence of flavonoids, guaiacol POD can participate in scavenging H_2O_2 both *in vivo* and *in vitro* by catalyzing the H_2O_2 dependent oxidation of flavonoids. In the flavonol-guaiacol POD reaction, ascorbate had the potential to regenerate flavonols by reducing the oxidized product (Yamasaki et al., 1997). The sensitivity of ascorbate POD to thiol reagents (Chen and Asada, 1992) and *p*-chloromercuribenzoate (Amako et al., 1994) distinguishes it from guaiacol POD. Complex I formed by APX in a POD peroxidatic reaction pathway is extremely unstable and is rapidly converted to complex II without the addition of a reducing substrate (Patterson et al., 1995), whereas guaiacol POD are characterized by stable complex I.

EFFECT OF PEROXIDASE ON RIPENING AND SENESCENCE

An important function of POD is related to its role in indole acetic acid (IAA) oxidation action, by which it participates in growth regulation (Acosta et al., 1986; Grambow, 1986). Peroxidases are considered to be indices of ripening and senescence (Haard, 1973;

Scott, 1975; Vamos-Vigyazo et al., 1981). The induction of POD isoenzymes during ethylene-induced senescence is a common response in cultivars of *C. sativus*, other species of *Cucumis* and other genera of Cucurbitaceae (Abeles et al., 1989). In fruits of climacteric ripening, POD and IAA oxidase isozymes were reinforced with progressing maturity, while in nonclimacteric fruits, where ethylene did not noticeably change during ripening, only IAA oxidase isozyme concentration increased, while the POD isoenzyme concentration decreased (Vamos-Vigyazo, 1981). In developing peaches, two POD activity peaks corresponded to the two periods of weight gain (Flurkey and Jen, 1978). The role played by POD in the cell wall degradation of tomato was demonstrated by Araujo (1985). Application of POD substrates including IAA, NADH, KI and Na_2SO_3 led to an increase in weight loss of ripening tomato tissue. 7-Hydroxy-2,2-dimethyl-2,3-dihydrobenzofuran inhibited tissue degradation. POD activity increased and IAA content decreased during development and senescence of Chinese cabbage, cabbage and spinach (Zhang and Zong, 1988). In mangoes, POD activity increased in pulp tissues up to the climacteric stage of ripening. In a parallel way, the quantity of extractable proteins decreased from preclimacteric to ripe or senescent fruits. Changes in the three POD isozymes appear to be related to the ripening process (Marin and Cano, 1992). The activities of POD and chlorophyllase in the peel of the ripe green mango fruit were also about half of the ripe yellow fruit (Ketsa et al., 1999). Changes in POD activities corresponded to the climacteric and the onset of senescence in 'Golden Delicious' apples (Gorin and Heidema, 1976). Maximum specific POD activities also occur at the "small green" and "large green" ripening stages of strawberry (Civello et al., 1995). Kinetin, a cytokinin involved in respiration and ethylene production, however, had no catalytic effect on cantaloupe melon POD (Lamikanra and Watson, 2000). Guaiacol POD appears to be the main POD class involved in lignification of the cell wall, degradation of IAA, biosynthesis of ethylene, wound healing and defense against pathogens (Gazaryan et al., 1996; Kobayashi et al., 1996).

EFFECT OF PEROXIDASE ON PLANT DEFENSE RESPONSES

POD is thought to be important in a variety of plant defense responses against pathogens. The involvement in POD plant defense mechanism is related to its role in the shikimate intermediate pathway in the synthesis of aromatic amino acids, indole acetic acid (auxins), cinnamic acids (precursors of phenylpropanoid phytoalexins), coumarins and lignins (Biles and Martyn, 1993). Other enzymes involved in the shikimate pathway, such as PAL, chalcone isomerase, polyphenol oxidase and shikimate dehydrogenase, are also associated with plant defense mechanisms or senescence. POD activity increased more rapidly in resistant than susceptible cucumber hypocotyls after inoculation with the pathogens *Clasodopodium cucumerinum* Ellis and Arth. (Svalheim and Robertsen, 1990) and *Colletotrichum lagenarium* (Pass.) Ell. and Hals. (Hammerschmidt and Kue, 1982). The pattern of isozymes that was induced by fungal infection or resistant hypocotyls was similar to the pattern of isozymes induced by wounding. POD enzymes from watermelon, muskmelon and cucumber induced with *C. lagenarium* were found to be antigenically and electrophoretically similar (Smith and Hammerschmidt, 1988). However, based on the inability of ethylene to induce resistance, and the inability of silver thiosulfate to increase susceptibility, Biles

et al. (1990) concluded that POD does not appear to play a role in limiting disease development in the cucumber-*C. lagenarium* system, although ethylene action still appeared to be necessary for lesion development and senescence. Pathogen and wound-induced POD activity have been demonstrated in fruits and vegetables (Biles and Martyn, 1993; Mohan et al., 1993; Madi and Katan, 1998; Mayer and Harel, 1979; Miyazawa et al., 1998; Moran, 1998; Sutha et al., 1996). Storage stability appears to be related to POD activity in cut vegetables. Baardseth and Slinde (1980) reported a correlation between shelf lives of cut vegetables and their POD and catalase (CAT) activities. Swede (*Brassica napus* L.), which has long storage stability in the cut state, has a high activity of POD and CAT relative to cut carrot with medium POD and CAT activity. Activity of POD and CAT were relatively low in Brussels sprouts. In addition to the relative activities of the enzymes in these vegetables, swede also contains the highest amount of ascorbic acid per milligram of protein. Ascorbic acid, acting as a reducing agent and endogenous substrate to the respiratory chains that give ATP, could also contribute significantly to the stability of cut swede. Increased POD activity has also been correlated with lignification, pathogenesis-related proteins, hydroxyproline-rich glycoproteins and suberization (Biles and Martyn, 1993). POD activity, particularly APX activity, could be indicative of oxidative stress in plant tissues (Gueta-Dahan et al., 1997; Kampfenkel et al., 1995). POD activities in cantaloupe melon, strawberry and lettuce were also suggested to be indicative of the relative levels of oxidative stress in the fresh-cut products (Lamikanra and Watson, 2000).

PEROXIDASE-CATALYZED BROWNING

The ability of POD to contribute to enzymatic browning is related to its affinity to accept a wide range of hydrogen donors, such as polyphenols (Richard-Forget and Gauillard, 1997). They are able to oxidize catechins (Lopez-Serrano and Ros-Barcelo, 1997), hydroxycinnamic acid derivatives and flavans (Robinson, 1991; Nicholas et al., 1994) and flavonoids (Miller and Schreier, 1985; Richard and Nicolas, 1989; Richard-Forget and Gauillard, 1997). Two possible mechanisms are proposed for POD-catalyzed browning reactions. One involves the generation of H_2O_2 during the oxidation of some phenolic compounds that is used as in a normal peroxidatic action to further oxidize the phenol, while the second involves the use of quinonic forms as substrate by POD. Both mechanisms indicate that the presence of polyphenol oxidase enzyme (EC 1.14.18.1) would enhance POD-mediated browning reactions (Richard-Forget and Gauillard, 1997). POD oxidizes (+) catechin at low H_2O_2 concentrations via the formation of complex I and complex II. In this reaction, (+) catechin is also an excellent *o*-diphenol for complex II reduction (Lopez-Serrano and Ros-Barcelo, 1997).

PEROXIDASE IN FRUITS AND VEGETABLES

Apple

POD activity increases with ripeness of apples with a peak in activity around the climacteric stage of ripening in the peel and pulp tissues (Prabha and Patwardhan, 1986; Kumar and Goswami, 1986). Activity also increases during controlled atmosphere storage (Gorin and Heidema, 1976) and apparently during normal cold storage

of apples, although variations occur with cultivar (Vamos-Vigyazo, 1981). The isozyme patterns differ among cultivars and could be used for cultivar differentiation (Barnes, 1993; Manganaris and Alston, 1993).

Asparagus

Basic isozymes of isoelectric point (pI) greater than nine predominate in asparagus POD. The more acidic fractions are found in the spear tip (Powers et al., 1984). Optimum activity for green asparagus POD occurred at pH 7. The enzyme regenerated after being subjected to temperatures between 90 and 125°C for short periods of time. The regenerated asparagus POD reached its maximum activity after being stored six days at 25°C (Rodrigo et al., 1996, 1997). The basic and acidic fractions are similar in heat stability, although the acidic fraction is more readily regenerated after heat treatment at 70°C (Powers et al., 1984).

Broccoli

Toivonen and Sweeney (1998) demonstrated the importance of antioxidant protection offered by POD and superoxide dismutase (SOD) to retention of green color in broccoli. POD and SOD activities were approximately 30% higher in 'Greenbelt,' a cultivar with a low yellowing susceptibility, than in the more susceptible 'Emperor' cultivar. The ratio of superoxide dismutase to peroxidase activity was also lower in 'Greenbelt.' POD and SOD are also involved in the enhanced lipid peroxidation in cell membranes that occurs under drought stress. With the time and intensity of drought stress, activities of SOD and POD decreased during the first two days, increased during days two through six, then decreased during days six through eight of drought stress (Yang and Yang, 1998).

There are no differences in POD, ascorbate oxidase and texture between MAP packed and nonpacked broccoli (Barth et al., 1993a). Reduced ascorbic acid retention, moisture content, total chlorophyll and color retention in broccoli are, however, greater when packaged in an atmosphere containing 8% CO₂ and 10% O₂. Barth et al. (1993b) observed lower POD activity in broccoli spears packaged using a semipermeable polymeric film and stored 96 h at 20°C relative to nonpackaged broccoli spears. Ascorbic acid, chlorophyll and moisture retention were also greater in packaged broccoli. A 1.5% O₂ atmosphere relative to normal air inhibits ionically bound POD activity in iceberg lettuce (Ke and Saltveit, 1989a). Packaging of stored lettuce under this atmosphere also inhibits ethylene and respiration, ethylene-induced russet spot development and PAL and indoleacetic acid (IAA) activities, and it reduces soluble phenolic content in the vegetable. Exposure of lettuce tissue to elevated CO₂ increased ionically bound insoluble POD and ionically bound IAA oxidase activity but reduced soluble IAA oxidase activity (Ke and Saltveit, 1989b). The use of a test strip ethanol biosensor composed of a chromogen and immobilized enzymes (POD and alcohol oxidase) to detect quality changes in improperly designed modified atmosphere packages that result in low injurious O₂ levels or temperature abuse was reported by Smyth et al. (1999). The biosensor was effective in detecting low O₂ injury in MAP samples of cut broccoli, cauliflower, lettuce and shredded cabbage packages.

Carrot

Surface discoloration on cut carrots occurs concurrently with increased levels of soluble phenolics, lignin POD, phenylalanine ammonia lyase (PAL) and syringaldazine oxidase (SOX) activities over time. Steam treatment and subsequent storage at 2°C retard surface discoloration by inhibiting phenylpropanoid metabolism. Soluble phenolic and isocoumarin production and lignin formation were inhibited, and POD, PAL and SOX were inactivated in treated cut carrots (Howard et al., 1994). In a study to determine the effects of an ethylene absorbent and storage at 2°C on surface discoloration and phenolic changes in MAP carrot sticks, lignin formation also occurred and corresponded with development of white discoloration in storage. Slicing stimulated PAL and POD activity, and both enzyme activities remained elevated during storage (Howard and Griffin, 1993).

Cucumber

Anodic POD isozymes from cucumber fruit vary widely in their substrate specificity but react preferentially with aromatic amines (Miller et al., 1990). In spinach, degradation of chlorophyll appears to be regulated through the peroxidase-hydrogen peroxide pathway, which opens the porphyrin ring, thus resulting in a colorless compound (Yamauchi and Watada, 1991). An anionic isozyme in cabbage with an isoelectric point (pI) of 3.7 and was relatively heat stable, while a cationic isozyme, pI 9.9, was more readily inactivated by heat (McLellan and Robinson, 1987b). Low-temperature storage reduces browning and PAL activity but has no effect on POD activity in shredded cabbage (Takahashi et al., 1996).

Mango

A number of POD isozymes have been extracted from mangoes. Khan and Robinson (1994) reported four POD isozymes—two anionic and the other two cationic. The anionic isozymes have higher molecular weights of 40 and 44 kDa, respectively, than cationic isozymes with molecular weights of 22 and 27 kDa (Khan and Robinson, 1994). Marin and Cano (1992) found three isoenzymes of POD in the extracts from ‘Smith’ mangoes that moved toward the anode at pH 8.3. The isoenzyme pattern of POD of ‘Lippens’ mango extracts was significantly different, showing four faster moving bands in addition to the three characteristic bands of ‘Smith’ mango fruits. Pulp POD activity increases with degree of ripeness in mangoes (Marin and Cano, 1992; Prabha and Patwardhan, 1986).

Melon

Cantaloupe melon POD activity appears to be consistent with that of ascorbate peroxidase based on the ascorbate inhibitor when guaiacol is used as substrate and inhibition by β -mercaptoethanol, L-cysteine and *p*-chloromercuribenzoate (Lamikanra and Watson, 2000, 2001). The optimum activity temperature for cantaloupe melon enzyme was 50–55°C. The enzyme was stable at temperatures below 40°C and at 50°C for up to 10 min. Over 90% of total activity was lost at 80°C within 5 min. The broad pH optima, 5.5–7.5 at 50°C and 6–7 at 30°C, are indicative of the presence

of more than one POD isozyme in the fruit (Lamikanra and Watson, 2000). Two native electrophoretic POD bands with approximate molecular weights of 240 and 170 kDa, respectively, comprised of six acidic subunits (pI 5.1–6.1) were present in cantaloupe (Lamikanra and Watson, 2001). POD activity in cut cantaloupe is inhibited by ascorbic acid. The inhibitory effect appears to be related to an intermediate ascorbate-POD complex. The ascorbate-POD exhibits antioxidant properties that involve trace metal ion cofactors. This enhanced antioxidant property protects β -carotene and extends product shelf life.

Honeydew melon and watermelon POD exhibit optimum activities at around pH 7 and 6.5, respectively. While the former has its maximum activity at 50°C, POD in watermelon is relatively more stable at higher temperatures than honeydew melon, pineapple and kiwifruit, and has an optimum activity temperature of about 70°C (Lamikanra and Watson, unpublished).

Orange

Orange contains a latent POD, which catalyzes the oxidation of a number of the fruit's constituents by H_2O_2 . Mn^{++} was required for the activity of POD as an oxidase with O_2 (Bruemmer et al., 1976). In the peroxidase H_2O_2 system, the most reactive constituent compounds were ascorbic, caffeic and gentisic acids. Quercetin was unreactive in this assay, unlike POD from some fruits and vegetables (Hemeda and Klein, 1991). In the O_2 and Mn^{++} system, ascorbic acid and catechol and *p*-phenylene diamine were unreactive. In orange juice, however, POD activity is very low, apparently because of the low H_2O_2 content of the fruit (Bruemmer et al., 1976). Cationic and anionic POD with pI values ranging between 4.5 and 9.0 and molecular weights 22–44 kDa were also isolated from oranges (Clemente, 1998).

Peach

POD activity changes with the three stages of development in peaches. The soluble POD activity is highest in the mesocarp and the exocarp at stage II, and isoenzymatic changes in these tissues corresponded to the transition from cationic isoenzymes, predominant at stage I, to anionic isoenzymes at stage III. The ionically bound peroxidase activity in these tissues is highest at stage I (Sanchez-Roldan et al., 1990). The endocarp of these drupes becomes lignified, while the mesocarp remains parenchymatous with fruit development. Acidic POD from lignifying endocarp are similar to those of the fleshy mesocarp. The endocarp has a larger amount and number of basic POD than the mesocarp (Abeles and Biles, 1991).

Pear

The influence of harvest date on the occurrence of physiological disorders in pears might be related to the nonenzymic and enzymic systems responsible for catabolism of active oxygen species. Pears (cv. 'Conference') harvested seven days after the ideal time for commercial harvest maintained a constant POD activity relative to those harvested on time, but APX increased 2.5-fold. Concomitantly, the activity of SOD and CAT fell about fivefold and twofold, respectively, when the fruit was

picked more mature, indicating a higher potential for the accumulation of cytotoxic O_2^- and H_2O_2 , respectively (Lentheric et al., 1999). POD activity is unevenly distributed in the flesh of pear cultivars, and the degree of inhomogeneity differs in each cultivar (Vamos-Vigyazo and Nadudvari-Markus, 1982). Localization of POD on the cellular particulate fraction is associated with sclereid development. Mineral nutrition in the fruit may profoundly alter lignin metabolism by affecting the localization and, hence, the metabolic control of POD (Ranadive and Haard, 1972). POD isozyme patterns in pear are cultivar specific, and this could be used for varietal identification (Santamour and Demuth, 1980; Hudina et al., 1996). Pear POD is actively involved in its enzymatic browning reactions (Richard-Forget and Gauillard, 1997), although the level of POD cannot be used as an indicator of browning potential (Vamos-Vigyazo and Nadudvari-Markus, 1982; Zhang et al., 1994).

Strawberry

Strawberry fruit POD activity decreases remarkably with fruit ripening, and the enzyme is found primarily in a membrane-bound form (Civello et al., 1995). The enzyme shows low thermal stability, and maximum enzyme activity occurs at 30°C and pH 6.0. Two basic POD isozymes of molecular masses 58.1 and 65.5 kDa were detected at different maturities of the fruit by Civello et al. (1995). Lopez-Serrano and Ros-Barcelo (1995, 1996, 1997), however, found one basic POD isozyme of high isoelectric point in process-ripe strawberry fruit, which is the only component of POD polymorphism in the whole fruit. This isozyme is capable of oxidizing phenols only in the presence of H_2O_2 and lacks catecholase, cresolase and laccase activities.

Zucchini Squash

Acclimation to chilling temperature in squash may be involved in the activities of POD, along with a number of free radical scavenging enzymes. The development of chilling injury symptoms in zucchini squash (*Cucurbita pepo* L.) stored at 5°C was delayed by preconditioning the fruit at a temperature of 15°C for two days (Wang, 1995). Temperature preconditioning treatment suppressed the increase in peroxidase activity and reduced the decline of CAT activity in squash during subsequent storage at 5°C. SOD activity also remained higher in temperature-conditioned squash than in the untreated squash throughout storage. The activities of ascorbate free radical reductase, ascorbate peroxidase and dehydroascorbate reductase increased initially after storage for four to eight days and declined thereafter in control and preconditioned fruits, but enzyme activities increased to a greater extent and remained higher in preconditioned fruits than in the controls (Wang, 1996).

Other Fruits and Vegetables

POD isozymes in carrot, tomato, kiwifruit, cauliflower, green beans and horseradish are comprised of three and six isozymes, respectively, in carrot (36–70 kDa) and tomato (38–62 kDa), one in cauliflower (70 kDa), two in kiwifruit (45–43 kDa) and a range of isozymes (36–120 kDa) in horseradish (Prestamo and Manzano, 1993). Ascorbic acid inhibits POD activity in the extracts.

POLYPHENOL OXIDASE

OCCURRENCE AND DISTRIBUTION

Polyphenol oxidase (PPO) is comprised of a group of copper protein complex enzymes that catalyze the oxidation of phenolic compounds to produce brown pigments at cut or damaged surfaces of fruits and vegetables. In plants, PPO is predominantly located in the chloroplast thylakoid membranes (Sherman et al., 1991; Hind et al., 1995) and can exist in an active state or a latent state (Mayer and Harel, 1979). The protein precursor of the enzyme contains the targeting sequence necessary for import into the chloroplast and insertion in the thylakoid membrane (Sommer et al., 1994). Lax and Cary (1995) reported that PPO is synthesized as precursor proteins having a transit sequence for transport into the chloroplast but appear to lack sequences for specific targeting into the thylakoid membrane. The distribution of PPO in different parts of fruits and vegetables as well as ratios of particle-bound and soluble enzymes with maturity vary considerably. Reports on the distribution of PPO in apples, for example, appear to be inconsistent. Although present in all parts of the fruit, some reports (Harel et al., 1964; Stelzig et al., 1972) indicate substantially higher levels in the peel than in the flesh, while others (Klein, 1987; Janovitz et al., 1989) found higher PPO in the cortex than in the peel. Murata et al. (1993) demonstrated that PPO enzymes in five apple cultivars are mainly localized near the core and secondarily near the skin. During ripening, the concentration of particulate enzymes decreased with the concurrent appearance of a soluble fraction. In mature apple fruits, where vacuoles occupy most of the cells, PPO was detected immunochemically near the cell walls with use of anti-apple PPO antibodies. In cells of immature fruits and tissue culture, PPO was detected in organelles other than the vacuoles, presumably in plastids (Murata et al., 1997). Clarified juices from pear were practically devoid of PPO activity, and PPO activity remained almost entirely in the pulp (Vamos-Vigyazo, 1981). In green leaves, PPO is predominantly located in the chloroplasts (Golbeck and Cammarata, 1981; Chazarra et al., 1999).

POLYPHENOL OXIDASE-MEDIATED BROWNING REACTIONS

PPO action usually results in the formation of highly reactive quinones that can then react with amino and sulfhydryl groups of proteins and enzymes as well as with other substrates, such as chlorogenic acid derivatives and flavonoids (catechins, anthocyanins, leucoanthocyanidins, flavonols and cinnamic acid derivatives). These secondary reactions may bring about changes in physical, chemical and nutritional characteristics and may also affect the sensory properties of fruits and vegetables. Quinones also contribute to the formation of brown pigments by participating in polymerization and condensation reactions with proteins (Shahidi and Naczki, 1995; Mayer and Harel, 1979; Mathew and Parpia, 1971; Vamos-Vigyazo, 1981).

PPO enzymes are classified based on substrate specificity. They include monophenol monooxygenase, cresolase or tyrosinase (EC 1.14.18.1), diphenol oxidase, catechol oxidase or diphenol oxygen oxidoreductase (EC 1.10.3.2) and laccase or *p*-diphenol oxygen oxidoreductase (EC 1.10.3.1). Formation of *o*-quinones from

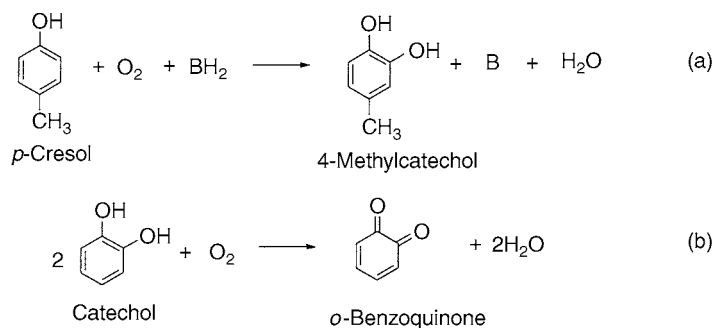


FIGURE 6.2 (a) Oxidation of monophenol to diphenol and (b) oxidation of diphenol to a quinone (Whitaker and Lee, 1995).

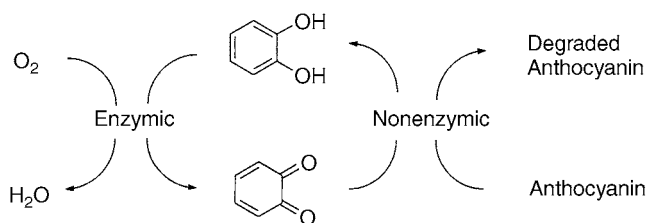


FIGURE 6.3 Mechanism of catechol and phenolase induced degradation of anthocyanin (Shahidi and Naczki, 1995).

monophenols, for example, proceeds by way of monophenolase (creolase) catalyzed hydroxylation to *o*-diphenols followed by diphenolase (catecholase) catalyzed oxidation of *o*-phenols to *o*-quinones (Figure 6.2). BH₂ stands for an *o*-diphenolic compound. The first activity shows a lag period (Chazarra et al., 1999; Perez-Gilabert and Garcia-Carmona, 2000) that has been explained by taking into account the chemical steps of tyrosinase action that are necessary for the production of *o*-diphenol (Cabanes et al., 1987), whereas the catecholase activity shows no slow transition phenomena (Perez-Gilabert and Garcia-Carmona, 2000). Depending on the phenol, *o*-quinones also show great differences in stability, and color intensities vary widely from one phenol to the other (Lee and Jaworski, 1988; Rouet-Mayer et al., 1990). Action of PPO on substrates results in decrease of activity, some loss of copper from the active site of PPO, and modification of one or more of the histidine residues that ligand copper (Osuga and Whitaker, 1995). Peach, pear and banana PPOs do not have creolase activity (Osuga and Whitaker, 1995). *o*-Quinones may also contribute to PPO-accelerated degradation of anthocyanins into colorless products. The degradation mechanism involves PPO and catechol in a sequential process (Figure 6.3). Eggplant anthocyanins, for example, are rapidly degraded by phenolases in the presence of catechin and chlorogenic acid (Sakamura et al., 1966). PPO extract from strawberry and *D*-catechin together also caused a 50–60% loss in anthocyanin pigments after 24 h at room temperature (Wesche-Ebeling and Montgomery, 1990).

Wounding and ethylene production induce PAL activities apparently by separate mechanisms (Abeles et al., 1992; Lopez-Galvez et al., 1996; Hyodo and Fujinami, 1989; Ke and Saltveit, 1989a). PAL is the first committed enzyme and catalyzes the rate-limiting step in phenylpropanoid metabolism that produces phenolase-catalyzed oxidizable substrates (Ke and Saltveit, 1989a; Martinez and Whitaker, 1995). PPO is activated as a result of disruption of cell integrity and when the content of the plasid and vacuole are mixed. Wounding apparently first induces an increase in PAL activity and, consequently, an increase in oxidizable substrates.

EFFECT OF POLYPHENOL OXIDASE ON PLANT TISSUE DEFENSE MECHANISM

Active PPO appears to be present in all photosynthetic organisms and performs some essential functions in plants, including deterrence of insects and fungal pathogens (Manibhushanrao et al., 1988; Hoagland, 1990; Sherman et al., 1995). The antisense downregulation of constitutive and induced PPO expression results in hypersusceptibility to pathogens in tomato, suggesting a critical role for PPO-mediated phenolic oxidation in plant defense (Thipyapong and Steffens, 1997). Increased activities of PPO, POD, LOX, chitinase and alpha-glucosidase were also detected in cucumber leaves in the vicinity of lesions caused by pathogens or phosphate application (Avdiushko et al., 1993b). Evidence of PPO involvement in the deterrence of pathogens in other fruits and vegetables include apple (Sharma and Kaul, 1996), mushroom (Jolivet et al., 1998), pepper and eggplant (Ouf et al., 1998).

INHIBITION OF POLYPHENOL OXIDASE-MEDIATED BROWNING REACTIONS

Considerable research on the conditions under which PPO action is inhibited or retarded in food systems and on the compounds that have inhibitive effects on PPO activity have been conducted. Inhibitors of PPO-catalyzed browning can act by way of a combination of reaction pathways. Some compounds prevent melanosis by direct reducing action on PPO (Kahn and Andrawis, 1986; Sayavedra-Soto and Montgomery, 1986), by reducing *o*-quinones to diphenols (Golan-Goldhirsh and Whitaker, 1984; Richard-Forget et al., 1992) or Cu^{2+} to Cu^+ (Hsu et al., 1988) by interaction with the formation of *o*-quinone products (Embs and Markakis, 1965; Sanchez-Ferrer et al., 1989) or by decreasing uptake of O_2 for the reaction (Embs and Markakis, 1965; Chen et al., 1991a). A number of PPO-catalyzed reaction inhibitors can act by more than one mechanism. Thiol compounds, for example, can act by reducing the active PPO site Cu^{2+} to Cu^+ that is more readily lost from PPO (Osuga and Whitaker, 1995), direct inhibitory actions on PPO (Dudley and Hotchkiss, 1989; Robert et al., 1996), reduction of *o*-quinone (Kahn, 1985; Richard-Forget et al., 1992) or by complexing with quinones to form colorless compounds (Richard et al., 1991; Richard-Forget et al., 1992). Ascorbate acts as a less-effective reducing agent than cysteine (Janovitz-Klapp et al., 1990) and converts *o*-quinones back to their corresponding *o*-diphenols. It also inactivates PPO directly and, in the presence of low levels of copper, can aerobically destroy histidine residues of PPO, releasing copper by a free radical mechanism (Osuga and Whitaker, 1995). Sodium erythorbate shows no inhibitory effect on PPO, but its

prevention of browning is due to reduction of the quinones formed in the enzymatic reaction back to original phenolic compounds (Gopalan et al., 1999). Benzoic acid, kojic acid [5-hydroxy-2-(hydroxymethyl)- γ -pyrone] and carboxylic acids of the cinnamic series have also been demonstrated to show inhibitory effects on PPO in fruits and vegetables (Vamos-Vigyazo, 1981; Chen et al., 1991b).

The inhibitory effect of calcium appears to be related to its preservation of membrane structure or direct inhibition of PPO. Stem browning in lettuce can be reduced by washing stem disks with solutions of 0.3 M calcium chloride, 0.1 mM 2,4-dichlorophenoxyacetic acid (2,4-D) or 0.5 M acetic acid (Thomas-Barberan et al., 1997). Calcium also caused a 60% decrease in PAL activity but had no substantial effect on the accumulation of phenolic compounds. Unlike acetic acid that irreversibly inhibited PAL activity, 2,4-D had a 60% inhibition. It also had an inhibitive effect on the biosynthesis of phenolic compounds. A mixture of vinegar and acetic acid solution could also be used to prevent lettuce butt discoloration (Castaner et al., 1996). Cysteine, resorcinol, EDTA and citric acid are also effective in the prevention of lettuce browning during storage.

Kahn (1985) demonstrated the inability of casein hydrolyzate and bovine serum albumin to inhibit mushroom (*Agaricus bisporus*) PPO activity. L-Lysine, glycine, L-histidine and L-phenylalanine (in increasing order of effectiveness) inhibited activity by up to 60%; L-cysteine at 0.4 mM gave full inhibition. Glycine, diglycine and triglycine (in increasing order) were effective in lowering the final level of colored melanin formed by the action of mushroom PPO on dehydroxyphenylalanine. Treatment of mushrooms with 4-hexylresorcinol was reported to induce discoloration of mushrooms. The combination of 4.5% sodium erythorbate, 0.1% cysteine HCl and 100 ppm EDTA, adjusted to pH 5.5, was quite effective in preventing browning (Sapers et al., 1995). Monophenolase and diphenolase activities of mushroom PPO are inhibited by agaritine [β -N-(γ -L(+)-glutamyl)-4-hydroxymethylphenylhydrazine]. The inhibitory effect is not as much as tropolone but is comparable with those of L-mimosine and benzoic acid, and more potent than azelaic acid (Cabanes et al., 1996). Agaritine, an abundant and characteristic compound from the *Agaricus* genus also removes the enzymatically generated *o*-quinones. It could thus be suggested that agaritine plays a role *in vivo* in the endogenous regulation of mushroom PPO activity and *o*-quinone concentration (Espin et al., 1998a).

The following is a recent review (Beaulieu and Gorny, 2002) on the effectiveness of some anti-browning compounds on fresh-cut fruits. Ascorbic acid was found to be more effective than erythorbic acid in preventing surface browning in 'Winesap' and 'Red Delicious' apple plugs stored 24 h at room temperature, and 1% citric acid enhanced their effectiveness (Sapers and Zoilkowski, 1987). Browning was restricted effectively in stored (4.4°C) vacuum-packed carambola slices that were treated with 1 or 2.5% citric acid plus 0.25% ascorbic acid (Weller et al., 1997). Ascorbic acid-2-phosphate and ascorbic acid-2-triphosphate treatments also decreased browning in 'Red Delicious' apple plugs for 24 hours at room temperature (Sapers et al., 1989). 'Fuji' apple slices treated with 2% ascorbic acid had no significant browning or loss of visual quality for up to 15 days when stored at 10°C in 0.25 kPa O₂ (Gil et al., 1998). Calcium, in combination with ascorbic acid (both generally applied as 1% dips), was highly effective in preventing discoloration of fresh-cut apples

(Ponting et al., 1972) and pears (Gorny et al., 1998; Rosen and Kader, 1989). Browning was retarded in slightly under-ripe 'Bartlett' and 'd'Anjou' pears treated with sodium erythorbate, CaCl_2 and 4-hexylresorcinol after 14 days of storage at 4°C, however, fresh-cut 'Bosc' pears suffered severe browning irrespective of inhibitor treatment (Sapers and Miller, 1998). A post-cutting dip with 0.01% 4-hexylresorcinol, 0.5% ascorbic acid and 1% calcium lactate extended shelf-life of 'Anjou', 'Bartlett' and 'Bosc' pear slices for 15 to 30 days (Dong et al., 2000). 'Red Delicious' apple slices treated with a combined anti-browning dip (4-hexylresorcinol, isoascorbic acid, *N*-acetylcysteine and calcium propionate) and held at 5°C maintained visual quality for five weeks, yet microbial decay was evident after four weeks (Buta et al., 1999). Analyses of organic acids and the major sugars revealed that the slices treated with the combinations of anti-browning compounds retained higher levels of malic acid and had no deterioration in sugar levels at 5 and 10°C, indicating that higher quality was maintained during storage. Browning was significantly reduced in fresh-cut banana slices treated with citric acid (0.5 M) and *N*-acetylcysteine (0.05 M) that were stored at 5°C or 15°C for seven days, and no microbial decay was observed during the seven-day storage (Moline et al., 1999). A combination of 0.5% carrageenan and 0.5% citric acid also inhibited browning in stored diced 'Granny Smith' and 'Red Delicious' apples for seven to nine days at 3°C (Tong and Hicks, 1991). When cysteine is used as an inhibitor of enzymatic browning on sliced apples (Walker and Reddish, 1964) or pears (Sapers and Miller, 1998), pinkish-red off-colored compounds are formed due to phenol regeneration with deep color formation (Richard-Forget et al., 1992). If off-color formation can be prevented, cysteine may prove to be an effective replacement to bisulfites, because cysteine is a naturally occurring amino acid that has GRAS status for use as a dough conditioner (Code of Federal Regulations 21:184.1271 and 21:184.1272). Browning was reduced for only one day at 0°C in 'Golden Delicious' fresh-cut apples treated with 0.1% cysteine (Nicoli et al., 1994). Ineffectiveness of the cysteine treatment was attributed to oxidation in the package and was likely due to the low concentration applied. Recently, Gorny et al. (2000) reported that a post-cutting dip (pH 7) of 2% ascorbic acid, 1% calcium lactate, plus 0.5% (w/v) cysteine significantly extended shelf life of 'Bartlett' pear slices by inhibiting loss of slice firmness and preventing cut surface browning. Consumers could not distinguish between pear slices treated with the preservative solution and control pear slices. After 10 days of storage in air at 0°C, 82% of consumers judged treated pear slices to be acceptable in appearance, and 70% judged flavor to be acceptable.

When used in combination with ascorbic acid, 4-hexylresorcinol has been reported to be a very effective inhibitor of cut surface browning on many fresh-cut fruits, including apples and pears especially (Dong et al., 2000; Luo and Barbosa-Cánovas, 1996, 1997; Moline et al., 1999; Monslave-Gonzalez et al., 1993; Sapers and Miller, 1998). Between 1 and 7 ppm of residual 4-hexylresorcinol was necessary to prevent browning on fresh-cut pear slices, stored up to 14 days at 2–5°C (Dong et al., 2000). Although 4-hexylresorcinol is effective in preventing cut surface browning in fresh-cut pear slices, it is not currently considered GRAS by the FDA and may not currently be used commercially on fresh-cut fruit products. However, 4-hexylresorcinol may also impart an unacceptable off-flavor on fruit products.

POLYPHENOL OXIDASE IN FRUITS AND VEGETABLES

Apple

Hydroxycinnamic acid derivatives (caffeoyl, coumaroyl and feruloyl) are the most important phenolics in various apple cultivars at maturity (Amiot et al., 1992). Apple fruit are characterized by a predominance of chlorogenic acid or 5'caffeoylquinic acid. Flavan-3-ols (catechins and procyanidins) are the second most important. The relative amounts of these compounds have been correlated with PPO-induced browning in apples (Vamos-Vigyazo, 1981; Amiot et al., 1992). Phenolic compounds and polyphenol oxidase (PPO) activity in the fruits of 11 apple cultivars, before and after bruising, showed that the extent of browning closely correlated with the amount of phenolics (hydroxycinnamic derivatives and flavan-3-ols) degraded. Maturity did not appear to greatly influence the development of browning (Amiot et al., 1992). Prabha and Patwardhan (1986), however, noted that during ripening, PPO activity increases in the peel tissue but decreases in the pulp.

Barrett et al. (1991) reported that shifts in subcellular location of PPO occur during controlled atmosphere storage (CA) of 'Delicious' apples. These shifts occurred sooner in apples that were stored under high CO₂ conditions (2.5–6% O₂, 8–12% CO₂) than those stored under normal CA conditions (2% O₂, 3% CO₂). PPO fractions obtained by centrifugation at 4000 × g and 100,000 × g decreased, while soluble and 200 × g fractions increased with storage. Isozyme patterns of PPO also varied in different subcellular fractions.

Cucumber

PPO activity in cucumber fruit is present only in the skin, unlike the fruit's POD activity in that it is present in the skin and the flesh, although POD activity is higher in the skin. Both enzymes in cucumber exhibit pH optima near 7.0 but exhibit different temperature optima and thermostabilities (Miller et al., 1990). PPO activity correlates with resistance to downy mildew (Yun et al., 1995) and *Pseudoperonospora cubensis* in cucumber (Li et al., 1991).

Lettuce

Ethylene-induced PAL activity is a good predictor of fresh-cut lettuce storage life (Couture et al., 1993). PPO enzyme in lettuce with an estimated molecular weight of 56 kDa quickly oxidized chlorogenic acid (5-caffeoyl quinic acid) and (–)-epicatechin. The optimal pH of chlorogenic acid oxidase and (–)-epicatechin oxidase activities are 4.5 and 7.8, respectively, and both activities are stable in the pH range 6–8 at 5°C for 20 hours (Fujita et al., 1991). Heimdal et al. (1994), however, found that PPO in photosynthetic and vascular tissues of lettuce had native molecular weights of approximately 150 and 136 kDa, respectively. The enzyme in vascular and photosynthetic tissues in lettuce was classified as a 1,2-benzenediol:oxygen oxidoreductase (EC 1.10.3.1). In addition, two PPO-active bands (40 and 46 kDa), possibly subunits of the enzyme, were found in vascular tissue only. The isoelectric point for lettuce PPO was 3.6. PPO-induced browning of lettuce can be correlated

with the soluble phenolic content (Ke and Saltveit, 1989b). The photosynthetic tissue has a higher phenolic content than the midrib tissue. For both types of tissue, an increase in PPO activity occurs during storage. The photosynthetic tissue in lettuce, however, seems to be better adapted for fresh-cut processing (Castaner et al., 1999).

Controlled atmospheres containing air + 11% CO₂ caused tissue injury and induced PAL activity in iceberg lettuce midrib tissue. Injury symptoms included brown stain (browning of epidermal tissue) and sunken epidermal areas (pitting) a few millimeters in diameter. Pitting occurred in high CO₂ atmospheres at 5°C with no increase in phenolic content, but browning did not develop until the tissue had been transferred to air at 25°C. Lettuce tissue exposed to 1.5% of O₂ + 11% CO₂ had reduced PAL activity and lower soluble phenolic content than lettuce exposed to air + 11% CO₂ (Ke and Saltveit, 1989b). The slight PPO activity inhibition by low O₂ appears to be related to the inhibition of ethylene action, the attendant effects on phenolic metabolism and IAA oxidase activity (Ke and Saltveit, 1989a). An elevated CO₂ environment inhibits cinnamic acid-4-hydroxylase activity in lettuce, presumably by inhibiting production of phenolic compounds (Siriphanich and Kader, 1985). However, susceptibility to CO₂ injury varies with cultivar. 'Climax' lettuce exhibited more severe CO₂ injury symptoms than 'Salinas' and 'Winterhaven' cultivars (Siriphanich and Kader, 1986). Moderate vacuum packaging (mvp) in 80 μ m polyethylene inhibited enzymatic browning over 10-day storage at 5°C. When packaged in 80% O₂, 20% CO₂ (80/20), more browning occurred in SL3-bags (59 μ m multilayer co-extruded film) than in polyethylene bags. Endogenous ascorbic acid was ineffective as an antioxidant in delaying browning (Heimdal et al., 1995). The initial physiological attributes of eight lettuce cultivars ('Calmar,' 'El Toro,' 'Sea Green,' 'Pacific,' 'Monterey,' 'Salinas 88,' 'Salinas 86-13,' and 'Nerone') and three maturity stages (immature, mature and overmature) did not correlate with storage quality when processed lettuce was kept in air or air plus ethylene for 1–4 days prior to air storage at 2.5–20°C. However, ethylene-induced PPO and PAL activities and browning intensity significantly correlated with the final visual quality of the ethylene-treated, minimally processed lettuce after 6–10 days of storage (Couture et al., 1993).

Mango

It appears that quality maintenance of fresh-cut mangoes is more related to particular combinations of anti-browning agents rather than the modified atmosphere created inside the package. Combinations of anti-browning agents resulted in a reduction of browning and deterioration of fresh-cut mangoes stored at 10°C and were more effective than those applied individually (Gonzalez-Aguilar et al., 2000). Treatments containing 4-hexylresorcinol and potassium sorbate, or D-isoascorbic acid reduced changes in color and microbial growth and did not affect sensory characteristics of fresh-cut mangoes.

Mushroom

Mushrooms are subject to severe enzymatic browning during handling and storage and may not respond well to treatment with browning inhibitor dips. Washing and

application of dips greatly increase perishability, primarily as a result of water absorption during treatment that increases an internal environment favorable to bacterial growth (Sapers et al., 1995). Increased browning of mushrooms at higher storage temperatures also occurs due to the direct effect of temperature on enzyme activity. Catechol oxidase activity in button mushrooms at 15°C was almost twice that at 10° and 5°C (Rai et al., 1989). The skin tissue has greater tyrosinase activity and higher concentrations of protein and phenol than the flesh tissue (Burton et al., 1993).

Melon

Lamikanra and Watson (2001) determined that the cantaloupe melon's PPO content was relatively weak compared with those in apple and lettuce. The fruit's content of oxidizable phenolic compounds was also negligible. This explains the lack of significant browning reactions in cut cantaloupe melon (Lamikanra et al., 2000).

Pear

Reported pH optimum for PPO from pear range from 4.3–7.0 (Espin et al., 1997; Saddiq et al., 1994; Zhou and Feng, 1991; Haruta et al., 1999). The enzyme shows monophenolase activity (Espin et al., 1997), and the presence of particle-bound PPO in pears is evidenced by the reduced browning in filtered and centrifuged pear juices (Sapers, 1992). The rate-limiting step in the monophenolase reaction mechanism in pears and apples appears to be a nucleophilic attack of the oxygen atom belonging to the hydroxyl group at the carbon atom in the 4-position on the copper atoms of the enzyme's active site (Espin et al., 1998b). Catechol, 4-methyl catechol and dopamine are good pear PPO substrates, but monohydroxy substrates show no activity (Siddiq et al., 1994). The enzyme could be assayed in a continuous spectrophotometric method using *p*-hydroxyphenyl propionic acid (PHPPA) with 3-methyl-2-benzothiazolinone hydrazone (MBTH) (Espin et al., 1997).

Flesh browning induced by CO₂ treatment was closely related to PPO and PAL activity in 'Nīitaka' pear (Park, 1999). PPO, PAL and cinnamate 4-hydroxylase (CA4H) activity in the flesh were considerably higher in fruits stored in 2% O₂ + 2.5% or 5% CO₂ than air or 2% O₂ at 5°C, but there was no difference in these enzyme activities at 0°C. Contents of total phenolics in flesh significantly increased with enriched CO₂ atmospheres.

Pineapple

The major PPO isozyme from pineapple appears to be a tetramer of identical subunits, each with a molecular mass of 25 kDa. The amino acid sequence of this isozyme indicates the presence of a high content of glutamic acid, glycine and serine and a low content of sulfur-containing amino acids. The PPO did not show creolase activity, and preferred substrates were diphenols. Ascorbic acid and L-cysteine were potent pineapple PPO inhibitors (Das et al., 1997).

A physiological disorder usually caused by chilling or gibberellic acid (GA-3) treatment of whole pineapple is the internal browning of fruit known as blackheart

or endogenous brown spot. This is mainly due to the oxidation of phenolic compounds catalyzed by PPO to form brown products. Low temperature or GA-3 markedly increases PPO and PAL activities and the content of catechol, chlorogenic acid and caffeic acid (Zhou and Tan, 1992). The relationship between PPO content and fruit size with susceptibility to internal browning in 'Smooth Cayenne' pineapple was demonstrated by Botrel et al. (1993). Fruits most susceptible to internal browning were those in the second largest category (1500–1799 g) in weight and those showing the highest PPO activity. The smallest fruits were the least susceptible. Fruits most resistant to internal browning had a high content of phenolic compounds and high POD activity. Heat treatment of fruit in an incubator (32–50°C for 24 hours) followed by storage in a cold room at 15°C decreases PPO, POD, ascorbic acid, acidity and spoilage rate. Dipping fruits in hot water (55°C for 10 minutes) followed by storage at 15°C also reduces the development of internal browning but induced loss of firmness and weight during the later stages of storage (Selvarajah et al., 1998).

Prunus Fruits

PPO from five *Prunus* species fruits (peach, apricot, almond, plum and cherry) show optimum pH ranges from 4–5.5, and the enzymes do not occur in a latent form (Fraignier et al., 1995). Multiple active forms occur as a result of proteolysis of one major form. Under denaturing conditions, the main active and proteolyzed forms are, respectively, monomers of 63 and 43 kDa. Peach PPO enzyme acts primarily on the orthodihydroxy configuration with optimum pH range of 4.0–6.0 (Reyes and Luh, 1960; Haruta et al., 1999). A correlation exists between peach genotypes for phenolic content and enzymatic browning (Gradziel and Wang, 1993). Four PPO isozymes with different heat stabilities were isolated from clingstone peach. None of the isozymes had monophenolase activity, and they varied in their specificity for several diphenols (Wong et al., 1971a). In a study to determine the effect of PPO inhibitors phloroglucinol and resorcinol on clingstone peach PPO-catalyzed oxidation of 4-methylcatechol, Wong et al. (1971b) determined that while PPO catalyzed the formation of 4-methyl-*o*-quinone, it did not play a role in the reaction with phloroglucinol, resorcinol, *d*-catechin or orcinol. The reaction between these phenols and 4-methyl-*o*-quinone to produce red-brown color takes place nonenzymatically.

Chlorogenic acid content decreased rapidly during enzymatic browning, but the susceptibility to browning seemed to be more strongly correlated with the initial amount of flavan-3-ols in several apricot cultivars. Chlorogenic and neochlorogenic acids, (+)-catechin and (–)-epicatechin, and rutin (or quercetin-3-rutinoside) are the major phenolic compounds in apricots (Radi et al., 1997).

PECTIC ENZYMES

SOFTENING OF FRUITS AND VEGETABLES

Pectic enzymes have received considerable attention regarding their involvement in ripening and softening of cell wall components. Firmness retention is an important quality parameter in fresh-cut fruit and vegetable products (Agar et al., 1999; Gorny

et al., 1999; Ji and Gross, 1998; Senesi and Pastine, 1996). Pectic materials are made up of chains of 1-4 linked D-galacturonic acid units that are usually esterified to varying degrees with methanol. These enzymes can be cross-linked in various ways, and they exhibit a wide range of solubilities from the highly soluble, extensively cross-linked molecules of native protopectin to readily soluble short, unbranched chains of low molecular weight (El-Ashwah et al., 1977; Legentil et al., 1995; Martin-Cabrejas et al., 1995). Pectins are important components of the cell wall and middle lamella in higher plants. They are essentially linear α -1,4-galacturonan chains with some esterified carboxyl groups. The chains are interrupted by linked α -1,2-rhamnose residues, and covalently bonded arabinose and galactose as α -1,5-arabinan and β -1,4-galactan side chains linked to rhamnose, respectively (Pressey, 1986). The nature and relative amounts of branching in pectin vary from one source to the other (Fischer et al., 1994; Massiot et al., 1988; Hoff and Castro, 1969; Ross et al., 1993). Branching of the galacturonan typically occurs more in the cell walls than in the middle lamella (Fischer et al., 1994; MacDougall et al., 1997; Knee et al., 1975). Free carboxylic acids in galacturonic acid are involved in intermolecular linkages that act as cross-bridges that influence cell wall strength. Calcium appears to be involved in forming intermolecular bridges by interaction with free carboxyl groups of pectic acid polymers to form insoluble salts that form ionic linkages between pectin molecules (Kohn, 1969; McFeeters and Fleming, 1991). Pectin occurs in most plant materials, particularly in young and fruit tissues, and is a major constituent of the cell wall. Pectin molecules are involved in cross-linking other polysaccharides and proteins in the cell wall (Cutsem et al., 1993; Qi et al., 1995). The cells in fruits and vegetables are connected through the middle lamella that is pectinous in nature. Pectin impacts firmness to plant organs by providing adhesion to juxtaposed cell walls in the middle lamella (Chen et al., 1998a; Crooks and Grierson, 1983; Luza et al., 1992; Rihouey et al., 1995).

One of the most obvious changes that occurs during the softening of fruits and vegetables is the progressive solubilization and depolymerization of pectic substances (Aspinall, 1980; McNeil et al., 1984; Bacic et al., 1988). Calcium ions and pectic enzymes play important roles in the softening process. There are a number of ways by which calcium ions could affect softening. Loss of calcium from the middle lamella could reduce ionic linkages between pectin molecules (Femenia et al., 1998; Hong et al., 1995; Tandon et al., 1984), and changes in ionic strength, resulting from calcium loss, could reduce its regulatory activity on cell wall hydrolase (Almeida and Huber, 1999), reduce cell turgor (Stow, 1993; Mignani et al., 1995) and, consequently, its stabilizing properties on membranes (Shackel et al., 1991; Picchioni et al., 1995). Cell turgor regulates the tissue tension, affecting structural firmness. Enzymatic breakdown of pectins in tomatoes affected the relative amounts of calcium bound to the pericarp, placenta and gel parenchyma (Burns and Pressey, 1987). There are two principal types of enzymes responsible for pectin degradation in fruits and vegetables. These are depolymerases (polygalacturonase and pectic lyase) and pectinesterase, also known as pectase, pectinmethylesterase or pectinmethoxylase. Depolymerizers hydrolyze the glycosidic bonds (hydrolases) or break them by β -elimination (lyases). Pectinesterase (EC 3.1.1.11) catalyzes the de-esterification of pectin.

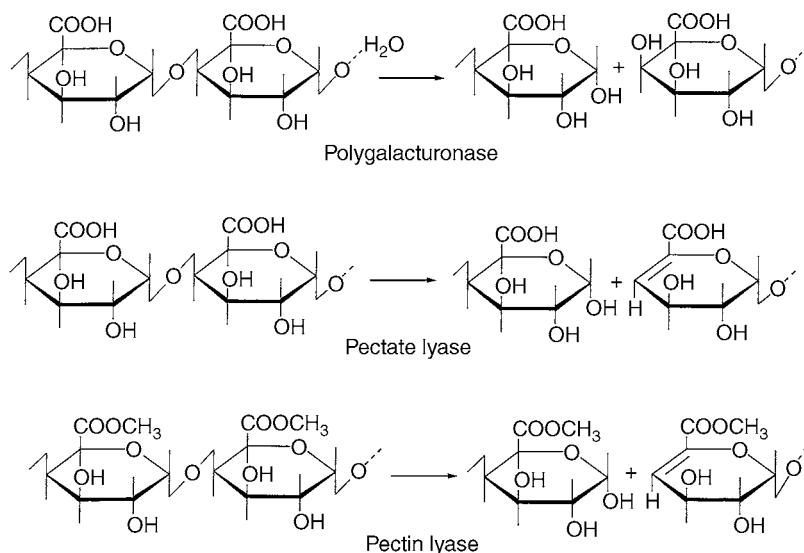


FIGURE 6.4 Splitting of glycosidic bonds in pectin by hydrolysis (polygalacturonase) and by β -elimination (pectate lyase and pectin lyase) (Pilnik and Voragen, 1989).

POLYGALACTURONASE

Poly- α -1,4-galacturonide glycanohydrolase, commonly referred to as polygalacturonase (PG), hydrolyzes glucosidic linkages as shown in Figure 6.4. Polygalacturonases are specific for de-esterified galacturonans. The rate and extent of hydrolysis are dependent on the degree of pectin esterification (Jain et al., 1990; Le-Cam et al., 1994). Polygalacturonases can be classified into endozymes that randomly cleave glycosidic bonds of pectic acids and polygalacturonates within the molecule at the α -1,4 linkages and exozymes that catalyze stepwise hydrolysis of galacturonic acid from the nonreducing end of the chain. Endopolygalacturonases (EC 3.2.1.15) occur in fruit and filamentous fungi but not in yeast or bacteria (Baldwin and Pressey, 1988; Hadfield et al., 1998; Kawano et al., 1999). Exopolygalacturonases (EC 3.2.1.67) occur in fungi, bacteria and plants (Bartley and Knee, 1982; Downs et al., 1992; Konno et al., 1986; Tae et al., 1997). In general, endopolygalacturonase (endo-PG) enzymes account for most of the polygalacturonase activity in ripe fruit (Brady, 1987; Downs et al., 1992; Nogata et al., 1993), although both endo- and exo-enzymes are active in some ripening fruits (Bartley and Knee, 1982; Nogata et al., 1993). Endo-PG appears to catalyze solubilization of pectins within the cells followed by further hydrolysis by exo-PG (Pressey and Avants, 1973, 1976). Some fruits that soften markedly during ripening (e.g., pears and freestone peaches) contain not only endo-PG but also exo-PG. Other fruits (e.g., apples and clingstone peaches) contain only exo-PG, consistent with slow softening characteristics. Low levels of exo-PG are found in many vegetative and storage tissues (Pressey, 1986).

LYASES

Poly(methoxygalacturonide) lyase (pectin lyase; EC 4.2.2.10) and pectate *trans* eliminase (pectate lyase; EC 4.2.2.2) cleave the α -1,4-galacturonosidic bond by *trans* elimination of hydrogen on carbon 5 of the galacturonic acid with the oxygen on the glycosidic bond. An unsaturated C-C bond is created between the 4- and 5-positions of the galacturonic acid residue at the nonreducing end of the fragment released (Figure 6.4). Glycosidic bonds in pectin are highly susceptible to this reaction. Pectin lyase depolymerizes highly esterified carboxyl pectin by splitting glycosidic linkages next to methyl esterified carboxyl groups by β -elimination, while pectate lyase attacks glycosidic linkages next to a free carboxyl group. Most lyases are, however, specific for esterified galacturonans (Bruchlmann, 1995; Chen et al., 1998b). The enzymes are almost exclusively from microorganisms, although there are indications of their natural occurrence in some fruits (Albersheim and Killias, 1962; Medina-Escobar et al., 1997). Lyases produce unsaturated monomers that rearrange to the 2-keto-uronic acid (Glover and Brady, 1995; Renard et al., 1991; Rong et al., 1994).

PECTINESTERASE

Pectinesterase (PE), also known as pectin pectyl-hydrolase, catalyzes the demethylation of esterified pectin (Figure 6.5). The enzyme specifically hydrolyzes the methylester groups of the C6 position of galacturonic acid and plays a crucial role in the degradation of cell walls in higher plants, rendering highly polymerized pectin susceptible to further degradation by PG (Fischer and Bennett, 1991). For example, in tomato, pretreatment of the wall with purified PE rendered walls from all ripening stages equally susceptible to PG (Koch, 1989). Early ripening tomato varieties also show higher PG and PE activity at all the stages, as compared to the late ripening varieties, where PG and PE activity increases during ripening (Young, 1995; Thakur and

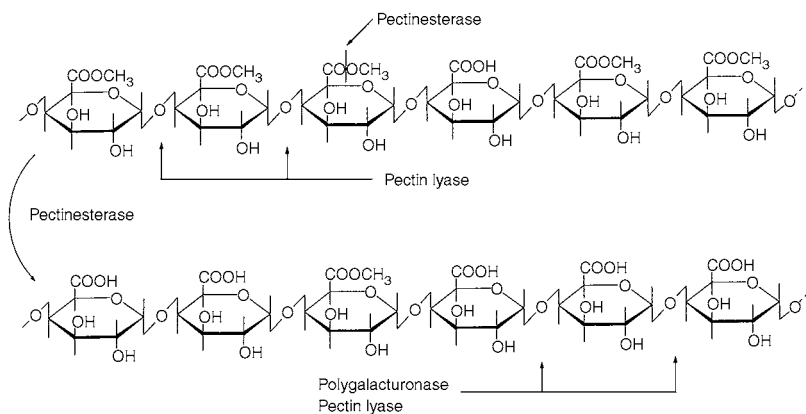


FIGURE 6.5 Fragment of pectin molecule and points of attack by pectic enzymes (Pilnik and Voragen, 1989).

Pandey, 1999). Unlike PG, PE is more commonly present in large amounts in unripe fruit (Tucker and Grierson, 1982). PE can also prepare a substrate for pectate lyase activity. The enzyme is specific for polygalacturonide esters and will not hydrolyze nongalacturonide methyl esters or those in short-chain galacturonans to a large extent. They are activated by divalent or monovalent cations at high concentrations and have a pH optimum activity range of 5–8 (Pressey, 1977). The distribution of methoxy groups apparently affects the reaction rate of the enzyme. Methoxy groups adjacent to free carboxyl groups are removed at a more rapid rate than those next to esterified residues on pectin molecules (MacMillian and Sheiman, 1974). PE activity presumably requires a free carboxyl group next to an esterified group on the galacturonide chain and proceeds linearly as methoxy groups are removed along the pectin chain until an obstruction is reached. Blocks of free carboxyl groups are thus produced as a result of PE action (Contreras-Esquivel et al., 1999; Grasdalen et al., 1996; Hou and Chang, 1997; Kohn et al., 1985).

PECTIC ENZYMES IN FRUITS AND VEGETABLES

Apple

The physiologically active cell wall degrading enzymes in apple are PE, exo-PG, β -galactosidase and β -1,4 glucanase (Pollard, 1975; Knee, 1993; Abeles and Takeda, 1990). Endo-PG is absent in the fruit. This makes the mechanism of ripening different from fruits like peach and pear, in which the endo- and exo-PG are present in the mature fruits. Softening of the cortical tissue of ripening apples is typically characterized by the loss of galactose residues from the cell wall and an increase in soluble pectin. Apple exo-PG has a pH optimum of 4.5–5, is inhibited by EDTA and citrate and is activated by Ca^{2+} and, to a lesser extent, by Sr^{2+} . The enzyme which has a molecular weight of approximately 58 kDa, degrades apple cortical cell wall preparations, releasing low molecular weight uronic acid and polyuronide residues (Bartley, 1978). Softening of apples during cold storage is accompanied by increased PG and Cx activities (Mahajan, 1994). On the tree, however, the process occurs concurrently with a decrease in Cx activity (Abeles and Takeda, 1990). Organic solvent extracts of apples inhibit β -galactosidase (Dick et al., 1984; Lidseter et al., 1985). These compounds that inhibit β -galactosidase have been identified to be chlorogenic acid, catechins and quercetin glycosides (Dick et al., 1985). β -Galactosidase in apple is believed to be responsible for the loss of galactose residues that result from hydrolysis of the galactan of the primary cell wall. Soluble polyuronide is derived from the middle lamella region of the wall (Knee, 1993). Solubilization of polyuronides is the main degradative activity that has been correlated with softening of apples (Bartley and Knee, 1982). β -Galactosidase activity increased, and cell wall galactose content decreased during softening of 'Lodi,' 'McIntosh,' 'Golden Delicious' and 'York Imperial' apples. The decrease in wall galactose was, however, least in 'Lodi,' which contained the lowest β -galactosidase activity. 'York Imperial,' which softened most slowly, showed the highest β -galactosidase activity throughout storage (Wallner, 1978).

The lack of PG involvement as a determinant in the softening of apple has also been suggested (Kang et al., 1999; Yoshioka et al., 1992). Kang et al. (1999) found

no detectable PG during the softening of 'Fuji' and 'Tsugaru' apple cultivars. Soluble enzyme activities involved are in decreasing order α -mannosidase > β -arabinosidase > β -galactosidase > α -galactosidase during softening of 'Fuji' apples, while the activities are in the order of β -galactosidase > α -mannosidase > β -arabinosidase > α -galactosidase during softening of 'Tsugaru' apples. In particular, β -galactosidase activity increased rapidly in both cultivars. Yoshioka et al. (1992) also detected no PG activity during softening of apples and suggested de-esterification of polyuronides with a high degree of methoxylation rather than depolymerization of polyuronides in the solubilization of polyuronides during ripening of the fruit. Irradiating apples (20 krd) accelerates pectin degradation and increases PE activity causing premature softening of fruit (Flores et al., 1971).

Two forms of PE are present in apples. They differ both in charge and molecular weights. Their molecular weights are 55 and 28 kDa, respectively, and the heavier form is stable up to 40°C. Optimum activity is in the pH range 6.5–7.5 (Castaldo et al., 1989). Storage of apples at 38°C for four days and subsequent storage at 0°C decreased softening of 'Golden Delicious' apples in spite of comparable PE levels with the unheated fruit, suggesting the lack of involvement of PE in apple ripening and softening when preheated (Klein et al., 1995). Heat treatment also inhibited PG activity but not that of PE. At the end of the ripening period, heated apples retained more insoluble pectin and were crisper than controls (Lurie and Klein, 1991).

Kiwifruit

Kiwifruit (*Actinidia deliciosa*) offers a useful alternative to tomato in which to study fruit softening. Once harvested, there is an extended period during which most of the fruit softening occurs. Starch degradation and cell wall changes occur, including pectin solubilization, loss of galactose from the pectin side chains, reduction in molecular weight of the xyloglucan and cell wall swelling. Tensile tests and ultra-structural studies have shown that a loss of cell-cell adhesion at the middle lamella occurs toward the end of fruit softening (MacRae and Redgwell, 1992). Sutherland et al. (1999) reported the predominance of pectin compared with cellulose also occurring at the middle lamella wedge near intercellular spaces of a number of kiwifruit cultivars. Negatively charged groups and, to a lesser extent, galacturonic acid residues were preferentially located near the cell wall/plasma membrane boundary. Cellulose remained intact across the cell wall at all stages of fruit ripening, while distribution of xyloglucan was scattered throughout the wall later in ripening.

Mature kiwifruit shows a rapid drop in firmness after harvest to approximately 2.5 kgf, after which softening slows down considerably. The outer pericarp softens more rapidly than the core of the fruit (MacRae et al., 1989; Wegrzyn and MacRae, 1992). Wang et al. (1995a) reported two stages of softening in 'Qinmei' kiwifruit. The first stage, the rapid softening phase, involves starch degradation, with amylase as the key enzyme involved. Water insoluble pectin and cellulose apparently were reduced, and the activities of PG and Cx were markedly increased in the second phase with no change in PE. Most cell wall galactose appears to be lost before pectic solubilization during ripening (Redgwell and Percy, 1992; Redgwell and Harker, 1995), apparently due to an endo- β -galactosidase enzyme system (Ross et al., 1993).

Endo-PG activity increases during kiwifruit softening, but its action alone cannot explain the pectin solubilization process (Redgwell et al., 1991). Miceli et al. (1995) found that cell wall changes during the softening of kiwifruit cv. *Hayward* are accompanied by a decrease in the total structural polysaccharides due, in particular, to a reduction in the amounts of pectin and hemicellulose. Significant variations in the quality were mainly due to an increase in highly methylated pectins and to a loss in protopectins. The amounts of arabinose, xylose and uronic acid in the cell wall polysaccharides also changed considerably during ripening (Miceli et al., 1995).

β -Galactosidase in ripening kiwifruit consists of several basic isoforms with molecular weights ranging between 33 and 67 kDa. The optimum activity of the enzyme for *p*-nitrophenyl- β -D-galactopyranoside occurs at pH 3.2 but shifts to pH 4.9 for a galactan purified from kiwifruit cell walls. The enzyme, which is specific for galactosyl residues in the β -configuration, releases galactose from a variety of kiwifruit cell-wall polysaccharide fractions, including cell wall material, Na₂CO₃-soluble pectin, high molecular weight galactan, xyloglucan and galactoglucomannan. It attacks the nonreducing end of galactose side chains, cleaving single galactose residues that may be attached to the 2, 3, 4 or 6 position of the aglycone (Ross et al., 1993). The loss of cell wall-associated galactose and pectin solubilization in kiwifruit cannot be correlated (Wegrzyn and MacRae, 1992; Redgwell and Harker, 1995). However, the ability of β -galactosidase to cause solubilization of pectin and decrease the galactosyl residues of galactans associated with cellulose could partially explain how fruits soften independent of PG activity (Gallego and Zarra, 1998).

Rapid softening of kiwifruit occurs in response to ethylene treatment that appears to be initiated by an induction of pectinesterase activity, causing increased de-esterification of cell wall pectins, followed by degradation of solubilized pectin. Ethylene treatment also causes a slight increase in PG activity during fruit softening, while β -galactosidase (EC 3.2.1.23) activity remains constant (Wegrzyn and MacRae, 1992).

Pectin solubilization, galactose loss and cell wall swelling that occur in kiwifruit are general features of cell wall changes that are not specific to postharvest ethylene treatments (Redgwell and Percy, 1992). Application of CaCl₂ reduces PG activity, leading to less degradation of protopectin, a smaller increase in water-soluble pectin and firmer fruits (Wang et al., 1995b; Xie et al., 1996).

Mango

Softening of mango causes an apparent overall loss of galactosyl and deoxyhexosyl residues, the latter indicating degradation of the pectin component of the cell wall. The loss of galactose appears to be restricted to the chelator-soluble fraction of the wall pectin, while loss of deoxyhexose seems to be more evenly distributed among the pectin regions. The chelator-soluble pectin fraction is progressively depolymerized and becomes more polydisperse (Muda et al., 1995). The inner mesocarp of the fruit is softer than the outer at each stage of maturity (Mitcham and McDonald, 1992). Cell wall neutral sugars, particularly arabinosyl, rhamnosyl and galactosyl residues, decrease concurrently with a reduced size of hemicellulose and increased PG activity during ripening (Mitcham and McDonald, 1992). β -Galactosidase activity

also increases, while PE activity decreases continuously during softening (Abu-Sarra and Abu-Goukh, 1992; Ketsa et al., 1998). Abu-Sarra and Abu-Goukh (1992) reported decreases in PE activity of three mango cultivars ('Kitchner,' 'Dr Knight' and 'Abu-Samaka') with ripening and were able to correlate decreased PE activity with softening better than PG. The firmer mango cultivar 'Keitt,' exhibits more loosely associated chelator-soluble pectin, accumulates more soluble polyuronides and retains more total pectin at the ripe stage than the less firm 'Tommy Atkins' cultivar. Both cultivars have similar PG activity that increases with ripening. The amount and molecular weight of cell wall hemicellulose decreased with ripening in both cultivars (Mitcham and McDonald, 1992). Increased quantities of calcium are released into the fruit flesh as ripening progresses. This also contributes to loss of texture and consequent softening (Tandon and Kalra, 1984).

β -Galactosidase apparently plays an important role in the softening of mangoes. Softening of ripening mango fruits was more closely related to changes in β -galactosidase activity than to PG and PE activities (Ketsa et al., 1998). Fruit extracts of ripening 'Harumanis' mango contained a number of glycosidases and glycanases. Among the glycosidases, β -D-galactosidase appeared to be the most significant. Mango β -galactosidase has at least three isoforms: β -galactosidase I, II and III, with apparent K_m values for *p*-nitrophenyl β -D-galactoside as 3.7, 3.3 and 2.7 mM. Optimum activities are at pH 3.2 for β -galactosidase I and II and pH 3.6 for β -galactosidase III (Ali et al., 1995).

Cold storage (4°C) reduces softening of fruit. Chilled fruit contain higher levels of ammonium oxalate-soluble pectin and less water- and alkali-soluble pectin than nonchilled fruit. Correspondingly, PG and β -galactosidase activities are reduced, and PE is increased compared to nonchilled fruit (Saichol et al., 1999). Roe and Bruemmer (1981) reported an increase in both PG and Cx activities in mangoes stored at 4°C, and that Cx activity correlated better with softening than PG. PE activity also decreased with time. In chilled 'Keitt' mangoes, water- and alkali-soluble pectin declines, and ammonium oxalate-soluble pectin increases as the fruit becomes soft. The decline in alkali-soluble pectin, which takes place slowly in the cold fruit, correlates well with loss of firmness.

Melon

Loss of flesh firmness in cantaloupe melon (*Cucumis melo reticulatus*) occurs with modifications of pectic and hemicellulosic polysaccharides, and a net loss of non-cellulosic neutral sugars. Increase in solubility and decrease in molecular size of polyuronides appear to be unrelated to PG activity (Gross and Sams, 1984; Lester and Dunlap, 1985; McCollum et al., 1989). Luo et al. (1996), however, correlated increase in PG with ethylene production and fruit softening in the 'Hetao' muskmelon cultivar. Reduction in size of hemicelluloses is accompanied by increase in neutral sugar composition with xylose as the dominant sugar. Xylose also predominates in the smaller polymers that are found in cantaloupe melon during ripening (McCollum et al., 1989). Glucose may, however, suppress PG production *in vivo* (Zhang et al., 1999a). Changes in several glycosidase activities in the mesocarp tissue take place with ripening and softening (Fils-Lycaon and Buret, 1991; Ranwala et al., 1992).

Glycosidase of melon mesocarp can be classified into three groups based on their specific activity patterns during fruit development and ripening. The first group (A) is composed of α -D-galactosidase, α -D-mannosidase and α -L-arabinofuranosidase. Their specific activities decrease during ripening. In the second group (B), β -D-galactosidase, β -D-glucosidase and α -L-arabinopyranosidase show increasing specific activities toward the end of ripening and overripening. The third group (C) is composed of only β -D-xylosidase, whose activity remains constant from the pre-ripe until the overripe stage. A large increase in β -D-galactosidase, α -L-arabinopyranosidase and a limited increase in β -D-glucosidase occur during later stages of ripening, which could degrade the arabinogalactan side chains on the pectic fraction of the fruit cell walls (Fils-Lycaon and Buret, 1991). Watermelon fruit are rich in α -mannosidase and β -N-acetyl-hexosaminidase, and changes in their cell walls appear to be less pronounced than in muskmelons (Nakagawa et al., 1988).

PG isozymes play an important role in postharvest fruit decay caused by microbial pathogens. Eight PG isozymes, with pIs ranging from 3.7–8.6, were isolated in *Phomopsis cucurbitae* infected fruit (Zhang et al., 1997). The most prominent isozyme has endo-PG activity, a molecular weight of 54 kDa, a pI of 4.2 and an optimum activity pH of 5 (Zhang et al., 1999a). One of 14 PG isozymes potentially produced by *Fusarium solani* was detected in infected muskmelon tissue (Zhang et al., 1999b). The endo-PG isozyme has a molecular weight of 38 kDa and pI of 9.5.

Pear

Endo- and exo-PG are present in pear (Pressey and Avants, 1976). A rise in endo-PG activity during ripening correlates with the decrease in polymerization of pectin in the fruit and release of uronic acid in the fruit (Pressey and Avants, 1976; Bartley et al., 1982). Yoshioka et al. (1992) found that de-esterification of polyuronides with a high degree of methoxylation is mainly responsible for the solubilization of polyuronides in softening of pears. The softening characteristics of pear, however, vary with cultivar. A comparison of enzymatic activities during the softening of Chinese pear cv. 'Yali' (*Pyrus bretschneideri*) fruits, Japanese pear cv. 'Nijisseiki' (*P. pyrifolia*) and European pears 'La France' and 'Bartlett' (*P. communis*) by Ning et al., (1997) demonstrates differences that are cultivar specific. Rapid increases of PE and PG activities and water-soluble pectins occurred with a decrease in HCl-soluble pectins in 'La France' and 'Bartlett' fruits, whereas enzyme concentrations and an increase in water-soluble pectins did not occur to the same extent in 'Yali' and 'Nijisseiki' fruits. The latter cultivars also had slight decreases in HCl-soluble pectin. Cellulase activity increased in 'La France' and 'Yali' fruits, whereas its increase was slight during ripening in 'Nijisseiki' and 'Bartlett' fruits. Ahmed and Labavitch (1980) did not find Cx activity in ripening Bartlett pears. Yamaki and Matsuda (1977) reported endo-PG isozymes with pH optima of 5.5 and 7.0 in oriental pears (*P. serotina*). The neutral PG isozyme increased with ripening and so did β -glucosidase.

Two PG isozymes are present in ripening 'D'Anjou' (*Pyrus communis* L.) pears. One of these hydrolyzes the pectate chain randomly with a pH optimum of 4.5, while the major PG catalyzes a stepwise removal of monomer units from the

nonreducing ends of the substrate molecules. This endo-PG that exhibits high affinity for pectate has a pH optimum of 5.5 and is activated by Ca^{2+} and Sr^{2+} (Pressey and Avants, 1976). Three isoforms of β -galactosidase also appear to have distinct regulatory mechanisms during ripening and softening of 'D' Anjou' pear. β -Galactosidase measured in cell walls at various stages of ripening at 20°C exhibits three distinct regions of cell wall β -galactosidase activity between pH 8.6–8.7, pH 7.1–7.7 and pH 5.6–5.7. The activity of the basic pI group declines slowly, while the neutral pI group declines sharply after peaking at four days. The activity of the acidic pI group that is initially low, steadily increases with ripening (Perdue et al., 1998).

Two general types of polyuronides, the major type being a homogalacturonan (HGA) whose molecular weight decreases upon ripening, are found in 'Bartlett' pears. The other type comprises heteropolymers composed of various amounts of arabinose, rhamnose and galactose. Glycosyl-linkage analysis of the arabinosyl-polyuronide gave results indicating a rhamnogalacturonan I-like polysaccharide with large, highly branched araban side chains (RG-I). There does not appear to be a linkage between HGA and RG-I, but highly branched araban RG-I in ripening pears appears to be degraded with the initial loss of much of its arabinose side chains (Dick and Labavitch, 1989). PG, α -galactosidase and α -mannosidase activities also increase with softening of 'Bartlett' pears (Ahmed and Labavitch, 1980).

Peach

Peach softening seems to result mainly from PG activity. Clingstone varieties that remain firm when ripe exhibit only exo-PG, while freestones that develop a soft mesocarp during ripening show increased exo-PG and endo-PG activity (Downs and Brady, 1990), as well as cellulase (Cx) activity (Hinton and Pressey, 1974). The correlation suggests that the extensive softening of the mesocarp tissue of freestone peaches is related to the presence of endo-PG activity (Pressey and Avants, 1978). Cx, exo- and endo-PG activities are very low at the preclimacteric stage of freestone peaches, and significant increases occur only after the ethylene rise. During fruit development, cellulase and exo-PG activity are high at the first stage of fast growth, while endo-PG activity increases during the last growth stage when the fruit has reached its final size and ripening has started (Zanchin et al., 1994; Bonghi et al., 1998; Ruzzon et al., 1998). Endo- β -1,4 glucanase (Egase) appears to play a primary role during early growth and at the beginning of softening, while PG may be involved during melting (Bonghi et al., 1998; Ruzzon et al., 1998). Propylene treatments reduce EGase activity during the early stage of fruit growth but then dramatically enhance this enzyme activity with the onset of ripeness and ultimately accelerate loss of firmness (Bonghi et al., 1998). This observation and the presence of two isoforms (pI 6.5 and 9.5) suggest that different EGase genes operate during the early and late developmental stages in peach fruit. The isoform with the higher pI predominates during late development. α -1,4-Galacturonase (EC 3.2.1.15) is also involved in softening of "melting flesh" peaches during the latter stages of ripening, causing the destruction of cell wall polymers containing long, thin pectin aggregates, while leaving cell wall polymers containing short, thick pectin aggregates intact (Fishman et al., 1993). Orr and Brady (1993) reported that the pattern of accumulation

of endo-PD activity in peach differed from that observed in tomato, but like in tomato, endo-PG is not the sole determinant for textural changes. A small level of activity was detected in fruit that was substantially softer than mature unripe fruit in which little activity was detected. In very soft fruits with the “melting soft” character, however, enzyme activity increased sharply.

Cellulose activity in preclimacteric fruits is attributed to one molecular form (pI 6.5). Two main forms are present at the postclimacteric stage (pI 6.5 and 9.5, the latter being the most abundant). Endo-PG is present at the preclimacteric stage in two molecular forms (pI 5.2 and 8.4). At the postclimacteric stage, the acidic form disappears, and activity is due exclusively to the pI 8.4 form. Exo-PG activity at the preclimacteric stage is present as one form (pI 4.9). A reduction in the activity of the acidic form occurs at the postclimacteric stage concurrently with the appearance of another isoform (pI 8.8) (Tonutti et al., 1994). The more abundant form of the enzyme is a polypeptide with a molecular weight of 66 kDa and with a substantial excess of basic over-acidic amino acids (Downs et al., 1992).

Storage temperature influences pectic enzyme activity and peach fruit firmness. Firmness decreases more rapidly at lower temperatures. Initial storage at 1°C followed by 20°C also causes a decrease in PE and an increase in PG activity during storage and ripening (Salmeron and Artes, 1991). The poor texture described as “woolliness” is apparently caused by altered pectic polymer breakdown by inhibited PG activity that does not affect PE activity. Cold-stored fruits show lower PG activity, which likely causes the lowering of lower water-soluble pectin content and increases sodium carbonate soluble pectin content. PE activity does not appear to be affected by cold storage (Choi and Lee, 1999; Sonogo et al., 1998). The onset of woolly breakdown could be delayed, and its severity could be reduced by exposure to ethylene at 1 or 10 $\mu\text{L/L}$, and this does not affect fruit softening. Sonogo et al. (1998) reported undetectable PG activity in control fruits during storage at 0°C, but PG activity was induced by ethylene at 0°C and enhanced five- to 10-fold after transfer to 20°C. However, PG protein content was unaffected by ethylene treatment. PE content and activity, which increased during cold storage, were also unaffected by ethylene treatment. ‘Yumyeong’ peach fruits stored at 0°C, with or without a modified atmosphere (MA, using polyethylene film, 0.03 mm thickness), for four weeks followed by a ripening period at 20°C indicated a lack of relationship between PE activity and EDTA-soluble pectin content with woolliness. Reduction in woolliness occurred with MA storage and appeared to be based on an increase in water-soluble pectin and a decrease in sodium-carbonate-soluble pectin by increased PG activity (Choi et al., 1997).

Chang et al. (1999) recently demonstrated the involvement of an array of enzymes in the rate of softening of peach fruits using a fast-softening cultivar (‘Mibaekdo’) and a slow-softening cultivar (‘Yumyung’). They found activities of soluble and cell-wall-bound PG to be similar in both cultivars. Activities of soluble and cell-wall-bound α -galactosidase, β -galactosidase, β -arabinosidase and α -mannosidase were maintained at high levels in both cultivars, while those of β -glucosidase and β -xylosidase were low during softening. However, higher activities of soluble α -galactosidase, β -arabinosidase and α -mannosidase were observed in ‘Yumyung’ cultivar relative to ‘Mibaekdo.’ ‘Yumyung’ fruit exhibited lower cell-wall-bound α -galactosidase and

β -arabinosidase activities than 'Mibaekdo' throughout softening. The cell-wall-bound α -mannosidase activity was similar in both cultivars, but cell-wall-bound β -galactosidase was higher in 'Mibaekdo' cultivar than in 'Yumyung' cultivar.

