Microbiological Spoilage of Fruits and Vegetables

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Introduction

Consumption of fruit and vegetable products has dramatically increased in the United States by more than 30% during the past few decades. It is also estimated that about 20% of all fruits and vegetables produced is lost each year due to spoilage. The focus of this chapter is to provide a general background on microbiological spoilage of fruit and vegetable products that are organized in three categories: fresh whole fruits and vegetables, fresh-cut fruits and vegetables, and fermented or acidified vegetable products. This chapter will address characteristics of spoilage microorganisms associated with each of these fruit and vegetable categories including spoilage mechanisms, spoilage defects, prevention and control of spoilage, and methods for detecting spoilage microorganisms.

Microbiological Spoilage of Fresh Whole Fruits and Vegetables

Introduction

During the period 1970–2004, US per capita consumption of fruits and vegetables increased by 19.9%, to 694.3 pounds per capita per year (ERS, 2007). Fresh fruit and vegetable consumption increased by 25.8 and 32.6%, respectively, and far exceeded the increases observed for processed fruit and vegetable products. If US consumption patterns continue in this direction, total per capita consumption of fresh fruits and vegetables would surpass consumption of processed fruits and vegetables within the next decade.

This shift toward overall increased produce consumption can be attributed, at least in part, to increased awareness in healthy eating habits as revealed by a broad field of research addressing food consumption and health and promoted by the

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National Cancer Institute with the 5-A-Day Challenge along with the USDA-revised Food Pyramid. Additional factors influencing greater fresh produce consumption are the increased availability of fresh produce throughout the country throughout the year, increased diversity of selection at the retail level (Kaufman, Handy, McLaughlin, Park & Green, 2001), and rapid growth in the fresh-cut, ready-to-eat produce sector (see "Microbiological Spoilage of Fresh-Cut Fruits and Vegetables").

According to a USDA-Economic Research Service study in 1995, 18.9 billion pounds of fresh fruits and vegetables were lost annually due to spoilage, which was 19.6% of all US losses of edible foods that year (Kantor, Lipton, Manchester, & Oliveira, 1997). The portion of loss specifically due to microbiological spoilage was not reported.

Most microorganisms that are initially observed on whole fruit or vegetable surfaces are soil inhabitants, members of a very large and diverse community of microbes that collectively are responsible for maintaining a dynamic ecological balance within most agricultural systems. Vectors for disseminating these microbes include soil particles, airborne spores, and irrigation water. Most bacteria and fungi that arrive on the developing crop plant either are completely benign to the crop's health or, in many instances, provide a natural biological barrier to infestation by the subset of microorganisms responsible for crop damage (Janisiewicz & Korsten, 2002, Andrews & Harris, 2000). The even smaller subset of bacteria and fungi responsible for causing spoilage to the edible portion of the crop plant is the subject of this section.

Spoilage microorganisms can be introduced to the crop on the seed itself, during crop growth in the field, during harvesting and postharvest handling, or during storage and distribution. Those same types of soil-borne spoilage microbes that occur on produce are the same spoilage microorganisms that are present on harvesting equipment, on handling equipment in the packinghouse, in the storage facility, and on food contact surfaces throughout the distribution chain. Therefore, early intervention measures during crop development and harvesting through the use of good agricultural practices (GAP) will provide dramatic reductions in yield loss due to spoilage at all subsequent steps in the food-to-fork continuum (Eckert & Ogawa, 1988). Examples of GAPs include foliar fungicide application in the field, cross-contamination prevention measures (stringent sanitation standard operating procedures) in the packinghouse and storage facility, and use of postharvest fungicides. These practices also will enhance substantially the food safety and shelf life of fresh-cut produce (see "Microbiological Spoilage of Fresh-Cut Fruits and Vegetables").

In 1998, FDA published the *Guide to Minimize Microbial Food Safety Hazards for Fresh Fruits and Vegetables*, recommending GAPs that growers, packers, and shippers implement to address the common microbiological hazards that may be associated with their operations (FDA, 1998). These GAPs are organized in eight categories:

I. Water
II. Manure and municipal biosolids

III. Worker health and hygiene
IV. Sanitary facilities
V. Field sanitation
VI. Packing facilities sanitation
VII. Transportation
VIII. Traceback

In addition, FDA worked with the produce industry to develop commodity-specific food safety guidelines for sprouts, lettuce and leafy greens, melons, and tomatoes that provided metrics for soil and water amendments as well as adjacent land usage. In March 2007, FDA issued a draft final version of its "guide" (FDA, 2007). These should also improve substantially the food safety and shelf life of fresh-cut produce (see "Microbiological Spoilage of Fresh-Cut Fruits and Vegetables").

Unusual Characteristics of Spoilage Microorganisms

Many fruits and vegetables present nearly ideal conditions for the survival and growth of many types of microorganisms. The internal tissues are nutrient rich and many, especially vegetables, have a pH near neutrality. Their structure is comprised mainly of the polysaccharides cellulose, hemicellulose, and pectin. The principal storage polymer is starch. Spoilage microorganisms exploit the host using extracellular lytic enzymes that degrade these polymers to release water and the plant's other intracellular constituents for use as nutrients for their growth. Fungi in particular produce an abundance of extracellular pectinases and hemicellulases that are important factors for fungal spoilage (Miedes & Lorences, 2004). Some spoilage microbes are capable of colonizing and creating lesions on healthy, undamaged plant tissue (Tournas, 2005b). Spoilage microorganisms also can enter plant tissues during fruit development, either through the calyx (flower end) or along the stem, or through various specialized water and gas exchange structures of leafy matter. Successful establishment, however, requires the spoilage microbe to overcome multiple natural protective barriers. Fruits and vegetables possess an outer protective epidermis, typically covered by a natural waxy cuticle layer containing the polymer cutin (Lequeu, Faucconnier, Chamma, Bronner, & Blee, 2003). A diverse community of epiphytic microorganisms that present a further competitive barrier to the spoilage organism also typically colonizes the outermost fruit surface. Overcoming these barriers requires an exquisite set of biochemical tools that allow the spoilage microorganism to (1) identify and recognize the plant surface; (2) employ one or more strategies to achieve irreversible attachment to the plant surface; and (3) initiate steps leading to internalization of the tissue (Mandrell, Gorski & Brandl, 2006). On plant structures other than the fruit, internalization can be achieved through a number of specialized vessels and surface structures employed by the plant to absorb and release water and to provide CO_2 and O_2 exchange (Bartz, 2006).

Table 1 Bacterial fruit pathogens[a]

	2004 Annual US per capita consumption (lbs)[b]	*Pseudomonas*	*Erwinia*	*Xanthomonas*	*Acidovorax*
Apples	18.8		+		
Bananas	25.8				
Berries	6.1				
Citrus	22.7	+		+	
Grapes	7.9				
Melons	14.7				+
Peaches	5.1				
Pears	3.1		+		
Pineapple	4.4				

[a]Important postharvest diseases retrieved from Sholberg et al. (2004) and other sources.
[b]85.2% of all fresh fruits consumed per capita in the United States in 2004 (ERS, 2007).

However, the fruit of the plant lacks many of these structures, requiring the spoilage microbe to employ other methods to become internalized (Lindow & Brandl, 2003; Agrios, 1997). This may partially explain the rather limited success of bacteria to spoil fruits (Table 1) and an improved ability to spoil vegetables that are not the fruit of the plant (Table 3). The natural acidity of most fruits also serves as a barrier to many spoilage microbes, especially bacteria. By contrast, spoilage fungi that typically produce more diverse and greater amounts of extracellular depolymerases successfully attack and spoil both fruits and vegetables (Tables 2 and 4).

Colonization and lesion development more typically and more rapidly occurs within damaged or otherwise compromised plant tissue. External damage such as bruising, cracks, and punctures creates sites for establishment and outgrowth of the spoilage microbes. Lesion development can be relatively rapid, occurring within days or weeks. This presents the risk that rapidly reproducing spoilage microorganisms will arrive within open wound sites at the packing facility, and thereby, through shedding from the asymptomatic wound, present the potential for cross-contamination within the facility during handling, culling, washing, sorting, and packing before storage. Such cross-contamination to some degree is inevitable and, if not carefully managed with a robust facility sanitation program, could lead to the establishment of a population of spoilage microbes endemic to the facility that may be difficult to eradicate. A further and potentially more serious complication is the introduction into the cold storage facility of spoilage microorganisms already established in wound sites on product, whether the product is in bins or boxed and palletized. Depending upon storage conditions and storage time (greater than 12 months for certain robust crops), and if not carefully managed, these “primed” spoilage microorganisms can have a devastating impact on the stored product. Apples, for example, are stored in very large, controlled atmosphere storage rooms, either in wooden bins or boxed and ready for distribution (Watkins, Kupferman, & Rosenberger, 2004).

Table 2 Fungal fruit pathogens[a]

	2004 Annual U.S. per capita consumption (lbs)[b]	*Penicillium*	*Geotrichum*	*Fusarium*	*Botrytis*	*Colletotrichum*	*Mucor*	*Monilinia*	*Rhizopus*	*Phtyophthora*
Apples	18.8	+			+	+	+	+		
Bananas	25.8			+		+				
Berries	6.1	+			+	+	+	+		+
Citrus	22.7	+	+			+				+
Grapes	7.9	+			+				+	
Melons	14.7									
Peaches	5.1	+			+			+	+	
Pears	3.1	+			+		+			
Pineapple	4.4			+						

[a]Important postharvest diseases retrieved from Sholberg et al. (2004) and other sources.
[b]85.2% of all fresh fruits consumed per capita in the United States in 2004 (ERS, 2007).