Tomato

Most of the reported work on ripening and softening of fruits has been on tomato, because the fruit provides the most obvious change in color from green to red, ripens uniformly in a manner that could easily and consistently be staged using morphological markers and is a rich source of pectic enzymes. The softening of tomato has been well reviewed (Gray et al., 1992; Gross, 1990; Giovannoni et al., 1992; Kramer et al., 1989; Pressey, 1986). The richest plant source of PG is ripe tomato fruit. Preclimacteric tomatoes have very low levels of exo-PG and no detectable endo-PG (Pressey, 1987). As fruit begins to change color, PG activity increases. Endo-PG appears at the onset of ripening and increases sharply during ripening. In ripe tomatoes, the level of endo-PG is about 600 times higher than that of exo-PG in green fruit (Pressey, 1986) and becomes a major protein in the ripe fruit (Brady et al., 1982). PG accumulation parallels pectin degradation and fruit softening. Consequently, endo-PG has been implicated as a primary determinant of pectin degradation and softening of tomato fruit (Huber, 1983; Grierson, 1985; Brady et al., 1987; Giovannoni et al., 1989). PG activity is highest in the outer locule wall of the pericarp tissue followed by the inner locule walls and the placental tissue. The enzyme is not present in the locular contents. Activity first appears in the placenta and then develops in both the inner and outer locule walls as the change in color spreads to the pericarp (Pressey, 1977). The deep red color in tomatoes has been associated with high PG levels. The inability of accumulated PG early in ripening to depolymerize pectin appears to result from the fact that PG deposition during early ripening is uniform and mobile (Brady et al., 1987). There is less correlation between other cell wall degrading enzymes and the rate of tomato fruit softening (Tigchelaar et al., 1978; Wallner and Walker, 1975). PE for example, increases severalfold during ripening, and several isozymes exist, with one being the dominant isozyme (Pressey and Woods, 1992), but there is no significant correlation between firmness and pectinase in tomato. A number of other factors contribute to softening in tomatoes, and there is considerable evidence that PG alone cannot be used as a determinant of softening. Cellulotic enzymes degrade cellulose and hemicellulose, and the amounts of these enzymes have been correlated with softening (Pressey, 1977; Huber, 1983). Endo-PG-dependent pectin hydrolysis may generate oligosaccharide molecules capable of influencing other aspects of the softening process (Brady et al., 1987; Bennett and DellaPenna, 1987; Baldwin and Pressey, 1988). This includes the production of ethylene by cell wall fragments (Baldwin and Pressey, 1990). In very ripe fruit, pectin depolymerization could result from leakage of calcium chelating compounds such as citrate and malate into available free space. PG action *in vitro* is severely limited by the presence of calcium in the wall (Brady et al., 1987). Using a pleiotropic genetic mutation, *rin*, that blocks many aspects of ripening including PG synthesis, and inserting another PG gene under an inducible promoter, Giovannoni et al. (1989) demonstrated that accumulated PG had no significant effect on fruit softening, ethylene evolution or color

development, in spite of its role as the primary determinant in cell wall pectin degradation. This observation might, however, be the result of the pleiotropic effects of the *rin* that inhibit other normal PG-induced changes as they occur in the normal fruit (Langley et al., 1994; Fenwick et al., 1996).

Three main PG isozymes with different physical properties have been identified in tomato (Ali and Brady, 1982). The isozymes have in common only one polypeptide, but differ in glycosylation and composition of the inactive units (Moshrefi and Luh, 1983; Pressey, 1984). During early ripening, a PG isoform (PG1) of approximately 110 kDa develops. As fruit development continues, two smaller isoforms (PG2a and PG2b) of approximately 42 and 46 kDa, respectively, accumulate (Brady et al., 1982). Both have the same isoelectric point (9.4), whereas PG1 has a pI of 8.6. PG1 is also more thermostable than PG2 (Knegt et al., 1988; Pogson and Brady, 1993). The relative amounts of PG isozymes vary widely. PG1 is thought to be a complex of PG2a or PG2b with a further polypeptide known as the β -chain and can be broken down to yield PG2 and the β -subunit in a 1:1 ratio (Hobson and Grierson, 1993). It has been suggested that the softening occurs from the combination of PG2 with the β -form to form PG1. PG1 content appears to be better correlated with the maceration process (Brady et al., 1985; Pogson and Brady, 1993). A nonspecific converter glycoprotein, capable of converting PG2a and PG2b into PG1, that is localized in the plant cell wall has been purified (Pressey, 1994). Alkaline hydrolysis of PG1 results in the release of PG2. This suggests that the converter is the associated molecule (Pressey, 1986). The purified converter can also be reacted with PG2 enzymes to form an enzyme similar to PG1 (Knegt et al., 1988). Thus, tomato softening appears to be related to the interactions between PG2 polypeptide and the immobile converter peptide to form the physiologically active PG1 enzyme. Chilling-associated softening, however, is more related to PE activity. Marangoni et al. (1995) demonstrated that while softening of nonchilled fruit was well correlated with extracted PG1 activity, chilling-associated softening correlated with higher initial extracted PE activity. The loss of turgor from translocation of water to the PE-modified cell wall was suggested to be responsible for softening as a result of chilling.

Several reports correlate PG activity with shelf life and survivability of the fruit. Low PG levels increase the average molecular weight of pectin and improve firmness throughout ripening (Murray et al., 1993; Young, 1995; Poole, 1993). They also render the fruit much less susceptible to mechanical damage and cracking (Schuch et al., 1991). Fruits with reduced PG also have increased resistance to *Geotrichum candidum* and *Rhizopus stolonifer*, fungi that normally infect ripening fruits (Kramer et al., 1992).

CONCLUSION

Biochemical and physiological consequences of fresh-cut processing are related to the wounding of tissue that occurs. The destruction of surface cells and injury stress of underlying tissues cause reactions that result in sensory deteriorations such as off-flavor, discoloration and loss of firmness. Many factors potentially influence the nature and extent of enzymatic activities and their effect on the flavor and texture

of fresh-cut fruit and vegetable products. These include growing conditions and cultural practices, cultivar and maturity at harvest, harvesting and handling methods and storage conditions. It is evident from this review that the continued growth of the fresh-cut industry will demand a better understanding of enzymatic changes and how they affect sensory and shelf life properties of processed products. It is also important that interactions of the enzymes with other food components be understood so that beneficial properties could be optimized and detrimental effects could be minimized. Most of the reported properties and characteristics of enzymes in common fresh-cut processed fruits and vegetables are from the uncut produce. While these serve as a valuable pool of information for the fresh-cut industry, continued industry-specific research will ensure improved sensory quality and shelf life and consistency of fresh-cut fruit and vegetable products.

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7 Microbiology of Fresh-cut Produce

Gillian M. Heard

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INTRODUCTION

Microorganisms are natural contaminants of fresh produce and minimally processed fresh-cut products, and contamination arises from a number of sources, including the farm environment, postharvest handling and processing (Beuchat, 1996; Heard, 1999b). Fresh-cut products are particularly susceptible to microbial attack because of the changes occurring to the tissues during processing. Processing operations such as cutting, shredding and slicing not only provide opportunities for contamination but also cause damage to fruit and vegetable tissues and cellular structure, leading to leakage of nutrients and cellular fluids. Unlike other types of processing, such as freezing or canning, no heat treatment is given to the produce to reduce microbial populations. Fresh-cut products are also packaged under modified atmosphere

conditions and stored refrigerated for up to 10–15 days. This creates a favorable environment and time for proliferation of spoilage organisms and microorganisms of public health significance (Ahvenainen, 1996; Francis et al., 1999). Microorganisms impact the economic value of fresh-cut products by decreasing product shelf life, through spoilage, and by posing a risk to public health by causing foodborne disease (Doyle, 1990; Lund, 1992; Brackett, 1994; Nguyen-The and Carlin, 1994, 2000). It has been known for more than a century that raw fruits and vegetables can act as vehicles for outbreaks of human disease. However, until recently, there has been little interest in documenting evidence of the incidence of foodborne pathogens on fresh produce. Additionally, outbreaks of foodborne disease associated with fruits and vegetables have rarely been documented (Beuchat, 1998). Subsequently, the ecological development and activity of microorganisms on produce and, in particular, on fresh-cut products is still poorly understood. Current knowledge is mostly limited to qualitative descriptions of the microbial species isolated at the time of spoilage or after an outbreak of foodborne disease (Tauxe et al., 1997; Heard, 1999b). Also lacking is our understanding of the factors affecting microbial contamination and colonization of fresh produce and processed fresh cuts, microbial interactions on these products and biochemical changes occurring. What do the microorganisms grow on and what metabolites are produced and do these metabolites contribute to the spoilage process? Does the growth of spoilage bacteria such as pseudomonads and lactic acid bacteria influence the growth of pathogenic species? Are there natural biocontrol mechanisms that can assist in controlling the microflora? This information, as well as knowledge of the factors affecting the growth of microorganisms on minimally processed fruits and vegetables, is necessary if we are to control the quality and safety of fresh-cut products.

The aims of this chapter are to review the current available knowledge on the diversity of the microflora of fresh-cut products, the source and significance of the predominant microbial species, the conditions that encourage microbial growth and the consequences of microbial growth. Plus, recommendations for future research will be made.

GENERAL MICROBIOLOGY OF FRESH-CUT PRODUCE

TOTAL MICROFLORA

Fruits and vegetables become contaminated with microorganisms while on the plant, in the field, during harvest and transport to market and during processing and packaging. Microorganisms may be present as chance contaminants, or they may possess characteristics enabling colonization of the plant. They may cause spoilage or may be of public health significance. Hence, the microflora associated with fresh-cut produce is diverse. Much of the literature reporting the occurrence of microorganisms in these products has, unfortunately, only focused on total bacterial populations and microbial groups, such as coliforms, fecal coliforms, pectinolytic species and yeast and mold counts. Although we know which pathogens may occur in fresh-cut products, rather than monitoring pathogen populations directly, coliforms are often used as indicators of contamination with pathogens from fecal sources. Unfortunately, many nonpathogenic

bacterial species normally present on the surface of fresh produce, such as species of *Enterobacter*, will give positive results on coliform detection. It is now increasingly acknowledged that coliform populations of raw and freshly processed vegetables should not be used to indicate contamination with fecal pathogens (Beuchat, 1998; Nguyen-The and Carlin, 2000). Nevertheless, total count and coliform tests are simple to perform, and they are used by the fresh-cut produce industry as indicators of hygiene and quality. For this reason, such data have been summarized for a wide range of fresh-cut products and are shown in Table 7.1. Numbers of mesophilic organisms reported on fresh-cut salad vegetables at the time of processing are similar to the numbers present on unprocessed produce. Microbial counts are within the range 10^1 – 10^9 cfu/g, varying with fruit and vegetable type (Table 7.1). Approximately 80–90% of these organisms are reported to be gram-negative rods, which are predominantly pseudomonads. Approximately 10–60% of these organisms are fluorescent, pectinolytic pseudomonads, varying from 10–20% of isolates from shredded lettuce to 20–60% of fluorescent pseudomonads isolated from carrots and endives (Nguyen-The and Carlin, 1994, 2000; Carlin et al., 1989; Nguyen-The and Prunier, 1989; Magnusson et al., 1990; Bennick et al., 1998; Jayasekara, 1999). Fluorescent pseudomonads are the dominant group isolated from endive (Jacques and Morris, 1995), spinach, cauliflower and carrots (Garg et al., 1990).

A recent study of the microflora of chicory and mung bean sprouts (Bennick et al., 1998) also reported predominance by pseudomonads. Enterobacteriaceae are also present on minimally processed vegetables, however, their presence is often summarized as coliform or fecal coliform counts (Nguyen-The and Carlin, 1994; Bennick et al., 1998). Other microbial groups reported on fresh-cut vegetables include yeast and molds (Table 7.1). For example, molds are reported at populations varying from 10^2 cfu/g on cut lettuce to 10^8 cfu/g in shredded vegetable packs and shredded carrots. In mayonnaise-based salads, yeast populations as high as 10^6 have been reported, the low pH favoring yeast growth over other microorganisms (Christiansen and King, 1971; Fowler and Clark, 1975; Brocklehurst et al., 1983; Brocklehurst and Lund, 1984; Lindroth et al., 1985; Hunter et al., 1994). Lactic acid bacteria are present on selected fresh-cut vegetables and have been reported to occur in populations ranging from 10^2 cfu/g in shredded chicory to 10^6 cfu/g in mixed vegetables (Manzano et al., 1995; Jacxsens et al., 1999).

There are few reports of the predominant microflora of fresh-cut fruit products. Unlike on whole fruits, growth of molds on fresh-cut products does not appear to be a major problem, and it may be assumed that the high moisture content of ready-to-eat fruit products may encourage faster-growing bacteria and yeast. Yeast populations of 10^3 – 10^4 have been reported for processed fruits (Nguyen-The and Carlin, 1994), and total bacterial populations of 10^6 cfu/g have been reported for cantaloupe (Sapers and Simmons, 1998). More detailed investigations of the occurrence of microorganisms in processed fruit products are needed.

ORIGINS OF THE MICROFLORA OF FRESH-CUT PRODUCE

Microbial contamination of fruits and vegetables is reported to arise during growth; from the soil, organic matter, organic fertilizers, irrigation processes, insects, animals and human contact; and from postharvest practices, including washing, trimming

TABLE 7.1
Total Microbial Populations and Predominant Microbial Groups Present on Fresh-cut Fruits and Vegetables¹

Fresh-cut Product	Microbial Populations (Log cfu g ⁻¹)				Ref.
	Total Count (Mesophilic)	Coliform Count	Lactic Acid Bacteria	Yeast and Molds	
		Individual Commodities			
Broccoli florets	6.5	5.9 ²	—	5.2	Brackett (1989)
Broccoli florets	4.7	2.2	—	3.3	Mohd-Som et al. (1994)
Broccoli florets	4.7	2.1	—	3.25	Jacques and Morris (1995)
Cantaloupe	6.11	—	—	—	Sapers and Simmons (1998)
Minimally processed cantaloupe	1.05	—	ND	—	Lamikanra et al. (2000)
Cut carrots	4.4	—	—	—	Priepke et al. (1976)
Carrot sticks	4.99–5.77	—	ND–3.1	ND–4.25	Garg et al. (1990)
Shredded carrots	2.9	—	1.1	—	Chervin and Boisseau (1994)
Shredded carrots	6.00	—	4.00	<2.00	Carlin et al. (1990)
Carrot sticks	4.98	2.84	ND	—	Garg et al. (1993)
Shredded carrots	4.88	—	3.52	3.15	Kakiomenou et al. (1996)
Carrot sticks	5.13	—	—	—	Odumeru et al. (1997)
Shredded carrots	2.84–3.85	1.54–2.3 (fecal)	1.65–2.69	2.00–2.12	Sinigaglia et al. (1999)
Minimally processed broad-leaf endive	3.83–4.82	—	—	—	Carlin et al. (1996)
Cut chicory endive	4.00	—	—	—	Bennick et al. (1998)
Chicory endive (shredded)	5.2	—	2.63	3.0	Jacxsens et al. (1999)
Coleslaw	<4.00–>8.00	<2.00–>6.00	—	—	Fowler and Foster (1976)
Cabbage (coleslaw)	4.07–7.08	—	ND–2.4	ND–2.2	Garg et al. (1990)
Coleslaw mix	5.14	—	—	—	Odumeru et al. (1997)
Coleslaw (dryslaw)	7.32–7.84	—	—	—	Jayasekara (1999)
Cut celery	5.7	—	—	—	Priepke et al. (1976)
Cut lettuce	5.3	—	—	—	Priepke et al. (1976)
Prepared lettuce for caterers	5.6	3.9	—	—	Maxcy (1978)
Lettuce	5.41	—	ND	ND	Garg et al. (1990)

(continued)

TABLE 7.1**Total Microbial Populations and Predominant Microbial Groups Present on Fresh-cut Fruits and Vegetables¹ (Continued)**

Fresh-cut Product	Microbial Populations (Log cfu g ⁻¹)				Ref.
	Total Count (Mesophilic)	Coliform Count	Lactic Acid Bacteria	Yeast and Molds	
Lettuce	6.39–7.69	4.14–5.29	—	—	Gras et al. (1994)
Chopped lettuce	4.85	—	—	—	Odumeru et al. (1997)
Processed lettuce	2.5–6.2	—	—	—	Francis and O'Beirne (1998)
Shredded lettuce	4.28	—	<1	2.07	Delaquis et al. (1999)
Lettuce salad	7.23–7.61	—	—	—	Jayasekara (1999)
Chopped bell peppers	3.5	—	—	—	Izumi (1999)
Fresh-cut mushrooms	8.3	—	—	—	Sapers and Simmons (1998)
Potato strips	2.00	—	—	—	Gunes et al. (1997)
Sliced potatoes	2.01–2.6	<0.7	<0.7	—	Laurila et al. (1998)
Diced potatoes	5.00	—	—	—	Izumi (1999)
Potato salad	5.41–4.98	—	—	—	Jayasekara (1999)
Japanese radish shreds	3.9	—	—	—	Izumi (1999)
Trimmed spinach leaves	4.00	—	—	—	Izumi (1999)
Mixed Products					
Chicken and vegetable salad	5.8	3.27	—	4.00	Christiansen and King (1971)
Green salad	<4.00–7.00	<2.00–>6.00	—	—	Fowler and Foster (1976)
Ham and vegetable salad	6.17	2.47	—	3.08	Christiansen and King (1971)
Mixed green salad	<4.00–8.00	<2.00–6.00	—	—	Fowler and Foster (1976)
Mixed vegetable salad	5.00	4.00	3.00	—	Manvell and Ackland (1986)
Mixed vegetables	8	—	5.5–6.3	4–4.2	Manzano et al. (1995)
Mixed salad in school kitchens	1.84–2.99	0.7–1.90	—	—	Martínez-Tomé et al. (2000)
Packaged garden salad (iceberg lettuce, carrot, red cabbage)	5.3–8.9	—	—	0.9–3.85 yeasts <0.3–2.2 molds	Hagenmaier and Baker (1998)
Prepackaged ready-to-serve salad	5.5–8.3	—	—	<3–6.75	Lack et al. (1996)
Raw vegetables	5.7	2.3	—	—	Kaneko et al. (1999)

TABLE 7.1
Total Microbial Populations and Predominant Microbial Groups Present on Fresh-cut Fruits and Vegetables¹ (Continued)

Fresh-cut Product	Microbial Populations (Log cfu g ⁻¹)				Ref.
	Total Count (Mesophilic)	Coliform Count	Lactic Acid Bacteria	Yeast and Molds	
Ready-to-use mixed salad	7.18	6.60	5.3	—	Vescovo et al. (1995)
Salad mix	5.35	—	—	—	Odumeru et al. (1997)
Vegetable salad	4–7	—	—	—	Wright et al. (1976)

¹Products were sampled prior to storage.

²Reported as Enterobacteriaceae counts.

—Not sampled.

and packing (Beuchat, 1996). Fresh-cut produce may be further contaminated during transport, from processing steps and during packing (Heard, 1999b). This section discusses the source of the microflora and highlights the gaps in our knowledge about where the contamination actually occurs along the production chain.

Contamination on the Farm

Preharvest

Fruits and vegetables become contaminated in the field during growth. The microflora may arise from within the plant, such as from the seed or tuber or from the environment, during growth. Seeds are a source of foodborne pathogens such as *Bacillus cereus* and *Salmonella* as well as bacteria and fungi that cause postharvest diseases (Portnoy et al., 1976; Maud, 1983; Harmon et al., 1987; O'Mahony et al., 1990; Nguyen-The and Carlin, 2000). Thus, the first leaves emerging from contaminated seeds will also be contaminated. For example, Morris and Lucotte (1993) reported total populations of 10³ cfu/cm⁻² on the first leaves of a green endive plant. Most contamination occurs on the outside or surface of plants, although in some fruits and vegetables, the inner tissues may be invaded in the early stages of fruit development (ICMSF, 1998). The predominant microbial species present on fresh-cut produce (Table 7.1) are also present in soil, irrigation water and the general farm environment. Table 7.2 summarizes the sources of microbial contamination during the production of fresh-cut produce, as reported in the literature. As mentioned previously, pectinolytic and fluorescent bacteria, particularly the pseudomonads, are the dominant flora of many plant products at the time of harvest. These organisms as well as coryneform bacteria, lactic acid bacteria, yeasts and molds are derived from air, water and soil and contaminate the leaves and outer surfaces of plants during growth (Lund, 1983, 1992; ICMSF, 1998).

Crops can become contaminated with spoilage organisms from a number of sources on the farm. The presence of rotting organic material in soil should be

TABLE 7.2**Sources of Microbial Contamination of Fresh-cut Salad Products—from the Farm and during Processing**

Source of Contamination	Product Example	Microflora	Ref.
On the Farm—Pre- and Postharvest			
Grazing domestic animals, manure from cattle (unprocessed organic fertilizer)	Vegetables	Spoilage flora and pathogens— <i>Salmonella</i> , <i>E. coli</i> O157:H7, <i>Cryptosporidium parvum</i>	Tauxe et al. (1997), Nguyen-The and Carlin (2000)
Hygienic practices of farm workers	Sliced melon	<i>Vibrio cholerae</i>	Ackers et al. (1997)
	Raspberries and sliced melons	Pathogens	Ackers et al. (1997), Lund and Snowden (2000)
Insects	Damaged fruits and vegetables	Spoilage or pathogenic microflora	Lund (1983)
Overhead irrigation systems	Tomatoes	Bacterial contamination	Samish and Etinger-Tulczynska (1963)
Packing shed design	All produce	—	Troller (1983), Coghlan (2000)
Pesticides (mixed up using contaminated water)	Fresh fruits and vegetables, e.g., raspberries (inoculated with pathogens)	<i>Salmonella</i> , <i>E. coli</i> O157:H7, <i>Shigella</i> survival and growth in the pesticide, <i>Cyclospora cayetanensis</i> on raspberries	Hertwaldt et al. (1997), Tauxe et al. (1997), Coghlan (2000)
Rainfall and temperature	Vegetables	Lactic acid bacteria numbers are low in hot, dry conditions	Mundt et al. (1967), Mundt (1970)
Rhizospheres	Root crops	Fungal spores, Pectolytic pseudomonads, spoilage microflora	Droby et al. (1984), Sands and Hankin (1975)
Seeds, tubers	—	—	—
	Endive plants	Total microflora	Morris and Lucotte (1983)
	Sprouts	<i>Salmonella</i> , <i>Bacillus cereus</i>	Portnoy et al. (1976), Maud (1983), Harmon et al. (1987), O’Mahony et al. (1990)
Soil, organic material	Rhizospheres	<i>Cl. perfringens</i>	Lund (1992)
Transport—trucks, pallets, boxes, crates	—	—	No reports
Wastewater, sewage	Vegetables	<i>Vibrio cholerae</i> , <i>Listeria monocytogenes</i>	Beuchat (1998), Nguyen-The and Carlin (2000)

TABLE 7.2
Sources of Microbial Contamination of Fresh-cut Salad Products—from the Farm and during Processing (Continued)

Source of Contamination	Product Example	Microflora	Ref.
Wind, dust	Cucumbers —	Fecal coliforms —	No reports*
During Processing			
Equipment—trimming, shredding, cutting, surfaces	Vegetables	Total microflora and coliforms	Garg et al. (1990), Barry-Ryan and O’Beirne (1998)
Hygiene of handlers	—	—	No reports*
Non-vegetable ingredients	Chicken or meat added to salads	Total microflora increase	Christiansen and King (1971)
Packaging	Lettuce	Wider variety of microorganisms present in unsealed packages than in sealed packages	Magnuson et al. (1990), King et al. (1991)
Use of chlorine	All produce	Total counts reduced by 1–3 log	Garg et al. (1990), Nguyen-The and Carlin (1994)
	Broccoli	Delayed spoilage by pseudomonads in modified atmosphere packages	Brackett (1989)
Wash water, water bath used by the packer	Tomatoes	Total microflora increase, <i>Salmonella</i> (caused an outbreak of foodborne disease)	Tamplin (1997)

* There is little evidence to document the effect of these factors as sources of contamination, but they are commonly referred to in reviews by Beuchat (1996, 1998), ICMSF (1998) and Heard (1999b).

avoided, as it may provide a source of contamination for nearby growing crops. For example, pectolytic pseudomonads have been shown to survive in soil containing the rhizospheres of previous crops (Sands and Hankin, 1975). This may result in spoilage of subsequent crops (Nguyen-The and Carlin, 2000). In a review by Lund (1992), it was suggested that rhizospheres may also support the survival of clostridia up to 10⁶cfu/g. *Clostridium perfringens* has been reported as a common species in rhizospheres. Harvesting equipment can become contaminated with fungal spores and bacteria, from the soil and from decaying organic matter, subsequently, contaminating new crops. For example, Droby et al. (1984) reported that potato tubers became contaminated with fungal spores originating from infected foliage.

Domestic animals may also disseminate spoilage organisms ingested with plant fodder (Nguyen-The and Carlin, 2000). Insect activity is encouraged by the presence of decaying organic matter remaining in fields, and the insects may disseminate

microorganisms to other crops, resulting in contamination (Lund, 1983). The use of overhead irrigation systems has been linked with bacterial contamination of fruit such as tomatoes. Bacteria enter the fruit through the sepals (Samish and Etinger-Tulczynska, 1963). The role played by the wind for contaminating fruits and vegetables is not reported in the literature and warrants investigation. Wind may transfer dust contaminated with mold or bacterial spores on the surface of plants.

Pathogens of public health significance, including *Listeria monocytogenes*, thermotolerant *Campylobacter* and the opportunistic pathogen *Pseudomonas aeruginosa*, have also been isolated from soil or water, bird and animal droppings (Geldreich and Bordner, 1971; Colburn et al., 1990; Park and Sander, 1992; Nguyen-The and Carlin, 2000) and farm workers in the fields. Farmers and agricultural laborers often assume that because raw produce is soiled during growth, personal hygiene and equipment cleanliness is not necessary. Inadequate hand washing, disposal of domestic waste and inadequate cleaning of farm equipment can result in contamination of produce with spoilage organisms and possibly with microorganisms of public health significance (Geldreich and Bordner, 1971; Beuchat, 1996, 1998; Brackett, 1999). The *Guide to Minimize Microbial Food Safety Hazards for Fresh Fruits and Vegetables* (Guidance for Industry, 1998) has recently been published by the U.S. Department of Agriculture to assist in educating farmers and processors in producing safe produce. These guidelines address the hygiene issues mentioned above.

Wastewater or water polluted with fecal material is also a source of contamination. Similarly, the use of untreated organic fertilizers and direct application of human fecal material to growing crops may result in contamination with pathogens (ICMSF, 1998). Pathogens, including members of the Enterobacteriaceae, viruses, protozoa and nematodes and *L. monocytogenes* may be transmitted to fresh fruits and vegetables from sewage water and untreated wastewater and fecal matter. For example, wastewater-irrigated vegetables are reported to be responsible for cholera outbreaks in Chile and Costa Rica in the early 1990s (Nguyen-The and Carlin, 2000). Several reports of pathogen contamination of fruits document the source of the pathogens, *Salmonella*, *Escherichia coli* O157:H7 and *Cryptosporidium parvum*, as manure from grazing cattle (Tauxe et al., 1997). Several investigations of the influence of wastewater on microbial populations of irrigated vegetables have been reported in the literature, although there are no recent studies. Sadowski et al. (1978), during an evaluation of methods for irrigating crops, recorded fecal coliform counts of 10^3 cfu/100 g on cucumbers taken from sewage-irrigated plots. Rosas et al. (1984) investigated the bacteriological quality of crops irrigated with wastewater in an area of Mexico City.

Postharvest

Fruits and vegetables can become further contaminated during harvest and from postharvest handling from the handlers, the work surfaces, wash water, packaging crates and pallets and trucks during transport (Table 7.2). Improper hygiene practices may influence the microbial safety of produce during harvest (Geldreich and Bordner, 1971). Toilets should provide adequate hand washing facilities, and sewage should not be in contact with crops (Beuchat, 1998). Contamination of fruits such as raspberries and sliced melons has been linked to pickers (Ackers et al., 1997; Lund and

Snowden, 2000). For example, Ackers et al. (1997) report the probable connection between hygiene of agricultural workers and an outbreak of cholera associated with sliced melon. Cross-contamination between crops may occur during handling and harvest and postharvest operations, and contamination with pathogens is possible from handlers' hands or from polluted wash water (Lund, 1992; ICMSF, 1998). Control measures include the use of clean water and sanitizers for washing fruits and cleaning work surfaces, refrigeration of packing sheds and training of agricultural workers in good manufacturing and hygiene practices.

Contamination During Processing

The main sources of contamination during the processing of fresh-cut fruits and vegetables are most probably the general factory environment and the processing equipment (Table 7.2). However, there is currently little information available documenting the risks of the contamination with individual organisms at this stage of production. Factory workers are also a possible source of contamination, but there is no supporting evidence in the literature. One study conducted by Garg et al. (1990) during the processing of vegetables such as cabbage, lettuce and onions established that the shredders and slicers were major sources of contamination. More information is needed about the microflora of processing plants and the extent of contamination in this environment. Future studies should also investigate the role of biofilm formation in contamination of fresh-cut products. Recent studies have suggested that biofilm formation on processing equipment may provide contamination points (Carmichael et al., 1999).

Other non-vegetable ingredients may also be sources of contamination with microorganisms for fresh-cuts. In the case of low pH, dressed salads, processors may combine fresh-cut fruits and vegetables with meat, chicken or seafood, thus increasing the range of potential spoilage microflora and the introduction of organisms of public health significance. Christiansen and King (1971) examined meat-based salads, including ham, chicken and barbecue pork. Total counts for the ham salad ranged from 10^2 – 10^6 counts/g salad, and counts for the other salads were slightly higher, up to 10^7 counts/g salad. The contribution of the meat to the microflora of the salads was not investigated. Little is known about the spoilage microflora of these salads.

FACTORS AFFECTING THE GROWTH OF MICROORGANISMS

Factors affecting microbial stability and quality of fresh-cut vegetables can be simplified into the four main categories listed below:

1. Intrinsic properties of the food—pH, water content, nutrients and protecting biological structures such as skin or cuticle
2. Processing factors—washing, blanching, cutting, shredding, packaging, conditions of temperature during the process and addition of preservatives
3. Extrinsic factors—storage temperature and use of modified atmospheres
4. Implicit properties of the microbial species—growth rate, temperature and pH tolerance and interactions (Heard, 1999b)

Table 7.3 relates these factors to the production of fruit and vegetables, from the farm through to the processed, packaged product, and the main factors are discussed below.

HANDLING PRACTICES ON THE FARM

Environmental conditions such as temperature and rainfall, farm practices and the standard of hygiene on the farm are all acknowledged as factors affecting the microbial quality of fresh produce. During harvest and postharvest storage, microbial numbers are believed to be influenced by the temperature, the hygiene of storage and transport facilities and the degree of damage of the produce during the harvest (Lund, 1992; Nguyen-The and Carlin, 1994; ICMSF, 1998; Brackett, 1999; Nguyen-The and Carlin, 2000). Surprisingly, although these factors are reported to be of significance for the safe production of fresh produce, there is little documented evidence of their effects on the individual microbial populations present throughout growth, harvest and transport. The available literature is summarized below.

Water Quality

Water quality is an important factor influencing the microbial contamination of fresh produce during growth. Water is used for irrigation, washing, hand washing, cooling and for pesticide or foliar application (Pabrua, 1999). The methods used for irrigation can significantly influence the extent of contamination. Sadovski et al. (1978), in a study of the practice of wastewater irrigation, showed that contamination can be minimized if covered drip irrigation is used rather than spray irrigation. Contamination with fecal coliforms was 38-fold higher on vegetables irrigated with sewage effluent with an uncovered drip system than those irrigated with fresh water, but vegetables irrigated with contaminated water through a drip system covered by soil were contaminated with populations only 10-fold higher than the control. They also found that there was more risk of contamination if crops were watered with wastewater just prior to harvest rather than earlier in the growth cycle. Irrigation frequency may influence the bacterial populations of crops during growth. Ludy et al. (1997) established that reduction of irrigation frequency from two to eight days reduced the incidence of soft rot in broccoli heads from 30–15% in one year and from 22–10% in another year. Use of contaminated water to prepare pesticides has also been linked to outbreaks of foodborne disease. Recent studies have shown that the pathogens *Salmonella*, *Shigella* and *E. coli* O157:H7 can survive and grow in pesticides (Coghlan, 2000) (Table 7.2). Washing the harvested crop can further increase microbial populations, particularly if wash water is not clean (ICMSF, 1998), and storage and transport of crops in water can further encourage microbial growth. Segall and Dow (1973) reported contamination with species of *Erwinia* from potatoes transported in water.

Good agricultural practices require knowledge of the source and safety of the water and, if possible, knowledge of microbial populations, to prevent use of contaminated sources. In many countries, health authorities have banned the use of untreated waters for irrigation. However, in many countries, there is no legislation

TABLE 7.3
Factors Affecting the Microbial Stability and Quality of Minimally Processed Vegetable Salads from Farm to Retail

Intrinsic Factors

pH

- pH of salads vary depending on the type of vegetable used, e.g., tomatoes are low pH, lettuce is at a neutral pH

Nutrient availability

- mixed salads contain a wider variety of nutrients than single packs of vegetables
- addition of non-vegetable ingredients such as dairy products, meat, seafood and chicken provides fat and protein

Biological structure

- the skin or cuticle of minimally processed vegetables is damaged and, therefore, not an obstacle for microbial growth
- the physiology of various vegetables, e.g., broccoli structure is complex, including tightly packed florets and a stem with a waxy cuticle
- the biochemical, physiological changes and interactions that occur in the salad during processing and storage

Other factors

- antimicrobial effects of vegetables, e.g., the antilisterial effect of carrots

Processing Factors

Farm practices

- use of fertilizers
- use of pesticides
- contamination from handlers, farm animals and insects
- damage during harvest
- use of contaminated water
- type of harvesting, i.e., manual or mechanical
- condition of packing sheds and trucks

Washing

- washing at the time of harvest may contaminate the vegetables with waterborne microorganisms
- washing in the processing plant should reduce the microbial load (if the water is clean)

Temperature

- temperature during harvest and trimming and washing on the farm
- blanching or cooking of ingredients such as pasta and potato reduce or destroy vegetative microbial cells
- low temperature (0–5°C) during preparation, processing and storage limits the growth of microorganisms

Other processes

- processing operations such as chopping, shredding and slicing may contaminate the vegetables
- assembly of salads may result in contamination from process workers and work surfaces
- use of low pH dressings such as sour cream and mayonnaise lower salad pH
- use of packaging to act as oxygen and water barriers and to prevent microbial contamination

(continued)

TABLE 7.3**Factors Affecting the Microbial Stability and Quality of Minimally Processed Vegetable Salads from Farm to Retail (Continued)****Extrinsic Factors**

Temperature

- temperature fluctuations during transport and retailing

Modified atmospheres

- modified atmosphere packaging influences survival of microorganisms during storage

Implicit Factors

- competition between predominant microbial groups, e.g., lactic acid bacteria and pseudomonads
- antagonistic relationships between microbial groups
- synergism between microbial groups

Source: Adapted from Heard (1999b).

controlling water quality in agriculture, and water remains a major source of contamination (Bryan, 1977; Beuchat, 1996, 1998; Pabrua, 1999).

Disinfectants

Washing fruits and vegetables in clean water can remove organisms from the surface, and the addition of a disinfectant (the use of chlorine, surfactants or acids such as peroxyacetic acid) can achieve additional 1–2 log reductions (Cherry, 1999). Beuchat (1998) reviewed processes for the surface decontamination of fruits and vegetables. Despite the lack of extensive scientific data, Beuchat (1998) makes a number of conclusions about the efficacy of washing treatments and, in particular, the use of disinfectants. The efficacy of treatment varies with the following:

1. The type and pH of the disinfectant—disinfectants should be used within the pH range in which they are most active. For example, chlorine is most effective at a slightly acid pH, where the predominant and most effective species is hypochlorous acid.
2. The time and type of contact—disinfectants such as chlorine are most effective within the first few seconds of treatment.
3. Water temperature—the temperature of the wash water should be higher than that of the produce to avoid uptake of microbial cells by the tissue.
4. The properties of the produce—different surface structures can influence interactions with disinfectants.
5. The properties of the microorganisms—types of cells and stress influence response to disinfectants. Resistance of pathogens to chlorine varies, and it is not known how effective disinfectants are in killing parasites and viruses on fruits and vegetables.
6. The level of contamination—heavily contaminated produce should be washed twice, first to remove heavy soil and second to sanitize.

Produce should also be dried after treatment to prevent growth of remaining organisms. Beuchat (1998) also suggested that organic acids such as peroxyacetic acid and ozonation treatment showed good potential as disinfectants, but the conditions of use require more investigation.

Fertilizers

The choice of fertilizer can also influence the degree of contamination. The use of inorganic fertilizers or composted, treated manure can reduce the risk of contaminating crops with pathogens (ICMSF, 1998). Organic material can attach to outer leaves of produce, effectively enmeshing organisms on the surface. Bovine feces have been reported as a source of *E. coli* O157:H7, and avian feces contain *Salmonella* spp. (Beuchat, 1996).

Damage

Injury of fruits and vegetables can occur during growth, for example, during hailstorms. Fruits and vegetables also often become injured during harvest resulting in release of nutrients and allowing entry of microorganisms to the internal tissues (ICMSF, 1998). Prior to injury or damage occurring, most microorganisms are present on the outside of fruits and vegetables and cannot enter the inner tissues due to the cuticular layer covering the epidermis of aerial organs such as leaves, stems and fruits (Nguyen-The and Carlin, 2000). Only true plant pathogens can invade the tissues of uninjured fruits and vegetables. Once the cuticular layer is broken, microorganisms are exposed to cellular fluids and moisture, and microbial growth is encouraged. The release of juices containing sugars also encourages attack by insects, further damaging the produce and allowing dissemination of microorganisms. Thus, hygienic handling conditions and cool temperatures during transport of harvested produce to the processing plant are desirable to avoid excessive microbial contamination (ICMSF, 1998; Heard, 1999b). Use of pesticides or use of organic farming practices to deter insects is also desirable to reduce insect activity (Lund and Snowden, 2000).

Rainfall and Temperature

Warm, moist conditions during harvest are known to increase the overall microbial load of crops such as tomatoes (Senter et al., 1985). The rainfall during growth of vegetables has also been shown to affect the microbial population. Mundt and co-workers (1967, 1970) studied the numbers and types of lactic acid bacteria on vegetables and reported that populations of these organisms are likely to be low and difficult to recover in conditions of near drought. Numbers of the predominant species, *Leuconostoc mesenteroides*, reached as high as 10^4 cfu/g on green vegetables when rainfall was more abundant.

Temperature is also an important factor influencing microbial numbers during postharvest handling. Splittstoesser (1970) observed that fresh vegetables, such as spinach and peas, harvested and transported to the processor under warm, humid

conditions carried up to 10^7 cfu/g total bacteria. Rapid transport of produce under cool conditions should be encouraged to minimize microbial growth.

The full extent of the influence of farm practices on the quality of fruits and vegetables used for fresh-cuts and the effect on the final processed product is yet to be determined. However, certain control measures may be taken to reduce contamination, including use of refrigeration and clean water during postharvest operations. Detailed ecological investigation is required for us to determine the most important microbial species present and the most important influencing factors. With evidence for the importance of hygienic practices on the farm, we can educate and train farm workers in the safe production of fresh produce for all applications.

CONDITIONS DURING PROCESSING/PACKAGING

What are the factors influencing the survival and growth of the microflora of fresh produce during the processing stages? The predominant species at the time of harvest and transport will not necessarily dominate during processing, and it may not be these original species that cause the spoilage symptoms (Nguyen-The and Prunier, 1989; Bennick et al., 1998) or cause outbreaks of foodborne disease (Beuchat, 1998). Optimization of conditions to extend the shelf life of these products is the main priority of processors, as microbial spoilage is of major commercial significance and will continue to be so as the market expands worldwide. Currently, the main hurdles applied to control microbial growth are the use of low-temperature and modified atmospheres. Unfortunately, the effects of processing, low-temperature storage and modified atmosphere storage on individual species or microbial groups present on the raw produce are yet to be studied. We also lack information about biochemical reactions that may assist in the development of microorganisms and interactions between species that may occur during processing as the structures and intrinsic properties of the produce are altered. The following section mainly examines the processing factors and conditions that influence the growth of microorganisms in salads, as reported in the literature.

Processes

On arrival at the processing plant, raw fruits and vegetables are trimmed and peeled. Trimming processes are the first steps taken toward reducing microbial load of the produce by removing the most heavily contaminated outer layers. Decay of leafy vegetables such as endive occurs more frequently on the outer green leaves than on the inner yellow leaves (Carlin et al., 1995). However, the actual process of removal may result in contamination of the edible portion of the fruit or vegetable, thus increasing the risk of microbial contamination during subsequent processing steps (Garg et al., 1990; Beuchat, 1998). Thus, washing after trimming and cutting is necessary to remove microbes (Sinigaglia et al., 1999). Washing may not always remove microbial cells, and processors should be aware that cells may attach to the tissues of produce. For example, Babic et al. (1996), while examining the surface of processed spinach leaves using scanning electron microscopy, found that microorganisms were embedded in damaged spinach cells or in cells adjacent to the damaged tissue. Garg et al. (1990) investigated the effect of processing on the total microbial counts of

vegetables including cabbage, lettuce and onions. The shredders and slicers were found to be major factors influencing the level of contamination. The aerobic plate count of lettuce increased from 1.8×10^4 cfu/g to 140×10^4 cfu/g after shredding. Similarly, the aerobic plate count of onions increased from 4.0×10^3 to 1.2×10^5 cfu/g during slicing. More recently, Barry-Ryan and O'Beirne (1998) determined that the method of slicing also influences the microbial load of vegetables. Microbial counts of carrot slices sliced using a razor and a slicing machine were compared (both blunt and sharp blades were used in the machine). Total microbial counts, coliforms, lactic acid bacteria and yeast and mold counts were all higher on slices prepared with the machine than slices cut with a razor. For example, after the carrots had been stored for one day, the total aerobic counts on razor-sliced carrots were $5.77 \log_{10}/g$, and the counts on carrots cut with a blunt blade were $6.27 \log_{10}/g$. However, the effects of the machine blade, blunt or sharp, were only significant for population of *Pseudomonas* and coliforms. Advances in size reduction technology may be used in the future to reduce contamination of vegetables. For example, high-pressure water jets and CO₂ lasers may be used as tools for cutting (Sanguansri, 1997). These methods result in cleaner cuts and minimum dust generation, and the CO₂ laser partially sterilizes the cut surface. The effect of such technologies on the microbial ecology of cut vegetables is yet to be determined.

Sanitation

How important are sanitation and the application of hygienic principles during the processing of fruits and vegetables? All handlers of produce in the processing environment must ensure the highest level of hygiene. Cleaning and sanitation of equipment and processing surfaces is also of utmost importance to prevent buildup of organic residues that might encourage growth of microorganisms and formation of microcolonies or biofilms (Brackett, 1989; Carmichael et al., 1999). The significance of biofilm formation in processing environments is yet to be determined. Other processes, including washing, may contribute to the spoilage microflora of the processed vegetables, especially where recycled water is used. Buildup of organic residues in the water can result in growth of spoilage microorganisms, increasing the potential for contamination (Brackett, 1992).

Washing fruits and vegetables with water alone only achieves a small (1 log) reduction in microbial numbers (Nguyen-The and Carlin, 1994). Chlorinated water (100–200 mg/L) is widely used for washing and sanitizing minimally processed fruits and vegetables (Ahvenainen, 1996), although the effect of chlorine on microorganisms attached to surfaces is limited. As mentioned previously, the efficacy of disinfection treatments varies with the concentration of the disinfectant used, time taken to process vegetables and temperature during processing and level of contamination of the produce with organic matter and microbial cells. Table 7.4 summarizes a number of reports of commonly used disinfectants and their efficacy for reducing populations of microorganisms on processed fruits and vegetables. Most workers have concentrated their research efforts on the reduction of total microbial populations and *L. monocytogenes* in salads. It is possible to achieve a 3 log reduction in total count on lettuce using 300 mg/l-1 sodium hypochlorite (Garg et al., 1990),

TABLE 7.4**Effect of Common Disinfecting Treatments of Microbial Populations on Fresh-cut Fruit and Vegetable Products (at the Time of Treatment)**

Disinfecting Treatment	Product	Effect on Microbial Count	Ref.
Chlorine (300 mg/L free) pH 9.4	Lettuce leaves	0.7% of the unwashed count (approximately 10^7 cfu/g)	Adams et al. (1989)
Chlorine (100 mg/L free) pH 5	Lettuce leaves	6.22 log reduction	Adams et al. (1989)
• sulphuric acid		5.83 log reduction	
• acetic acid		5.58 log reduction	
• citric acid		5.77 log reduction	
• lactic acid		5.6 log reduction	
• propionic acid		5.6 log reduction	
Chlorine (210–289 μ g/ml) water	Chopped tomatoes	No reduction in counts of <i>L. monocytogenes</i> or aerobic plate counts when compared to untreated product	Beuchat and Brackett (1991)
Chlorine 200 ppm (10 min)	Lettuce	1.79 log reduction in <i>Salmonella</i> populations (compared to untreated) 2.48 log reduction in <i>E. coli</i> O157:H7 population (compared to untreated) 0.33 log reduction in aerobic mesophile population (compared to untreated)	Beuchat et al. (1998)
Cl ₂ wash (concentration not specified, no treatment control)	Cantaloupe (fresh cut)	Approximately 0.12 log reduction in fluorescent pseudomonads when compared to untreated product	Sapers and Simmons (1998)
Cl ₂ wash (concentration not specified, no treatment control)	Zucchini (fresh cut)	Approximately 1.5 log reduction in fluorescent pseudomonads when compared to untreated product	Sapers and Simmons (1998)
Chlorine wash (100 μ g/mL) • warm wash at 47°C • chilled wash at 4°C	Shredded lettuce	3 log reduction after warm wash 1 log reduction after chilled wash	Delaquis et al. (1999)
Chlorine dioxide (5 mg/l, 10 min, 40°C, pH 7.4)	Shredded lettuce	1.1 log reduction of <i>L. monocytogenes</i>	Zhang and Farber (1996)

TABLE 7.4
Effect of Common Disinfecting Treatments of Microbial Populations on Fresh-cut Fruit and Vegetable Products (at the Time of Treatment) (Continued)

Disinfecting Treatment	Product	Effect on Microbial Count	Ref.
Dichloroisocyanurate (equivalent to 40–320 ppm free chlorine)	Vegetables	1.69–2.42 log reduction compared to wash with water alone	Nicholl and Prendergast (1998)
Electrolyzed water (20 ppm available chlorine)	Fresh-cut vegetables	Log reduction (compared to untreated)	Izumi (1999)
		0.3	
	• carrot	1.8	
	• spinach	0.4	
	• bell pepper	0.2	
	• Japanese radish potato	0.4	
H ₂ O ₂ (no treatment controls)	Cantaloupe (fresh-cut)	Approximately 0.68 log reduction in fluorescent pseudomonads when compared to untreated product	Sapers and Simmons (1998)
H ₂ O ₂ (water and no treatment controls)	Mushrooms (fresh cut)	Approximately 1 log reduction in fluorescent pseudomonads when compared to untreated or water-washed product	Sapers and Simmons (1998)
H ₂ O ₂ (no treatment controls)	Zucchini (fresh cut)	Approximately 1 log reduction in fluorescent pseudomonads when compared to untreated product	Sapers and Simmons (1998)
H ₂ O ₂ (5%)	Fresh-cut fruits and vegetables	3 log kills	Cherry (1999)
Isothiocyanate 400 µL allyl isothiocyanate (2–4 days treatment)	Iceberg lettuce	Up to 8 log reduction of <i>E. coli</i> O157:H7	Lin et al. (2000)
Ozone (1.3 mM ozone, injected at 1.5 L/min for 3 min)	Shredded lettuce	2 log reduction in total count	Kim et al. (1999)
Peroxyacetic acid (200 ppm)	Fresh-cut fruits and vegetables	2 log kills	Cherry (1999)

Count taken immediately after treatment.

although commercially, chlorine concentrations are generally lower, 100–200 ppm. At these concentrations, a 1–2 log reduction in total aerobic count would be expected on processed lettuce and endive (Adams et al., 1989; Beuchat and Brackett, 1990b). The effect of chlorine on several pathogens has been established on only a limited

range of processed products. Zhang and Farber (1996) reported reduction of *L. monocytogenes* on shredded lettuce and cabbage. They achieved a 1.3–1.7 log₁₀ cfu/g on lettuce and 0.9–1.2 log₁₀ cfu/g on cabbage after treatment with 200 ppm chlorine for 10 min. Beuchat et al. (1998) measured the reduction of *E. coli* O157:H7, *Salmonella* and *L. monocytogenes* on whole and cut lettuce sprayed and rinsed with chlorine solutions of 200 ppm. Compared with control samples that were washed in water, reductions of only 1 log cfu/g were achieved by treatment with chlorine. Further investigation is needed of the effect of disinfectants on pathogenic species on a wider range of products.

Recent controversy over the safety and effectiveness of chlorine (Sapers and Simmons, 1998) has encouraged interest in alternative sanitizers, including peroxy-acetic acid, chlorine dioxide, ozone, trisodium phosphate and hydrogen peroxide (Beuchat, 1998; Sapers and Simmons, 1998; Xu, 1999). Detailed studies are needed to determine the effectiveness of these compounds for reducing the initial load of the produce and also to determine their role in shelf life extension of the cut produce.

Temperature

Temperature control is commonly used to prevent or minimize the microbial spoilage of foods. Although refrigeration of fruits and vegetables does not completely inhibit microorganisms, it reduces the growth rates of some spoilage organisms and food-borne pathogens (Nguyen-The and Carlin, 2000). However, we need to establish to what extent temperature control is important during minimal processing of vegetables. The key issue is how does the temperature profile of the produce from receipt at the factory and throughout processing influence the growth of individual species' microbial interactions? Is it crucial for the quality of the final product to refrigerate produce immediately after harvest and to maintain refrigeration conditions at all stages of processing? Ideally, to answer these questions, we should study the microbial ecology of vegetables and the effect of temperature on this ecology throughout all stages of the production chain. Unfortunately, there are no reports of the temperature effect during processing, but most processors maintain lower than ambient temperatures during processing operations. From the time of arrival, processors attempt to reduce the temperature of raw fruits and vegetables to refrigeration temperatures, 5°C. The environment in which trimming and peeling processes are performed is maintained at around 10–15°C, and wash water is generally refrigerated. After processing, fresh-cut products are cooled to 2–5°C (Ahvenainen, 1996).

There are several publications investigating the effect of storage temperature on microbial development in fresh-cut products. Storage at refrigeration temperatures generally selects for growth of psychrotrophic organisms (Nguyen-The and Carlin, 1994, 2000), even though mesophilic and psychrotrophic counts may be of similar magnitude prior to processing (Garg et al., 1990). Refrigeration prevents the growth of *Erwinia* spp. and several spoilage fungi, including *Fusarium* and *Phytophthora* spp. but does not prevent the growth of *Pseudomonas fluorescens* (Lund, 1983; Nguyen-The and Carlin, 2000). Mesophilic organisms may also grow at refrigeration temperatures, at reduced growth rates (Marth, 1998). Manvell and Ackland (1986) used the presence of lactic acid in salads as an indicator of temperature abuse. Gram-negative

organisms were found to predominate in mixed salads containing leafy vegetables and in carrots at 7°C, whereas lactic acid bacteria could proliferate in salads at 30°C. Growth of mesophilic organisms is reduced on leafy vegetables as the storage temperature decreases (2°C) (Bolin et al., 1977; Carlin and Nguyen-The, 1989; Magnusson et al., 1990; Beuchat and Brackett, 1990b), presumably because the temperature is below optimum growth temperature. However, spoilage of salads at low temperatures may not be the result of psychrotrophic bacteria alone. For example, Vescovo et al. (1996) observed a 2 log increase in mesophilic counts in mixed salads stored at 8°C for six days. King et al. (1991) investigated the effect of temperature on bacterial growth on lettuce. Bacterial counts increased significantly on lettuces stored at 2, 5 and 7°C, although growth rates were similar at all temperatures. It was assumed that the bacteria were growing below their optima at all storage temperatures used in the study. Growth rates of lactic acid bacteria on shredded carrots were found to decrease as storage temperatures were lowered (Carlin et al., 1990; Kakiomenou et al., 1996). Similarly, Guerzoni et al. (1996) showed that processing time and temperature directly influenced the proliferation of contaminants on lettuce. Lettuces treated with chlorine solution (165 ppm) were stored at either 5 or 12°C for six hours after treatment, then subsequently stored at 5°C for up to 10 days. Lettuces exposed to the higher temperature were more heavily contaminated with bacteria (10^7 cfu/g coliforms) than those stored at 5°C (10^5 cfu/g coliforms).

The effect of temperature on the survival and activity of pathogens on fresh-cut produce is not widely reported in the literature, and most reports focus on *L. monocytogenes*. Storage of processed fruits and vegetables at refrigeration temperatures may deter the growth of mesophilic pathogens but will not necessarily prevent survival and growth of *L. monocytogenes* or *Aeromonas hydrophila*. However, refrigerated storage may reduce the growth rate of these pathogens, thus reducing the risk of their development during the storage life of fresh-cut products. *Listeria monocytogenes* was reported to survive on shredded lettuce at 5°C, although no significant growth was recorded for up to eight days of storage (Beuchat and Brackett, 1990b). By increasing storage temperature to 10°C, growth was significant after three days of storage, and at 10 days, 10^8 – 10^9 cfu/g were recorded. Similarly, Aytaç and Gorris (1994), Carlin and Nguyen-The (1994) and Jacxsens et al. (1999) reported slow growth of *L. monocytogenes* on a range of lettuce and chicory endives at temperatures under 10°C. Kallander et al. (1991) found that *L. monocytogenes* grew rapidly (1 log increase by day two) on cabbage for the first few days of storage at 5°C, but numbers decreased after six days, at which time the cabbage was spoiled and pH was reduced. *Aeromonas* was reported by Aytaç and Gorris (1994) and Jacxsens et al. (1999) to exhibit a higher growth rate than *Listeria*, increasing on chicory endive by 4 logs after seven days of storage at 6.5°C. *Clostridium botulinum* can also survive at refrigeration temperatures, although toxin is generally not produced if the temperature is lower than 15°C (Petran et al., 1995). Enteric viruses can also survive in processed vegetables at 40°C. Poliovirus decreased by only 1 log cycle on cut vegetables stored at 4°C for 10 days (Crocì et al., 1991). There are limited reports of survival of other pathogens in refrigerated processed fruits and vegetables. The main risks from pathogens such as *Staphylococcus aureus* and *Salmonella* spp. arise from products that may undergo temperature abuse. These organisms are known to survive on vegetables,

including tomatoes and mushrooms, stored at 20–35°C. Further studies of the effects of temperature fluctuations throughout processing and storage of fresh-cut products will allow us to better assess the risks of the growth of pathogens.

There are few reports investigating the effect of temperature on the spoilage of low pH, dressed salads. King et al. (1976) studied the effect of low temperature on the microflora of coleslaw dressed with sour cream and mayonnaise. Deterioration of salad quality was observed for coleslaw stored at two temperatures, 7 and 14°C. At 14°C, the total microbial count increased logarithmically during storage, while at 7°C, the microbial count gradually decreased. No spoilage organisms were detected in the coleslaw stored at the lower temperature, and it was assumed that deterioration of the salads was due to physiological changes occurring in the cabbage tissue. Brocklehurst et al. (1983) studied the microflora of coleslaw stored at 5 and 10°C. Coleslaw dressed with mayonnaise supported growth of *Saccharomyces exiguus* during storage at 5°C and *Saccharomyces dairensis* during storage at 10°C. Lactic acid bacteria could not grow during storage at 5 or 10°C.

pH

Lowering the pH of foods to within the range 3.0–5.0 restricts the types of microorganisms able to grow, thus reducing the risk of spoilage or the growth of organisms of public health significance. Traditionally, this has been achieved by fermentation or addition of acidic ingredients. We are currently unsure of the effect of minimal processing on the pH of fruits and vegetables. Can the pH be altered during minimal processing of fruits and vegetables to create hurdles for spoilage organisms and microorganisms of public health significance? In the case of many fruits, the pH may already be sufficiently low to deter spoilage bacteria. Can the acidity of fruits be relied on to reduce the risk of spoilage or growth of pathogens in mixed fresh-cut products? The influence of pH changes during processing on the microflora should also be investigated. Only a few studies have reported pH changes for minimally processed vegetables, including dressed salads. King et al. (1991) noted that pH of lettuce increased during storage, concurrently with an increase in bacterial population that was predominantly Gram-negative. A decrease in pH was observed for vegetables such as shredded carrots, where the predominant flora is lactic acid bacteria (Kakiomenou et al., 1996). Growth of spoilage bacteria, presumably lactic acid bacteria, on shredded cabbage and the resulting reduction in pH prevented the proliferation of *L. monocytogenes* (Kallander et al., 1991). Marchetti et al. (1992) observed that development of microbial flora on minimally processed salads was not simply related to pH and the presence of organic acids. They concluded that spoilage patterns were also related to characteristics of the raw materials. This further emphasizes the need to investigate interactions between pH and other factors affecting the shelf life of ready-to-eat salads.

Fresh-cuts may be dressed with acidic sauces or creams such as mayonnaise or sour cream to lower the pH. This creates a selective environment that favors growth of acid-tolerant microorganisms. The predominant flora of dressed, low-pH salads are bacteria such as acid-tolerant lactobacilli and yeasts (Hildebrandt et al., 1989; Hunter et al., 1994). Growth of other microorganisms is either inhibited by the acetic

acid present in mayonnaise or by acids produced by the sour cream culture or by competition, in the case of sour cream dressings. For example, King et al. (1976) concluded that the microflora of coleslaw prepared with sour cream was the same as that of the cream, replacing the cabbage microorganisms.

Brocklehurst et al. (1983) and Brocklehurst and Lund (1994) studied the effect of pH on spoilage of salads. They reported that acetic acid in mayonnaise was inhibitory to lactic acid bacteria and the spoilage yeasts, *S. dairensis* and *S. exiguus*, in potato salads and coleslaw. However, the addition of salad ingredients reduced inhibitory effects of mayonnaise. It was hypothesized that salad ingredients absorbed acetic acid, resulting in an overall increase in salad pH. The combined hurdles of low temperature and low pH during storage also influenced growth. The acid environment is more inhibitive at refrigeration temperatures (5°C) than at higher temperatures (10°C), emphasizing the importance of controlling temperature and pH during processing. Fresh-cut produce are now mixed with ingredients such as pasta and a range of salads based on low-fat and sour cream dressings and low-acid dressings that are now available. The significance of using less acid and the changes to pH during production and storage of salads should be investigated, focusing on the survival of individual microbial species.

Packaging

The final stage in production of fresh-cut vegetables is packaging. The package provides protection for the fresh-cut product from damage and further contamination with microorganisms. The use of controlled and modified atmosphere packaging also provides, to some extent, a hurdle against the growth of the remaining spoilage microflora and foodborne pathogens (Phillips, 1996). However, the microbial ecology of packaged fresh-cut products is poorly understood. Investigation is needed of the influence of packaging methods on the growth of individual species, how packaging and controlled atmospheres influence interactions between species and, ultimately, how they influence the microbial quality and safety of the products. Fresh-cut fruits and vegetables are either packaged unsealed, in the presence of air or under a modified atmosphere (MA) (Ngyuen-The and Carlin, 1994). If a package is sealed under air, the MA results from the respiration of the product. Alternatively, the package may be filled with a specific gas mixture, for example, 5–10% CO₂ and 2–5% O₂ as is used to extend shelf life of whole vegetables (King and Bolin, 1989). Further reduction of oxygen or increase in carbon dioxide concentrations may be deleterious to the physiology of the product, for example, browning reactions may be encouraged (Kader et al., 1989; Phillips, 1996).

The influence of MA packaging on the physiology and microbiology of fruits and vegetables has been reviewed (Nguyen-The and Carlin, 1994, 2000; Phillips, 1996; Bennick et al., 1996, 1998; Kader and Watkins, 2000), and it is clear that the benefits of MA cannot be explained solely by a reduction in total microbial load. Other influencing factors include the packaging material used, the use of microperforations in the material, relative humidity during storage, time prior to packaging, temperature of storage, type of produce and type and number of microorganisms present and the nutrients available to support microbial growth. A combination of low-temperature

storage and MA packaging is recommended to enhance quality of fresh-cut fruits and vegetables and to extend shelf life. There have been few attempts to relate the effect of packaging on the individual spoilage organisms and on the overall acceptability of vegetable products throughout storage. However, a number of studies investigated the use of modified atmosphere packaging and the effect of increasing CO₂ concentrations on the survival of *L. monocytogenes* and other pathogens (Table 7.5).

The effect of modified atmosphere packaging on the microbial ecology and organoleptic qualities of lettuce has been studied by Magnuson et al. (1990) and King et al. (1991) (Table 7.5). Modified atmosphere was created passively in both studies. The major difference between lettuces stored unsealed and sealed in bags was the influence of the MAP on the microflora. A wider variety of microorganisms grew on unsealed packaged lettuce than on the packaged lettuce. Although bacteria initiated spoilage, the yeasts *Pichia fermentans* and *Torulaspora delbrueckii* developed as the conditions became anaerobic in the sealed bags. Higher microbial counts, including *Pseudomonas* counts, were reported in unpackaged and shrink-wrapped cut broccoli than in gas-packed samples by Brackett (1989). However, populations of Enterobacteriaceae were similar regardless of packing treatment. Gas-packed samples remained fresh after six weeks of storage, unlike other treatments in which the broccoli was spoiled, suggesting that modified atmosphere packaging may delay the spoilage of a product. Kakiomenou et al. (1996) observed that the onset of spoilage of shredded carrots by lactic acid bacteria was delayed in carrots stored at 5% CO₂. Lactic acid bacteria predominated in packaged and unpacked samples, although organic acid production was higher under MA packaging, suggesting greater metabolic activity under these conditions.

Bennick et al. (1996, 1998) presented a detailed ecological study of the effect of MA packaging on the predominant flora of chicory and mung bean sprouts. The numbers and types of organisms and the growth rates of these organisms were recorded during storage. Overall, MA did not influence maximum population densities, but the increase in CO₂ in packages resulted in the reduction of maximum specific growth rates, particularly for *Pseudomonas* spp. Changes in predominance of microbial species were specific to different vegetables, suggesting specific effects of the MA on microorganisms, thus reinforcing the need to investigate factors affecting the growth of individual organisms present on vegetables.

Packaging under modified atmosphere generally does not inhibit the growth of *L. monocytogenes*, although other factors influence its survival (Beuchat and Brackett, 1990b; Kallander et al., 1991; Aytaç and Gorris, 1994; Carlin et al., 1996; Jacxsens et al., 1999). *Listeria monocytogenes* grows well on lettuce and chicory endive under modified atmosphere. Carlin et al. (1996) observed stored chicory endive under MA increasing CO₂ concentration as high as 50%. They observed that *Listeria* grew better as the CO₂ increased. Kallander et al. (1991) showed that temperature affected the growth of *L. monocytogenes* under MA conditions. At 5°C, no difference was observed in growth of the organisms on shredded cabbage, but at 25°C, initial growth was reduced, and a rapid decline was observed after six days of growth. This was most probably due to the excessive growth of spoilage flora under the MA conditions. Jacxsens et al. (1999) observed the effect of an equilibrium atmosphere of 2–3% O₂:3% CO₂:94–96% N₂ on the growth of *L. monocytogenes* and *Aeromonas* spp. on

TABLE 7.5
Influence of Packaging on the Growth of Microorganisms in Salads/Vegetables

Salads/ Vegetables	Packaging Treatment	Growth of Spoilage Microorganisms	Spoilage Characteristics	Ref.
Broccoli	1. No package 2. Shrink-wrap 3. MAP pouches (5% O ₂ , 10% CO ₂ , 85% N ₂)	Higher total counts in treatment 1 than in other treatments. No differences between counts of Enterobacteriaceae. Pseudomonas counts were higher in treatment 1 than in other treatments. Coryneform bacteria predominated in shrink-wrap packages. MAP broccoli had a longer shelf life than in other treatments.	Broccoli in no packaging was spoiled after six weeks storage—yellowing, slimes, wet lesions. No spoilage symptoms in other treatments at six weeks.	Brackett (1989)
Broccoli florets	1. No package 2. Packaged	Aerobic plate count and coliform counts were consistently higher in unpackaged than in packaged during storage at 8°C for seven days.	Moisture loss in unpackaged florets, less crisp than packaged florets.	Mohd-Som et al. (1994)
Shredded cabbage	Packages 1. Normal atmosphere 2. Modified atmosphere (70% CO ₂ , 30% N ₂)	After 20 days storage at 5°C, population of <i>Listeria monocytogenes</i> gradually increased up to 13 days followed by a rapid decline, 2.0 log, under normal atmosphere conditions. Under modified atmosphere, there was an approximate 1.0 log increase.	—	Kallander et al. (1991)
Chicory/endive	Packaged 1. No vacuum 2. Half vacuum	<i>Aeromonas hydrophila</i> population increases 4 log units, seven days storage with no vacuum, gradual loss of viability under vacuum. <i>Listeria monocytogenes</i> maintained viability in both packages (approximate 2 log increase under vacuum).	—	Aytaç and Gorris (1994)

(continued)

TABLE 7.5
Influence of Packaging on the Growth of Microorganisms in Salads/Vegetables (Continued)

Salads/ Vegetables	Packaging Treatment	Growth of Spoilage Microorganisms	Spoilage Characteristics	Ref.
Chicory/endive	1. Control—21% O ₂ , 0% CO ₂ , 78% N ₂	Control—50% higher maximum growth rate of the predominant spoilage organisms than in the MAP treatment.	Control—discoloration and softening, rotting.	Bennick et al. (1996)
	2. MAP—1.5% O ₂ , 20% CO ₂ , 78% N ₂	MAP—Predominant organisms include <i>Ps. fluorescens</i> , <i>Ps. corrugata</i> , <i>Ps. putida</i> , species from the family Enterobacteriaceae.	MAP—good appearance, acceptable quality after 13 days of storage.	
Shredded chicory/endive	1. Air	Changes (increase) in microbial populations over 6–7 days storage (7°C)	Less enzymic discoloration, longer retention of rigidity, beneficial to physiological state of the vegetable in EMA	Jacxsens et al. (1999)
	2. Equilibrium modified atmosphere (EMA) (2–3% O ₂ , 3% CO ₂ , 94–96% N ₂)	Total count: Air—2.36, EMA—2.52 <i>Aeromonas</i> : Air—0.5, EMA—2.5 <i>Listeria monocytogenes</i> : Air and EMA—0–0.1		
Lettuce	Polyethylene (38 µm) bags	Initial spoilage of all samples by bacteria. Wider variety of yeast species in unsealed bags. Growth of fermentative yeasts (<i>Pichia fermentans</i> and <i>Torulaspora delbrueckii</i>) was encouraged.	Discoloration and softening. (Lettuce stored for 11 weeks.)	Magnuson et al. (1990)
	1. Unsealed 2. Sealed			
Lettuce	Polyethylene bags	Slower bacterial growth in sealed bags, wider variety of yeast species in unsealed bags	Discoloration and softening	King et al. (1991)
	1. Sealed 2. Unsealed			

Shredded iceberg lettuce	1. Air 2. Equilibrium modified atmosphere (EMA) (2–3% O ₂ , 3% CO ₂ , 94–96% N ₂)	Changes (increase) in microbial populations over six to seven days storage (7°C) Total count: Air—2.45, EMA—1.16 <i>Aeromonas</i> : Air—0.2, EMA—0.1 <i>Listeria monocytogenes</i> : Air—1.5	Less enzymic discoloration, longer retention of rigidity, beneficial to physiological state of the vegetable in EMA.	Jacxsens et al. (1999)
Mixed vegetable salad	Plastic film Initial headspace 1. Air 2. MA—10.5% CO ₂ , 2.25% O ₂ , 87.25% N ₂	Total microbial counts—no difference between packaging treatments.	Acceptable in MAP after 10 days.	Priepke et al. (1971)
Shredded carrots	Bags 1. Air 2. MAP—5% CO ₂ , 95% N ₂ 3. Polyethylene (60 µm)—4.9% CO ₂ , 2.1% O ₂ , 93% N ₂	Lactic acid bacteria predominated in all treatments. Organic acid production increased for carrots stored under MAP.	Better texture, color and odor under MAP. Delayed spoilage.	Kakiomenou et al. (1996)

Source: Adapted from Heard (1999b).

a range of vegetables, including shredded lettuce, chicory endive, Brussels sprouts and carrots. Brussels sprouts and carrots were found to inhibit the growth of the pathogens, but both organisms grew under the MA conditions. There is also a general concern that anaerobic conditions of MA-packaged fresh-cuts will support the growth of anaerobic clostridia, in particular, *Cl. botulinum* (Nguyen-The and Carlin, 2000); however, there is little information available concerning the risks.

CONDITIONS DURING RETAILING

There is no scientific evidence documenting the influence of retail conditions on the microbial quality of fresh-cut products. Nevertheless, if we apply the main factors influencing the microbial ecology of foods as summarized in Table 7.3, the main influencing factors can be predicted. The shelf life and microbial quality of fresh-cut products during transport and retailing are dependent on the following:

1. Method of transport—refrigerated transport should be used to maintain temperatures below 5°C.
2. Time—transport and loading times should be minimized, e.g., temperature fluctuations occur when products are left on loading docks or in storage rooms at supermarkets.
3. Temperature of storage cabinets—refrigerated cabinets should be maintained at temperatures below 5°C.

The final factor affecting the quality during retailing is handling by the consumer. Fresh-cut products are highly perishable products and should be stored under refrigeration after purchase. Education on proper food handling practices should be provided for supermarket handlers and consumers.

OTHER FACTORS

The intrinsic properties of some vegetables and implicit characteristics of the microorganisms may influence development of the microflora during the production and storage of fresh-cut produce. For example, carrots are reported to exhibit antilisterial effects (Beuchat and Brackett, 1990a) and may be used in combination with other ingredients as an extra hurdle to prevent growth of *L. monocytogenes*. Marchetti et al. (1992) observed that red chicory and carrot juices exhibited antimicrobial activity against *Ps. fluorescens* and *L. monocytogenes*. Background microflora of spinach, endive and lettuce have also been reported to restrict the growth of *Listeria* spp. (Carlin et al., 1996; Babic et al., 1997; Francis and O'Beirne, 1998). For example, *Enterobacter* spp. were found to compete with *L. innocua* on processed lettuce (Francis and O'Beirne, 1998). *Listeria monocytogenes* may also be inhibited in foods by *Ps. fluorescens* and lactic acid bacteria. *Pseudomonas* spp. produce siderophores to bind essential nutrients, and lactic acid bacteria may produce antimicrobial bacteriocins in addition to lactic acid and hydrogen peroxide (Harris et al., 1989).

Finally, it is essential to ask if there are interactions in fresh-cut products between the indigenous spoilage flora such as the pseudomonads and foodborne pathogens. It has been suggested that removal of the indigenous spoilage microflora of fresh

foods such as fruits and vegetables “opens the door” for pathogens and allows them to proliferate. Preliminary investigations have indicated that biofilms occur on vegetable leaves (Carmichael et al., 1999). Biofilms formed by nonpathogenic species may form a natural barrier to prevent attachment of pathogens to surfaces of fruits and vegetables. Further study is required to better understand leaf ecology and interactions. Seo and Frank (1999) investigated the colonization of lettuce leaves by *E. coli* O157:H7 and *Ps. fluorescens*. The use of confocal scanning laser microscopy and dual staining techniques enabled detection of the different organisms on the leaf surface. The pseudomonad preferentially adhered to the intact leaf surfaces, while the pathogen colonized cut edges and stomata. Interestingly, the pathogen did not adhere to the biofilm formed by the pseudomonad on leaf surfaces.

MICROBIAL SPOILAGE OF FRESH-CUT PRODUCTS

EFFECT OF MICROBIAL GROWTH ON THE QUALITY AND SHELF LIFE OF FRESH-CUT SALADS

Spoilage symptoms appear as microbial numbers increase, and as a result, quality is reduced and shelf life shortened. However, the degree of spoilage does not always correlate with large total populations (Nguyen-The and Carlin, 2000). One example, reported by Nguyen-The and Prunier (1989), was the spoilage of chicory endive salad by *Pseudomonas marginalis*. After initial investigation, it was concluded that deterioration of the product with this organism occurred because it was the predominant species. However, treatment of the vegetable with pure cultures of other species (10^8 cfu/g leaves) failed to induce spoilage. As described in the previous section, environmental factors, the types of fruits or vegetables and the types of organisms present determine what type of spoilage occurs and how quickly the quality of the product deteriorates. For example, pectinolytic pseudomonads present on chicory endive leaves will cause spoilage (Nguyen-The and Prunier, 1989). However, carrots stored at 10°C and contaminated with equal numbers of pseudomonads and lactic acid bacteria exhibit spoilage symptoms of the latter (Carlin et al., 1989). Unlike whole vegetables, fresh-cuts are not spoiled by the soft rot bacteria *Erwinia*, suggesting that either the environment does not encourage their growth or that other bacteria outgrow these species (Nguyen-The and Carlin, 2000).

Despite these observations, our knowledge of the spoilage patterns of fresh-cut products is limited. Most manufacturers base shelf life predictions on total microbial counts or groups of microorganisms and observation of the associated spoilage defects as well as the extent of enzymatic degradation of the tissues. We do not fully understand the microbial interactions occurring or the factors influencing spoilage by individual species in the finished product, and further study is required.

SPOILAGE CHARACTERISTICS

Spoilage of fresh-cut vegetables by bacteria is characterized by brown discoloration, production of off-odors, loss of texture and, to a lesser extent, soft rot. Fruit products undergo fermentative spoilage by lactic acid bacteria or yeasts, resulting in the

production of acids, alcohol and CO₂, although pseudomonads may spoil less acidic fruits such as cantaloupe. Lipase activity and utilization of amino acids can alter the flavor of fruits, resulting in a loss of quality. The main types of spoilage responsible for deterioration of quality and the mechanisms of spoilage are summarized as follows:

1. Soft rots—maceration of the vegetable tissue caused by enzymatic degradation of the plant cell wall by pectinolytic enzymes (Liao and Wells, 1987)
2. Formation of off-odors and off-flavors—activity of lipolytic and proteolytic enzymes and fermentation reactions (Kato et al., 1989; Wyllie and Leach, 1992; Zhuang et al., 1994; Lamikanra et al., 2000)
3. Wilting—brought on by vascular infections (Schroth et al., 1992)
4. Brown discoloration—polyphenol oxidase activity of the microflora may contribute to browning (Padaga et al., 1999)
5. Fermentative spoilage—fermentation of carbohydrates to produce acid, gas or alcohol

OCCURRENCE OF SPOILAGE ORGANISMS IN FRESH-CUT PRODUCTS

Deterioration of fresh-cut products occurs after packaging, during retailing, and although this may be due to the natural, physiological response of the plant tissue, microorganisms make a significant contribution to onset of spoilage and decrease in product shelf life (Lund, 1971, 1992; Nguyen-The and Carlin, 1994; Brackett, 1997; Heard, 1999b). To be able to predict when and where spoilage will occur, we need to know which organisms are present and which organisms are responsible for onset of spoilage. And, we need to understand some of the characteristics of the organisms.

Relatively few studies have reported the occurrence of individual, including spoilage, organisms in fresh-cut fruits and vegetables. Table 7.6 summarizes the predominant microorganisms present in fresh-cut products, including salads that are mixed with mayonnaise and that may contain other ingredients such as cheese, seafood or meat. Although not strictly considered to be fresh-cuts, these are common ways of packaging and retailing minimally processed salad vegetables, and the microbiological consequences of adding such ingredients should be considered.

Pseudomonads are the most common organisms isolated from fresh-cut vegetable salads. Biovars of *Pseudomonas fluorescens* are generally the main species (Denis and Picoche, 1986; Marchetti et al., 1992) and have been reported in mixed salads as well as lettuce salads at populations of up to 10⁷ cfu/g salad (Denis and Picoche, 1986; Geiges et al., 1990). Marchetti et al. (1992) found that *P. fluorescens* occurred at higher frequency (23% of 162 isolates) than all other bacteria present on both lettuce and mixed salads. Other species often present on leafy vegetables include *Pseudomonas putida*, *Pseudomonas chloraphis*, *Pseudomonas corrugata*, *Pseudomonas cepacia*, *Pseudomonas paucimobilis*, *Pseudomonas marginalis* (*P. fluorescens* biotype II) and *Pseudomonas viridiflava* (Table 7.6). Nguyen-The and Prunier (1989) observed that *Ps. marginalis* was the predominant organism on chicory-endive leaves. They concluded that it was a weak plant pathogen only causing spoilage if

TABLE 7.6
Predominant Spoilage Microflora in Salads

Spoilage Organism	Salad	Ref.
Pseudomonads		
<i>Pseudomonas fluorescens</i>	Carrots, lettuce, mixed salad, chicory, potato salad, dryslaw, tabouli	1,2,3,4,5,6,9,11
<i>Pseudomonas fragi</i>	Potato salad, dryslaw, tabouli	11
<i>Pseudomonas putida</i>	Carrots, lettuce, mixed salad, alfalfa sprouts, potato salad, dryslaw, tabouli	1,2,3,4,9,11
<i>Pseudomonas marginalis</i>	Carrots, lettuce, mixed salad, chicory leaf, potato salad	1,3,9,11
<i>Pseudomonas cepacia</i>	Carrots, mixed salads, chicory	5
<i>Pseudomonas chicorii</i>	Chicory	9
<i>Pseudomonas fulva</i>	Chicory	9
<i>Pseudomonas paucimobilis</i>	Carrots, mixed salad, chicory	5
<i>Methylobacterium mesophilica</i>	Carrots, mixed salad, chicory	5
<i>Pseudomonas viridiflava</i>	Carrots, lettuce, mixed salad	1
<i>Stenotrophomonas maltophilia</i>	Carrots, lettuce, mixed salad, potato salad, dryslaw	1,11
<i>Pseudomonas chloroaphis</i>	Prepared salad	4
<i>Pseudomonas corrugata</i>	Chicory, sprouts, potato salad, dryslaw, tabouli	9,11
<i>Flavimonas oryzihabitans</i>	Tabouli	11
Other Bacteria		
<i>Agrobacterium radiobacter</i>	Potato salad	11
<i>Acinetobacter</i> spp.	Tabouli	11
Coryneform bacteria	Carrots, lettuce, mixed salad	1
<i>Flavobacterium</i> sp.	Carrots, lettuce, mixed salad	1
<i>Enterobacter agglomerans</i>	Mixed salad, chicory leaf, salad leaves, prepared salad, dryslaw, tabouli	2,3,4,6,7,11
<i>Enterobacter amnigenus</i>	Tabouli	11
<i>Enterobacter gervoidae</i>	Dryslaw	11
<i>Erwinia carotovora</i>	Mixed salad	2,6
Enterobacteriaceae**	Chicory, sprouts	9
<i>Klebsiella terrigena</i>	Dryslaw	11
<i>Lactobacillus</i> spp.	Carrots, lettuce, mixed salad	1,6
<i>Leuconostoc</i> spp.	Carrots, lettuce, mixed salad, potato salad, dryslaw, tabouli	1,2,11
<i>Rahnella aquatilis</i>	Potato salad, dryslaw	11
<i>Serratia marcescens</i>	Tabouli	11
<i>Yersinia intermediata</i>	Potato salad	11
Yeasts		
<i>Candida</i> spp.	Salad mix, mayonnaise-based salads,* carrots, chicory	5,6,8,10
<i>Cryptococcus albidus</i>	Lettuce	6
<i>Cryptococcus laurentii</i>	Lettuce, carrots, mixed salad, chicory	5,6

(continued)

TABLE 7.6
Predominant Spoilage Microflora in Salads (Continued)

Spoilage Organism	Salad	Ref.
<i>Debaryomyces hansenii</i>	Salad mix, mayonnaise-based salads*	6,8
<i>Pichia fermentans</i>	Lettuce, salad mix	6
<i>Pichia membranifaciens</i>	Mayonnaise-based salads*	8,10
<i>Saccharomyces cerevisiae</i>	Salad mix, mayonnaise-based salads*	6,8
<i>Saccharomyces dairensis</i>	Mayonnaise-based salads*	8
<i>Saccharomyces exiguus</i>	Mayonnaise-based salads*	8
<i>Torulaspora delbrueckii</i>	Salad mix, mayonnaise-based salads*	8,10
<i>Trichosporon cutaneum</i>	Lettuce	6
<i>Yarrowia lipolytica</i>	Mayonnaise-based salads*	8
<i>Zygosaccharomyces bailii</i>	Mayonnaise-based salads*	8
Molds		
<i>Aspergillus niger</i>	Lettuce, mayonnaise-based salads*	6,8
<i>Botrytis allii</i>	Lettuce	6
<i>Penicillium chrysogenum</i>	Mayonnaise-based salads*	8

References—1: Denis and Pioche (1986); 2: Brocklehurst et al. (1987); 3: Nguyen-The and Prunier (1989); 4: Geiges et al. (1990); 5: Marchetti et al. (1992); 6: Magnuson et al. (1990); 7: Gras et al. (1994); 8: Hunter et al. (1994); 9: Bennick et al. (1998); 10: Birzele et al. (1997); and 11: Jayasekara (1999).

* Mayonnaise-based salads include coleslaw, rice salads, potato salads, fruit and nut salads, prawn and pasta salads and other miscellaneous salads.

** Enterobacteriaceae isolates include *Rahnella aquatilis*, *Serratia odorifera*, *Escherichia vulneris*, *Klebsiella oxytoca*, *Enterobacter cloacae*, *Erwinia amylovora*, *Enterobacter intermedius*, *Kluyvera cryocrescens*, *Serratia proteamaculans*, *Buttiauxella agrestis* and *Enterobacter cloacae*.

present on leaves in excess of 10^8 cfu/g. Jayasekara (1999) enumerated pseudomonads and related species on several salads, including tabouli, dryslaw mix and potato salad. Most commonly isolated species included *Ps. fluorescens*, *Ps. corrugata*, *Ps. putida* and *Ps. marginalis*. Other commonly isolated organisms included species from the Enterobacteriaceae and lactic acid bacteria. Coryneform bacteria and species of *Lactobacillus* and *Leuconostoc* are commonly isolated from whole vegetables and may contribute to the spoilage of processed salads during storage (Brackett, 1994, 1997). Of the lactic acid bacteria, *Leuconostoc* are reported to predominate, in particular, *Leuconostoc mesenteroides* (Carlin et al., 1989). Populations of *Lactobacilli* of around 10^4 cfu/g have been reported on fresh-cut pineapple and cantaloupe after processing, increasing to 10^7 cfu/g after 12 days of refrigerated storage (O'Conner-Shaw et al., 1994; Portella et al., 1997 cited in Lamikanra et al., 2000).

Bennick et al. (1998) reported the occurrence of various species of Enterobacteriaceae, including *Enterobacter cloacae*, *Pantoea agglomerans* and *Rahnella aquatilis* on mung bean sprouts and chicory endive. *Pseudomonas* species such as *Ps. fluorescens*, *Ps. corrugata* and *Ps. viridiflava* also were detected on fresh and spoiling

vegetables. Predominant species reported on vegetables such as broccoli, endive and sprouts include the fluorescent pseudomonads and species of *Klebsiella*, *Serratia*, *Flavobacterium*, *Xanthomonas*, *Chromobacterium* and *Alcaligenes* (Table 7.6).

A number of yeast species have been associated with dry and dressed, low-pH salads. Although yeasts have been implicated in the spoilage of fermented vegetable products, their role in the spoilage of fresh vegetables is not well studied. Spoilage defects reported in the literature include soft rot formation in onions, surface defects of vegetables such as bell peppers and discoloration of cabbages caused by *Kluyveromyces* spp. (Fleet, 1992). The main yeasts associated with vegetable spoilage are *Cryptococcus* species, in particular, *Cryptococcus laurentii* and species of *Candida*. The more fermentative species, *S. exiguus*, *Saccharomyces cerevisiae* and *S. dairenensis* occur mainly in salads dressed with mayonnaise. Spoilage defects caused by fermentative yeasts are off-odors, gassiness and growth on the surface of the salad (Denis and Buhagiar, 1980; Fleet, 1992). The origin of *Saccharomyces* species in processed salads is not clear but may be the result of contamination from the processing environment, as these species are not commonly found in unprocessed fruits and vegetables (Deak and Beuchat, 1987). Spoilage as a result of mold growth does not appear to be a major problem for ready-to-eat salads, although the problem is not well documented in the literature. A wide variety of fungi is commonly found on the surface of other vegetables and fruits at the time of harvest (Lund and Snowden, 2000; Nguyen-The and Carlin, 2000). However, the high moisture content of the processed fruits and vegetables and modified atmospheres in packaged salads favor proliferation of the faster-growing bacteria and yeast species, thus reducing or inhibiting the growth of molds (Nguyen-The and Carlin, 2000). *Botrytis*, *Aspergillus* and *Penicillium* spp. have been reported to occur on salad vegetables such as lettuce (Magnuson et al., 1990; Hunter et al., 1994). In a recent study of the quality of minimally processed cantaloupe, Lamikanra et al. (2000) reported growth of Gram-negative and Gram-positive bacteria with only minimal presence of mold.

CHARACTERISTICS OF SPOILAGE ORGANISMS

Our understanding of the microbial spoilage of fresh-cut products is incomplete, but we can draw on our knowledge of spoilage of whole fruits and vegetables and other foods to predict spoilage patterns and microbial interactions. Some characteristics of those organisms known to occur on fresh-cut products are presented in this section.

Pseudomonads and Related Species

The family Pseudomonadaceae consists of the four genera: *Pseudomonas*, *Xanthomonas*, *Zoogloea* and *Frauteuria* (Palleroni, 1992). The type genus is *Pseudomonas*, and members are often referred to as pseudomonads. They are Gram-negative rods, occurring singly or in pairs, motile with one or more polar flagella, strictly aerobic catalase positive and oxidase positive or negative. Pseudomonads are also characterized by their ability to grow in simple media. In recent years, species of this genus have been characterized for taxonomic purposes by their RNA group (five RNA groups).

Ps. aeruginosa (type species) and fluorescent species commonly associated with plants *Ps. fluorescens*, *Ps. putida*, *Ps. chlororaphis*, *Ps. syringae* and *Ps. viridiflava* belong to RNA group I. *Pseudomonas fluorescens* is a heterogeneous species that has been subdivided into groups known as subspecies or biotypes/bivars A, B, C, D, E, F and G. They are grouped according to biochemical, physiological and nutritional characteristics (Stanier et al., 1966; Barrett et al., 1986). *Pseudomonas fluorescens* G is the most heterogeneous of all the biotypes and includes strains with irregular nutritional properties and characteristics. No methods for identification and subdivision can entirely classify strains of this biotype (Palleroni, 1992, 1993). The other major group of plant pathogens, the *Pseudomonas cepacia*-*Pseudomonas solanacearum* belongs to RNA group II.

The role of pseudomonads as spoilage organisms is well acknowledged in the food industry. They are capable of synthesizing enzymes, even under refrigeration conditions that facilitate the breakdown of food components and cause spoilage (Cousin, 1982; Jayasekara, 1999). During the spoilage of fruits and vegetables, pseudomonads produce pectolytic enzymes to degrade the cell walls of the host tissue. This results in maceration of the tissue. Other tissue-degrading enzymes produced include cellulases, xylanases and glycoside hydrolases and lipoxygenase (Gross and Cody, 1985; Zhuang et al., 1994). Padaga et al. (1999) tested 165 bacterial isolates from broccoli florets for production of pectinolytic enzymes and lipolytic and proteolytic activity. Strains of *Ps. fluorescens* B were predominantly pectinolytic, producing pectate lyase, pectolytic and polygalacturonase activity. Proteolytic activity and lipolytic activity were noted for pseudomonad isolates. Biosurfactants produced by pseudomonads also assist in degradation of plant tissue. An example is viscosin, a potent peptolipid produced by a strain of *Ps. fluorescens* B. Viscosin production facilitates bacterial infection and spread of decay on unwounded broccoli florets (Laycock et al., 1991). Padaga et al. (1999) reported that 50% of the strains of *Ps. fluorescens* A, *Ps. viridiflava*, *Ps. mendocina* and *Ps. fragii* isolated from broccoli were capable of producing biosurfactant. However, only 10% were strong producers. Similar studies should be conducted for isolates from fresh-cut products to determine the role of pseudomonads in onset of spoilage.

Pseudomonads may also contribute to the yellowing of vegetable products during storage, through the production of the ripening hormone ethylene. Weingart and Völksch (1997) and Weingart et al. (1999) studied the production of ethylene by *Pseudomonas syringae*. When inoculated into a weed, it was shown that enhanced ethylene production during onset of disease was due to ethylene production by the bacteria. Padaga et al. (1999) also observed ethylene production by various strains of *Ps. fluorescens* A and G from broccoli origin.

Lactic Acid Bacteria

The term lactic acid bacteria describes a number of genera of Gram-positive bacteria (rods and cocci) that are traditionally known as fermentative organisms associated with fermented food products and food spoilage. Those genera commonly associated with spoilage of foods include *Lactobacillus*, *Leuconostoc* and *Pediococcus*. Based on chemotaxonomic and phylogenetic studies, these three genera are closely related,

with overlap between them. There are three main groups: the *Lactobacillus delbrueckii* group, which includes mainly homofermentative lactobacilli; the *Lactobacillus casei*/*Pediococcus* group; and the *Leuconostoc* group, including some obligate heterofermentative lactobacilli (Stiles and Holzapel, 1997). The habitats of species of the genera *Lactobacillus* and *Leuconostoc* include plants and plant material, soil, water and sewage and fruit and grain mashes (Stiles and Holzapel, 1997). *Leuconostoc* spp. may be present on growing, undamaged plants in relatively low numbers, but numbers increase during maturation and later during harvest (Daeschel et al., 1987). Although considered desirable during the production of fermented foods, fermentation of sugars to produce acid and gas is undesirable in fresh-cut products. Their fermentative metabolism and ability to grow in anaerobic conditions enables lactic acid bacteria to cause spoilage, such as souring of the product, gas production and slime formation (Carlin et al., 1990; Stiles and Holzapel, 1997).

Enterobacteriaceae

The family Enterobacteriaceae consists of a number of genera of Gram-negative rod-shaped bacteria. The different genera with a variety of ecological niches, including plants, insects, animals and humans, may contaminate fresh-cut products on the farm and during processing. The pathogenic species of this group are usually enumerated using selective isolation media, but the nonpathogens are generally isolated as a total group termed “coliforms.” With the exception of *Erw. caratovora*, a known postharvest pathogen (Nguyen-The and Carlin, 2000), reports of the occurrence of individual species in foods are rare. One such report by Bennick et al. (1998) described the presence of several species on fresh vegetables. They included *Ent. cloacae*, *Pant. agglomerans*, *Rah. aquatilis*, *Erw. caratovora*, *Erw. amylovora*, *Kleb. oxytoca* and *Serratia. oderifera*. The main characteristics of these organisms allowing growth and potential spoilage of fresh-cut vegetables are as follows:

- their ability to grow as facultative anaerobes, thus they can survive in the modified atmosphere of packaged salads (Bennick et al., 1998)
- their ability to ferment glucose to produce acids, alcohols and esters (Adams and Moss, 1995)

The role of these organisms in the spoilage of fresh-cut products is not well understood and is an area for future research.

Coryneform Bacteria

The term “coryneform bacteria” is a general term used for practical purposes to describe a large, diverse group of bacterial taxa that are Gram positive, nonsporing, irregular-shaped rods. They are mostly aerobic, and many of the genera are pigmented. Some of the genera, including *Arthrobacter*, *Rhodococcus* and *Brevibacterium* may have a distinct rod-coccus growth cycle, while others do not display such obvious irregularity (Coyle and Lipsky, 1990). The coryneform groups are well distributed in nature and are found in soil, on plants and in food processing environments.

Taxonomic classification of the group is confusing, and identification is difficult, because characteristics used in conventional identification of bacteria such as morphology and physiology are of little value to separate the taxa. The main approaches to identification of organisms within the coryneform group are the use of chemotaxonomic methods, DND-DNA homology and 16sRNA cataloging (Minnikin and Goodfellow, 1980; Gobbetti and Smacchi, 2000). However, little is known about the occurrence and activity of individual genera or species of coryneform bacteria in foods. Currently, the group consists of the following genera—*Arthrobacter*, *Brevibacterium*, *Rhodococcus*, *Curtobacterium*, *Micobacterium*, *Aureobacterium*, *Corynebacterium*, *Agromyces*, *Cellulomonas* and *Oerskovia*. One genus known to occur in vegetable products is *Arthrobacter*. Some of the characteristics of this species will be discussed.

Arthrobacter

Arthrobacter spp. have a rod-coccus growth cycle, as mentioned previously, do not form endospores and may be either motile or nonmotile by one subpolar or few lateral flagella. They have a respiratory metabolism and are never fermentative. They often occur in soil and in the rhizospheres of plants, exhibiting extreme resistance to dry conditions and periods of starvation. Isolates from soils often have the ability to degrade polymeric compounds, although they do not often produce pectolytic enzymes (Gobbetti and Smacchi, 2000). *Arthrobacter* spp. are recognized as playing a role in the ripening of smear cheeses, contributing to aroma, flavor and texture development. These changes are facilitated by the production of lipolytic and proteolytic enzymes. There is less information available describing the activity of *Arthrobacter* spp. on vegetables. Gobbetti and Smacchi (2000), reviewing the role of *Arthrobacter* spp. in foods, report that they are chitinolytic bacteria, able to degrade fungal hyphae, thus destroying some soilborne fungal pathogens. They also report that *Arthrobacter* spp. have been known to degrade pesticides in polluted and cold environments. *Arthrobacter* spp. isolated from broccoli florets were reported by Padaga et al. (1999) to produce biosurfactants and tissue-degrading enzymes including pectolytic, lipolytic and proteolytic enzymes. These species also produced polyphenol oxidase activity and were capable of forming unpleasant aroma compounds such as methanethiol. Further investigation is necessary to determine the significance of coryneform bacteria such as *Arthrobacter* spp. in the fresh-cut environment.

Yeasts and Molds

A wide variety of species of molds are known to cause postharvest disease in fruits and vegetables. However, in the fresh-cut environment, faster-growing yeasts tend to outgrow molds to cause spoilage. Characteristics of yeasts will be discussed. Yeasts are single-celled eukaryotic organisms of which many genera are associated with the fermentation and spoilage of foods. Fermentative species of yeasts such as *Kloeckera* and *Hanseniaspora* occur naturally on the surfaces of fruits and are capable of causing fermentative spoilage (Barnett et al., 2000). Other fermentative species such as *S. cerevisiae* and *S. exiguus* may contaminate fruits during processing and cause explosive fermentative spoilage. Growth and fermentation do not usually

occur until the fruit is damaged, allowing leakage of juices and sugars. Thus, in the fresh-cut environment (and in low-pH dressed salads), yeast growth and spoilage of fruits is predictable. The characteristics of yeasts that allow growth are their ability to ferment simple carbohydrates to produce alcohol, gas and flavor components, such as esters, acids and higher alcohols, and the ability of some species to grow at relatively low temperatures (10–15°C). Less fermentative species, such as *P. membranifaciens*, *Candida krusei* and *Kluyveromyces*, may also spoil fresh-cut products through the formation of films or off-odors (Fleet and Heard, 1992; Fleet, 1993; Heard 1999a).

FOODBORNE PATHOGENS

THE ASSOCIATION OF FOODBORNE PATHOGENS WITH FRESH-CUT SALADS

Fresh-cut products have been linked with outbreaks of foodborne disease, and foodborne pathogens may form a part of the microflora of these products. Historically, epidemiological surveillance of foods for the presence of pathogens has concentrated on foods of animal origin. Although fruits and vegetables have long been known as sources of infectious microbial agents, there is very little evidence documenting the risks to public health. Increased consumption of produce and the growth of the fresh-cut industry have prompted interest to investigate the association of pathogens with these products. This section will briefly describe the pathogens of concern, the diseases they cause, their origin and some of the characteristics that enable them to survive in fresh-cuts and the processing environment. Current approaches to detection of pathogens will be mentioned, and gaps in knowledge will be highlighted.

In recent years, a number of review papers have discussed the occurrence of foodborne pathogens in fresh produce and fresh-cut products (Fain, 1996; Beuchat, 1996, 1998; Francis et al., 1999). These reports have highlighted the gaps in knowledge regarding the organisms of most concern and emphasized the need for full risk analysis of fresh-cut production from the farm to the consumer (Beuchat, 1998). It is generally accepted that the pathogens of concern in fresh-cut salads are similar to those present on raw vegetables (Nguyen-The and Carlin, 2000). Due to the worldwide concern over listeriosis, many studies have focused on the incidence of *L. monocytogenes* in processed, refrigerated vegetable products (Nguyen-The and Carlin, 2000). However, other bacterial pathogens reported to be of concern are *Cl. botulinum*, enterohemorrhagic *E. coli* including serotypes O157:H7 and O111, *Shigella* spp., *B. cereus*, *Aeromonas hydrophila* and *Yersinia enterocolitica* (Fain, 1996). Pathogens also associated with raw vegetables include *Vibrio cholerae* (isolated from cabbage), *St. aureus* and *Salmonella* spp. (isolated from salad vegetables) and *Campylobacter jejuni* (found in retail mushrooms) (Doyle and Schoeni, 1986; Satchell et al., 1990; Fain, 1996; Francis et al., 1999). Some examples of foodborne pathogens isolated from fresh-cut salad products are listed in Table 7.7. Table 7.8 lists a number of outbreaks of foodborne disease associated with fresh-cuts. Nonbacterial pathogens may also be transmitted by fresh produce, originating from wash water and being

TABLE 7.7
Some Examples of the Occurrence of Foodborne Pathogens in Fresh-cut Salad Products

Pathogenic Species	Product (+ Origin)	Occurrence	Ref.
<i>Aeromonas hydrophila</i>	Prepared salads (U.K.)	21.6%	Fricker and Tompsett (1989)
<i>Campylobacter</i>	Vegetable salads (Germany)	0% (20 samples)	Karib and Seeger (1994)
<i>Cryptosporidium</i> oocysts	Lettuce (Costa Rica)	2.5% (2/80 samples)	Monge and Chinchilla (1996)
<i>E. coli</i> O157:H7	Cabbage (Mexico)	25%	Beuchat (1996)
<i>Listeria monocytogenes</i>	Coleslaw (Canada)	2.2% (2/92 samples)	Schlech et al. (1983)
<i>Listeria monocytogenes</i>	Prepacked salad (N. Ireland)	14.3%	Harvey and Gilmour (1993)
<i>Listeria monocytogenes</i>	Coleslaw (U.K.)	7.7% (3/39 samples)	MacGowan et al. (1994)
<i>Listeria monocytogenes</i>	Prepared vegetables (U.K.)	3.8% (1/26 samples)	MacGowan et al. (1994)
<i>Salmonella</i>	Mixed salad vegetables for retail use (U.S.)	0% (0/63)	Lin et al. (1996)
<i>Shigella</i>	Salad vegetables (Egypt)	1.2%	Satchell et al. (1990)
<i>Staphylococcus</i> spp.	Salad vegetables (Egypt)	8.3%	Satchell et al. (1990)
<i>Yersinia enterocolitica</i>	Packaged vegetable products (France)	22–56% (100 samples)	Manzano et al. (1995)

transmitted from the food to food handlers. These include viruses, such as the Norwalk virus and hepatitis A, and the parasites *Giardia* and *Cryptosporidium*. Characteristics of selected pathogens of concern will now be discussed in more detail.

Pathogens of Concern

Listeria Monocytogenes

Listeria monocytogenes is a Gram-positive bacteria capable of causing foodborne disease in humans. The infective dose of this organism is not yet defined, although it is currently thought that a dose of greater than 10³ cfu/g is necessary to cause disease. Illness usually occurs in those who are immunosuppressed, such as in pregnant women, neonates, cancer patients and the elderly, and although many people may be without symptoms, the clinical manifestations of the disease include mild febrile gastroenteritis, conjunctivitis, meningitis, septicemia and spontaneous abortion and death (Sutherland and Porritt, 1997; Farber and Peterkin, 2000).

Although the risk of listeriosis is considered minor by many authors, it is the severity of the disease that causes concern. Foods identified as high risk include refrigerated, minimally processed products such as fresh-cut salads (Sutherland and Porritt, 1997). *Listeria monocytogenes* can survive and grow at both ambient and refrigeration temperatures, and it is facultatively anaerobic, enabling it to persist in

TABLE 7.8
Examples of Outbreaks of Foodborne Disease Associated with
Consumption of Fresh-cut Products

Pathogenic Species	Product (+ Origin)	Source of Contamination	Number of Cases of Disease	Ref.
<i>Cyclospora cayetanensis</i>	Raspberries (U.S., Canada—produce imported from Guatemala)	Spraying with fungicides prepared with contaminated water	>1000	Hertwaldt et al. (1997), Tauxe et al. (1997)
<i>Escherichia coli</i> O157:H7	Mixed lettuce (U.S.)	Bad manufacturing practice, possibly fecal contamination	26 people	De Roever (1998)
Hepatitis A virus	Diced tomatoes	Food handler	92	Williams et al. (1994) cited by Lund and Snowden (2000)
Norwalk virus	Fresh-cut fruit	Food handler	>217	Herwaldt et al. (1997)
<i>Listeria monocytogenes</i>	Coleslaw (Canada)	Sheep manure	34 cases	Schlech et al. (1983)
<i>Salmonella</i> spp.	Precut watermelons (U.S.)	Stored without refrigeration	18 cases	Blostein (1993), CDC (1979, 1991)
	Cantaloupe (Mexico and Central America)	Not known, obtained from salad bars	>245 (estimated 25,000)	CDC (1991), Tamplin (1997)
<i>Shigella sonnei</i>	Shredded lettuce	Processing	347 people	Davis et al. (1988)
<i>Shigella</i> spp.	Fruit salad (U.K.)	Not known	36	O'Brien (1998)
<i>Vibrio cholerae</i>	Cabbage (Peru)	Organic fertilizer of polluted water	—	Swerdlow et al. (1992)

these products. The organism may enter the processing chain from the farm or from the processing environment. It is present in the intestinal tract of animals and humans, and it is found to be widespread in soil and in sewage. It is also a saprophyte and can survive on decaying plant material (Beuchat, 1998), and it is disseminated on farms by animals grazing on decaying plants, spreading their feces onto fresh fields. If it contaminates the processing environment, it may colonize processing surfaces, surviving in drains, cracks in floors and walls and in crevices in equipment. There are reports describing survival of the organism and formation of biofilms on surfaces in food-processing environments, particularly in drains. It may also be transmitted by aerosols and on workers' hands (Sutherland and Porritt, 1997; Beuchat, 1998).

Listeria monocytogenes has been isolated from prepackaged mixed vegetable products, chicory endive and lettuce fresh-cuts, sliced cucumber and fruits, such as tomatoes and cantaloupe, and has also been implicated in foodborne disease outbreaks in several countries (Beuchat and Brackett 1991, Beuchat 1998). For example, vegetable mix for coleslaw was implicated as the vehicle for *L. monocytogenes*, causing an outbreak of listeriosis in Canada in 1981. However, most reports of listeriosis associated with fresh produce are linked to the consumption of whole fruits and vegetables. Further investigation is required to quantify the risks of listeriosis associated with fresh-cut products.

Enteric Pathogens (Family Enterobacteriaceae)

Escherichia Coli

Escherichia coli is part of the natural microflora of the intestinal tract of warm-blooded animals and humans. However, there are also strains capable of causing gastrointestinal disease in humans. These strains are grouped as the enterotoxigenic, enterohemorrhagic, enteropathogenic and enteroinvasive strains of *E. coli* (Doyle et al., 1997). Enterotoxigenic *E. coli* causes traveller's diarrhea, and contaminated produce may well be a source of this organism (Beuchat, 1998). Similarly, enteropathogenic *E. coli* causes gastroenteritis symptoms in adults and infants, and enteroinvasive *E. coli* invades the colonic epithelial tissue lining the colon, resulting in the onset of bloody diarrhea (Doyle and Padhye, 1989; Desmarchelier and Grau, 1997). The final group, enterohemorrhagic *E. coli* is probably the most dangerous to humans. The infectious dose of this organism has been shown to be as low as two cells in 25 g of food, and it is now believed that the infectious dose is less than 100 cells/g food (Willshaw et al., 1994; Griffin et al., 1994; Doyle et al., 1997). Although the pathogenicity is not fully understood, it produces a number of verotoxins (cytotoxins to the African green monkey kidney cells), depending on the strain and enterohemolysin (Desmarchelier and Grau, 1997). The most severe signs are seen in the elderly and in children. It causes hemorrhagic colitis, hemolytic uremic syndrome (usually in children) and thrombocytopenia purpura (in adults). Deaths have been reported mainly in the elderly (Doyle et al., 1997).

Fresh produce can become contaminated with any one of these organisms in the field, through contact with contaminated animal droppings, particularly from ruminants, or from organic fertilizers, such as uncomposted manure. Other potential contamination sources are the water used, workers' hands and wind and dust contamination, as described in a previous section (pages 190–197). Survival of the organism and mechanisms of contamination in the processing environment have not been studied. Enterohemorrhagic *E. coli* O157:H7 has been recognized in recent years as a food-related pathogen and has been responsible for outbreaks linked to a wide range of foods, including fresh produce. This organism may grow on processed fruits such as watermelon and cantaloupe (del Rosario and Beuchat, 1995), shredded lettuce, sliced cucumbers and sprouts (Abdul-Raouf et al., 1993; Zhao et al., 1993; Diaz and Hotchkiss et al., 1996; Nathan, 1997). The organism can grow at temperatures down to around 7–8°C and has been shown to survive under acidic

conditions, in apple juice, at this temperature for up to 12 days (Zhao et al., 1993). Fluctuations in handling and storage temperatures of fresh-cut products, including cut fruits such as cantaloupe and other melons, may provide opportunity for this organism to survive, creating a public health risk. It is of some concern that most research on survival, detection and enumeration of Enterohemorrhagic *E. coli* has focused on just one serotype, O157:H7. Other serotypes, including O111:NM, O26:H11 and O26:HNM are also frequently implicated in outbreaks of disease (Desmarchelier and Grau, 1997), yet there is little evidence to document their incidence in foods. This is an area for future study.

Shigella

The genus *Shigella* is closely related to *E. coli* (ICMSF, 1996). The genus is divided into four species: *Shigella dysenteriae*, *Shigella sonnei*, *Shigella flexneri* and *Shigella boydii*, all of which can cause shigellosis or bacillary dysentery in humans. Invasive serovars of *Sh. dysenteriae* produce a cytotoxin called a Shiga toxin. Noninvasive serovars and other *Shigella* species produce only low levels of cytotoxicity and show endotoxic and neurotoxic activity (Lightfoot, 1997; Lampel et al., 2000). Outbreaks of shigellosis are generally linked to water of food contaminated with human feces. Thus, fresh produce can become contaminated through the use of contaminated irrigation water, the use of raw sewage as fertilizers, insect transfer or human contact (Beuchat, 1998). *Shigella* species can survive on shredded lettuce under refrigeration for up to three days without populations decreasing and can also survive on sliced fruits, including watermelon and raw papaya (Escartin et al., 1989; Satchell et al., 1990). Processed fruits and vegetables have been implicated in a number of outbreaks of shigellosis. Salad vegetables, cantaloupe and potato salad are examples of the associated products (Formal et al., 1965; Frelund et al., 1987; Dunn et al., 1995).

Salmonella

Within the genus *Salmonella*, differentiation into species is based on antigenic differences. There are currently over 2370 serovars recognized, however, only 200 are known to cause disease in humans, including *Salmonella typhi*, the causative agent in the disease typhoid (Jay et al., 1997; Beuchat, 1998; D'Aoust, 2000). Food-borne disease caused by nontyphoid serovars of *Salmonella* includes gastroenteritis and enterocolitis, with symptoms appearing from 8–72 h after food consumption. More severe complications include septicemia and onset of reactive arthritis (Jay et al., 1997). Salmonellae have been isolated from fresh produce, and fruits and vegetables have been linked to outbreaks of salmonellosis (Hedburg and Olsterholm, 1993; Beuchat, 1998). Fresh produce may become contaminated with salmonellae either from sewage and contaminated water or from handling by infected workers. Although there are no reported cases of salmonellosis from fresh-cut products, *Salmonella* can grow on the surface of alfalfa sprouts (Jaquette et al., 1996), and these are sometimes used as ingredients in mixed packaged products. Salmonellae do not grow in foods at less than 7°C and, therefore, should not pose a risk to public health in fresh-cut products, provided they are maintained at refrigeration temperatures. Further investigation of the factors influencing survival of this pathogen in the salad environment are recommended.

SPORE-FORMING BACTERIA

Spore-forming pathogens such as *Clos. perfringens*, *Clos. botulinum* and *B. cereus* may be isolated from soil and have been isolated from fresh and minimally processed vegetables and raw seed vegetable sprouts (Beuchat, 1996). *Clostridium botulinum* produces potent neurotoxins that produce a range of symptoms in humans, including nausea, diarrhea and vomiting and neurological symptoms such as blurred vision, dilated pupils, paralysis of motor nerves, loss of mouth and throat normal functions, lack of muscle coordination and other complications and possible death. Modified atmosphere conditions favor the development of the organism, and although toxin production has not been detected in vegetables stored at refrigeration temperatures (Beuchat, 1996), care should be taken to avoid temperature increases, to prevent germination of spores, to prevent growth of vegetative cells and to prevent toxin production. *Clostridium perfringens* and *B. cereus* produce enterotoxins responsible for causing abdominal cramps and diarrhea (Bates, 1997; Jensen and Moir, 1997; Labbé, 2000; Lund and Peck, 2000). There is also an emetic strain of *B. cereus* that causes rapid onset of disease characterized by acute nausea and vomiting but usually no diarrhea. *Clostridium perfringens* cells die at temperatures below 10°C but can grow at 15°C. The risk to public health arises if products contaminated with these organisms are handled in such a way as to enable spore germination and outgrowth of the vegetative cell, for example, when temperature fluctuations occur during handling, transport and retailing of the finished product. Psychrotrophic strains of *B. cereus* have been isolated from foods (Jensen and Moir, 1997) and may pose a risk to public health if present in refrigerated fresh-cut products.

Staphylococcus Aureus

Staphylococcus aureus (Gram-positive cocci) has been isolated from vegetables and fresh-cut products (Abdelnoor et al., 1983; Houang et al., 1991), but there have been no reports of staphylococcal food poisoning from such products. This organism is thought to originate from handling by workers. It is often present in the nasal passages and on the hands of humans. It is generally accepted that *Staph. aureus* does not compete well in fresh foods, where there is a diverse microflora (Baird-Parker, 2000). However, there have been no reports of the factors influencing the growth of the organisms in the fresh-cut environment.

Campylobacter

Campylobacter jejuni (Gram-positive spiral rods) is found in the intestinal tract of a wide variety of wild and domestic animals. It is a common cause of bacterial enteritis in many countries and is generally associated with food poisoning outbreaks involving animal products. Symptoms of the disease include acute diarrhea lasting for up to five days accompanied by fever and abdominal pain. There are some incidences of infection arising from contamination of fruits and vegetables (Bean and Griffin, 1990; Castillo and Escartin, 1994; Harris et al., 1989) presumably contaminated from animal waste. Cross-contamination between animal and vegetable products may occur where non-vegetable ingredients are added to salads. Although the optimum growth temperature is 42°C, it grows under microaerophilic conditions similar to those of packaged fresh-cuts.

Yersinia Enterocolitica

Yersinia spp. are Gram-negative rods or coccobacilli. *Yersinia enterocolitica* is part of the natural intestinal flora of swine, although it is also a psychrotroph that can grow at temperatures as low as 0°C (Barton et al., 1997). This pathogen is recognized to produce a wide range of clinical and immunological symptoms. The most common is enterocolitis, seen mainly in young children, and pseudoappendicitis, occurring in older children and adolescents (Barton et al., 1997). It may contaminate fresh produce from feces and through cross-contamination in processing establishments. *Yersinia* spp. have been isolated from fresh fruits and vegetables, including salad vegetables such as lettuce and grated carrots, although it has not been implicated in disease outbreaks from eating produce (Beuchat, 1996; Nesbakken, 2000). They pose a potential risk to public health in fresh-cut products because of their ability to grow at low temperatures. An investigation of their incidence in fresh-cuts is warranted.

Aeromonas Species

Aeromonas are Gram-negative rod-shaped coccoid bacteria that are ubiquitous to most aquatic environments and occur in a wide variety of foods (Palumbo et al., 2000). Not all strains are pathogenic, and aeromonads are often responsible for spoilage of foods. Aeromonads may contaminate fresh fruits and vegetables from wash water and possibly through cross-contamination from seafood, meat or poultry. Some strains can grow at refrigeration temperatures, reaching up to 10⁶ cfu/g on vegetables such as asparagus, broccoli and cauliflower after two weeks of storage at 4°C (Berrang et al., 1989). They can also survive under modified atmosphere conditions. The group most affected by pathogenic *Aeromonas* are the young. The organism causes a self-limiting illness characterized by diarrhea and mild fever. There are also reports of cholera-like symptoms (Kirov, 1997).

Vibrio Species

Vibrio species (Gram-negative vibrio-shaped rods) occur predominantly in estuarine waters, and foodborne disease from these organisms are usually associated with fish and seafood. Of the 12 pathogenic species, *Vibrio cholera* causes the most severe disease, cholera (Kaysner, 2000). *Vibrio parahaemolyticus* is often associated with disease outbreaks from under-cooked seafood. Onset of disease may be up to 96 hours after food consumption and is characterized by symptoms including diarrhea, nausea, vomiting, abdominal cramps and fever (ICMSF, 1996). The potential risk of disease from this organism in association with fresh-cut products is from cross-contamination during handling and mixing in retail establishments.

Viruses

Viruses can be responsible for outbreaks of foodborne disease. They are excreted by infected individuals, and although they do not grow on food, they can survive in water and sewage and may subsequently contaminate food such as fruits and vegetables. Over 150 types of enteric viruses representing four viral families can be present in raw sewage, and they cause a range of diseases including respiratory infections, skin disorders, meningitis and gastroenteritis (Grohmann, 1997; Owen Caul, 2000). Diseases caused by hepatitis A, rotavirus and Norwalk-like viruses (small, round-structured viruses) have most commonly been linked to fresh produce,

the vehicles included lettuce, chopped tomatoes and strawberries (Beuchat, 1998). Other viruses linked to foodborne disease are astroviruses, enteroviruses, parvoviruses and adenoviruses. The factors affecting the survival of viruses on fruits and vegetables are not known and should be studied in the future.

Parasites

Parasites are defined as eukaryotic organisms and may be classified in two main groups, protozoa and helminths. Parasites are dependent on host organisms for survival, and although their life cycles vary, they must all pass through an animal or human host to survive and reproduce (Goldsmid and Speare, 1997). They may infect food from contaminated water or sewage, from food handlers or insects, or the parasite may be ingested by animals and be present in animal flesh at the time of slaughter. Many parasites are worldwide in their distribution and are prevalent especially in Third World countries where sanitation and hygiene conditions are poor (Goldsmid and Speare, 1997). Protozoa most commonly associated with human infections are *Giardia*, *Cryptosporidium*, *Cyclospora*, *Entamoeba*, *Toxoplasma*, *Sarcocystis* and *Isopora* (Goldsmid and Speare, 1997; Beuchat, 1998; Taylor, 2000). All of these parasites cause diarrhea-like symptoms except *Toxoplasma* which causes fetal damage and glandular fever-like syndrome (Goldsmid and Speare, 1997). A number of helminths have also been associated with foods, including liver and intestinal flukes, particularly, the *Trematoda*. The epidemiology of these protozoa is not well understood and requires more detailed surveillance. Protozoa such as *Giardia lamblia* and *Cyclospora cayetanensis* have been linked with foodborne disease, where the food vehicle was fresh produce (Beuchat, 1998). *Cryptosporidium* has been found on a range of vegetables, including lettuce, cucumbers, carrots, and tomatoes (Monge and Chinchilla, 1996). The factors affecting the survival of parasites in processed fruits and vegetables are not well understood and warrant investigation.

DETECTION OF PATHOGENS IN FRESH-CUT PRODUCTS

Surveillance programs in a number of countries have investigated the incidence of pathogens in fresh-cuts and the involvement of fresh-cut salads in outbreaks of foodborne disease. Overall, the incidence of pathogens in these products is low, and pathogens such as *Salmonella*, often found on whole fruits and vegetables, have not been isolated from processed products. Nguyen-The and Carlin (2000) attribute the low incidence of pathogens in these products to improved processing techniques and the implementation of quality assurance or good manufacturing practices. However, because there are reported outbreaks of foodborne disease associated with fresh-cuts, and because we have not yet fully investigated the microbial ecology of these products, we cannot assume that pathogens are not present. This is especially important in countries where there is no legislation enforcing the use of quality assurance programs or prohibiting the use of contaminated water for irrigation of crops (Beuchat, 1998). The adequacy of methods for the detection of foodborne pathogens in the fresh-cut salad environment should be investigated, and where necessary, improved methods should be sought after to increase our ability to target and detect pathogens. These issues must be addressed if we are to fully assess the risk to human health from the consumption of salads. Current knowledge of the

pathogens associated with fresh-cut products is incomplete and largely qualitative. Questions to be answered include the following:

1. Does the background flora of these products influence the survival of pathogens?
2. Does the background flora interfere with the detection of pathogens in these products?
3. Can pathogens be detected in the fresh-cut environment using current detection methods?

Methods are also needed to facilitate monitoring of viruses and parasites in fresh-cut products to determine the extent of the risk to public health.

Pathogens are often present in foods in low numbers, or they may be trapped in a biofilm or in pores of the food and are subsequently difficult to detect. To facilitate detection, enrichment techniques, requiring up to four to five days to complete, are used to allow growth of the pathogen within the food. Such techniques are used to detect organisms such as *L. monocytogenes*, *Shigella*, *Salmonella*, *E. coli* O157:H7, *C. jejuni* and *Yersinia* (Barton et al., 1997; Desmarchelier and Grau, 1997; Jay et al., 1997; Lightfoot, 1997; Sutherland and Porritt, 1997; Wallace, 1997). However, selective enrichment techniques for isolation of pathogens from foods provide only qualitative information about the presence or absence of the organisms. Also of concern, many countries use standardized methods for detection of pathogens in foods that have not been tested for validity or reliability for use in the salad environment. Two recent studies (unpublished work) showed that the microbial ecology of vegetable products is so complex that conventional methods alone cannot sufficiently reveal the diversity of the microbial species present. These studies revealed that when fresh-cut salads were inoculated with either *L. monocytogenes* or *E. coli* O157:H7 at 1–10 cells/g and 10–100 cells/g, detection of these pathogens using the appropriate standard method was obscured by the indigenous flora.

Future considerations for microbiological methods for detection of pathogens in fresh-cut salads are as follows:

1. Validation of methods for detection of pathogens in fresh-cut salads
2. Reduction of time for sampling and detection (may include adoption of rapid methods)
3. Development of quantitative sampling and detection methods to enable study of the complete microbial ecology of these products

BIOCONTROL

There are very few hurdles in packaged fresh-cut products to prevent the growth of microorganisms. The products are washed to remove excessive contamination, but after processing, the main controls used are storage at refrigeration temperature and packaging in modified atmospheres. Current approaches to food preservation advocate the hurdle concept, in which multiple factors are used to prevent microbial growth

(Leistner, 1995). Unfortunately the “fresh” nature of minimally processed fruits and vegetables prevents the use of traditional processing such as cooking/heating, and consumers are demanding that our foods contain no chemical preservatives. Thus, application of biocontrol concepts may be useful to create extra preservation hurdles for fresh-cut products. Biocontrol methods include the use of the following:

1. Antagonistic organisms to control growth of either spoilage or pathogenic species (biopreservation)
2. Natural antimicrobial compounds to control microbial growth
3. Natural plant defenses to reduce microbial attack-induced resistance

BIOPRESERVATION

The influence of naturally occurring microorganisms of vegetables on pathogenic bacteria has been described in the literature. For example, Carlin et al. (1996) reported that the indigenous microflora isolated from endive leaves could competitively inhibit *L. monocytogenes*. Similarly, Babic et al. (1997) reported that freeze-dried spinach powder, containing a mixture of mesophilic aerobic microorganisms, was inhibitory to *L. monocytogenes*. Francis and O’Beirne (1998) found that mixed populations of bacteria isolated from shredded lettuce generally diminished the growth of *Listeria innocua*, when mixed in model media, and they concluded that species of *Enterobacter* competed with the *Listeria* species. These authors suggested that the natural background flora could be an important influence on growth of *Listeria* species on lettuce.

Pseudomonas spp. have been used as antagonists for postharvest applications. Species such as *Ps. chlororaphis*, *Ps. fluorescens*, *Ps. putida* and *Ps. syringae* have also been tested for siderophore production and antimicrobial activity against food-borne pathogens (Freedman et al., 1989; Laine et al., 1996; Janisiewicz et al., 1999). Almost all fluorescent pseudomonads and some nonfluorescent species produce siderophores, and these compounds are considered to be the main factors influencing the biocontrol potential of pseudomonads (Jayasekara, 1999). Pseudomonads also produce antifungal compounds [chitinase and laminarinase enzymes (Lim et al., 1991), syringomycin (Vassilev et al., 1996) and antimicrobial pigments (Dakhama et al., 1993)] that may be applied to foods such as fresh-cut fruits. We may potentially be able to use selected spoilage flora of fresh-cut products to our advantage, through exploitation of biocontrol characteristics. This will only be possible after complete investigation of the microbial ecology of the system.

Microorganisms such as lactic acid bacteria are used as biopreservative agents in foods to inhibit the growth of other undesirable species. Mechanisms of antagonism include competition for nutrients, binding of nutrients and production of metabolic products with antimicrobial activity. Fermentation with lactic acid bacteria (LAB) is a traditional biopreservation method employed to increase the safety and quality of foods, including fruits and vegetables. Examples include fermented olives, sauerkraut and pickles (Stiles, 1996). In recent years, lactic acid bacteria have also been used as competitive biocontrol agents and antagonists in nonfermented foods (Breidt and Fleming, 1997). These organisms are often present on the surface of fruits and vegetables and, if encouraged, may reduce the growth of other indigenous

spoilage organisms or foodborne pathogens. Lactic acid bacteria are known to produce antimicrobial metabolites such as lactic and acetic acids, hydrogen peroxide and enzymes including lysozyme. Some strains of lactic acid bacteria also produce bacteriocins (Holzapfel et al., 1995; Breidt and Fleming, 1997). These are described as groups of potent antimicrobial peptides or proteins that are active against other microorganisms (Holzapfel et al., 1995). Several studies have described the use of bacteriocin-producing lactic acid bacteria to improve the safety of ready-to-eat salads. Vescovo et al. (1995) investigated the effect of lactic acid bacteria on the mesophilic microflora during refrigerated storage of ready-to-eat vegetables. Strains of lactic acid bacteria selected for their ability to grow and produce antimicrobial compounds at refrigeration temperatures inhibited the mesophilic flora, including enterococci and other coliforms, within three days of storage. In a subsequent study, Vescovo et al. (1996) applied antimicrobial-producing lactic acid bacteria to ready-to-eat vegetables to prevent the growth of pathogens. Psychrotrophic strains of *Lactobacillus casei*, *Lactobacillus plantarum* and *Pediococcus* spp. inhibited *A. hydrophila*, *L. monocytogenes*, *Salmonella typhimurium* and *S. aureus* in a range of vegetable salads. Further investigation with one strain, *L. casei* IMPC LC34, revealed that addition of this strain to ready-to-use vegetables resulted in reduction of total mesophilic counts and coliforms counts and the disappearance of the pathogens *A. hydrophila*, *Sal. typhimurium* and *Staph. aureus* after six days of storage. A culture permeate of this strain also reduced counts of total flora, coliforms, enterococci and *A. hydrophila* in ready-to-eat vegetables (Torriani et al., 1997).

USE OF NATURAL ANTIMICROBIAL COMPOUNDS

Bacteriocins

Purified antimicrobial compounds such as bacteriocins may be added to fresh-cut vegetables to achieve a protective effect as an alternative to adding live cultures. Choi and Beuchat (1994) used a bacteriocin from *Pediococcus acidilactici* M to inhibit the growth of *L. monocytogenes* during kimchi fermentation. Addition of crude bacteriocin powder (10 mg bacteriocin/150 g salad) was initially lethal to *L. monocytogenes* and controlled growth of the pathogen for the duration of fermentation (16 days). A bacteriocin, Plantaricin D, has been isolated from *Lactobacillus plantarum* BFE 905, found in Waldorf salad (Franz et al., 1998). Plantaricin D was found to exhibit antilisterial activity, and the authors suggested use of the compound or the biopreservative culture to improve the safety of ready-to-eat vegetables. The commercially available bacteriocin, nisin (from *Lactobacillus lactis*), has been used in foods such as pasteurized cheese spreads to inhibit outgrowth of *Clostridia* spores, and it is approved in a number of European countries for addition to fresh cheese, processed vegetables and canned foods (Holzapfel et al., 1995). Antilisterial activity of bacteriocins nisin and ALTATM2341 were tested by Szabo and Cahill (1998) under varying conditions of temperature and modified atmosphere. At 4°C and in a CO₂-rich atmosphere, growth of *L. monocytogenes* was controlled in a broth medium. At 12°C (abuse temperature), addition of bacteriocins was necessary to prevent growth of the pathogen. The authors expressed concern over the development of

bacteriocin-resistant isolates and recommend investigation of resistance mechanisms prior to development of food applications for bacteriocins. Other considerations include the following:

1. The factors affecting bacteriocin activity in foods.
2. Are bacteriocins active in the fresh-cut environment?

Natural Plant Volatiles

Volatile compounds not only comprise the aroma and flavor compounds of fruits and vegetables but may also play a functional role in plant-microorganism interactions. Interest in the use of these compounds is encouraged by consumer demands for “natural foods.” The natural origin of plant volatiles and the fact that they are consumed normally in fresh fruits and vegetables in the diet may enhance consumer acceptance of their use to control microbial spoilage. To date, interest in application of volatiles to fresh produce has focused on treatment of fruits to inhibit the growth of postharvest decay fungi. For example, Vaughn et al. (1993) analyzed volatiles from raspberries and strawberry fruits, including benzaldehyde, 1-hexanol and 2-nonanone, for their ability to inhibit *Alternaria alternata*, *Botrytis cinerea* and *Colletotrichum gloesporoides* *in vitro*. Benzaldehyde inhibited all three fungi when added to growth media at 4 μ L/mL. Similarly, Archbold et al. (1997) identified volatile compounds to inhibit the growth of *Botrytis*. Ten compounds including hexanal, 1-hexanol, methyl salicylate and methyl benzoate, prevented the growth of *Botrytis* on blackberries, strawberries and grapes. Volatiles from stone fruit origin including benzaldehyde (5000–10,000 ppm) and hexanal (2500 ppm) were shown by Caccioni et al. (1995) to exhibit a fungistatic effect against *Monilia laxa* and *Rhizopus stolonifer*.

Application of natural plant volatiles to fresh-cut salads to improve quality and safety has been investigated by Dawson et al. (1999). In this study, a number of volatile compounds (acetic acid, ethanol and several pyruvates) were added to fresh-cut, mixed lettuce and cabbage to control the growth of pathogens such as *L. monocytogenes* and *B. cereus*. Acetic acid and the pyruvates reduced the total count of these vegetables by up to 2 log cycles, and populations of the pathogens were also reduced. Disadvantages of fumigation with volatiles include tainting effects resulting from compounds such as acetic acid, corrosive effects and toxic effects.

Nonvolatile compounds such as essential oils may also be used to inhibit spoilage flora and foodborne pathogens. Wan et al. (1998) found that washing lettuce with a component of basil oil (0.1–1.0% v/v) was as effective as washing with 125 ppm chlorine. The oil components were effective against *Pseudomonas* spp. and *A. hydrophila*.

INDUCED RESISTANCE

Plant tissues possess inherent defense mechanisms to enable resistance to microbiological invasion. Plant defense mechanisms and methods to induce resistance were recently reviewed by Forbes-Smith (1999). To date, there are no published reports examining induced resistance and fresh-cut fruits and vegetables. The following section aims to introduce the concepts of plant defense mechanisms and induced resistance.

Synthesis of Phenolic Secondary Metabolites—Lignin and Phytoalexins

Lignification of tissues occurs to assist plants in resisting disease. Lignin is a polymeric polyphenol that combines with cellulose and pectin in the plant cell wall to increase resistance to pathogen penetration (Forbes-Smith, 1999). Phytoalexins or phenolic “antibiotics” are also produced as a response to infection by microbial plant pathogens. An example is the phytoalexin glycinol, produced by soybeans. Glycinol was found to inhibit DNA, RNA and protein synthesis of the soft rot bacteria *Erwinia caratovora* (Weinstein and Albersheim, 1983).

Synthesis of Pathogenesis-Related Proteins

Some plant tissues produce antifungal proteins in response to invasion by microorganisms or as a response to exposure to ethylene (Schlumbaum et al., 1986; Enyedi et al., 1992). For example, plant cells produce the lytic enzymes chitinase and chitosinase to degrade fungal cell walls (Baldwin et al., 1995).

Induced Resistance

It is possible to treat plant tissues with substances to elicit the natural defense mechanisms. Elicitors may be biotic (signal molecules produced by the plant) or abiotic (treatment with radiation such as UV radiation). Compounds such as methyl salicylate and chitosan are known to be antifungal and have been used to elicit resistance in fruits, including strawberries (Forbes-Smith, 1999). UV-C radiation has been used to treat processed carrots. Treatment with low-dose UV-C was found by Mercier et al. (1993a and b) to enhance resistance of the carrots to infection by *Botrytis cinerea* and *Sclerotinia sclerotiorum* at refrigeration temperatures. In a recent study, Lamikanra et al. (2002) reported the production of cyclic and acyclic terpenoid phytoalexins (β -ionone, geranylacetone, and terpinyl acetate) in cut cantaloupe melons exposed to UV radiation. Geranylacetone and terpinyl acetate, when added to cut cantaloupe (0.01% w/w), reduced microbial population from 6.2×10^8 in the untreated control to 1.2×10^8 and 3.1×10^7 cfu/g, respectively, over a period of 24 h at 20°C. β -ionone completely inhibited microbial growth under similar conditions. Application of induced resistance methods to fresh-cut produce should be considered as an alternative biocontrol hurdle, as the cutting processes involved in fresh-cut processing may induce an elicitor response. Areas for future research include the effectiveness of elicitors in this environment and the effects of treatment on the sensory quality of fresh-cut products.

CONCLUSION

Although the role of microorganisms in determining the safety and spoilage of fresh-cut produce is acknowledged in the literature, our understanding of the microbial ecology is still limited. Optimization of processes to ensure freshness and safety and the application of innovative biocontrol techniques will rely on fundamental investigation of the growth, interactions and biochemical activity of associated microbial species and the mechanisms underlying their development.

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8 Microbial Enzymes Associated with Fresh-cut Produce

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INTRODUCTION

Plants harbor a diverse microflora ranging from the three primary domains of life: Bacteria, Archaea, and Eucarya (Andrews and Harris, 2000). Microorganisms on plant surfaces are also commonly associated with, or are contaminants from, soils, insects, mammals, and other animals (Jay, 1997). For agricultural produce, microbial

contamination occurs at every stage of the production chain, from cultivation to processing. With the exception of plant pathogens, most microorganisms are generally prevented from penetrating produce tissue by outer protective layers such as the epidermis. Plant pathogenic microorganisms can directly attack plant tissues and destroy the whole plant or plant parts. Fresh-cut produce presents an additional dimension of the issue. Cutting destroys the internal cell compartment and creates wounds on the plant organs. The wounded tissue releases plant juice or cell contents that serve as nutrients for microorganisms. Thus, cut surfaces, in most cases, are ideal for the growth of microorganisms, including human pathogens.

Once in contact with plant, microorganisms are subjected to various levels of microbial-plant interactions or interrelationships for their population expansion. At the low end, plants only provide a physical space or nutrients for microorganisms. At the higher level, plants and microbes recognize specific molecular signals from each other and trigger a series of biochemical reactions. The interaction depends on the nature of the microorganisms, the host plant condition and the occurrence of favorable environmental factors. A response to these parameters could involve the coordination expression of the microbial genes. With facultative plant pathogens, the interaction switches between the saprophytic and the pathogenic phases. To participate in the microbe-plant interaction and explore the food sources, microorganisms may secrete enzymes, toxins, growth regulators and polysaccharides (Agrios, 1997), and the host plants may react to these substances accordingly. Among these substances, microbial enzymes have received the most attention.

Cell cytoplasm contains nutrients that are readily used by microorganisms (such as simple sugars and amino acids) and carbon and energy reserves (such as starches, proteins and fats). A larger food source for microorganisms is plant biopolymers. Plant tissues are primarily cells and their metabolites. The undisrupted surface of a fruit or vegetable is covered with a protective layer called cuticle consisting of cutins. Plant cell walls consist of cellulose, pectins and structural proteins. The middle lamella that is primarily pectins holds plant cells together. Inside a cell wall, cell membrane consists of lipids and proteins. Microorganisms can convert cell polymers to soluble products that are transportable into the microbial cell. This degradation process is brought about by the action of one or more sets of enzymes secreted by microorganisms.

This chapter is not intended to be a comprehensive review of all microbial enzymes related to fresh-cut produce. In fact, few publications emphasize the perspective of microbial enzymes associated with fresh-cut produce, although there are a number of reviews related to the characteristics of microorganisms associated with produce (Beuchat, 1996; Brackett, 1999; Nguyen-The and Carlin, 1994). Most commonly, microbial enzymes were studied because of their roles in rot or spoilage of vegetables and fruits. A more recent trend is the focus on a group of microorganisms that are significant in terms of food safety (Liao et al., 1999). The chapter briefly discusses a few enzymes commonly produced by microorganisms related to fresh-cut produce or wounded plant tissues. Because of the large volume of data, enzymes from plant pathogens have inevitably received more emphases despite of the fact that most of the plant microorganisms are not plant pathogenic. A wide range of enzymes are known to be involved in the degradation of plant cell polymers. These often include

enzymes such as pectinases, cellulases, amylases, lipases and proteinases. Because of the interest in plant-pathogen interactions, and the industrial applications, pectinases have been extensively studied (Bateman and Miller, 1996; Barras et al., 1994; Blanco et al., 1999; Collmer and Keen, 1986; Herron et al., 2000; Hugouvieux-Cotte-Pattat et al., 1996; Lang and Dornenburg, 2000; Sakai et al., 1993).

THE MICROBIAL ENZYMES

PECTINASES

Pectic substances are one of the most abundant polysaccharides in cell walls of higher plants. They are composed primarily of linear polymeric chains of D-galacturonic acid linked as an α -1,4 glycoside that contains carboxyl groups either esterified (pectin) or nonesterified (pectic acid) to different degrees with methanol. Natural pectins are present at various levels of methylesterification. Pectins are the basic constituents of the intercellular cement in the middle lamella. Pectin degradation results in liquefaction of the pectic substances leading to tissue maceration or softening. Pectic enzymes or pectinases degrade pectic substances. Degradation of pectic substances is the main cause of rots in fruits or vegetables. Many microorganisms can utilize the monomers and oligomers of pectin as a food source. In the case of plant pathogens, the additional role of pectin degradation is its participation in the process of pathogenicity. Pectic enzyme activity enhances the capability of a pathogen to penetrate and colonize its hosts.

The complexity of the pectin biopolymer is indicated by the wide variety of pectin-degrading enzymes available. Pectinases can be classified according to their preferential substrate: pectin or polygalacturonate, their reaction mechanism through β -elimination or hydrolysis, and the cleavage position in the polymer chain (*endo*- or *exo*-) (Sakai et al., 1993). In general, they are divided into two groups: pectinesterases and depolymerizing enzymes. The pectinesterases or pectin methylhydrolase (EC 3.1.1.11) remove short branches of the pectin chains by deesterifying the methoxyl group of pectin resulting in pectic acid. These enzymes have no effect on the overall chain length, but they may alter the solubility of the pectins and affect the activities of the enzymes in the second group. The second group of enzymes is the chain-splitting pectinases that cleave the pectinic chain and release shorter chain portions containing one or a few molecules of galacturonan. There are two subgroups. One subgroup involves enzymes that hydrolyze glycosidic linkages including polymethylgalacturonase (PMG) and polygalacturonase (PG). Enzymes in the other subgroup cleave β -1,4 glycosidic linkages by transelimination, which results in galacturonide with an unsaturated bond between C4 and C5 at the nonreducing end of the galacturonic acid formed. Similarly, two types of enzymes are in this subgroup: polymethylgalacturonate lyase (PMGL) or pectin lyase and polygalacturonate lyase (PGL) or pectate lyase (PL). The properties and assays of pectic enzymes have been comprehensively reviewed (Bateman and Miller, 1996; Barras et al., 1994; Sakai et al., 1993).

Production of pectolytic enzymes by microorganisms is known to be both constitutive and inductive. For plant pathogenic bacteria, the extracellular pectinases are regulated by the availability of the pectin polymer and the release of galacturonan units.

A general model for pectinase induction is that the microorganism always produces a base-level amount of pectolytic enzymes. In the presence of pectin, this will release a small number of galacturonan monomers, dimers or oligomers. These molecules serve as inducers for enhanced synthesis and release of pectolytic enzymes. The increase in pectolytic enzyme concentration further increases the degradation of the pectin polymer. After some time, however, high concentrations of generated monomers, dimers or oligomers then decrease the production of pectolytic enzymes. The production of pectolytic enzymes is also repressed when pathogens grow in the presence of glucose (Agrios, 1997).

CELLULASES

Cellulose is a linear chain of glucose linked by β -1,4-glucosidic bonds up to several thousand monomers. Because chains of six or more monomers are already insoluble, cellulose is insoluble in water. In general, enzymatic degradation of cellulose is a slow process. The major limiting factor in the hydrolysis of such materials is probably the sequestration of single molecules of substrate by the enzymes involved (Warren, 1996). Fruits and vegetables contain much less cellulose relative to woody plants. Cellulolytic enzymes secreted by pathogens play a role in the softening and disintegration of cell wall material (Agrios, 1997). Cellulolytic enzymes may further participate indirectly in spoilage by releasing soluble sugars from cellulose chains. These soluble sugars can serve as food for the pathogen. Nonpathogenic microorganisms may also participate in cellulolysis for food resources.

Cellulases are commonly secreted by microorganisms to attack the cellulose polymer. In addition to the common hydrolytic enzymes, oxidative and phosphorolytic enzymes are also involved in cellulose depolymerization (Warren, 1996). The three major types of hydrolytic cellulases that participate in the degradation of cellulose to glucose are endoglucanases (*endo*-1,4- β -D-glucanohydrolase, EC 3.2.1.4) that randomly attack the cellulose chain and split the β -1,4-glucosidic bond; exoglucanases (*exo*-1,4- β -D-glucan 4-cellobiohydrolase, EC 3.2.1.91) that release either cellobiose or glucose from the nonreducing end of cellulose; and β -glucosidase or cellobiase (EC 3.2.1.21) that hydrolyze cellobiose and other water-soluble cellodextrins to glucose (Singh and Hayashi, 1995). While fungi produce all of the three types of cellulases, all cellulolytic bacteria secrete a variety of endoglucanases (Beguín, 1990).

CUTINASES

The natural plant surface of leaves, flowers, fruits and young stems are covered by cuticle, which is a barrier protecting plants from pathogen invasion. The structural component of plant cuticle, called cutin, is an insoluble aliphatic biopolymer composed of hydroxy and hydroxyepoxy fatty acids. Many fungi and a few bacteria are able to produce cutinases. Fungal cutinase is composed of a single peptide with a molecular weight near 25,000 (Kolattukudy, 1985). As part of the pathogenicity, many fungal pathogens can penetrate the intact barriers (Kolattukudy, 1985). With the

production of cutinase, some fungi could grow on cutin as the sole source of carbon (Purdy and Kolattukudy, 1973).

PROTEINASES

In addition to its enzymatic function, proteins are constituents of cell membranes and structural components of plant cell walls. Proteinases or proteases catalyze the hydrolysis of peptide bonds in proteins or peptides. Proteinases produced by bacteria and fungi are predominantly extracellular and can be classified into four groups based on the essential catalytic residue at their active site. They include serine proteases (EC 3.4.21), cysteine proteases (also called thiol proteases) (EC 3.4.22), aspartate proteases (EC 3.4.23) and the metalloproteases (EC 3.4.24) (Hase and Finkelstein, 1994). Degradation of host proteins by proteinase secreted by microorganisms can profoundly affect the organization and function of the host cells. However, few investigations have been done on the nature and extent of degradation of plant tissues (Agrios, 1997). It has been documented that *Erwinia* spp. produces and secretes several proteases that have been associated with virulence in plants (Wandersman et al., 1987).

STARCH-HYDROLYZING ENZYMES

Starch contains two kinds of glucose polymer: α -amylose and amylopectin. The former consists of long, unbranched chains of D-glucose in a unit connected by α -1,4 glycosidic bonds. The glycosidic linkage of an amylopectin chain is α -1,4, but the branch points are α -1,6 glycosidic bonds. Starch can be hydrolyzed to smaller units that serve as nutrients for microorganisms. Starch hydrolysis requires enzymes hydrolyzing α -1,4 and, to a lesser extent, α -1,6 glucosidic bonds. The degradation of starch is brought about by the combined action of several types of enzymes called amylases. α -Amylases cleave long starch molecules to oligosaccharides that are hydrolyzed by glucoamylases, β -amylases, and other *exo*- α -1,4-glucanases (Nigam and Singh, 1995). The end product of starch breakdown is glucose that microorganisms use directly.

LIPOLYTIC ENZYMES

The common characteristic of all lipids is that they contain saturated or unsaturated fatty acids. Oils and fats are found in many cells as energy storage compounds. Wax lipids are common on aerial epidermal cells. Phospholipids and glycolipids, along with proteins, are the main constituents of all plant cell membranes. Lipolytic enzymes or lipases hydrolyze lipids and liberate fatty acids. The microorganisms presumably utilize the fatty acids directly (Agrios, 1997).

OTHER ENZYMES

The biochemical diversity suggests the potential of a large number of enzymes produced by microorganisms. In addition to the enzymes mentioned above, some of the enzymes of importance in food production include anthocyanase, catalase, dextranase,

glucose oxidase, invertase, lactase, etc. (Taylor and Richardson, 1979). In most cases, the roles of these enzymes in the microbe-host interaction remain to be studied.

THE MICROORGANISMS

Many microorganisms isolated from produce are known to produce extracellular enzymes. Magnuson et al. (1990) reported that approximately 10–20% of isolates among the mesophilic bacteria from shredded lettuce were pectinolytic. In many samples of shredded carrots and shredded chicory salads, 20–60% of the isolated pseudomonads were pectinolytic (Nguyen-The and Prunier, 1989). Recent investigations have revealed that sophisticated mechanisms often exist with plant pathogens that can actively attack plant tissues for their population prosperity. One of the best-studied models is probably *Erwinia* species that cause soft rots of vegetables.

In general, microorganisms on fresh-cut products fall in the category of bacteria, yeasts and molds or filamentous fungi (Barriga et al., 1991; Lamikanra et al., 2000; Magnuson et al., 1990; Zagory, 1999). Yeasts and lactic acid bacteria are common microflora on fruits. They do not actively attack plant tissues. However, they are responsible for the spoilage of many fruit products, particularly in wounded or cut tissues where cell contents are released. Yeasts and lactic acid bacteria often use simple sugars found in fruits to bring about fermentation, resulting in the production of alcohol, organic acid and carbon dioxide. On the cut surface of fresh-cut produce, yeasts and lactic acid bacteria grow faster and often precede molds in the spoilage process. This was clearly demonstrated during an experiment of monitoring the microbial change on fresh-cut cantaloupe (Lamikanra et al., 2000). As spoilage progresses, degradation of the high molecular weight plant polymers is later brought about by molds and other pectinolytic and cellulolytic bacteria (Jay, 1997).

THE BACTERIA

Fresh vegetables are all subject to bacterial soft rots. The rotting process could be as short as three to five days under favorable conditions. Repulsive odor can usually be found with rotten cruciferous plants and onions. Through wounds, the soft rot bacteria enter plants and multiply quickly in the intercellular spaces. They produce a set of enzymes such as pectinases, cellulases and proteases, dissolve the middle lamella and separate the cells. This causes maceration and softening of the affected tissues. Several species in the bacterial genera of *Erwinia*, *Pseudomonas*, *Bacillus* and *Clostridium* are primary rotting pathogens of vegetables (Agrios, 1997). The role of *Xanthomonas* in the soft rots of fruits and vegetables was also discussed by Liao and Wells (1987a). The soft rot bacteria can grow and are active over a range of temperatures from 5–35°C. They are killed with extended exposure at about 50°C (Agrios, 1997).

The Erwiniae

The soft rot *Erwiniae* produces large quantities of extracellular plant cell wall-degrading enzymes. Pectin methylesterases, pectate lyases, pectin lyase, polygalacturonase, cellulases, proteases and a phospholipase have been identified in *E. chrysanthemi*

(Collmer and Keen, 1986). Among all of these degrading enzymes, pectate lyases have a predominant role in plant-tissue maceration. Three soft rot erwinias, *E. carotovora* var. *carotovora*, *E. carotovora* var. *atroseptica* and *E. chrysanthemi* have been extensively studied (Barras et al., 1994). The main characteristic distinguishing soft rot erwinias from other *Erwinia* species is the ability to produce large quantities of pectic lyases. The enzyme macerates parenchymatous tissue of a wide range of plant species. The three soft rot erwinias have a worldwide distribution. The host range of *E. carotovora* var. *atroseptica* is mostly potatoes, a cool climate crop. *E. chrysanthemi* causes diseases in a wide range of tropical and subtropical crops. *E. carotovora* var. *carotovora*, however, has a wide distribution in both the temperate and tropical zones (Barras et al., 1994).

Wounds on the plant host are required for penetration of the soft rot bacteria. The pathogens feed and multiply on the plant juice from the wound surface. The production of large amounts of pectolytic enzymes leads to further maceration of the tissues. The bacteria continue to multiply and advance in the intercellular spaces, while the surrounding plant cells plasmolyze, collapse and die. The invaded tissues soon become soft with the appearance of a slimy mass consisting of innumerable bacteria swimming about in the liquefied substances (Agrios, 1997).

At the molecular level, the genes encoding pectic enzymes from penetrating pathogens are expressed in a characteristic manner in the infected tissue. The enzymes are exported from the pathogen cytoplasm to the host tissue milieu. Under favorable conditions, the enzymes are active and cleave structural polymers in the primary cell wall and middle lamella. The pectic fragments released by the enzymes may also have effects on the interaction, including the elicitation of host defense reactions. The results of the enzyme activity facilitate pathogen penetration, colonization and the appearance of rot symptoms (Collmer and Keen, 1986).

Like *Escherichia coli*, *Erwinia* species also belong the family of Enterobacteriaceae. The genetic tools developed for the *E. coli* system could be applied to study *Erwinia* with minimum modifications. Thus, *E. chrysanthemi* and *E. carotovora* were selected as model systems for the analysis of micro-plant interactions. These models have generated a great amount of data regarding the enzymes associated with soft rot diseases (Barras et al., 1994; Herron et al., 2000; Hugouvieux-Cotte-Pattat et al., 1996).

As summarized by Hugouvieux-Cotte-Pattat et al. (1996), the *E. chrysanthemi* strain 3937 produces five types of pectinases and multiple isoenzymes: at least six endopectate lyases (PelA to PelE and PelL), an exopectate lyase (PelX), two pectin methyl esterases (PemA and PemB), a pectin lyase (PnlA) and an exopolysaccharuronase (PehX). While PelX, PemB and Ogl are intracellular enzymes, the other pectinases are secreted into the extracellular medium by *E. chrysanthemi* cells. Among the six genes of endopectate lyases, five (*pelA*, *pelB*, *pelC*, *pelD* and *pelE*) play a major role in pectate lyase activity (Kotoujansky, 1987). These genes are organized in two clusters: *pelB* and *pelC* in one cluster and *pelA*, *pelE* and *pelD* in another. The two clusters are widely separated on the bacterial chromosome (Hugouvieux-Cotte-Pattat et al., 1996). The PelL protein shows no homology with other pectinases except for PelX, restricted to the C-terminal region (Brooks et al., 1990). In contrast to these endopectate lyases, which degrade long polymeric chains, oligogalacturonate lyase (Ogl) recognizes pectic oligomers of two to four residues. This cytoplasmic

enzyme cleaves the α -1,4 glycosidic bond by transelimination. The exopeptidase lyase PelX can utilize PGA and also methylated pectins as substrates (Brooks et al., 1990). It is expected that this type of enzyme is present in the bacterial periplasm because it acts better on oligomers produced by endopeptidase lyases than on polymeric substrates. Most soft rot *Erwinia* produce an endopeptidase lyase (PnlA) activity in response to DNA-damaging agents (Tsuyumu and Chatterjee, 1984).

In contrast to lyase, hydrolase does not appear to be the predominant pectin depolymerase in *E. chrysanthemi*. An exo-cleaving polygalacturonase (PehX), the only hydrolase, was found in *E. chrysanthemi*. The gene *pemA*, which encodes an extracellular pectin methylesterase, is linked to the *pelA*, *pelE*, *pelD* locus encoding three major pectate lyases (Kotoujansky, 1987). The gene *pemB*, which encodes a novel pectin methylesterase, is an outer membrane lipoprotein. The activity of this enzyme is approximately 100-fold higher on pectic oligomers than on natural pectins. The action of extracellular pectinases on pectin probably liberates small methylated oligogalacturonides that can enter the periplasm by diffusion, and the role of *pemB* might be to degrade such oligomers (Hugouvieux-Cotte-Pattat et al., 1996).

Cellulases produced by *E. chrysanthemi* were secreted to the culture medium. Mutation in the secretory machinery resulted in the accumulation of cellulase and pectinase in the periplasmic space (Andro et al., 1984). The genetic locus of the secretory machinery was later determined to consist of 15 genes organized in five transcriptional units (Salmond, 1994) to form a type II secretion machinery. The genes of two cellulases, CelY (EGY) and CelZ (EGZ) were later cloned and sequenced (Guisseppi et al., 1988, 1991). The two genes do not appear to be homologous. While CelZ represents approximately 95% of the total carboxymethyl cellulase activity, synergistic hydrolysis was observed from the two enzymes (Zhou and Ingram, 2000). Synergy did not require the simultaneous presence of both enzymes. But, it is important that CelY was used as the first enzyme (Zhou and Ingram, 2000).

The export pathway of proteinases is different from those of cellulases and pectinases. The extracellular state of an *E. chrysanthemi* protease was not affected by the Out mutation(s) (Andro et al., 1984). Wandersman et al. (1987) studied three closely related metalloproteases—A, B and C. They localized the structural genes for proteases B and C (*prtB* and *prtC*) as two distinct adjacent transcription units. Three genes, *prtD*, *prtE* and *prtF*, are needed for the specific secretion of the *prtB*- and *prtC*-encoded proteases. The *prtD*, *prtE* and *prtF* genes are part of an operon located immediately upstream of *prtB* and *prtC*. Expression of these genes in *Escherichia coli* allows the specific and efficient secretion of protease B and protease C (Letoffe et al., 1990). Marits et al. (1999) isolated an extracellular protease gene (*prtW*) from a strain of *E. carotovora* subsp. *carotovora*. The *prtW* gene was found to be strongly induced in the presence of plant extracts. An insertion mutation in this gene exhibited reduced virulence, indicating that the proteinase gene plays an important role in maceration of plant tissue.

The Pseudomonads

The production of pectolytic enzymes by pseudomonads that are involved in maceration of host tissues is mainly the nonfluorescent species such as *P. cepacia*, *P. caryophylli* and *P. gladioli* (Gross and Cody, 1985). These enzymes are secreted

directly into the plant tissue and cause rotting. Endo-polygalacturonase was reported to be the principal enzyme produced during infection of *P. cepacia* (Ulrich, 1975). Tissue degradation resulted in the decrease of pH from 5.5 to as low as 4.0. Such a lower pH value is favorable to the high endo-polygalacturonase activity. It is also well known that a fluorescent species, *P. marginalis* causes soft rots of various vegetables. However, pectate lyase (polygalacturonate trans-eliminase) is responsible for maceration of host tissues (Zucker et al., 1972). In contrast to that of *P. cepacia*, the infection from *P. marginalis* led to the increase of pH value, corresponding to the high pH optima of the pectate lyase. In addition, *P. cepacia* also produces a pectate lyase (Ulrich, 1975). *Pseudomonas cepacia* is currently under the name of *Burkholderia cepacia*.

Among the 128 strains of pectolytic bacteria studied, Liao and Wells (1987b) identified 55 strains (43%) as fluorescent *Pseudomonas* spp., only second to *Erwinia carotovora* (64 strains or 50%). More importantly, the *Pseudomonas* strains could grow at 4°C. The psychrotrophic characteristics of the pectolytic *Pseudomonas* undoubtedly represent a threat to fresh-cut produce, because the usual recommendation is for fresh-cut produce to be stored under low temperature conditions. Psychrotrophic pseudomonads have been known to cause spoilage in vegetables stored at low temperatures (Brocklehurst and Lund, 1981; Magnusson et al., 1990; Nguyen-The and Prunier, 1989). Only a few pseudomonads have been identified to produce cellulases compared to pectolytic enzymes (Gross and Cody, 1985). Cellulase activity was not detected in all of the *Pseudomonas* strains investigated by Liao and Wells (1987b).

Bacteria of Food Safety Concern

The pectic enzymes from foodborne human pathogenic bacteria are becoming the focus of some researchers. The pectic enzymes from these bacteria are not extracellular (Chatterjee et al., 1979). Knowledge about pectic enzymes from these saprophytic but human pathogenic bacteria becomes more relevant with the growth of the fresh-cut produce industry. These bacteria can utilize the readily available pectic substrates of plant produce for growth and survival purposes.

Pectic enzymes from *Yersinia* species have received some investigation. The first report came from Von Riesen (1975), who observed that the human and animal pathogenic *Y. enterocolitica* and *Y. pseudotuberculosis* could digest polypectate gel. Later, Starr et al. (1977) demonstrated the production of pectate lyase from *Yersinia* species. The intracellular pectate lyase was then further characterized (Bagley and Starr, 1979). In strains of *Y. enterocolitica* and *Y. pseudotuberculosis*, pectate lyase is a periplasmic and cytoplasmic enzyme. In *Klebsiella pneumoniae*, another species of enteric bacteria, pectate lyase is entirely cytoplasmic. These contrast to the soft rot *Erwinia* species, where a large quantity of extracellular pectate lyase is produced and almost totally excreted. A much higher level of pectate lyase activity was detected in cells of *K. pneumoniae*, but not culture supernatants, when grown on polygalacturonate than when grown on other carbon sources, indicating the catabolic function of pectic enzymes in this bacterium (Chatterjee et al., 1979).

Liao et al. (1999) recently characterized an exopolygalacturonase and a pectate lyase from *Y. enterocolitica*. A 1803 bp gene of a polygalacturonase *pehY* was cloned.

Purified polygalacturonase was unable to macerate plant tissue. The deduced amino acid sequence of *pehY* showed 59% identity to the exopolygalacturonase (exoPG) of *E. chrysanthemi* (He and Collmer, 1990) and 43% identity to the exopolygalacturonase of *Ralstonia solanacearum* (Huang and Allen, 1997). The polygalacturonase was determined to be exolytic, whereas the pectate lyase was endolytic. Both *pehY* and *pel* genes of *Y. enterocolitica* are possibly encoded in the chromosome rather than plasmid-borne.

The Lactic Acid Bacteria

The lactic acid group of bacteria is loosely defined with no precise boundaries. A common characteristic that they all share is the production of lactic acid from hexose fermentation. Bacteria in this group do not have functional Krebs cycle and heme-linked electron transport systems or cytochromes. They obtain their energy by substrate-level phosphorylation (Jay, 1997). The common genera of lactic acid bacteria include *Carnobacterium*, *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Oenococcus*, *Pediococcus*, *Streptococcus*, *Tetragenococcus*, *Vagococcus* and *Weissella* (Stiles and Hozapfel, 1997). Lactic acid bacteria are widely used in food biotechnology, including food or feed production from raw plant material. Karam and Balarbi (1995) reported that polygalacturonases and pectin esterases were also present in lactic acid bacteria. Lactic acid bacteria are not necessarily destructive to plant tissue. The ability of lactic acid bacteria to alter food flavor is, however, well known. A possible pathway of fruit flavor deterioration by lactic acid bacteria is by way of an increased lipase production (Chandler and Ranganathan, 1975; Meyers et al., 1996). The delicate balance of flavors in fruits could be more severely affected by the growth of lactic acid bacteria than vegetables, and this might contribute to the relatively rapid flavor loss in minimally processed fruits (Lamikanra et al., 2000).

In a recent investigation, Lamikanra et al. (2000) reported the decay of fresh-cut cantaloupe stored at 20°C with the dominant microflora being Gram-positive bacteria, and the concurrent increase in lactic acid production. However, it is generally believed that lactic acid bacteria do not directly attack plant cell wall polymers. At 4°C, fruit stored did not show significant degradation over 14 days. Gram-negative rods were the dominant microflora with no lactic acid present. The Gram-negative bacteria were believed to be psychrotrophic pseudomonads. These pseudomonads are capable of producing pectic enzymes that would have been expected to degrade the fruit tissue. The observation is an indication of the need for further research on the effects of microorganisms on fresh-cut fruit tissue surfaces.