Table 3 Bacterial vegetable pathogens[a]

	2004 Annual US per capita consumption (lbs)[b]	*Pseudomonas*	*Erwinia*	*Xanthomonas*	*Bacillus*	*Clostridium*	Lactic acid bacteria
Broccoli	5.9	+	+	+			
Cabbage	8.3	+	+	+			
Carrots	8.9	+	+		+		
Corn, sweet	9.6						
Cucumbers	6.3		+		+		
Lettuce, head	22.5	+	+	+			
Lettuce, leaf	12.0	+	+	+			
Mushrooms	2.6	+					
Onions	21.7		+		+		
Potatoes	46.5	+	+		+	+	
Spinach	2.1						
Tomatoes	19.3	+	+	+	+		+

[a]Important postharvest diseases retrieved from Sholberg et al. (2004) and other sources.
[b]81.0% of all fresh vegetables consumed per capita in the United States in 2004 (ERS, 2007).

Fig. 1 Extensive *blue* mold infestation on apples

Two wound pathogens, *Penicillium expansum* and *Botrytis cinerea*, if not scrupulously cleaned from fruits prior to storage or if fruits with infected wounds have not thoroughly been culled from the lot, can cause significant crop loss as these spoilage fungi eventually degrade the wound sites, create lesions, and cross-contaminate adjacent fruits. If fruits receive improper preharvest fungicide application, poor washing, and/or inadequate culling, an expanding infestation of spoilage microorganisms can destroy a substantial portion of a stored lot of fruits (Figs. 1 and 4). *P. expansum* (Miedes & Lorences, 2004) and *B. cinerea* (van Kan, 2006) are pathogens of apples, pears, and a number of other pectin-rich fruits. *B. cinerea* is an especially sophisticated and selective plant pathogen that possesses multiple cutinases and lipases that are capable of degrading plants rich in pectin (van Kan 2006).

The bacterium *Erwinia carotovora* subsp. *carotovora* is a highly effective spoilage microbe that causes soft rot across a broad host range of vegetables and some fruits (Lund, Baird-Parker, & Gould, 1983; Table 4). One of six known genera of soft-rot bacteria (including *Xanthomonas*, *Pseudomonas*, *Clostridium*, *Cytophaga,* and *Bacillus*), *E. carotovora* subsp. *carotovora* is one of several species of *Erwinia* that infect and destroy plant tissues both pre- and postharvest and is the species that causes the greatest damage to harvested vegetables. Soft rot is a form of decay characterized by a watery transparency in infected leafy plant parts and

Table 4 Fungal vegetable pathogens[a]

	2004 Annual US per capita consumption (lbs)[b]	*Geotrichum*	*Rhizopus*	*Phytophthora*	*Fusarium*	*Pythium*	*Alternaria*	*Colletotrichum*	*Botrytis*	*Sclerotinia*
Broccoli	5.9		+				+		+	+
Cabbage	8.3		+				+			+
Carrots	8.9	+							+	+
Corn, sweet	9.6				+					
Cucumbers	6.3		+		+	+		+		
Lettuce, head	22.5	+							+	+
Lettuce, leaf	12.0	+							+	+
Mushrooms	2.6									
Onions	21.7	+			+		+	+	+	
Potatoes	46.5		+	+	+	+				
Spinach	2.1									
Tomatoes	19.3	+	+	+	+		+	+	+	+

[a]Important postharvest diseases retrieved from Sholberg et al. (2004) and other sources.
[b]81.0% of all fresh vegetables consumed per capita in the United States in 2004 (ERS, 2007).

watery disintegration of nonleafy plant materials. "Soft-rot erwinia" tend to initiate infection and decay at wound sites and, once established, can quickly advance to total destruction of the product. Soft-rot erwinia express four pectin-degrading extracellular enzymes: pectin lyase, polygalacturonase, pectin methylesterase, and pectate lyase. Of these enzymes, pectate lyase is primarily responsible for extensive decay. *E. carotovora* has built-in redundancy for this apparently critical pathogenicity factor, expressing four distinct extracellular pectate lyase isozymes (Barras, van Gijsegem, & Chatterjee, 1994).

Soft-rot erwinia are active only at temperatures of 20°C and above, which reinforces the need to maintain a continuous cold chain from immediately postharvest to retail to successfully manage this ubiquitous spoilage bacterium. Another group of soft-rotting bacteria, the fluorescent pseudomonads (i.e., *Pseudomonas fluorescens* and *Pseudomonas viridiflava*), can decay plant tissue at temperatures at or below 4°C. This is one explanation for the high prevalence of these bacteria on decayed vegetables at wholesale and retail markets (Liao & Wells, 1987). Liao, Hung, & Chatterjee (1988) revealed through several *P. viridiflava* mutants defective in pectate lyase expression that these mutants completely lost the ability to induce soft rot on potato tuber slices. The soft-rotting fluorescent pseudomonads, when considered together with soft-rot erwinia, present a formidable challenge to commercial fresh product operations, and fresh vegetables in particular, from the farm to retail and wholesale outlets.

Pseudomonas tolaasii, another fluorescent pseudomonad and fresh produce spoilage bacterium, has a much narrower range of host-specificity than *P. fluorescens* and *P. viridiflava*. *P. tolaasii* causes spoilage of the white mushroom, *Agaricus bisporus*. Similar to *P. fluorescens* and *P. viridiflava*, *P. tolaasii* produces siderophores that fluoresce under ultraviolet light (Munsch, Geoffroy, Alatossans, & Meyer, 2000). However, unlike the soft-rot pseudomonads, *P. tolaasii* does not cause soft rot on plants (i.e., it does not produce pectin depolymerases) but instead creates unsightly blemishes on the caps and stems of the *Agaricus* fruiting body as a result of localized infection and decay of those parts of the mushroom. Wells, Sapers, Fett, Butterfield, Jones, Bouzar, & Miller (1996) identified three pathotypes of mushrooms based on pathology and fatty acid analysis: *P. tolaasii* and *P. gingeri* which cause severe and yellowed lesions and *P. reactans* which causes a mild discoloration of the infected area.

Prevention and Control Measures

Preharvest and Harvest Factors

Fresh fruits and vegetables are among the more challenging of food products to commercially produce and distribute. Fresh produce remains metabolically and developmentally active as it proceeds from the commercially appropriate time to harvest (horticultural maturity), to physiological maturity, to senescence and complete deterioration. During this period of development, several physiological and

compositional changes occur. This process can be summarized chronologically as growth, maturation, physiological maturity, ripening, and senescence (Watada, Herner, Kader, Romani, & Staby, 1984). Although infection and microbiological spoilage can proceed at any time during this developmental continuum, the period of greatest susceptibility to decay onset is during ripening and senescence. Prior to ripening, fruits and vegetables are equipped with defensive barriers to infection including active wound healing and the production of phytoalexins which are phenolic substances that are toxic to fungi (Kader, 1992; Sommer, Fortlagae, & Edwards, 1992).

Losses due to postharvest spoilage or pathological decay are a result either of latent infections in the field that become active following harvest or of cross-contamination during harvest, cleaning, storage, and distribution. Presence of the pathogen on a susceptible host fruit or vegetable, combined with suitable environmental conditions such as high temperature, provides the three components required for disease expression such as host, environment, and pathogen (Sommer et al., 1992). Therefore, spoilage management should begin in the field using an integrated strategy of GAPs. Balanced crop nutrition influences susceptibility to spoilage. For example, Sugar, Righetti, Sanchez, and Khemira (1992) determined that adjusting pear orchard nutrition, specifically for low nitrogen and high calcium, reduced fruit decay postharvest. High nitrogen in plant tissues generally increases susceptibility to decay, whereas high calcium content reduces postharvest decay on several crops (Conway, 1984, 1989; Conway, Janisiewicz, Klein, & Sams, 1999). Removing dead and decaying plant matter and other organic material from the crop plant and soil surface will eliminate a major harborage for spoilage microbes as well as other crop pests. To the extent possible, isolating the agricultural field from wild and domestic animals will not only reduce total microbial pressure on the crop, but also reduce food safety risks. Aerial fungicide applications preharvest also will reduce postharvest spoilage in storage. For example, a single application of the fungicide ziram to pome fruit reduced postharvest decay by 25–50% (Sugar & Spotts, 1995). Other preharvest fungicides are also available (e.g., iprodione and cypronidil) and several new fungicides are under development (Sholberg & Conway, 2004). In addition, insect pest management will reduce insect damage to crops and also will reduce microbial cross-contamination by the insect vector. This is especially important for chewing insects that create wounds on the fruit or vegetable and can simultaneously inoculate the wound site (Mahovic, Sargent, & Bartz, 2005).

At time of harvest and throughout handling before storage and distribution, it is important to minimize wounds and bruising and to cull all damaged and diseased product. A few spoilage microbes, primarily fungi, can infect healthy tissues by forming appressoria, external structures that enable the pathogen to penetrate the cuticle and epidermis (Sommer et al., 1992). The developing appressorium ramifies through these protective layers and into the pulp through a combination of mechanical pressure and tissue destruction by extracellular enzymes (Collmer & Keen, 1986). However, most spoilage microbes infect and initiate decay at punctures and splits in the epidermal layer or, in far fewer cases, through natural openings such as stomata and lenticels.