THE FUNGI

Fungi can produce a wide array of enzymes, such as amylase, protease, lipase, cellulase and pectinase, that degrade plant polymers. From the industrial application point of view, *Aspergillus oryzae* and *Aspergillus niger*, together with a bacterium, *Bacillus subtilis*, are the three most useful, well-known and safe microbial sources for enzymes (Talyor and Richardson, 1979). Pectic enzymes have received considerable attention regarding their involvement in fruit and vegetable rots.

The role of pectic enzymes in plant-fungi interaction was actually postulated more than a century ago by De Bary in 1886 (Lang and Dornenburg, 2000). Many filamentous fungi such as *Aspergillus*, *Cercospora*, *Fusarium*, *Penicillium*, *Rhizoctonia* and *Trichoderma* are known to produce large amounts of extracellular pectic enzymes. Although yeasts are not considered to be capable of actively attacking plant tissue, the production of pectic enzymes by yeasts has long been known. In 1951, Luh and Phaff first described pectic enzymes in *Saccharomyces fragilis*. Since then, a few yeast species have been reported to produce pectic enzymes (Blanco et al., 1999).

The Filamentous Fungi

The degradation process of plant polymers by filamentous fungi involves the action of a number of extracellular pectic enzymes. In contrast to the widespread occurrence of endopectate lyase in bacteria, fungi mostly produce polygalacturonases and pectin esterases (Sakai et al., 1993). Most fungal polygalacturonases are endoenzymes. However, some fungi also produce exoenzymes. Polygalacturonase genes have been described in a large number of phytopathogenic and nonphytopathogenic fungi, including some of the fruit rot species in *Aspergillus*, *Botrytis*, *Colletorichum* and *Penicillium*. In most of these cases, a detailed genetic analysis reveals the existence of polygalacturonase gene families rather than a single polygalacturonase gene (Lang and Dornenburg, 2000). The synthesized pectinases are generally secreted from intact cells into the surrounding tissue. However, some enzymes might remain inside the cell, obviously for their catabolic function. In a comparative study, the *Penicillium frequentans* synthesized 11 polygalacturonases and two pectinesterases when grown in liquid culture supplemented with pectin. Seven polygalacturonases and the two pectinesterases were secreted in the medium, whereas four polygalacturonases were not secreted (Kawano et al., 1999).

In a study with grape bitter rot caused by *Greenaria uvicola*, Ridings and Clayton (1970) related the production of pectic enzymes by the pathogen to the rot symptom. *G. uvicola* produced polygalacturonase in four- and seven-day-old cultures and pectate lyase (trans-eliminase) in 12-day-old cultures. Pectin methylesterase or cellulolytic enzymes were absent. They further found that *G. uvicola* is not restricted in its pathogenicity to the fruits of *Vitis* spp. Based on the inoculation experiment through wounded fruits or vegetables, they reported that *G. uvicola* induces rots of several fruits, including peach, apple, strawberry, banana and blueberry. The importance of pectolytic enzymes for fungal virulence was further demonstrated by the mutagenesis experiments of endopolygalacturonase genes in *Aspergillus flavus* (Shieh et al., 1997) and *Botrytis cinerea* (Ten Have et al., 1998). The inactivations of these genes were directly associated with the loss of virulence.

It should, however, be noted that not all of the PG genes are involved in virulence. The regulation of the polygalacturonase gene expressions is apparently of great importance on the enzyme biological function. Similar to the bacterial counterpart, pectic enzymes in fungi are produced both inductively and constitutively. Whitehead et al. (1995) reported that the polygalacturonase genes of *Aspergillus flavus* were induced in pectin-containing media but not in glucose. The regulation is at the transcriptional

level, suggesting that endopolygalacturonases participate in host penetration by degrading the pectin layer.

Botrytis

Botrytis cinerea is an important pre- and postharvest pathogen of many fruits. In the early 1900s, several biochemical studies showed that endopolygalacturonase production by *B. cinerea* was affected by the carbon source available, particularly in the presence or absence of pectin in the culture medium (Johnston and Williamson, 1992; Leone, 1990). The endopolygalacturonase genes from *B. cinerea* were later cloned (Ten Have et al., 1998; Wubben et al., 1999). *B. cinerea* produces a set of endopolygalacturonase isozymes. The different endopolygalacturonase isoforms of *B. cinerea* are encoded by a gene family of at least six genes (*Bcpg1*–*6*) (Ten Have et al., 1998; Wubben et al., 1999). The expression of the different endopolygalacturonase-encoding genes of *B. cinerea* was affected in liquid culture by the carbon source available (Wubben et al., 1999) as well as by changes in pH of the culture medium (Wubben et al., 2000). A basic constitutive expression level was observed for two genes, *Bcpg1* and *Bcpg2* which encode basic isozymes. Galacturonic acid was shown to induce the expression of *Bcpg4* and *Bcpg6*. Low pH of the culture medium resulted in induced expression of the *Bcpg3* gene. Expression of the *Bcpg5* gene was inducible; however, the inducing factors could not be identified. There is evidence that *Bcpg5* gene expression is favored by a combination of low pH and galacturonic acid induction. Finally, galacturonic acid-induced expression of the *Bcpg4* gene was repressed by the presence of more favorable carbon sources, such as glucose (Wubben et al., 2000).

Urbanek and Kaczmarek (1985) reported that an apple strain of *B. cinerea* produced extracellular acid proteinases, aspartic proteinase and carboxypeptidase. They noted that isolated aspartic proteinase hydrolyzed proteins in the preparations of apple cell walls and that the excretion of aspartic proteinase preceded that of carboxypeptidase. Doss (1999) analyzed the composition and enzymatic activity of the extracellular matrix secreted by germlings of *B. cinerea* to serve in part in their attachment. Cellulase, pectin lyase and pectin methylesterase activities were noted, but proteinase activity was not detected.

Aspergillus

Most of the currently used pectinolytic enzymes in food processing are derived from *Aspergillus* species. *A. niger* is probably one of the best analyzed fungi with respect to polygalacturonase synthesis. It possesses a complete family of endopolygalacturonase encoding genes and produces several polygalacturonase isoenzymes that display considerable differences with respect to substrate specificity, cleavage rate and optimal pH for activity (Parenicova et al., 1998). Polygalacturonases are abundant among the saprophytic fungi using dead plant tissue as a food source. They are mostly present together with other pectinases and are the enzymatic machinery for degrading the complex plant material and their conversion to readily metabolizable carbohydrates. *A. niger* also produces other plant cell wall degradation enzymes. Gherbawy (1998) surveyed the presence of fungi on plums, pears and apples in Egypt. *A. niger* was found to be the dominant fungus. He also reported that the low

dose of gamma irradiation (1 Mci for 10 min) enhanced the *A. niger* isolates to produce more biomass and polygalacturonase, pectinmethylesterase, cellulase and protease.

For plant pathogenic species, it is clear that pectic enzymes are involved in pathogenicity or virulence. *A. flavus* harbors two glucose-repressible and one constitutive endopolygalacturonase gene (Cleveland and Cotty, 1991). The gene *pecA* was shown to be related to pathogenicity. In another study (Shieh et al., 1997), the involvement of one of the endopolygalacturonases (P2c) in fungal aggressiveness was positively demonstrated by using genetic manipulation methods. Deleting the P2c gene significantly decreases the ability of the fungus to spread in cotton balls, while introducing the specific gene into a P2c null mutant increased the aggressiveness of the strain. It is interesting to note that in the nonpathogenic *A. nidulans*, a phytopathogenic potential could be demonstrated under conditions when polygalacturonase synthesis was induced (Dean and Timberlake, 1989).

YEASTS

Although yeasts are common microflora of plant surface, particularly on fruits, they are not plant pathogens. Yeasts are highly efficient in metabolizing simple sugars. When the cell content is released, yeasts can multiply quickly in fruit juice by the fermentation of simple sugars. Therefore, yeasts play an important role in fruit spoilage under favorable conditions. Pectic enzymes have been reported in several yeast species. These yeasts belong to the genera *Canidia*, *Cryptococcus*, *Fabospora*, *Kluyveromyces*, *Pichia*, *Saccharomyces* and *Zygosaccharomyces* (Blanco et al., 1999). With the exception of a few cases, pectic enzymes from yeasts are mainly endopolygalacturonases (Gainvors et al., 1994). The end products of endopolygalacturonases reactions are always oligosaccharides with a varying number of galacturonic residues. Moreover, all of these enzymes preferentially attack pectate over pectin, and their activities decrease as the degree of methylation increases (Blanco et al., 1999). Barnby et al. (1990) found that the activity of endo-polygalacturonase from *K. marxianus* with 37.8% esterified pectin is about 95%, and with 61% pectin esterification, the activity decreases to 25%.

The function of pectic enzymes in yeasts is largely unknown. Blanco et al. (1999) proposed two possible functions. For the endopolygalacturonase producing group, most authors have attributed an ecological role rather than a trophic one to yeast polygalacturonases. These enzymes could be involved in substrate colonization on fruits, causing the breakdown of plant tissues with a concomitant release of sugars from plant cells. This, in turn, can be utilized for yeast growth and can, consequently, cause further spoilage. The other group encompasses the yeasts that, like filamentous fungi, have the ability to grow using pectic substances as the sole carbon source (Federici, 1985).

The production of pectic enzymes by yeasts is usually constitutive. However, the pectolytic capacity of a few species such as *C. albidus* has been reported to be inducible (Blanco et al., 1999). *Cryptococcus albidus*, together with some filamentous fungi, was reported to produce an inducible endopolygalacturonase that is involved in the spoilage of preserved fruit (Federici, 1985).

Using classical genetic techniques, Blanco et al. (1997) demonstrated that the pectolytic capacity of *S. cerevisiae* had at least two structural genes in the wild-type strain 1389, whereas the genetic strain IM108b was monogenic. Iguchi et al. (1997) reported the cloning of a protopectinase-encoding gene (PSE3) from *Trichosporon penicillatum*. The PSE3 gene contains an ORF encoding a 367 amino acid protein. The deduced amino acid sequence for this gene shows a high homology (65.4%) with the polygalacturonases from *Aspergillus oryzae* and other filamentous fungi.

FURTHER CONSIDERATION

It is evident that further research on the effects of microorganisms and microbial enzymes on plant tissue as they relate to fresh-cut produce is needed. The presence of a large area of wound surface or large number of wounded cells and the refrigeration requirements are factors for consideration. In fruits, for example, flavor changes occur rapidly regardless of refrigeration. Although the cause of such changes in flavor is a subject for research, activities of pectic enzymes as well as other depolymerases could contribute significantly enough to cause changes in flavor. Microorganisms are usually considered to be the source of these enzymes. The precise biological role of some depolymerases such as polygalacturonases, however, remains unclear even in the tomato fruit, which has been extensively studied (Lang and Dornenburg, 2000). It is also possible that deterioration caused by other non-microbial physiological changes precedes microbial activity. Such changes could be responsible for high microbial activities that further affect quality. Thus, it is conceivable that physiological changes, including nonmicrobial changes that degrade cells, could precede an increase in microbial activity (Zagory, 1999). Increased microbial activity could then be the result of the contents of the ruptured cells serving as substrates for microorganisms. In such cases, the presence of the microbial enzymes is the result, and not the cause, of spoilage. However, enzymes produced through this pathway would be expected to further degrade produce quality. Regardless of the mode of product deterioration, it is evident that interactions between microorganisms and the plant host play significant roles in the quality of fresh-cut fruit and vegetable products. Knowledge of the microbe-plant interactions in fresh-cut produce will help to develop strategies to improve their sensory quality and shelf life.

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9 Preservative Treatments for Fresh-cut Fruits and Vegetables

Elisabeth Garcia and Diane M. Barrett

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INTRODUCTION

From the quality standpoint, it is desirable to preserve the characteristics of fresh-cut fruits and vegetables at their peak. What the consumer perceives as the most appealing attributes of these products include their fresh-like appearance, taste and flavor, in addition to convenience. Obviously, any food product should be safe for consumption, and fresh-cut products are very sensitive to contamination. Among the limitations to shelf life of fresh-cut products are microbial spoilage, desiccation, discoloration or browning, bleaching, textural changes and development of off-flavor or off-odor. Nevertheless, safety aspects are not discussed in this chapter, but were reviewed in Chapter 4. The primary quality attributes of a food product include color, texture, flavor and nutritional value. When assessing plant product quality, consumers take product appearance into consideration as a primary criterion, and color is probably the main factor considered (Kays, 1999).

While conventional food-processing methods extend the shelf life of fruits and vegetables, the minimal processing to which fresh-cut fruits and vegetables are submitted renders products highly perishable, requiring chilled storage to ensure a reasonable shelf life. Preparation steps such as peeling or scrubbing, slicing, shredding, etc., remove the natural protection (peel or skin) of fruits and vegetables and cause bruises, rendering them susceptible to desiccation and wilting. This also exposes internal tissues to microbes and potentially deleterious endogenous enzymes. Among the possible consequences of mechanical injuries to produce are increase in respiration rate and ethylene production, accelerated senescence and enzymatic browning (Rosen and Kader, 1989). In conventional types of fruit and vegetable processing, such as canning and freezing, many of these problems are prevented or controlled by heat processing and consequent inactivation of enzymes by the use of protective packaging materials, or through the application of various additives. In the production of fresh-cut products, the use of heat is avoided in order to prevent cooking of the product and, consequently, loss of fresh-like characteristics. Several chemical preservatives can be used, depending on what is to be prevented; often, chemical preservatives are applied in the control of enzymatic browning, firmness and decay (Brecht, 1995). Other important applications include the use of controlled modified atmosphere packaging, and edible films also have many potential applications.

A survey on consumer perception of convenience products revealed the desire that such products maintain fresh characteristics longer without the use of preservatives (Bruhn, 1994). Unfortunately, depending on the type of quality defect to be prevented or controlled, it is not always possible to avoid the use of chemical treatments. One important aspect to consider is the establishment of conditions that allow

for quality optimization at a reasonable shelf life, rather than extending shelf life at an acceptable quality (Shewfelt, 1994).

In this chapter, we review the most common treatments used to preserve the color and texture of fresh-cut products. Color preservation is, after safety, the most important attribute to be preserved, because frequently, a product is selected for its appearance, particularly its color. Color has been considered to have a key role in food choice, food preference and acceptability, and may even influence taste thresholds, sweetness perception and pleasantness (Clydesdale, 1993). Second, texture loss and preservation in fresh-cut products will be discussed, due to its important impact on product appearance and sensory quality.

FRESH-CUT PRODUCTS AND COLOR PRESERVATION

Fruits and vegetables are attractive and eye-catching to a large degree because of the richness of pigments that they contain. Preservation of chlorophyll in vegetables, red to purple anthocyanins, and yellow, orange and red carotenoids in fruits and vegetables is of vital importance to maintain quality. Color changes (Figure 9.1) in fresh-cut fruits and vegetables may have different origins, for example, decreased green pigmentation in fresh-cut lettuce may result from senescence, heat exposure or acidification; discoloration or browning of sliced mushrooms, apples and pears is brought about through the action of polyphenol oxidases; and white blush development in carrots is initially caused by desiccation and later, by lignification. The main focus of this chapter is on prevention of enzyme-catalyzed browning, although some of the other color changes will be briefly discussed.

ENZYMATIC BROWNING

Enzymatic browning is one of the most limiting factors on the shelf life of fresh-cut products. During the preparation stages, produce is submitted to operations where cells are broken, causing enzymes to be liberated from tissues and put in contact with their substrates. Enzymatic browning is the discoloration that results from the action of a group of enzymes called polyphenol oxidases (PPOs), which have been reported to occur in all plants and exist in particularly high amounts in mushroom, banana, apple, pear, potato, avocado and peach. Enzymatic browning must be distinguished from nonenzymatic browning, which results upon heating or storage after processing of foods. Types of nonenzymatic browning include the Maillard reaction, caramelization and ascorbic acid oxidation.

Enzymatic browning is a complex process that can be subdivided in two parts. The first part is mediated by PPO (Figure 9.2), resulting in the formation of *o*-quinones (slightly colored), which through nonenzymatic reactions, lead to the formation of complex brown pigments. *o*-Quinones are highly reactive and can rapidly undergo oxidation and polymerization. *o*-Quinones react with other quinone molecules, with other phenolic compounds, with the amino groups of proteins, peptides and amino acids, with aromatic amines, thiol compounds, ascorbic acid, etc. (Whitaker and Lee, 1995; Nicolas et al., 1993). Usually, brown pigments are formed, but in addition, reddish-brown, blue-gray and even black discolorations can be produced on some bruised plant tissues. Color variation in

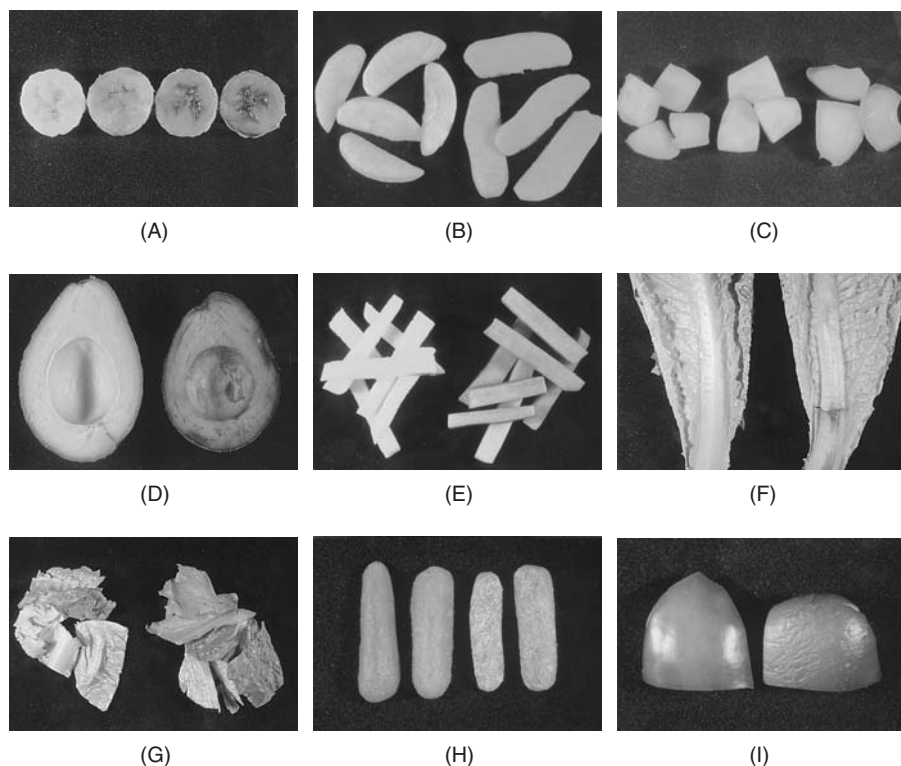


FIGURE 9.1 Some examples of changes in appearance of cut fruits and vegetables. Browning is shown in photos A–G. (A) Banana slices after 0, 1, 2, and 3 days of cutting. (B) Apple wedges; left: immediately after cutting, right: 24 hr after cutting. (C) Comparison of browning development among three pear varieties: ‘Red D’Anjou’ (left), ‘Hi-Up’ (center), ‘Bartlett’ (right). (D) Avocado halves. (E) Potato sticks. (F and G) Romaine lettuce. (H) White blush (right) in baby carrots. (I) Wrinkling on green bell peppers (right).

products of enzymatic oxidation is related to the phenolic compounds involved in the reaction (Amiot et al., 1997), and both color intensity and hue of pigments formed vary widely (Nicolas et al., 1993). Consequences of enzymatic browning are not restricted to discoloration—undesirable tastes can also be produced, and loss of nutrient quality may result (Vámos-Vigyázó, 1981). Biochemical details on PPO action were reviewed in Chapter 6. PPO has been considered one of the most damaging enzymes to quality maintenance of fresh produce (Whitaker and Lee, 1995), and the prevention of enzymatic browning has always been considered a challenge to food scientists (Ponting, 1960).

Preharvest Factors

Several parameters may contribute to the development of enzymatic browning. Agricultural practices, soil, fertilizers, climate and harvesting conditions all affect the final quality of fresh-cut products (Ahvenainen, 1996). High nitrogen levels have been related to a greater tendency to brown in potatoes (Mondy et al., 1979).

TABLE 9.1
Enzymatic Browning in Purées
Prepared with Various Apricot
Cultivars at Commercial Maturity

Cultivar	DL*
'Henderson'	26.3
'Moniqui'	21.4
'Rouge de Roussillon'	17.8
'Rouge de Fournes'	17.8
'Polonais'	16.8
'Canino'	16.7
'Cafona'	11.8
'Bebeco'	5.3
'Precoce de Tyrinthe'	3.7

DL* = Difference in lightness between oxidized and nonoxidized apricot purées.

Source: Adapted from Radi et al., 1997.

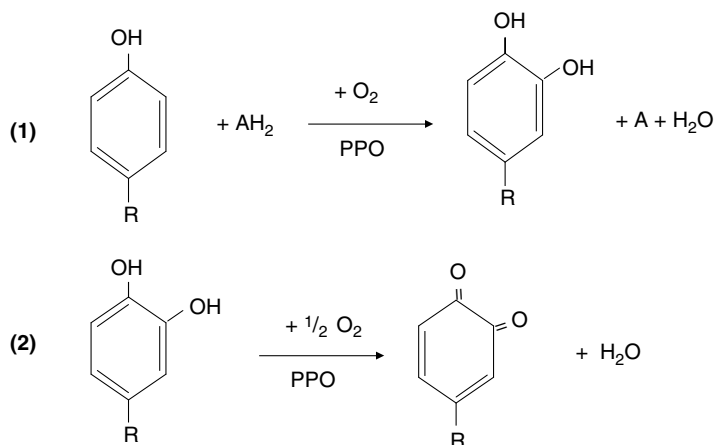


FIGURE 9.2 Reactions that can be catalyzed by polyphenol oxidase: (1) hydroxylation of monophenols to *o*-diphenols and (2) oxidation of *o*-diphenols to *o*-quinones.

The selection of raw material for processing needs to be carefully evaluated. The susceptibility to browning may differ from cultivar to cultivar, as exemplified in Tables 9.1 and 9.2. Some tissues may have high PPO activity and/or high concentration or types of phenolic PPO substrates which, under appropriate conditions, lead to a higher tendency to brown. In pears, it was found that although the phenolic content tended to decrease with delayed harvest time, phenolic levels did not always correlate with the susceptibility to browning (Amiot et al., 1995). In general, high concentrations

TABLE 9.2
Susceptibility of Potato Varieties to Enzymatic
Browning after Storage (of Whole Unpeeled Tubers)
at 5°C and 75% RH

Storage Time	Browning Index		
	var. 'Bintje'	var. 'Van Gogh'	var. 'Nicola'
1 mo.			
30 min*	6	27	44
60 min	15	40	75
120 min	21	52	88
5 mo.			
30 min	16	26	21
60 min	23	56	58
120 min	30	78	98
8 mo.			
30 min	10	28	66
60 min	32	74	112
120 min	62	104	145

Note: Browning evaluation was carried out on 5 mm slices cut from the center of the tubers and left at 23°C for observation at 30 min, 60 min and 120 min after cutting.

Source: Adapted from Mattila et al., 1993.

of phenolic compounds are found in young fruits. While in bananas, PPO activity is higher in the pulp than in the peel, in pear and apple, PPO activity is higher in the peel than in the flesh (Macheix et al., 1990). In addition, PPO activity may vary widely between cultivars of the same crop and at different maturity stages. Examples of such variations are shown in Table 9.3. Ideally, produce varieties with either low levels of PPO or phenolic substrates, or both, should be selected for fresh-cut processing. New varieties with desirable traits for fresh-cut processing may be developed by conventional breeding techniques and potentially through biotechnology (Chapter 13). Nevertheless, it is important to point out that not only PPO activity and concentration of substrates are important, but also, individual phenolics exhibit different degrees of browning, and the rate of enzymatic browning is also affected by other polyphenol compounds present in the tissue (Lee, 1992).

Postharvest and Processing Factors

Processing operations such as washing, scrubbing, peeling, trimming, cutting, shredding, etc., carried out during the initial stages of fresh-cut preparation cause mechanical injury to the plant tissues. Moreover, even prior to processing, produce manipulation may bring mechanical shocks resulting in cracks and bruises, which can elicit physiological and biochemical responses in the wounded tissue as well as in unwounded distant cells (Saltveit, 1997). Peel removal and loss of tissue integrity with cell

TABLE 9.3
Relative PPO Activity in Different
Apple Cultivars

Cultivar	Relative PPO Activity	
	Peel	Cortex
'Red Delicious'	100	100
'Golden Delicious'	33	30
'McIntosh'	46	80
'Fuji'	57	71
'Gala'	30	48
'Granny Smith'	43	73
'Jonagold'	43	43
'Elstar'	10	20

Source: Adapted from Janovitz-Klapp et al., 1989.

breakage facilitate microbial contamination. In addition, exposure to air and release of endogenous enzymes that are put in contact with their substrates, originally in different cell compartments, may lead to detrimental consequences. Living tissues are still physiologically active and respond to wounding. The first responses to mechanical injury relate to respiration rate increase and possibly increased ethylene production (see Chapter 5). In general, respiration rates are inversely related to the shelf life of produce. Quality deterioration may result from increased ethylene production, which may induce higher cellular metabolism and higher enzymatic activity (Reyes, 1996). Another consequence of wounding is the induction of secondary product synthesis, including a variety of phenolic compounds. Among the enzymes that may have deleterious effects, polyphenol oxidase (PPO) can be the most damaging enzyme with regard to color deterioration of plant foods (Whitaker and Lee, 1995).

During peeling and cutting operations, if the equipment used is not in the best condition, for example, if dull knives and blades are used, bruising and damage occurs in more tissue layers than intended; thus, the sharpness of knife blades can significantly affect product storage life (Bolin et al., 1977). An increase of 15% in the respiration rate of hand-peeled carrots was detected when compared to unpeeled carrots. In contrast, abrasion peeling, which is more destructive than hand peeling, led to almost doubled respiration rates. For stored carrots, respiration rates increased two- and threefold when fine-abrasion vs. coarse-abrasion peeling were used, respectively, in comparison with the rates observed for hand-peeled carrots. Shredded iceberg lettuce had a 35–40% increase in respiration rate in relation to quartered lettuce heads. The type of equipment used may also affect the physiological response of the tissues—sharp rotating blades gave better results in cutting lettuce (lower respiration and lower microbial count during storage) than sharp stationary blades (O’Beirne, 1995). Evidently, the tissue response to mechanical injury is expected to be more pronounced when extensive wounding is inflicted on the produce, such as

the grating of carrots vs. preparation of carrot sticks. Moreover, the direction of the cut also affects the tissue response to wounding (Zhou et al., 1992).

As a result of cutting, there is accumulation of cell fluids on the cut surface, and, in general, washing of cut produce may be helpful. Removal of cellular fluids (which carry potentially deleterious enzymes such as PPO, peroxidase, etc.) released during the cutting operation is important and can be accomplished by simple rinsing procedures. In addition, such fluids are nutrient-rich and facilitate microbial growth. Although washed mushrooms had 15% less soluble phenolics and showed leaching of PPO (two out of four isoforms) and, therefore, less enzymatic activity, there was also water uptake during washing. Consequently, a more rapid deterioration of mushrooms, due to microbial spoilage and mechanical damage (Choi and Sapers, 1994), was shown. Other commodities, such as lettuce, do not benefit from rinsing. Rinsed and drained shredded lettuce may retain 0.5–1% water on the surface, a residual amount that can decrease product quality by facilitating decay; thus, dewatering has to be carried out (Bolin et al., 1977). However, centrifugation of lettuce to remove residual cold water may require spin conditions (speed, time) that result in mechanical damage of the produce.

For many fruits and vegetables utilized by the fresh-cut industry, processing is carried out shortly after harvest, but in some instances, the seasonality of harvesting may not allow for this. Potatoes are an example of a vegetable that can be stored before use in the preparation of pre-peeled products. A Finnish study evaluated the tendency to brown of three potato varieties stored for different periods (Table 9.2). Results showed that only one variety ('Bintje') stored for one month would pass the requirements of the local industry, which establishes a maximum browning index of 10 as acceptable for fresh-cut processing (Mattila et al., 1993).

Browning and Enzymes Other Than Polyphenoloxidase

Mechanical injury (wounding) and ethylene can stimulate phenolic metabolism in fresh-cut tissue. Wounding and ethylene induce the activity of the enzyme phenylalanine ammonia lyase (PAL), a key enzyme for phenolic biosynthesis. Accumulated phenolic compounds can be used as substrates by PPO, leading to browning. It has been suggested that lettuce storage life is related to the activity of stress-induced PAL (Couture et al., 1993; Saltveit, 1997). In fresh-cut lettuce, browning of pieces is also a major detriment to quality. Different types of browning defects can be observed in lettuce, such as russet spotting (RS) (which is characterized by brown spots on the lettuce midribs), browning of cut leaf edges (LEB) and browning of the leaf surface (LSB). In wounded air-stored lettuce pieces, the major defects described are EB and LSB, while RS is most apparent in wounded ethylene-stored samples. A comparison of the response of five types of lettuce (iceberg, romaine, green leaf, red leaf and butterhead) revealed differences in the maximum level of wound-induced PAL, which were also affected by the storage of the whole lettuce heads before processing. Maximum levels of PAL decreased with increased storage time (López-Gálvez et al., 1996). In addition, in harvested lettuce heads, the stem tissue near the harvesting cut may develop browning or so-called butt discoloration, when the cut stem initially becomes yellow, later develops a reddish-brown color, and

finally develops an intense brown pigmentation. PAL activity is induced by cutting the lettuce stem, with subsequent synthesis and accumulation of soluble phenolic compounds (mainly caffeic acid derivatives), supplying substrates for PPO (Tomás-Barberán et al., 1997). PAL activity is believed to be proportional to the extent of wounding.

Peroxidase is an enzyme widely distributed in plants. Changes in peroxidase may be brought about by wounding, physiological stress and infections. Many reactions can be promoted by peroxidase, and in the presence of small amounts of hydrogen peroxide, it can oxidize a number of naturally occurring phenolics. Mono- and diphenols are potential substrates for peroxidase (Robinson, 1991). It is believed that although peroxidase may also contribute to enzymatic browning, its role remains questionable (Nicolas et al., 1993) and limited by hydrogen peroxide availability (Amiot et al., 1997).

CONTROL OF ENZYMATIC BROWNING

Enzymatic browning may be controlled through the use of physical and chemical methods, and, in most cases, both are employed. Physical methods may include reduction of temperature and/or oxygen, use of modified atmosphere packaging or edible coatings or treatment with gamma irradiation or high pressure. Chemical methods utilize compounds that act to inhibit the enzyme, remove its substrates (oxygen and phenolics) or function as preferred substrate. Chemical means of controlling browning will be discussed first.

Prior to having their GRAS status revoked by the FDA in 1986 due to potential health risks posed to sensitive consumers (Taylor, 1993), sulfites had a widespread application in controlling both enzymatic and nonenzymatic browning. Following their ban for use in fruits and vegetables to be consumed raw, other chemicals have been sought for prevention of enzymatic browning. Regardless of the fact that many different PPO inhibitors have been used in research (Vámos-Vigyázó, 1981; McEvily et al., 1992; Iyengar and McEvily, 1992; Sapers, 1993), in this chapter, only inhibitors with potential application for fresh-cut fruits and vegetables will be discussed. It is important to point out that some chemicals used in research may not meet the safety standards and pose toxic risks, others may impart undesirable sensory effects to foods and others have shown effectiveness only in fruit juices but not on cut surfaces.

Traditionally, conventional food processing achieves the prevention of browning through heat inactivation of PPO, as with blanching and cooking. Heat inactivation is an effective method of browning prevention, and PPO is considered an enzyme of low thermostability, although differences in heat stability are reported for different cultivars and PPO isoforms (Zawistowski et al., 1991). Nevertheless, use of heat also has the potential to cause destruction of some food quality attributes, such as texture and flavor, and to result in nutritional losses. It is considered that in fresh-cut products, if heat treatments are applied, they should be minimized and should not cause a cessation of respiration. Rather than, or in addition to, the use of heat, the control of enzymatic browning is frequently achieved through the use of different types of chemicals, generally referred to as antibrowning agents.

For an enzymatic browning reaction to occur, essential elements are required: the presence of active PPO, oxygen and phenolic substrates. Browning prevention is possible, at least temporarily, through elimination of substrates and/or enzyme inhibition.

Antibrowning Agents

Several types of chemicals are used in the control of browning (Table 9.4). Some types act directly as inhibitors of PPO, others render the medium inadequate for the development of the browning reaction, and still others react with the products of the PPO reaction before these can lead to the formation of dark pigments.

Acidulants

While the optimum pH for PPO has been reported as ranging from acid to neutral, in most fruits and vegetables, optimum PPO activity is observed at pH 6.0–6.5, while little activity is detected below pH 4.5 (Whitaker, 1994). It has also been reported that irreversible inactivation of PPO can be achieved below pH 3.0 (Richardson and Hyslop, 1985). Nevertheless, it has also been reported that apple PPO is quite tolerant to acidity, and at pH 3.0, it retains 40% of its maximum activity (Nicolas et al., 1994).

The use of chemicals that lower the product pH, or acidulants, finds widespread application in the control of enzymatic browning. The most commonly used acidulant is citric acid. Acidulants are frequently used in combination with other types of antibrowning agents, because it is difficult to achieve efficient browning inhibition solely through pH control. In addition, there are variations in the effect of different acids on PPO; as an example, malic acid has been reported to be more efficient in preventing apple juice browning than citric acid (Ponting, 1960).

Reducing Agents

This type of antibrowning agent causes chemical reduction of colorless *o*-quinones resulting from the PPO reaction back to *o*-diphenols (Iyengar and McEvily, 1992). Reductants are irreversibly oxidized during the reaction, which means that the protection they confer is only temporary, because they are consumed in the reaction. When all the reducing agent added is oxidized, the *o*-quinones from the PPO reaction may undergo further oxidation reactions (not involving PPO) and finally rapid polymerization leading to the formation of brown pigments (Figure 9.2). Due to the oxidative nature of enzymatic browning, reducing agents can also be applied in the prevention of discoloration.

Ascorbic acid is probably the most widely used antibrowning agent, and in addition to its reducing properties, it also slightly lowers pH. Ascorbic acid reduces the *o*-benzoquinones back to *o*-diphenols, and it also has a direct effect on PPO (Whitaker, 1994; Golan-Goldhirsh et al., 1992).

Thiol-containing compounds, such as cysteine, are also reducing agents that inhibit enzymatic browning. However, for complete browning control, the amount of cysteine required (cysteine-to-phenol ratios above 1) is often incompatible with product taste (Richard-Forget et al., 1992).

Chelating Agents

By complexing copper from the PPO active site, chelating compounds, such as ethylenediamine tetraacetic acid (EDTA) can inhibit PPO, which is a metalloenzyme containing copper in the active site. Sporix is a powerful chelator, and also an acidulant. Browning prevention in apple juice and cut surfaces was obtained with combinations of Sporix and ascorbic acid (Sapers et al., 1989).

Complexing Agents

This category includes agents capable of entrapping or forming complexes with PPO substrates or reaction products. Examples of this category are cyclodextrins or cyclic nonreducing oligosaccharides of six or more D-glucose residues. In aqueous solution, the central cavity of cyclodextrins can form inclusion complexes with phenolics, consequently depleting PPO substrates. β -Cyclodextrin has the most appropriate cavity size for complexing phenolic compounds, but its water solubility is low (Billaud et al., 1995). β -Cyclodextrin was not effective in controlling browning of diced apples, presumably due to its low diffusion (Sapers and Hicks, 1989). Large variations in the inhibitory properties of cyclodextrins have been found with different phenols tested. β -Cyclodextrin binding strength varies with different phenols. In model systems containing a single phenolic compound, β -cyclodextrin always works as a PPO inhibitor. When mixtures of phenolic compounds were tested, the results were variable, and the balance among the PPO substrates present can be modified, resulting in color changes after enzymatic oxidation catalyzed by PPO (Billaud et al., 1995).

Enzyme Inhibitors

One of the antibrowning agents with the most potential for application to fresh-cut products is 4-hexylresorcinol, a chemical that has been safely used in medications for a long time and has been granted FDA GRASS (generally regarded as safe) status for use in the prevention of shrimp discoloration (melanosis), where it proved to be more effective than sulfite on a weight-to-weight basis (McEvily et al., 1992). Currently, its use on fruit and vegetable products has been delayed while awaiting FDA approval. The efficiency of 4-hexylresorcinol has been demonstrated in preliminary tests carried out using cut apples and potatoes (McEvily et al., 1991). The combination of 4-hexylresorcinol with ascorbic acid improved browning control in apple slices (Luo and Barbosa-Canovas, 1995).

Other Antibrowning Agents

Sodium chloride (as other halides) is known to inhibit PPO; its inhibition increases as pH decreases. Chloride is a weak inhibitor; some authors report that the chloride levels required for PPO inhibition are elevated and may compromise product taste (Mayer and Harel, 1991). Nevertheless, other authors believe that browning control may be possible provided that the dipping solutions are acidic; a pH of at least 3.5 has been suggested (Rouet-Mayer and Philippon, 1986).

Calcium treatments used for tissue firming have also been reported to reduce browning (Drake and Spayd, 1983; Hopfinger et al., 1984; Bolin and Huxsoll, 1989). Although citric acid and/or ascorbic acid dips were not effective in preventing browning of pear, slices dipped in 1% CaCl_2 and stored for a week at 2.5°C appeared to be lighter in color than water-treated control slices (Rosen and Kader, 1989). In fact, this could be due to the PPO inhibition by the chloride ion (Table 9.4). Nevertheless, the firming action of calcium (pages 292–293) could contribute to a reduced leakage of PPO and its substrates at the exposed cut surfaces (Sapers and Miller, 1992).

Antibrowning activity has been attributed to a small peptide isolated from honey. Browning inhibition (62%) in slices of peeled apples has been achieved by dipping in a 10% honey solution for 30 minutes at room temperature. Comparison with a

TABLE 9.4
Chemical Agents with Inhibitory Action on Enzymatic Browning

Browning Inhibitor	Effect/Action	Shortcomings	Comments	Examples of Tested Concentrations
Acidulants				
Citric acid	Possible dual effect: lowering pH and chelating Cu from PPO active site		Frequently used in combination with other agents Theoretically, inhibition of enzymatic browning can be achieved by lowering the pH 2 or more units below the PPO optimum pH ⁽¹⁾	0.5–2% (w/v) ⁽²⁾
Other organic acids: Tartaric acid, malic acid, lactic acid	Lower pH	Cost Limited availability		
Inorganic acids: Phosphoric acid, hydrochloric acid	Lower pH	Sensory effects: taste		
Reductants (Reducing Agents; Antioxidants)				
Ascorbic acid (AA)	Reduction of <i>o</i> -quinones to colorless diphenols	Temporary effect; ascorbic acid is also consumed Nonspecific; can cause formation of off-colors and/or off-flavor Insufficient penetration into the food tissues		0.5%–1% (AA, EA) ⁽³⁾
Erythorbic acid (EA)	Same as ascorbic acid	Some authors reported that erythorbic acid is destroyed at a faster rate than ascorbic acid		0.8%–1.6% (AA, EA) ⁽⁴⁾

Ascorbyl-phosphate esters AA-2-phosphate (AAP) AA-triphosphate (AATP)	Release ascorbic acid upon hydrolysis by acid phosphatase present in plant tissues React with <i>o</i> -quinones producing stable adducts (colorless)	Less effective than ascorbic acid in some applications, more effective in others (depends on the phosphatase activity of each tissue) Expensive Potential formation of off-flavors at the required concentration	More stable to oxidation than ascorbic acid More effective than ascorbic acid	45.4 mM (0.8% AA) ⁽⁵⁾ 230 mM ⁽⁶⁾
Sulphydryl compounds: L-cysteine				
Complexing Agents				
Cyclodextrins (cyclic oligosaccharides)	Formation of complexes with PPO substrates Entrapment of PPO substrates or products	Complex formation is not specific Potential removal of color and/or flavor compounds Cost: Not approved yet	Suggested use also in combination with other agents (acidulants, chelators, reductants) ⁽⁹⁾	
β -cyclodextrin (β -CD)		Suggested use in juices	Water soluble Lower levels were required when combined with phosphates ⁽¹⁰⁾ More soluble than β -CD	1–4% ⁽²⁾ 4% ⁽¹⁰⁾ 10% ⁽¹⁰⁾
maltosyl- β -CD hydroxyethyl- β -CD				
Chelating Agents				
EDTA	Metal chelator: Binds copper at the PPO active site and copper available in the tissue		Commonly used in combination with other antibrowning chemicals Levels up to 500 ppm are permitted for disodium EDTA and calcium and disodium EDTA	
Polyphosphate	Chelators	Low solubility in cold water	Used in combination with other agents	0.5–2% ⁽²⁾

(continued)

TABLE 9.4
Chemical Agents with Inhibitory Action on Enzymatic Browning (Continued)

Browning Inhibitor	Effect/Action	Shortcomings	Comments	Examples of Tested Concentrations
Sporix TM (acidic polyphosphate)	Chelator and acidulant	Not approved in the U.S. for food use	Although ineffective when tested alone on apple plugs, in combination with ascorbic acid, it was very effective (apparent synergism)	0.24% Sporix + 1% ascorbic acid ⁽⁸⁾
Sodium acid pyrophosphate, Sodium Hexametaphosphate				
Enzyme Inhibitors				
4-hexyl resorcinol	PPO inhibitor	Not approved for use in fruits and vegetables	Specific action on PPO Water soluble Chemically stable Safely used in the prevention of shrimp pigmentation (GRAS status) Other potential food uses	
Anions: Chloride (NaCl, CaCl ₂) (ZnCl ₂)	Interaction with copper at the PPO active site	Weak inhibitor at low to moderate concentration	Inhibition increases at lower pHs	2–4% ⁽²⁾

Miscellaneous		
Enzymatic treatment with proteases	Proteolysis	Fig preparations revealed compounds other than ficin, resorcinol derivatives, which are PPO inhibitors
Ficin (from fig), Bromelain (from pineapple), Papain (from papaya)		Cost
Honey	Contains a small peptide that inhibits PPO	0.5% w/v solution ⁽¹¹⁾ 20% solution ⁽¹²⁾

Note: References: (1) Whitaker and Lee, 1995; (2) McEvily et al., 1992; (3) Santerre et al., 1988; (4) Sapers and Ziolkowski, 1987; (5) Sapers et al., 1989; (6) Kahn, 1985; (7) Montgomery, 1983; (8) Gardner et al., 1991; (9) Hicks et al., 1990; (10) Hicks et al., 1996; (11) Labuza et al., 1992; (12) Oszmianski and Lee, 1990.

control sucrose solution at the same sugar level as the honey preparation showed only a 23% inhibition of browning (Oszmianski and Lee, 1990).

Enzymatic treatments with proteases that attack PPO have been suggested as alternative prevention treatments for enzymatic browning. It was presumed that PPO inhibition by proteases was due to proteolysis or to binding at specific sites required for activation. Another possible mechanism of action suggested was related to the presence of sulfhydryl groups (such as cysteine) in the proteases. Enzymatic treatment of PPO could potentially be carried out with bromelain (extracted from pineapple), papain (from papaya) and ficin (from figs). Preliminary tests were done using small pieces of apples and potatoes that were dipped for 5 minutes in a 2% enzyme solution in citrate buffer at pH 4.5. Results showed that papain worked best on apples, while ficin worked better on potatoes. Parallel tests on untreated samples and control citrate buffer-dipped samples developed comparable discoloration (Labuza et al., 1992). However, partially purified ficin preparations, where the protease was heat inactivated, were comparable to preparations containing active ficin as PPO inhibitors (McEvily, 1991). Later, it was found that ficin preparations contain, in addition to the protease, other antibrowning agents that are analogs of 4-substituted resorcinol (McEvily et al., 1992). Extracts prepared from papaya contain cysteine and another "quinone-trapping" substance identified as a dipeptide cysteine-glutamic acid (Richard-Forget et al., 1998).

Although benzoic and cinnamic acids (aromatic carboxylic acids) are PPO inhibitors (Walker, 1975), they have not given prolonged protection as antibrowning agents. When solutions of sodium cinnamate were used to dip apple plugs, browning prevention was obtained on a short term, but over prolonged storage (>24 hr), a severe browning developed (Sapers et al., 1989). It has been suggested that cinnamates and benzoates may undergo a slow but gradual conversion to PPO substrates (Sapers et al., 1989; McEvily et al., 1992).

There are consumers who want to avoid any type of food preservative (Bruhn, 1995). It is recognized that the consumer perceives fresh-cut products as minimally processed products with characteristics close to their raw unprocessed material. Flavor, color and texture characteristics are probably an added appeal of fresh-cut products, and as a consequence, some processors would rather not use chemical additives that could change that perception of a "natural" product. This may be one of the reasons that ascorbic acid, which may be labeled as vitamin C, is frequently preferred as an antibrowning agent, an added value to the product. Other chemicals of natural origin or identical to natural compounds are also frequently preferred, an example of which is citric acid. With this in mind, some authors have tested the efficiency of other natural products, such as pineapple juice, in the control of enzymatic browning. Among the constituents of pineapple juice, antibrowning activity could be attributed to both ascorbic acid, but in addition, the juice contains a low molecular weight inhibitor which is as yet uncharacterized (Lozano-de-Gonzalez et al., 1993).

Application of Antibrowning Agents

In general, chemicals used to prevent or control enzymatic browning are used in solutions, frequently as formulations containing one or more compounds that are used for dipping the fruit or vegetable pieces. It has been reported that with some

chemicals, such as ascorbic and erythorbic acid or their salts, limited penetration into the plant tissue is an issue. A comparison of the effect of dipping vs. pressure or vacuum infiltration on the penetration of ascorbic and erythorbic acids showed that pressure infiltration was ineffective with potato dice but extended the shelf life of potato plugs by two to four days when compared to dipping (Sapers et al., 1990). The variation in response of potato plugs and dice to pressure infiltration was attributed to the smaller surface-to-volume ratio in the plugs. The authors of the study suggested that the technique could be applied to larger pieces, even peeled tubers. With apple plugs and dice, the pressure infiltration method was superior to dipping, providing an increase of three to seven days in the storage life of apple pieces. Nevertheless, infiltrated dice can become waterlogged and require dewatering by centrifugation or partial dehydration to overcome that defect. In addition, if too much pressure is applied, cell rupture can occur leading to loss of textural integrity and perhaps reduced shelf life.

Combined Treatments

More effective preservation of fresh-cut products can frequently be achieved by using a combination of treatments. A common treatment combination includes ascorbic acid and calcium chloride, such as presented in Table 9.5 (Ponting et al., 1972). In the case of two apple varieties, e.g., ‘Newton Pippin’ and ‘Golden Delicious,’ the highest concentrations of ascorbic acid (1%) and CaCl₂ (0.1%) utilized resulted in the lowest loss of reflectance or browning readings. It is interesting that the use of CaCl₂ alone caused almost as much inhibition on ‘Newton Pippin’ apples, but this was not so for ‘Golden Delicious.’ Table 9.6 shows some results from a

TABLE 9.5
Effect of Treatments with Ascorbic Acid (AA) and Calcium Chloride on the Prevention of Discoloration in Apple Slices

Treatment ¹	Loss of Reflectance (%) Compared to Freshly Sliced Apples	
	var. ‘Newton Pippin’	var. ‘Golden Delicious’
Control—water dip	62.5	60.5
0.05% CaCl ₂	24.8	58.9
0.1% CaCl ₂	23.3	51.2
0.5% AA	57.9	59.2
0.5% AA + 0.05% CaCl ₂	26.9	48.0
0.5% AA + 0.1% CaCl ₂	24.2	25.6
1% AA	25.5	45.6
1% AA + 0.05% CaCl ₂	20.5	39.2
1% AA + 0.1% CaCl ₂	4.2	17.0

¹Three-minute dip in 1 L of antibrowning solution, followed by 1 min draining and packaging in plastic bags prior to storage at ~1°C for 11 weeks.

Source: Adapted from Ponting et al., 1972.

study using different combinations of antibrowning agents on slices prepared from three different potato varieties stored varying lengths of time (Mattila et al., 1993). Other combination treatments may include the use of antibrowning agents and physical methods, such as a heat treatment or controlled atmosphere, such as the combination of 0.5% O₂ and 1% CaCl₂, which was effective in minimizing browning in sliced pears (Rosen and Kader, 1989). In the preparation of pre-peeled potatoes, the damage inflicted by the peeling method has a significant effect on product discoloration. Unstable tissue from peeled potatoes can be removed by lye digestion or hot ascorbic acid/citric acid solutions prior to the treatment with browning inhibitors (Sapers et al., 1995).

Physical Treatments and Browning Control

One of the most commonly used approaches to controlling enzymatic activity in fresh-cut products is the use of low temperatures during handling, processing and storage. At low temperatures, not only is enzymatic activity reduced, but general metabolic rates are also lower, which assists in extending product shelf life.

Some of the physical methods suggested for application in postharvest handling of fruits and vegetables have also been proposed for fresh-cut products. These include the use of modified/controlled atmospheres and gamma irradiation. Nonthermal methods currently being investigated by food processors that may have application for fresh-cut products include high-pressure treatments or treatment with high electric field pulses (Ohlsson, 1994).

Reducing Oxygen Availability

It is important to consider that as a requirement of living tissues, fresh-cut products cannot be exposed to environments with complete removal of oxygen. Nevertheless, enzymatic browning can be delayed (in the presence of active enzyme and phenolic substrates) if oxygen is not available for the reaction to take place. In fruits and vegetables used for either conventional or fresh-cut processing, it is a common practice to hold preprepared produce (already peeled, cut, etc.) immersed in water, brine or syrup to retard diffusion of oxygen. However, tissue will brown when it is re-exposed to air. In addition, during the time the tissue is held, osmotic equilibrium may result in loss of solutes and imbibition of the storage solution.

Modified atmospheres are frequently used in packaging and/or storage of fruits and vegetables. These conditions as well as edible coatings can also be successfully adapted to fresh-cut fruits and vegetables (see Chapter 10).

Among other benefits, the use of modified or controlled atmospheres retards senescence and, consequently, extends the storage life of products. Modified or controlled atmospheres should be seen as a supplements to adequate management of temperature and controlled humidity (Kader, 1992).

Modified atmosphere packaging aims at the creation of an ideal gas composition in the package that can be achieved through commodity-generated modified atmosphere in the package and through the establishment of an active modified atmosphere in the package. However, it is important to avoid damaging low levels of oxygen or high levels of carbon dioxide which lead to anaerobic respiration, resulting in the

development of off-flavors and off-odors and increasing the susceptibility to decay. Appropriate gas compositions, of modified atmosphere need to be experimentally determined for each particular product (Wills et al., 1998). Using a moderate vacuum packaging with polyethylene (80 μ m) for the storage of shredded iceberg lettuce at 5°C, browning was inhibited over a 10-day period (Heimdal et al., 1995). Browning of commercially prepared cut lettuce was retarded in packaged product, where the atmosphere was altered by the respiring product. Visual quality of the cut lettuce packaged in sealed bags received an original score of 9 (excellent), and after storage for two weeks at 2.8°C, the score dropped to 7 (good). Samples stored in an unsealed package received a score of 3 (poor). Modified atmosphere packaging was also efficient in controlling microbial buildup during storage (King et al., 1991).

Shelf-life extension has also been investigated by enrobing fresh-cut products in edible coatings. Such thin layers of protective materials are applied to the surface of the fruit or vegetable as a replacement for the natural protective tissue (epidermis, peel). Edible coatings are used as a semipermeable barrier that helps reduce respiration, retard water loss and color changes, improve texture and mechanical integrity, improve handling characteristics, help retain volatile flavor compounds and reduce microbial growth. It is possible to create a modified atmosphere enrobing fresh-cut produce in edible coating (Baldwin et al., 1995a; Baldwin et al., 1996). Detailed information on edible coatings is presented in several reviews (Krochta et al., 1994; Baldwin et al., 1995a,b; Nisperos and Baldwin, 1996).

Basically, edible coatings are comprised of one or more major components (polysaccharides, proteins, resins, waxes or oils), which may be improved by the addition of plasticizers, surfactants and emulsifiers. Appropriate selection of edible coatings is important due to the hydrophilic nature of cut surfaces of many fresh-cut products. Some coatings may not adhere to such surfaces, others may offer good adherence but may be poor barriers to moisture or not resist water vapor diffusion (Baldwin et al., 1995a,b). Lipid components confer important water barrier characteristics to some coatings, however, they may present a drawback, because they may give a waxy or gummy mouth feel to the product (Wong et al., 1994). On the other hand, hydrophilic polymers (such as carboxymethyl cellulose) do not work well in reducing water loss of coated products, due to their poor moisture barrier characteristics (Baldwin et al., 1996). Emulsion coatings containing mixed components seem to have better performance, such as coatings of casein and acetylated monoglyceride; when the pH is adequately adjusted, a tight matrix is formed, trapping the lipid molecules (Krochta et al., 1994). In addition, some lipid components (such as acetylated monoglyceride) are solid at room temperature, and without an emulsifier (such as calcium caseinate), they could not be used as a coating for fresh fruits and vegetables (Avena-Bustillos et al., 1997). In the application of some coatings, it is possible to induce the formation of cross-links between pectin molecules of the fresh-cut product surface and the coating (Wong et al., 1994). Interestingly, different food additives can be incorporated into coating formulations, such as coatings with antioxidants (Baldwin et al., 1995a). The efficiency of ascorbic acid in delaying enzymatic browning in cut apple and potato was improved when incorporated in an edible coating formulation in comparison to dipping. A carboxymethylcellulose-based coating did not control enzymatic browning of cut apples and potatoes, but when such a coating was combined

with additives (antioxidant, acidulant and preservative), browning control was superior than dipping the fresh-cut produce in solutions with the same additives (Baldwin et al., 1996). Examples of browning inhibition of apple slices have been described with different edible coatings, such as formulations containing casein and lipid (Avena-Bustillos and Krochta, 1993) or soybean protein (Kinzel, 1992).

Reducing Temperature

Temperature management during handling is essential in minimizing the damaging effects of mechanical injury because of the ability of low temperatures to reduce metabolic reactions. Temperature has a tremendous effect on respiration rates; moreover, it affects permeability of gases through the packaging films and also slows microbial growth. Fresh-cut products generally have higher respiration rates than the same intact produce—the respiration increase may vary from a few percent to over 100%. Moreover, the degree of respiration increase varies with temperature and commodity (Watada et al., 1996). Storage temperature is a critical parameter in achieving maximum shelf life of products. Refrigeration throughout the production chain to consumption is of fundamental importance in extending the shelf life of fresh-cut products. To ensure high-quality products, it is recommended that fresh-cut products be kept at temperatures just above freezing; nevertheless, temperature needs to be adequately chosen in order to avoid damage such as chilling injury in sensitive commodities. A common practice in the preparation of fresh-cut products is rinsing the peeled and/or cut product in cold water, which helps lower the temperature in addition to removing cellular exudates released during the peeling and/or cutting of produce. Dewatering of rinsed products is normally required to control decay. This is done commercially through centrifugation but can also be achieved with forced air.

Although emphasis is normally placed on the use of low temperatures, there are examples of benefits of some heat treatments on browning control. Heat shock treatment (45°C for 105 min) of whole apples later used for preparing slices resulted in product with less browning and firmer texture than product prepared from non-heated fruit (Kim et al., 1993). In conventional food processing, the most widely used methods for enzyme inactivation rely on heat application. Optimum PPO activity has been reported to vary with the source of the enzyme and reaction conditions (pH, substrate, etc.). PPO from several plant sources exhibits maximum activity in the temperature range of 20–35°C. Many factors affect PPO heat stability, among them are enzyme source, plant cultivar, molecular form (isozyme) and heat penetration into the tissue (Vámos-Vigyázó, 1981). PPO is not a very heat-stable enzyme; thermal inactivation occurs at temperatures higher than 40°C. Temperature stability of PPO depends on the source of the enzyme. Moreover, PPO thermostability is also influenced by cultivar, growing location and pH (Vámos-Vigyázó, 1981; Nicolas et al., 1994). Banana PPO is inactivated in 15 min at 80°C (Galeazzi and Sgarbieri, 1978), while green pea PPO required 29 min at 80°C or 2.5 min at 90°C and only 1 min at 95°C (Krotov et al., 1971). Low-temperature blanching may be effective in preventing or controlling enzymatic activity in fresh-cut products. Blanching (95°C for 3 min) of ready-to-use pear cubes under aseptic conditions resulted in complete inhibition of enzymatic browning with an acceptable texture reduction, as judged

by a trained panel (Pittia et al., 1999). Recently, heat shock treatment has been suggested as a new way to control browning in fresh-cut products. The mechanical injury caused by tissue wounding induces synthesis of enzymes, such as phenylalanine ammonia lyase (PAL), involved in phenolic metabolism, leading to accumulation of phenolic compounds, which in turn can be potential substrates for PPO. Within 24 hours of cutting, iceberg lettuce cut into 2×2 cm pieces showed a six- to 12-fold increase in PAL activity. A heat shock treatment on cut iceberg lettuce for 90 seconds at 45°C prevented such increase in PAL activity, which might offer a new alternative to control browning in fresh-cut products (Saltveit, 2000).

Applying Gamma Radiation

Application of gamma radiation to fruits and vegetables has been used for insect and disease disinfestation, as well as to retard ripening and sprouting. Irradiation applied to fresh-cut carrots stored in microporous plastic bags resulted in limited respiration increase due to wounding and reduced ethylene production. Treatment was considered to increase the shelf life of the product (Chervin et al., 1992). Nevertheless, the application of irradiation may bring about undesirable biochemical changes. In fact, enzymatic browning may be aggravated by irradiation treatments, which may alter the permeability of cell compartments favoring contact between PPO and its substrates (Mayer and Harel, 1991). Apples and pears irradiated as a quarantine treatment showed decreased firmness, which was cultivar dependent, and changes in internal color of 'Gala' and 'Granny Smith' apples (Drake et al., 1999). Endive samples that were irradiated revealed longitudinal internal pink-brown lines, which progressed to the entire vegetable piece becoming pink-brown. In contrast, the cut control discolored only on cut surfaces (Hanotel et al., 1995). Such alterations may be an indication of cell damage, release of PPO and browning in the irradiated endive.

Use of Other Nonthermal Technologies

High-pressure processing has applications in food preservation due to its potential effect on microorganisms and enzymes. Inactivation of deleterious enzymes has been achieved through application of high-pressure technology (Hendrickx et al., 1998; Seyderhelm et al., 1996; Weemaes et al., 1999). An important advantage of this new technology is that high-pressure treatments at low temperatures have either no effect or a minimal effect on flavor and nutritional value of foods. However, high-pressure processing may create new textures or tastes (Messens et al., 1997), and cause discoloration of some commodities (Asaka and Hayashi, 1991).

While bacterial spores are highly resistant to pressure treatment, and over 1200 MPa is required for their inactivation, yeasts, molds and vegetative cells are pressure sensitive and can be inactivated by milder treatments at ~300–600 MPa. When aiming at enzyme inactivation, pressure requirements vary depending on the enzyme; some enzymes are resistant to 1000 MPa, others can be inactivated by a few hundred MPa at room temperature. High pressure has been considered an alternative for irreversible inactivation of PPO (Hendrickx et al., 1998). It has been observed that the application of low pressure results in pressure-induced membrane damage with consequent decompartmentalization and enzyme activation. In fact, pear PPO (cell-free extracts) was activated after pressure treatment at 400 MPa for 10 min at 25°C (Asaka and

Hayashi, 1991). PPO activation was also described in low-pressure treatments of crude carrot and apple extracts (Anese et al., 1995).

PPO sensitivity to pressure varies with the enzyme source—while apricot PPO has been inactivated at ~100 MPa, and strawberry PPO at 400 MPa, potato and mushroom PPO required much higher pressures (~800–900 MPa). In addition, PPO inactivation by pressure is affected by pH (Anese et al., 1995).

Many studies have been carried out in model systems, cell-free or crude extracts, not real foods. Experiments carried out on whole foods revealed that high-pressure treatment caused browning of mushrooms, apples and potatoes (Gomes and Ledward, 1996). It is known that food ingredients have a protective effect on enzyme pressure stability, and the efficiency of the pressure treatment depends on pH, temperature and treatment. When comparing the barostability of different food enzymes, PPO was second, only after peroxidase, as the most tolerant to pressure treatment duration (Seyderhelm et al., 1996). Due to the poor effectiveness of lower pressure treatments on PPO activity, it has been suggested that its inhibition would require a combination of pressure treatments with one or more additional methods, such as blanching, modified atmospheres and/or refrigeration (Anese et al., 1995). Complete inactivation of enzymes is not expected with the application of hydrostatic pressures compatible with maintenance of food tissue integrity (Whitaker, 1996).

The use of high-intensity pulsed electric fields is a new technology that has been suggested to inactivate microorganisms and enzymes with minimal resultant temperature increase (Qin et al., 1996). Application of high-intensity electric field pulses on a culture of potato cells increased the release of PPO into the medium, with both increased intensity and duration of treatment (Knorr and Angersbach, 1998). Preliminary results using model systems (enzyme solutions) resulted in large variations among enzymes. Although a reduction of 88% in pectinesterase activity has been reported in treated orange juice (Hye et al., 2000), a moderate activity reduction of 30–40% was described for PPO and peroxidase treated in buffer solutions (Ho et al., 1997). Although this technology seems to offer potential applications to liquid foods, it still seems premature to recommend its use in fresh-cut products.

OTHER COLOR CHANGES

White Blush in Carrots

The bright orange color of fresh carrots can disappear in stored fresh-cut products, particularly when abrasion peeling is used. Carrots may develop “white blush,” also known as “white bloom,” a discoloration defect that results in the formation of a white layer of material on the surface of peeled carrots, giving a poor appearance to the product. Upon peeling, the protective superficial layer (epidermis) of carrots is removed, generally by abrasion, leaving cell debris and an irregular surface, which while moist, presents the natural orange color of carrots. Once the carrots are exposed to air, they easily dehydrate, and the dried cell debris acquires a whitish color, forming a white layer on the carrot surface. The disruption of surface tissues followed by dehydration in white blush formation was confirmed by scanning electron microscopy, when comparing carrots peeled with a knife and a razor-sharp blade. Knife-peeled

carrot surfaces appeared severely damaged, compressed, sloughed and separated from underlying tissue, and therefore, prone to dehydration. Razor-peeled carrot surfaces were cleaner, and apparently, only a thin layer of cells had been removed, resulting in a product that upon drying did not acquire the whitish appearance (Tatsumi et al., 1991). At this stage, the quality defect can be reversed by dipping the carrots in water and allowing for rehydration (Cisneros-Zevallos et al., 1995).

It has been suggested that with time, phenolic metabolism may be activated, inducing increases in lignin, phenolic compounds and phenylalanine ammonia lyase activity, and irreversible color change takes place (Cisneros-Zevallos et al., 1995; Howard and Griffin, 1993). A positive test for lignin was described in the white abraded material. The severity of the lignification will depend on the harshness of the peeling process (coarse sandpaper > fine sandpaper > stainless steel pad). Although hand peeling of carrots with a razor blade has been reported to result in no development of "white blush," even after prolonged storage at 1°C (Bolin and Huxsoll, 1991), the extent of injury of fresh-cut carrot discs depends on the blade type and sharpness, and storage conditions. Carrot discs prepared by hand slicing with a razor blade resulted in better quality products as compared to mechanical slicing. Great damage was inflicted by a blunt machine blade; after 10 days of storage at 8°C, carrot discs sliced with a blunt machine blade revealed thickened cell walls and the presence of lignin (Barry-Ryan et al., 2000). As the lignification process is enzyme mediated, some dipping treatments directed to inactivate the responsible enzymes have been tested. A successful result was obtained with a treatment combining heat inactivation and an acidic environment. Carrots peeled with coarse sandpaper and dipped for 20–30 sec in a 2% citric acid solution at 70°C did not develop the defect for at least five weeks in cold storage; product taste was not affected by the treatment (Bolin and Huxsoll, 1991). Edible films have also been shown to protect carrots from this quality defect (Sargent et al., 1994). Sensory results showed preference for carrots coated with an edible cellulose-based coating due to a fresh appearance (Howard and Dewi, 1995), because consumers perceive white blush carrots as not fresh or aged. Losses of carotenes have been described in fresh-cut carrots. With the application of an edible coating, a 50% retention of β -carotene was obtained after 28 days of storage, compared to 33% retention in the control (Li and Barth, 1998). Edible coating emulsions containing caseinate-stearic acid were effective in reducing the white blush defect of carrots (Avena-Bustillos et al., 1994).

Yellowing or Degreening

Reduction of green pigmentation and, therefore, the predominance of yellow pigments is a normal process in ripening or senescence of many fruits and vegetables, and such changes can be accelerated by ethylene. In fresh-cut products the stress imparted by wounding results in increased respiration, ethylene production and other alterations. In fact, degreening is also observed during storage of leafy and other green fresh-cut products. Shredded iceberg lettuce darkens during storage, particularly at high temperatures. Simultaneously, loss of green pigmentation was observed (Bolin et al., 1977; Bolin and Huxsoll, 1991). Studying the susceptibility of fresh-cut baby and romaine lettuces to browning, it was observed that samples of photosynthetic tissue

became lighter during storage. In fact, while there is mid-rib discoloration, the photosynthetic tissues also develop browning and loss of green pigments (Castañer et al., 1999). In a study on coleslaw color, over a period of cold storage, changes were from green to a lighter white color, suggesting chlorophyll degradation resulting in colorless compounds (Heaton et al., 1996). The reactions involved in the loss of chlorophyll in green fresh-cut products are still unclear. During the preparation steps of fresh-cut products, acids and enzymes are released, and both could be involved in the loss of green pigmentation.

The visual quality of broccoli is lost when florets turn yellow. The retention of green color has been attained with the use of modified atmosphere packaging and storage at 10°C (Barth et al., 1993). These authors found that within 48 hours, the carbon dioxide concentration inside broccoli packages reached equilibrium at ~8% and oxygen content was 10%, causing a reduction of respiration rate. Modified atmosphere packaging contributed to significantly higher retention of green color in broccoli, as indicated by the total chlorophyll levels and color determination (hue angle). In contrast, in less than 72 hours, nonpackaged samples lost about 20% of initial chlorophyll content. Although there was ethylene accumulation during storage, it is suggested that the elevated carbon dioxide atmosphere counteracted ethylene effects, thus preventing chlorophyll degradation. Furthermore, the packaging of broccoli spears resulted in improved retention of vitamin C.

In a study of texture improvement, calcium-chloride-treated fresh-cut green pepper and nontreated control stored at 10°C had significant losses in green color after four days of storage. Calcium-treated samples stored at 5°C were significantly better in all sensory attributes by day four, and their superiority was maintained throughout the eight-day storage period (Barrett, unpublished).

PREVENTION OF TEXTURE LOSS IN FRESH-CUT PRODUCTS

Appearance of a food product plays an important role in consumer evaluation. It has been estimated that 95% of American consumers take appearance into account in their purchases of fruits and vegetables (Shewfelt, 1994). As mentioned earlier, color has a great impact on appearance consideration, but quality loss is also observed with changes in texture (Figure 9.1), another important quality criteria for many fruit and vegetable products.

While genetic background is the major contributor to the texture of a plant food, other factors, such as morphology, cell wall-middle lamella structure, cell turgor, water content and biochemical components, all affect texture (Harker et al., 1997). In addition, texture is also affected by growing conditions, including environmental factors and production practices (Sams, 1999). After harvesting, it is important to store fruits and vegetables at the appropriate temperature and relative humidity to preserve their quality. Storage temperature has a major effect on water, weight loss and metabolic activity.

In general, perishability of intact fruits and vegetables correlates well with respiration rates—produce with a high respiration rate tends to be more perishable. In fresh-cut products, as a result of wounding, respiration is elevated compared to the

intact produce. Moreover, the extent of wounding also affects the shelf life of products. Hand tearing of lettuce has been shown to be less damaging than slicing with rotating knives, and reduction of lettuce piece size shortens shelf life (Bolin and Huxsoll, 1991).

FRUIT AND VEGETABLE TISSUE FIRING

During fruit ripening, one of the most notable changes is softening, which is related to biochemical alterations at the cell wall, middle lamella and membrane levels. Although pectic enzymes, polygalacturonase and pectin methylesterase have been attributed to a significant role in the softening process, the precise mechanism is still unclear.

Calcium and/or Heat Treatments

It is well known that calcium is involved in maintaining the textural quality of produce. Calcium ions form cross-links or bridges between free carboxyl groups of the pectin chains, resulting in strengthening of the cell wall. A common treatment used to improve tissue firmness is to dip fruit or vegetable pieces in calcium solutions, as described for strawberries (Main et al., 1986), pears (Rosen and Kader, 1989) and shredded carrots (Izumi and Watada, 1994), among others. In contrast, calcium treatment was not effective in carrot slices and sticks, a fact attributed to insufficient calcium absorption by the tissue, because the levels of calcium were two and three times higher in shredded carrots than in sticks and slices, respectively. In addition, increasing the concentration of CaCl_2 in the dip solution (0.5% or 1%) brought an increase in the tissue calcium content of treated samples, without a subsequent correlation with product texture (Izumi and Watada, 1994).

A combined treatment associating low-temperature blanching to activate the enzyme pectinesterase (PE) prior to the calcium dip is helpful in preserving fruit texture. PE brings about the de-esterification of pectin, thus increasing the number of calcium-binding sites. To such mechanism has been attributed the firming effect observed in apple slices kept at 38°C for six days immediately after harvest, and sliced and dipped in calcium solution after six months of cold storage (Lidster et al., 1979). In fresh-cut melon cylinders dipped in calcium chloride solutions at different temperatures (Luna-Guzmán et al., 1999), texture was firmer in samples treated at 60°C (77% improvement in firmness) than at 40°C (58% improvement) and 20°C (45% improvement).

Frequently, calcium chloride has been used as a firming agent, however, it may confer undesirable bitterness to the product. Fresh-cut cantaloupe cylinders dipped in calcium lactate solutions resulted in a textural improvement similar to calcium-chloride-treated fruit cylinders. Sensory evaluation indicated that results were better, e.g., less bitterness and a more detectable melon flavor was perceived. Fresh-cut cantaloupe cylinders treated by a combination of heat treatment (60°C) and calcium lactate dip were not significantly different either in bitterness or firmness in relation to fruit treated at 25°C (Luna-Guzmán and Barrett, 2000).

Heat treatment alone has been shown to have the potential to benefit product texture. In a comparison of 11 apple cultivars, heat treatment of whole fruit resulted in firmer products when compared with nonheated fruit. The best firmness improvement was obtained with 'Golden Delicious' and 'Delicious' apples (Kim et al., 1993). Heat treatment of whole apples improved apple slice firmness, but the storage temperature of whole fruit after heating had a significant effect on product firmness, except for fruit of the cultivar 'McIntosh.' Slices prepared from previously heat-treated whole apples stored at 2°C were firmer than products from fruit kept at 10, 18 and 25°C for seven days. Slices prepared from heat-treated apples showed increased firmness during storage of up to seven days for 'Golden Delicious' (firmness 34% higher than on day zero of storage) and up to 14 days for 'Delicious' apple (48% higher firmness than at the beginning of storage). With longer storage times, there was a decrease in firmness for both cultivars (Kim et al., 1994).

Gamma-irradiated apple slices showed firmness reduction dependent on the irradiation dose applied. Significant softening was observed at doses above 0.34 kGy. Although the total pectin content was unaltered, there was an increase in the content of water-soluble pectin in the irradiated slices. Calcium treatment of thick ($\frac{1}{8}$ of an apple) fruit wedges prior to irradiation led to a small firmness improvement in comparison to the softening brought about by radiation at 1 kGy. The inefficiency of calcium in preventing irradiation-induced softening could be due to the limited penetration of calcium into the treated cut apples. When thin (3–4 mm) apple rings were treated with calcium chloride (2 to 4%) and then irradiated, softening was reduced proportionally to the calcium levels, although firmness was still lower than non-irradiated controls (Gunes et al., 2001).

Use of Modified Atmosphere Packaging

Controlled atmospheres retard senescence, lower respiration rates and slow the rate of tissue softening (Kader, 1992). Texture loss has been reported to decrease in controlled atmosphere packaging of fruit. Strawberry slices kept under controlled atmosphere for a week had comparable firmness to whole and freshly sliced fruit (Rosen and Kader, 1989). The authors suggest that the effect of controlled atmosphere on firmness appeared to be cultivar dependent.

Storage stability of halved fruits was evaluated in a combination treatment that included chemical dips to prevent browning and retard texture loss, complemented by different storage conditions. Table 9.7 presents the texture results for peach halves stored in sealed packages where oxygen was being consumed with accumulation of carbon dioxide and sealed packages containing an oxygen scavenger; the latter treatment gave better results. Interestingly, a peach cultivar with soft texture, 'Suncrest,' even showed an increase (+0.5 N/wk) in fruit firmness during the first few weeks of storage with oxygen scavengers. Similar storage conditions were not successful in the treatment of pears. The authors suggest as an optimum treatment for halved peaches and apricots a combination of a dip in 2% calcium chloride and 1% zinc chloride, followed by packaging with an oxygen scavenger and storage at 0–2°C (Bolin and Huxsoll, 1989).

TABLE 9.7

Texture Loss in Fresh-cut Freestone Peach Halves Stored for Seven Weeks at 2°C

Peach Variety	Initial Texture	Modified Atmosphere		With O ₂ Scavenger	
	(N)	Texture (N)	Rate of Loss (N/Week)	Texture (N)	Rate of Loss (N/Week)
'Fairmont'	>21	8.0	1.7	17.0	0.5
'Suncrest'	7	4.0	0.5	10.5	+0.5
'Flamecrest'	21	3.9	2.4	7.6	1.9

Note: Texture measurement units in Newtons (N).

Source: From Bolin, H.R. and Huxsoll, C.C. (1989). *J. Food Process. Preserv.* 13:281–292. With permission.

WATER LOSS PREVENTION

After harvest, produce must utilize internal moisture solely; water lost through transpiration cannot be replaced. Although plant tissues are mainly composed of water, even small changes in water content may have a large impact on produce quality, causing losses that may occur in a few hours under dry and warm conditions. Water losses of 3 and 5% in spinach and apple, respectively, render these commodities unmarketable (Sams, 1999). Crispness of fresh produce is related to turgor pressure, whose loss may also contribute to softening. Leafy vegetables are particularly susceptible to desiccation because of their large surface-to-volume ratios; moreover, loose leaves, such as spinach, are more prone to desiccation than a compact head, such as a whole lettuce head (Salunkhe and Desai, 1984). As a consequence of water loss, appearance changes such as wilting and reduced crispness may occur.

Fresh-cut products tend to be more vulnerable to water losses because they are no longer intact after peeling and cutting or shredding, slicing, etc. Peel or skin is a very important barrier to loss of turgor and desiccation. Many commodities have a protective waxy coating that is highly resistant to water loss. Evidently, peel removal renders commodities more perishable. The mechanical injury brought on by cutting and the method used, directly expose the internal tissues to the atmosphere, promoting desiccation. The shredding or slicing operations result in increased surface area, an additional problem. Moreover, mechanical injury brings about physiological responses, such as respiration increase and, potentially, ethylene production, responses that shorten the life of a commodity. When rinsing of products is done after cutting, this is frequently followed by centrifugation. If accelerated centrifugation speed or long centrifugation times are applied, increased desiccation can result, as reported for cut lettuce (Bolin and Huxsoll, 1989). Appropriate handling techniques including temperature and relative humidity control can help minimize the rate of water loss. Reduction of water loss can be achieved basically by decreasing the capacity of the surrounding air to hold water, which can be obtained by lowering the temperature and/or increasing the relative humidity. To reduce the rate of water loss in cool storage, it is also important to restrict the air movement around the commodities

(Wills et al., 1998). The primary parameter affecting celery quality is water loss, small, reductions in moisture (2.5–5%) may lead to flaccidity, shriveling, wrinkling and pithiness. Significant increases in moisture retention by celery sticks were described with the application of a caseinate-acetylated monoglyceride coating (Avena-Bustillos et al., 1997). Additionally, it is important to point out that appropriate packaging is of enormous importance in preserving fresh-cut products.

APPENDIX: EVALUATION OF ENZYMATIC BROWNING

Different authors have used somewhat different methods to measure the intensity of enzymatic browning, and some tried to establish correlations of browning with PPO activity and/or phenolic substrates content. Frequently, browning evaluation is based on reflectance measurements on exposed surfaces, such as cut fruits and vegetables or produce homogenates. Laboratory assays commonly involve extraction of browning products and measurement of absorbance at particular wavelengths. Nevertheless, as not all PPO products are soluble, some authors have developed methods that also evaluate the insoluble colored products.

Following, we present a brief description of some of the methods that were used by authors whose results are displayed in tables included in this chapter. A more complete approach for assessing susceptibility to browning is also included, and at last, we present a visual assay that can be used in assessing produce varieties tendency to discoloration.

REFLECTANCE MEASUREMENTS

Table 9.1 presents results in DL^* (decrease in L^* indicates darkening of the samples) (Radi et al., 1997).

Homogenates (purees) of previously dried apricots were poured in small petri dishes, and reflectance measurements (L^* lightness; a^* green/red; b^* blue/yellow chromaticity) were taken with a Minolta CR300 chromameter. The authors determined the difference between measurements taken from oxidized and nonoxidized (addition of enzyme inhibitors) samples, and expressed the results in DL^* , Da^* and Db^* .

BROWNING INDEX

These results are expressed in Tables 9.2 and 9.6 (Mattila et al., 1993). Evaluation was based on sensory evaluation by a trained panel.

Twenty potato slices (5 mm thick) were left to stand at 23°C for 30, 60 and 120 min, and discoloration was evaluated by comparison with slices that had just been cut. Results were scored by browning grades from “0” (no color change) to “3” (strong change). To each browning grade a coefficient was attributed as follows: “0” browning grade $\Rightarrow$ coefficient 0; “1” browning grade $\Rightarrow$ 1; “2” browning grade $\Rightarrow$ 5; and “3” browning grade $\Rightarrow$ 10.

As an example of a browning index calculation, consider that from the 20 potato slices evaluated, 10 received a grade “0”, five a grade 1, three a grade 2 and two a grade 3. The final Browning Index would then be as follows:

$$(10 \times 0) + (5 \times 1) + (3 \times 5) + (2 \times 10) = 40$$

LOSS OF REFLECTANCE

These data are presented in Table 9.5 (Ponting et al., 1972). Reflectance measurements were made by reading total reflectance from apple slices rotated to three positions (~120° apart) and then averaging these readings to obtain the final reflectance value. Such a result was compared to readings from fresh-cut apple slices to calculate percent reflectance loss. The authors found that for apple slices, loss of reflectance correlated better with the subjective evaluation of color than the “a” or “b” values.

ESTIMATE OF APPLE SUSCEPTIBILITY TO BROWNING

Amiot et al. (1992) measured the following:

1. Absorbance at 400 nm of an apple extract containing soluble pigment formed during the browning reaction. [For details on the methods used, refer to Amiot et al. (1992).]
2. Lightness (L^*) of the pellets obtained after centrifugation during the preparation of the soluble pigments extract. (L^* results were related to the insoluble brown pigments.)

The authors suggested that the normalized sum of A_{400} and L^* be used to express the degree of browning.

Visual observation of browning is poorly correlated with measurements of absorbance at only one wavelength (Nicolas et al., 1993). Depending on the pigments formed during browning, there may be a wide variation (360–500 nm) of maximum optical absorption (Amiot et al., 1997). For a detailed discussion on measurements of browning, refer to Nicolas et al. (1993) and Macheix et al. (1990).

VISUAL EVALUATION OF BROWNING

For a visual evaluation of browning potential, which can be helpful in selecting cultivars with lower browning tendency, a quick assay was described by Kader and Chordas (1984).

1. *PPO activity evaluation*: to slices (3–4 cm diameter) of fruit or vegetable, add one drop of a freshly prepared 0.1 M solution of catechol in 0.1 M citric acid-phosphate buffer pH 6.2 (PPO substrate). Let rest for 6 min and then compare the samples and score the browning intensity. The discoloration is rated on a progressive 1 to 5 scale.

According to the authors, the natural PPO substrate content does not interfere with the test within the 6 min duration of the assay.

2. *PPO substrates evaluation*: to slices (3–4 cm diameter) of fruit or vegetable, add one drop of each of the following solutions in succession: a 10% sodium nitrite, 20% urea and 10% acetic acid. Let rest for 4 min and then apply two drops of 8% sodium hydroxide solution.

This test is based on a color reaction developed by endogenous phenolic compounds with the reactives added to the fruit or vegetable slice. The intensity of the deep cherry-red color developed during the reaction depends on the amount of phenolic compounds present in the tissue. The result is rated on a 1 to 5 scale, from the less colored to the most intensely colored sample, according to the chart presented in the cited paper.

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10 Application of Packaging and Modified Atmosphere to Fresh-cut Fruits and Vegetables

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INTRODUCTION

As market demand for fresh and minimally processed fruits increases, quality and shelf life issues increase in importance. A key requirement is to ensure that product is able to reach the consumer with minimal quality deterioration and safety risks. This becomes problematic when we recognize that fresh-cut fruit (FCF) products constitute a delicate, dynamic system that is prone to many forms of deterioration. In order to investigate how packaging impacts deterioration, we must first understand its causes.

The inherent quality deterioration of FCF is largely due to cutting, because it initiates the physiological and biochemical changes at a faster rate than in intact raw fruits (Kim et al., 1993a). Generally, quality deterioration (color, flavor and texture) is attributed to the combined effect of endogenous enzymes, enhanced respiration, microbial growth (Gil et al., 1998; Kim et al., 1993b), physical abuse and environmental factors (Chau and Talasila, 1994). The surrounding environmental factors such as temperature, humidity, atmospheric composition and ethylene concentration directly influence the deterioration process. Mechanical bruises and damage caused by harvesting, handling, storage and transportation are also detrimental to the FCF shelf life (Chau and Talasila, 1994).

There are no simple answers and no single treatment is known to limit overall quality deterioration. However, there are several strategies that are being implemented in order to reduce the rate of deterioration for FCF. These include, but are not limited to starting with high-quality raw produce, implementing sanitation practices, controlling temperature, lowering respiration rate, lowering ethylene production, and preventing mechanical abuse. Packaging technology is the common denominator that allows us to implement these strategies and, thus, is key to quality preservation.

MODIFIED ATMOSPHERE PACKAGING

INTRODUCTION

A promising packaging technology for addressing quality deterioration issues of FCF is modified atmosphere packaging (MAP). This technology is targeted at reducing the respiration rate of fresh produce and slowing senescence. The nineteenth century French chemist Berard is reported to be the first to study the effect of modified atmosphere on the shelf life of horticultural products. The result of his work showed that fruits do not ripen in anaerobic conditions. In the 1920s, Kidd and West studied how O₂ and CO₂ affect the shelf life of apples, pears and berries. Their research led to the important finding that low O₂ and moderately high CO₂ storage conditions could extend the shelf life of fruits.

“Modified atmosphere packaging” describes altering the gases surrounding a commodity producing an atmospheric composition different from that of air. The purpose of modified atmosphere is not necessarily to create a fixed gas composition throughout

the shelf life, as in controlled atmosphere storage. MAP creates a predetermined gas composition, which may change over time. Variables that are related to produce physiology (respiration rate, etc.), physical factors of the environment (temperature, RH, etc.) and barrier properties of the packaging material determine the specific gas composition at equilibrium. MAP has been commercially applied for a variety of whole fresh produce and minimally processed vegetables but has not achieved comparable success for FCF for reasons that will be discussed below.

Generally, modified atmospheres can be achieved passively or actively. A passive MAP occurs when fresh produce is hermetically sealed in a semipermeable container. The respiration process of the produce and, to a certain extent, the microbial growth, combined with container permeability alters the gas composition. Because of the respiration process, the produce consumes the surrounding O_2 and produces CO_2 , and therefore, the O_2 level is reduced, while the CO_2 level is increased. After a period of adjustment between respiration rate and permeation rate, a steady state is established inside the package. At this stage, the amount of O_2 consumed and CO_2 produced inside the package equals the O_2 and CO_2 amount permeating through the film. Thus, passive atmosphere modification is a complex process with many interactions among different components and variables. If the container is impermeable to gases, then theoretically, O_2 concentrations may be lowered (from atmospheric 21%) to near 0%, while CO_2 (initially 0.03% in the container) concentrations can reach 20% or higher.

On the other hand, an active MAP can be achieved by flushing out the air within the package with a precise mixture of gases to create an initial atmosphere. Usually, nitrogen serves as a filler gas to provide a precise concentration of other gases in the package, as well as to prevent the package from collapsing.

A fundamental knowledge of gas permeability characteristics of the film as well as of the produce is essential for the development of both types of MAP systems. A successful MAP design requires the determination of respiration rate and respiration quotient (R_{CO_2}/R_{O_2}) of the produce, an appropriate polymeric film with a suitable barrier properties, appropriate film area, appropriate produce weight, and appropriate headspace volume.

MAP technology is an interactive system that permits interplay between the physiological parameters of the commodity and the film characteristics. In such a system, there are four main processes occurring simultaneously: respiration of the produce, transpiration of the produce, permeation of gases through the packaging material and heat transfer (Chau and Talasila, 1994). Respiration changes according to the following factors: temperature; produce maturity; and CO_2 , O_2 and ethylene levels within the package. The temperature of the produce is also altered due to the heat generated by the respiration process. Transpiration depends on the produce surface temperature and the temperature and relative humidity (RH) of the surroundings. Permeability properties of the polymeric film depend on the chemical makeup of the material, ambient temperature, film thickness, permeating gas and the difference in gas concentrations across the film. Understanding the extent of all of these variables is necessary to optimize the effect of MAP on extending the shelf life of a chosen FCF commodity.

Current Status

Advances in the chemistry and engineering of polymers contribute in the development of materials for MAP applications. By far, oriented polypropylene (OPP) is the most used material for MAP, and the majority of MAP of fresh produce is marketed in pouches. However, MAP systems have been utilizing perforated, thin, low-density polyethylene (LDPE) for bagged produce for a long time, as well as monolayer polyvinyl chloride (PVC) for tray overwrapped produce. Coextrusion technology has improved material design and properties to meet some of the MAP system needs. Blends of linear low- and medium-density PE with ethylene-vinyl acetate (EVA) copolymer are acceptable candidates for MAP application, because PE resins provide excellent shrink and are good moisture barriers, while the EVA copolymer provides sealability and a higher O₂ permeability than PE resin (Robertson, 1993). Nonetheless, recent research focuses on the effect of perforation on MAP application to fresh produce. Some studies suggest the application of perforation and microperforated films to prevent anaerobic conditions (Hirata et al., 1996; Lee et al., 1996; Emond et al., 1991; Hobson and Burton, 1989), while others suggest ceramic-filled plastic films (Wang et al., 1998; Lee et al., 1992). Because of the limited range of gas permeability of plastic films and permselectivity, which do not satisfy and match the wider ranges of produce respiration, all of these materials, and others, have had only limited success in MAP, especially when the produce packaged is FCF.

Shortcomings of MAP

Developments in materials science and engineering have not fully met the packaging requirements of fresh produce in general, and FCF in particular. For example, commonly available polymeric films have a wide range of permeability coefficients for each individual gas that can be in the range of 1000-fold difference. However, the range of respiration rates of fresh fruit is in the range of only 10-fold. Therefore, it is possible for the films to satisfy the need for either O₂ or CO₂ alone but not both of them at the same time. This presents one of the major disadvantages of polymeric films: they permeate CO₂ at higher rates than O₂. The ratio of CO₂-to-O₂ permeability coefficient is 4–6:1 which can contribute to shortening the shelf life of the products by not maintaining the required gas atmosphere inside the package, and thus, it defeats the purpose of the modified atmosphere created in the packages. Engineered properties of most recently developed polymeric materials do not address the CO₂-to-O₂ permeability ratio, which is a crucial component for successful MAP systems, and especially so for MAP of FCF. To our knowledge, no successful solution of this problem has been introduced. The ultimate solution is to develop a systematic approach that can be followed during the synthesis of polymeric materials that can produce a wide range of permeabilities and CO₂:O₂ permeability ratios. This would lead to successful materials for specific MAP-FCF products.

MAP EFFECT ON MICROORGANISMS

Effects of gas composition created in MAP systems on microflora has been reviewed and studied intensively by many researchers (Varoquaux and Wiley, 1994; Brackett, 1994). Microbial growth is a leading mechanism of deterioration and a primary

safety factor for fresh produce products and is affected by the gas composition created in a MAP system. Under inappropriate gas composition, spoilage is characterized by undesirable sensory changes in color, texture, flavor or odor and the potential for growth of pathogenic microorganisms. MAP, however, can delay and arrest microbial spoilage but may not necessarily improve the produce quality. Parameters affecting microbial growth include the intrinsic properties of the food (nutrient availability, pH and a_w) and external factors imposed by the surrounding environment, including the gaseous composition of the surrounding environment and temperature (Parry, 1993).

O₂ Effects

When a sufficient O₂ level is maintained in the package to prevent anaerobic respiration in the produce, aerobic pathogens may grow if neither inhibitors nor competitors are present. The O₂ level in the package is affected by processing conditions, produce temperature, packaging permeability to O₂, O₂ consumption rates due to the microbial growth and produce respiration. Figure 10.1 shows how the internal O₂ pressure affects the O₂ permeability of a polymeric film and respiration rate. O₂ effects on microbial growth depend on microorganism type. In the absence of O₂, growth of Gram-negative, aerobic spoilage organisms such as *Pseudomonas* is restricted, while growth of Gram-positive, microaerophilic species such as *Lactobacillus* or *Brothothrix* flourishes. Anaerobic conditions have little effect on facultative anaerobes (Gram-positive or Gram-negative) (Labuza et al., 1992).

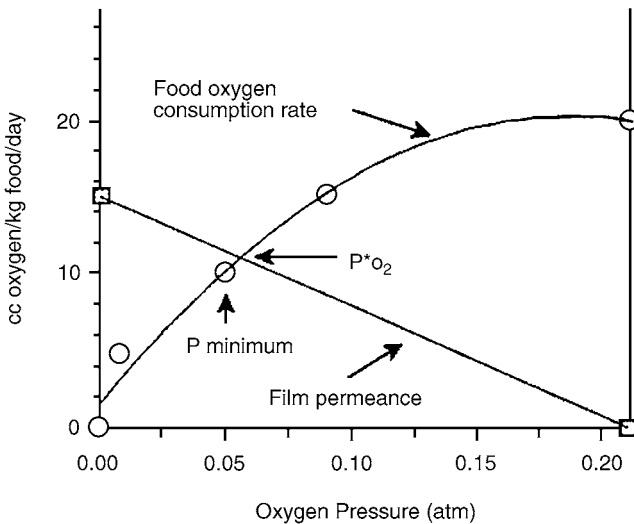


FIGURE 10.1 Oxygen consumption and permeation rates as a function of internal package oxygen pressure. $P^*_{O_2}$ represents the critical oxygen level the package will attain. (Reproduced with permission from Labuza, T. et al., 1992.)

CO₂ Effects

CO₂ is the most important gas in MAP applications. The inhibitory effect of CO₂ on microorganisms' growth is a complex phenomenon and is not completely understood. Microorganisms found under CO₂ atmosphere are different from those found in air. As temperature increases (for example, under abuse), CO₂ protection against microbial growth decreases. CO₂ in excess of 5% v/v inhibits growth of many food spoilage bacteria, especially psychotropic species, which grow on wide range of refrigerated foods (Hendricks and Hotchkiss, 1997). Gram-negative bacteria are generally more sensitive to CO₂ than are Gram-positive bacteria. Most mold species require O₂ and are sensitive to high levels of CO₂. Many yeasts grow anaerobically and are relatively resistant to CO₂. The extent of CO₂ activity depends on the type, number and age of microorganisms, as well as CO₂ concentration, *a_w* and pH of the product, and storage temperature. However, *C. botulinum* and *C. perfringen* are not greatly affected by the presence of CO₂ and are found to grow if MAP provides anaerobic conditions. Redox potential is not often considered in MAP, mainly because it is difficult to measure. Redox potential effects on microorganisms are mainly due to the presence or absence of O₂ and/or CO₂ (Hanlin et al., 1995).

MAP EFFECTS ON RESPIRATION

Fresh fruits deteriorate as a consequence of respiration (Zagory, 1995). In the absence of O₂, anaerobic respiration occurs and generates off-flavors, off-odors and metabolic tissue damage, and eventually, the tissue dies from substantial O₂ deprivation. When the partial pressure of O₂ drops around 10 KPa, anaerobic respiration occurs to produce CO₂ and ethanol. Reduced metabolites associated with offensive odors are produced by reoxidation of reduced pyridine nucleotides, NADH and NADPH (Flodin et al., 1999). Aerobic metabolism, on the other hand, results in undesirable textural and flavor changes due to the consumption of sugar, starch or other energy storage products of the fruit tissues. Also, one of the respiration by-products is water vapor, which upon condensation in the package, becomes free water promoting the growth of spoilage and pathogenic microorganisms. Another by-product of respiration is heat, which may reduce the effectiveness of all the measures taken for temperature control during processing, distribution, marketing, etc. So, the higher the respiration rate, the faster the release of energy required to drive the metabolic processes, and therefore, the shorter the shelf life.

Successful MAP balances reduction in respiration against anaerobic metabolism. The O₂ consumption rate is balanced with the O₂ permeability of the packaging film, while maintaining the steady state O₂ at high enough levels to prevent anaerobic respiration and at low enough levels to inhibit the respiration rate of the produce. However, in MAP of FCF, the consequences resulting from processing must also be taken into account. Because of wound response, respiration rate of apple slices, for instance, is considerably higher than that of whole apples (Gunes et al., 2001). When the respiration rates of different cultivars of apples were studied, cut apples always showed higher respiration rates than intact apples (Kim et al., 1993b). This increased respiration rate of FCF imposes many challenges for the MAP systems and packaging materials.

PERMEABILITY

BACKGROUND

Nonporous polymeric films allow gases and vapors to pass through them as a function of partial pressure differences of permeants across the films. This property of films is important, because it determines the atmosphere composition inside the container with MAP. Permeation is considered to be a solution-diffusion process. During the permeation process, a permeant goes through three steps in the following order: condensation and mixing (solution) of the permeant in the surface of a film, migration (diffusion) to the opposite surface of the film under a concentration gradient and evaporation from the surface into the ambient surrounding (Rogers, 1985).

The permeability coefficient (P) is the product of a solubility coefficient (S), the thermodynamic parameter, and diffusion parameter (D), the kinetic parameter. Therefore,

$$P = DS \quad (1)$$

The solubility coefficient indicates how much gas a polymeric film can take up, and it is measured by the concentration (c) of the sorbed gas per unit volume of the film. The polymer-permeant interactions, inherent condensibility of the permeant and the amount of free volume in a glassy polymer determine the solubility coefficient (Kim et al., 1988). The diffusion coefficient (D) accounts for the mobility of the permeant molecules in the film. In the macroscopic picture, the sorbed gas molecules move under the force of the chemical potential gradient of the permeating gas in the membrane ($\partial\mu/\partial x$). At position x within the film matrix ($0 \leq x \leq l$, where l = thickness of the membrane), the flux density of the permeant (J) is expressed by Fick's law according to the following: $J_x = -D (\partial C/\partial x)$ where D = constant.

Fick's Law

According to Fick's law, gas diffuses, under steady state, through polymeric materials at a constant rate as long as a constant pressure difference is applied across the material. The amount of the permeant moving through the film of unit area in a unit time, is diffusive flux or transport rate J :

$$J = Q/A t \quad (2)$$

where (Q) is the total permeant amount passing through area (A) during time (t). However, the concentration gradient is proportionally related to the (J) in Fick's law as follows:

$$J = -D \frac{\partial c}{\partial x} \quad (3)$$

where (D) is diffusion coefficient, c is the concentration of the permeant and $\frac{\partial c}{\partial x}$ is the concentration gradient of the permeant across a given thickness (∂x).

Given that (D) is a constant and is independent from (c), then once the steady state is established, (J) becomes a constant, and Equation (3) can be integrated across the total thickness of the polymer film (x) and between the two concentrations (c_1 and c_2), so:

$$Jx = -D(c_2 - c_1) \quad \text{and} \quad J = D \frac{(c_1 - c_2)}{x} \quad (4)$$

Substituting for J [Equation (2)] into Equation (4), the permeant amount diffusing through a film of area (A) and time t is as follows:

$$Q = D \frac{(c_1 - c_2) A t}{x} \quad (5)$$

Henry's Law

Because the permeant is a gas, it is more convenient to replace concentration with partial pressure (p) of the permeant gas. So, at low concentrations, (c) can be expressed, according to Henry's law, as follows:

$$c = S p \quad (6)$$

where (S) is the solubility coefficient of the permeant in the polymer film. Equations (5) and (6) can be combined as follows:

$$Q = D S \frac{(p_1 - p_2) A t}{x} \quad (7)$$

where p_1 is the pressure of permeant in the high-pressure side of the film, and p_2 is the pressure in the lower-pressure side of the film.

Permeability coefficient (P) is shown in Equation (7) as the product (DS), so:

$$P = \frac{Qx}{A t(p_1 - p_2)} \quad (8)$$

$$\frac{Q}{t} = \frac{P}{x} A(\Delta p) \quad (9)$$

Time Lag

Permeability measurement can be carried out experimentally using the time lag method. From the typical permeation curve (Figure 10.2), during the steady state, the amount of gas permeating through the polymeric film increases linearly with time. The linear portion of the steady state line AB can be extrapolated back to $Q = 0$,

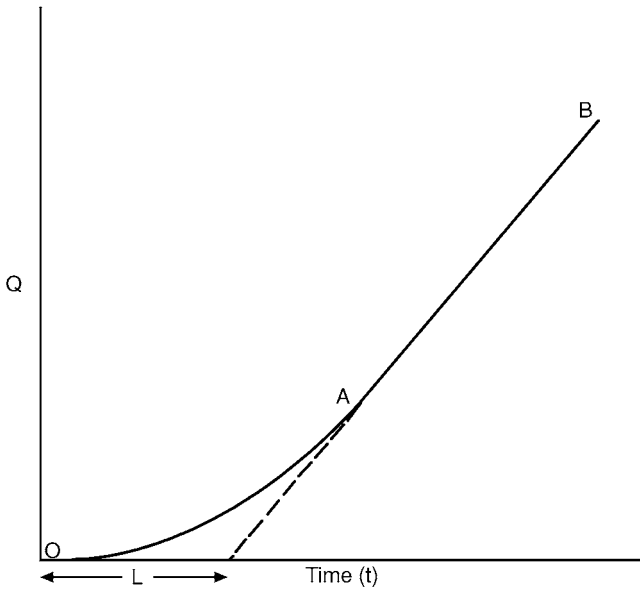


FIGURE 10.2 Typical permeation and time lag curve from a permeability experiment. Amount of permeated penetrant, Q , as a function of time, t . Extrapolation of the steady state line AB to the time axis gives the lag time as intercept L . (With permission from Rogers, C.E. 1985.)

where the intercept $t = L$. However, when the film initially is penetrant-free, the following has been shown:

$$D = \frac{x^2}{6L} \quad \text{and} \quad L = \frac{x^2}{6D} \quad (10)$$

The extrapolated steady state line intercepts with the time axis at a point represented in the graph by value L , referred to as time lag. All three parameters of the permeation process (P , D , S) can be calculated from one permeation measurement test. The slope of the line (A - B) represents the permeation rate at steady state, i.e., (Q/t) . If Q/t is substituted in Equation (9), (P) can be calculated. From the time lag L , (D) can be calculated according to Equation (10), and (S) can be calculated from ($S = P/D$). It has been found that the steady state is almost always reached after a period of time that is equivalent to approximately $2.7 L$ (Rogers, 1985).

FACTORS AFFECTING PERMEABILITY

Film barrier properties combined with respiration determine gas composition inside MAP systems of FCF and are, in turn, affected by the basic structural and chemical properties of polymeric films and, thus, play important roles in controlling the permeation process. Understanding the influence of polymer structure aids in understanding

the fundamental causes of the limitations of current polymer films (see “Shortcomings of MAP” presented earlier). More research can then be directed toward engineering better-suited polymeric films to meet the challenges imposed by MAP of FCF.

Several factors affect permeability of polymeric films (Ashley, 1985). Such chemical groups as nitrile, fluoride, chloride, acrylic and ester induce polarity in chain segments, which increases chain packing and, thus, reduces permeability. For example, polar polymers that contain hydroxyl groups as polyvinyl alcohol have very low gas permeability, whereas those with nonpolar groups as polyethylene have higher gas permeability. Linear, simple molecules promote chain packing, and thus, the polymer would acquire lower permeability, whereas polymers in which the backbones contain bulky side groups have weaker chain packing ability and, thus, higher permeability. Crystallinity also plays an important role in affecting the permeability, as crystallites are impervious to gases, making the amorphous areas of polymers the only regions for the permeation process to occur. The higher the crystallinity, the lower the permeability. In addition, orientation can play an important role for amorphous polymers. Orientation of polymer chains in the amorphous region may lead to about 10–15% reduction in permeability. Cross-linking between polymer chains inhibits permeant transport, as cross-links reduce the diffusion coefficients (Chodak, 1995).

Polymeric materials can exhibit either rubbery or glassy state depending on the temperatures at which the materials are used. The transition from one state to another occurs at a glass transition temperature, T_g (Vieth, 1991; Ganesh et al., 1992; Mandelkern, 1972). At a high enough temperature, a polymer exhibits a rubber, liquid-like state with oscillated molecular chains occupying the amorphous region. Rubbery polymers are tough and flexible due to the free mobility of the polymer chains with random conformations. As the temperature is lowered, polymer molecules exhibit a well-organized crystalline structure. At low enough temperatures, there is no adequate mobility for the polymer chains to achieve their equilibrium configurations; hence, the polymer exhibits a glass phase. Glassy polymers are brittle and hard due to the restricted segmental mobility. T_g temperatures for common polymers vary widely, and for many, the T_g is well above room temperature.

The permeability of rubbery and glassy polymers is a function of the molecular mass of the permeant (Figure 10.3) (Bell et al., 1988). Rubbery polymers behave differently from glassy polymers in terms of their permeability to different gases. There are two important phenomena observed in this figure. The permeability coefficients of rubbery polymers increase as the permeant molecular mass increases, and meanwhile, the permeability coefficient of glassy polymers decreases as the permeant molecular mass increases. This phenomenon can be explained as follows. With increasing molecular mass of gases, while gas solubility coefficients increase in both polymers, the diffusion coefficient of gases decreases more rapidly in glassy polymers than in rubbery polymers. However, the increase in solubility coefficient has more effect than the decrease in diffusion coefficient for rubbery polymers, but it has less profound effects than the decrease in diffusion coefficient for glassy polymers. Therefore, gases with smaller molecular mass would have higher diffusion coefficients and, therefore, would permeate preferentially in glassy polymers. While in glassy polymer, gas molecules with higher solubility coefficients would permeate preferentially.

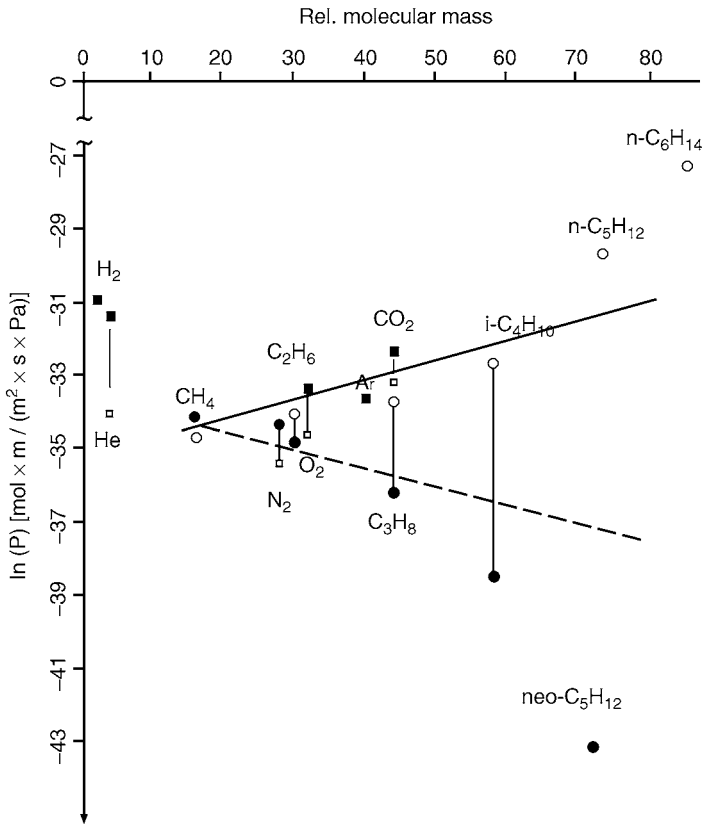


FIGURE 10.3 Permeability coefficient, P , of gases in —, ○, □: LDPE, and ---, ●, ■: polycarbonate. (Reprinted from *Journal of Membrane Science*, Bell et al., “Selection of Polymers for Pervaporation Membranes,” 36: 315–329, Copyright 1988, with permission from Elsevier Science.)

TEMPERATURE EFFECTS ON MAP OF FCF SYSTEMS

The effect of temperature on MAP of FCF is twofold. It affects the respiration rate of FCF and the permeability of the packaging materials. Due to the unpredictability of temperature changes and difficulties in maintaining an optimum temperature, temperature often plays a detrimental role on shelf life of FCF under MAP conditions. Both permeability and respiration rate follow an Arrhenius-type relationship as a function of temperature (Mannapperuma and Singh, 1994) as follows:

$$P = P_o \exp (-E_p/R^*T)$$

$$R = P_o \exp (-E_R/R^*T)$$

where P = permeability, P_0 = Arrhenius constant, R = respiration rate, R_0 = Arrhenius constant, E_p = activation energy of permeation, E_R = activation energy of respiration, R^* = universal gas constant (J/mol-K) and T = temperature (K).

According to the Arrhenius equation, both respiration and permeability have a proportional relationship with temperature. The equations further suggest that temperature would have a negligible effect on gas composition within MAP of fresh produce if both permeation and respiration rates are increased by the same magnitude. However, this relationship may not hold true for MAP of FCF due to the fact that cut fruits usually have higher respiration rates and therefore, the response to temperature is expected to be more profound than the effect on permeability. Therefore, as the temperature increases, the O_2 level inside the package is expected to decrease to a greater extent than the Arrhenius equation predicts. In this case, the respiration rate of FCF consumes more O_2 than the film can permeate into the package, even with increased permeability coefficient. This raises concerns associated with anaerobic development and importance of temperature control for MAP of FCF. Other elements of deterioration such as ethylene production, transpiration and microbial growth are also affected by temperature (Chau and Talasila, 1994). Cooling fresh produce immediately after harvest and holding at an appropriate low temperature throughout the processing of the FCF, transportation, marketing and postpurchasing are among the most important steps to minimize losses and preserve quality. Equally important, the barrier properties should function to counteract the effect of the increased respiration rate of FCF for a given MAP system.

PERMSELECTIVITY

INTRODUCTION

Film CO_2/O_2 permselectivity (β) is the ratio of the CO_2 permeability coefficient (P_{CO_2}) to O_2 permeability coefficient (P_{O_2}):

$$\beta = P_{CO_2}/P_{O_2} \quad (11)$$

As pointed out above, the dynamics of permeability and permselectivity characteristics of a gas mixture/polymer system are eventually determined by the diffusion and solution properties of a polymeric film. Differences in permeability coefficients among different films are generally attributed to differences in their diffusion coefficients more than solubility coefficients. However, permselectivity of films to gases is mainly dependent on diffusion coefficients for glassy polymers and on solubility coefficients in the case of rubbery films. Figure 10.4 shows the typical behavior of polymers in which permeability is inversely related to permselectivity. Rubbery and glassy polymers with low CO_2 and O_2 permeability coefficients have relatively higher CO_2/O_2 permselectivity than those polymers with higher permeability coefficients for this gas pair (Petroopoulos, 1990).

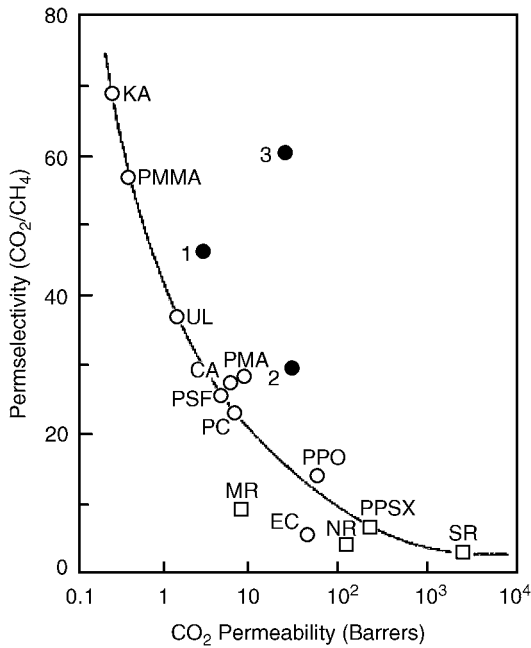


FIGURE 10.4 Permselectivity (CO_2/CH_4)-permeability (CO_2) relation for some common glassy (○) and rubbery (□) polymers (KA = Kapton polyimide; UL = Ultem polyetherimide; PMA = polymethylacrylate; CA = cellulose acetate; EC = ethyl cellulose; NR = natural rubber; SR = silicone rubber; MR = methyl rubber; PPSX = polyphenyl siloxane; PC = polycarbonate; PSF = polysulfone; PPO = polyphenylene oxide; PMMA = polymethyl methacrylate. Filled points: PMDA-ODA (1), PMDA-IPDA (2) and 6FDA-ODA (3). (Reprinted from *Journal of Membrane Science*, Petropoulos, J.H. “Some Fundamental Approaches to Membrane Gas Permeability and Permselectivity,” 53: 229–258, Copyright 1990, with permission from Elsevier Science.)

FACTORS AFFECTING PERMSELECTIVITY

For a pair of gases, permselectivity is based on the diffusivity selectivity ($D_{\text{CO}_2}/D_{\text{O}_2}$) and solubility selectivity ($S_{\text{CO}_2}/S_{\text{O}_2}$) of a polymeric film, and according to Equations (1) and (11):

$$\beta = \frac{P_{\text{CO}_2}}{P_{\text{O}_2}} = \frac{D_{\text{CO}_2}}{D_{\text{O}_2}} \frac{S_{\text{CO}_2}}{S_{\text{O}_2}} \quad (12)$$

The diffusivity selectivity relies on the polymer segmental mobility and intersegmental packing of the polymer chains and the differences in size and shape of the two penetrants. The film functions as a selective media for the size and shape of the penetrant pair, whereas the solubility selectivity relies on the differences of the condensibility between the gas pair and the physical interactions between the gases

and the polymer chains (Kim et al., 1988). It is important to understand the differences between glassy and rubbery polymeric films in order to explore the permselectivity phenomenon. Glassy polymers have a low intrasegmental mobility and long relaxation times, which is not true for rubbery polymers. Rubbery polymers have low solubility coefficients for gases with low critical temperatures, so the diffusion, solubility and permeability coefficients are independent of the gas pressure or concentrations (assuming no plasticization). This explains, for instance, the much higher solubility coefficient for CO₂ in LDPE relative to O₂ that even higher O₂ diffusion coefficients cannot overcome; therefore, CO₂ always has a higher permeability coefficient than O₂ in most rubbery and glassy polymers. However solubility, diffusion and permeability coefficients are functions of gas pressures for glassy polymers. The structure/permeability relationship can explain the differences between the glassy and rubbery polymers. Most of the differences in behaviors are attributed to the fact that glassy polymers are not usually in the true thermodynamic equilibrium state, and so the structure effects on permeability of rubbery and glassy polymers are different (Stern, 1994).

Examples of studies aimed at altering CO₂/O₂ permselectivity for MAP purposes are rare. The difficulty arises from the inherent high solubility of CO₂ in most of the plastic films relative to O₂, despite the higher O₂ diffusion coefficient. However, Kim et al. (1987) have attempted successfully to reverse permselectivity of N₂ and CH₄. Except for polysulfone, with N₂/CH₄ permselectivity of 1.0, most plastic films have permselectivity values less than 1.0 for this pair. For instance, the N₂/CH₄ is 0.27 for natural rubber and 0.93 for polycarbonate. Because CH₄ has a higher critical temperature (191°K) than N₂ (126°K), it is more condensible than N₂; therefore, CH₄ has a higher solubility coefficient than N₂. So, the CH₄ permeability is higher than the N₂ permeability, despite the fact that the diffusion coefficient of N₂ is slightly higher than CH₄ in both rubbery and glassy polymers. However, manipulation of the chemical structure of the polyimide family leads to an increase in the N₂ permeability so that polyimides can have a range of N₂/CH₄ permselectivity all of which is higher than 1. For example, PMDA-ODA has an N₂/CH₄ permselectivity of 1.75, 6FDA-IPDA, 1.9, and 6FDA-ODA, 2.18, etc. (Kim et al., 1987).

Additionally, although not investigated for MAP applications, there are several factors believed to affect the permselectivity (Stern, 1994). For instance, chain stiffness of the backbone chains can be increased by introducing bulky groups that inhibit intrasegmental mobility (rotation), thus reducing the permeability and increasing the permselectivity. Another approach involves reducing chain packing by introducing charges, which increases permeability. Optimizing gas atmosphere evolution inside MAP applications of different FCF products must rely on investigating such approaches and properties of polymeric films, which so far has not been comprehensively carried out.

CO₂/O₂ PERMSELECTIVITY AND RESPIRATION QUOTIENT

The respiration quotient (RQ) is the ratio of CO₂ production rate to O₂ consumption rate and can be stated as follows:

$$RQ = R_{CO_2}/R_{O_2} \quad (13)$$

Where R_{CO_2} is the CO_2 production rate, and R_{O_2} is the O_2 consumption rate. If for each molecule of CO_2 produced during aerobic respiration, one molecule of O_2 is consumed, the respiration quotient (RQ) = 1. CO_2/O_2 permselectivity is an important property of polymeric films, especially those for MAP application of FCF. Therefore, to match a film permselectivity with a given produce respiration quotient (RQ) is a challenge that would contribute to a successful application of MAP for FCF products.

If a polymeric film permeates O_2 and CO_2 equally (i.e., $P_{CO_2}:P_{O_2}$ ratio is 1:1), then when the O_2 level is reduced due to produce respiration from, say, 21–3% (18% change), the package would also create an equal amount of change for CO_2 as well, i.e., CO_2 would increase to 18% within the package. Because different produce items require different O_2 and CO_2 levels, both high and low levels of the gases can be beneficial or harmful, depending on the variety and type of produce. In this case, permselectivity of CO_2/O_2 is as important as the absolute permeability values for MAP of FCF (Zagory, 1995).

ROLE OF PERMSELECTIVITY IN MAP

Despite the critical importance of permselectivity for the successful application of MAP to whole and cut produce, little progress has been made in developing materials with optimum permselectivities. Use of a polymeric film with a high β (e.g., >4–6) results in an equilibrium atmosphere that is low in CO_2 , and films with low β (e.g., <2) tend to accumulate high levels of CO_2 without regard to absolute permeation rates. The effect of β on the package atmosphere cannot be overridden by any other means, including gas flushing, vacuum packaging, changing the size of the bag or changing the amount of the product in the bag. Thus, selection of optimal β will be necessary to optimize any packaging system for FCF.

β values can be used to predict the equilibrium concentration of CO_2 and O_2 inside a package, which in turn, can be compared to the optimal mixture, once the respiration rate of the produce is known. An illustration of the significance of β on MAP systems is given in Figure 10.5. The optimal ranges for O_2 and CO_2 concentration needed in a modified atmosphere for different produce commodities are plotted based on literature values (Zagory, 1995). For example, the optimal CO_2 range for grapefruit is 5–9% and the O_2 is 4–10%. Atmospheres within these values will prolong product quality. PVC, which has a β value of 6, would result in CO_2 and O_2 mixtures represented by line E in Figure 10.5. Thus, the maximum amount of CO_2 achievable would be approximately 3%. This is well below the optimum for grapefruit. It would be possible, however, to achieve an optimum gas atmosphere for oranges (Figure 10.5).

The range of values for β in common films is more limited than the range of recommended O_2 and CO_2 concentrations, meaning that most produce items do not have a matching suitable film to produce an optimal atmosphere. Currently available films satisfy only whole fresh produce with relatively low O_2 and CO_2 requirements. Thus, commonly available polymeric films fail to provide the recommended atmosphere for most fresh whole produce items and are woefully inadequate for FCF products due to their higher respiration rates. For instance, a film with β value of 3.4 (e.g., PET) passes through recommended areas O_2 and CO_2 for orange and

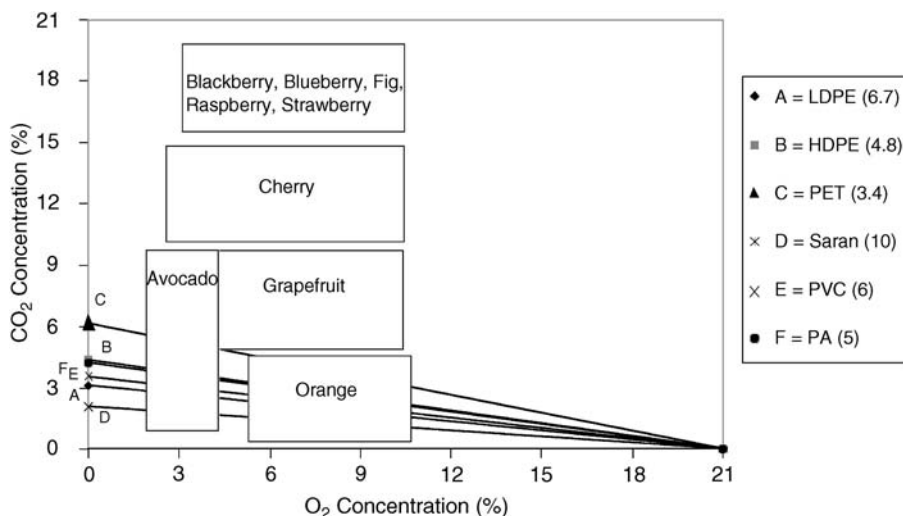


FIGURE 10.5 Recommended gas composition for fruits and permselectivity of various polymer films. (Reproduced with permission from Zagory, D. 1995.)

avocado (Figure 10.5) and would be appropriate for those two commodities. Because the β line of PET does not pass through cherries, the film is not the appropriate film with which to package this commodity.

Perusal of the permselectivities for a large number of polymeric films (Table 10.1) show a relatively limited selectivity of 2–10, with most common films in the range of 4–6. This means that the produce in a MAP system must have a respiration rate that would match β values of 4–6 to produce an optimum atmosphere. The range of available selectivities is too limited for the much wider range of respiration rates among produce.

MATHEMATICAL PREDICTIVE MODELS

INTRODUCTION

To design an optimal MAP system for a given FCF (or whole fruits or vegetables for that matter), it is necessary to predict changes in atmosphere composition over time inside the package. Such prediction is possible with the help of mathematical models. Such models facilitate selecting an appropriate packaging material for a given FCF, once packaging dimensions and respiration rate are known, by calculating the required permeability coefficients of the packaging material and then choosing materials with comparable gas permeability. If the permeability and respiration characteristics are provided, the dimension of the package can be calculated as well.

Several models have been published (Senesi et al., 1999; Cameron et al., 1995; Piergiovanni et al., 1999; Lee and Renault, 1998; Lakakul et al., 1999; McLaughlin and O'Beirne, 1999; Ishitani and Inoue, 1993; Chinnan 1989; and Mannapperuma and Singh, 1994). For any MAP system, what determines O_2 influxes and CO_2

TABLE 10.1**CO₂ and O₂ Permeability Coefficients and CO₂/O₂ Permselectivity of Various Polymeric Materials**

No.	Material Type	Permeability Coefficient ²			Permselectivity CO ₂ /O ₂	Ref.
		CO ₂	O ₂	Units $\frac{10^{-15} \text{ mol m}}{\text{m}^2 \text{ s kPa}}$		
1	LDPE	99	27		3.7	Cameron
2	PP	58	9		6.2	et al., 1995
3	PVC	0.65	0.19		3.4	
4	Cellulose acetate	348	10		34	
5	PSS-Na ¹	14.1	2.9	Barrer*	4.9	Chen and
6	PSS-Mg ²	8.9	1.8		4.9	Martin, 1994
7	Poly(ethyl methacrylate)	7×10^{-10}	1.9×10^{-10}	$\frac{\text{cm}^3 (\text{STP}) \text{ cm}}{\text{cm}^2 \text{ s cmHg}}$	3.7	Chiou and Paul, 1989
8	TMPC/SAN ³	6.56×10^{-6}	7.9×10^{-7}	$\frac{\text{cm}^3 (\text{STP}) \text{ cm}}{\text{cm}^2 \text{ s cmHg}}$	8.3	Chiou and Paul, 1987a
9	Cellulose acetate	9	0.92	Barrer		Feldman et al., 1987
10	PSF ⁴	5.5	1.29	Barrer	4.3	Ghosal
11	PSF-NO ₂ (50%)	3.4	0.88		3.9	et al., 1995
12	PSF-NO ₂ (98%)	2.3	0.66		3.5	
13	PSF-NO ₂ (192%)	1.5	0.44		3.4	
14	PC ⁵	6.8	1.6	Barrer	4.3	Hellmus
15	TMPC	18.6	5.6		3.3	et al., 1989
16	HFPC ⁶	24	6.9		3.4	
17	TMHF-PC ⁷	111	32		3.5	
18	Polyimide	1357	213	Barrer	6.4	Hofman, et al., 1996
19	PSF	5.6	1.4	Barrer	4	Houde et al., 1994
20	PSF-PPHA ⁸	6.5	2		3.3	
21	PMMA	3.1×10^{-11}	8.75×10^{-12}	$\frac{\text{cm}^3 (\text{STP}) \text{ cm}}{\text{cm}^2 \text{ s cmHg}}$	3.5	Chiou and
22	CO ₂ -PMMA ⁹	4.91×10^{-11}	1.26×10^{-11}		3.9	Paul, 1987b
23	Vulcanized rubber	0.132×10^{-6}	0.02×10^{-6}	$\frac{\text{cc mm}}{\text{cm}^2 \text{ s cmHg}}$	6.6	Barrer, 1939
24	PE @ 15°C	130	27.5	$\frac{10^{10} \text{ cc (STP) mm}}{\text{cm}^2 \text{ s cmHg}}$	4.7	Soboley
25	10 ⁸ r PE ¹⁰	72.7	15.3		4.75	et al., 1955
26	PET	53	6.1	$\frac{10^{-12} \text{ cc (STP) mm}}{\text{cm}^2 \text{ s cmHg}}$	8.7	Pye et al., 1976
27	PE (1.2 mil thick)	12.9	3.4	Barrer	3.8	Pasternak et al., 1970
28	Cellophane	0.0008×10^{-6}	0.0002×10^{-6}	$\frac{\text{c cm}}{\text{cm}^2 \text{ min cmHg}}$	4	Davis, 1946
29	Koroseal ¹¹	0.26×10^{-6}	0.055×10^{-6}		4.7	
30	Vynilite ¹²	0.14×10^{-6}	0.036×10^{-6}		3.9	
31	Pliofilm ¹³	0.56×10^{-6}	0.11×10^{-6}		5.1	

(continued)

TABLE 10.1
CO₂ and O₂ Permeability Coefficients and CO₂/O₂ Permselectivity
of Various Polymeric Materials (Continued)

No.	Material Type	Permeability Coefficient			Permselectivity CO ₂ /O ₂	Ref.
		CO ₂	O ₂	Units		
32	Mylar	346	74	$\frac{\text{cc mil}}{\text{m}^2 \text{ day atm}}$	4.7	Karel et al., 1963
33	PP	11,200	4110		2.7	
34	PMSF ¹⁴	280	77.3	$\frac{10^8 \text{ cm}^3 (\text{STP}) \text{ cm}}{\text{cm}^2 \text{ s cmHg}}$	3.6	Ichiraku et al., 1987
35	21% S-PE ¹⁵	14.9	3.45	$\frac{10^9 \text{ ccmm}}{\text{cm}^2 \text{ s cmHg}}$	4.3	Myers et al., 1960
36	41% S-PE ¹⁶	20.2	4.26		4.7	
37	1.8% A-PE ¹⁷	25.4	5.73		4.4	
38	31% A-PE ¹⁸	9.7	2.24		4.3	
39	34% V-PE ¹⁹	11.3	2.33		4.8	
40	60% V-PE ²⁰	2.47	0.57		4.3	
41	Epoxy-diacrylate-co ²¹	1.53×10^{-3}	1.19×10^{-3}	$\frac{\text{cm}^3 (\text{STP}) \text{ cm}}{\text{cm}^2 \text{ s cmHg}}$	1.3	Bellobono et al., 1991
42	Al ₃ -Sil-A/U ²² (@200°C)	5.5	4	$\frac{10^{-7} \text{ mol}}{\text{m}^2 \text{ s Pa}}$	1.4	De Lang et al., 1995
43	PMP ²³	10,700	2700	Barrer	4	Morisato and Pinnau, 1996
44	PTBA ²⁴	560	130		4.3	
45	PC ²⁵	6	1.5	Barrer	4	Muruga-nandam and Paul, 1987
46	TCPC ²⁶	6.7	2.3		2.9	
47	TMPC ²⁷	4.2	1.4		3	
48	Teflon AF-2400 ²⁸	3900	1600	Barrer	2.4	Pinnau and Toy, 1996
49	37% DMS ²⁹	2.2	1.04	$\frac{10^9 \text{ cm}^3 (\text{STP}) \text{ cm}}{\text{cm}^2 \text{ s cmHg}}$	2.1	Suzuki et al., 1995
50	57.4% DMS ³⁰	61.7	11.3		5.5	
51	65% DMS ³¹	298	49.4		6	
52	<i>t</i> -Bu acetylene ³²	13.6×10^{-8}	3×10^{-8}	$\frac{\text{cm}^3 (\text{STP}) \text{ cm}}{\text{cm}^2 \text{ s cmHg}}$	4.5	Takada and Matsuya, 1985
53	Cl-Ph acetylene ³³	61×10^{-10}	8×10^{-10}		7.6	
54	6FDA-3,3'-ODA ³⁴	2.1	0.68	Barrer	3.1	Mi et al., 1993
55	6FDA-4,4'-ODA ³⁵	22	5.05		4.4	
56	pp-PFP/PDMS ³⁶	20	6	Barrer	3.3	Oh and Zurawsky, 1996
57	pp-PFT/PDMS ³⁷	21.7	4.9		4.4	

TABLE 10.1

CO₂ and O₂ Permeability Coefficients and CO₂/O₂ Permselectivity of Various Polymeric Materials (Continued)

No.	Material Type	Permeability Coefficient			Permselectivity	Ref.
		CO ₂	O ₂	Units	CO ₂ /O ₂	
58	Uncoated PE ³⁸	9.3	3.3	Barrer	2.8	Surya-narayanan et al., 1974
59	PP coated PE ³⁹	1.9	1		2	
60	Polyethylene methacrylate				4.3	
61	Polyvinylbenzoate				5.8	Talasila and Cameron, 1997
62	PVC (unplasticized)				3.5	
63	Polytetrafluoroethylene				2.3	
64	Polybutadiene				7.3	
65	Ethyl cellulose				7.7	
66	Cellulose cetate (plasticized)				18.7	
67	Polystyrene	10,000–26,000	2600–7700	$\frac{\text{mL mil}}{\text{m}^2 \text{ day atm}}$	3.4–3.8	Kader et al., 1989
68	Polyvinylidene	59	15.5		3.8	
69	Polycarbonate	23,250–26,350	13,950–14,725		1.7–1.8	
70	Methylcellulose	6200	1240		5	
71	Ionomer ⁴⁰	7.73×10^{-8}	1.85×10^{-8}	$\frac{\text{cm}^3 (\text{STP}) \text{ cm}}{\text{cm}^2 \text{ s cmHg}}$	4.2	
72	Ionomer ⁴¹	5.64×10^{-8}	1.44×10^{-8}		3.9	
73	6FDA-DAF ⁴²	19.5	5.1	Barrer	3.8	Kim and Koros, 1989
74	6FDA-IPDA ⁴³	24.3	5.1		4.8	
75	6F-TADPO ⁴⁴	27.6	7.9	Barrer	3.5	Koros and Fleming, 1993
76	6F-ODA ⁴⁵	23	4.3		5.3	
77	LLDPE ⁴⁶ @25°C	N/A	N/A		4.4	Villaluenga et al., 1998
78	LLDPE ⁴⁷ @80°C	N/A	N/A		3.0	
79	PPO ⁴⁸	58	18	Barrer	3.2	Koros et al., 1987
80	Ionomer ⁴⁹	1.21×10^{-7}	3.15×10^{-8}	$\frac{\text{cm}^3 (\text{STP}) \text{ cm}}{\text{cm}^2 \text{ s atm}}$	3.8	
81	Ionomer ⁵⁰	1.03×10^{-7}	2.61×10^{-8}	$\frac{\text{cm}^3 (\text{STP}) \text{ cm}}{\text{cm}^2 \text{ s atm}}$	3.9	Mensitieri et al., 1996
82	Ionomer ⁵¹	1.13×10^{-7}	3.01×10^{-8}		3.8	
83	PS	88	11	$\frac{10^{11} \text{ cm}^3 (\text{STP}) \text{ cm}}{\text{cm}^2 \text{ s cmHg}}$	8	Robertson, 1993
84	Nylon 6	1.6	0.38		4.2	

(continued)

TABLE 10.1**CO₂ and O₂ Permeability Coefficients and CO₂/O₂ Permselectivity of Various Polymeric Materials (Continued)**

No.	Material Type	Permeability Coefficient			Permselectivity	Ref.
		CO ₂	O ₂	Units	CO ₂ /O ₂	
85	PMDA-ODA ⁵²	2.7	0.61	Barrer	4.4	Kim et al., 1988
86	PMDA-MDA ⁵³	4.3	0.98		4.4	
87	PMDA-IPDA ⁵⁴	26.8	7.1		3.8	
88	PVC/MSN ⁵⁵				9	Miltz et al., 1992
89	PVC/PA ⁵⁶				2.4	
90	Poly(butadiene-styrene)	6.5×10^{-1}	7×10^{-2}	$\frac{\text{mL mil}}{\text{cm}^2 \cdot \text{h} \cdot \text{atm}}$	9.2	Exama et al., 1993a
91	Butyl rubber	1.6×10^{-2}	3.6×10^{-3}		4.4	
92	Saran	4.63×10^{-5}	4.54×10^{-6}		10.2	
93	Silicone rubber	7.13	1.1	$\frac{\text{mL mil}}{\text{cm}^2 \cdot \text{h} \cdot \text{atm}}$	6.5	Exama et al., 1993b
94	Ethyl cellulose	0.378	0.155		2.4	
95	Natural rubber	0.672	0.095		7.1	
96	HDPE	7.4×10^{-4}	1.53×10^{-4}		4.8	
97	Rubber hydrochloride	3.67×10^{-3}	1.5×10^{-3}		2.4	
98	Poly (vinylchloride-vinyl acetate)	1.74×10^{-1}	2.62×10^{-2}		6.6	

* Barrer = $10^{-10} \text{ cm}^3 \text{ (STP) cm/cm}^2 \text{ s cmHg}$

¹ PSS-Na: Na counterion of poly(styrene-co-styrenesulfonic acid)

² PSS-Mg = Mg counterion of poly(styrene-co-styrenesulfonic acid)

³ TMPC/SAN = tetramethyl bisphenol-A polycarbonate/styrene-acrylonitrile copolymer

⁴ PSF = polysulfone; nitrate with different levels (50%, 98% and 192%)

⁵ PC = polycarbonate

⁶ HFPC = hexafluorobisphenol-A polycarbonate

⁷ TMHF-PC = tetramethylhexafluorobisphenol-A polycarbonate

⁸ PSF-PPHA = phenolphthalein-based polysulfone

⁹ Conditioned extruded poly(methyl methacrylate) under 25 atm CO₂

¹⁰ 10⁸r PE = irradiated PE with a dose of 10⁸ roentgens

¹¹ Koroseal = polyvinyl chloride composition

¹² Vinylite = polyvinyl chloride

¹³ Pliofilm = rubber-wax composition

¹⁴ PMSP = poly(1-trimethylsilyl-1-propylene)

¹⁵ 21% S-PE = 20.9% grafted styrene-polyethylene copolymer

¹⁶ 41% S-PE = 41.3% grafted styrene-polyethylene copolymer

¹⁷ 1.8% A-PE = 1.8% grafted acrylonitrile-polyethylene copolymer

¹⁸ 31% A-PE = 31.3% grafted acrylonitrile-polyethylene copolymer

¹⁹ 34% V-PE = 34% grafted vinylpyridine-polyethylene copolymer

²⁰ 60% V-PE = 60% grafted vinylpyridine-polyethylene copolymer

TABLE 10.1**CO₂ and O₂ Permeability Coefficients and CO₂/O₂ Permselectivity of Various Polymeric Materials (Continued)**

²¹ Epoxy-diacrylate-co = photochemically grafting an epoxy-diacrylate copolymer containing 30 wt.% of active carbon onto cellulose

²² Al₃-Sil-A/U = silica-modified γ -alumina membrane

²³ PMP = poly(4-methyl-2-pentyne)

²⁴ PTBA = poly(*tert*-butylacetylene)

²⁵ PC = bisphenol-A polycarbonate

²⁶ TCPC = tetrachloro-bisphenol-A polycarbonate

²⁷ TMPC = tetramethylbisphenol-A polycarbonate

²⁸ Teflon AF-2400 = an amorphous PDD-PTFE copolymer containing 87 mol% 2,2-bis(trifluoromethyl)-4,5-difluoro-1,3-dioxole and 13 mol% tetrafluoroethylene

²⁹ 37% DMS = poly(styrene-*co*-maleic anhydride) with 37% dimethylsiloxane (DMS MW = 10,000)

³⁰ 57.4% DMS = poly(styrene-*co*-maleic anhydride) with 57.4% dimethylsiloxane (DMS MW = 10,000)

³¹ 65% DMS = poly(styrene-*co*-maleic anhydride) with 65% dimethylsiloxane (DMS MW = 10,000)

³² *t*-Bu acetylene = *tert*-butylacetylene

³³ Cl-Ph acetylene = 1-chloro-2-phenylacetylene

³⁴ 6FDA-3,3'-ODA = polyimide isomer (5,5'-[2,2,2-trifluoro-1(trifluoromethyl)-ethylidene]-bis-1,3-isobenzofurandione)-diamine

³⁵ 6FDA-4,4'-ODA = polyimide isomer (5,5'-[2,2,2-trifluoro-1(trifluoromethyl)-ethylidene]-bis-1,3-isobenzofurandione)-diamine

³⁶ pp-PFP/PDMS = plasma polymerized pentafluoropyridine poly(dimethylsiloxane)

³⁷ pp-PFT/PDMS = plasma polymerized pentafluorotoluene poly(dimethylsiloxane)

³⁸ Uncoated PE = uncoated 1-mil PE film

³⁹ PP coated PE = 1-mil PE film coated with 1–2 microns PP

⁴⁰ Ionomer¹ = E-0.046AA-0.12Zn [ethylene-acrylic acid (0.046%) with 0.12% Zn] @25°C

⁴¹ Ionomer² = E-0.065AA-0.25Zn [ethylene-acrylic acid (0.065%) with 0.25% Zn] @25°C

⁴² 6FDA-DAF = 5,5'-[2,2,2-trifluoro-1 (trifluoromethyl)-ethylidene]-bis-1,3-isobenzofurandione)-diamine,2,7-fluorenediamine

⁴³ 6FDA-IPDA = 5,5'-[2,2,2-trifluoro-1 (trifluoromethyl)-ethylidene]-bis-1,3-isobenzofurandione) -isopropylidenedianiline

⁴⁴ 6F-TADPO = ether connected polypyrrolone polyimide

⁴⁵ 6F-ODA = ether connected polyimide

⁴⁶ LLDPE⁴⁶ = linear low-density polyethylene extruded with 15 mm distance between die and chill role 2 cmHg vacuum, at 25°C

⁴⁷ LLDPE⁴⁷ = linear low-density polyethylene extruded with: 5 mm distance between die and chill role, 5 cmHg vacuum, at 80°C

⁴⁸ PPO = polyphenylene oxide

⁴⁹ Ionomer⁴⁹ = E-0.046AA-0.12Zn [ethylene-acrylic acid (0.046%) with 0.12% Zn]

⁵⁰ Ionomer⁵⁰ = E-0.065AA-0.25Zn [ethylene-acrylic acid (0.065%) with 0.25% Zn]

⁵¹ Ionomer⁵¹ = E-0.037AA-0.35Zn [ethylene-acrylic acid (0.037%) with 0.35% Zn]

⁵² PMDA-ODA = polypyromellitim of oxydianiline

⁵³ PMDA-MDA = polypyromellitim of methylenedianiline

⁵⁴ PMDA-IPDA = polypyromellitim of isopropylidenedianiline

⁵⁵ PVC-MSN⁵⁵ = the difference between 55 and 56 is only plasticizer content

⁵⁶ PVC/PA⁵⁶ = the difference between 55 and 56 is only plasticizer content

effluxes from a given polymeric package, at a given temperature, are the permeability coefficient, thickness of the film, surface area and partial pressure gradient of O₂ and CO₂ inside and outside of the film. There is control over the first three parameters, but the optimum partial pressure gradients are determined by the produce and are dictated by the optimum atmosphere composition for a given produce item. Therefore, an appropriate mathematical model must be carefully designed and tested taking into account these parameters. The models are similar and are generally developed with the same basic steps as follows.

STEPS OF DEVELOPING MATHEMATICAL MODELS

The mathematical models combine mathematical descriptions of gas fluxes through both polymeric film and the fresh produce (Robertson, 1993). For the film, Fick's law is applied. From Equation (9), CO₂ and O₂ flux across the film are as follows:

$$Q_{O_2} = \frac{P_{O_2}}{X} A(p_{O_{20}} - p_{O_{2i}}); \quad (14)$$

$$Q_{CO_2} = \frac{P_{CO_2}}{X} A(p_{CO_{2i}} - p_{CO_{20}}); \quad (15)$$

where Q is the diffusive flux of O₂ and CO₂ through a film in a unit time, $p_{O_{20}}$ is the partial pressure of O₂ outside the package, $p_{O_{2i}}$ is the O₂ partial pressure inside the package, $p_{CO_{20}}$ is the CO₂ partial pressure outside the package and $p_{CO_{2i}}$ is the CO₂ partial pressure inside the package.

The O₂ flux into the fruit is a function of respiration rate, expressed as either (R_{O_2}) or (R_{CO_2}):

$$Q_f = R_{O_2} W \quad (16)$$

where Q_f is the flux of O₂ into the fruit per unit time, R_{O_2} is the respiration rate (or oxygen consumption) of the fruit (mL/Kg.h) and W is the weight of fruit in the package. Gas exchange in a MAP of a fresh produce system reaches equilibrium after some time, when the amount of gas consumed (or produced) by the produce equals the amount of gas flux (influx and efflux) through the package. Since at this steady state, the O₂ flux through the film and into the fruit are equal, then

$$Q_{O_2} = Q_f$$

$$\frac{P_{O_2}}{X} A(p_{O_{20}} - p_{O_{2i}}) = R_{O_2} W \quad (17)$$

Once the relationship between the respiration rate (R_{O_2}), weight of produce, package dimensions and O₂ concentration are known, Equation (17) provides a mathematical means to predict the O₂ permeation requirement for a film to successfully

perform in this MAP system. Similarly, a predictive mathematical equation to model CO₂ evolution inside the package can be developed as follows:

$$\frac{P_{CO_2}}{x} A(p_{CO_{2i}} - p_{CO_{2o}}) = R_{CO_2} W \quad (18)$$

PUBLISHED MATHEMATICAL MODELS IN MAP STUDIES

The published models for respiring produce packaged under modified atmosphere conditions reveal an interesting fact—all models are very similar.

Jurin and Karel (1963) published one of the earliest models, which addressed the respiration rates of 'McIntosh' apples as a function of oxygen concentration. They used the relationships obtained in respiration studies to predict optimum packaging conditions for apples. The packaging parameters considered were volume, surface area and permeability to O₂ and CO₂. At steady state, concentrations of O₂ and of CO₂ [assuming the respiration quotient (RQ) = 1] were obtained by the following equations:

$$V_c = \int (c_2) \quad (19)$$

$$V_d = (P_{O_2})(A)(x^{-1})(p_1 - p_2) \quad (20)$$

Where

V_c = volume of O₂ consumed (cc/package x day)

c_2 = O₂ concentration in the package expressed as a fraction

V_d = O₂ diffusion into the package (cc/package x day)

P_{O_2} = O₂ permeability (cc.mil)/(m².day.atm)

A = area of the package (m²)

x = thickness of the packaging material (mil)

p_1 = partial pressure of O₂ outside the package

p_2 = O₂ inside

At steady state, both Equations (19) and (20) become equal as follows:

$$V_c = V_d = \int (c_2) = (P_{O_2})(A)(x^{-1})(p_1 - p_2) \quad (21)$$

Equation (21) can be rewritten as follows:

$$P_{O_2} = (V_c)(x)/(A)(c_1 - c_2) \quad (22)$$

This equation suggests that the O₂ permeability coefficient of a given MAP for fresh produce is a function of produce consumption rate of O₂ (V_c), film thickness (x), area of the film (A) and the O₂ concentration difference across the film ($c_1 - c_2$).

Hayakawa et al. (1975) developed a mathematical model for simulating gas exchange of packaged fresh produce and then obtained analytical solutions for the model.

From these solutions, they derived simple algebraic formulae for the optimization of packaging parameters.

$$\frac{y_{eqi} - y}{y_{eqi} - y_{oi}} = \exp[-1/V(SK_y/t + W_{oi})\tau] \quad (23)$$

$$y_{eqi} = (y_a k_y / t - q_{iw} / s) / (k_y / t + W_{oi} / s) \quad (24)$$

$$\frac{z_{eqi} - z}{z_{eqi} - z_{oi}} = \exp[-1/V(SK_z/t + W_{oi})\tau] \quad (25)$$

$$z_{eqi} = (z_a k_z / t + f_{iw} / s) / (k_z / t + W_{ei} / s) \quad (26)$$

where

y_{eqi} = volumetric concentration of O₂ in fresh produce package (atm) at hypothetical equilibrium state

z_{eqi} = volumetric concentration of CO₂ in fresh produce package (atm) at hypothetical equilibrium state

y = volumetric concentration of O₂

z = volumetric concentration of CO₂

y_{oi} = volumetric concentration of O₂ when $\tau = 0$

W = weight of fresh produce per one package (Kg)

V = inside free volume of a package (cc)

S = surface area of fresh produce package through which gas permeates

K_y = permeability of polymeric film to O₂ (cc.mil/hr.in².atm)

K_z = permeability of polymeric film to CO₂ (cc.mil/hr.in².atm)

t = thickness of polymeric film (mil)

τ = time after packaging (hr)

W_{ei} = lower quantity of CO₂ from CO₂ evolution rate curve

W_{oi} = weight when $\tau = 0$

$\tau = t - t_i$: time after y becomes equal to y_i or after z becomes equal to z_i ;
(e.g., y_i = the limit of $(i - 1)$ th line segment for approximating a curve of R_y values (same for z))

f = constant used for approximating a curve for CO₂ evolution rate of fresh produce

q = constant used for approximating a curve for O₂ consumption rate of fresh produce

Equations (24) and (26) are similar to Equation (22) developed by Jurin and Karel (1963).

Deily and Rizvi (1981) integrated variables such as respiration rate, weight and optimum gaseous composition requirements with packaging parameters such as permeability, surface area and free volume into a set of analytical equations. These equations can be solved to provide prediction of transient and equilibrium time values for O₂ and CO₂ concentrations within a produce package or conversely, can be used

to design a package that will help set up a known gaseous composition. The equations are as follows:

$$y' = y_a - (W/SK_y)R_y \quad (27)$$

$$y(t) = y' + (y_a - y')e^{-SK_y t/V} \quad (28)$$

$$z' = z + (W/SK_z)R_z \quad (29)$$

$$z(t) = z' + (z_a - z')e^{-SK_z t/V} \quad (30)$$

Equation (27) can be rewritten as follows:

$$K_y = WR_y/A(y_a - y') \quad (31)$$

where

y' = O₂ concentration at hypothetical equilibrium state (atm)

z' = CO₂ concentration at hypothetical equilibrium state (atm)

y_a = O₂ concentration outside the package (atm)

z_a = CO₂ concentration outside the package (atm)

z = volumetric concentration of CO₂ gas inside fresh produce package (in atm)

y = volumetric concentration of O₂ gas inside fresh produce package (in atm)

K_y = O₂ transmission rate of a polymeric film (cc of O₂/m²·h·atm)

K_z = CO₂ transmission rate of a polymeric film (cc of O₂/m²·h·atm)

R_y = rate of consumption of O₂ (cc/Kg)

R_z = rate of evolution of CO₂ (cc/kg)

W = weight of fresh produce per one package (kg)

S = surface area of produce-package through which O₂ and CO₂ can permeate (m²)

t = time after package (h)

V = inside free volume of a package (cc)

Equation (31) is likewise, similar to Equation (22) of Jurin and Karel (1963).

The authors suggest that Equations (27) through (30) can be used to predict equilibrium and transient state concentrations of O₂ and CO₂ at constant temperature. Since the time to reach equilibrium will be dependent upon the free volume inside the package, they proposed that equilibrium concentration is independent of time, as indicated by Equations (27) and (29), even though the equations do not account for volume. In addition, they provided Equations (28) and (30) to calculate the time required for equilibrium to be reached.

Cameron (1990) calculated the rates of O₂ uptake and CO₂ production, assuming that they equaled the rates of flux of the respective gases across the film as follows:

$$R_{O_2} = P_{O_2} A x^{-1} ([O_2]_{atm} - [O_2]_{pkg}) W^{-1} \quad (32)$$

$$R_{CO_2} = P_{CO_2} A x^{-1} ([CO_2]_{pkg} - [CO_2]_{atm}) W^{-1} \quad (33)$$

These equations are again similar to Jurin and Karel's (1963). For instance, Equation (32) (oxygen uptake) can be rewritten as follows:

$$P_{O_2} = R_{O_2} x W/A([O_2]_{atm} - [O_2]_{pkg}) \quad (34)$$

Cameron et al. (1989) developed mathematical equations describing O_2 consumption as a function of O_2 concentration (for tomato fruit) and modeled film characteristics (O_2 permeability coefficient, surface area, thickness) as a function of fruit weight. To predict film permeability characteristics for a sealed package containing a given weight of fruit to yield a desired package O_2 concentration, they developed the following equation:

$$P_{O_2} A/\Delta x = R_{O_2} [(O_2)_{pkg}] W/[O_2]_{atm} - [O_2]_{pkg}$$

This equation is equivalent to the following:

$$P_{O_2} = \Delta x R_{O_2} [(O_2)_{pkg}] W/A([O_2]_{atm} - [O_2]_{pkg}) \quad (35)$$

This equation is again similar to Jurin and Karel's (1963).

Cameron et al. (1995) examined the factors that limit the ability to control gas levels in MA packages. They suggested that while many researchers have recognized that gas partial pressures in the package atmosphere can be modeled, there are no correct models that use information on modifying atmosphere inside the plant tissue. Their solution was to model O_2 uptake based on the Michaelis-Menton equation to take into account the enzymatic rate of O_2 uptake in plant tissues. They suggested the following equation for estimating O_2 permeability ($P_i^{film,T}$):

$$P_i^{film,T} = [\Delta x (W/A_{film}) R_{O_2}^{max,T}] / [(O_2)_{ext} - (O_2)_{pkg}] \quad (36)$$

R_{O_2} = the consumption rate of O_2 . The equation once rewritten as follows:

$$P_i = W R_{O_2} x/A[(O_2)_{ext} - (O_2)_{pkg}]$$

is identical to Jurin and Karel's (1963) Equation (22).

Talasila et al. (1995) developed a procedure to design MA packages for fresh produce that accounts for changes in total pressure inside the package. Their model is also affected by surrounding temperatures, product respiration and film permeability. They developed steady state equations to determine partial pressure of oxygen and carbon dioxide as follows:

$$AP_{MA}/22.414E = (P_{A2} - P_{A1}) = R_{AW} \quad (37)$$

$$AP_{MB}/22.414E = (P_{B1} - P_{B2}) = R_{BW} \quad (38)$$

where

- A = surface area of the film (m²)
- P_{MA} = oxygen permeation rate
- R_{AW} = oxygen consumption
- P_{A1} = atmospheric oxygen concentration (0.21 atm)
- P_{B1} = CO₂ inside the package
- P_{A2} = oxygen inside the package
- P_{B2} = atmospheric CO₂ (0.0 atm)
- E = film thickness

Equations (37) and (38) can be written as follows:

$$P_{MA} = R_{AW} 22.44E/A(P_{A2} - P_{A1})$$

which is again similar to Jurin and Karel's (1963) Equation (22). They also suggest that if the product respiration rate does not vary with time, the partial pressures of gases inside the package at any given time are as follows:

$$P_{A1}(t) = P_{A1}(t = t_{ss}) - [P_{A1}(t = t_{ss}) - P_{A1}(t = 0)] \exp[(-AP_{MA}GT_1/22.414 EV_1)t] \quad (39)$$

$$P_{B1}(t) = P_{B1}(t = t_{ss}) - [P_{B1}(t = t_{ss}) - P_{B1}(t = 0)] \exp[(-AP_{MB}GT_1/22.414 EV_1)t] \quad (40)$$

These latter two equations also determine the times needed to reach any specific O₂ and CO₂ partial pressures.

Exama et al. (1993b) suggest that in order to obtain the maximum benefit from MAP systems, the steady state gas concentration should correspond to the storage optima of the packaged commodity. They computed the film permeability requirements by analyzing the kinetics of the MA process. Evolution of the volume fraction of O₂ inside MA package (yiO₂) as a function of time (t) was determined by the following:

$$dyiO_2/dt = [(AP_{O_2} p/VL)(yeO_2 - yiO_2)] - (WR_{O_2}/V) \quad (41)$$

Because at steady state, yiO₂ remains constant [i.e., the left side of Equation (41) equals zero], and because it is desirable that at this state the internal O₂ concentration is at the optimum for the packaged produce (yiO₂ = yoO₂), Equation (41) can be rearranged to give the required O₂ permeability (P_{O₂}^R):

$$(AP_{O_2} p/VL)(yeO_2 - yoO_2) = WR_{O_2}/V \quad (42)$$

so that

$$P_{O_2}^R = WR_{O_2} L / Ap(yeO_2 - yoO_2) \quad (43)$$

Similarly, the permeation required to provide optimum CO₂ concentration is as follows:

$$P_{\text{CO}_2}^R = WR_{\text{CO}_2}L/Ap(y_{\text{O}_2}\text{CO}_2 - y_{\text{eCO}_2}) \quad (44)$$

where

A = surface area of the film (m²)

L = film thickness (mil)

V = void volume inside the package (mL)

P_{O₂} = O₂ permeability of the film

y_{iO₂} = volume fraction of O₂ inside package

y_{eO₂} = external O₂ volume fraction

y_{oO₂} = internal optimum O₂ volume fraction

W = weight of produce (Kg)

R_{O₂} = O₂ consumption (respiration) rate

R_{CO₂} = CO₂ production rate

p = atmospheric pressure (1 atm)

Both Equations (43) and (44) are similar to Jurin and Karel's (1963) Equation (22).

Generally, all the models are based on mathematically combining respiration and permeability characteristics of a given MAP/fresh produce system, and therefore, the outcomes are similar. However, this does not limit the power of such mathematical models in facilitating material and product selection or package design in order to optimize gas concentration through the shelf life of a MAP system. Most importantly, they represent a scientifically sound tool as opposed to a trial and error approach to develop MAP applications. Due to the mathematical nature of such models, it is possible to further advance the equations to include additional parameters and future developments in packaging materials or produce.

CASE STUDY OF FRESH-CUT APPLES

Gunes et al. (2001) studied cut 'Red Delicious' apple wedges stored at 5°C and treated under the following MA conditions: O₂ (0.5, 1, 10, 21%) and CO₂ (0, 7.5, 15, 30%). The samples that were treated with 30% CO₂ and 0.5% O₂ yielded the lowest fermentation products and ethylene production. The respiration rate measured for those samples was R_{CO₂} = 2.1 Kg/hr and RQ = 1.9. According to Exama et al. (1993b), calculations of the required gas permeabilities for whole 'Delicious' apples, the dimensions of the packages used are as follows:

Area of the film (A) = 1320 cm², film thickness = 1 mil, weight of apples = 2.27 Kg, bulk volume of apples = 3818 cm³, container volume = 5090 cm³, and headspace volume = 1272 cm³. Gas concentrations information is optimum CO₂ concentration is y_{oCO₂} = 0.30, optimum O₂ is y_{oO₂} = 0.005, external CO₂ and O₂ concentrations are y_{eCO₂} = 0, and y_{eO₂} = 0.21, respectively. Using Equations (43) and (44), the calculated required permeability for fresh-cut apples is as follows:

$$\begin{aligned} P_{\text{CO}_2}^R &= WR_{\text{CO}_2}L/Ap(y_{\text{O}_2}\text{CO}_2 - y_{\text{eCO}_2}) \\ &= \frac{(2.27 \text{ Kg})(2.1 \text{ ml/Kg} \cdot \text{hr})(1 \text{ mil})}{(1320 \text{ cm}^2)(0.3 \text{ atm} - 0.0 \text{ atm})} \end{aligned}$$

$$\begin{aligned} P_{CO_2}^R &= 0.012 \text{ mL mil/cm}^2 \text{ hr atm} \\ P_{O_2}^R &= \frac{WR_{O_2}L}{Ap(yeO_2 - yoO_2)} \\ &= \frac{(2.27 \text{ Kg})(1.1 \text{ ml/Kg} \cdot \text{hr})(1 \text{ mil})}{(1320 \text{ cm}^2)(0.21 \text{ atm} - 0.005 \text{ atm})} \\ P_{O_2}^R &= 9.228 \times 10^{-3} \text{ mL mil/cm}^2 \text{ hr atm} \end{aligned}$$

Therefore, the permselectivity required for the product if stored under the studied MA conditions would be

$$\begin{aligned} P_{CO_2}^R/P_{O_2}^R &= \frac{0.012 \text{ mL mil/cm}^2 \text{ hr atm}}{9.228 \times 10^{-3} \text{ mL mil/cm}^2 \text{ hr atm}} \\ P_{CO_2}^R/P_{O_2}^R &= 1.3 \end{aligned}$$

Figure 10.6 shows how permselectivity can be applied to cut fruit, ‘Red Delicious’ wedges in this case, given the recommended MA conditions (i.e., 30% CO₂ and 0.5% O₂). Figure 10.6, generated using the same approach as Figure 10.5, plots

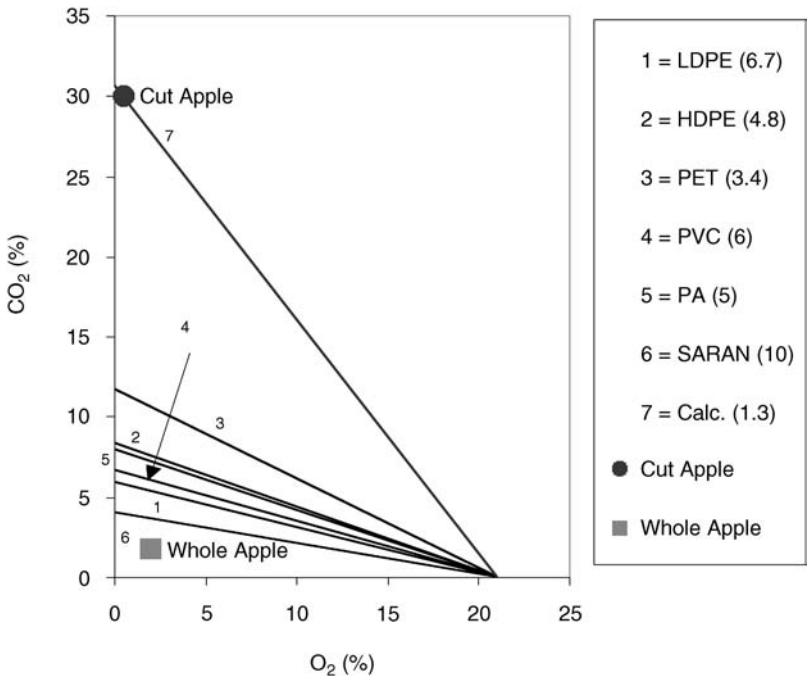


FIGURE 10.6 Calculated optimum permselectivity for fresh-cut apple vs. fresh whole apple.

optimum atmospheres for both whole and cut apple wedges along with the calculated permselectivity required for cut apples (line 7). The permselectivities provided by several commonly available polymeric films are also plotted. None of the films presented in the figure pass through the "cut apple" area, and thus, none perform satisfactorily to create the recommended MA for the cut apples (30% CO₂ and 0.5% O₂). Only the calculated (1.3) permselectivity line (7) passes through "cut apples." This supports the theoretical findings discussed earlier and demonstrates the need to develop films with lower β values than currently available polymers provide.

CONCLUSIONS

This example shows that the challenges facing the MAP of FCF cannot be overcome without considering the barrier properties of packaging material. Successful design and application of MAP for FCF must consider the CO₂/O₂ permselectivity as an important property as gas permeability of a polymeric film. If gas permeability coefficients can be viewed as a property that must match the respiration rate requirement of fresh produce, then permselectivity must be viewed as a property that must match the respiration quotient of the fresh produce. However, because most of the MAP application is used for fresh whole produce, the permselectivity of common films may satisfy the required permselectivity with limited success that is not usually detected by the consumer. On the other hand, common films fail what is required by fresh-cut fruits, and therefore, rapid deterioration of the produce is evident. Therefore, material scientists and engineers must cooperate with plant physiologists to innovate polymeric films that can meet and satisfy the permeability and CO₂/O₂ permselectivity required by FCF.