Postharvest Factors

Product integrity at time of harvest and stringent temperature management from harvest to consumption are two critically important factors contributing to acceptable storage and shelf life of all fresh fruits and vegetables. Upon harvest, fresh fruits and vegetables benefit from immediate surface sanitation and rapid cooling to slow product metabolism and growth of spoilage microbes. Reducing the rate of metabolism likewise reduces product respiration which, in turn, reduces the rate of deterioration, or perishability, of the crop (Kader, 1992). In many instances, product cooling and sanitation are accomplished simultaneously through one or more washings with chilled water amended with a sanitizing chemical. Chlorine, as sodium hypochlorite, calcium hypochlorite, or chlorine gas, is the most commonly used sanitizing chemical in the produce industry. Chlorine at a rate of between 50 and 200 ppm is added to prechilled water which is then applied to harvested fruit as a dip or as a spray or as some combination of these two methods. Concentrations below 50 ppm may not be particularly effective on some fruit, and concentrations above 200 ppm may damage the product and also create a potential worker safety issue due to off-gassing. To achieve and maintain maximum sanitizing efficacy, it is important to maintain water pH at or slightly below neutrality (pH 6.5–7.0). This can be achieved using any of a number of food-grade acids such as citric acid. It is also important to maintain as low an organic load as possible in the wash water because chlorine is unstable in the presence of organic matter and is rapidly inactivated. Other sanitizing chemicals such as ozone, chlorine dioxide, and peroxyacetic acid also are approved for use on fresh produce and are available commercially (Sapers, Miller, Pilizota, & Mattrazzo, 2001). Methods for monitoring sanitizer concentration are available for all commercially available sanitizers, and it is also strongly recommended to perform routine treatment efficacy assessments. This can be determined by collecting a minimum of three, and preferably five, individual samples immediately prior to washing and another three or five samples immediately following washing, and determining the total aerobic plate count on each sample. A properly functioning wash system should reduce the average total aerobic plate count by 10- to 100-fold. Sequential wash steps will further improve product sanitation by providing greater reductions in microbial load on the product.

Some additional commonly used methods for removing the field heat of harvested produce include forced air refrigeration, vacuum cooling, and immersion in ice. Mushrooms, for example, are not as amenable to water washing as many other products, and hence forced air and vacuum cooling are common in the mushroom industry (although mushroom wash systems are beginning to see increasing use). Selecting the optimum cooling method or combination of methods for a given product is beyond the scope of this chapter, but there are excellent resources available that provide specific technical guidance in this area (Kader, 1992).

Methods for Detection and Isolation of Spoilage Microorganisms

Methods to detect and isolate spoilage microbes from fresh fruits and vegetables depend largely on whether the sample of interest is currently infected with a visible

lesion or the sample has no visible lesions. If the sample has no visible signs of disease, it is reasonable to assume any spoilage microbes present will be residing at or near the outer surfaces of the sample. In this case the objective of sample preparation is to dislodge as many of the viable microorganisms as possible from the sample surface for subsequent isolation and detection. Several different strategies may be used to release microorganisms, and all typically begin by adding the sample to a volume of sterile diluent to obtain a 1:10 dilution in a sterile Whirlpak® or Stomacher® bag. Sterile, deionized water can be used for this purpose, but this is not recommended as osmotic shock may inactivate a portion of the total microbial population. Phosphate-buffered saline, Butterfield's buffer, and 1% buffered peptone water are all acceptable diluents for this purpose and can be prepared easily in the laboratory or purchased preformulated. Physically dislodging the microbes can be accomplished by palpating the sample in a Stomacher for up to 2 min, or by pulping the sample in a sterile, commercial food blender for up to 60 s, or by vigorous shaking on a wrist-action shaker for up to 30 min. Indeed, when sample preparation must be conducted outside the laboratory setting, dislodging surface microbes can be accomplished, albeit less efficiently and with lower yields than the aforementioned methods, by hand shaking the sample bag for up to 2 min. Each of these mechanical methods has advantages and disadvantages. The Stomacher method, probably the most widely applied in the food industry, is rapid, does not come into physical contact with the diluted sample (does not require re-sterilization between samples), and reportedly provides a high rate of recovery of viable microbes from the sample (Sharpe, Hearn, & Kovacs-Nolan, 2000; Wu, Jitareerat, & Fung, 2003). Blending the sample is rapid and efficient, but the blender jar and blades must be re-sterilized between samples (or multiple blender jars must be used). Shaking the diluted sample on a wrist-action shaker is efficient and, depending upon the length of the shaker arms, up to 16 samples can be prepared simultaneously, and the shaker reused immediately as the sample does not come into direct contact with the equipment. Another advantage of agitation by a wrist-action shaker is that the sample remains more or less intact. This is unlike either the Stomacher or blender that macerates the tissue and makes subsequent sample handling steps such as pipetting more difficult. A relatively new piece of equipment, the Pulsifier, offers the same advantage as the wrist-action shaker by preparing the sample with very little maceration of the sample (Fung, 2006). Wu et al. (2003) compared total viable bacterial cell and total coliform, recovered from samples of 30 different fresh vegetables, with the Stomacher and with the Pulsifier and found no difference in viable recovery between the two methods. Irrespective of the initial sample preparation step, the next step depends on whether the investigator is interested in attempting quantitative recovery of a specific pathogen (or pathogens) or simply desires to determine if the microbe of interest is present on the sample. Quantitative recovery can be difficult if a suitably selective medium for the pathogen of interest does not exist. In this instance, it is typically necessary to streak the plate directly onto several nutritionally different media and subsequently identify those colonies resembling the microbe of interest. However, if a suitable selective medium does exist, the next step after sample preparation is serial dilution, followed by spread-plating (0.1 ml)

and incubation. Incubation time and temperature depend very much on the temperature range of the pathogen of interest, compared with the typical temperature range of the background flora the investigator wishes to suppress.

Several texts are available that describe routine diagnosis of fungal and bacterial diseases of fresh fruits and vegetables. Many spoilage microbes develop very distinctive lesions depending upon the fruit or vegetable afflicted. For this reason, initial diagnosis often is conducted in the field or in the packing facility based on macroscopic appearance of the lesion. *A Color Atlas of Post-Harvest Diseases and Disorders of Fruits and Vegetables* (Snowdon, 1990) is a two-volume set of texts that provides a comprehensive review of the biology, epidemiology, and physical appearance of a large number of fruit and vegetable spoilage microorganisms. Included are several photomicrographs of the pathogens described, illustrating their appearance either as single cells, mycelia, and fruiting bodies.

Basic Plant Pathology Methods (Dhingra & Sinclair, 1985) is a very comprehensive reference text that includes not only methods for enrichment, isolation and identification of most plant pathogens, but also provides several chapters on methods for pathogen isolation from soils and other strata, manipulation and handling of pure cultures in the laboratory, microscopy methods (including several staining techniques), fungicide efficacy assays and biological control assays, and histological techniques.

Two other manuals that address specific methods for the diagnosis of plant bacterial diseases are *Methods for the Diagnosis of Bacterial Diseases of Plants* (Lelliott & Stead, 1987) and *Laboratory Guide for Identification of Plant Pathogenic Bacteria* (Schaad, 1988). The former text provides both macroscopic diagnosis on plant and fruit tissues and isolation and identification methods, whereas the latter focuses more on laboratory procedures. Supplemental information can be found in more general references (Downes & Ito, 2001; Jackson, 1998; Gerhardt, et al., 1981),

Microbiological Spoilage of Fresh-Cut Fruits and Vegetables

Introduction

Fresh-cut fruits and vegetables are “any fresh fruit or vegetable or any combination thereof that has been physically altered from its original form, but remains in a fresh state” (IFPA, 2001). Fresh-cut fruits and vegetables offer consumers ready-to-eat produce that is one of several convenient, nutritious and fresh-like tasting, and are a rapidly growing category of value-added produce products that are minimally or lightly processed. Processing of fresh-cut products involves sorting, cleaning, washing, heating/pasteurization (such as Biosteam®), trimming/peeling, coring, slicing, shredding, packaging and/or other related steps, depending on the product. The processing can be as simple as fresh-cut grape tomatoes for which raw tomato fruit is only sorted and washed with sanitized water or as complicated as cut cantaloupe, for which cantaloupe is sorted, cleaned with brush and spray water, heat treated

with hot water or steam, peeled, deseeded, chunked, and rinsed with sanitized water before packaging in rigid containers. The fresh-cut fruits include melon chunks and slices; cored and sliced pineapple; apple wedges treated with antibrowning preservatives; peeled citrus fruits and segments; de-capped strawberry; de-stemmed and washed grapes; sliced kiwifruits, and fruit salads. Examples of fresh-cut vegetables are shredded lettuce, shredded and diced cabbage, washed and trimmed spinach, peeled "baby" carrots, cauliflower and broccoli florets, sliced or diced tomatoes, peeled and sliced potatoes, snapped green beans, trimmed green onions, cleaned and diced onions, and mixed salads. Compared with whole fresh produce, fresh-cut produce is ready-to-use (ready-to-eat), contains 100% usable product, and always requires processing, refrigeration (including chilling-sensitive fruits and vegetables that can be injured after a period of exposure to chilling temperatures below 10–15°C (50–59°F) but above their freezing points), and packaging, specifically modified atmosphere packaging (MAP, a packaging technique that utilizes package atmosphere other than air, <0.1% CO_2, 20.9% O_2, 78% N_2, in a sealed package to extend shelf life of foods). Fresh-cut products are in a raw state or fresh-like, nutritious, and contain live tissues without freezing, canning (heat sterilization), dehydrating, fermentation, acidification, or treatments with additives or preservatives to prevent spoilage. Fresh-cut is also described as precut, minimally processed, minimally processed refrigerated, lightly processed, partially processed, fresh processed, and pre-prepared in literature and application communications. Fresh-cut fruit and vegetable sales are approximately $12 billion per year in the North American foodservice and retail market and account for nearly 15% of all produce sales (IFPA, 2001). Fresh-cut products offer produce growers/shippers an opportunity to increase sales by adding value to raw agricultural commodities. The largest portion of US fresh-cut vegetable sales at retail is fresh-cut salads, with sales of $2.4 billion. Retail fresh-cut fruit products are the fast growing fresh-cut produce category. In 2004, 3.5 million units of fresh-cut fruits were sold for $719 million in sales. Between January and February of 2005, those numbers were up 17% over 2004 (Warren, 2005).