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11 Biotechnology and the Fresh-cut Produce Industry

Jennylynd A. James

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INTRODUCTION

Biotechnology can be defined as the manipulation of biological systems, living organisms or their derivatives to make products or modify processes for a determined end use. This technology has been implemented for centuries with the discovery of fermentation techniques and plant breeding by ancient civilizations. In its modern

form, biotechnology has assumed new methods of altering genetic information of organisms, the most significant of these being genetic engineering. The tools of biotechnology have been used in recent times in food processing and medicine to produce enzymes at commercial levels. Biotechnology has enhanced life and will continue to do so by providing useful tools for human health and nutrition.

Plant breeding has been conducted by many cultures over centuries to improve crop yields and produce varieties of crops with new and improved qualities. It is only within the last two decades, with the development of biotechnology tools, that new technologies have been included in plant breeding. Scientists have found a way to transfer genes coding for desired characteristics into plants to produce viable new crops. Most of the success has been achieved in sustainable agricultural crops like corn, wheat and other grain. In recent years, scientists have been investigating ways to enhance sensory qualities of perishable crops like fruits and vegetables, to extend shelf life for the fresh market and processing and to improve color, flavor and other qualities. The FLAVR SAVRTM tomato was the first fresh produce item to get attention for commercial production (Calgene, Inc., Davis, California).

The use of biotechnology to improve the food supply has had mixed reactions worldwide. It is seen by some as a fast and efficient improvement on traditional breeding techniques. Purists have discredited the technology saying that scientists are tampering with nature. Several methods are used to identify genetically modified organisms (GMOs) including polymerase chain reaction (PCR) technology and enzyme-linked immunosorbent assay (ELISA). Before GMOs can be commercialized, they are supposed to be tested extensively to ensure that no harmful effects have been introduced and the new product is "substantially equivalent" to the old. Are safety assessment methods thorough and statistically sound or are the methods outdated and in need of revision as new technologies are introduced? There is no sufficient data available to disprove long-term damage to humans or the environment.

The fear of the unknown has triggered hysteria in some regions of the world. Trade barriers are being instituted to prevent entry of genetically modified foods. Calls are being made to growers and manufacturing operations who use GMOs to label products as "Genetically Modified." Labeling of a product is thought to be one way of giving consumers the opportunity to choose and be informed. Labeling is, however, very expensive to implement, and the benefits of labels have been disputed by many in the produce industry. The labeling of a GMO may have negative connotation for a crop that is perfectly sound. Also, new laws are being formulated by government regulators to encompass GMOs that were not, until recently, given any separate regulatory attention, apart from any new food being introduced. No uniformity in regulation yet exists worldwide. Thus, future trade will become more and more complicated.

This review seeks to focus on some of the prominent applications of agricultural biotechnology. Mention is made of the work done to develop new crops and of the laws and regulations used as guidelines in the production of genetically engineered foods. The possibilities of agricultural biotechnology are limited only by one's imagination. It is hoped that this technology will not be overregulated

so as to stifle the production of ingenious food products or the solution of problems faced in the food industry and agriculture. Because human subjects cannot be used for safety assessment studies, as with other new technologies, only time will tell the effects.

APPLICATIONS OF BIOTECHNOLOGY IN AGRICULTURE

HISTORICAL PERSPECTIVE AND CONVENTIONAL PLANT BREEDING

Agricultural biotechnology in the fundamental sense could be traced to human activity for thousands of years. Traditional breeding methods have introduced substantial improvements to generate crops that would not otherwise occur in nature. One successful example of the use of traditional breeding methods that evolved through history was the work done by Native Americans over 8000 years to develop modern corn from a wild plant called teosinte. The ancient ancestor of corn was small and did not possess the large, succulent grain of the modern variety. However, through years of crossbreeding and selection of viable plants that bore these large grains, the modern variety evolved into what we know today. The history of agricultural biotechnology is summarized in Table 11.1 (IFT Expert Committee, 2000a,c).

The origin of the concept of inheritance may be traced back to the times of ancient Greece when Theophrastus, a student of Aristotle, first recognized the analogy between animal and plant reproduction and coined the words male and female to describe the participants in sexual reproduction. However, the concept of a gene began with Gregor Mendel in the 1860s, although the word itself was not formed until scientists repeated and extended Mendel's work during the early twentieth century. The term "gene" was introduced by W. Johannsen in 1910 and referred to a hypothetical unit of information that determines the inheritance of an individual characteristic in an organism. The existence of genes was inferred from the statistical distribution of simple heritable traits as studied by Mendel in plant breeding experiments. The Mendelian view of inheritance in eukaryotes was defined by the occurrence of independent segregation and the independent assortment of different allelic pairs of genes, each specifying a different trait.

Crossbreeding methods are used to transfer the complete set of genes from the parent plants to the new offspring. Not only are one or two desired genes introduced, but thousands of other genes, some benign and some undesirable, are also exchanged. Expensive, time-consuming methods are used to remove unwanted genes and to retain the desired improvements. One conventional breeding method used to reshuffle genes is the production of "double-cross hybrids." The breeder crosses a variety X with Y to produce Z, variety A is crossed with B to make C, then C and Z are crossed to produce the desired seed. Another conventional breeding method that is widely accepted is "mutation breeding," in which ionizing radiation or other mutagenic agents are used to create genetic changes. Conventional breeding methods are limited in that if a desired gene is not available or cannot be mutated from an existing gene, then the desired trait cannot be achieved (McHughen, 2000).

TABLE 11.1
History of Agricultural Biotechnology and Recent Applications
of New Technologies

Date	Discovery
2000 BC	Cultivation
19th Century	Selective crossbreeding (in Europe—Gregor Mendel)
Early 20th Century	Mutagenesis and selection
Mid 20th Century	Cell culture
1930s	Somoclonal variation
1940s	Embryo rescue
1950s	Polyembryogenesis
1970s	Anther culture
1970	Norman Borlaug, first plant breeder to win a Nobel Prize for his work in Green Revolution wheat varieties (high yield)
1980	Recombinant DNA
1980s	Marker-assisted selection
1980s and 1990s	Chloroplast transformation to increase transformation efficiency and control gene flow Cobombardment using the gene gun to add multiple genes simultaneously Transposon tagging, positive selection exclusive energy sources, an effective alternative to antibiotic selection. Targeted site-specific recombination to target gene inserts to specific sites in plant tissue Chimeraplasty to create subtle alterations in the plant's own genes, for example, to produce herbicide tolerance without introducing novel genes
1990s	Genomics
1994	The FLAVR SAVR TM tomato, first product of agricultural biotechnology approved for U.S. grocery stores
1995	Soybeans, products of agricultural biotechnology, introduced on the market
1997	U.S. government accepted 18 crop applications of biotechnology
1999	Development of 'Golden Rice' which contained beta-carotene, a precursor of vitamin A
2000 and beyond	Bioinformatics Designing plants for herbicide tolerance, insect protection, disease protection, improved nutrition profiles

Source: Adapted from McGloughlin (2000) and Council for Biotechnology Information (2000).

Organisms have been genetically engineered to overproduce bioingredients and improve food fermentations, especially in the making of wine, beer and cheese. Genetic engineering has also been used in the detection of food pathogens, using DNA probes and monoclonal antibodies, and for the effective utilization of food-processing wastes. Recombinant DNA technology has provided powerful and novel approaches to understanding the complex mechanisms by which eukaryotic gene expression is regulated, and this technology could provide countless benefits and improvements in food production.

DEFINITIONS — TRANSGENIC CROPS AND GMOs

Transgenic crops are those plants that are derived using recombinant DNA technology and other biotechnologies to form a new variety that expresses the trait coded by the inserted gene(s). Recombinant DNA methods enable breeders to select, transfer or modify single genes thereby eliminating the need to “select out” undesirable genes. R-DNA technology allows the insertion of useful genes from any other species. Genetically modified (GM) refers to the fact that the plant genome has been altered by the addition or removal of a gene. In order to successfully transfer a gene or genes coding for a specific trait, the plant genome should be sequenced and the gene function elucidated, an area of study called genomics. There is functional genomics that studies specific traits from gene codes, as well as structural genomics that includes the genetic mapping, physical mapping and sequencing of entire genomes. Many databases already exist to distribute molecular information. However, the post-genomic era will require many more to collect, manage and publish the influx of new research results. The future of agricultural biotechnology will depend in part on advances in sequencing, genome analysis and information technology to characterize beneficial genes for crop improvement.

COMPARISON OF TRADITIONAL PLANT BREEDING AND GENETIC MODIFICATION OF CROPS

Traditional biotechnology has been used for thousands of years, since the advent of the first agricultural practices for the improvement of plants, animals and microbes. Selective breeding was used to exchange genetic information between two related plant parents, producing progeny which had desired properties, for example, increased yields and improved taste. Traditional breeding, however, requires that the two plants being crossed be closely related or of the same species. Thus, active plant breeding has led to the development of superior plant varieties over centuries, far more rapidly than random mating in the wild. Traditional breeding is, however, time-consuming and many times the characteristic of interest does not occur in a related species. This is where modern biotechnology methods have assisted traditional breeding methods.

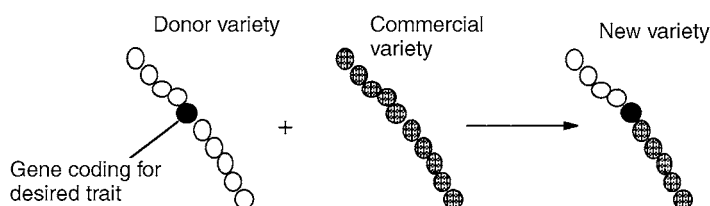
Some scientists consider genetic engineering an extension of conventional breeding, while others hold the view that it differs profoundly. Conventional breeding develops a new plant variety by the process of selection, and genetic material that is already present within a species is expressed. The exception to this would be hybridization, wide crosses and horizontal gene transfer (Hansen, 2000). Genetic engineering allows the insertion of a gene, and this must be followed up by selection. A promoter gene from a virus is usually inserted to make the new gene express itself. This whole process is significantly different from conventional breeding, even if the goal is to insert genetic material from the same species. There is an increase in precision when the gene carrying the trait of interest is known. Also using genetic engineering techniques, the potential sources from which desirable traits may be obtained are increased. The entire span of genetic capabilities available among all biological organisms can potentially be used in any other organism.

There are major differences in the success rates of conventional breeding vs. use of genetic engineering. In nature, most offspring are viable, and in conventional breeding, scientists grow many plants and keep only a few with the most desirable traits. In the early development of genetic engineering, although cells containing the inserted gene of interest were selected, it was still necessary to grow whole plants from these cells to determine whether the gene was expressed giving the desired trait. A large percent of engineered cells were not viable or failed to produce the desired trait. In successive plant generations, only one in thousands (or millions) of cells is able to incorporate the desired trait and express this for generations without producing undesirable side effects. This indicates that genetic engineering is not as efficient as it is advertised to be (Hansen, 2000; Walden and Wingender, 1995).

Genetic engineering controls the trait that is to be introduced, yet cannot control the location of introduction. Traditional breeding, however, occurs between organisms that share a recent evolutionary history, so shuffling occurs around alleles, different versions of the same gene. These genes are usually fixed in location on the chromosome by evolution. A foreign promoter, usually from a plant virus, is used to enhance the expression of transgenes in genetic engineering, and the introduction of foreign DNA is not used in traditional breeding (Meyer, 1995).

Figure 11.1 shows a schematic comparison of conventional breeding methods vs. genetic engineering techniques. The precision of transforming DNA with the gene of interest eliminates the need to do a series of back crossing to remove unwanted properties in traditional breeding methods. Table 11.2 summarizes the differences between conventional breeding and genetic engineering. One of the main technical

Conventional Breeding



Agricultural Biotechnology

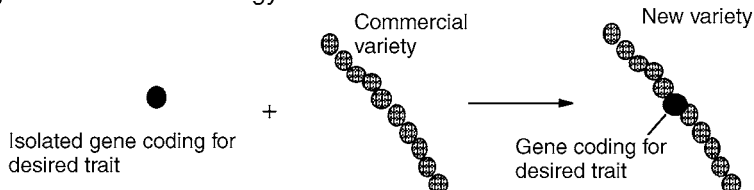


FIGURE 11.1 Classical plant breeding vs. genetic engineering. Traditional plant breeding combines many genes at one time, while in plant biotechnology, a single gene may be added to the commercial variety.

TABLE 11.2
Classical Breeding vs. Genetic Engineering

Classical Breeding	Genetic Engineering
(A) Use living plant machinery	Use plant machinery in the laboratory
(B) Random genetic exchange using entire genomes of both plants	There is an exchange of a specific gene or genes
(C) Breeding must occur between closely related species	Genes from plants or other organism may be used to transform the plant
(D) Control of gene expression depends on source of genetic material	When and where the gene is expressed can be controlled
(E) Inclusion of ancillary, unwanted traits that must be eliminated by generations of back crossing	Precise traits are transferred
(F) Progress is lengthy	If the gene coding for a trait is known, there is rapid development of varieties with new and desirable traits

limitations in the improvement of plants using r-DNA technology is that there is not enough genomic data for all commercially produced crops. The DNA sequences of all plants must be studied to identify the location and function of all genes. Experiments have shown that many sequences are conserved among species, and the same gene confers the same trait in different species (Persley and Siedow, 1999).

There are, thus, key scientific differences between genetic engineering and conventional breeding, in terms of the process and the genetic makeup of the product. Recoverable DNA from genetically engineered plants would usually reveal a viral promoter, genetic material from *Agrobacterium* and, in many cases, a bacterial antibiotic marker gene. These are never deliberately introduced in conventional breeding of plants.

COMMERCIALY AVAILABLE GENETICALLY MODIFIED CROPS

Agricultural biotechnology has been applied in the improvement of agronomic and quality traits, as well as the production of novel crop products and renewable resources. This technology has been used to develop crops with pesticide resistance, improved yield, ability to use marginalized land, improved nutritional benefits, reduced environmental impact and pharmaceutical benefits like vaccines (Third World Academy of Sciences, 2000). Early products of agricultural biotechnology focused mainly on agronomic traits like those related to biotic stress: insect resistance, disease resistance (viral, bacteria, fungal, nematode) and weed-herbicide tolerance. Work has been done to provide relief from abiotic stresses like drought, cold, heat and poor soils, and to improve yields by nitrogen assimilation, starch biosynthesis and oxygen assimilation (Wilkinson, 1997).

Research to improve quality traits has been developed in the areas of processing properties: extension of shelf life; altering reproductive methods by creating sex barriers, male sterility and seedlessness; production of nutraceutical plants with improved protein, carbohydrates, fats, vitamins, etc.; and plants with the ability to

remove toxins and phytase. Improvements in taste, architecture, fiber content and in ornamentals, changing color, shelf life, morphology and fragrance are all quality traits geared for improvement using biotechnology. Novel crop products like oils with special properties, proteins and polymer production, as well as vaccine production in plants, are some of the future applications of agricultural biotechnology.

Products found initially on the market would not be specialty items but staples like flour, meal or oil extracted from genetically modified crops. Commercial production of novel transgenic crops first focused on agronomic benefits like increased productivity of crops with reduction of production by decreasing the need for inputs of pesticides. This has been studied mostly in crops grown in temperate zones. The intense and expensive research and development of transgenic plants over two decades has led to the commercial production of new varieties over the last four years (Persley, 1999).

Herbicide resistance has allowed the possibility of reducing chemical application of herbicides during large-scale farming. The application of agricultural biotechnology could mean an improvement in the quality of life, because new strains of plants, giving higher yields with fewer inputs, will be grown in a broader range of environments. Natural resources will be conserved, providing more nutritious products with a longer shelf life at an economical cost to consumers. Multiple benefits for growers include more flexibility in terms of crop management (especially for herbicide-tolerant crops), decreased dependency on conventional insecticides and herbicides, higher yields and cleaner and higher grades of grain/end products (Vasil et al., 1992).

Commercial products that have been enhanced using biotechnology can be found in Table 11.3. Preliminary work focused on large-volume, sustainable agricultural crops like corn, soybeans and potatoes that require fewer applications of herbicides. Biotechnology-enhanced soybeans have been created with a lower saturated fat content and higher oleic acid content, thus offering better frying stability of soya bean oil without further processing. 'Roundup Ready' soybean is one example of a transgenic crop with herbicide tolerance. U.S. farmers are said to have saved an estimated \$330 million in 1998 because of lower herbicide costs in growing "Roundup Ready" soybeans. There was a reduction in crop injury and an improvement in weed control. The application also encourages adoption of no-till, which saves the environment from erosion. Herbicide-tolerant crops also benefit water quality. "Roundup Ready" corn was planted at five Illinois watersheds in 1999. All sheds had past problems of atrazine in excess of 50 ppb, however, samples collected in 1999 were above the 3 ppb standard for the five watersheds using "Roundup Ready" corn (McGloughlin, 2000). The U.S. farmer benefited with 76% of the \$100 million saved in 1997 using "Roundup Ready" soybean. The consumer benefited only 4% (Falck-Zepeda et al., 1999).

The problems of the Colorado potato beetle (CPB) and corn rootworms (CRWs) caused billions of dollars in losses to farmers in the United States in the past because of loss of crops and increased pesticide application costs. Thus, using agricultural biotechnology to create insect resistance in crops was a very exciting prospect for farmers (NRC, 2000a; Vaek et al., 1987). The first insect-resistant crops were tobacco and tomato plants that used a native truncated lepidopteran-specific insect toxin gene from *Bacillus thuringiensis* subsp. *kurstaki* (Btk). The field testing conducted in

TABLE 11.3
Beneficial Crops Derived Using Biotechnology

Year/Firm	New Variety	Trait Gene and Source
2000		
Aventis	Male-sterile corn	The barnase gene from <i>Bacillus amyloliquefaciens</i>
1999		
Agripto Inc.	Modified fruit-ripening cantaloupe	S-adenosylmethionine hydrolase gene from <i>Escherichia coli</i> bacteriophage T3
BASF AG	Phytaseed canola	The phytase gene from <i>Aspergillus niger</i> var. van Tieghem
Rhone-Poulenc Ag. Co.	Bromynil-tolerant canola	The nitrase gene from <i>Klesiella pneumoniae</i> subsp. <i>ozaenae</i>
1998		
AgrEvo, Inc.	Glufosinate-tolerant soybean	Phosphinothricin acetyltransferase gene from <i>Streptomyces viridochromogenes</i>
	Glufosinate-tolerant sugar beet	Phosphinothricin acetyltransferase gene from <i>S. viridochromogenes</i>
	Insect-protected and glufosinate-tolerant corn	The cry9C gene from <i>Bacillus thuringiensis</i> (Bt) subsp. <i>tolworthi</i> and the bar gene from <i>Streptomyces hygroscopicus</i>
	Male-sterile or fertility-restorer and glufosinate-tolerant canola	The male-sterile canola contains the barnase gene, and the fertility-restorer canola contains the barstar gene from <i>B. amyloliquefaciens</i> , both lines have the phosphinothricin acetyltransferase gene from <i>S. viridochromogenes</i>
Calgene, Inc.	Bromoxynil-tolerant/insect-protected cotton	Nitrilase gene from <i>Klebsiella pneumoniae</i> and the cryIA(c) gene from <i>B. thuringiensis</i> subsp. <i>kurstaki</i>
	Insect-protected tomato	The cryIA(c) gene from <i>B. thuringiensis</i> subsp. <i>kurstaki</i>
Monsanto Co.	Glyphosate-tolerant corn	A modified enolpyruvylshikimate-3-phosphate synthase gene from corn
	Insect- and virus-protected potato	The cryIIIA gene from <i>B. thuringiensis</i> sp. <i>tenebrionis</i> and the Potato Leaf Roll Virus replicase gene
	Insect- and virus-protected potato	The cryIIIA gene from <i>B. thuringiensis</i> sp. <i>tenebrionis</i> and the Potato Virus Y coat protein gene
Monsanto Co./Novartis	Glyphosate-tolerant sugar beet	The enolpyruvylshikimate-3-phosphate synthase gene from <i>Agrobacterium</i> sp. strain CP4, and a truncated glyphosate oxidoreductase gene from <i>Ochrobactrum anthropi</i>
Pioneer Hi-Bred University of Saskatchewan	Male-sterile corn	The DNA adenine methylase gene from <i>E. coli</i>
	Sulfonylurea-tolerant flax	Acetolactase synthase gene from <i>Arabidopsis</i>
1997		
AgrEvo, Inc.	Glufosinate-tolerant canola	Phosphinothricin acetyltransferase gene from <i>S. viridochromogenes</i>

(continued)

TABLE 11.3
Beneficial Crops Derived Using Biotechnology (Continued)

Year/Firm	New Variety	Trait Gene and Source
Bejo Zaden BV	Male-sterile radichio rosso	The barnase gene from <i>B. amyloliquefaciens</i>
Dekalb Genetics Corp.	Insect-protected corn	The cryIA(c) gene from <i>B. thuringiensis</i>
DuPont	High-oleic-acid soybean	Sense suppression of the GmFad2-1 gene which encodes a delta-12 desaturase enzyme
Seminis Vegetable Seeds	Virus-resistant squash	Coat protein genes of Cucumber Mosaic Virus Zucchini Yellow Mosaic Virus and Watermelon Mosaic Virus
University of Hawaii/Cornell University	Virus-resistant papaya	Coat protein gene of the Papaya Ringspot Virus
1996		
AgriPro Inc.	Modified fruit ripening tomato	S-adenosylmethionine hydrolase gene from <i>E. coli</i> bacteriophage T3
Dekalb Genetic Corp.	Glufosinate-tolerant corn	Phosphinothricin acetyl transferase gene from <i>S. hygroscopicus</i>
DuPont	Sulfonylurea-tolerant cotton	Acetolactate synthase gene from tobacco, <i>Nicotiana tabacum</i> cv. <i>Xanthi</i>
Monsanto Co.	Insect-protected potato	The cryIIIA gene from <i>B. thuringiensis</i>
	Insect-protected corn	The cryIA(b) gene from <i>B. thuringiensis</i> subsp. <i>kurstaki</i>
	Insect-protected corn	The cryIA(b) gene from <i>B. thuringiensis</i> subsp. <i>kurstaki</i>
Northrup King Co.	Glyphosate-tolerant/insect-protected corn	The endopyruvylshikimate-3-phosphate synthase gene from <i>Agrobacterium</i> sp. strain CP4 and the glyphosate oxidoreductase gene from <i>O. anthropi</i> in the glyphosate tolerant lines; the cryIA(b) gene from <i>B. thuringiensis</i> subsp. <i>kurstaki</i> in lines that are also insect protected
Plant Genetic Systems NV	Insect-protected corn	The cryIA(b) gene from <i>B. thuringiensis</i> subsp. <i>kurstaki</i>
	Male-sterile and fertility-restorer oilseed rape	The male-sterile oilseed rape contains the barnase gene from <i>B. amyloliquefaciens</i> , the fertility-restorer lines express the barstar gene from <i>B. amyloliquefaciens</i>
	Male-sterile corn	The barnase gene from <i>B. amyloliquefaciens</i>
1995		
AgriEvo Inc.	Glufosinate-tolerant canola	Phosphinothricin acetyltransferase gene from <i>S. viridochromogenes</i>
	Glufosinate-tolerant corn	Phosphinothricin acetyltransferase gene from <i>S. viridochromogenes</i>
Calgene, Inc.	Laurate canola	The 12:0 acyl carrier protein thioesterase gene from California bay, <i>Umbellularia californica</i>

TABLE 11.3
Beneficial Crops Derived Using Biotechnology (Continued)

Year/Firm	New Variety	Trait Gene and Source
Ciba-Geigy Corp.	Insect-protected corn	The cryIA(b) gene from <i>B. thuringiensis</i> subsp. <i>kurstaki</i>
Monsanto Co.	Glyphosate-tolerant corn	Enolpyruvylshikimate-3-phosphate synthase gene from <i>Agrobacterium</i> sp. strain CP4
	Glyphosate-tolerant canola	Enolpyruvylshikimate-3-phosphate synthase gene from <i>Agrobacterium</i> sp. strain CP4
	Insect-protected cotton	The cryIA(c) gene from <i>B. thuringiensis</i> subsp. <i>kurstaki</i>
1994		
Asgrow Seed Co.	Virus-resistant squash	Coat protein genes of Watermelon Mosaic Virus 2 and Zucchini Yellow Mosaic Virus
Calgene, Inc.	FLAVR SAVR™ tomato	Antisense polygalacturonase gene from tomato
DNA Plant Technology Corp.	Bromoxynil-tolerant cotton	A nitrilase gene isolated from <i>Klebsiella ozaenae</i>
Monsanto Co.	Improved-ripening tomato	A fragment of the aminocyclopropane carboxylic acid synthase gene from tomato
Zeneca Plant Science	Glyphosate-tolerant soybean	Enolpyruvylshikimate-3-phosphate synthase gene from <i>Agrobacterium</i> sp. strain CP4
	Improved-ripening tomato	Aminocyclopropane carboxylic acid deaminase gene from <i>Pseudomonas chloraphis</i> strain 6G5
	Insect-protected potato	The cryIIIA gene from <i>B. thuringiensis</i> sp. <i>tenebrionis</i>
	Delayed-softening tomato	A fragment of the polygalacturonase gene from tomato

Source: Reprinted with permission from the Institute of Food Technologists (IFT Expert Committee, 2000c).

1987 and 1988 showed a reduction in damage caused by the tomato fruit worm. However, the expression of the Bt protein was too low for commercial use. A redesigned synthetic Bt gene was created with a 500-fold increase in expression as compared to the wild type. Transgenic potato plants expressing the synthetic gene from *B. thuringiensis* subsp. *tenebrionis* at high levels exhibited strong resistance to CPB in a large number of field trials. This new potato crop introduced in the mid 1990s significantly reduced the use of undesirable insecticides in the environment, as well as saved farmers millions in insecticide application (Shah et al., 1995).

Insect damage was also a serious problem for cotton crops with lepidopteran insects such as cotton bollworm, being responsible for US \$216 million in losses in the mid 1990s. Genetically modified cotton plants with an agronomically useful level of resistance to bollworm were developed through the expression of synthetic Btk gene at high levels. Also, the European corn borer (ECB) was a major corn pest in North America and Europe, causing loss in yield of 3–7% annually (Shah et al., 1995). The economic losses in Illinois alone amounted to US \$50 million in 1995.

A synthetic Btk gene was introduced into corn to provide resistance to ECB. Commercialization of high-valued, insect-resistant crops decreased the use of chemical insecticides and chemical residues in the environment. There was the concern, however, that the use of Bt proteins for the control of insects would develop insect resistance to Bt toxins. Thus, resistance-management strategies are combined with integrated pest management (IPM) procedures. Some strategies include using the high-dose expression of Bt genes, using host plants for sensitive insects (Koziel et al., 1993, 1996). Also, agronomic practices that minimize insect exposure to Bt and an IPM system are important. Long-term strategies use the multiple genes encoding insect-control proteins with unique modes of action in the same crop. Research has been conducted to find non-Bt proteins to control insect pests. Examples of insecticidal plant proteins include lectins, amylase inhibitors and protease inhibitors that can retard insect growth and development when ingested at high levels. These compounds, however, do not provide the same mortality rate of Bt.

An indirect benefit of cultivating Bt corn is a decreased risk of low grain quality by mycotoxins. Research showed that ears and grain from 'YieldGard' corn were less contaminated by fusarium and fumonisin than conventional corn. Bt crops had improved insect control, improved farm efficiency, reduced crop injury with improved quality and reduced insecticide exposure. A large percent of the benefits (42%) of growing Bt corn accrued to the farmer in 1997. In 1998, 22 of the 75 million acres of corn planted were Bt corn. In a November 1999 Iowa State study, 26% of the farmers planting Bt corn were able to reduce their pesticide use, and there were at least 6 million acres of land with little or no pesticide application [International Food Information Council (IFIC), 1999]. Also in 1999, adopters of genetically engineered corn, soybeans and cotton combined used 7.6 million fewer acre treatments (2.5%) of pesticide than nonadopters in 1997 (Falck-Zepeda et al., 1999). Fewer pounds of insecticide (2 million) were used in 1998 to control bollworm and budworm in 1998 than in 1995, before the introduction of Bt cotton. Cotton farmers increased their yields by 85 million pounds and made \$92 million more than those who did not adopt the new technology (McGloughlin, 2000).

Annual losses of over \$100 billion are incurred because of nematode damage to crops (Atkinson et al., 1995). Plant-parasitic nematodes are quite numerous and have adopted subtle yet damaging interactions with host plants. Nematicides are used to control these pests, but in many cases, these hazardous chemicals are not effective. Agricultural technology may offer one means of controlling nematodes. One approach requires promoters that direct a specific pattern for genes encoding effector proteins. Effectors may act directly against the nematode or disrupt modification of the plant cell by the parasite (Atkinson et al., 1995).

Seed companies and produce growers have taken on the challenge of using agricultural biotechnology to improve crops in the United States, and according to U.S. Secretary of State, Dan Glickman, more than 70 million acres of genetically modified crops were grown in 1999. Field corn and soybeans make up the vast majority of agricultural biotech products. However, sweet corn, squash and potatoes are also being produced (Shee, 2000).

In terms of international growth, in 1999, an estimated 40 million hectares of land worldwide, were cultivated with transgenic plants of over 20 species, the most important

TABLE 11.4
Global Area of Transgenic Crops in 1998 and 1999:
By Crop (Millions of Hectares)

Crop	1998	%	1999	%	Increase	(Ratio)
Soybean	14.5	52	21.6	54	7.1	(0.5)
Corn	8.3	30	11.1	28	2.8	(0.3)
Cotton	2.5	9	3.7	9	1.2	(0.5)
Canola	2.4	9	3.4	9	1.0	(0.4)
Potato	<0.1	<1	<0.1	<1	<0.1	(—)
Squash	0.0	0	<0.1	<1	(—)	(—)
Papaya	0.0	0	<0.1	<1	(—)	(—)
Total	27.8	100	39.9	100	12.1	(0.4)

Source: James (1999), ISAAA.

TABLE 11.5
Global Area of Transgenic Crops in 1998 and 1999:
By Country (Millions of Hectares)

Country	1998	%	1999	%	Increase 1998 to 1999 (Ratio)	
Argentina	4.3	15	6.7	17	2.4	(0.6)
Canada	2.8	10	4.0	10	1.2	(0.4)
China	<0.1	<1	0.3	1	0.2	(3.0)
Australia	0.1	1	0.1	<1	<0.1	(—)
South Africa	<0.1	<1	0.1	<1	<0.1	(—)
Mexico	<0.1	<1	<0.1	<1	<0.1	(—)
Spain	<0.1	<1	<0.1	<1	<0.1	(—)
France	<0.1	<1	<0.1	<1	<0.1	(—)
Portugal	0.0	0	<0.1	<1	<0.1	(—)
Romania	0.0	0	<0.1	<1	<0.1	(—)
Ukraine	0.0	0	<0.1	<1	<0.1	(—)
United States	20.5	74	28.7	72	8.2	(0.4)
Total	27.8	100	39.9	100	12.1	(0.4)

Source: James (1999), ISAAA.

being cotton, corn, soybean and rapeseed (James, 1999). Summaries of the global area of transgenic crops, the types of crops commercialized and the new traits promoted in transgenic crops, are contained in Tables 11.4, 11.5 and 11.6. Twelve countries, eight industrial and four developing, have contributed to a more than 20-fold ($\times 23.5$) increase in the global area of transgenic crops between 1996 and 1999. High adoption rates of new transgenic crops reflect grower satisfaction with the products that offer significant benefits ranging from more convenient and flexible crop management,

TABLE 11.6
Global Area of Transgenic Crops in 1998 and 1999: By Trait (Millions of Hectares)

Trait	1998	%	1999	%	Increase	(Ratio)
Herbicide tolerance	19.8	71	28.1	71	8.3	(0.4)
Insect resistance (Bt)	7.7	28	8.9	22	1.2	(0.2)
Bt/herbicide tolerance	0.3	1	2.9	7	2.6	(8.7)
Virus resistance/other	<0.1	<1	<0.1	<1	<0.1	(—)
Global totals	27.8	100	39.9	100	12.1	(0.4)

Source: James (1999), ISAAA.

higher productivity or net returns/hectare and a safer environment through decreased use of conventional pesticides, leading to more sustainable agriculture (James, 1999).

Seven major transgenic crops were commercialized in 1999. They were soybean, corn/maize, cotton, canola/rapeseed, potato, squash and papaya (Table 11.4). The traits of significant importance were herbicide tolerance and insect resistance. There was also an increase in production of crops with virus resistance traits in 1999, mainly potatoes, squash and papaya, less than 1% annual acreage. The global market for transgenic crops has grown rapidly over five years, from \$75 million in sales in 1995 to an estimated \$3 billion in sales in the year 2000. This is projected to increase to \$25 billion in 2010 (James, 1999). The global production of transgenic crops increased from 4.3 million acres in 1996 to 98.6 million acres in 1999. The United States accounted for 72% of the global area, followed by Argentina (17%), Canada (10%), China (1%) and Australia and South Africa (0.23 million acres).

As agricultural biotechnology applications increase, there will be a change of focus from beneficial profiles for growers to benefits for consumers in the high-value-added markets. The pace of biotechnology-driven consolidations in industry was slower in 1999 than in the previous three years. Most of the large multinational companies with investments in seeds, crop biotechnology and crop protection are restructuring and downsizing programs, and this could lead to new alliances and mergers. A global review of commercialized transgenic crops was written by James (1999).

BIOTECHNOLOGY APPLICATIONS IN FRUIT AND VEGETABLE PRODUCTION

As expansion of transgenic crops continues, there will be a shift from the current generation of “input” agronomic traits to the next generation of “output” quality traits, which will result in improved and specialized nutritional food and feed products to satisfy the high-value-added market. This movement will broaden the beneficiary profile from growers to consumers, perhaps improving public acceptance (James, 1999).

Enhancement of Product Quality and Shelf Life

Improvements targeted in the fresh fruits and vegetables and fresh-cut industry include improved sweetness in peas and producing higher crop yields, smaller seedless melons

for use as single servings, delayed ripening in bananas and pineapples and fungal resistance in bananas. Recent successes in enhancing fruits and vegetables for the fresh fruit and processing markets include the biotechnological modification of tomatoes to soften slower and remain on the vine longer, resulting in more flavor and color. Tomatoes are one of the most important world crops in terms of sales and their overall contribution to nutrition. Tomatoes are used either fresh or for processing, and thus, distinct qualities are required for the different markets. Fresh tomatoes should have an acceptable flavor and firm structure, while processing tomatoes require suitable rheological characteristics for production of juice, ketchup and other products. A systematic approach was undertaken to understanding tomato ripening at the molecular level, and ripening was controlled to improve quality and reduce postharvest losses. Two methods to achieve this were by altering the activity of cell wall enzymes that are involved in softening or blocking the biosynthesis of ethylene, the fruit-ripening hormone (Schuch, 1994).

Scientists at Zeneca Plant Science in Germany were able to study the hormones responsible for the rate of ripening and the biosynthesis and deposition of carotenoids in chromoplasts determining color pigment production. They also studied the metabolism of sugars and acids involved in flavor development and factors affecting fruit firmness in modifying the structure and composition of cell walls. Genes controlling these quality parameters were identified. Polygalacturonase, which hydrolyzes α -1,4 linkages in the polygalacturonic (PG) acid component of cell walls, and pectinesterase (PE) were identified as major cell-wall-modifying enzymes formed during tomato ripening. The plant genome was modified to introduce genes in an antisense orientation (Smith et al., 1988, 1990), thus reducing levels of these enzymes in ripened tomatoes. Genetically modified tomatoes had improved processing characteristics for Bostwick viscosity, serum consistency and soluble solids content. The fresh market attributes of low PG tomatoes led to improved postharvest handling (Kramer et al., 1992; Schuch et al., 1992; Gray et al., 1993). They were able to last on the vines for a longer time, thus developing more flavor. Calgene, a California-based biotechnology company, developed and commercialized a product called the FLAVR SAVRTM tomato for the fresh market using this technology. The product was not commercially successful, however, mainly because of problems in marketing and ability to supply large volumes.

Tomatoes have also been cloned with disease resistance genes. The Pto gene that confers resistance to bacterial speck has been cloned from wild tomato species. Programmed cell death in plants (apoptosis) has been studied, elucidating the pathways linking PCD to disease, expression of novel genes or signal molecules to alter pathogen invasion or symptom expression. Tomato plant resistance to the fungus *Alternaria alternata* sp. *lycopersici* was achieved by the transformation of tomato plants with an inhibitor of PCD, the baculovirus p35 gene. Genetically modified plants thrived in the presence of the fungus, compared to the susceptible wild type. Lesions on inoculated fruit were much smaller. Also, the p35 expressing plant had increased resistance to bacteria, showing decreased infection in response to infection by *Pseudomonas syringae* pv. *tomato* and *Colletotrichum coccodes* (McGloughlin, 2000).

Another example of consumer benefits from agricultural biotechnology is in the development of new strains of papaya. Agricultural biotechnology has been used to create virus-resistant papayas, thus saving the Hawaiian papaya industry. The papaya ring spot virus (PRSV) has affected papaya production for decades, and traditional

breeding methods have proven unsuccessful in solving the problem. However, in 1986, with the discovery of a parasite-derived resistance gene that interfered with the reproductive cycle of the virus, a modified crop was developed. After years of testing, two seed varieties, 'UH Sunup' and 'UH Rainbow', were released to the industry. Field trials of transgenic 'UH Rainbow' and 'UH Sunup' were established in Puna, in October, 1995, and they make up 1600 of the 3200 acres of papayas grown today (Gonsalves, 1998; Shee, 2000).

Asgrow Vegetable Seeds, a California-based company, introduced a disease-resistant green-stemmed squash hybrid in June, 2000 (Packer, 2000). Three plant viruses—cucumber mosaic virus, watermelon virus and zucchini yellow mosaic virus—destroy up to 80% of U.S. squash crops during the growing seasons. Growers have planted a yellow-stemmed variety that is very susceptible to virus attack. University studies have shown that the green-stemmed variety is more suitable for the fresh-cut industry, because it does not oxidize as quickly as the yellow-stemmed variety. The first two hybrids introduced, Destiny III and Liberator III, were produced by inserting genes that improved the plant's defense against viruses, interfering with the virus' ability to reproduce. According to the Asgrow developers, the nutritional quality, taste and texture of the hybrids remain unchanged, and U.S. regulatory agencies (the USDA, FDA and EPA) have already approved the products for consumer use (Shee, 2000).

Seminis Vegetable Seeds, of Oxnard, California, is using agricultural biotechnology techniques to improve weed, insect and disease control in squash, tomatoes, melons, lettuce, peas and sweet corn. Traits that extend shelf life, allowing growers to harvest later in the growing cycle, while still obtaining product that can withstand abuses in distribution, will add value for growers, distributors, retailers and consumers (Shee, 2000). Seminis Vegetable Seeds continues work on shelf life stability of tomatoes and melons and nutritional content of tomatoes and broccoli. The company plans to introduce these new products in 2003–2005 after extensive safety evaluation and field testing and after fulfilling regulatory requirements for approval. More genetically enhanced crops will be on the market in the future, as scientists continue to improve the properties of existing produce.

Other novel produce items being developed using agricultural biotechnology are fruits without fertilization. Focus has been placed on cherry, raisin tomato, squash, eggplant, pepper, strawberry, melon and watermelon. The benefits of this are improved taste, because seedless fruits often have higher total soluble sugar (TSS) levels than seeded fruit. Benefits also include reliable crop yield, because pollination is eliminated as a factor in fruit development. In greenhouse operations, there would be a decrease in pollination-related expenses. Processing plants benefit, because there is a longer harvesting period enabling processing equipment to be used more efficiently. The seed removal stage is eliminated, thus reducing expenses. Eggplant has been produced using the auxin gene (*iaaM*) under the control of *Antirrhinum majus* ovule-specific promoter.

Nutritional and Biomedical Benefits

Agricultural biotechnology will be used to improve nutrition and quality of peppers, strawberries, raspberries, bananas, sweet potatoes and melons. A major application will be in strawberries, with a higher crop yield and improved freshness, flavor and texture.

Reduced allergens, a reduction in natural toxin levels in plants and peanuts with an improved protein balance reducing allergic reaction are some of the many benefits that are projected to be derived using agricultural biotechnology. Other potential crops that will provide health benefits include the following (IFIC, 2000a):

- tomatoes with a higher lycopene content (antioxidant)
- fruits and vegetables fortified with or containing higher levels of vitamins, such as C and E, to potentially protect against the risk of chronic diseases such as cancer and heart disease
- enhanced allicin production in garlic cloves to help lower cholesterol levels
- increased ellagic acid, a natural cancer-fighting agent, in strawberries
- improved rice proteins, using genes transferred from pea plants
- simple, fast methods for detection of pathogens, toxins and contaminants

Rice, a staple in many countries of the world, has been improved, from a nutritional standpoint, by the introduction of genes from daffodil and a bacterial strain. Swiss professor Ingo Potrykus developed a rice strain that is able to produce β -carotene, the precursor to vitamin A. This 'golden rice', as it has been named, is said to have the potential of saving millions of children worldwide from blindness and other illnesses associated with vitamin A deficiency. Potrykus collaborated with Peter Beyer of the University of Freiburg in Germany to make golden rice. Beyer, an expert in the biochemistry of daffodils, was able to provide the genes for making β -carotene. The scientific challenge of this development was the transfer of a group of genes that represented a key part of a biochemical pathway, and not just a single gene, as in previous work with transgenic crops (Nash, 2000). The genes that give golden rice its ability to make β -carotene in its endosperm come from daffodils and a bacterium called *Erwinia uredovora*. These genes and various promoters are inserted into plasmids of the bacterium *Agrobacterium tumefaciens*. The agrobacteria are used to transfect rice embryos, transferring the gene coding for β -carotene. The transgenic rice plants must then be crossed with strains of rice that are grown locally and are suited to a particular region's climate and growing conditions (Nash, 2000).

Starting with the precursor geranylgeranyl diphosphate, enzymes added included phytoene synthase, from daffodil. Phytoene is the first carotenoid precursor in the biosynthetic pathway leading to the production of β -carotene. Phytoene desaturase from *Erwinia* and lycopene β -cyclase from daffodil react with the precursor to produce β -carotene. Also, ferritin, an iron-rich bean storage protein and phytase, an enzyme that breaks down phytate making Fe available, were added to rice. A gene for a cysteine-rich metallothioneine-like protein, for reabsorption of iron, has also been engineered into rice (Potrykus et al., 1998; Ye et al., 2000).

Funding for this project was initiated by Gary Toenniessen, director of food security for the Rockefeller Foundation. He identified the lack of β -carotene in polished rice grains as an appropriate target for geneticists, because traditional plant breeding seemed unable to address the problem. Later, funding was also supplied by the Swiss government and the European Union. Because the work was carried out without industrial support, Potrykus hoped to distribute the technology freely to

countries that needed it the most. There are still major hurdles to cross, however, like patents and proprietary rights of genes used, as well as criticism by public interest groups like the Rural Advancement Foundation International of Canada and others. The affiliation of golden rice with a large commercial enterprise, AstraZeneca, which will market the product, is felt to be contrary to public trust, because the crop may not have been tested thoroughly for adverse human effects (Nash, 2000).

Another recent advancement in agricultural technology is the development of potatoes with higher starch content. The bacterial gene coding for enzyme ADP-glucose pyrophosphorylase was inserted into the potato genome under the control of the patatin promoter, resulting in 30–60% greater accumulation of starch. High-starch potatoes have less moisture and absorb less fat during frying. This product, if commercialized, would be very beneficial in lowering the calories in french fries, one of the most heavily consumed foods in the United States, thus helping to alleviate the national challenge of obesity.

Transgenic Plants as Vaccine Production Systems

Traditionally, fermentation-based systems have been used for industrial production of vaccines, but genetically modified plants that express foreign proteins offer an economical alternative. Large amounts of antigens could be produced using agriculture instead of complex cell culture-based expression systems (Weksler, 2000). The concept of genetically modified plants being used for vaccine production emerged in 1992 when an investigation was conducted to produce different classes of proteins of pharmaceutical value in plants. Also, the announcement of the Children's Vaccine Initiative in 1993 documented the need for vaccine technology to combat infectious diseases. For a review of the progress made by several groups of scientists in vaccine research using genetically modified plants, the reader is referred to Mason and Arntzen (1995).

Diseases have been extremely challenging in developing countries, where antigens from enteric pathogens were early targets for plant-based expression studies. Convenience and the need to evaluate genetic constructs quickly, determined the initial plant systems used in testing recombinant antigen production. Tobacco plants were used in early studies, but high levels of toxic alkaloids in the leaves inhibited animal feeding studies. Many scientists used potato, because mice accepted raw potato tubers instead of feed, and these could be generated in a relatively short time after transformation.

Genes coding for antigens from pathogenic viruses, bacteria or parasites that have been characterized, and for which antibodies exist, can be manipulated in two ways. One method is transient expression using viral vectors. If epitopes within the antigen are identified, DNA fragments encoding these can be used to construct chimeric genes by fusion with coat protein genes from a plant virus, e.g., tobacco mosaic virus (TMV) or cowpea mosaic virus (CPMV). The recombinant virus, or in the case of TMV and CPMV, even the viral RNA made *in vitro* from the plasmid clone, is then used to infect established plants. Virus replication and systematic spread allow high-level transient expression of the chimeric coat protein in most plant tissues. The viral particles expressing the foreign epitope on their surfaces are then purified and used for immunogenicity studies (Mason and Arntzen, 1995).

Alternatively, the entire structural gene may be inserted into a plant transformation vector between 5' and 3' regulatory elements. This allows the transcription and accumulation of the coding sequence in all or selected plant tissues. The vector is then used for the *Agrobacterium*-mediated transformation of plant cells, or for stable integration of the expression cassette by other means, with regeneration of transgenic plants. The resulting plants contain the expression cassette with stable integration into the nuclear chromosomal DNA and can be used either for extraction and partial purification of the foreign antigen or for direct feeding of plant tissues for assessment of immunogenicity (Figure 11.2) (Mason and Arntzen, 1995). Thus, eating fruit can then induce antibodies just like a vaccination, rendering the person immune to the disease. Dr. Charles Arntzen at Cornell University is actively pursuing research to allow children to be immunized against debilitating diseases like hepatitis B, simply by eating a modified banana.

Examples of stable genomic transformation using genes encoding foreign antigens include *Streptococcus* mutans *spaA* protein, hepatitis B surface antigen (HbsAg) and *E. coli* heat-labile enterotoxin B subunit and cholera-toxin B subunit. A patent application published under the International Patent Cooperation Treaty in 1990 was the first report of an edible vaccine. The surface protein (*spaA*) of *Streptococcus* mutans was expressed in tobacco plants to a level of about 0.02% of the total leaf protein. Oral immunogenicity of *spaA* produced in *E. coli* stimulated the production of S-IgA in saliva. Examples of transient expression of candidate vaccines using viral vectors include malarial epitope fusions with tobacco mosaic virus (TMV) capsid protein, *Zona pellucida* protein fusion with TMV capsid protein and cowpea mosaic virus capsid protein fusion (Mason and Arntzen, 1995).

Because vaccine antigens can be produced in plants in their native form, great possibilities exist in using this technology for food-based, "edible vaccines" that would be more economical for delivery in developing countries. Some concerns and challenges that must be overcome before commercialization of this process include maximizing the expression of antigenic proteins, stabilizing the foreign protein during postharvest storage in plant tissues and enhancing the oral immunogenicity of some antigens. Also, the possibility of allergy and immune tolerance of orally applied antigens must be addressed. A delivery system is needed to deliver the edible vaccine as a medicinal product at the correct dosage and not as a routine food source. Also, a thorough study of the immunogenic dose response and antigen levels in various foods is needed, so initially, animals may be the more likely target for edible-vaccine technology than humans (Arntzen, 1997a,b).

ENVIRONMENTAL BENEFITS

Agricultural biotechnology may be used to improve the environment, for example, in the development of plants that produce biodegradable plastics or plants that are able to absorb harmful substances from the soil. Phytoremediation of contaminated soils could be tackled by several methods: pollutant stabilization, containment and decontamination. In pollutant stabilization, vegetative cover and soil conditions are manipulated to reduce the environmental hazard, while decontamination occurs when plants and their associated microflora are used to eliminate the contaminant

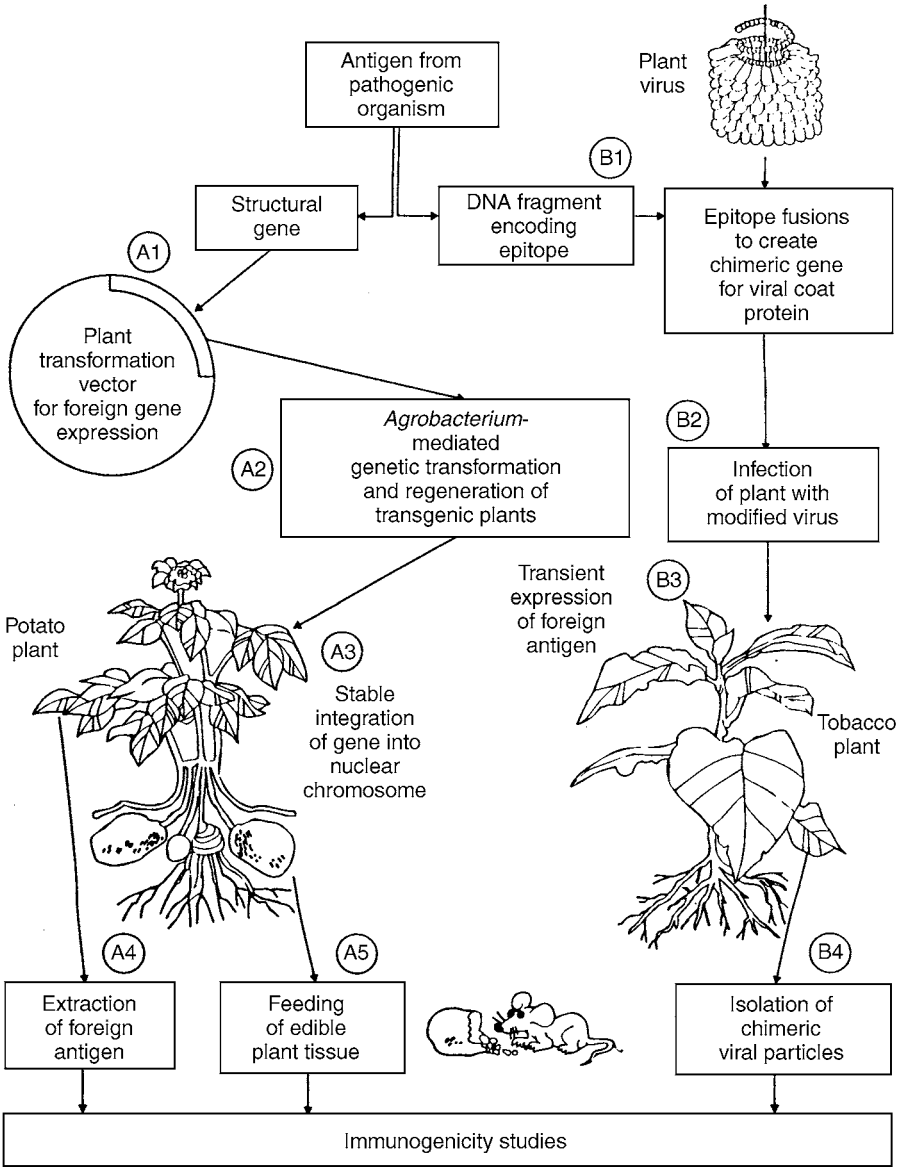


FIGURE 11.2 Strategies for the production of candidate vaccine antigens in plant tissues. (Adapted from Mason and Arntzen, 1995.)

from the soil. The reader is referred to Cunningham et al. (1995) for a review of this topic. Figure 11.3 summarizes the processes involved in phytoextraction of contaminants from soil, removal of organic material and the phytostabilization of soil contaminants.

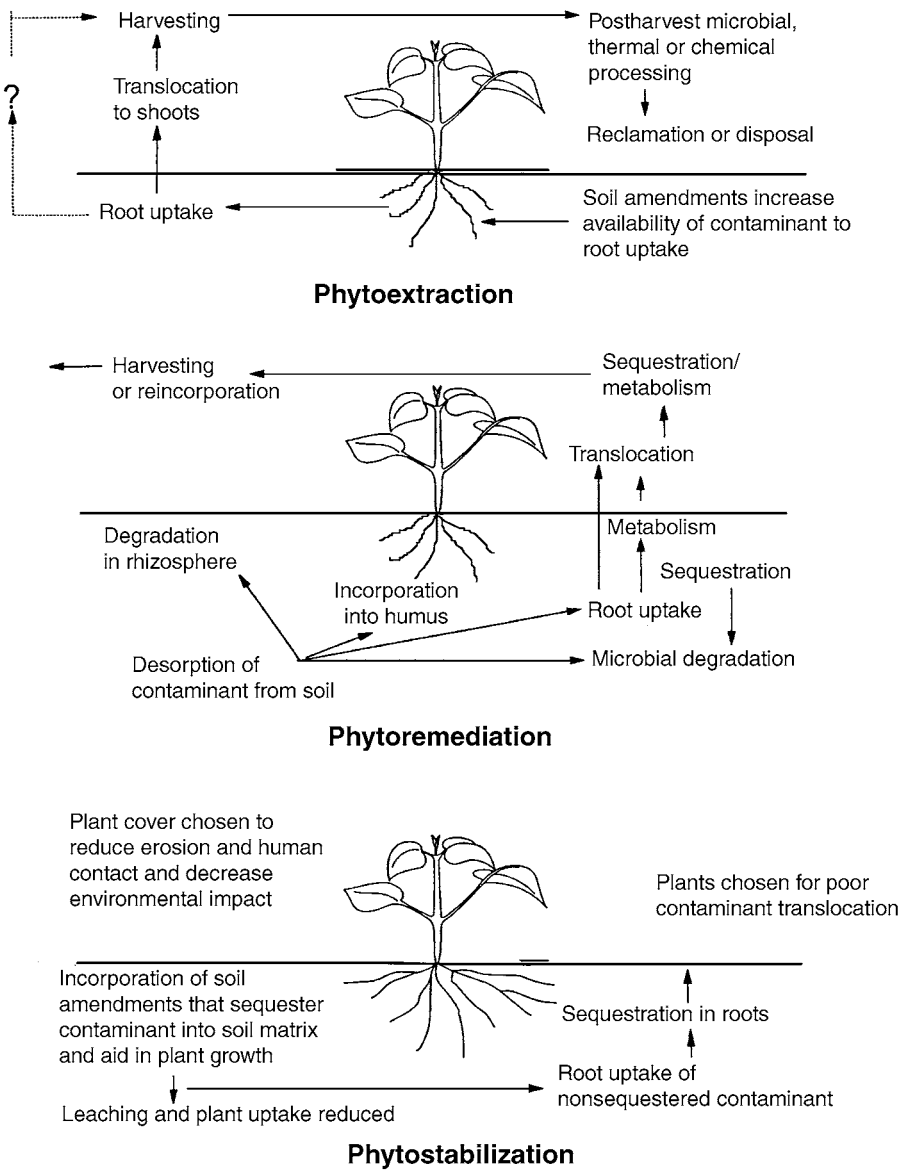


FIGURE 11.3 Processes involved in the phytoextraction of contaminants from soils. (Adapted from Cunningham et al., 1995.)

Green-plant-based remediation has been used for years in the form of constructed wetlands, floating-plant systems in wastewater treatment and reed beds. Because plants are living organisms that require oxygen, water and nutrients in order to grow, certain limitations exist in plant tolerance to the toxin that must be removed, as well as soil conditions of pH, salinity and texture. Phytoremediation is typically slower

than chemical or physical processes used to remove toxins. However, large areas of immobile contaminants may be amenable to phytoremediation that is far more cost effective than landfilling or thermal treatment.

Sebertia accuminata is a rare plant that hyperaccumulates toxic metals. This plant grows on outcroppings of metalliferous soils, having a sap that is 25% nickel by dry weight. Also, *Thlaspi caerulescens*, a member of the *Brassica* family, can accumulate up to 4% zinc in its tissue without apparent damage. Limitations in the use of hyperaccumulators for remediation are that these plants usually accumulate one specific element, they grow slowly and have a small biomass, and agronomic characteristics of these plants are not yet well established. Effective genetic engineering of metal transport and tolerance plants requires a good understanding of the mechanisms involved. However, metal tolerance has been obtained by introducing metallothioneins and by the introduction of a semisynthetic gene encoding MerA (bacterial mercuric ion reductase). Genetically modified *Arabidopsis* can reduce the toxic mercuric ion to mercury (Hg), which is then volatilized, with tolerance levels up to 100 μM . Alterations in phytochelatins and metal-binding peptides also offer possible tolerance mechanisms. However, mechanisms to increase the translocation of metals from the root to the shoot are needed. Phytoextraction could be quite profitable if metals of economic value, like nickel or copper, are targeted. In cases where volatile forms of inorganic contaminants (e.g., selenium as dimethyl selenide) are produced, this would eliminate the costs of harvesting and processing, making the process more economical (Cunningham et al., 1995).

Phytodegradation may only take place if organic contaminants are biologically available for absorption or uptake by plants, followed by metabolism of plant or plant-associated microbial systems. Investigations of plant-associated microbial systems have shown bioremediation through the plant rhizosphere. The rhizosphere accelerates rates of degradation for many pesticides, as well as trichloroethylene and petroleum hydrocarbons. Research has been conducted to accelerate the degradation process of the plant-microbe interface. However, more work is needed to increase the rate of metal phytoextraction and to develop new plant and soil management practices. In phytostabilization, the role of the plants is to increase the sequestration of the contaminant by altering water flux through the soil, preventing wind and rain erosion and incorporating residual-free contaminants into roots. Future benefits of agricultural biotechnology in phytoremediation will require the combination of traditional disciplines in waste management with the tools of biotechnology.

OTHER BENEFICIAL CROPS — PLANTS AS BIOREACTORS

Genetically modified plants provide an economical alternative to using microbial systems for the production of biomolecules. Synthetic saccharides and fatty acids of nonplant origin may be synthesized in plants. However, plants may also be manipulated to overproduce plant metabolites. One benefit to the overproduction of heterologous proteins in plants is that expensive purification could be avoided if the plant material is used in human food or animal feed, allowing oral or topical ingestion. Table 11.7 depicts a summary of the use of plants as bioreactors for the production of lipids, carbohydrates and proteins (Goddijn and Pen, 1995).

TABLE 11.7
The Use of Plants as Bioreactors for the Production of Lipids, Carbohydrates and Proteins

Compound	Origin of Gene(s)	Application	Plant Species Used
<i>Lipids</i>			
Medium-chain fatty acids	California bay tree	Food detergent, industrial	Oilseed rape
Monounsaturated fatty acids	Rat	Food	Tobacco
Polyhydroxybutyric acid	<i>Alcaligenes eutrophus</i>	Biodegradable plastics	Arabidopsis, oilseed rape, soybean
Saturated fatty acids	<i>Brassica rapa</i>	Food, confectioneries	Oilseed rape
<i>Carbohydrates</i>			
Amylose-free starch	<i>Solanum tuberosum</i>	Food, industrial	Potato
Fructans	<i>Klebsiella pneumoniae</i>	Food, pharmaceutical	Potato
Increased amount of starch	<i>Bacillus subtilis</i>	Industrial, food	Tobacco, potato
Trehalose	<i>Escherichia coli</i>	Food, industrial	Potato
	<i>E. coli</i>	Food stabilizer	Tobacco
<i>Pharmaceutical polypeptides</i>			
Alpha-trichosantin	Chinese medicinal plant	Inhibition of HIV replication	<i>Nicotiana benthamiana</i>
Angiotensin-I-converting enzyme inhibitor	Milk	Antihypersensitive effect	Tobacco, tomato
Antibodies	Mouse	Various	
Antigens	Bacteria, viruses	Orally administered vaccines	Mainly tobacco
			Tobacco, tomato, potato, lettuce
Antigens	Pathogens	Subunit vaccines	
Enkephalin	Human	Opiate activity	Tobacco
Epidermal growth factor	Human	Proliferation of specific cells	Oilseed rape, Arabidopsis
Erythropoietin	Human	Regulation of erythrocyte levels	Tobacco
Growth hormone	Trout	Growth stimulation	Tobacco
Hirudin	Synthetic	Thrombin inhibitor	
Human serum albumin	Human	Plasma expander	Tobacco, Arabidopsis
Interferon	Human	Antiviral	Oilseed rape
			Tobacco, potato
			Turnip

(continued)

TABLE 11.7**The Use of Plants as Bioreactors for the Production of Lipids, Carbohydrates and Proteins (Continued)**

Compound	Origin of Gene(s)	Application	Plant Species Used
<i>Industrial enzymes</i>			
Alpha-amylase	<i>Bacillus licheniformis</i>	Liquefaction of starch	Tobacco, alfalfa
(1-3,1-4)- β -Glucanase	<i>Trichoderma reesei</i> , hybrid of two <i>Bacillus</i> species	Brewing	Barley cells
Manganese-dependent lignin peroxidase	<i>Phanerochaete chrysosporium</i>	Bleaching and pulping of paper Animal feed	Alfalfa
Phytase	<i>Aspergillus niger</i>	Animal feed, paper and pulp, baking	Tobacco
Xylanase	<i>Clostridium thermocellum</i> <i>Cryptococcus albidus</i>		Tobacco

Source: Adapted from Goddijn and Pen (1995).

Experiments with the *Agrobacterium rhizogenes* (hairy roots) have led to the production of bioactive compounds. They express root-specific pathways and have shown stable production of alkaloids, polyacetylene, sesquiterpenes, naphthoquinones and other natural products. Hairy roots have been adapted for the overproduction of secondary metabolites and biotransformation of chemicals (McGloughlin, 2000). A short-day flowering plant was used to make the Ma-1 gene that would offer the benefits to biomass and forage crops in which flowering is undesirable. Agricultural biotechnology may be used to create new plant genotypes with attributes that can be turned on and off at various stages in the growth season. Phytofluors of different colors will be used to "tag" proteins, enabling scientists to study interactions between molecules within the cells (Clark Lagarias, University of California, Davis), but few tools are available at present for this research.

Applied Phytologics, Inc., Sacramento, California, has prepared a malting grain model using rice. The rice is transformed with the desired protein, controlled by an α -amylase promoter in the aleurone layer. This crop is grown in the field, and grain is harvested at maturity. Seeds are imbibed to induce expression of the α -amylase promoter and production of the desired protein. Proteins are then extracted from germinating seeds and purified (McGloughlin, 2000). This novel method of protein expression has been implemented by Applied Phytologics, Inc., for a wide range of biotechnology products, including blood proteins, bioactive therapeutic proteins, price-sensitive industrial enzymes, animal health products and enzyme-based bioremediation.

Environmental stresses like drought, salt loading, freezing and elevated temperatures affect plant productivity. A gene that was transformed into wild-type *Arabidopsis* has been shown to confer to the seeds the ability to complete germination at colder temperatures. The choline esterase gene from a soil bacterium was used to transform

Arabidopsis (McGloughlin, 2000). Choline oxidase catalyzes the conversion of choline to betaine, a potent protectant molecule in bacteria, plants and animals, thus enhancing stress tolerance. Glycine betaine insulates plant cells against the ravages of salt by preserving the osmotic balance, by stabilizing the structure of proteins and by protecting the photosynthetic apparatus. Transformed plants were able to germinate at low temperatures and grow at elevated and low temperatures that severely limit wild-type growth rates. Plants engineered with the COR15a transcription factor may indeed have better cold tolerance. Betaine overproducing plants were also able to grow under conditions of elevated salt. Choline oxidase (codA) from a soil bacterium tolerated saline and cold conditions. A salt tolerance gene from mangroves (*Avicennia marina*) has been identified, cloned and transferred to other plants that were found to be tolerant to high salt concentrations; and a gene from *E. coli* was also used to generate salt-tolerant transgenic maize (Liu et al., 1999; Third World Academy of Sciences, 2000).

Plants have been used to synthesize biodegradable plastics. Genetically modified *Arabidopsis* expressed the polyhydroxybutyrate (PHB) pathway in the cytoplasm. Plants are, in general, suitable for the production of industrial enzymes. However, it is important that the protein of plant origin is more economical to produce than the product of microbial fermentation. Agricultural biotechnology will increase yields of rice crops with the properties of improved nitrogen assimilation, increased sucrose hydrolysis, starch biosynthesis, increased O₂ availability and modified photosynthesis. Success in the use of plants as bioreactors will rely on the availability of structural and biosynthetic genes for specific biosynthetic pathways, improvement of production levels and increased knowledge of downstream processing (Goddijn and Pen, 1995).

FOOD SAFETY CONCERNS

HUMAN HEALTH AND ENVIRONMENTAL RISKS

Recombinant DNA technology is no longer obscure, because there are many practical applications and even high school kits for gene splicing experiments. Two major nonbiochemical realities or constraints that govern both the rate and the amount of penetration the products of biotechnology will have in the food industry are economics and regulatory approval. New components will have to be more cost effective than the ingredients they are replacing, and the reformulated and totally new products will have to pass the tests of the consumer marketplace. Food safety concerns with transgenic crops include the introduction of novel proteins that are allergenic, and genetic exchange of traits like insect and herbicide resistance from transgenic crops to weeds and other wild-type species. Unintended effects of genetic modification may be manifested as the expression of unknown or unexpected toxic or antinutrient factors or increased production of known toxic constituents (Royal Society, 1999). Other important issues in applying genetic engineering to food include nutritional equivalency of genetically altered foods compared to their traditional counterparts and sensory acceptability of genetically altered foods compared to their traditional counterparts.

Food allergens are caused by immunological responses to substances in foods, usually natural proteins in commonly allergenic foods like peanuts, milk and seafood.

Symptoms of allergic reactions can be mild cutaneous or gastrointestinal symptoms or life-threatening anaphylactic shock. Because new proteins are formed when genes are introduced in GMOs, potential allergenicity of the new food should be assessed (IFT Expert Committee, 2000a,b). Release of GMOs into the environment allows the possibility of plants breeding with related species in the wild. This has been documented in canola fields of Saskatchewan, Canada. Canola shipped to France from Canada was banned early in the year 2000 because it contained greater than 1% GM grain. It was then discovered that the shipment was contaminated by pollen from a genetically modified crop that was grown more than 100 meters away. Another example of contamination of “GMO-free” product occurred in a shipment of wheat that was transported with genetically modified product. The wheat flour was used to prepare breeding for turkey cutlets, and when tested, the breeding was positive for genetic modification (Stram et al., 2000).

Consumer concern about the safety of genetically engineered organisms is not unfounded. In the late 1980s, the unregulated, genetically engineered nutritional supplement, L-tryptophan, produced by Showa Denko, Tokyo, caused some 27 deaths by eosinophilia-myalgia syndrome (EMS) (Hoyle, 1992). The problem was traced back to changes in a bacterial fermentation process involving a recombinant strain of *Bacillus amyloliquefaciens*. The actual changes that caused the problem are still unknown, so the importance of safety assessment cannot be stressed enough.

It was felt by some organizations that the environmental issue is not addressed sufficiently in the FAO/WHO safety assessment of food from biotechnology. A pilot plant without containment in the former German Democratic Republic produced modified *Bacillus subtilis* strains carrying α -amylase genes from *Bacillus amyloliquefaciens* on a nontransmissible plasmid derived from *Streptococcus*. Biomass from the plant was dispersed in a sewage pond, exhaust air was not filtered and bacteria in the enzyme solution were not inactivated (Teuber, 1993). When the plant closed down, it was found that 5–18% of *Bacillus*, *Streptococcus*, *Micrococcus* and *Staphylococcus* strains isolated from the environment carried erythromycin resistance. This occurred because the modified plasmid pSB20 was maintained in bacteria that survived in sterile soil and river water. However, selection pressure may also explain the persistence of resistance genes in bacterial populations (Teuber, 1993).

Hansen (2000) compared the development of agricultural biotechnology with the creation of synthetic chemicals in the early 1900s. Synthetic organic chemistry may be an extension of basic chemistry, yet the distribution of some novel chemicals like PCBs, organochlorine pesticides and vinyl chloride into the environment yielded unexpected results. These chemicals were found to be carcinogens, reproductive toxins, endocrine disruptors or causative agents for other medical conditions, and the Toxic Substances Control Act was passed by the EPA, requiring premarket screening of synthetic chemicals. It is possible that the experience with synthetic organic chemicals could be repeated with the introduction of novel genetically engineered food into the biosphere.

Key areas for concern include the scope of genetic material transferred, unnatural recombination, location of the genetic insertions and use of vectors designed to move and express genes across species barriers. Foreign promoters and foreign marker genes, particularly genes coding for antibiotic resistance, are used. Also, genetic engineering

allows the insertion of genes not only from widely different plant families, but also from any organism or synthetic genes. This is felt to introduce new elements of uncertainty. The genome is made up of genes that interact in complex regulatory pathways to maintain the organism, so the addition of new genetic material may end up destabilizing pathways. Likewise, the introduction of a new species into the environment may cause little or no change or have a catastrophic effect on the ecosystem. Unfortunately, these changes cannot be predicted reliably with the limited knowledge of the biology of the introduced species (Hansen, 2000; Matzke and Matzke, 1995).

The effect of a gene on the whole organism is significantly governed by its location. Thus, the lack of control over location in genetic engineering is cause for unexpected effects. Conventional breeding involves the reshuffling of alleles of organisms that share a recent evolutionary history, so the genes most likely function in the same location. One example of the unpredictable effects of genetic engineering and the location of gene insertion is an experiment by Bergelson et al. (1998) with *Arabidopsis thaliana*, a plant from the mustard seed family. Several lines that exhibited the same trait of herbicide tolerance were compared: one developed by conventional breeding methods and two using genetic engineering. Researchers at the University of Chicago induced herbicide (chlorsulphuron) tolerance (HT) into *A. thaliana* via mutation breeding and genetic engineering. The surprising results were that the per-plant out-crossing rate was 0.3% for mutant fathers (mutation breeding) and 5.98% for transgenic fathers, a 20-fold difference. They attributed this difference to the difference in location of the insertions of the gene, because the genetic construct was the same in all of the plants. The act of genetic engineering had transformed a species that was normally an in-breeder to an out-crosser (Hansen, 2000).

Another example of an unexpected effect that may have been a result of the location of insertion is the experiment by Inose and Murata (1995), who inserted not a transgene, but multiple copies of a naturally occurring yeast gene. Scientists found that a threefold increase in the enzyme phosphofructokinase resulted in 40-fold to 200-fold increase in methylglyoxal (MG), a toxic substance that is known to be mutagenic, ending on the yeast line. The genetically engineered yeast cells had significantly altered metabolism, resulting in the accumulation of a toxic substance, and the conclusion was that the position of the insertion was the cause.

Environmental concerns also include the introduction of herbicide-tolerance genes to plants that then increases its weediness. A general agreement on what defines a weed includes (APHIS, 2000) the ability to germinate in many different environments; discontinuous germination and great longevity of seed; rapid growth through vegetative phase to flowering; continuous seed production for the length of time of growth; self-compatibility but partially autogamous and apomictic; ease in cross-pollination, by either unspecialized visitors or wind pollination; rapid growth in favorable environments and seed production in a wide variety of environments; short- and long-distance dispersal capabilities; and vegetative production and easy regeneration from fragments. Vertical transfer of the new genes, i.e., out-crossing from the transformed cultivar to other domestic plants, occurs naturally in nature.

Scientists have also recently discovered lateral movement of genetic material, called horizontal gene flow. This is thought to be one way in which antibiotic resistance or pathogenicity is passed around among bacteria. Cho et al. (1998) reported evidence

that genes from a fungus had invaded 48 out of 335 genera of land plants surveyed. Jakowitsch et al. (1999) demonstrated that sequences from a previously unidentified tobacco pararetrovirus had repeatedly integrated itself into tobacco chromosomes. In the past, it was thought that plant viral sequences rarely, if at all, integrated into host genomes. Thus, it is now recognized that genetic material can move laterally between species and exist for extended periods of time. Horizontal gene flow in nature is only limited to a few microorganisms, and plants have evolved defenses against this, but genetic engineering can be compared as an enhanced version of this natural phenomenon. Numerous marker genes are used in genetic engineering to facilitate the identification of transferred genes. Markers are typically antibiotic resistant. One concern is that these antibiotic genes may move horizontally to widespread bacteria in nature, rendering them resistant to the antibiotic in question.

Hoffman et al. (1994) reported horizontal gene transfer from higher transgenic plants via the soil to a soil microorganism (*Aspergillus niger*). Scientists reported cases of genetic transfer across taxa of eukaryotes. The main example suggesting a transfer over evolutionary time from unrelated taxa to higher plants was the case of vertebrate hemoglobin and legume hemoglobin. The fate of the Monarch butterfly received a great deal of publicity, because their larvae died after feeding on milkweed patches adjacent to cornfields that had been genetically engineered for insect resistance. Laboratory studies showed that when fed milkweed coated with pollen containing the insecticidal protein, the butterflies die. *In vitro* studies, however, did not account for the fact that the pollination cycle and the migration of Monarch butterflies occur at different times (Gorny, 2000). Also, pollen is not typically carried long distances, because it is too dense to float well in air currents. Horizontal (nonsexual) transfer of transgenes from genetically engineered plants into other organisms is not yet well documented or proven.

Vectors have been designed to move and express genes across species and ecological barriers. No special genetic elements are required to facilitate movement of genes in conventional breeding, because it involves the mixing of genetic material from species that are sexually compatible. In genetic engineering, vectors are usually derived from efficient genetic parasites like viruses or genetic elements that can enter cell barriers. Plasmids move readily between barriers, and in plants, the tumor-inducing plasmid (the Ti-plasmid) of *Agrobacterium* is used for agricultural biotechnology. The genes of *Agrobacterium* are not found in crops in nature except in those infected plants with crown gall or hairy root disease. Vectors used in agricultural biotechnology usually contain a powerful promoter derived from the cauliflower mosaic virus (CaMV 35S promoter). This causes disease in plants of the mustard family. Virus promoters enhance the hyperexpression of transgenes to a higher magnitude than the organism's own gene. The naturally occurring promoters in plants would not effectively express desired traits of a transgene if one relied solely on this mechanism. The CaMV promoter is so strong that one safety concern is that it would not only turn "on" the transgenes, but that it would also affect other genes thousands of base pairs upstream and downstream of the insertion site and even affect the behavior of genes on other chromosomes. It is possible for a gene that codes for a toxin to be turned "on" (Hansen, 2000).

SAFETY ASSESSMENT

Consumer groups feel that the FDA has a legal obligation to require mandatory reviews of all genetically engineered foods before they go on the market and to develop ways to screen for unexpected effects that could have health consequences. The predictable risks and potential risks of toxins, allergens, nutritional changes and antibiotic marker genes should also be addressed. Safety review should also be developed through a process of notice and comment (Hansen, 2000). In general, the criteria used in safety assessment in foods include the chemical composition, specification of the product, nutritional/metabolic data and toxicological data. If a genetically altered organism is found to be significantly different from a traditional one, more comprehensive testing is required, i.e., toxicological tests and nutritional tests of the food product. Where potential allergenicity is suspected, comprehensive animal and laboratory tests should be conducted and, if necessary, limited human volunteer studies should be conducted.

Allergenicity of products of agricultural biotechnology is assessed following a decision tree process outlined by the International Food Biotechnology Council (IFBC) and the Allergy and Immunology Institute of the International Life Sciences Institute (ILSI) (Metcalfe et al., 1996). Focus is placed on the source of the gene(s), sequence homology of the newly introduced protein(s) to known allergens, the immunochemical reactivity of newly introduced protein(s) with immunoglobulin E (IgE) antibodies from the blood serum of individuals with known allergies to the source from which the genetic material was obtained and physicochemical properties like digestive stability of the new protein (IFT Expert Committee, 2000b). Other assessment criteria that have been suggested by the FAO/WHO (1991, 2000) include the evaluation of the functional category for the protein introduced and the level of expression of the newly introduced protein(s) in the edible portions of the improved variety. Allergy assessment is a key part of the overall food safety assessment of genetically modified food, and strategies for assessment should be updated continuously as new technology and methods become available (FAO, 1996; IFT Expert Committee, 2000b).

The Animal and Plant Health Inspection Service (APHIS), a branch of the United States Department of Agriculture is responsible for the evaluation of genetically engineered plants. Guidelines have been prepared for submitting petitions to APHIS, and a petitioner is required to submit in detail the following information on new, genetically modified crops:

1. Rationale or development of the petitioned crop
2. The biology of the petitioned crop
3. Description of the transformation system
4. Donor genes and regulatory sequences
5. Genetic analysis and agronomic performance
6. Environmental consequences of introduction of transformed cultivar
7. Adverse consequences of new cultivar introduction
8. References

Description of the biology of the nonmodified recipient organism should include taxonomy, genetics, pollination, evidence of reported weediness and discussion of sexual compatibility with wild and weedy free-living relatives in natural crosses or crosses with human intervention. Applications should include whether the crop or sexually compatible species is listed in the relevant publications of the Weed Society of America. The source of recipient (cultivar name or accession number) and the weed status of its sexually compatible relatives is also required (APHIS, 2000). Applications should also include the identification of the lines that are to be considered in the petition and the cultivars from which they are derived. If there are multiple lines, each line must be identified. In virus-resistant plants, applications should also include a section for information on the nature of the virus that provided the sequences encoding the resistance phenotype.

Because some plants have known toxicants that may affect nontarget organisms and beneficial insects, e.g., tomatin in tomatoes, cucurbitin in cucurbits or gossypol in cotton, the applicant should determine whether the introduction of new genes in these plants altered the level of toxicants. If the plants produce no known toxicants, a reference should be provided to support the claim. The toxicological data on effects of the plant on nontarget organisms and threat to endangered species is required if there is a notable difference between the transgenic and nontransgenic plant levels of toxicants. During field testing, it should be determined whether adjacent nonsexually compatible plants developed weediness, in the case of the transfer of an herbicide-tolerant gene. When a single plant has more than one phenotype modification, then only one petition should be submitted. For a complete review of the APHIS requirements in filing a petition for approval of a genetically modified plant, the reader is referred to APHIS (2000).

Health Canada has primary responsibility for food safety, under the broad authority of the Food and Drugs Act. Health Canada reviews products for safety and sets data requirements that will allow them to make safety assessments of products. Agriculture and Agri-Food Canada works closely with Health Canada to ensure that food risks are identified in order to prevent a threat to human health and safety (Agriculture and Agri-Food Canada, 1993). Criteria to assess food safety of novel foods were revised in September, 1994, in publications of Health and Welfare Canada. Detailed guidelines are provided in two volumes to assist the researcher in safety assessment and guidance in notification of novel food products (Health and Welfare Canada, 1994). New food products produced by genetic engineering were dealt with on a case-by-case basis to establish the nature of regulation required. Genetically modified microorganisms and their products when submitted for approval should compare favorably with the unmodified organism, should not be pathogenic or produce toxic substances, the DNA introduced should not have harmful sequences, the vector used should be characterized and potentially harmful markers (e.g., antibiotic resistance) should be absent or inactivated (Health and Welfare Canada, 1994).

Health risks of biotechnology products are also assessed under the Canadian Environmental Protection Act (CEPA), which came into force in June, 1988. The goal of CEPA is to protect the environment and human health from "potentially toxic" substances. Products of biotechnology and genetically modified organisms are assessed and controlled by CEPA under a proactive program that prevents manufacture or

entry into Canada until the federal government assesses potential effects on human health and the environment. Scientists at Health Canada and Environment Canada assess whether or not a product is “toxic,” if its use should be banned or controlled. Under CEPA, a product of biotechnology may be deemed “toxic” if it poses a risk to human health and the environment (i.e., wildlife and flora). Organisms involved and any genetic modifications must be accurately identified. Biotechnology companies are required to provide information on the organism used, its past history of adverse effects on human health, if any, and antibiotic resistance profile. Firms may also be required to test GMOs for pathogenicity using procedures that are valid for the specific organism. The federal government continues to monitor the health effects of products of biotechnology long after they have been initially approved for use (Health Canada, 2000).

In terms of international safety assessment of GMOs, the World Health Organization (WHO), since its inception in 1948, has promoted scientific research in food safety and the development of principles and guidelines to be used by its Member States. In 1991, a joint FAO/WHO Consultation resulted in the publication of specific recommendations for assessing the safety of foods produced by biotechnology, including genetically modified organisms. The safety assessment was to be based on sound, scientific principles and data, and be flexible enough to accommodate scientific advances. The organisms that contributed genetic material should be identified taxonomically and genotypically. Vectors should be constructed to minimize transfer to other microbes and selectable markers genes should not encode resistance to clinically useful antibiotics. Pathogenic organisms should not be introduced into food.

In May, 2000, the WHO introduced a series of consultations addressing the safety of foods derived from biotechnology, co-sponsored by the Food and Agricultural Organization (FAO). The first joint consultation held in Geneva, Switzerland, May 29–June 2, 2000, was followed by another joint consultation that focused on allergenicity, from January 22–25, 2001, in Rome, Italy. The WHO joined with the Italian Environmental Protection Agency to host a seminar on the potential health hazards of the release of GMOs in the environment. Previous WHO consultations related to foods derived from biotechnology include the 1996 meeting to provide practical recommendations for safety assessment, the 1995 meeting for guidance on the concept of substantial equivalence, the 1993 meeting on health aspects of antibiotic resistance marker genes and the 1990 consultation on safety assessment strategies.

The Codex Alimentarius Commission (CAC), a subgroup of WHO, is charged with establishing standards and guidelines to protect consumer health. In the twenty-third session of CAC held in June, 1999, the Medium-Term Plan for 1998 to 2002 was adopted. This plan allows for the development of standards for foods derived from biotechnology or traits introduced into foods by biotechnology, where justified scientifically. An ad hoc intergovernmental task force was established to implement the Medium-Term Plan, and this group first met in Japan in March, 2000. Also, the Codex Committee on Food Labeling has discussed recommendations for the labeling of foods derived through biotechnology, and these will be included in the Codex General Standards for Labeling of Prepackaged Foods. For a summary of the activities of the FAO/WHO consultations and international policy making on foods derived from biotechnology, the reader is referred to WHO (1990, 2000a,b).

FAO supports a science-based evaluation system that would objectively determine the benefits and risks of each individual GMO on a case-by-case basis prior to release. The possible effects of biodiversity, the environment and food safety should be evaluated, and the benefits of the product or process vs. the risks should be assessed. FAO expects that the national regulatory authority in each region be consulted when releasing GM foods. After release of GM foods, careful monitoring should continue to ensure continued safety to human beings, animals and the environment (FAO/WHO, 1991; FAO, 1996, 2000). Another international group, the Organization for Economic Cooperation and Development (OECD), was formed in 1960 to promote policies to achieve the highest sustainable economic growth of its member nations and to promote trade on a multilateral basis. In 1992, the OECD published a report on Safety Considerations for Biotechnology that was intended for those carrying out safety evaluations of new foods or food components derived by means of modern biotechnology. The report is based on comparison of GM food with traditional foods that are safe for consumption and the principles considered in making evaluations. Also, a task force was created for the safety of novel foods and feeds (OECD, 1993, 1998, 2000).

THE U.S. PERSPECTIVE

Genetically engineered foods were the number six concern in the United States according to a series of surveys conducted by the Food Marketing Institute from 1995–1997. Topics ranked in order of “serious hazard” include bacterial contamination, general food safety, chemical pesticides, nutritional quality, artificial preservatives then genetically modified foods. Consumer telephone surveys were conducted by scientists at North Carolina State University in 1992 when bovine somatotrophin (BST) was a serious food safety concern (Hoban, 1994). Investigations were also conducted with the Food Marketing Institute, Canadian researchers and the International Food Information Council (IFIC) over the years in North America, Japan and throughout Europe to determine consumer attitudes about biotechnology (Hoban, 1996a,b, 1997, 1998; Hoban and Katic, 1998). Support for biotechnology has wavered between 70% in 1992 to 72% in 1998 in the United States. Demographic differences show that men are more positive than women in their evaluation of biotechnology.

In a May, 2000, IFIC survey of 1000 randomly selected consumers, 79% said they had heard or read about biotechnology, while only 2% admitted to being well informed about the technology (IFIC, 2000a). Forty percent thought that there were foods produced through biotechnology in the supermarket now, and 54% said they would be likely to buy produce if it had been modified by biotechnology to taste better or fresher. A higher percentage (69%) said that they would purchase produce genetically modified to resist insect damage and require fewer pesticides, and 40% supported biotechnology to enhance plants that yielded reduced saturated fat. The FDA policy to only label products of agricultural biotechnology if allergens were introduced or if the food’s nutritional content was altered was opposed by 28% of those surveyed. When informed that critics of the FDA policy felt that labeling of GMOs should be mandatory, even if no harmful effects were introduced, consumers tended to support critics 43%. However, it was felt by 55% that simply labeling that

products contained ingredients of biotechnology did not provide sufficient information to make an informed decision (Hoban, 1996b; IFIC, 2000a).

Agricultural biotechnology may gain greater acceptance with the 40% who are in opposition, if it were not veiled in secrecy. Consumers should be exposed to the benefits and disadvantages of the technology in order to make informed decisions (Jungmeyer, 2000). A Food Marketing Institute survey of consumer confidence in food safety over the period of 1996–2000 has shown a gradual decrease from 84% with complete confidence to 74% confident in the year 2000. According to a survey conducted by GAP Research for Philip Morris and the American Farm Bureau, 57% of consumers support the use of biotechnology to improve the taste of foods, 65% support its use to improve the nutritional value of foods, 69% support its use to increase food production and 73% support biotech to reduce pesticide use (IFIC, 2000a). Consumer surveys in North America show a high degree of acceptance of foods derived from agricultural biotechnology. Consumers also have high confidence in the government and regulatory agencies to assure the safety of food.

From an economical standpoint, many experts believe that farmers face an uncertain future in terms of profiting from biotechnology. In the year 2000, farmers were reported as receiving only \$0.24 on every food dollar invested (Gorny, 2000). Annual produce sales total \$80 million representing over 300 commodities in the U.S., while the produce seed business is valued at \$500 million a year (IFPA, 2000). Thus, the large investment for agricultural biotechnology research is difficult to justify for such a small annual income. Increased farm revenue could be offset by higher costs of seed, especially if farmers have to segregate genetically modified plants from those derived from conventional methods. Retailers have the most influence on price, and increased production yields may not be beneficial to farmers in areas where a glut has already depressed produce prices. Most of the benefits so far have been to seed companies that developed new product lines. The consumer has not necessarily benefited from lower prices, thus, informative decisions must be made on how to apply agricultural biotechnology to benefit the consumer (Harvey, 2000).

The major issues that determine acceptance of products of biotechnology are public acceptance, which drives market demand, and regulation. These topics and labeling of foods derived from genetically modified plants continue to dominate and impact commercial planting of transgenic crops and consumption of genetically modified foods. The FDA's May 2000 policy that requires mandatory assessment of products of agricultural biotechnology, a review of methods used in detecting these products and the setting of labeling standards, make the whole process more transparent, and the industry hopes this will boost consumer confidence in biotechnology.

AN INTERNATIONAL PERSPECTIVE

A United Nations' estimate of the world population by the year 2050 is 7.8 billion or as many as 12.5 billion people, as compared to about 5.9 billion in 1997. Unfortunately, the surface area of the earth will remain the same, and we are left with the choices of securing more land for agriculture or increasing output on the land that is used at present for farming. Agricultural biotechnology may hold the most promise

to increasing crop yield without damaging environmentally sensitive areas or clearing more rain forests for farming.

In 1984, the Rockefeller Foundation, a private philanthropic organization with the aim of increasing crop yield of small farmers in developing countries, introduced the International Rice Biotechnology Program, focusing on Asia. Rice is the most important crop in developing countries, accounting for 80% of all calories consumed in Asian countries (IFIC, 1999; NRC, 2000b). The Foundation hopes to increase rice yields by 20% in Asia by the year 2005. Similar work was introduced in Africa in 1988, where the problems of soil-nutrient depletion and yield losses caused by pests and diseases take priority. According to Gary Toenniessen, director of agricultural sciences for the Rockefeller Foundation, "The tools of biotechnology should be developed for all major food crops, including those primarily grown in developing countries and on marginal lands" (IFIC, 1999).

Another organization, the International Laboratory for Tropical Agricultural Biotechnology (ILTAB), is developing transformation methods for application in viral disease control in tropical plants like rice, cassava and tomato. The first transfer of a resistance gene from a wild rice species to a susceptible cultivated rice variety was reported in 1995. The resistance gene for the bacterium *Xanthomonas oryzae*, which causes disease in rice crop, was transferred to several useful rice varieties that are cultivated on more than 24 million hectares worldwide. The hope of ILTAB is that this research will assist farmers in developing countries to increase rice yields through the development of disease-resistant strains (IFIC, 1999).

Agricultural biotechnology research is being established and strengthened at the national level and within international agricultural research centers. Several developing nations have an emerging private-sector crop-biotechnology industry that produces hybrid seeds and micropropagated plants for commercial farmers. These molecular biology research facilities are limited, however, and collaborate heavily with the public sector and international partners. Table 11.8 summarizes international organizations that transfer plant biotechnologies to developing countries. The Consultative Group on International Agricultural Research (CGIAR), with its Secretariat at the World Bank, helps to coordinate the efforts of several organizations to conduct strategic and applied research, facilitate technology transfer as well as deliver advanced technology to farmers in the form of improved seed (CGIAR, 1999; Cook, 1999; Toenniessen, 1995).

The CGIAR network has only had marginal success because of numerous challenges. One challenge is the ability to produce improved varieties for highly variable land areas with limited agronomic potential, for example, in parts of Africa, Latin America and Asia. On-farm research is needed to develop sustainable cropping systems that allow performance under local conditions. Also, in densely populated areas where high yield varieties are widely used, there is little land left for expansion. Development of yield-enhancing and resource-conserving technologies will help to solve these challenges. Seed multiplication is relatively straightforward for cereals, but vegetatively propagated crops such as cassava, potatoes, yams, plantains and bananas require more technical biotechnology methods for improvement (Gould, 1999).

Traditionally, free exchange of materials and information has assisted in international agricultural research. However, applied crop biotechnology research in

TABLE 11.8
International Organizations Facilitating the Transfer of Plant
Biotechnology to Developing Countries

International Organization	Description
Food and Agriculture Organization (FAO) of the United Nations, Rome, Italy	Conducts research and facilitates transfer of plant biotechnology that can benefit developing countries through its Plant Production and Protection Division in Rome, its regional offices and the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture in Vienna
International Atomic Energy Agency (IAEA), Vienna, Austria	Conducts collaborative research national agencies and provides training in mutation breeding and other plant biotechnology through the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture
International Laboratory for Tropical Agricultural Biotechnology (ILTAB), La Jolla, CA, United States	An advanced-research laboratory, developed through a collaboration between the Scripps Research Institute and the French Technical Assistance organization ORSTOM, which conducts research and offers training on development of disease-resistant tropical plants through genetic engineering
International Center for Genetic Engineering and Biotechnology (ICGEB), Trieste, Italy and New Delhi, India	Originally established by the United Nations Industrial Development Organization and now an independent research and training organization with crop biotechnology programs in New Delhi and information dissemination provided through Trieste
Center for the Application of Molecular Biology to International Agriculture (CAMBIA), Canberra, Australia	A research and technology transfer organization specializing in the production and dissemination of inexpensive biotechnology tools that can be employed in developing countries
International Service for the Acquisition of Agribiotech Applications (ISAAA), Ithaca, NY, United States	An international organization committed to the acquisition and transfer of proprietary agricultural biotechnologies from the industrial countries for the benefit of the developing world
Intermediary Biotechnology Service (IBS), The Hague, the Netherlands	A unit of the International Service for National Agricultural Research that provides national agricultural research agencies with information, advice and assistance to help strengthen their biotechnology capacities
Biotechnology Advisory Commission (BAC), Stockholm, Sweden	A unit of the Stockholm Environment Institute that provides biosafety advice and helps developing countries assess the possible environmental, health and socioeconomic impacts of proposed biotechnology introductions
Technical Center for Agricultural and Rural Development (CTA), Wageningen, the Netherlands	A unit of the European Union that collects, disseminates and facilitates exchange of information on research innovations including plant biotechnologies for the benefit of Asian, Caribbean and Pacific states
International Institute for Co-operation in Agriculture (IICA), San José, Costa Rica	Assists countries in Latin America and the Caribbean with policy issues related to biotechnology including the formulation and harmonization of biosafety procedures

(continued)

TABLE 11.8**International Organizations Facilitating the Transfer of Plant Biotechnology to Developing Countries (Continued)**

International Organization	Description
Biotechnology Research Center, Chinese Academy of Agricultural Sciences, Beijing, China	Carries out research in crop breeding using tools of biotechnology; use marker-aided selection
Biotechnology Center, Indian Agricultural Research Institute, New Delhi, India	
National Center for Research on Genetic Resources and Biotechnology, EMBRAPA, Brasilia, Brazil	

Source: Toenniessen (1995), with permission.

industrial countries has increasingly become the function of “for-profit” organizations. There is a significant increase in research that is protected under many forms of intellectual property rights, including patents, plant breeders’ rights and trade secrets. Now, even public sector plant scientists are being encouraged to seek intellectual property rights for their inventions and to license technology to the corporate sector. Even if information is shared, results needed for further distribution and commercialization are retarded by one or more material transfer agreements. The International Service for the Acquisition of Agri-biotech Applications (ISAAA) was formed to serve as an agency to facilitate transfers of proprietary agricultural biotechnologies from industrial countries to the developing world (Toenniessen, 1995).

Developing countries may be faced with the dilemma of a second generation of dependency on industrialized nations through the appropriation of germ plasm by the latter and through socioeconomic dislocation resulting from substitution of biosynthetic products for natural ones. But, as former U.S. president Jimmy Carter stated: “Responsible biotechnology is not the enemy; starvation is. Without adequate food supplies at affordable prices, we cannot expect world health or peace” (IFIC, 1999). In order to meet the challenge of feeding the ever-growing populations in developing countries, it will be necessary to deliver low-cost, high-value seeds to poor farmers and to ensure that crop germplasm can continue to be distributed and shared among the developing countries without restrictions. The sociopolitical and socioeconomic obstacles limiting food distribution may be the real barriers to food distribution to the hungry (Gorny, 2000).

The United States is the leader in the growing of crops derived from agricultural biotechnology—the most predominant crops being GM corn, soy and cottonseed. During the year 2000, there was a modest reversal to traditional crops with a 9–24% decrease in biotech crops from the year 1999, especially in corn (Barach, 2000). Transgenic corn production went from 33% of total corn crop to 25%. This decrease has been attributed to adjustments made to accommodate sales to Europe and countries

that have a non-GMO policy, as well as a reduced demand because of less insect pressure. Numerous processed and formulated foods contain GM soy, corn and cottonseed oil in products like salad dressings. Soy ingredients may be found in up to 60% of all formulated foods in the U.S. today, with consumers estimated to have ingested GM foods since 1996. The European market is very important to the United States, estimating \$4 billion in sales of value-added food products in 1999. The recent policy of non-GM foods would make export of food products to this region extremely difficult. Most U.S. food processors do not formulate products exclusively for the European marketplace. Recent restrictions in labeling and difficulties in reformulation or ingredient segregation have caused some U.S. processors to cease exports to the European Union (EU). It has proven easier for some companies to sell to other markets than to attempt to comply with EU labeling regulations.

Bulk items like soy that are processed into ingredients are handled without intent or opportunity for segregation of GM crops from identity preserved (IP) crops. Only small, niche markets of specialty crops would have the service available to segregate IP crops. However, isolated grower and handling and certification and testing incur costs that must be recovered in the finished product. The EU and Japan, as well as the organic market, may be niche markets for IP crops. This mode of handling would not be practical for commodity items. Only labeling may satisfy the consumer's "right to know."

There are social and cultural reasons for the greater opposition in Europe to agricultural biotechnology. Consumers are more skeptical and concerned, so new products face significant challenges to achieve a level of consumer confidence. Northern European Union nations and Spain view GMOs with less scorn than Italy and France, but the presence of Greenpeace has caused governments to be cautious. There is a general lack of confidence in government food authorities in the EU. This was probably fueled by the highly publicized food safety crisis of mad cow disease (bovine spongiform encephalopathy or BSE) in the United Kingdom and dioxin-contaminated feed in Belgium. Also, there are few scientific and academic voices supporting the safety of agricultural biotechnology (Anonymous, 1998). Anti-GM lobbyists have been more successful at communicating the risks of GM foods than biotechnology companies promoting the benefits. Consumer activist groups like Greenpeace have gained a great deal of support in their activities against genetically modified food. Political action has raised awareness of GM food with some EU nations restricting the use and sale of GM corn and enforcing the labeling of GM foods. Retailers have also influenced consumers by eliminating GM product when possible.

Reaction from consumers and industry across Europe is not uniform, however. Northern European nations tended to be more aware of the environment and focused on consumers, thus tending to create some resistance to biotechnology, while southern Europe has shown an indifference. A survey conducted in Europe, Canada, the United States and Japan in 1995 showed that above 50% of consumers were willing to buy GM insect-protected produce. Two countries where acceptance was low, were Austria (22%) and Germany (30%). Austrian consumers were some of the most negative toward agricultural biotechnology. But, Austria has a large number of organic farmers and low government support for biotechnology, hence, the negative

reaction is understandable (Hoban, 1997). Consumers in these countries were the lowest in their willingness to buy products of biotechnology; however, they had a relatively high awareness of the technology, because those interviewed responded as having spoken to someone about GMOs. When consumers were asked to evaluate applications of biotechnology in food, animal health and human healthcare, 85% of people around the world found applications in healthcare acceptable.

Media analysis in countries that showed the most resistance to GMOs revealed that the opponents to biotechnology were able to voice their opinions relatively unchallenged by biotechnology companies. More positive media coverage only came afterwards. Consumers seemed to need a basic understanding of how food was produced. When asked if ordinary tomatoes did not contain genes while genetically modified ones did, there was a great deal of uncertainty. Fifty percent of Americans did not know, while there was considerable variation in knowledge among European countries. Some of the perceived fears of GMOs became evident when consumers were asked if consuming GMOs would change a person's genes. There was a better understanding in the Netherlands, Canada and the United States, while 40% of respondents from Austria thought this was true (Hoban, 1997).

Also, there were significant differences in the ability of various groups to educate consumers. U.S. consumers trusted organizations like the American Medical Association, the National Institutes of Health, the Food and Drug Administration and university scientists to supply trustworthy information. The media, biotechnology companies, packaged food manufacturers, chefs, and activist groups and retailers tended to have lower credibility. However, in Europe, the sources of consumer education were the opposite. European consumers trusted environmental and consumer groups more than the government and industry to supply trustworthy information. Ironically, these educators have been the strongest opponents of agricultural biotechnology.

There is a major philosophical disagreement as to what agriculture should be: natural selection vs. genetic manipulation using new technology (Nuffield Council on Bioethics, 1999). China is moving ahead with research in agricultural biotechnology, with more than 1 million farmers planting genetically modified crops from about 200 seed varieties in the year 2000. The European Union, however, has added only 10 million hectares in Eastern Europe to its subsidy-dependent agricultural system, refusing to accept agricultural biotechnology and putting restrictions on farm trade (Karst, 2000). One concern in developed nations is that the proponents of organic growth may impose the belief that this is the only acceptable method of breeding new plant species, thereby preventing novel research using the methods of biotechnology that could be of benefit.

LEGAL CONSIDERATIONS AND LIMITATIONS

REGULATION OF GMOs

New technologies present new safety concerns, and the products of modern biotechnology inevitably will be subjected to regulation by the federal government for the purpose of assuring safety. A basic general principle of food safety and nutrition is that food should be safe, sound, wholesome and fit for human consumption.

Consumers and scientists are concerned that genetically modified organisms or products might harbor unknown risks for human health and the environment.

Setting up regulating procedures was a vital first step in controlling “novel” food products, which were defined as food that had not been used previously to any significant degree for human consumption. In the United States, the federal government approved rules and guidelines for regulating the biotechnology industry in June, 1986. Regulation of safety of new products was divided among five federal agencies. The Food and Drug Administration (FDA) was responsible for genetically engineered organisms in foods and drugs. The USDA was responsible for engineered organisms used with crop plants and animals. The National Institutes of Health and the Occupational Safety and Health Administration (OSHA) were responsible for engineered organisms that could affect public health and the workplace, respectively. The Environmental Protection Agency (EPA) was responsible for engineered organisms released into the environment for pest and pollution control and related activities.

The principal statute administered by the FDA was the Federal Food, Drug and Cosmetic Act (FD&C Act) (1982). There was also the Federal Meat and Poultry Products Inspection Acts. Genetically altered plants were subjected to the Federal Plant Pest Act and other laws depending on how they were constructed and their intended use (Wasserman, 1988). FDA believed its existing requirements and procedures with respect to food additives and generally recognized as safe (GRAS) food substances were sufficient. The FDA stated that it would apply the existing requirements and procedures to the products of modern biotechnology on a “case-by-case” basis. Under the FD&C Act, the FDA’s principal power to regulate food applications of modern biotechnological methods was found in its premarket clearance authority. Such clearance powers were tied to legal classification of the food ingredient, namely, whether it was a food additive, a GRAS substance, a prior-sanctioned ingredient or simply a food. The FDA’s authority to require clearance before entry in the marketplace was confined to food additives only.

The FDA published its policy on genetically modified foods in 1992, establishing that the FDA would treat as equivalent, food derived from plants modified by older breeding techniques and those derived from plants modified by genetic engineering. According to the policy, certain foods would be considered food additives under the FD&C Act. Under this policy, however, the FDA did not require premarket review if the food constituents of the new plant variety were the same or substantially similar to those in other foods. The FDA required that the following concerns of novel genetically modified foods be addressed:

1. Does the food contain genes from known allergenic sources?
2. Have novel toxins been introduced or have endogenous toxins increased?
3. Has the nutrient content changed?
4. Does the food contain a substance that is new to the food supply?

Premarket approval was required when the characteristics of the new varieties posed food safety questions from novel ingredients or toxicants. The policy also addressed the introduction of allergens that were not present previously. In cases of serious allergenicity risks, such foods would be banned from the food supply. All genetically

modified foods introduced from 1992 to 1999 went through the premarket approval process voluntarily.

Recent lobbying by antibiotechnology groups has forced a change in U.S. Food and Drug Administration (FDA) policies. All new genetically modified foods must face mandatory assessment, even when there is little or no scientific basis for a formal assessment. The Clinton administration announced in May, 2000, that food biotechnology rules would be developed and implemented that would allow for more government oversight of genetically modified crops. Also, labeling standards would be set for foods marketed as "biotech-free." It would become mandatory to provide the FDA information about new genetically engineered crops and notify the agency at least four months before introducing the product on the market. The USDA, which oversees the growing of genetically engineered crops, requires a buffer zone around crops to make sure pollen drift does not occur.

The USDA will be responsible for building a certificate program for new scientific tests that detect products of agricultural biotechnology (Barach, 2000). Senator Dick Durbin of Illinois in October, 2000, began promoting the regulation of food produced using techniques of biotechnology. The proposition would allow the FDA to have the authority to approve new foods and determine if they contained genetically modified elements. Labeling of genetically modified foods is now voluntary in the FDA's policy and is only considered necessary if foods contain allergens or toxins. The proposed legislation does not call for mandatory labeling, and the topic would be reviewed by Congress. In Canada, the Committee on Voluntary Labeling, an initiative by the Canadian Council of Grocery Distributors, drafted a document that included topics such as requirements for claims, claim templates, compliance measures and verification (Waterfield, 2000). For a thorough review of this topic, the reader is referred to the Institute of Food Technologists, Expert Report on Biotechnology and Foods (IFT Expert Committee, 2000c).

An important legal consideration is the evaluation of food for the presence of genetically modified organisms. At present, a number of European nations have declared that they will not accept products derived using agricultural biotechnology methods. Testing is conducted to ensure a less than 0.1% GMO content (DNA and/or protein content). There is no validated method or common international standard of measure, and, in some cases, test results have proved to be inconsistent between labs in the United States and abroad (Roseboro, 2000). The most common method used to detect GMOs is the polymerase chain reaction (PCR), which analyzes at the DNA level. This method is thought to be sensitive and able to quantify GM content at low levels. There are, however, no common protocols to address the many factors influencing PCR reliability, like sample preparation, DNA extraction, PCR amplification of genetically modified sequences and electrophoretic analysis of PCR reaction products to determine the presence and concentration of genetically modified DNA. Enzyme-linked immunosorbent assay (ELISA) is used to detect target proteins.

PCR is not easily adapted for rapid on-site testing at elevators or processing plants, because specific DNA sequence information is needed to prepare primers, and this may be proprietary. Also, an initial investment of \$20–30,000 for conventional PCR, to \$60–100,000 for real-time PCR is quite costly and requires highly skilled technicians for operation. ELISA tests analyze for a specific antibody reaction marking

the presence of the expected protein. This method is less expensive and can be carried out by nontechnical personnel, allowing testing at the point of sale. Several private labs have been established for GMO testing. One of the pioneers, Genetic ID, received accreditation from the United Kingdom Accreditation Service (UKAS) for all GMO testing methods. The European Union, one of the major markets requiring GMO testing, accepts UKAS accreditation. The USDA's Grain Inspection Packers and Stockyard Administration (GIPSA) plans to accredit U.S. PCR laboratories for testing grains. This is a move to ensure that participants in the trade of grain feel more confident about their transactions. Also, collaborative studies between the American Association of Cereal Chemists and the Association of Official Analytical Chemists will be conducted to evaluate PCR methods and laboratories' ability to quantify the GM content of grain samples and processed food (Roseboro, 2000).

The American Crop Protection Agency (ACPA) will support the U.S. Department of Agriculture's initiatives to develop processes and procedures for the identification of biotechnology-derived crops by providing reference materials and methods that will allow for more accurate testing. Also, plant biotechnology companies will assist the USDA in efforts to validate tests for detection of biotech crops. In May, 2000, the USDA announced that it would seek public comments on proposed testing validation and accreditation standards. Plant material containing specific proteins expressed by transgenic genes and the DNA sequence for the novel trait have typically been considered proprietary, so a consensus is needed from plant biotech companies to make standard reference material for transgenic crops accessible for testing. ACPA member companies have agreed to assist in this process. For an updated report on the methods of detection of GMO grain in commerce, testing costs and verification of grain as "GMO free," the reader is referred to ACPA (2000).

Several European bodies have published guidelines for the detection of genetically engineered foods. The International Life Sciences Institute Europe (ILSI) held a workshop in Belgium in 1998 to review current knowledge of detection methods for GMOs. Also, the Joint Research Center in Ispra, Italy, validated a PCR screening method for detection of DNA of GMOs that year. Members of the Analytical Environmental Immunochemical Consortium (AEIC) have also developed a guideline document for the use of immunoassays to detect proteins of GM crops. Current regulations are based on quantitative PCR for DNA detection or ELISA for protein detection. Food-processing steps like heat, pH treatment or enzyme reactions may affect the quantities of DNA and protein in food.

A new brand of identity-preserved (IP) corn products was launched by Cargill's Illinois Cereal Mills, with a system called InnovaSureTM for producing such products. InnovaSureTM is a system designed to maintain the integrity of specialty whole corn, yellow goods and masa through comprehensive procedures that start with the seed and continue through to the customer's doorsteps (Giese, 2000). The system requires working with seed companies, growing all crop on contract, providing IP protocol training and continuous in-house testing.

A food safety crisis in the United States involving the detection of 'StarLink' corn in taco shells, has raised questions about the feasibility of segregating genetically engineered foods from those produced using traditional methods of breeding. The Food and Drug Administration had approved the use of StarLink corn for animal

feed but had not moved in a timely manner to approve its use for human consumption. This may have been the cause for the confusion. About 2600 growers produced the corn, and officials blamed Aventis, the developers of the new strain, for allowing it to enter the food chain. The USDA was forced to “arrest” millions of bushels of corn until it could be channeled for nonfood use. There has been a long record of safety of biotech foods since about 40 new items have been approved and in use for the last 10 years. But, the USDA has made efforts to remove StarLink corn from certain export markets and out of the organic food market and other niche markets that do not want products of agricultural biotechnology (Waterfield, 2000).

Several international organizations are also involved in the regulation of products of agricultural biotechnology in their respective countries. Scientists at the National Center for Research on Genetic Resources and Biotechnology (Empraba) in Brazil have proposed a statistically significant method for evaluation of substantial equivalence between a genetically modified crop and its conventional analog or wild type. The method expands on that proposed by the U.S. Department of Agriculture and is thought to provide a more significant comparison (Belem, 2000). Substantial equivalence is required to ensure the health and well being of the consumer, rationalize the cost of the investment in developing the new product through biotechnology and provide the basis for regulation of products of agricultural biotechnology.

LABELING OF GMOs — THE DEBATE

Labels are used to convey information on nutritional or health-related concerns of the contents of a package. The FDA requires labeling in two instances: if the food characteristics significantly differ from what is normally expected or if safety issues arise because of the new technology. The principal criteria considered are as follows:

1. The presence of novel allergens
2. Introduction of novel toxins or the increase of endogenous toxins
3. The change of levels of important nutrients
4. Significant alterations in composition

If the product of genetic engineering is identical to the normal version of the same food, then the label could be misleading.

Consumer groups advise that labels will allow the consumer the right to choose which product he wants. They feel that the FDA decision over the term “significant difference” should be irrelevant. They say a small difference or a large difference in products of agricultural biotechnology should not alter a decision in labeling. Labeling should not be a deviation from previous FDA policy. The FDA requires the labeling of frozen peas and fresh peas so that consumers can make a choice about what to buy. Thus, food produced by or derived from a process of genetic engineering where foreign DNA has been introduced into an organism would also be labeled, allowing the consumer the opportunity of choice (Consumer Union’s comments to FDA on Docket No. 99N-4282) (Hansen, 2000).

Some consumers justify labeling because they believe that the insertion of foreign DNA into food is an adulteration or contamination of food with a chemical.

It is found that consumer concern is often quieted when they are educated that all living things contain DNA; DNA is an ingredient in almost all foods. Proponents of labeling could argue that all foods containing chemicals carry a generic label stating that the products contain chemicals. However, this would be meaningless and would not provide information to make an informed choice (McHuguen, 2000).

In October, 2000, 'StarLink' corn product was discovered in food exported to Japan where mandatory labeling and import notification for foods containing GMOs will soon be a requirement. Media exposure over the commingling of StarLink corn with varieties approved for food may well prompt the mandatory labeling of all genetically modified foods in the United States, and this labeling of genetically modified foods could increase product costs 16–18%. The government would also be challenged to determine how much labeling should be done, what should be put on the labels, and how to enforce labeling. In an IFIC survey, it was found that consumers did not place much value on labeling and said that they should not have to pay more to keep food segregated or labeled. However, this cost would not be borne by food companies, but indeed passed on to the consumer. If educational information is not provided on labels, they are thought to be useless, but labeling remains a focus point for activist groups. A review of the debate on food labeling was published by the Institute of Food Technologists (IFT), Expert Committee on Biotechnology (2000d).

International debate on the labeling of GMOs has continued for a decade. In 1990, the announcement of a genetically modified baker's yeast in Great Britain caused a public outcry. The Food Advisory Committee (FAC) of the UK subsequently designated four basic food categories as a primary screening mechanism to determine when specific food labeling might be required. The groupings were as follows (Teuber, 1993):

1. Nature-identical food products of genetically modified organisms
2. Food from intraspecies genetically modified organisms
3. Novel food products of genetically modified organisms
4. Foods from transpecies genetically modified organisms

Several barriers to the trade of genetically modified crops now exist in Europe. The European Union safety assessment review process was put on hold since early 1998. No new products have been approved, and this has impacted trade with the United States. In addition, the EU requires mandatory labeling of products containing genetically modified organisms. This labeling has been viewed by some as a protectionist move to steer consumers away from this technology, rather than to educate. Since the institution of mandatory labeling, retailers in the EU have removed product from the shelves or reformulated product to exclude genetically modified ingredients. This has left the EU consumer with a decrease in choices. The elimination of trade opportunities for commodity corn and soy has raised questions about these technical barriers to trade with U.S. companies doing business in the EU (Barach, 2000).

Other countries are also moving toward mandatory labeling as seen in Table 11.9. Australia, Japan, Korea, Russia and others are all working on labeling regulations. Each country has its own labeling criteria and unique labeling language, thus compounding the complexity of the situation. There is also a lack of infrastructure for

TABLE 11.9
International Status Summary in June, 2000, on Labeling for Products
Containing Genetically Modified Organisms (GMOs)

Country	Labeling	Status	Special Provisions
United States	For safety, nutrition or allergen reasons (only when not substantially equivalent)	FDA policy adopted 5/92	Labeling of characteristic (not of process or GM origin)
Canada	Similar to U.S.	Policy adopted 12/95	9/99 initiative to develop guidelines for voluntary labeling
Mexico		Senate passes bill to mandate labeling in 4/00 (requires approval by the House and Presidential Signature)	
Australia and New Zealand	Proposal to mandate labeling released 11/99	Health Ministers postpone "decision" on labeling	Proposing strict verification standards for "GMO-free" or "sourced from non-GMO"
European Union	Mandatory labeling EU Directive 258/97, 1139/98	Effective 9/1/98 Implemented 4/9/00	Does not permit "may contain" (no action on "negative list," or analytical standards)
	Standing Committee amendments to 1139/98 to establish de minimis threshold		10/99—1% de minimis threshold for identity preserved non-GMO product
Japan	Proposal to mandate labeling released 11/29/99	Implementation date 4/1/2001	Requires verifiable system for segregation (no final threshold established) Provides labeling for 24 products/product categories
China	No labeling required		
Russia	Labeling proposal released 12/1/99	Implementation date 7/1/00	Provides for a "negative" list

Source: Reprinted from Barach (2000), *Food Testing and Analysis*, June/July, with permission of the publisher ©2000. The Target Group.

compliance and education of consumers at present. The Cartagena Biosafety Protocol was an agreement among 130 nations passed early in the year 2000 to regulate the transboundary movement of living genetically modified organisms. The ratification of the agreement by 50 countries will establish an international framework for countries to use when making decisions on introducing GM crops to farmland. However, because a “precautionary principle” has been adopted in the Biosafety Protocol agreement, some countries may decide to exclude GM crops without any scientific evidence of harm (Barach, 2000).

The Cartagena Protocol may not take effect in individual countries for another two years, but it does not override rights and obligations under other international agreements like that of the World Trade Organization (WTO). Several key areas in the agreement that may be problematic to trade with the U.S. include the fact that exporters must obtain advance permission from the importing country prior to shipping products of agricultural biotechnology that will be released into the environment, commodity shipments that contain living modified organisms (LMOs) must be labeled and international labeling initiatives may force the United States to institute labeling laws.

Some nations are interested in factors other than science on which to base food regulation, for example, ethical, social and cultural issues. Many are focused on the consumer “right-to-know” concept. The Codex Alimentarius, the agency of the United Nations that deals with food safety, quality and labeling standards and guidelines, has a subcommittee on food labeling, which is working on standards for labeling foods from biotechnology. U.S. trade associations like the National Food Processors Association (NFPA) believe that labeling should only be used if it refers to safety, nutritional value, health or composition of the food. They also support voluntary labeling, provided the information is truthful and not misleading. Voluntary labels could state “Biotech Free” or “Contains Biotech Ingredients.” The EU consumer “right-to-know” provision goes one step further in claiming that the consumer must know if the process of genetic engineering was used in developing the crop, and thus, any ingredient derivatives must be labeled.

The EU Novel Foods Regulation (358/97) is the route for mandatory labeling, with the requirement that recombinant DNA and/or detectable protein products from modified DNA are present. This ruling has been changed by EC Regulation 1139/98 to require labeling of foods containing GM soy or corn. The EC Regulation 1139/98 was further amended to establish a 1% de minimus threshold for inadvertent contamination of non-GM food. Labeling of such foods and ingredients containing additives and flavorings is required (Barach, 2000).

There are, however, flaws in the labeling approach, because no threshold has been established for the point at which labeling becomes unnecessary for DNA or protein. Refined ingredients that are known not to contain DNA and/or protein, e.g., oils and sugar, should form a list of products that do not require labeling. Standards are needed for “Biotech Free” claims, and testing methods for determining the presence of GMOs should be standardized (Barach, 2000). The impact of the apparent deficiencies in labeling rules and regulations will be compounded when other countries introduce their regulations. One example is the Australian-New Zealand Food Authority’s (ANZFA) five-level labeling approach with choices including “GM free,” “not sourced

from GMO,” “may contain GM ingredients,” “contains GM ingredients” and “genetically modified.” As detection methods advance, the industry may continue to require lower detection levels, and the labeling may become more complicated.

CONCLUSIONS

Agricultural biotechnology brings together the diverse fields of molecular biology, practical agriculture, sustainable agriculture and chemical engineering. Scientists are now able to manipulate living organisms with greater precision than ever before. New advances in biotechnology have improved food production, increasing yields and making production more cost effective. Agricultural biotechnology has assisted farmers with the introduction of pest-resistant crops that eliminate the need to apply pesticides, saving the environment from harmful chemicals as well as reducing production costs. This new technology has also introduced herbicide resistance in crops, so that herbicides can be applied without the fear of crop destruction. These two main benefits of agricultural biotechnology have been used widely in sustainable agricultural crops like corn, soybean, wheat and potatoes. Experiments that benefit the fruit and vegetable industry are in the early stages of development. Viral disease resistance in papaya and delayed fruit ripening in tomatoes are early examples of how biotechnology can be used to provide consumer benefits. In the long run, genetically engineered foods may minimize seasonal and geographic variations. Consumer trends are dictating the direction in which research should go. The accent these days is on a more healthy and nutrition-conscious lifestyle. This has encouraged the development of natural food products with enhanced nutritional benefits like “golden rice” which produces β -carotene. Extension of shelf life for the fresh-cut produce industry will also make demands on current technology. Longer shipping times are required to further markets than traditionally.

Genetic engineering in plants differs from conventional breeding in that conventional breeding relies on selection, using natural processes of sexual and asexual reproduction. Genetic engineering utilizes a process of insertion of genetic material via a method of direct gene introduction. However, genetic engineering relies on the insertion of the gene in a random location, which may disrupt the natural functions and complex interactions in a plant cell, exhibiting unexpected effects. The use of viral promoters, genetic material from *Agrobacterium* and bacterial antibiotic marker genes also introduces new variables with a cause for concern, for example, the introduction of new proteins that cause human allergic reactions. Release of transgenic crops into the environment concerns consumer advocate groups and organic farmers. Increased weediness may develop if herbicide-resistant genes are transferred to wild-type plants, and the transfer of antibiotic resistance to organisms in nature could cause future problems.

No evidence has yet been presented to prove that consumption of foods derived from biotechnology produce adverse human health consequences. Since the application of the technology is relatively new, only after several generations may we become aware of the health risks, if any. Most science experts agree that biotechnology derived foods are safe provided that they are “substantially equivalent” to the original products. The USDA and FDA have set guidelines for the safety assessment of foods

derived from agricultural biotechnology in the United States. Various international organizations like FAO, WHO, OECD and the Codex Alimentarius Commission have proposed guidelines for safety assessment and labeling of these novel foods. Genetically modified foods may be the most highly regulated and observed foods of all time. Ironically, crops produced by conventional breeding could technically be considered genetically modified, because the genes of the final product are significantly altered compared to the parent plant. Yet, these crops have traditionally been assumed safe for consumption.

Consumer acceptance of products of agricultural biotechnology vary from country to country. The United States at present has the highest acceptance of genetically modified foods. In the European Union, there is a greater distrust of this new technology, fueled by numerous presentations of the negative aspects of the technology by consumer advocate groups. The media in Europe has played a large role in distribution of information on the dangers of biotechnology. Because there has not been an equally active campaign on the benefits of biotechnology, a great deal of fear and distrust has evolved. Consumer education is thought to be the main key to acceptance of biotechnology, however, the issue becomes one of ethics vs. science. Is it ethical to tamper with nature and rearrange genetic information in organisms for the benefit of man? There is no winner in this argument.

The European Union was the first to restrict the imports of genetically modified foods. The member nations have introduced mandatory labeling laws so that the consumer has the right to choose which product to consume. Many feel that the labeling laws are unjust, because they create a fear of genetically modified food without proof that there is a food safety hazard. There is an increase cost to labeling, and labeling laws are not yet clear on wording or enforcement methods. Other countries have followed the EU by restricting the entry of GM foods. This has created problems in trade with the United States, the largest producer of GM crops for sustainable agriculture. Fresh produce is at the head of the organics market, for example, in the United Kingdom, it accounts for about 40% of the market. There has been little focus on fresh produce in the debate of genetically modified foods. Price, quality and appearance still dominate; however, the fresh produce industry would have to place great emphasis on consumer and retailer education in order to promote the benefits of genetic modification when the need arises. The fresh produce industry will eventually be pulled into the debate of genetically modified foods as whole foods replace processed foods as an area of concern.

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12 Flavor and Aroma of Fresh-cut Fruits and Vegetables

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INTRODUCTION AND OVERVIEW

Throughout the last half of the 20th century, numerous investigations set out to identify volatile compounds in many horticultural crops. Massive amounts of volatile data have been generated for most commodities. However, we only have a relatively complete understanding or knowledge of the essential flavor compounds for some of our most popular fruits and vegetables. Much less work has been performed to elucidate the aroma-forming mechanisms. In this chapter, we discuss the genesis and importance of flavor attributes, considering the effects of processing and storage on selected compounds in some fresh-cut market products. Also reviewed are flavor attributes in relation to sugars, organic acids and titratable acidity (TA) in certain commodities where these compounds are essential contributors to flavor. There is currently little analytical and sensory data available concerning aroma and flavor changes for fresh-cut commodities. Therefore, we took the liberty to discuss numerous items in regard to unprocessed fruits and vegetables and their storage, and extrapolated to confer logical physiological consequences of processing plus storage in fresh-cuts. Various preharvest and postharvest factors that may affect fresh-cut flavor quality will be addressed, where data are available. Flavor and sensory information in the literature will be used in conjunction with recent fresh-cut data emerging in the literature and our laboratories.

FRESH-CUT PHYSIOLOGY AND FLAVOR AND AROMA

Fresh-cut processing causes major tissue disruption as vacuolar, cytoplasmic and nucleic enzymes and substrates become mixed (Watada and Qi, 1999; Wiley, 1994; Watada et al., 1990). Processing increases wound-induced C_2H_4 and respiration rates, surface area per unit volume and water activity (King and Bolin, 1989). Additional details concerning the physiological effects resulting from processing can be found in Chapter 5. Physiological changes may be accompanied by browning, flavor loss, rapid softening, shrinkage and a shorter storage life. Accelerated water loss and increased water activity and carbon supply from freed soluble sugars enhance potential microbial attack, especially in fruits. Therefore, flavor and texture changes/loss during and after processing are especially of concern in fresh-cuts, yet little research has occurred in this area. This review subsequently outlines important pathways and enzymes believed to be critical concerning genesis of volatile flavor compounds or classes of compounds. Thus, information regarding flavor genesis will be more readily available to the fresh-cut industry.

FLAVOR PERCEPTION

Flavor is an important internal quality factor for fresh produce. Consumers often buy the first time based on appearance, but repeat purchases are driven by internal quality factors such as flavor and texture. Flavor is comprised of taste and aroma relating mainly to sugars, acids and volatile components (DeRovira, 1996). Human perception of flavor is exceedingly complex. Aroma compounds are detected by olfactory nerve endings in the nose (in parts per billion) (DeRovira, 1997). In contrast, taste is the detection of nonvolatile compounds by several types of receptors in the

tongue (in parts per hundred). The brain processes all of this information to give an integrated flavor experience. However, the brain may interpret changes in aroma as changes in taste (O'Mahony, 1995), or vice versa. This was evidenced by studies where levels of aroma compounds influenced panelist perception of sweetness and sourness for tomatoes (Baldwin et al., 1998), and levels of taste components influenced panelist perception of aromatic descriptors in mangoes (Malundo et al., 2001).

The volatiles in foods that can be perceived by the human nose are assumed to contribute to the flavor of a food. Odor thresholds can be established (the level at which a compound can be detected by smell) as described by the Ascending Method of Limits of the American Society for Testing and Materials, ASTM 1991 (Meilgaard et al., 1991). Log odor units can then be calculated from the ratio of the concentration of a component in a food to its odor threshold. Compounds with positive odor units are likely to contribute to the flavor of a food (Buttery, 1989). This has been done for tomato aroma compounds, for volatiles present at levels of one ppb or more (Buttery, 1993) (about 30 of the more than 400 identified compounds).

DOES FRESH-CUT QUALITY INDICATE FLAVOR QUALITY?

Fresh-cut vegetable salads have great consumer appeal due to their convenience, flexibility of use and probably due to the fact that their desirable flavor often comes about via condiments (croutons, spices or dressing), or because numerous products make up a medley mixture. Nonetheless, certain vegetables have specific characteristic aromas (mainly S-compounds) that must be perceived by the consumer. Consumer acceptance of fresh-cut fruits most often relies upon the inherent flavor and textural quality of the product, seldom with accompaniments. Unfortunately, in the fresh-cut industry, it is generally assumed that "if it looks good, it tastes good." Slow market growth for fresh-cut fruits may be attributed to the consumer's apprehension to repeatedly purchase products with inconsistent or unsatisfactory aroma and flavor quality.

Optimum harvest quality, postharvest quality and cutting quality are essential for maximizing fresh-cut shelf life. Harvest indices used for optimizing whole fruit shipping and or storage oftentimes should not be used for fruits destined to be processed. For example, an optimally mature load of cantaloupe may be shipped to a processor, received and then rejected because fruits are too soft or ripe for cutting. Additionally, controlled atmosphere (CA) stored apples with optimum visual appearance and firmness often have inferior aroma quality after long-term storage (Fellman and Mattheis, 1995; Harada et al., 1985). Likewise, *Crucifers* stored under low O₂ may develop off-odors (Kaji et al., 1993; Lipton and Harris, 1974) that may not readily dissipate after processing. The processor must, therefore, understand the physiology of each commodity, their packaging and end product to judge accurately when and what to process. Commercially, fresh-cut "quality" is generally only assessed visually, and flavor quality is seldom assessed (aside from °Brix or acidity) before or after processing. Most fresh-cut research in the last decade focused on quality retention based upon visual and subjective appearance and rapid common biochemical analyses. This approach (often guided by empirical observation) is often a physiologically reliable tool for root and thick leafy tissues but is suspect regarding flavor of high water content fruits and vegetables. "Fresh-cut" should imply that a product

is relatively fresh in terms of days since processing, and this should also help safeguard against inevitable flavor and aroma loss. Consequently, choice of variety, harvest condition, maturity, storage and shelf life with regard to flavor quality are becoming active areas of research in fresh-cuts.

FLAVOR COMPOUNDS IN FRESH-CUT PRODUCE

The edible and fresh-cut portions of vegetables and fruits are derived from numerous botanically different plant tissues. Subsequently, a plethora of compounds and compound classes may be important in fresh-cut flavor, depending upon which tissue(s) was used. For the most part, volatile compounds discussed in this chapter are those classified as naturally occurring (endogenous). However, because fresh-cuts are “processed,” a discussion of secondary (reaction products) compounds is relevant. Secondary metabolites may also have flavor contributions including bitterness, e.g., that related to sesquiterpene lactones in chicory (Peters and Amerongen, 1998), saltiness due to various natural salts, astringency related to flavonoids or alkaloids (DeRovira, 1997; Zitnak and Filadelfi, 1985) and tannins (Taylor, 1993). Natural flavor compounds are intrinsic entities within a given tissue, whereas the secondary compounds are generally elicited products of enzymatic action (oxidative and hydrolytic degradation of lipids and their by-products) attributed to processing.

Although fresh-cuts are processed, and secondary compound production is invoked, very little research has addressed the impact of secondary compounds upon fresh-cut flavor quality through storage. This is an interesting point, especially considering the fact that the act of mastication during consumption produces flavors and characteristic flavor compounds in many commodities. For example, the characteristic flavor of garlic is due to 2-propenyl 2-propenethiosulfinate, however, this compound is only produced upon tissue rupturing (Carson, 1987). Many of the characteristic aromas in cabbage (*Crucifers*, in general), cucumber, green bean, tomato, olive, some melons, etc., are only produced after cutting, chewing or tissue disruption. Because enzyme-mediated secondary compounds have been reported to be both desirable and undesirable flavor compounds in edible plant products, it is safe to assume that a far greater quantity of “flavor” compounds in fresh-cuts may in fact be “secondary compounds.” To date, little flavor and sensory work has been performed on fresh-cut fruits and vegetables. Two recent articles have reviewed numerous compounds considered to be important regarding flavor and aroma for numerous fruits (Baldwin, 2002; Beaulieu and Gorny, 2002).

VOLATILE PRECURSORS AND BIOGENESIS

Flavor perception relies upon our sense of taste and smell. The tastes, generally due to sugars, organic acids and sometimes phenols, tannins and other minor compounds (e.g., terpenoids and carotenoids), are “sweet,” “sour,” “bitter” and “salty.” In most fruits, a certain level of sugars (sweetness) or a balanced sugar-to-acid ratio determines consumer satisfaction. Oftentimes, the most important characteristic flavors for fruits and vegetables are attributed to specific aroma compounds. The flavor volatiles for most commodities are complex, including a range of molecular weight alcohols, aldehydes, esters,

ketones, lactones, sulfur-containing compounds and other compounds. Thus, numerous biosynthetic pathways are responsible for flavor production in a given fruit or vegetable.

Various references will be made illustrating the likely biosynthetic pathways leading to many characteristic aroma and flavor compounds in fruits and vegetables. Numerous pathways involving different enzymes and substrates are involved and are regulated under vastly different physiological conditions. Therefore, we will not attempt to thoroughly cover genesis of flavor compounds for all fresh-cut commodities currently marketed, because it is out of the scope of this review. For schematic overviews of flavor genesis, the reader is referred to the literature (Baldwin et al., 2000; Fellman et al., 2000; Sanz et al., 1997; Olías et al., 1993; Galliard et al., 1977; Yabumoto et al., 1977; Hatanaka et al., 1975).

Esters

Most fruits do not have characteristic aromas or flavors until they begin ripening. As ripening commences in climacteric melon fruit, methionine levels rise and ethylene synthesis increases. Ethylene initiates and coordinates numerous diverse physiological pathways that essentially trigger a cascade of catabolic processes. Increased ethylene production is often associated with an increasing free amino acid pool [for example, alanine, leucine, isoleucine, valine and methionine in muskmelons (Wyllie et al., 1996a)], membrane-mediated softening events, and as fruit maturity approaches, this is accompanied by increased volatile production. The energy sources behind most respiratory events during ripening are sugars and organic acids, which are also highly important concerning flavor, and sometimes reserve starch. Most primary aroma compounds are generated through β -oxidation of fatty acids, and secondary compounds are mainly formed as fatty acids that are oxidized via the lipoxygenase (LOX) pathway. As previously mentioned, secondary metabolites often have significant flavor roles in certain commodities. Although exhaustive information exists concerning fruit volatile composition, there has been limited work performed on aroma biosynthesis and the relative contribution to flavor formation for the three main classes of aroma precursors: amino acids, fatty acids and carbohydrates. Characterization of volatile and flavor formation and/or change occurring during preparation and storage of fresh-cuts is even more deficient.

Fatty acid and amino acid biosynthesis have long been shown to be important in aroma biosynthesis (Drawert et al., 1973; Tressl and Drawert, 1973; Myers et al., 1970). In the 1960s, the typical flavor compounds in pears were believed to be generated through β -oxidation of linoleic and linolenic acids (Jennings and Tressl, 1964). Most unripe fruits synthesize and catabolize a variety of C_1 – C_{20} fatty acids and produce both primary and secondary alcohols. Together, these compounds are precursors to one of the most important flavor and aroma compound classes in fruits—esters. In banana, simple amino acids such as [^{14}C]leucine and [^{14}C]valine were converted into the corresponding methyl-branched alkyl and acyl esters, alcohols and acids, whereas C_6 and C_9 aldehydes and C_9 and C_{12} oxo acids resulted from oxidation of fatty acids (Tressl and Drawert, 1973; Myers et al., 1970). In apples, straight-chain fatty acid esters can be formed from β -oxidation of long-chain fatty acids, and the branched-chain acid moieties arise from amino acids (Brackmann et al., 1993).

Quantitatively speaking, fatty acids are the predominant precursors responsible for the characteristic flavor and odor volatile compounds in fruits. The first reaction activating the β -oxidation spiral is when a saturated or unsaturated (Goodwin and Mercer, 1988) fatty acyl CoA (even number of C atoms) is oxidized via acyl CoA dehydrogenase. Each cycle through, and the final step of β -oxidation, produces acetyl CoA, and a fatty acyl CoA, which is catalyzed by acetyl CoA acyltransferase. Subsequently, straight-chain acid backbone moieties for many esters are readily available in plants, because their immediate precursors are intermediates of fatty acid β -oxidation (Lehninger, 1975; Conn and Stumpf, 1973).

Ethyl esters share a common substrate (the ethyl moiety from ethanol), and the acetate esters share another, the acetyl group (from acetyl-CoA), and both moieties can be glycolytically derived from pyruvic acid (Yabumoto et al., 1978). Pyruvic acid is decarboxylated via pyruvate decarboxylase, forming acetaldehyde, which is reduced via ADH to ethanol. The oxidative decarboxylation of pyruvic acid by means of coenzyme A (CoA) also yields acetyl-CoA, which is thought of as the direct precursor of esters and acetates. It is generally believed that an ester is enzymatically formed by combining an alcohol with an acyl group such as acetyl CoA (White et al., 1973; Forss, 1972).

The alcohol precursors for straight-chain ester biosynthesis are thought to be derived from oxidation of long-chain fatty acids through several cycles of the β -oxidation pathway resulting in a short-chain acyl CoA. Acyl CoAs are reduced to corresponding aldehydes via acyl CoA reductase that is reduced further to the alcohol via ADH (Bartley et al., 1985). In addition, research with deuterium-labeled precursors such as linoleic acid in apples showed that straight-chain esters are synthesized via the β -oxidation of fatty acids to give acetic, butanoic and hexanoic acids, which may be reduced to the corresponding alcohols before transesterification (Rowan et al., 1999). Furthermore, the use of deuterated precursors indicated that hexyl esters were formed via a hexanoate intermediate, rather than hexanal (Rowan et al., 1999).

It is generally believed that low molecular weight branched chain esters (C_3 – C_{12}) are synthesized by enzymatic combination of an amino acid and alcohol moiety (Yabumoto et al., 1977). Radio tracer techniques demonstrated that amino acids such as leucine, isoleucine or valine were converted into branched-chain alcohols and esters in muskmelon. Valine was converted into esters containing the 2-methylpropyl structure (2-methylpropanoates), leucine was converted into 3-methylbutyl esters (3-methylbutanoates) and isoleucine was transformed into 2-methylbutanoates (isobutyrate) (Yabumoto et al., 1977). Apples infiltrated with L-isoleucine had increased 2-methylbut-2-enyl and 2/3-methylbutyl esters (Hansen and Poll, 1993). Alanine was found to be the most important amino acid in terms of ester formation in strawberries (Pérez et al., 1992; Drawert, 1981). Alanine is an especially interesting amino acid in that it can supply both the ethyl group and acetate group found in many muskmelon aroma volatiles (Wyllie et al., 1995).

Aroma esters are believed to be synthesized enzymatically from alcohols and acyl CoA via alcohol acetyltransferase (AAT). AAT catalyzes the transfer of an acyl moiety from acyl-CoA onto the corresponding alcohol to form an ester. AAT is widely distributed in fruits and has been investigated in apple (Fellman and Mattheis, 1995; Fellman et al., 1993a), banana (Ueda et al., 1992; Harada et al., 1985), melons

(Ueda et al., 1997) and strawberry (Ke et al., 1994; Pérez et al., 1993). Many studies infer the presence of the enzyme via esterification of exogenous alcohol in fruit tissue; however, numerous cucurbit fruit lacked this enzymatic capacity (Ueda et al., 1997). Regardless, little work has been carried out to elucidate the properties of AAT, especially in fresh-cut products.

The first step in the conversion of an amino acid to an ester is deamination. This is followed by decarboxylation, various reductions and finally esterification (Pérez et al., 1992). The important enzymes in the conversion of isoleucine to 2-methylbutyl esters and 2-methylbutanoate are α -aminotransferase, α -ketoacid decarboxylase, α -ketoacid dehydrogenase, ADH and AAT (Wyllie et al., 1996b). In banana, the concentration of leucine and valine increased after the climacteric rise in respiration, as did the corresponding branched-chain esters and alcohols (Tressl and Drawert, 1973; Myers et al., 1970). Based on the above examples, it is clear that a large proportion of aroma compounds contain structural elements derived from various amino acids (Wyllie et al., 1996b) and fatty acids.

Aromatic Compounds

Aromatic volatile compounds are formed chiefly by the amino acid L-phenylalanine. D-glucose is converted into phenolic compounds via the shikimic acid pathway, yielding the active precursor amino acid, L-phenylalanine. Glucose becomes phosphorylated and condenses with phosphoenolpyruvate, and through a series of enzymatic transformations, shikimic acid is derived, which is phosphorylated (via shikimate kinase) to 5-phosphoshikimic acid. 5-Phosphoshikimic acid is ultimately deaminated (via phenylalanine ammonia lyase, PAL) into *trans*-cinnamic acid and *trans-p*-coumaric acid, which is further transformed into ferulic acid and sinapic acid. Cinnamic acid can undergo ring substitution in a series of hydroxylation and methylation steps, resulting in various acids that can be activated, as their corresponding esters of CoA. These activated esters can enter various pathways leading to lignin, flavonoids, stilbenes, benzoic acids and other compounds (Schreier, 1984; Grisebach, 1981). The biosynthetic pathway forming lignin (through *p*-coumaric and ferulic acids) is responsible for various aromatic compounds such as alcohols, esters, flavonoids, hydroxyacids and amides (Gross, 1981).

Cinnamyl alcohols constitute the substrate pool leading to numerous characteristic aromatic compounds via side-chain elongations and degradations (Gross, 1981). After side-chain elongation, it is assumed that cinnamic acids proceed through β -oxidation to form benzoyl-CoA esters that could form benzoic acid esters or benzaldehyde and alcohols (Gross, 1981). Side-chain degradation, effectively removing an acetate unit from cinnamic acids, is proposed to be an important pathway resulting in the formation of benzoic acids (Schreier, 1984). Although there are several different pathways for the formation of benzoic acids, side-chain degradation of cinnamic acids is the most important mechanism (Gross, 1981). Characteristic aromatic compound production depends on the substitution pattern of the individual benzoic acids based on their respective phenylpropane precursors (Schreier, 1984). In tomato, phenylalanine can be converted to 1-nitro phenylethane and then to phenylacetaldehyde *in vitro* by pH reduction (Buttery, 1993). [^{14}C]phenylalanine was the precursor for phenylethanol, phenethylacetate, phenethyl butanoate and phenolic ethers in banana (Tressl and

Drawert, 1973). The conversion of labeled phenylalanine into phenolic ethers such as eugenol, eugenol methyl ether and elimicin was catalyzed by PAL, cinnamic acid-4-hydroxylase, phenolase and methyltransferase (Tressl and Drawert, 1973). After processing, increased PAL activity leads to browning and phenolic metabolism in lettuce (Ke and Saltveit, 1989). Browning-related phenolic oxidation compounds have been implicated to contribute to off-flavors (Whitaker and Lee, 1995).

The mevalonic acid pathway forms terpenoids, carotenoids and geraniol (geranium oil). Aromatic terpenes are generally considered secondary defense compounds, yet some such as limonene and menthol have aroma attributes as well. The oxygenated terpenoid volatiles linalool, neral and geraniol, which are important for flavor in some fruits, have been identified in ripe tomatoes (Buttery and Ling, 1993). Lactones that are important flavors of peaches and apricots are produced via LOX activity (Crouzet et al., 1990). Extensive information concerning formation of chiral δ -lactone and γ -lactone and the pathways has been published (Tressl et al., 1996).

Certain aromatic compounds are believed to be formed as breakdown products from various aromatic pigments such as lycopene, carotene, etc., and these compounds apparently occur as oxidation products as well (Buttery, 1981). For example, β -ionone probably results from the oxidative breakdown of β -carotene. Therefore, during wounding or processing (cutting or chewing), the induction of certain volatiles (predominately secondary metabolites or oxidation products) may affect flavor attributes, especially in fruits and vegetables with high concentrations of readily oxidizable aromatic pigments (i.e., carotene) and fatty acids.

Aldehydes and the Lipoxygenase Pathway

The pleasant odor in cucumber was attributed to 2,6-nonadienal, and two unsaturated aldehydes (2-hexenal and 2-nonenal) and three saturated aldehydes (ethanal, propanal and hexanal) were considered to contribute secondarily to overall flavor (Forss et al., 1962). Using cucumber homogenates radiolabeled with ^{14}C -linolenic (18:3) and ^{14}C -linoleic (18:2), the flavor active aldehydes propanal (*E*) 2-hexenal and (*E,Z*) 2,6-nonadienal were related to linolenic acid, whereas hexanal and (*E*) 2-nonenal were related to linoleic acid (Grosch and Schwarz, 1971). However, cutting or mechanically rupturing cucumber fruit tissue was associated with enzymatically produced aldehydes (Fleming et al., 1968). Production of green-apple-like odors, known to be related to hexanal and (*E*) 2-hexenal, increased upon crushing or cutting fruit cells (Drawert et al., 1966). C_6 aldehydes and alcohols have since been recovered after tissue disruption in numerous crops (e.g., apple, banana, grape, green leafy tissue, olive and tomato) and are related to the LOX pathway.

Lipoxygenase (Galliard and Phillips, 1976), a hydroperoxide lyase (HPL) (Galliard et al., 1976) and (*Z*)-3: (*E*)-2-enal isomerase (Phillips et al., 1979) enzymes are involved in the formation of volatiles from fatty acid precursors. The LOX pathway is also responsible for production of C_6 aroma compounds [i.e., (*Z*)-3-hexenal, (*Z*)-3-hexenol and (*Z*)-3-hexenyl acetate] in green leafy and fruit tissue (Gardner, 1989). Acyl hydrolases (AH) effectively catalyze the hydrolysis of ester bonds in monoglycerides, monogalactosyldiglycerides, lysoglycerophospholipides, diglycerides, digalactosyldiglycerides and glycerophospholipids but not ester bonds

in triglycerides. AH enzymes are very active and destroy most membrane-bound polar lipids that lead to the free fatty acid pool ($C_{16:0}$, $C_{18:2}$, $C_{18:3}$) when tissue is disrupted (Goodwin and Mercer, 1988). Lipid AH breaks down linolenic acid, which is hydroperoxygenated by LOX into hydroperoxylinolenic acid, then HPL cleaves this into (Z)-3-hexenal and 12-oxo-(Z) dodecenoic acid. The (Z)-3-hexenal is subsequently reduced via ADH to (Z)-3-hexenol, which is then esterified to (Z)-3-hexenyl acetate (Hatanaka, 1993; Anderson, 1989; Sekiya et al., 1982). The LOX and HPL enzyme system involved is bound to the thylakoid membrane of chloroplasts in green leaves. Lipoxygenase adds oxygen stereoselectively to unsaturated fatty acids having a (1Z, 4Z)-pentadiene moiety (e.g., alpha-linolenic and linoleic acids), to produce 13-(S)-hydroperoxides that are next cleaved by HPL at the bond between C_{12} and C_{13} of these hydroperoxides to form C_6 aldehydes (Hatanaka, 1993).

“Green” and “grassy” food flavors are generally due to C_6 compounds formed from unsaturated aliphatic C_{18} fatty acids oxidized by LOX and intermediary substrates converted into various organoleptic compounds via HPL. For example, the characteristic tomato flavor compounds hexenal and (Z)-3-hexenal are thought to be secondary compounds (Riley and Thompson, 1998). Likewise, the characteristic flavor compounds of bell peppers [hexanol, hexanal, (Z)-3-hexen-1-ol, (E)-2-hexenal, (E)-2-hexen-1-ol] and cucumbers [(E,Z)-2, 6-nonadienal, (E)-2-nonenal and 2-hexenal] are generated enzymtically via LOX, as a consequence of cutting or homogenization (Matsui et al., 1997; Wu and Liou, 1986; Fleming et al., 1968). LOX and HPL have been found in many fruits commonly fresh cut (Pérez et al., 1999b; Matsui et al., 1997; Riley et al., 1996; Olías et al., 1993; Kim and Grosch, 1981; Vick and Zimmerman, 1976). In bell peppers, both HPL and LOX activities decreased with maturation, and the amounts of C_6 aldehydes and alcohols formed from homogenization of mature fruit also decreased (Matsui et al., 1997). Subsequently, selection for varieties with low LOX and HPL activity may be critical in crops where cutting may provoke off-flavors.

Hexyl acetate arising from olive crushing has been associated with lipid degrading enzymes as well as a complete enzyme system leading to green odor notes (Olías et al., 1993). Triacylglycerols and phospholipids (mainly polyunsaturated) are hydrolyzed by AH. LOX then cleaves the resulting linoleic and linolenic acids into 9- and 13-hydroperoxides, and then HPL selectively cleaves the 13-hydroperoxide to form hexanal and (Z)-3-hexenal. Hexanal and hexenal (oftentimes including isomerizations) are reduced via ADH to their corresponding alcohols. Finally, AAT, with the participation of acetyl-CoA (hence, not a direct esterification), produces an ester from the alcohol (Olías et al., 1993; Pérez et al., 1993). Similar results concerning ester formation (oftentimes utilizing precursor feeding regimes) via LOX and β -oxidation, dependent upon available acetyl CoA, have been reported in apple (Rowan et al., 1996, 1999; Berger and Drawert, 1984), banana (Harada et al., 1985; Myers et al., 1970) and strawberry (Yamashita et al., 1975).

IMPORTANT AROMAS AND FLAVORS

Although wounding tissue invokes secondary aldehyde and alcohol production, many aldehydes have been considered to impart characteristic and desirable odors to foods so long as their concentrations are extremely low. Furthermore, cell disruption is

sometimes necessary to allow enzymes and substrates that were formerly compartmentalized to interact (Buttery, 1993). For example, some aroma compounds are bound to sugars as glycosides (celery, lettuce) or glucosinolates (cabbage, radish). Glycosidically bound furaneol (2,5-dimethyl-4-hydroxy-2H-furan-3-one) and its methyl ether mesifurane are important aroma components in strawberry that appear to have D-fructose as a precursor (Pérez et al., 1999a; Zabetakis et al., 1996). This linkage can be cleaved by enzyme action or heat (cooking). Bound volatiles were also found in fruits such as apricot, mango, grape and passion fruit (Chassagne and Crouzet, 1995). Others are breakdown products of lipids, amino acids, lignin or pigments (Buttery and Ling, 1993). Secondary metabolites can also be produced due to wounding of tissue that occurs during processing (Wong, 1994). For example, there is an accumulation of glycoalkaloids in damaged potato tubers and an increase in polyphenols in many tissues that brown on the cut surface, such as apples and pears, for which the flavor impact is unknown.

Thioesters have been reported to be probable flavor notes responsible for the “earthy” and “musky” notes in muskmelon (Wyllie et al., 1994). In addition, methionine can be converted into thioether esters (Wyllie et al., 1995). Glucosinolates (thioglucosides) are naturally occurring secondary plant metabolites that have been found in the Cruciferae family. These metabolites (mainly sulfur containing) are responsible for the taste and odor, termed “mustard oils,” in these vegetables (Ju et al., 1982), especially upon cell rupture. When Cruciferae cells are ruptured, glucosinolates undergo enzymatic hydrolysis with the endogenous myrosinase enzymes (thioglucosidase), releasing thiocyanates, isothiocyanates (Wattenberg, 1978) sulfate and glucose (Ju et al., 1982). Therefore, extra precautions must be taken to insure that off-odors and off-flavors do not jeopardize the marketability of shredded Crucifer products.

SUGARS (SOLUBLE SOLIDS), ORGANIC ACIDS AND TITRATABLE ACIDITY

Important flavor contributions for many fruits and vegetable are attributed to specific organic acids and various sugars. Glucose, sucrose and fructose are the most important sugars that affect the perception of sweetness. Of these, glucose is perceived as less sweet and fructose more sweet than sucrose. Thus, a weighting of these sugars in relation to sucrose and subsequent combining can give a single value “sucrose equivalent” (SE) for a sample (Koehler and Kays, 1991). Sugars are commonly thought to be synonymous with soluble solids (SS). However, the real proportion of sugars measured in SS depends on the fruit or vegetable, and this may still not indicate clearly the contribution to flavor. For example, in orange, SS appear to relate to sweetness, while in tomato and mango, the relationship is not clear (Malundo et al., 2001; Baldwin et al., 1998, 1999a). It is possible that cut produce that exhibits high respiration due to wounding may catabolize sugars or acids as a carbon source during storage. Sweetness, flesh firmness and taste are very important characteristics for fresh-cut melon quality. It is a well-established fact in the food industry that sugar content is generally positively correlated with desirable flavor quality. However, occasionally, too much sugar can be perceived negatively. The best sugar range for storage of fresh-cut cantaloupe was found to be 10–13°Brix, however, some people judged the fruit as

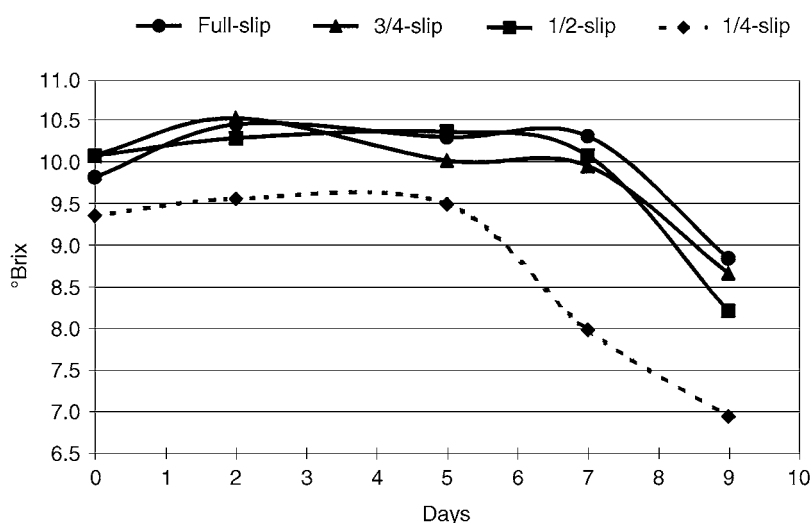


FIGURE 12.1 °Brix change (Atago PR-101) in fresh-cut cantaloupe prepared from fruit harvested at different maturities, held at 4°C ($n = 30$). All quarter-slip means, except day 0, were significantly different (0.05 level) from other maturities according to an analysis of variance performed with Duncan's multiple-range test.

being too sweet at 13°Brix (Anonymous, 2000). In 17 Western cantaloupe varieties, there was an average 5% decrease in SS content (range 0–11%) and an average 8% decrease in sugar concentration (range 0–21%) when cubes were stored 12 days (in air) at 5°C (Cantwell and Portela, 1997). Likewise, SS were found to decrease in cut peaches during storage at 2°C in either air or 2% O₂, which affected flavor (Mencarelli et al., 1998). °Brix decreased 9.7% (range 2.3–13%) in cantaloupe balls prepared from four Eastern varieties stored 8 days at 0°C (Lange, 1998). However, CA-stored melon pieces had higher SS concentrations than air-stored (10.3% vs. 9.5% and 10.2% vs. 9.1%, at 10°C and 5°C, respectively), after 9 days at 10°C and 15 days at 5°C (Cantwell and Portela, 1997). Soluble solids remained somewhat constant for 7 days storage (4°C) in fresh-cut cantaloupe when harvest maturity was at least half-slip, but rapidly declined after only 5 days of storage in quarter-slip cubes (Figure 12.1). Cubes prepared from fruit harvested at quarter-slip had significantly lower (0.05 level) °Brix compared to all other harvest maturities on all days, except day 0. However, in fresh-cut 'Gala' apple, sugars remained constant during 14 days of storage at 1°C, although sweetness and sweet aromatic flavor increased then decreased (Bett et al., 2001).

Titrateable acidity and pH have also been used to assess the sugar-to-acidity ratio in some fresh-cut fruits. Changes in TA, pH and SS in apple slices from 12 cultivars that were stored at 2°C for 12 days were small and varied by cultivar (Kim et al., 1993). In cut 'Gala' apple, pH decreased during 2 weeks storage at 1°C, while %TA increased then decreased and sensory perception of sourness also fluctuated

(Bett et al., 2001). Fresh-cut persimmons stored in CA for 7 days at 5°C had increased SS for 3 days then decreased by day eight, and pH tended to increase through storage (except when stored under 2% O₂) (Wright and Kader, 1997). A 17% loss in SS and a twofold acidity increase occurred after only 2 days of storage at 20°C in cantaloupe slices, but acidity change was attributed to lactic acid bacteria (Lamikanra et al., 2000). Titratable acidity in fresh-cut oranges stored at 4°C for 8 days decreased 36% (Rocha et al., 1995).

The main organic acids regarding flavor notes for most fruits are malic, citric, tartaric, succinic and quinic acids (Kays, 1997). Sugars are the primary product of photosynthesis, whereas most organic acids are synthesized from glycolytic precursors in the tricarboxylic acid pathway as products of respiration. A characteristic flavor is often attributed to a high concentration of one organic acid (e.g., citric acid in lemon) or to the overall acidity or sugar-to-acid ratio inherent to the product/variety (e.g., tomato and apple). Acids such as citric in citrus and tomatoes, tartaric in grapes and malic in apples, give fruit and vegetables their sour flavor. Some fruits, like melon or banana, have very little acid (Wyllie et al., 1995). Sometimes, measurement of SS, the ratio of SS/TA or pH relate better to sourness than TA itself (Malundo et al., 2001; Baldwin et al., 1998).

FACTORS AFFECTING FRESH-CUT FLAVOR

Overall, flavor is affected by genetics, preharvest environment (Baldwin et al., 1995c; Kim et al., 1993; Romani et al., 1983), cultural practices (Romig, 1995; Wright and Harris, 1985), harvest maturity and postharvest handling or storage (Baldwin et al., 1999a,b; Gorny et al., 1998a; Maul et al., 1998a,b; Watada et al., 1996; Mattheis et al., 1991, 1995; Fellman et al., 1993b). Generally speaking, flavor of fresh produce will not improve after harvest (aside from the effect of continued ripening in climacteric fruit), and therefore, flavor deterioration should be minimized. This is an especially difficult task in fresh-cut products, where biochemical changes due to wounding can affect shelf life and flavor quality. Processing technique (Bolin et al., 1977; Saltveit, 1997; Wright and Kader, 1997), sanitation (Hurst, 1995), packaging (Cameron et al., 1995; Solomos, 1994) and temperature management during shipping, handling and marketing (Brecht, 1999) also play important roles in maintenance of fresh-cut quality.

Physical alterations and potential low O₂ atmospheres in packages may create significant negative changes in flavor and aroma. There are also synergistic interactions between numerous factors such as variety, source, season, initial maturity, optimum processing maturity, slicing and cutting equipment, GRAS treatments, container or bag [including modified atmosphere packaging (MAP)], temperature management, shipping, handling and length of shelf life. The synergistic interaction between the above factors may have negative consequences on flavor attributes and sensory acceptability. However, little information is available regarding the aggregate effects these factors have on flavor quality. Therefore, properly preparing, packaging and handling fresh-cut products is essential to avoid potential flavor loss or change that may actually cause a decrease in consumer satisfaction.

MORPHOLOGICAL CONSIDERATIONS

Fruit tissues, due to their unique anatomical nature, are very susceptible to bruising and mechanical injury. This is very different from most fresh-cut vegetables that may be derived from very durable root tissues (e.g., carrots, radishes) or pliable leaf tissues (e.g., iceberg lettuce, cabbage). Because a commodity's natural cuticle or skin barrier to gas diffusion is compromised during processing, accelerated flavor volatile loss most likely accompanies increased respiration and ethylene production.

CHILLING INJURY AND STORAGE TEMPERATURES

One type of chilling injury (CI) is the loss of aroma compounds. Chilling tomato fruit to 5°C for one week with subsequent ripening at 20°C affected the fruit flavor (Kader et al., 1978). In other studies, tomato fruit stored at 2, 5, 10 and 13°C were shown to have reduced levels of important volatiles (Maul et al., 2000; Buttery et al., 1987). Tomatoes stored at 2, 5, 10 or 12.5°C were also shown to have less ripe aroma and flavors as well as more off-flavors compared to fruit stored at 20°C by a trained descriptive panel (Maul et al., 2000). Cut tomato must be stored at 5°C or lower to prevent spoilage. If not ripened properly, >27–49 Newtons (N), prior to cutting, stone fruit are susceptible to aberrant ripening that negatively affected eating quality when fresh-cut product was stored at CI temperatures (Gorny et al., 1999a). A significant number of fruit are CI sensitive as whole intact fruit before processing. Examples include fruit such as pineapple, cantaloupe, honeydew, watermelon, peach, nectarine and mango. Therefore, in certain cases, chilling temperatures before processing and subsequent holding of fresh-cut products at low temperatures may have negative consequences on flavor quality. Intermittent warming and brief heat treatments have been employed in order to alleviate CI in some crops. However, heat treatment of apple fruit to reduce physiological and pathological disorders inhibited emission of volatile esters important to apple flavor (Fallik et al., 1997). Furthermore, commercially harvested 'Gala' apples that were heat treated after harvest and later sliced and stored showed lower volatile levels than unheated controls (Table 12.1).