Shelf Life of Fresh-Cut Fruits and Vegetables

The shelf life (i.e., the length of time that corresponds to a tolerable loss in quality of a processed food and other perishable items) of fresh-cut fruits and vegetables ranges from 1 to 35 days depending on types of shelf life (such as marketing shelf life, food safety shelf life, sensory shelf life, or microbiological shelf life), food safety concerns, marketing strategies of fresh-cut processors, produce commodities, raw materials, refrigerated storage temperatures, preparation methods, and packaging methods. Marketing shelf life of fresh-cut products, indicated by sell-by-date, best if use-by-date, use-by-date, or best use-by-day, is not necessarily the same as sensory shelf life or microbiological shelf life, depending on marketing concerns and competition. The average shelf lives of fresh-cut fruits and vegetables are typically 10–14 days (Cantwell & Suslow, 2002). However, the shelf life for kitchen- or

store-prepared fresh-cut fruits for the catering industry and restaurants is only 1–2 days (Ahvennainen, 1996). FDA (2000) has suggested a 7-day shelf life at 5°C for fresh-cut melons prepared at home for microbiological safety reasons. Sensory shelf life and microbiological shelf life are usually the same; however, they can differ significantly. For example, white surface formation on "baby" carrots can occur weeks before microbial spoilage is observed even though the peeled carrots are packaged in polyethylene pouches and stored at 2°C (Bolin & Huxsoll, 1991). Poubol and Izumi (2005) reported that fresh-cut mango made from cultivar "Nam Dorkmai" (50–60% yellow) had a shelf life as short as 2 days at 5°C due to browning discoloration and a water-soaked appearance. However, fresh-cut celery of 10-cm sticks or 1.9-cm crescents retained its fresh appearance and flavor up to 28 days when stored at 2°C (Robbs, Bartz, McFie, & Hodge, 1996a). O'Hare (1994) reported that cut pineapple had a shelf life of more than 5 weeks at 1°C. Fresh-cut melons (such as cantaloupe and watermelon) prepared by retail stores (store-cut) and displayed on retail shelves typically have a 2-day shelf life. The shelf life of cut cantaloupe that is prepared by fresh-cut processors could be as short as 5 days at 7°C. Cantaloupe washed with hydrogen peroxide, cut and packed at research laboratories could last for more than 18 days at 4°C (Sapers, Miller, Pilizota, et al., 2001). Sterile diced cantaloupe stored in controlled atmospheres at 4.5°C had a shelf life of up to 28 days (O'Connor-Shaw, Roberts, Ford, & Nottingham, 1996). Powrie, Wu, and Skura (1988) reported a shelf life of 12 weeks at 1°C for sliced melon, using modified atmosphere packaging (MAP) and gas-impermeable containers.

Impact of Microbiological Spoilage

As processing and packaging technologies have improved during the last decade, microbiological spoilage or microbiological shelf life has become a major reason for sensory quality shelf life failure for most packaged fresh-cut fruits and vegetables, followed by surface discoloration (e.g., pinking of cut lettuce, browning of cut potato, graying and browning with processed pineapple, and gray discoloration with cabbage), water-soaked appearance or translucency (e.g., cut watermelon, papaya, honeydew, and tomatoes), moisture loss (e.g., "baby" carrots and celery sticks), off-aroma (e.g., broccoli florets and diced cabbage in low % O_2 and high CO_2 packages), flavor changes (e.g., cut kiwifruit), and texture changes (e.g., processed strawberry, grated celery, kiwifruit, and papaya). Microbial spoilage including off-flavor (e.g., fermented aroma with cut lettuce, sour taste with cantaloupe and bell pepper) formation, slimy surface (e.g., "baby" carrots), wetness and soft rot (e.g., cut bell pepper), discoloration (e.g., apple wedges), and visual microbial growth/colonies (such as apple wedges, cantaloupe chunks, and cored pineapple) has been used as a main or exclusive objective criterion to determine shelf life of fresh-cut products (Sapers, Miller, Pilizota, et al., 2001, O'Connor-Shaw, Roberts, Ford, & Nottingham, 1994). Brackett (1994) concluded that microbial decay can be a major source of spoilage of fresh-cut produce. O'Connor-Shaw et al. (1996) reported that microbial spoilage is a limiting factor for shelf life of fruit pieces stored under con-

trolled atmosphere conditions. Shelf life, including microbial spoilage, results in 30–50% shrinkage of fresh-cut fruits (Warren, 2005). Microbial spoilage has been used by quality assurance departments in the fresh-cut industry as the objective indicator for quality failure for more than 50% of fresh-cut vegetable commodities and almost 100% of fresh-cut fruit products that have been treated with preservatives (such as antibrowning reagents) and/or packaged properly using MAP technologies. Under equilibrium modified atmosphere (MA) conditions, mixed fresh-cut bell pepper (including green, yellow, and red bell pepper) was unacceptable by day 6 of storage at 7°C due to acidic flavor, water loss, and texture change (Jacxsens, Devlieghere, Ragaert, Vanneste, & Debevere, 2003). Processed Lollo Rosso lettuce had a shelf life of shorter than 7 days at 5°C due to high microbial counts and off-odor formation under MAP (Allende, Aguayo, & Artes, 2004). Grated carrots became wet and slimy, lost firmness, and produced off-odors during storage at 10°C under MAP (Buick & Damoglou, 1987; Carlin, Nguyen-the, Cudennec, & Reich, 1989, 1990). The first indicator of changes in freshness for fresh-cut lettuce packed using active/passive MAP to prevent pinking or browning is fermented aroma formation (Table 5). Studies of cut cantaloupe revealed evidence of visual spoilage, including presence of microbial colonies, slime, and turbidity in juice, within 15 days of storage at 4°C (Sapers, Miller, Pilizota, et al., 2001).

Table 5 Changes in intensity of sensory attributes of fresh-cut lettuce during storage at refrigeration temperature (15-point universal scale was used in the test)

Shelf life (days)	Flavor	Hardness (texture)	Fermented aroma
0	6.3	5.3	3.8a
4	5.8	5.2	4.3a
5	5.6	5.8	6.1ab
6	4.6	7.3	5.9ab
7	6.0	6.7	4.6ab
10	4.3	7.6	6.3ab
11	6.2	6.3	6.8b
12	5.9	6.6	6.9b
P-value	0.671	0.131	0.047

Sources of Microbial Contamination

Contamination sources of fresh-cut fruits and vegetables include raw materials and contact with processing equipment. The microorganisms that exist on the surfaces of raw, whole produce appear to be the major source of microbial contamination and consequent spoilage of fresh-cut fruits and vegetables. Sapers, Miller, Jantschke, and Mattrazzo (2001) reported that, compared with good surface sanitization practices, no decontamination treatment or an ineffective antimicrobial treatment on whole cantaloupe resulted in premature microbiological spoilage of fresh-cut cantaloupe. Studies have also revealed over a 1-year period of sampling that there is a close relationship between the total mesophilic aerobic counts on lettuce

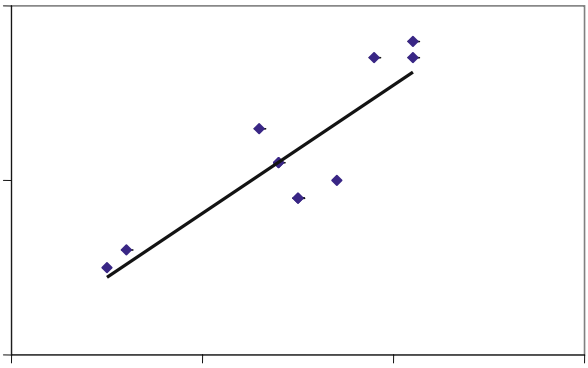

Fig. 2 Relationship between microbial population of raw lettuce and shredded finished product

raw material and those on finished shredded lettuce product (Fig. 2). Robbs et al. (1996a) determined that the most common bacteria on raw celery plants, including fluorescent *Pseudomonas* spp. and *Aeromonas* spp., were also the most common bacteria on cut celery products. Boyette, Ritchie, Carballo, Blankenship, and Sanders (1993) reported that the microbial decay of fresh-cut lettuce is largely due to the growth of microorganisms originating from preharvest environments. Delaquis, Stewart, Toivonen, and Moyles (1999) determined that the types of microorganisms found on shredded lettuce were highly associated with the microorganisms detected on lettuce before shedding. Several studies (Magnusson, King, & Torok, 1990; Geeson, Churey, & Splittstoesser, 1990; Torok & King, 1991) have revealed that yeast species identified on fresh-cut produce can also be isolated from raw materials prior to processing. Garg, Churey, and Splittstoesser (1990) concluded that peel is the major source of microbial contaminants on carrot sticks. Several outbreaks of salmonellosis that were associated with cut cantaloupe and watermelon have resulted from *Salmonella* present on the rind contaminated in the field or packinghouse (Harris et al., 2003). Inoculation of *Listeria monocytogenes* and *Salmonella* on the surface of entire cantaloupes resulted in the contamination to fresh-cut pieces during cutting (Ukuku & Sapers, 2001, Ukuku & Fett, 2002). These results indicate that the bacteria on the surface of whole produce are the same as those on fresh-cut produce and can contaminate finished product through processing. Fresh-cut products can also be contaminated by spoilage microorganisms through contact by people or equipment during processing possibly by air during processing and packaging steps, especially in facilities that have been used for produce processing over an extended period of time. Cantwell and Suslow (2002) found significantly higher bacterial counts during processing on automated cutters and package fillers of a lettuce processing line, indicating that clean product can become recontaminated after passing through operations where vegetable and fruit debris can accumulate, such as cutters and package-filling equipment. Shredding and slicing steps in fresh-cut processing resulted in increased microbial populations by 1–3 logs on cut cabbage, lettuce, and onions (Garg et al., 1990) and at least a 1-log increase for lettuce and chicory salads (Jockel & Otto, 1990). Legnani and Leoni (2004) found statistically significant differences for total plate counts and total and fecal coliforms in the last

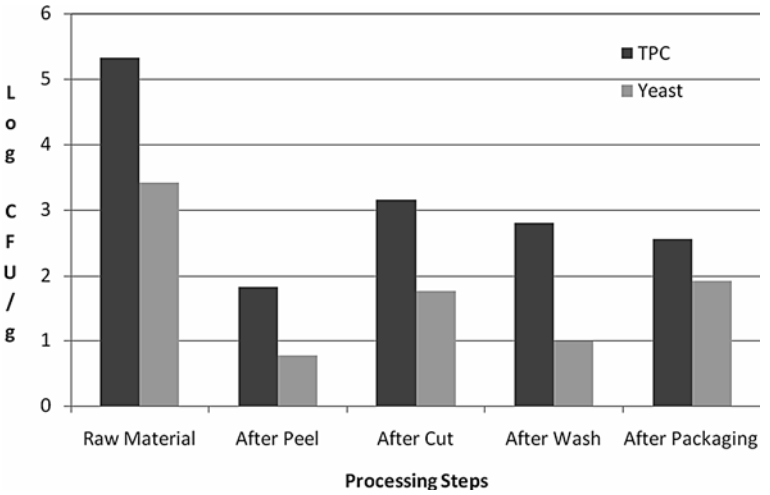

Fig. 3 Changes in mesophilic aerobic bacterial (TPC) and yeast populations of cantaloupe during processing

wash water step (rinsing water) compared to the water used during the previous disinfecting stage (3.52 $\log_{10}$ vs. 0.94 $\log_{10}$ for TPC, 1.86 $\log_{10}$ vs. 0.04 $\log_{10}$ for total coliforms). Zhuang, Barth, and Hankinson (2003) reported that the microbial population significantly increased on broccoli florets after the washing step. Allende et al. (2004) determined that shredding, rinsing, and centrifugation of red lettuce Lollo Rosso increased coliform, lactic acid bacteria, and psychrotrophic bacterial counts. Yeast populations on cut cantaloupes increased after packaging (Fig. 3). Several researchers have suggested that the large numbers of lactic acid bacteria and fungi present on fresh-cut products indicate likely contamination from processing, such as cutting machines (Brocklehurst, Zaman-Wong, & Lund, 1987; Dijk et al., 1999). For example, *Geotrichum candidum* has been termed "machinery mold" because it can accumulate on fruit-processing equipment.

Microbial Populations and Varieties on Fresh-Cut Fruits and Vegetables

Since raw materials can contribute to contamination of produce products during cultivation, harvesting, packaging, and shipping, and there are no definite decontamination steps during processing, it is not surprising that a variety of microbial populations can be present. Goepfert (1980) reported that mesophilic aerobic bacterial populations on vegetables sampled at processing plants ranged from 4.6 (carrots) to 7.5 (peas) $\log_{10}$ CFU/g fresh weight. The mesophilic aerobic bacterial counts ranged from 4 to 6 $\log_{10}$ CFU/g fresh weight on finished cut vegetables and from 2 to 5 $\log_{10}$ CFU/g fresh weight on finished cut fruits, depending on the commodities, seasons of the year, and growing regions (Zhuang et al., 2003). The mesophilic

aerobic bacterial counts on bagged salads from the retail market ranged from 4.0 to 9.0 $\log_{10}$ CFU/g (Heard, 2000). High numbers of yeast and mold populations were also present on many ready-to-eat packaged salads, including lettuce, coleslaw, celery chunks, and baby carrots, and salad bar items including broccoli, cauliflower, iceberg and romaine lettuce, spinach, sliced green peppers, cucumbers, and tomatoes, ranging from 1.6×10^3 cfu/g on iceberg lettuce to 9.2×10^6 cfu/g on sliced tomatoes (Tournas, 2005a).

Many types of microorganisms can be found on a cut fruit or vegetable, including Gram-negative bacteria, Gram-positive bacteria, and fungi (yeasts and molds). Some viruses have been identified as plant pathogens of whole produce and presumably result in quality loss of fresh-cut root or tuber vegetables. Parasites can be a food safety concern but do not affect the sensory qualities/spoilage of either whole or fresh-cut fruits and vegetables. Buick and Damoglou (1987) reported that the microflora isolated from chlorinated sliced carrots included 70% *Erwinia* spp., 20% *Pseudomonas* spp., and 10% *Bacillus* spp. Babic, Hilbert, Nguyen-the, and Giraud (1992) identified a variety of yeasts, including *Candida* spp., *Cryptococcus albidus*, *Rhodotorula* spp., *Trichosporon penicillatum*, and *Saccharomyces cerevisiae,* on packed grated carrots. Carlin et al. (1989) isolated lactic acid bacteria, specifically *Leuconostoc mesenteroides*, and yeasts from grated carrots stored in MAP. Liao and Fett (2001) isolated lactic acid bacteria, yeasts, and 48 strains of pectolytic bacteria of the genera *Pseudomonas, Erwinia, Bacillus*, *Xanthomonas,* and *Flavobacterium* from baby carrots. Poubol and Izumi (2005) reported that bacteria isolated from "Nam Dokmai" mango cubes were predominantly Gram-negative rods of which about 60% were Enterobacteriaceae*,* including the genera *Klebsiella* and *Pantoea*. Phytopathogenic bacteria that cause rot in vegetables such as *Pantoea agglomerans* (synonymous with *Erwinia herbicola* and *Enterobacter agglomerans*) and *Burkholderia cepacia* (synonymous with *Pseudomonas cepacia*) were also isolated frequently. The most common Gram-positive bacteria were of the genus *Curtobacterium*. Robbs et al. (1996a, 1996b) found Gram-negative bacteria fluorescent *Pseudomonas* spp., *Pantoea herbicola (E. herbicola)*, *P. agglomerans*, *Aeromonas, Arthrobacter*, *Aureobacterium,* and *E. carotovora*, Gram-positive cocci (*Leuconostoc*), and Gram-positive rods on decayed fresh-cut celery. Garg et al. (1990) detected large populations of Gram-negative psychrotrophic bacteria, particularly *Pseudomonas* spp., lactic acid bacteria, and fungi on freshly prepared carrot sticks. The filamentous fungi isolated from ready-to-eat salads included *Alternaria, Cladosporium and Penicillium, Alternaria, Cladosporium and Aspergillus, and Fusarium* (Tournas, 2005b; Acevedo, Mendoza, & Oyon, 2001).

Characteristics of Spoilage Microorganisms on Fresh-Cut Fruits and Vegetables

Fresh-cut produce differs substantially in physical properties (no protective epidermic tissues and high moisture), biochemical characteristics (wounding response), and handling environment (processed and stored under refrigerated temperatures

and packed under MAP) from whole produce. These differences likely influence the types of spoilage microflora present. Fresh-cut vegetables and melons, including cantaloupe, honeydew, and watermelon, have high water and nutrient contents (water activity, a_w>0.90), with pH values higher than 4.5. These characteristics make them suitable hosts for most microorganisms. Refrigerated conditions and high oxygen transmission rate film packages (in which equilibrium $\%O_2$ is >5% and $\%\ CO_2 < 5\%$) (Lund, 1982) enable bacteria to more successfully compete with the fungi in these foods. Hence, bacteria are more often responsible than fungi for postharvest spoilage of refrigerated fresh-cut vegetables and melons, with the majority being psychrotrophic and Gram-negative. The most common and important spoilage microorganisms of refrigerated fresh-cut vegetables are the fluorescent *Pseudomonas* species of which *P. marginalis* is an example. *Pseudomonas* spp. is a Gram-negative rod and strict aerobe. These species can be divided into four groups based on RNA homology and nine groups based on cellular fatty acid composition. Pseudomonads are widely distributed in nature and are found on both animal and plant products. They are able to utilize a wide variety of organic compounds and produce acids oxidatively from glucose and/or maltose. Some pseudomonads species produce pyoverdine or fluorescein that are water soluble, fluorescent pigments and can be observed in spoiled foods with an ultraviolet light. They are usually yellow-green but may appear blue or orange depending on the species and environmental factors. Pseudomonads produce catalase, oxidase (most), and enzymes that catalyze proteolytic and lipolytic reactions that contribute to spoilage of refrigerated fresh animal products, and pectolytic enzymes that can cause soft rot of fleshy vegetables. Pseudomonads are heat sensitive, are not found in heat-processed foods unless there is post-processing contamination, and are not very sensitive to drying or gamma irradiation. However, they are able to grow at refrigeration temperature (the minimal temperature for growth is ca. 4°C) and have been found in a variety of frozen and refrigerated foods, including fresh-cut produce. Pseudomonads can cause soft-rot decay of many types of vegetables including celery, potato, chicory, lettuce, Chinese chard, and cabbage (Brocklehurst & Lund, 1981). Many researchers (Brocklehurst et al., 1987; Carlin et al., 1989; Garg et al., 1990; Magnusson et al., 1990; Manvell & Ackland, 1986; Marchetti, Casadei, & Guerzoni, 1992; Nguyen-the & Prunier, 1989) have determined that 80–90% of mesophilic bacteria in the aerobic plate counts of vegetables are Gram-negative rods, *Pseudomonas* spp., *Enterobacter* spp., or *Erwinia* spp. with pseudomonads prevailing over other genera. Koek, De Witte, and De Maaker (1983) found that Pseudomonadaceae were 5–10 times more numerous than other families on prepared raw vegetables. King, Magnusson, Torok, and Goodman (1991) determined on cut lettuce the frequency of pseudomonads as 56.7% of total bacterial population; other species identified were *Flavobacterium* spp., *Xanthomonas* spp., *Chromobacterium* spp., *Chryseomonas* spp., *Rahnella aquatilis*, *Serratia* spp., *Alcaligenes* spp., and *Bacillus* spp. Robbs (1996a) found that the predominant bacteria in soft rot of fresh-cut celery were fluorescent *Pseudomonas* spp. *P. marginalis* and *P. chlororaphis* were isolated from 15 of the 16 samples, whereas *P. fluorescens* was found in 9 of the 16 samples. Large populations of *Pseudomonas cichorii*, *P. syringae*, and *P. viridiflava*