VARIETIES, GROWING REGION AND SEASON

Preharvest factors such as sunlight, water availability, fertilization and chemical applications undoubtedly affect the condition of the crop. This, in turn, can have an effect on the internal quality characteristics of the harvested product, including flavor. Preharvest treatment with aminoethoxyvinylglycine (AVG) suppressed volatile production in pears by approximately 50%, while ethylene exposure reversed the suppression (Romani et al., 1983). Heavy rains prior to harvest appeared to dilute flavor compounds in tomato (Baldwin et al., 1995a). Fruit from tomato plants treated with increased levels of nitrogen and potassium fertilizer scored lower in sensory analysis and showed increased levels of TA, SS and several volatiles (Wright and Harris, 1985). Greenhouse-grown tomatoes exhibited lower levels of numerous volatile components compared to their field-grown counterparts (Dalal et al., 1967). According to a trained sensory panel, mite control prior to harvest resulted in field-grown

TABLE 12.1
Effect of Heat-Treating Intact Commercially Harvested ‘Gala’ Apple Fruit on Important Volatile Components in Fresh-cuts during Storage at 8°C. Intact Apples were Subjected to 38°C at 98% RH for 4 Days Prior to Slicing

Volatile Compound (ng g ⁻¹)	Day 0		Day 7	
	Control	Heat	Control	Heat
Butanol	429 a ¹	73 c	200 b	60 c
Butyl acetate	499 a	14 c	148 b	17 c
Butyl butanoate	4 a	5 a	0 c	1 b
Butyl hexanoate	3 a	n.d. ²	1 b	n.d.
2-Methylbutyl acetate	15 a	4 b	4 b	3 b
2-Methylbutyl 3-methylbutanoate	4 a	3 a	3 a	3 a
Hexanal	n.d.	7 b	n.d.	14 a
Trans-2-hexenal	5 a	4 a	6 a	5 a
Hexyl acetate	19 a	1 c	5 b	1 c

¹ Mean values (*n* = 3) in the same row that are not followed by the same letter show significant difference (*p* < 0.05).

² n.d. = not detected.

strawberries with more sweetness and flavor intensity than those receiving no treatments (Podoski et al., 1997). Varieties of fruits and vegetables have been shown to differ in flavor based on sensory and chemical analysis, reflecting their genetic diversity. For example, aroma in apples was not the result of the same compounds in every cultivar, although some volatile compounds that seemed to be important were common to all 40 cultivars studied (Cunningham et al., 1985). Varieties perform optimally in certain growing regions and often have variable postharvest quality attributes depending on cultural practices, climate, season and harvest maturity. The proportions of dominant apple volatiles varied by season (López et al., 1998), and desirable pineapple flesh volatile oil content was higher in summer fruit than in winter fruit (Haagen-Smit et al., 1945). Several reports have documented clearly that certain cultivars outperform others with regard to fresh-cut shelf life and keeping quality (Anonymous, 2000; Gorny et al., 1998b, 1999b; Lange, 1998; Cantwell and Portela, 1997; Kim et al., 1993). However, little to no data are available concerning flavor and sensory quality for fresh-cuts produced from different varieties grown under different cultural conditions.

Harvest maturity can also affect the flavor of the ripened product. This is especially important for fresh-cut produce, where harvest maturity can also affect the shelf life of the product. Ideally, horticultural products are harvested at a stage that gives optimal eating quality. In reality, this optimal quality is often sacrificed to minimize physical damage during shipping, handling and processing to maximize shelf life. Harvest maturity affected ester formation in apples, depending on exact climacteric stage at time of harvest (Fellman et al., 1993a). Acid levels decreased as days after full bloom increased for apple, and this affected sensory responses for

tartness (Plotto et al., 1997). However, apples harvested later were found to be more fruity and sweet compared to apples harvested two weeks earlier (Cliff et al., 1998). Harvest maturity was shown to affect both the sensory and chemical analysis of ripened tomato fruit (Maul et al., 1998a). Tomatoes harvested at the immature green stage resulted in ripened fruit with lower volatile levels than mature green-harvested tomatoes, while tomatoes harvested table ripe displayed higher intensities for sweetness, saltiness and fruity floral aroma (due to levels of both volatile and nonvolatile components) than green or breaker-harvested fruit (Watada et al., 1979). Similarly, fruit harvested at the turning-red stage were sweeter, less sour and more tomato-like, with less off-flavor than earlier-harvested fruit (Kader et al., 1977). Harvest maturity also affected consumer acceptability ratings of mango and trained descriptive panel ratings for sweetness, sourness and various aroma descriptors. Fruit harvested later were sweeter, less sour and generally had more intense aroma characteristics (Baldwin et al., 1999a). It is not difficult to assume that these findings for intact fruit would apply to the fresh-cut product as well.

RIPENESS AT CUTTING, FIRMNESS AND PROCESSING

Many fruits are picked before they are fully ripe. Therefore, the question arises as to what maturity should climacteric fruit be when fresh-cut in order to optimize product shelf life and eating quality? Both the maturity at harvest and the ripeness stage at cutting will affect the postcutting quality and shelf life of fresh-cut fruit products. Mature-green tomato fruit ripened normally and attained comparable eating quality compared to those fruit that were sliced after the whole fruit ripened (Mencarelli and Saltveit, 1988). However, little research has addressed whether normal ripening will continue in other climacteric fruit if the cutting process is done on unripe fruit. In immature sliced pear and peach fruit, softening occurred, but other ripening-related processes such as flavor development and texture seemed aberrant when fruit were processed at an excessively immature stage (Gorny et al., 2000; Beaulieu et al., 1999; Gorny et al., 1998a; Mencarelli et al., 1998).

Maturity at cutting for many fruits can help to predict potential flavor quality through storage. For example, the optimum initial fruit firmness for 'Bartlett' pears, for maintenance of firmness without browning in fresh-cut slices, was found to be roughly 49 N (Dong et al., 2000; Sapers and Miller, 1998). Fresh-cut pear slices prepared from firm 'Bartlett' and 'Bosc' fruit (70–85 N) were excessively firm and lacked flavor (Dong et al., 2000). For climacteric fruits, initial fruit firmness may, therefore, be a good indicator of fruit ripeness for optimum postcutting flavor quality. A mature green cantaloupe will not have sugars or volatiles associated with a desirable ripe fruit (Pratt, 1971). Melon fruit harvested before fully ripe (full-slip) developed only about one quarter the total volatiles as compared to three-day-old fully ripe fruit (Wyllie et al., 1996a). Mature green cantaloupe (i.e., <half-slip) will deliver a fresh-cut product that has optimum visual shelf-life, but sugars (Figure 12.1) and volatiles (Beaulieu and Grimm, 2001) will be severely compromised, and this trend was conserved through 10 days fresh-cut storage at 4°C. As harvest maturity increased, the relative amount (SPME, GC-MS) of 29 esters increased, yet, quarter-slip fruit had roughly one-third the volatiles as compared to full-slip fruit (Figure 12.2). After five to seven days storage,

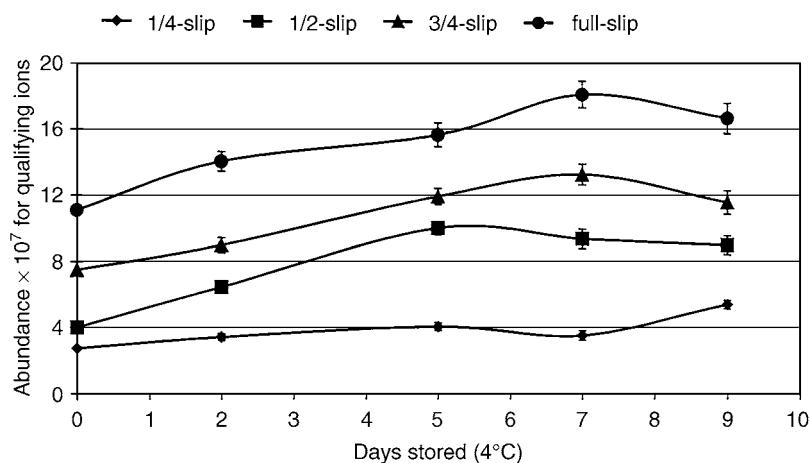


FIGURE 12.2 Change in total esters recovered (solid-phase microextraction and gas chromatography-mass spectrometry; SPME, GC-MS) from fresh-cut cantaloupe prepared with fruit harvested at four maturities, stored at 4°C ($n = 3 \pm$ standard deviation).

volatiles declined slightly in cubes that were prepared from >half-slip maturity fruit. These data may indicate that harvest maturity is critical for fresh-cut flavor quality.

CHEMICAL AND PHYSICAL TREATMENTS

Many physical and chemical treatments have been applied to whole produce, and the efficacy of some of these treatments is currently being investigated on fresh-cuts. However, as with numerous aforementioned protocols, very little aroma or flavor work has been accomplished where edible coatings, disinfection, natural plant products, ethylene absorbents, gamma irradiation, heat shock, microbial competition and pulsed-microwave irradiation have been used on fresh-cuts. For more physiological details and discussion regarding the use of treatments geared toward extending acceptable fresh-cut quality, please see Chapter 9.

Chlorination and Washes

Chlorination, as commonly used for fresh-cut salad sanitation, may not be desirable for all fresh-cut fruits. Postcutting washing and/or dipping may have negative consequences regarding increased water activity and the “washing away” of desirable flavor attributes. Numerous processors do not wash freshly cut fruits that have little or no browning (e.g., melons and strawberry), because GRAS treatments are seldom applied and because water removal (centrifugation or spinning) can severely damage the tissue.

Calcium Salts and Antibrowning

Application of aqueous solutions of calcium salts, ascorbate, citric acid, isoascorbic acid and sodium erythorbate (generally 0.5–1.0% solutions/dips) can help maintain

fresh-cut tissue firmness and reduce surface browning (Gil et al., 1998; Gorny et al., 1998b; Izumi and Watada, 1995; Rosen and Kader, 1989; Sapers and Zoilkowski, 1987; Morris et al., 1985; Ponting et al., 1971, 1972). Unfortunately, some treatments that reduce enzymatic browning or improve texture can impart off-flavors. For example, calcium chloride has been shown to impart a detectable off-flavor in cantaloupe slices at concentrations above 0.5%, whereas calcium lactate improved firmness without imparting bitter flavor (Luna-Guzmán and Barrett, 2000). After 10 days of storage in air at 0°C, 70% of consumers judged fresh-cut 'Bartlett' pear that were treated with 2% ascorbic acid, 1% calcium lactate and 0.5% (w/v) cysteine to have acceptable flavor that was undistinguishable from the controls (Gorny et al., 2002).

Numerous new experimental compounds (ascorbic acid-2-phosphate and ascorbic acid-2-triphosphate, calcium propionate, cysteine, N-acetylcysteine and 4-hexylresorcinol) are being tested for antibrowning capacities. Yet, we are unaware what effects most of these compounds will have upon flavor and aroma in fresh-cuts. It is suspected that 4-hexylresorcinol may impart an unacceptable off-flavor on fruit products. 'Red Delicious' apple slices treated with a combined antibrowning dip (4-hexylresorcinol, isoascorbic acid, N-acetylcysteine and calcium propionate) held at 5°C maintained visual quality for five weeks, yet microbial decay was evident after four weeks (Buta et al., 1999). Analyses of organic acids and the major sugars revealed that the slices treated with combined antibrowning compounds retained higher levels of malic acid and had no deterioration in sugar levels at 5 and 10°C, indicating that higher quality was maintained during storage. One would expect that these results should also translate into maintenance of flavor quality.

Antimicrobial, Edible Coating and Other Treatment Compounds

Treatment of fruits and vegetables with acetaldehyde, ethanol or low O₂ (which can result in production of acetaldehyde and ethanol) has resulted in flavor enhancement of pears, tomatoes and blueberries (Paz et al., 1982), grapes (Pesis and Frenkel, 1989), strawberry (Pesis and Avissar, 1990) and feijoa (Pesis et al., 1991). For example, in strawberry, an increase in acetaldehyde, ethanol, methyl acetate, ethyl acetate and ethyl butyrate was found after application of acetaldehyde (Pesis, 1996). Treatment of oranges with acetaldehyde resulted in induced synthesis of ethylbutyrate (Shaw et al., 1991). Treatment of tomatoes, blueberries and pears led to enhanced sensory quality, in part due to increased sugars (Paz et al., 1982), and reduced acidity in fig and orange (Pesis and Avissar, 1989; Hirai et al., 1968). 'Red Delicious' apples were stored in an atmosphere containing ethanol vapors for 24 hours, which resulted in a threefold increase in ethyl ester concentrations (Berger and Drawert, 1984). However, attempts to promote volatile and sensory attributes with vaporous acetaldehyde or alcohol in fresh-cut fruits may be quite challenging, because these compounds have a maturity- and concentration-dependent effect on inhibiting or promoting ripening (Beaulieu and Saltveit, 1997; Beaulieu et al., 1997).

Nevertheless, treatment of whole fruit with acetaldehyde, ethanol or low O₂ may improve flavor of the subsequent fresh-cut product. In addition, acetaldehyde and ethanol have antimicrobial properties. For example, acetaldehyde reduced *Botrytis cinerea*

TABLE 12.2

Effect of Exogenous Ethanol on Ethyl Ester and Other Major Volatile Ester Contents in Fresh-cut ‘Gala’ Apples during Storage at 8°C. Intact Fruits were Incubated in Saturated Ethanol Vapor at 23°C for 24 Hours before Slicing, or Cut Slices were Dipped in 70% Ethanol for 30 Seconds

Esters (ng g ⁻¹)	Day 0			Day 7		
	Control	Vapor	Dip	Control	Vapor	Dip
<i>Ethylesters</i>						
Acetate	22 e ¹	3386 a	145 d	1032 c	2437 b	2585 b
Propanoate	521 d	2912 c	9986 a	865 d	2270 c	5682 b
Butanoate	2 c	5 b	1 c	148 a	110 a	121 a
2-Methylbutanoate	2 c	11 b	1 c	21 a	16 a	22 a
Hexanoate	2 b	4 a	2 b	2 b	2 b	2 b
<i>Other major esters</i>						
Butyl acetate	535 a	240 c	399 b	158 d	104 e	63 f
Butyl butanoate	4 b	10 a	5 b	2 c	3 c	2 c
Butyl hexanoate	4 b	12 a	6 b	2 c	3 c	3 c
3-Methylbutyl 2-methylbutanoate	0 b	2 a	0 b	0 b	0 b	1 a
Hexyl acetate	19 a	16 a	17 a	5 b	4 b	3 b

¹ Mean values ($n = 3$) in the same row that are not followed by the same letter show significant difference ($p < 0.05$).

and *Rizopus stolonifer* on strawberries and grapes (Prasad and Stadelbacher, 1974). Whole apples were treated with ethanol vapor prior to slicing, and sliced apples received an ethanol dip. In both cases, after two weeks of storage, the ethanol-treated apples were higher in some volatiles, including several esters (Table 12.2).

Hexanal is a natural aroma precursor in apples that is readily converted to aroma volatiles *in vivo* by fresh-cut apple slices (Song et al., 1996). Hexanal not only enhanced the aroma of fresh-cut apple slices, but it also reduced enzymatic browning at the cut surface as well as inhibited molds, yeasts and mesophilic and psychrotrophic bacteria in ‘Ganny Smith’ slices stored at 15°C (Lanciotti et al., 1999). Research is currently in the initial stages for the use of this compound on fresh-cut fruit products. However, it is currently not approved for use.

Microbial spoilage of cut fruit products can affect their flavor quality and shelf life. Methyl jasmonate is a volatile, naturally occurring compound found in many plants and has been reported to have hormone-like activity at very low concentrations. Exogenously applied methyl jasmonate has been shown to be very effective in reducing mold growth on fresh-cut celery and peppers and may, in the future, have applications as a naturally derived fungicide (Buta and Moline, 1998). Citric acid is a GRAS-listed compound that is a natural organic acid and can be used as a preservative, acidulant or flavoring agent in foods. By acidifying the surface of cut products, citric acid can reduce the microbial load and thus improve flavor. The shelf life of peeled oranges was extended by 0.5–1.0% citric acid infusion treatments that

TABLE 12.3

Effect of Treating Intact ‘Gala’ Fruit with 1-Methylcyclopropene (MCP) on Major Volatile Contents in Fresh-cut Apples during Storage at 8°C. Treatment Consisted of 1 ppm MCP for 18 Hours at 20°C prior to Slicing

Volatile Compounds (ng g ⁻¹)	Day 0		Day 7	
	Control	MCP	Control	MCP
Butanol	201 b ¹	296 a	100 c	73 d
Butyl acetate	323 b	392 a	143 c	90 d
Butyl butanoate	5 a	5 a	1 b	1 b
Butyl hexanoate	2 a	3 a	1 b	n.d. ²
2-Methylbutyl acetate	28 a	29 a	3 b	3 b
2-Methylbutyl 3-methylbutanoate	4 a	3 a	3 a	3 a
Hexanal	n.d.	6 a	n.d.	n. d.
<i>Trans</i> -2-hexenal	6 b	9 a	5 b	2 c
Hexyl acetate	15 a	16 a	6 b	2 c

¹ Mean values ($n = 3$) in the same row that are not followed by the same letter show significant difference ($p < 0.05$).

² n.d. = not detected.

inhibited growth of spoilage bacteria (Pao and Petrcek, 1997). Diced celery flavor and shelf life were improved by treatment of samples with 0.5 and 1.0 kGy gamma irradiation compared to conventional treatments such as acidification, blanching and chlorination (Prakash et al., 2000). Although all samples lost flavor over 22 days of storage, irradiated samples maintained color, texture and aroma longer than samples from other treatments and had less off-flavor. Sensory shelf life was extended 7–29 days, in part due to reduced aerobic microbial plate counts. 1-Methylcyclopropene (MCP) has been shown to block ethylene action and, thus, inhibit many ethylene responses such as ripening, softening, etc., as has been shown on avocados (Feng et al., 2000), apricots (Fan et al., 2000) and bananas (Harris et al., 2000). The effects of MCP could be useful for fresh-cut products. Whole apples treated with MCP and later sliced and stored were firmer but contained less aroma compounds than nontreated fruit after one week (Table 12.3).

Use of edible coatings can improve fresh-cut fruit quality. Peeled packaged citrus products have a shelf life of approximately 17–21 days, but fluid leakage can be problematic. Edible wax microemulsion coatings (up to 12% solids) reduced leakage of dry-packed grapefruit segments by 80% after 2 weeks and 64% after 4 weeks of storage (Baker and Hagenmaier, 1997). Coatings with carnauba wax were found to be most effective, and coatings were not detected by informal taste panels before or after storage (Baker and Hagenmaier, 1997). Cut apples, treated with acidic coatings, exhibited lower microbial populations, without an overly acidic flavor (Baldwin et al., 1996). Research on use of edible coatings on fresh-cuts has recently escalated, but little work has been published concerning retention of flavor quality.

CONTROLLED ATMOSPHERE, MODIFIED ATMOSPHERE PACKAGING AND FLAVOR

The beneficial effects of CA storage for whole fruits and vegetables have been well documented, and CA storage is widely employed throughout the produce industry. However, CA storage alters the flavor of apples (Yahia, 1994), and if prolonged, reduces the volatile emission compared to air-stored fruit (Fellman et al., 1993a; Yahia, 1991; Lidster et al., 1983), especially lipid-derived esters (Mattheis et al., 1995; Yahia et al., 1990). Sensory analysis of CA-stored apples revealed that intensity of fruity and floral descriptors decreased after 10 weeks in CA, while sourness and astringency were higher compared to apples stored in air. Some recovery of aroma was noted after removal from CA to air (Plotto et al., 1999). CA storage also increased certain volatiles in tomato compared to air-stored fruit (Crouzet et al., 1986). Therefore, certain packaged fresh-cut products may require active modification of the atmosphere so as to insure desirable flavor at time of consumption.

High CO₂, low O₂ refrigerated CA storage is most likely associated with decreased ester volatile production, because respiration and the tricarboxylic acid cycle (TCA) are downregulated. The precursors to the alcohol and carboxylic acid moieties for ester formation in apples are thought to originate from fatty acid and amino acid metabolism during oxygen-dependent events (Hansen et al., 1992). Esterification of alcohols to the corresponding acetates proceeds via oxygen-dependent AAT, in the presence of acetyl CoA. AAT activity was suppressed by low O₂ (0.5–1.0%) CA storage in apples (Fellman et al., 1993a). Unlike melons, amino acids, which are the putative precursors for some esters, decrease during apple ripening and remain relatively constant in storage (Ackermann et al., 1992). Subsequently, the synthesis of important amino acids derived from the TCA and their backbone moieties will also be reduced (Brackmann et al., 1993), because reduced catabolic activity and β -oxidation limit the substrates (mainly acetyl CoA and amino acids) required to continuously produce aroma volatiles.

For fresh-cuts, the method of storage is more likely to include packaging that can create a modified atmosphere (MA) and possibly edible coatings that can enhance the MA with reduced O₂ and elevated CO₂ levels, similar to that of CA. Use of edible coatings has also been shown to affect flavor and levels of volatile flavor compounds in intact citrus (Baldwin et al., 1995b; Cohen et al., 1990), apple (Saftner, 1999; Saftner et al., 1999) and mango fruit (Baldwin et al., 1999b). This was likely due to anaerobic respiration, which induces synthesis of ethanol and acetaldehyde, and to entrapment of volatiles, including ethanol and acetaldehyde, by the coating barrier (Baldwin et al., 1995c, 1999b). Entrapment of volatiles may be desirable in cut fruit, because many will be otherwise lost due to off-gassing at the cut surface. Fresh-cut 'Gala' apples packaged in film pouches stored for up to 14 days lost volatiles including farnescene, hexyl hexanoate and 2-methylbutyl hexanoate, whereas hexyl acetate and hexane increased during storage (Bett et al., 2001).

MAP is widely used for fresh-cut vegetables and fruits; however, occasionally, undesirable atmospheres can reduce quality due to discoloration and off-flavors induced by anaerobic respiration (Mateos et al., 1993; Kader, 1986). Broccoli is particularly sensitive to MAP, creating sulfur-containing volatiles, including methanethiol and

dimethyl disulfide in anaerobic conditions (Dan et al., 1997) which could be problematic in fresh-cut florets. Foul odors were detected in broccoli florets packaged in MAP after one to three days at 5°C when O₂ concentrations dropped below 1% (Ballantyne et al., 1988b); yet, CA (6% CO₂ + 2% O₂) at 4°C maintained flavor quality (Bastrash et al., 1993). Sweet off-odors were detected in fresh-cut lettuce stored at 5°C under MAP (5% CO₂ + 5% O₂) by day 14 (Ballantyne et al., 1988a). Flavor loss was greatest and off-flavors were detected in fresh-cut lettuce stored at 5°C in polyethylene bags by day 10, and sweet odors were detected by 17 days in vacuum-sealed PE bags (Heimdal et al., 1995). Off-odors were detected in shredded cabbage stored under CA (0–15% CO₂ + 6% O₂) after six days at 5°C, and when product was stored in MA pouches (with 1.7% O₂), these off-odors were detected after just four days of storage (Kaji et al., 1993). Because fruit and vegetable tolerance to reduced O₂ and elevated CO₂ levels is mainly attributed to skin resistance to gas diffusion (Park et al., 1993; Theologis and Laties, 1982), a reexamination of optimum atmospheres in addition to physical and chemical treatments for fresh-cut fruits is underway.

Most fresh-cut apple research has focused on browning, and scant sensory and flavor analysis has been performed. In fresh-cut ‘Gala’ apples packaged in film pouches, flavor intensity increased the first few days after preparation and packaging, then dissipated after five to nine days (Bett et al., 2001). ‘Gala’ apples stored in CA had a decrease in volatile production as well as a decrease in fruity flavor (Plotto et al., 1999). We evaluated postharvest and flavor changes in browning-inhibited (BI = 2 or 4% sodium erythorbate + 0.1% calcium chloride) stored fresh-cut ‘Gala’ apples prepared immediately after harvest (pre-CA) or after CA storage (three months, 1.4% CO₂ and 3% O₂ + one month refrigeration at 4°C). All pre-CA Hunter L* values were higher than post-CA for all treatments on all sampling days, and both BI treatments maintained color for 14 days (Figure 12.3). In general, wedge color was superior in most pre-CA vs. post-CA treatments throughout the 14 days of storage. Although BI treatments maintained color, most wedges stored in linear low-density polyethylene (LLDPE) pouches or air generally experienced short-term ester increase, followed by a significant decrease and concomitant alcohol increases. Most recovered alcohols (e.g., ethanol, 1-butanol, 1-hexanol and 1-octanol) increased significantly after two days of storage in pouches [Figure 12.4(a)], whereas there were slight decreases in alcohol concentration in flow-through air. Most flavor-related esters generally increased by day two and decreased continuously if held in pouches or decreased until day seven then gradually increased through 14 days when stored in flow-through containers [Figure 12.4(b)]. With one exception (hexyl 2-methylbutanoate), BI-treated tissue stored in pouches generally had lower ester recovery after 14 days. Accumulation of esters in LLDPE pouches could be explained by a respiratory- or wound-induced burst associated with decreasing resistance for volatiles escaping the tissue, then general decline with ensuing catabolism of pyruvic acid into alcohols, as opposed to the TCA.

Numerous ester volatiles potentially having flavor impact in apples (e.g., butyl acetate, 2-methyl-1-butyl acetate, pentyl acetate, butyl hexanoate, hexyl acetate and butyl hexanoate) followed the trend illustrated in Figure 12.4(b), as pouches approached anaerobic conditions (data not shown). On the other hand, CA-stored fresh-cut ‘Bounty’ peach wedges stored in either air flow-through or CA atmosphere (1% O₂ + 5% CO₂) had extremely similar flavor volatile profiles. In preliminary

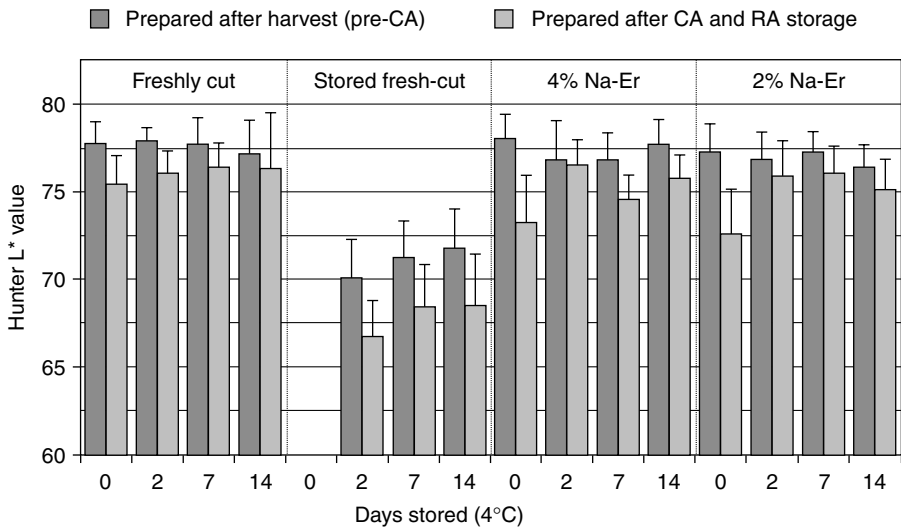


FIGURE 12.3 Color change in pre-CA and post-CA (three months in 1.4% CO₂ and 3% O₂ at 4°C) and RA (one month in air at 4°C) stored, browning-inhibited (2% Na-erythorbate + 0.1% CaCl₂), fresh-cut ‘Gala’ apple wedges ($n = 30 \pm$ standard deviation).

fresh-cut peach experiments, the concentration of linalool (3,6 dimethyl-1,6-octadien-3-ol) increased on day two in tree-ripe (TR = harvested at 29.7 N, processed at < 26.6 N) wedges but declined in commercially ripe (CR = firmer, less mature) wedges [Figure 12.5(a)]. The concentration of most characteristic flavor lactones (e.g., γ -decalactone) increased after two days of storage in wedges prepared from both TR and overripe (OR) fruit but declined faster in OR as compared to TR through seven or 12 days of storage, respectively [Figure 12.5(b)]. Poly-packed (MA) fresh-cut fruits may suffer substantial flavor loss after roughly one week of storage if films do not have adequate CO₂ and O₂ transmission rates or if fruits were previously CA-stored, especially if fresh-cut product is temperature abused.

FLAVOR LIFE VS. SHELF LIFE

Flavor and aroma qualities are most often the true indicators of shelf life from the consumer’s point of view. Unfortunately, “quality” of intact vegetables and fruits generally emphasizes maintenance of appearance, at times sacrificing flavor and texture (Sapers et al., 1997). Much variability exists in the fresh-cut literature regarding acceptability based on sensory evaluations, and this variability can often be attributed to different varied sensory analyses and experimental design. For example, sensory evaluation determined that fresh-cut honeydew, kiwi, papaya, pineapple and cantaloupe stored at 4°C were unacceptable after 7, 4, 2, ~7 and 4 days, respectively (O’Connor-Shaw et al., 1994). Yet, fruit were not sanitized and gloves were not worn during preparation, and subsequently, microbial decay and associated texture loss most likely limited postcutting life. Fresh-cut pineapple stored at 4°C had excellent

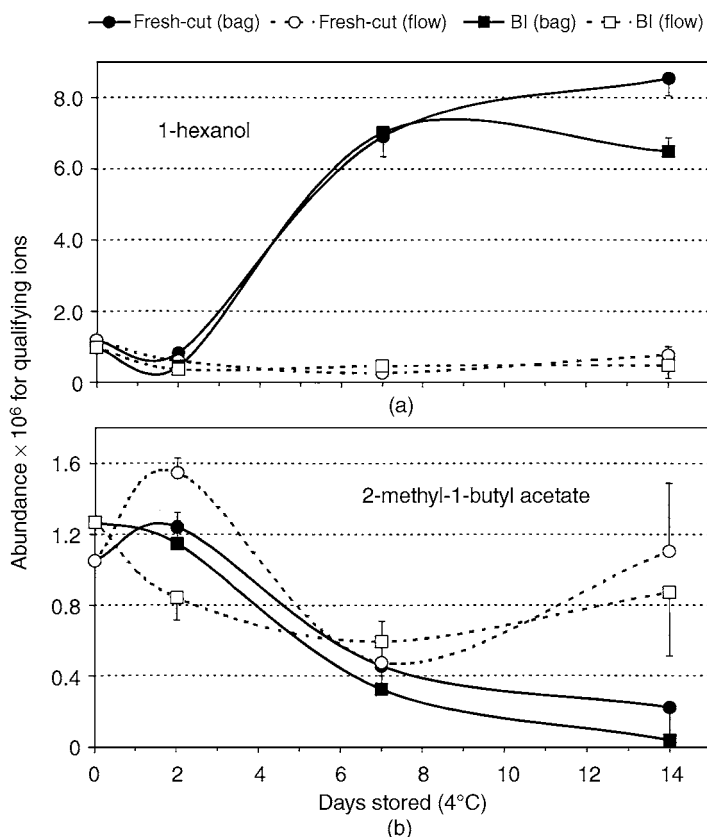


FIGURE 12.4 Typical alcohol (a) 1-hexanol and ester (b) 2-methyl-1-butyl acetate volatile profiles (SPME, GC-MS) for fresh-cut 'Gala' wedges $\pm$ browning inhibitor (2% Na-erythorbate + 0.1% CaCl_2) stored at 4°C in LLDPE pouches or flow-through air ($n = 3 \pm$ standard deviation).

visual appearance. However, chunks in the lower portion of containers developed off-flavors associated with microbial fermentation after seven to 10 days of storage (Spanier et al., 1998). Cantaloupe pieces stored at 2°C in ready-to-serve tray-packs were visually acceptable after 19 days, but flavor scores were low after 13 days (Silva et al., 1987). Fresh-cut honeydew melon stored in air at 5°C for six days were judged by an informal taste panel as being flat in flavor and lacking textural characteristics (Qi et al., 1998). Fresh-cut orange segments that had acceptable appearance after 14 days of storage were found to have unacceptable flavor quality after only five days of storage at 4°C (Rocha et al., 1995). Likewise, undesirable flavor was the limiting subjective factor in sliced, wrapped watermelon stored seven days at 5°C, even though aroma was still acceptable and microbial populations were not problematic until after eight days (Abbey et al., 1988).

An adequate postcutting subjective appraisal indicating acceptable postharvest quality does not necessarily imply that a product has satisfactory flavor quality.

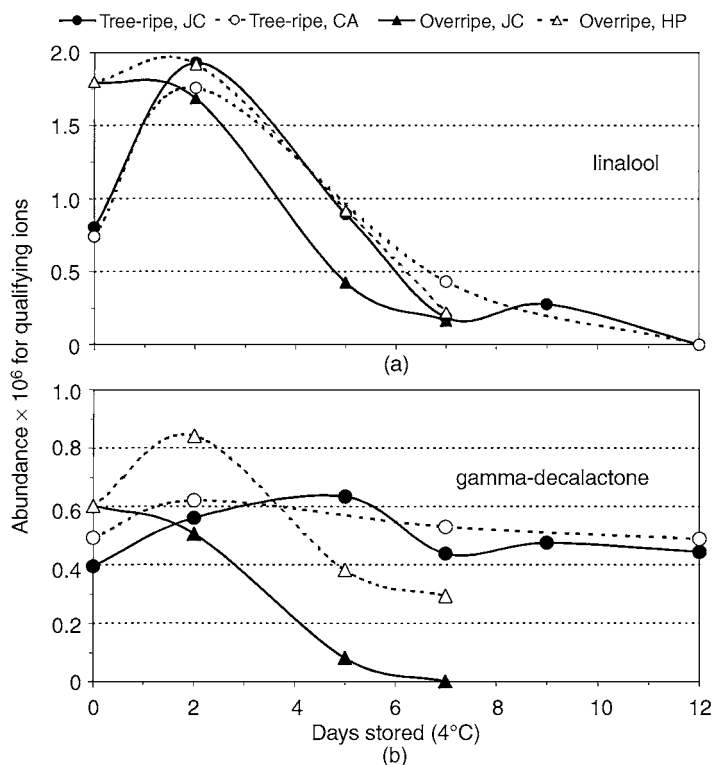


FIGURE 12.5 Preliminary SPME, GC-MS volatile profiles (a) (linalool) and (b) (gamma-decalactone) for tree-ripe or overripe fresh-cut 'Bounty' peaches stored in Juice Catcher (JC) or high-profile (HP) deli containers under air or CA (1% O₂ + 5% CO₂) at 4°C ($n = 2$).

Numerous fresh-cut articles are gradually accumulating evidence suggesting that flavor quality is normally compromised before visual quality. However, establishing overall shelf life limits for fresh-cut fruit while taking flavor quality into consideration is difficult, because initial product variability (e.g., seasonal or regional), potential postcutting treatments and/or packaging affect flavor attributes differently. Furthermore, little work has been performed to assess what effect storage temperature has upon volatile production in fresh-cuts. Subsequently, uniform flavor quality and consumer acceptance of fresh-cut fruits based on aroma and flavor remain a challenging area for the industry. Fresh-cut flavor quality has recently become an area of active research, and the industry needs to focus attention here as well.

CONCLUSION AND FUTURE RESEARCH

There remains a market niche for almost all value-added products in today's society, and therefore, production, marketing and research on fresh-cut fruits and vegetables are expanding. This chapter illustrates numerous areas of active research geared toward optimizing product quality and improving consumer acceptability of fresh-cut fruit

products. Researchers and the industry need to work together to overcome barriers that hamper national delivery of high-quality fresh-cut fruits throughout the year. As the foodservice industry and home meal replacement industry expand, there will be a greater demand for fresh-cut fruits and vegetables with acceptable flavor quality.

A fair amount of work has been performed concerning unveiling important fruit flavors and probable enzymatic pathways. Throughout this chapter, numerous enzymes have been mentioned that have critical roles regarding genesis of specific volatile flavor compounds or classes of compounds. Relatively speaking, little work has been completed concerning characterizing the important pathways and optimum enzyme conditions favoring production of desired volatile compounds. Likewise, little work has been performed to limit enzymatic production of undesirable aroma compounds. This is an area open for active research, especially because cutting products exacerbates respiration and secondary volatile production and may lead to further volatile loss or change.

Researchers and fresh-cut producers are becoming aware that flavor quality will become a major driving force within the fresh-cut industry. Seed companies are looking for means by which to increase their profits by having more participation in associated agricultural activities or industries. No longer will selling seed suffice. Rather, brand name products and patent rights may become important. With this in mind, more emphasis will likely be directed toward determining critical fresh-cut flavor volatiles and precursor compounds and elucidating their biosynthetic pathways for enzymatic regulation. A successful fresh-cut market, especially fruits, may indeed be the driving force required to breed flavor back into some of our important fruits and vegetables.

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13 Evaluating Sensory Quality of Fresh-cut Fruits and Vegetables

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INTRODUCTION

Lifestyle changes in the new millennium necessitate portable, safe, nutritious and high-quality fresh-cut produce. Because people want the fresh fruits and vegetables in their diet, but do not choose to prepare it, the market niche exists. Consistent fresh-like quality is what will keep consumers purchasing fresh-cut produce. Normally, flavor changes occur before the visual appearance deteriorates (Anonymous, 2000). Therefore, consumers have to feel confident they will be purchasing fresh-cut produce with good sensory quality for repeat purchasing. For the continued growth of this industry, flavor and texture changes have to be understood, and the quality must be monitored. Sensory evaluation is critical to understanding and monitoring flavor and texture changes. Such analysis can be expensive, but it is essential to understand what is happening in the mouth before correlation with instrumental methods should be attempted (Muños et al., 1991). Sensory evaluation can be used for quality assurance and quality control purposes, but instrumental methods, if available, are more economical on a routine basis. Consumer-based evaluations are subjective and depend on the pool of consumers being tested. Unless consumers are selected properly, the test can be biased (i.e., company employees do not represent the population as a whole).

Consumer or affective tests do not give information about the perceived difference (Poste et al., 1991). This chapter will focus on descriptive sensory analysis of flavor and texture, which is more effective for measuring quality changes than using untrained consumers. ASTM (1992) manual MNL 13 describes various methods of descriptive analysis. The purpose of this chapter is to give an overview of what is involved in sensory evaluation of fresh-cut produce. This is by no means a comprehensive discussion on training and operating a sensory panel, but it will aid in the understanding of what is involved.

FLAVOR

Flavors of various fruits and vegetables are unique. An apple is distinctly different from an orange. Some flavor characteristics are, however, common among fruits and/or vegetables. Green, sweet, sour, bitter and astringent are common characteristics in most fruits and vegetables (Tables 13.1 and 13.2). Flavors common in many fruits are caramelized/honey, chemical, estery or fruity and floral/perfumy. These flavors are defined in Table 13.3. Off-flavors common in fruits are deteriorated/rotten and fermented. Earthy/musty is less common. There are flavors that are unique to certain fruits. For example, there is a melon flavor in cantaloupe, apple flavor in apples and pineapple flavor in pineapple. We have sometimes observed the appearance of noncharacteristic fruit flavors in apples, and pumpkin and cucumber flavors in cantaloupe. This can occur because compounds that make up these flavors are present in other fruits and vegetables at different concentrations. Variations in climatic conditions, storage temperatures and maturity levels are examples of conditions that can result in the production of the atypical flavor observed. Pumpkin or cucumber flavors have been observed in immature cantaloupe but are less common in mature fruit (Bett, 2000, unpublished data).

Typically, vegetables are not considered sweet, with a few exceptions, such as carrots and sweet potatoes, but most have low-intensity sweet taste. Broccoli and cabbage have a strong sulfury note. Radishes have a pungent mouthfeel, and onions have an alliaceous aroma. Carrots are sweet and have a clove flavor. Sweet taste is used as a descriptor for each vegetable in Table 13.2, and green/grassy is used to describe several vegetables. Some attributes listed in Table 13.2 may occur in more vegetables than are recorded. Vegetables can have the off-flavor, deteriorated/rotten (not listed in Table 13.2), but it was noted in several fruits listed in Table 13.1.

Flavor intensity is the strength that a particular flavor has within a food. Intensity can be measured on a universal intensity scale, product-specific scales or attribute-specific scales. Universal scales have several advantages. A group of trained panelists can use the same scale for any fruits or vegetables, processed or raw. They can evaluate other products as well. In addition, one can literally compare apples and oranges (based on intensity of common descriptors). The universal scale needs to be broad enough to cover the full range of attribute intensities but discrete enough to pick up small differences in intensities. Universal scale references are food products that consistently exhibit specific flavor intensities. Many different foods can be used in one universal scale. Meilgaard et al. (1999) lists many commercially available foods that can be used as intensity references, along with the flavor and its intensity for

TABLE 13.1
Common Flavors and Tastes Reported in Fruit-Type Produce

	Blueberry	Cherry	Grape	Strawberry	Orange	Apple	Pear	Apricot	Peach	Plum	Papaya	Passion Fruit	Pineapple
Astringent	x	x	x	x	x	x	x	x	x	x		x	x
Bitter	x	x	x	x	x	x	x	x	x	x		x	x
Caramelized/honey	x	x	x	x	x	x	x	x	x				
Chemical	x	x	x	x	x	x	x	x	x				x
Deteriorated/rotten	x	x	x	x	x	x	x	x	x	x			x
Estery	x	x	x	x	x	x	x	x	x	x		x	x
Etheral	x	x	x	x	x		x	x	x	x			x
Fermented	x	x	x	x	x	x	x	x	x	x			x
Floral/perfumy	x	x	x	x	x	x	x	x	x	x			x
Green	x	x	x	x	x	x	x	x	x	x			x
Sour	x	x	x	x	x	x	x	x	x	x			x
Sweet	x	x	x	x	x	x	x	x	x	x			x
Woody	x	x	x			x	x	x	x		x		
Musty/earthy		x	x	x		x						x	
Dried fruit													
Citrus													
Artificial ²	x	x	x	x	x		x	x	x		x		x
Raw	x	x	x			x	x	x	x	x			
Fruity						x	x	x	x	x		x	x

¹Blank spaces mean the descriptor was not reported in this fruit in ASTM (1996) but could possibly be found in it.

²Not recommended as a descriptor, because it may be covered in the intensity rating of another descriptor.

Source: Adapted with permission from DS-66, *Aroma and Flavor Lexicon for Sensory Evaluation*, copyright 1996, American Society of Testing and Materials (ASTM).

TABLE 13.2
Common Flavors and Tastes Reported in Vegetables-Type Produce

	Broccoli ¹	Cabbage	Carrot	Celery	Cucumber	Onion	Bell Pepper	Radish	Spinach	Summer Squash	Tomato
Astringent				X	X					X	X
Bitter			X	X	X	X			X	X	X
Earthy/musty			X	X		X			X	X	X
Floral	X										
Green/grassy	X		X	X	X	X	X		X		X
Salty	X		X	X				X			X
Sour	X				X			X			X
Sweet	X	X	X	X	X	X	X	X	X	X	X
Sulfur/sulfide	X	X									
Umami			X	X		X					
Fermented		X				X					
Metallic				X		X			X		X
Brown caramel/caramelized				X		X			X	X	X
Woody			X								X
Buttery						X				X	
Tobacco/paprika							X				X
Viney							X				X
Burn		X						X			

¹Blank spaces mean the descriptor was not reported in this vegetables in ASTM (1996) but could possibly be found in it.

Source: Adapted with permission from DS-66, *Aroma and Flavor Lexicon for Sensory Evaluation*, copyright 1996, American Society of Testing and Materials (ASTM).

TABLE 13.3**List of Descriptors with Definitions and References for Flavor, Taste and Mouthfeel of Fruits and Vegetables**

Alcohol—aromatic characteristic of the chemical class of compounds known as alcohols. Ref: ethanol.

Alliaceous—reminiscent of garlic or onion, associated with chemical family of compounds allyls.

Ref: methyl allyl trisulfide at 0.05% in water or oil (aroma only and prepare under a hood).

Apple—aromatic characteristic of different apple varieties. Ref: apple concentrate to which no essence has been added.

Astringent—the chemical feeling factor on the tongue or other skin surfaces of the oral cavity described as puckering/dry and associated with tannins or alum. Ref: 1% alum in water.

Barny/barnyard—aromatic characteristic of barn or barnyard, combination of manure, urine, moldy hay, feed, livestock odors. Ref: Tincture of Civet, full strength.

Bell pepper—aromatic associated with green bell peppers. Ref: 0.01 ppm 2-isobutyl-3-methoxy pyrazine.

Bite—chemical burning, sensation felt on tongue or in the mouth and throat. Ref: 0.15% red pepper in water.

Bitter—taste on tongue stimulated by solutions of caffeine, quinine and certain other alkaloids. Ref: 0.1% solution of caffeine or quinine.

Burn/heat—chemical feeling factor associated with high concentrations of irritants to the mucous membranes of the oral cavity. Ref: 30% alcohol solution, 10% NaCl solution, white vinegar.

Buttery/diacetyl—aromatic associated with artificial butter. Ref: 0.5 ppm diacetyl in water.

Cabbage, raw—sweet, earthy aromatics associated with raw cabbage. Ref: fresh, washed cabbage.

Caramelized/browned caramel—sweet aromatic characteristic of browned sugars and other carbohydrates. Ref: caramelized sugar.

Cardboardy—aromatic associated with slightly oxidized fats and oils, reminiscent of wet cardboard packaging. Ref: malonaldehyde or wet cardboard or paper filters.

Chemical—a very general term associated with many different types of compounds, such as solvents, cleaning compounds and hydrocarbons. Ref: product specific, bleach, caustic cleaning solutions.

Citrus—aromatic associated with general impression of citrus fruits. Ref: citrus oils.

Cloves—a sweet, brown-spice, almost minty aromatic associated with cloves. Ref: eugenol.

Cucumber—flavor typical of fresh cucumber. Ref: raw cucumber.

Deteriorated/rotten—aroma associated with rotten, deteriorated, decayed fruit/material. Ref: rotten fruits (specific).

Dried fruit—flavor associated with dried fruits. Ref: dried apples, apricots, peaches, pears, prunes, figs.

Earthy/musty—aromatic characteristic of damp soil, wet foliage or slightly undercooked boiled potato. Ref: 0.3 µg geosmin/L water, 30 ppb 2-methyl isoborneol or 0.4 ppm alpha fenchol in water.

Estery—ripe fruit character associated with esters. Ref: 0.5 ppm ethyl butyrate in water or other fruity compound.

Etheral—flavor associated with chloroform. Ref: 5 ppm acetal in water.

Fermented—aromatic associated with fermented fruits or vegetables. Ref: fermented apple juice or WONF 3RA654 (McCormick).

Floral—a sweet, fragrant aromatic associated with flowers. Ref: rose oil, 1 ppm geraniol in water, 2 ppm phenyl ethyl alcohol in water.

Fruity—aromatic associated with a mixture of nonspecific fruits (berries, apples/pears, tropical, melons, usually not citrus). Ref: 2 ppm ethyl butyrate, ethyl caprilate and ethyl acetate.

(continued)

TABLE 13.3**List of Descriptors with Definitions and References for Flavor, Taste and Mouthfeel of Fruits and Vegetables (Continued)**

Green apple—the aromatic associated with freshly harvested green varieties of apples. Ref: 5.0 ppm *trans*-2-hexen-1-al.

Green/grassy—aromatic characteristic of freshly cut leaves, grass or green vegetables. Ref: 50 ppm *cis*-3-hexen-1-ol.

Metallic—a flat chemical feeling factor stimulated on the tongue by metals. Ref: 0.1–1% ferrous sulfate solution in water.

Moldy—aromatic characteristic of mold growth or mildew. Ref: 10,000 ppm 2-ethyl-1-hexanol in glycol.

Onion—aromatic associated with onion. Ref: 0.50 g onion powder in 200 ml water.

Overripe tomato—aromatic associated with overripe tomatoes.

Peely/peel oil—flavor associated with peel or skin flavor. Ref: apple peel, grape skin or orange oil.

Raw—aromatic associated with unprocessed and/or uncooked product. Ref: fresh fruit or vegetable.

Salty—taste on tongue stimulated by sodium salt, especially sodium chloride. Ref: 0.3% NaCl in water.

Seedy—character associated with chewing on seeds. Ref: raspberry and blackberry seeds.

Skunky/mercaptan—aromatic associated with sulfur compounds, which exhibit a skunk-like character. Ref: 0.1% furfuryl mercaptan in alcohol and water.

Soapy—aroma associated with unscented soap. Ref: deconoic or dodeconoic acid, Ivory Snow flakes.

Sour—basic taste on tongue stimulated by acids. Ref: citric acid, vinegar, lactic acid.

Sulfide, sulfur—aromatic associated with hydrogen sulfide, rotten egg. Ref: hydrogen sulfide bubbled through water (use caution) or old eggs hard boiled.

Sweet—taste on the tongue stimulated by sugars and high potency sweeteners. Ref: 5% sucrose in water.

Terpeny—aromatic associated with pine volatiles. Ref: 0.05% beta-pinene or alpha-terpineol in water (sniff carefully).

Tobacco/paprika—aromatic associated with paprika. Ref: paprika.

Tomato—a general term that combines those characteristics commonly associated with tomato. Ref: 2–5 ppm methional.

Umami/monosodium glutamate—specific chemical feeling factor stimulated by monosodium glutamate (MSG). Ref: 0.2% solution of MSG.

Viney—aromatic associated with green wood/small young branch or stems of plants. Ref: alpha-lonone on perfumer's stick.

Waxy—aromatic reminiscent of waxes. Ref: gamma-undecalactone on perfumer's stick.

Woody—aromatic associated with dry, fresh-cut wood; balsamic or bark-like. Ref: 10 ppb alpha-humulene in water or alcohol or wood chips.

Source: Adapted with permission from DS-66, *Aroma and Flavor Lexicon for Sensory Evaluation*, copyright 1996, American Society of Testing and Materials (ASTM).

the Spectrum Intensity scale for descriptive analysis. Unstructured line scales (6 inches or 15 cm) that usually have an anchor on each end have the advantage over numerical scales in that no steps or “favorite” numbers exist. Panelists’ repeatability can be more difficult to attain, because the position on the line is not stored in memory like the number in a numerical scale. The type of scale used is decided by the panel leader, taking into account management’s goals.



TEXTURE

Texture, as opposed to flavor, is the structure and orientation of the food and the reaction of the food to an applied force. As in flavor characteristics, the various texture attributes are labeled and defined, similar to flavor characteristics. The texture attributes of produce can be divided into four areas: surface properties, first bite properties, chew down and after swallowing properties (Meilgaard et al., 1999). Table 13.4 lists the textural properties with definitions with the high- and low-intensity references common to most fruits and vegetables. Surface moisture (wetness) and roughness are surface properties. Springiness, cohesiveness, denseness, hardness, moisture release, juiciness, crispness and uniformity of bite are all determined on the first bite. Chewiness and cohesiveness of mass are determined during chew down. Mouth coating is evaluated after swallowing.

The texture intensity scales are unique for each attribute. Many of the attribute scales for the spectrum method are published in Meilgaard et al. (1999). Some of the overall most important attributes are crispness, hardness and juiciness or moisture release (which measure virtually the same attribute). Crispiness is important in some fruits or vegetables, because it indicates the degree of turgor pressure within the cells or the amount of wilting that has taken place. Hardness indicates the senescence that has occurred. Juiciness or moisture release can be an indication of the amount of dehydration that has occurred. The moisture release scale was developed at Southern Regional Research Center (SRRC). The intensities included in Table 13.4 are representative of the low and high ends of the scale. These universal scales are applicable for all foods that have these characteristics. For number scales, panelists are encouraged to use tenths to help define differences between fruit. Therefore, scales specific for fruits and vegetables are not needed.

CONDUCTING DESCRIPTIVE SENSORY ANALYSIS

ROOM DESIGN

The sensory laboratory should be set up specifically for the purpose of evaluating produce samples. The lab should be accessible but away from congested areas where noise levels can be loud. If panelists are drawn from people outside the work site, the laboratory should be near an entrance but away from machine shops, production lines or cafeteria kitchens, where odors may interfere with sensory work. If employees are used as panelists, a good location would be to have the panelists pass by the sensory laboratory on their way to the cafeteria or break room. The test facility should consist of booths for individual evaluation of samples, a conference table for training and a separate sample preparation room. Special booth features that enhance operation include a light signal system and a data entry device (keypad, tablet digitizer or personal computer). The conference room should be large enough to accommodate

TABLE 13.4
Fruit and Vegetable Sensory Texture Descriptors*

Phase/Descriptor	Definition/Reference Scale
<i>Phase I. Hold sample to lips and pass the tongue over the surface.</i>	
Wetness	The amount of moisture due to an aqueous system on the surface. Low = unsalted soda cracker ==> high = Oscar Mayer ham luncheon meat
Roughness	The amount of particles in the surface. Low = gelatin dessert ==> high = Finn Crisp rye wafer
<i>Phase II. Evaluate before or at first bite.</i>	
Cohesiveness	The degree to which sample deforms rather than crumbles, cracks or breaks. Low = corn muffin ==> high = sun-dried seedless raisins
Crispness	The force and noise with which a product breaks or fractures when compressed with the molar teeth. Low = Quaker Low Fat Chewy Chunk granola bar ==> high = Pepperidge Farm Goldfish cheese cracker
Hardness	The force to attain a given deformation (force to compress between molars). Low = cream cheese ==> high = LifeSavers hard candy
Juiciness**	The amount of juice/moisture perceived in the mouth. Low = banana ==> high = watermelon
Moisture release**	Amount of wetness/juiciness released from the sample. Low = (Betty Crocker) Gusher ==> high = grape
Denseness	The compactness of the cross section. Low = General Foods Cool Whip topping ==> high = Farley Fruit slices
<i>Phase III. Evaluate during chewing.</i>	
Cohesiveness of mass	The degree to which a chewed sample holds together in a mass. Low = shoestring licorice ==> high = archway Cookies soft brownie
Chewiness	The amount of work to chew the sample. Count the number of chews required to prepare for swallowing.
<i>Phase IV. Evaluate after swallowing.</i>	
Mouth coating	The amount of residue left in the mouth and on teeth surfaces after expectorating or swallowing. Low = cooked cornstarch ==> high = tooth powder (any brand)

*Note: Most of these descriptors are in Meilgaard et al. (1999).
**These two descriptors are similar, but the latter was developed at SRRC, and the former was from Meilgaard et al. (1999).

all panelists (10–15 people). A conference table (or tables arranged in conference style) that seats a full panel and “electronic write board” are needed. Low-pressure sodium lamps, colored bulbs or theater gel filters over recessed lights can be used to mask color differences. The preparation room for fresh-cut produce should consist of a separate room from the booths and training room. If processing is done in a plant or food preparation laboratory, then the basic requirements of counter space, sink and a refrigeration unit would be sufficient. For more detailed information, refer to ASTM (1986) and ISO (1988) guidelines. Booths and conference room should be held at 22–24°C (72–75°F) and 45–55% R.H. A slight positive pressure should be maintained to prevent odors from coming from surrounding facilities or the preparation laboratory into the booth area.

SERVING PROTOCOL

Fresh-cut fruits and vegetables are cut into bite-size pieces and placed in 0.178 liters (6 oz) glass custard cups and covered with 125 mm watch glasses. Avoid custard cups with fluted rims to prevent loss of volatiles. Watch glasses allow the headspace to trap volatiles for sniffing. Refrigerated samples should be allowed to warm up to ambient temperature before being presented to the panelists to allow more volatiles to emanate. It is of utmost importance that *all* samples be served at the same temperature. The same number of approximately the same size cubes should be placed in each custard cup. A new plastic fork that does not emit an aroma or a clean metal fork is used for tasting each sample. The presentation order should be randomized to minimize order bias. The first sample should be a nonexperimental, warm-up sample to help standardize the panel and minimize position effect on the first sample. Panelists should smell the sample first and then evaluate oral flavor and/or texture. Texture has a logical order. Surface properties are evaluated first, followed by first bite, then chew down and last, the properties measured after swallowing. Flavor has an order, also. Some tastes such as sweet and salty and some flavor notes are perceived early, while others are perceived later.

FACTORS THAT AFFECT FRESH-CUT SENSORY QUALITY

Cultivar differences can have significant effects on flavor and texture. Cultural and environmental conditions (climate, fertilizer application, soil conditions) can also cause changes in a given cultivar that could affect flavor and texture. Some cultivars are typically sweeter, have a more intense characteristic flavor or a softer texture. Some cultivars retain quality during fresh-cut storage better than others. Gorny et al. (1999) observed 13 cultivars of peaches during fresh-cut storage and found that ‘Flavorcrest,’ ‘Elegant Lady,’ ‘Red Cal’ and ‘Cal Red’ retained quality during fresh-cut storage longer than nine other cultivars. This work focused on visual appearance and not flavor and texture. In unpublished data (Bett, 2000, unpublished data), in two years of cantaloupe cultivar comparisons at SRRC during fresh-cut storage, ‘Pacstart’ got harder and ‘Athena’ got softer one year and remained the same another year. Kim et al. (1993)

reported that firmness of fresh-cut apples decreases with storage, but 'Golden Delicious' apples remained firm longer than 'Monroe,' 'Rhode Island Greening' and 'New York 674.' Some cultivars endure fresh-cut storage better than others. Plant breeders are developing new breeding lines that focus on fresh-cut processing quality.

Crop maturity has a significant impact on quality of fresh-cut produce. Gorny et al. (1998) reported that overripe stone fruit had a short shelf life (two days for peaches and three to six days for nectarines), while mature-green and partially ripe fruit increases in firmness during fresh-cut storage. Fresh-cut mature cantaloupe evaluated at SRRC retained its fruity flavor, while the immature cantaloupe fruit increased in fruity intensity with storage. Immature fruit tend to get harder during storage than mature fruit. Fruit maturity affects fruit quality, significantly (Watada and Qi, 1999). Immature fruit lack good sensory quality, and over-mature fruit have limited shelf life capacity.

Processing environment is critical to flavor and texture quality as well as to minimizing microbial problems. The temperature of the processing room should be maintained at 2.8°C. Contact surfaces need to be routinely cleaned and sanitized. Personnel need to use clean gloves, clean aprons, hair covers and surgical masks (Sargent, 1998). Substandard processing conditions can contribute to heavy microbial loads that lead to quality deterioration as well as possible safety problems.

Cutting the produce typically causes the respiration rate to increase. Therefore, minimal cutting is better for shelf life because it keeps the respiration nearer to that of intact produce (Watada et al., 1996). Removal of stems from grapes and hulls from strawberries changes the respiration rate less than peeling and slicing apples. Ideally, produce should be processed enough to make it convenient, but with minimal tissue damage.

Sanitizers can impart a flavor on produce if allowed to remain on the surfaces. Chlorine has a familiar characteristic odor, hydrogen peroxide and ultraviolet rays impart little to no flavor or aroma changes. Ozone has a faint ozone aroma, but it dissipates quickly. Peroxyacetic acid has a mild acetic acid/vinegar flavor. Irradiation has been proposed as a means of extending shelf life, but in whole fruits and vegetables, the doses required for microbial spoilage prevention cause tissue softening (Maxie et al., 1971).

Browning of cut surfaces can be a problem in some produce. Browning in cut fruit is usually caused by oxidation of phenols catalyzed by polyphenol oxidase enzymes. Browning in apples can increase the sweet aroma or caramel flavor. It typically can be prevented in fresh fruit by coating cut surfaces with a browning inhibitor, which in some cases, could impart a different flavor to the produce. Sodium erythorbate and sodium lactate, for example, impart a salty taste on the surface. Calcium chloride or calcium lactate can impart a slight bitter taste, but also, can make the product slightly firmer (Agar et al., 1999; Luna-Guzmán and Barrett, 2000).

Packaging normally creates an atmosphere to maintain conditions that increase product storage time. Permeability of the film can be selected to retard or enhance chlorophyll degradation, control browning, delay mold development and retard growth of some microorganisms (Cartaxo et al., 1997; Senesi et al., 1999; Watada, 1997). All of these can affect the sensory properties of the fresh-cut product. Selecting the appropriate packaging material can control the relative humidity surrounding the fruit

that can prevent dehydration of tissue and control condensation (Watada, 1997; Watada and Qi, 1999), both of which cause deterioration in sensory quality.

Proper storage temperature is critical for microbial quality, but it also affects sensory quality. Elevated temperature hastens processes such as respiration, browning and microbial growth. Microbial growth can generate off-flavors such as fermented, deteriorated/rotten and moldy and can become more intense at a faster rate with higher temperatures (Abbey et al., 1988). The increased enzymatic activity that occurs at higher temperatures accelerates browning and tissue softening (Watada, 1997). Storage temperatures that are too low can cause chilling injury. Chilling injury weakens tissues, because they are unable to carry on normal metabolic processes. After chilling injury, produce may look sound, but upon warming up slightly, they develop symptoms such as pitting, skin blemishes, internal discoloration or failure to ripen. Temperatures that cause a slight amount of chilling injury are preferred over temperatures that cause rapid senescence and microbial deterioration (Watada and Qi, 1999).

SUMMARY

Flavor and texture of fresh-cut produce are critical to consumer satisfaction. Sensory quality can be affected at every step from production to storage conditions at the point of consumption, and descriptive analysis is an effective tool to measure and monitor these differences. Produce optimization needs to be considered at each point in the production, processing and distribution chain. When sensory evaluation is implemented on fresh-cut produce, it must be carried out under very controlled and consistent conditions. This chapter has given an overview of what is involved in descriptive sensory analysis and how different aspects of the processing and distribution chain can affect sensory properties.

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14 Future Economic and Marketing Considerations

Greg Pompelli

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BACKGROUND

The food-processing industry offers consumers a new convenience-oriented food product almost every day of the year (*Progressive Grocer*, 1999). Given the high cost of development and marketing, companies launching these products must believe their products will be well received by consumers who indicate their lives are made better by the added convenience incorporated in the goods they purchase. Most of the chapters in this book focus on the development of processes and materials that allow companies to convert highly perishable raw agricultural commodities into desirable branded products. The great story of the fresh-cut industry is its ability to match technological know-how to consumer wants. Unfortunately, with so many firms attempting the same strategy, providing added convenience is not a sufficient guarantee of success. Value, or benefits divided by price, is a critical element in determining a product's success with consumers, just as it is with wholesale and institutional buyers.

The assessment of value occurs all along the fresh-cut supply chain. As firms search for ways to provide desired attributes (e.g., ease-of-use or retained nutritional value) using new and established technologies, they have recognized the need to ensure quality and uniformity in their inputs. The application of a new membrane technology, for example, is useless if the produce going into the bag is poorly handled or stressed by pest damage or other natural pressures. The contest for consumers' food expenditures, both at-home and away-from-home, is a circular process of assessment that, depending on where one wishes to begin, starts in the field and ends with consumers, or starts with consumers and ends in the field. In the first case, analysts follow physical products, and in the latter case, they follow the dollars used as economic signals.

This chapter briefly outlines economic and market factors that the fresh-cut produce industry may face as it matures and strives to grow. An essential part of the industry's future growth and presence in the food market will depend on its ability to deliver value to consumers, develop and implement new technologies, and reward all participants in the supply chain. Much of the industry's recent success is due to the convergence of consumer awareness and needs with technological developments and raw product availability.

OPPORTUNITY COSTS

The industry's future prospects will rely on continuous gains in understanding consumer needs, technological developments in agricultural and nonagricultural industries, and the actions and offerings of competing industries. The primary influence of these factors on the future prospects of the fresh-cut produce industry arises from a very basic economic concept called "opportunity cost." The fresh-cut produce industry is by no means alone in facing this influence, because opportunity costs influence the economic decisions of participants in every market. Opportunity costs of consumers, firms, and growers define the economic environment within which the fresh-cut produce industry has grown and within which it will exist.

Opportunity costs are a common link throughout this description, which starts with farm production and its declining share of consumer food dollars. The use of new technologies and non-farm inputs in the creation of fresh-cut products are among the most important reasons farmers receive a declining proportion of each consumer dollar. As consumers demand more product transformations (packaging, processing, grading, transportation, etc.), the portion of the product's cost/price accounted for by the raw farm product diminishes. With that said, the return to growers needs to be sufficient to keep resources in fruit and vegetable production. Each season, growers must compare their fruit or vegetable returns to the next best (most profitable) enterprises that they could operate using the resources they employ in their fruit or vegetable operations.

CHARACTERISTICS OF AGRICULTURAL INDUSTRIES

One complicating factor that makes agricultural production unique among industries is the reliance on natural, often uncontrollable inputs. Production of fruits and vegetables represents a combination of biological and physical processes that are affected by input use decisions, weather, and other natural pressures. To varying degrees,

production is dependent on germination and biological processes that occur within specific seasons and over extended periods of time within the seasons but not under entirely certain conditions. Extended time lags, years in the case of some perennial crops, exist between production decisions and product harvests. Furthermore, when compared to manufacturing industries, agricultural production decisions are not nearly as closely linked to harvests or output decisions. As a result, producers make their decisions based, in part, on expectations about growing conditions, prices, and future market access. The use of expectations and the lagged supply response can lead to price and production cycles over time.

The intensive and extensive use of natural resources (e.g., land and water) plays an important role in production decisions. Historically, few uses existed for these resources outside of agricultural production. However, as rural/urban interfaces expand, alternative uses of these resources increase (subdivisions, manufacturing, etc.). In turn, the number of alternative uses, and the returns to those alternatives, may increase. While land resources have only slowly shifted away from agricultural uses, capital and labor face significant alternative opportunities. Returns from fruit and vegetable production for land, labor, and capital must be as attractive as the next best alternative, or those factors of production will shift to other industries.

A further complicating factor is that fluctuating supplies and prices lead to fluctuating producer incomes. In response to income risk, producers may diversify their production mix so that stable or increasing prices for one set of products might offset falling prices for other products. However, diversification reduces specialization, and that can lead to higher average costs. For all but the largest U.S. farms, diversification can, and often does, include off-farm income. The potential for off-farm employment raises the opportunity cost labor used to produce fruits and vegetables, not only because of the income stream, but also because of potential benefits such as health coverage.

Seasonal overproduction is especially difficult to offset for perishable fruits and vegetables. The lack of market alternatives combined with inelastic demand has a heavily downward pressure on prices. Even growers with honored contracts may receive lower prices in the long-run if overproduction persists. As prices fall, so do the marginal product values of resources, and without offsetting technological innovations or reallocation of resources, farm incomes decline.

Opportunity costs influence producers' resource use, production mix, and farm and off-farm employment decisions. In turn, growers evaluate these alternatives to decide what and how much to produce. Growers make these assessments by comparing the expected returns to the "next" best rate of return their resources could receive in another use. The comparison may be viewed from long- or short-term perspectives, but they nonetheless occur.

CONSUMERS

At the other end of the marketing chain, opportunity costs influence consumers as they maximize their "utility" by making choices about purchases given limited budgets, a host of household characteristics, and an ever-changing set of tastes and preferences. Opportunity costs come into play for consumers as they assess the expected benefits or utility derived from one basket of goods compared to another set of goods.

The satisfaction consumers derive from consumption is typically a function of income, background, lifestyle, family composition, and nutritional need factors. As a result, the assessment made by one consumer may be different from that of another given differing household or personal characteristics.

FORCES SHAPING FRESH-CUT PRODUCE MARKETS

TECHNOLOGY ADOPTION

One reason the assessment of opportunity costs is a constant process in the fresh-cut produce industry is that many forces affect or may affect the industry. These forces include changes in consumer demand, technological developments that enable firms to deliver desired product qualities, the application of new food technologies in other industries, industry consolidation across the supply chain, and changes in the regulatory environment.

As is the case for most industries, processing and marketing firms lead the development and utilization of new technologies. Not surprisingly, technological developments and market consolidation forces are often complementary. The costs associated with technological developments and resulting gains in output reduce the number of firms that can profitably operate in a specific market. Technology is a source of the economies of size that provide market advantages for larger firms. Technology also helps differentiate goods in the marketplace. Brands that use specific technologies may become known for fresher, greener, or safer products. Those characteristics become the basis on which brands become differentiated.

Important consequences of the adoption of new technologies are often the need for more consistent raw inputs and consolidation in the technology-adopting industry. Consistent product standards are required to fully exploit new technologies. The needs for consistent product deliveries and product standards affect the way in which growers produce fruits and vegetables and leads to greater reliance on contracts over open market transactions. Consolidation leads to fewer marketing alternatives for growers (e.g., Fresh Express, Inc. and Dole Fresh Vegetables, Inc. control over 70% of the packaged salad market). Fewer marketing choices often limit opportunities for price discovery, which limits market participation and increases the importance of contracts.

FRESH-CUT PRODUCE CONSUMERS

Almost two decades of rising U.S. per capita consumption of fresh fruits and vegetables represent a wonderful example of suppliers reading the consumers' signals. As shown in Chapter 1, changing dietary habits, hectic lifestyles, increased raw product availability, and an expanding selection of fresh-cut fruits and vegetables have made the fresh-cut produce market the fastest growing in the fruit and vegetable industry. In short, the rapid growth of the fresh-cut produce industry shows what can happen when convenience and variety foster consumers' good intentions, and the technology can be applied to meet those needs. Fresh-cut fruits and vegetables are excellent examples of products that offer as much convenience as they offer nutritional value.

Nonetheless, the fresh-cut produce industry is no different from most other industries. The industry bases its success on consumer and foodservice demand for its

products. Consumers, as households or individuals, represent a combination of needs and income. Foodservice demand derives from consumer demand for meal replacements both at- and away-from-home. Both foodservice and consumers look for new or unique products, convenience in preparation/use, and price. The influence of these wants on the market is weighted by consumer incomes that determine the capacity to purchase goods. The wants and ability to pay determine “effective” demand.

While income is a primary force driving changes in demand for fresh-cut products, demographic, lifestyle, workforce patterns, and health considerations also influence demand. Income is the element that effectively transforms wants and needs into effective demand. Demographic factors (e.g., age, gender, education, ethnic background, and household composition) significantly influence consumer wants. Single-parent households possess different product characteristic needs compared to seniors.

Factors such as a lifestyles and values play an important role in forming consumer wants. Consumer profiles often categorize consumers using predominantly lifestyle or value terms such as, “Strivers,” “Adapters,” and “Achievers,” because consumer consumption patterns are so heavily influenced by lifestyles and attitudes. Understanding the effects of these factors is especially important, as they are expected to be drivers of future consumer/foodservice trends and fresh-cut technological development. For example, as the U.S. population ages, older age groups are expected to desire products that offer greater dietary benefits or increased convenience.

Marketers can use these profiles to develop products to include desired characteristics behavior, such as greater convenience or health benefits. However, firms do not create these products unless the expected return is sufficient to warrant the investment and resource use needed to bring the product to market. The assessments used to make production decisions are based on expectations about consumer behavior and are limited by actual and anticipated technological developments. Although firms rarely know the opportunity costs associated with production decisions with certainty, they are forced to allocate their scarce resources/inputs among different business activities.

Convenience and nutrition will remain significant demand factors that will affect the characteristics of the products the fresh-cut produce industry delivers. Although most consumers may not know much about their next meal, except that it should be ready in less than 30 minutes, consumers are concerned about more than just convenience and ease of preparation. Nutritional value is important, but freshness, shelf life, packaging, availability, and functional properties, not the least of which is taste, heavily influence purchase decisions.

While characteristics, such as freshness, nutrition, and functional properties, are considered important, they are often only important to the extent that consumers are made aware of their importance. Consumers gather information from a variety of sources, including family, friends, healthcare providers, magazines, web-based sources, advertising, etc. The variety and availability of sources is good but may lead to consumer burnout on health and nutrition issues. To the extent that these sources identify new products or health/lifestyle concepts, the fresh-cut industry will need to work with these sources and listen to their changing messages.

Finally, variety, appearance, and taste remain essential influences on human diets. Health claims and convenience can only go so far to guide food preferences. Taste and appearance ultimately determine consumer appeal. Favorite foods that can

be reformulated to include other desired characteristics frequently become the “new” favorite foods. Consumers may wish to control fat and caloric intake or increase nutritional value, but they also want to enjoy their meals.

MARKET DEVELOPMENTS

Marketing serves as the primary means for assessing the ability of firms to meet consumer desires. Part of a firm’s ability to meet consumer needs is based on its ability to collect information about consumer desires and match the consumers’ willingness to pay for characteristics with the cost associated with providing those characteristics. Firms combine market information and their resources and technology to determine if they could expect sufficient returns to develop a product.

However, firms must also examine their ability to reach consumers through marketing channels, such as distributors and retailers. Although direct marketing opportunities exist, food distributors and retailers generally control access to consumers. Firms also face competitors who are also trying to meet consumer needs with their products and product lines.

One of the reasons so much attention has been given to the fresh-cut produce industry has to do with its ability to provide products consumers desire and with the changes in industry structure. This has led to increased diversity in composition and form of products available. Another reason for this interest relates to the extent to which goods differ from original commodities and the impact of technology.

Firms within the fresh-cut industry have shown a keen ability to meet and even anticipate growth to date. Part of this success is due to technological developments and applications that made it feasible to meet consumers’ and foodservice needs. An equally critical element was the availability of produce that met industry needs. Future success may be more difficult to attain as the fresh-cut industry grows. Technology and research expenses, both farm-level and processing, favor larger operations. Larger operations spread development and investment costs over larger volumes. To the extent that human stomachs/demands do not grow with industry, increased output reduces the need for firms and farms. Thus, one of the forces affecting the maturing fresh-cut industry may well be profitability related to structural change in the number and size of firms and farms in the industry.

Competition from traditional fresh-cut substitutes as well as new food offerings should motivate continued innovation by processors and growers. Given that the novelty of products such as bagged salads has and will continue to diminish, much of the competitive pressure will coincide with pressures to lower processing costs, improve quality, and increase product differentiation. These pressures will be enhanced by retailers through market power and their ability to control access to consumers.

COMPETITIVE PRESSURES

Competitive pressures will also change grower-processor and processor-supplier relationships. Changes in these relationships reflect the fact that concentration heightens the importance of business ties. In open markets, suppliers offer their goods to any buyer willing to meet their asking or going prices, which are most likely

known by all participants. Concentration may reduce the transaction costs associated with finding buyers on open markets, but it amplifies the importance of business relationships and contracts and masks market information. As concentration increases, market access of producers to participants further along the supply chain (e.g., wholesalers, retailers, and consumers) typically decreases.

Perhaps the most important force facing the fresh-cut industry will be the evolution of business relationships between growers and processors because of the obvious disparities in market power due to their relative sizes. The development of these relationships will be explored a bit further in the supply chain management section. However, before moving on to the next section, it is important to note a few other significant forces in the fresh-cut market.

Most industry participants understand the effects of income distribution, lifestyles, demographic changes, health concerns, and workforce changes on demand as well as they understand the effects of technological development and adoption on supply responses. The product safety, regulatory, and brand image issues will likely become as important as other demand and technological considerations. If consumers, retailers, or foodservice buyers question the safety or quality of the industry's products or the reputation of individual brands, the industry's growth will be hampered. While product conversion efficiency and increased product appeal initiated and initially sustained industry growth, the industry has already shifted from a production-driven system to a demand-driven system in which end users have come to expect more value-added traits and higher quality.

FUTURE SUPPLY CHAIN MANAGEMENT CONSIDERATIONS

The development and maintenance of supply chains involves the integration of steps required to provide the products that end users demand. Product specification, timeliness, quality, availability, and the minimization of total cost are all elements of modern supply chains. Functioning chains offer integrated management of materials and products from input sources to final consumers and minimize the time needed to convert inputs into goods. When successful, this "team" approach satisfies consumer demand and creates a competitive advantage for those in the chain.

The fresh-cut industry is no different from other manufacturing industries in that fresh-cut firms want to reduce inventory, spoilage, transportation, and distribution costs. Fresh-cut firms also compete based on their ability to match efficiently and accurately product characteristics with consumer preferences, and supply chain practices aid in this response. The operative considerations for the adoption of supply chain practices are the improved ability to meet changing consumer desires, the minimization of costs across the whole supply chain, and the provision of adequate returns to all participants in the chain.

The difficulty for growers is that supply chains generally serve the interests of the supplied much better than the interests of suppliers (growers). When processors apply supply chain practices, they typically start by seeking consistent inputs that possess specific product attributes that can be delivered as needed. Input consistency

is valued, because it improves processing efficiency, and delivery timing reduces inventory costs. Increased efficiency and reduced inventories also allow processors to focus on the “core” marketing elements of their business that help them determine consumer preferences and willingness to pay. The desire for improved raw product quality and delivery means that processors are less willing to sort, store, or condition fruits and vegetables and more likely to insist that growers provide these services if they want to continue supplying products. The shift to demand-driven supply chains means that growers will feel increased pressure to create traits in their produce that generate premium value for the firms and users of their products. Product specification, quality, and, in part, food safety will be heavily influenced by growers’ decisions. Their influence on these traits will not, however, mean that growers always benefit from commercial market appeal or that their produce will receive premiums for the desired traits. As a result, a critical force within the fresh-cut industry is the state of grower-processor relationships and the degree to which risks and rewards are shared.

If the fresh-cut industry is going to continue to grow, then supply chain management practices should benefit all participants in the chain. However, the extent to which benefits are distributed is a function of the extent to which information and risks are shared across the chain. If information and risks are not shared, then processors will gain greater market power, and growers will face fewer marketing opportunities, narrower profit margins, and less control over their production practices.

There are many reasons why fresh-cut firms might adopt supply chain practices. Some argue that these practices make firms and marketing channel participants more efficient without regard to size. They note that the performance gap between top firms and the food industry average is narrowing. Of course, this narrowing is also a consequence of consolidation. Nonetheless, accurate information exchanges and rapid responses to market changes are valuable capacities in consumer-oriented markets.

Given the natural production lags associated with fruit and vegetable production, the successful application of supply chain management practices places a greater reliance on demand forecasts, communication, and expectations about changes in consumer preferences. These forecasts need to be shared by input suppliers and producers, if the ability to respond quickly is truly important. Faster response times are important in increasingly fragmented consumer markets.

Supply chain management practices may help growth in the fresh-cut industry. However, the application of these practices may not follow the simpler lock-step approaches seen in manufacturing industries. First, growers’ concerns about the cost of increasing quality and production flexibility need to be addressed. The cost of increasing produce quality can be high and is typically unpredictable. Pest pressures, weather, and other problems can undo the best plans. The investment required to maintain or increase production flexibility may be equally prohibitive. If increased quality and flexibility are important, then growers need to be rewarded. Unfortunately, the primary reward for quality- and flexibility-oriented investments is often only market access. Going back to the earlier discussion of opportunity costs, the comparison of returns to fruit and vegetable production under these conditions may not be sufficient to keep growers’ resources in fruit and vegetable production. This may be especially important as domestic fruit and vegetable prices are increasingly influenced by international market developments. For any given grower, the options

for reducing production costs or expanding production choices are limited. As the fresh-cut industry moves forward, it should consider ways that it can enhance information exchanges among growers and other suppliers, and when appropriate, share technology and agricultural risks.

SUMMARY

The fresh-cut produce industry has experienced an extended period of growth and has entered a new century with justifiable expectations of continued growth and success. The focus on technology transfers and fresh-cut specific research and development efforts has yielded new products, new brands, improved packaging, and extended shelf life in the store and at home. Much of this growth can be traced to the industry's ability to focus on health and nutrition characteristics while offering consumers convenience and product consistency. As a result, the fresh-cut industry is, in many ways, ahead of other segments in the agricultural economy in that it recognizes that production decisions should begin with a good understanding of end users' (consumers and food-service) demands and preferences.

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