were also isolated from the celery. Brocklehurst and Lund (1981) found fluorescent *Pseudomonas* species to be the principal microbes on chopped salad 1 day after the listed "sell by" date. Marchetti et al. (1992) found that *P. fluorescens* represented 90% of the pseudomonads in ready-to-eat vegetables salads, whereas yeasts and molds and lactic acid bacterial populations generally remained low. Ukuku and Sapers (2005) reported that one of the most common types of spoilage microbes associated with cut melon were *Pseudomonas* spp. *Pseudomonas* spp., *Escherichia coli*, *Enterobacter* spp., and *Micrococci* were the predominant microflora on sliced watermelon (Abbey, Heaton, Golden, & Beuchat, 1988). Inoculation with either large cell numbers of *P. marginalis* or filtrated *P. marginalis* cultures isolated from spoiled shredded endives produced soft rot of endive leaves (Nguyen-the & Prunier, 1989).

Erwinia spp. are another common Gram-negative spoilage microbe associated with fresh-cut vegetables. *Erwinia*, a genus within the family Enterobacteriaceae, are small rods and facultative anaerobes. Their optimum growth temperature is 30°C, and they can ferment sugar anaerobically to form acids. *Erwinia* cause rapid necrosis, progressive tissue maceration called "soft-rot" occlusion of vessel elements called "vascular wilt," and hypertrophy leading to gall or tumor formation in plant tissues. The genus *Erwinia* spp. consists of three species or subspecies, including *E. carotovora* subsp. *carotovora*, *E. carotovora* subsp. *atroseptica*, and *E. chrysanthemi*. *Erwinia* spp. are the major single cause of microbial spoilage of whole vegetables (Liao, 2005; Lund, 1983). Brocklehurst et al. (1987) and Manvell and Ackland (1986) identified *E. carotovora* as a principal spoilage microbe of both fresh-cut and fresh vegetables. Buick and Damoglou (1987) found that *E. carotovora* was the dominant spoilage microorganism on sliced carrots packed in air, consisting of more than 80% of total detectable microflora. Robbs et al. (1996a) identified *Erwinia* in 5 of 16 soft-rot samples of fresh-cut celery.

Several Gram-positive bacteria, most notably the lactic acid bacteria, have been associated with spoilage of fresh-cut fruits and vegetables that are packaged under modified atmosphere with <2% O_2 and >10% CO_2 and stored at 7°C or above, regardless of the produce. Lactic acid bacteria are Gram-positive, usually nonmotile, nonspore forming rods and cocci. They lack the ability to synthesize cytochromes and porphyrins (components of respiratory chains) and therefore cannot generate ATP by creation of a proton gradient. The lactics can only obtain ATP by fermentation, usually of sugars. Since they do not use oxygen in their energy production, lactic acid bacteria grow well under anaerobic conditions, but they can also grow in the presence of oxygen. They are protected from oxygen byproducts (e.g., hydrogen peroxide) by peroxidases. These organisms are often termed aerotolerant anaerobes or microaerotrophic. They are differentiated from other microbes by their ability to ferment hexoses to lactic acid, hence their name. Because of the low energy yields, lactic acid bacteria often grow more slowly than microbes capable of respiration and produce smaller colonies. The genera of lactic acid bacteria include *Lactobacillus*, *Leuconostoc, Pediococcus, Lactococcus,* and *Enterococcus.* They are often associated with animal oral cavities and intestines (e.g., *Enterococcus faecalis*), plant leaves (*Lactobacillus, Leuconostoc*) as well as decaying plant or animal matter such

as rotting vegetables, fecal matter, and compost. Lactic acid bacterial fermentation lowers the pH due to lactic acid production and produces acetylmethylcarbinol and diacetyl that provide an off-flavor similar to buttermilk. Other fermentation products include acetic acid, ethanol, formic acid, and CO_2. Lactic acid bacteria were detected in almost every fresh-cut product, including honeydew, papaya, pineapple, cantaloupe, cabbage, carrots, chicory, celery, bell peppers, and various salad mixes (O'Connor-Shaw et al., 1994; Garg et al., 1990; Allende, Jacxsens, Devlieghere, Bebevere, & Artes, 2002; Carlin et al., 1989; Jacxsens et al., 2003). When packed under MAP and stored at 10°C, spoiled shredded carrots contained high numbers of lactic acid bacteria, identified as *L. mesenteroides,* and large concentrations of lactic acid, acetic acid, ethanol, and CO_2. Inoculation of shredded carrots with *L. mesenteroides* reproduced spoilage when the carrots were stored under conditions similar to those previously used (Carlin & Nguyen-the, 1989). Jacxsens et al. (2003) reported that the spoilage of mixed cut bell peppers and grated celery was related to the populations of lactic acid bacteria and yeasts, concluding that these microbes were responsible for spoilage during refrigerated storage.

The last group of major microorganisms that can cause spoilage of fresh-cut fruits and vegetables under refrigerated and MAP storage are fungi, including yeasts and molds. The optimum pH for growth of most microorganisms is near neutrality (pH 7.0). However, yeasts and molds are usually acid tolerant and are therefore associated with the spoilage of acidic foods. Yeasts can grow in a pH range of 3–10. Molds can grow from pH 2 to 11, but favor an acidic pH. In terms of water requirements for growth, yeasts are intermediate between bacteria and molds. Yeasts of the genera *Saccharomyces, Candida, Torulopsis,* and *Hansenula* have been associated with fermentation of fruits. In addition, other yeasts that can cause quality loss of produce include *Rhodotorula mucilaginosa*, *R. glutinis*, *Zygosaccharomyces bailii*, *Z. bisporus,* and *Z. rouxii* and have been isolated widely from fresh-cut fruits and vegetables, and salad mixes, even though the specific species were rarely identified (O'Connor-Shaw et al., 1994; Ukuku & Fett, 2002; Delaquis et al., 1999; Carlin et al., 1989; Jacxsens, Devlieghere, Ragaert, van der Steen, & Debevere, 2001). Yeasts have a slightly higher growth rate than molds, ferment sugars into alcohols, and are responsible for off-flavors and off-odors. Yeast growth has been responsible for shelf life failure of grated celery stored under MAP at 4°C and shredded chicory endives packed in high oxygen atmosphere with barrier film (Jacxsens et al., 2001).

Molds are fungi that cover surfaces as fluffy mycelia and usually produce masses of asexual, or sometimes sexual, spores. Mold is a growth of minute fungi forming on vegetable or animal matter, commonly as a downy or furry coating and associated with decay or dampness. Molds are overwhelmingly present in postharvest diseases of fruits and vegetables. These pathogens are commonly members of the class *Ascomycetes* and the associated Fungi *Imperfecti*. Mold spoilage of fresh produce, especially fresh fruit, is caused by species of *Penicillium, Phytophthora, Alternaria, Botrytis, Fusarium, Cladosporium, Phoma, Trichoderma, Aspergillus, Alternaria, Rhizopus, Aureobasidium, and Colletotrichum*. The symptoms include visible growth, rots and discoloration, such as blue mold rot, gray mold rot, botry-

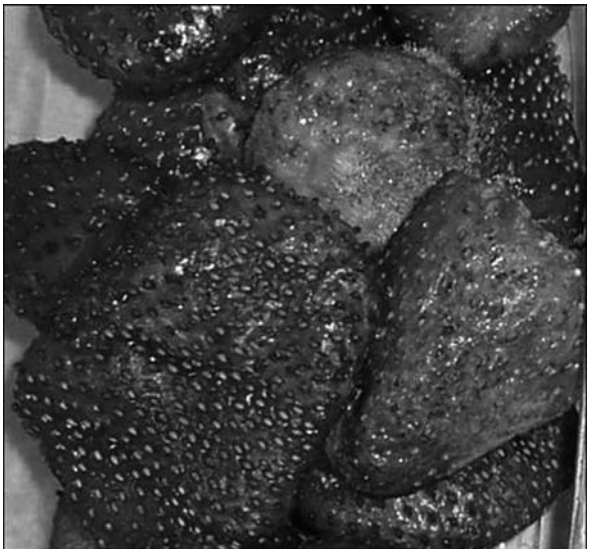

Fig. 4 Visible mold growth on cut strawberry

tis rot, and brown rot. Like yeasts, mold populations have been reported in various types of fresh-cut fruits and vegetables (Nguyen-the & Carlin, 1994; Tournas, 2005a; Hagenmaier & Baker, 1998) and visible molds have resulted in inedible fresh-cut fruits, such as strawberry, honeydew, pineapple, and cantaloupe (Fig. 4; O'Connor-Shaw et al., 1994; Ukuku & Fett, 2002). Since molds are usually detected and enumerated using the same plating media as yeasts and reported in the same category, their species most often are not identified and/or reported for contamination of fresh-cut produce.

Intrinsic and Extrinsic Factors of Fresh-Cut Fruit and Vegetable Products

Although microbes are largely responsible for the spoilage of fresh-cut produce, they can vary greatly for each type of fresh-cut product and accompanying storage conditions. The type of fresh-cut commodity and the pH of fresh-cut products are the two primary intrinsic factors that determine the microbiological spoilage profile of fresh-cut products. For example, under equilibrium-modified atmosphere spoilage of mixed lettuce (0.25 kg shredded lettuce mix with 20% each of endive, curled endive, radicchio lettuce, Lollo Rosso, and Lollo Bionda lettuces packed in 20 × 23.5 cm bags with 2,270 OTR, oxygen transmission rate) and chicory endive (0.15 kg of chicory endives packed in 19 × 15 cm bags with 3,704 OTR) leafy tissues was dominated by Gram-negative microorganisms at 7°C. However, spoilage of mixed bell peppers (0.15 kg of chopped green, red, and yellow bell peppers packed in 19 × 15 cm bags with 2,897 OTR) and grated celery (0.15 kg grated celery packed in 19 × 15 cm bags with 3,530 OTR) was dominated by lactic acid bacteria

and yeasts in the same study (Jacxsens et al., 2003). A relationship was observed between the number of pectinolytic *P. fluorescens* at the end of storage at 10°C and the extent of spoilage of shredded chicory; however, in similar experiments no relationship was noted between the presence of pectinolytic pseudomonads and spoilage with shredded carrots (Nguyen-the & Prunier 1989). Instead, spoilage of fresh-cut carrots in MAP at 10°C was highly associated with lactic acid bacteria and yeast populations (Carlin et al., 1989). In shredded cabbage stored at 4, 13, and 21°C, populations of aerobic bacteria were the dominant spoilage microbes. However, lactic acid bacterial populations increased to large cell numbers regardless of packaging treatment or storage temperature. The development of sourness and gassing of the packaging was related to extensive growth of these bacteria (Hao, Brackett, Beuchat, & Doyle, 1998). These illustrate that microbiological spoilage patterns are determined by the types of fresh-cut commodities. Intrinsic factors of plants affecting microbial spoilage can include the vegetable's nutrient content, biological structure, wounding response, self-defense system, and inherent antimicrobials. However, there are very few reports on the relationship between microbiological spoilage and these commodity-specific intrinsic variables. In addition to commodity differences, the pH of fresh-cut produce also significantly influences the microflora and the patterns of microbiological spoilage. Fresh-cut vegetables, melons, and some fruits, such as papaya, have a pH above 4.5 and the predominant spoilage microorganisms are mesophilic bacteria, specifically pseudomonads and *Erwinia.* Many reports indicate that fresh-cut lettuce packaged under oxygen-reduced atmospheres contain large populations of mesophilic and psychrotrophic bacteria, but small cell numbers of yeasts, molds, and lactic acid bacteria. The spoilage microflora of fresh-cut lettuce is largely dominated by *Pseudomonas* spp. (Garg et al., 1990; King et al., 1991; Delaquis et al., 1999). *Pseudomonas* spp., *E. coli, Enterobacter* spp., and *Micrococcus* spp. were predominant on watermelon slices during storage at 5°C (Abbey, et al., 1988). Mesophilic bacteria dominated the microflora of fresh-cut sweet potato slices, with lesser cell numbers of psychrotrophic bacteria and fungi, both initially and during storage (Erturk & Picha 2006). Mesophilic bacteria were also consistently predominant on finished cut honeydew and cantaloupe, including at the end of the shelf life (O'Connor-Shaw et al., 1994; Ukuku & Fett 2002). In contrast, the normal spoilage microflora of refrigerated, fresh-cut fruits differs markedly from that of vegetables. For most fresh-cut fruits such as apples, pineapples, strawberries, grapes, and a few fresh-cut vegetables, such as tomatoes, there is sufficient acid to limit spoilage primarily due to fungi (Splittstoesser, 1987) and aciduric bacteria (lactic acid bacteria, *Acetobacter, Gluconobacter*), *Leuconostoc* spp., and *Enterococci* spp. Martinez-Ferrer, Harper, Perez-Muroz, and Chaparro (2002) identified a relationship between increased shelf life and reduced populations of yeasts and molds on both cut mangoes and pineapples during storage. O'Connor-Shaw et al. (1994) reported that evidence of mold growth on cut pineapples stored in closed containers was the major quality defect at both 4 and 20°C. Under MAP and refrigerated storage (7°C), yeast and mold populations dominated the microflora of fresh-cut pineapples (Fig. 5) and strawberries

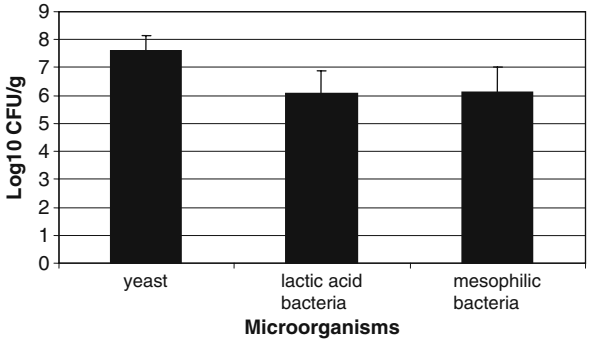

Fig. 5 Populations of microorganisms on cut pineapple held at 7°C for 9 days

Fig. 6 Microbial-related discoloration of fresh-cut apple

(Fig. 4). The most frequent reason for shelf life failure of antioxidant-treated apple wedges in MAP was fungi-related browning discoloration and decay (Fig. 6).

The major extrinsic factors that most significantly influence the microbial spoilage profile of fresh-cut produce are storage temperature and the modified atmosphere within the packages. Refrigerated storage temperature selects for psychrotrophic microbes over mesophilic microorganisms with high CO_2 (>10%) and low O_2 (<2%) content in fresh-cut packages favoring facultative and strict anaerobes instead of aerobic microbes. For example, cabbage (in coleslaw) deteriorated at the same rate at 7 and 14°C; however, at 7°C, the total mesophilic bacterial load was significantly reduced (King, Michener, Bayne, & Mihara, 1976). Similar phenomena have been reported for shredded chicory salads (Nguyen-the & Prunier, 1989) and shredded carrots (Carlin et al., 1989), in which the total mesophilic bacterial counts decreased with reduced storage temperatures. Characteristics of *P. marginalis* soft-rot spoilage are similar to those of *E. carotovora*. However, the more rapid growth of pseudomonads at refrigeration temperatures makes them more

likely to contribute to spoilage of refrigerated produce than would *E. carotovora* when the packaging atmosphere is not a limiting factor (Lund, 1982). *Erwinia* spp. (such as *E. carotovora* subsp. *carotovora* and *E. chrysanthemi*) grow poorly and fail to induce soft rot of fresh produce at 10°C or below, and the doubling time for *E. carotovora* was 15.4 h at 5.7°C (Lund, 1983). Growth of Gram-positive bacteria was more consistently affected by temperature. The optimal growth temperature range of these bacteria is 20–30°C, and they grow slowly, if at all, at refrigeration temperature. For example, the minimum growth temperature for a spoilage strain of *Clostridium puniceum* was ca. 7°C; however, most strains of *C. puniceum* are unable to grow at 10°C (Lund, Brockelhurst, & Wyatt, 1981). On shredded carrots, growth of lactic acid bacteria and subsequent spoilage was greatly delayed at 2°C compared to 10°C, but not significantly reduced at 6°C compared to 10°C (Carlin et al., 1989). Lactic acid bacteria predominated in vegetable salads held at 30°C and large amounts of lactic acid are produced. In salads held at 7°C even for prolonged periods, the lactic acid level remained low and Gram-negative bacteria predominated. The ratio of Gram-positive to Gram-negative bacteria in vegetable salads was consistently larger than 0 when held at 20 and 30°C; however, it was approximately 0 at 7°C. A Gram-positive to Gram-negative bacteria ratio of 0.5 or greater has been recommended as an indicator of temperature abuse for fresh-cut produce (Manvell & Ackland, 1986). Although many different types of fungi can be associated with spoilage of vegetables and fruits, only a few are able to spoil vegetables at refrigeration temperatures. Pitt and Hocking (1985) concluded that species of *Fusarium*, *Cladosporium*, *Penicillium*, and *Thamnidium* can grow and spoil foods at refrigeration temperatures. However, since psychrotrophic bacteria are more likely to cause spoilage in products with a neutral pH, fungal spoilage of vegetables and fruits is more likely to occur when adequate refrigeration is not maintained. Gimenez, Olarte, Sanz, Lomas, Echavarri, and Ayala (2003) found in their study of minimally processed artichokes packaged using different films that the populations of spore-forming bacteria remained virtually constant during storage at 4°C regardless of packaging films (ca. 2.5 and 2 log cfu g^{-1} of spore-forming aerobic and anaerobic bacteria, respectively). They concluded that the low storage temperature prevented germination of these spore-forming bacteria from occurring.

Pectolytic fluorescent pseudomonads that are involved in spoilage of cut produce are strictly aerobic bacteria. Gill and Tan (1979) and Wells (1974) reported that decreased O_2 content or increased CO_2 in the atmosphere reduced the growth of pectolytic fluorescent pseudomonads as well as reduced their capability of inducing soft rot on fresh produce. However, production of soft rot in potatoes by *Erwinia* and *Clostridium* is greatly enhanced by the depletion of oxygen (Perombelon, Cullings-Hander, & Kelman, 1978). The increased CO_2 and decreased O_2 concentrations formed in MAP generally favor the growth of lactic acid bacteria. Lactic acid bacterial populations were small on ready-to-use butterhead lettuce packaged with air as the initial headspace; however, higher counts were observed (with loss in quality) on samples packaged in a modified atmosphere containing 0% O_2 and 10% CO_2 as the initial headspace (Mazollier, Bardet, & Bonnafous, 1990). Growth of lactic acid bacteria on cut chicory leaves stored in modified atmosphere containing 20% CO_2 as the

initial headspace was more rapid than in air at 2, 6, and 10°C (Carlin & Nguyen-the, 1989). Generally, lactic acid bacteria grow slowly, if at all, at refrigeration temperature, and only become predominant under low O_2 and/or high CO_2 atmospheric storage. Studies have revealed that modified atmosphere storage of graded carrots at 10°C resulted in shelf life failure by *Leuconostoc*, a lactic acid bacterium (Babic et al., 1992; Carlin et al., 1989, 1990). Buick and Damoglou (1987) identified four different bacterial genera, i.e., *Erwinia*, *Pseudomonas*, *Bacillus,* and *Leuconostoc*, on sliced carrots and further study on the effect of vacuum packaging and nonvacuum packaging on microbial spoilage of sliced carrots revealed that in nonvacuum pouches, 100% of the bacteria were Gram-negative (90% was *Erwinia* and 10% was *Pseudomonas*) at 8 days of storage at 10 and 15°C, and 90% of bacteria was Gram-negative and 10% was *Bacillus* at 5°C storage. No *Leuconostoc* were detected as spoilage bacteria. Reducing the temperature had no effect on the percentage of *Pseudomonas* present, but reduced the percentage of *Erwinia* and increased the percentage of *Bacillus*. In the vacuum packages held at 15°C, 100% of the bacteria were *Leuconostoc*. Reducing the storage temperature to 5°C reduced the *Leuconostoc* population from 100 to 40% and increased the *Erwinia* population from 0 to 50%. No *Pseudomonas* spp. were detected.

Yeast populations on fresh-cut fruits and vegetables that have a neutral pH are generally not affected by modified atmospheres. Yeast growth on cut lettuce was unaffected by controlled atmosphere containing 3% O_2 with or without 10% CO_2 (Barriga et al., 1991). No differences in yeast counts were observed on grated carrots stored at 10°C under modified atmospheres containing 20–50% CO_2 and from 10 to 2% O_2 (Babic et al., 1992). However, yeasts can be facultatively anaerobic; hence in the absence of oxygen, fermentative yeasts can convert sugars into carbon dioxide and ethanol (alcohol) and result in spoilage of cut fruits and vegetables with pH values <4.5. In contrast, molds are strictly aerobic microorganisms and their spoilage depends on the availability of oxygen in the environment. MAP with high CO_2 (>10%) inhibits mold growth (Molin, 2000), although the effect is not fungicidal.

Based upon above research observations, the relationships between predominating spoilage microorganisms and the factors that influence the spoilage of fresh-cut produce are summarized in Table 6.

Table 6 Factors influencing spoilage of fresh-cut produce (Barriga, et al., 1991; Babic, et al., 1992, & Molin, et al., 2000).

pH of fresh-cut products	Storage atmosphere	Storage temperature	Predominating microorganisms
>4.5	Air	Refrigerated (<3°C)	Pseudomonads
<4.5	Air	Refrigerated and abuse temperature (>3°C)	Fungi and lactic Acid bacteria
>4.5	MAP (%O_2 < 1 and % CO_2 > 10)	Abuse temperature (>3°C)	*Erwinia*
<4.5	MAP (%O_2 < 1 and % CO_2 > 10)	Abuse temperature (>3°C)	Yeasts and lactic acid bacteria

Microbiological Spoilage Defects of Fresh-Cut Fruits and Vegetables

Microbiological spoilage defects of fresh-cut fruits and vegetables include microbial colony formation or visible microbial growth mainly due to microorganism proliferation, off-aroma and off-flavor formation mainly due to fermentation of sugar, soft-rot/water soak and sliminess due to enzymatic pectolyzation, and discoloration. For example, O'Connor-Shaw et al. (1994) observed mold growth at 14 days on cut pineapples held at 4°C and at 4 days when held at 20°C. White mold colony formation was observed at 11 days on cut cantaloupe held at 4°C and at 7 days on cut honeydew held at 8.5°C. Visual evidence of bacterial spoilage of cut cantaloupes results from the presence of bacterial colonies (Fig. 7), slime, juice turbidity, and off-odor (Sapers, Miller, Pilizota, et al., 2001). Mixed lettuce stored in bags with an oxygen transmission rate (OTR) of 15 mL $O_2/(M^2$ 24 h 1 atm) became inedible within 4 days at 7°C due to the off-odor and unacceptable taste that were described as alcoholic and fermented (Jacxsens et al., 2003). Mixed bell peppers held at 7°C were rejected by a trained sensory panel within 6 days due to an acid odor and taste. Fresh-cut cantaloupe held at 4°C developed an off-odor within 11 days (O'Connor-Shaw et al., 1994), and mixed vegetable salads held at 4°C were spoiled by off-odors within 7 days (Allende et al., 2002). Shredded carrots packaged in a modified atmosphere and held at 10°C developed off-flavors and became slimy (Carlin et al., 1989).

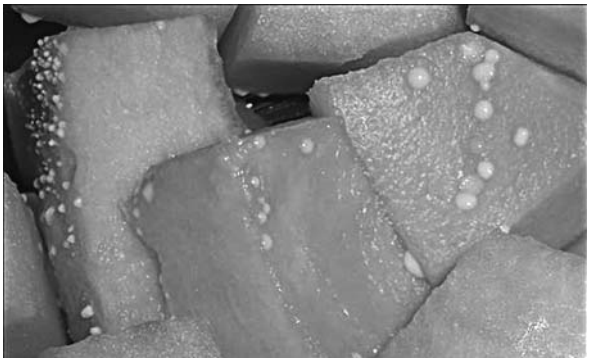

Fig. 7 Bacterial colony formation on fresh-cut cantaloupe

Another very common defect of fresh-cut vegetables attributed to microbiological spoilage is water soak/soft-rot. The decay of fresh-cut celery segments stored at <5°C in sealed film bags begins with water soaking at the cut surface and slimy moisture accumulation inside the bags (Robbs et al., 1996a). In a salad mix, endive and Lollo bionda developed soft rotting more rapidly than the other components (Artes & Martinez, 1996). Water soaking has been most commonly associated with spoilage of cut cantaloupe, honeydew, and watermelon (Ukuku & Fett, 2002; O'Connor-Shaw et al., 1994), especially under abusive storage temperature (>4°C), although there is no direct evidence that this results from the activity of pectolytic

bacteria. Studies have revealed that spoilage microbes such as *Gluconobacter and Acetobacter* can cause discoloration of whole produce, and fungal spoilage has discolored cut apples treated with antioxidants and packed in a modified atmosphere.

Microbiological Spoilage Mechanisms in Fresh-Cut Fruits and Vegetables

Growth of microorganisms subsequently forming visible colonies is a common cause of spoilage of fresh-cut fruits and vegetables. There is in general a linear relationship between the microbial cell numbers and spoilage of cut produce during refrigerated storage (Zhuang et al., 2003; Barriga, Richie, Willemot, & Simard, 1991). This relationship is stronger for fresh-cut melons and fruits compared with fresh-cut vegetables. In Europe, microbial specifications have been established for quality of fresh-cut produce. For example, in France and Germany, microbiological specifications for mesophilic aerobic bacterial populations or aerobic plate counts (APC) of salad vegetables at production (fresh) are 5×10^6 cfu/g, for separating good quality from marginally acceptable quality, and at use by date are 5×10^7 cfu/g (Francis, Thomas, & O'Beirne, 1999; Lund, 1993). Debevere (1996) proposed 10^8 cfu/g of aerobic psychrotrophic bacteria, 10^5 cfu/g of yeast, and 10^7 cfu/g for lactic acid bacteria as the limiting criteria for ready-to-eat vegetables. The Spanish legal limit (RD 3484/2000, 2001) for microbial populations on minimally fresh-processed fruit for safe consumption are 7, 5, and 3 $\log_{10}$ cfu/g for aerobic bacteria, yeasts, and molds, respectively.

Carlin, Nguyen-the, Abreu Da Silva, and Cochet (1996) and Nguyen-the and Carlin (2000) reported that the limits of microbial populations associated with spoilage of minimally processed endive packaged in sealed polypropylene film was associated with storage temperature. At storage temperature = 6°C, the bacterial counts when spoilage was first noticeable were $>10^8$ cfu/g; at 10°C, the bacterial counts were between 10^7 and 10^8 cfu/g; at 20°C, the average count was $10^{6.5}$ cfu/g. In cut cantaloupe, white colony formation was associated with high APC ($>10^8$ cfu/g) and yeast counts ($>10^5$ cfu/g) (O'Connor-Shaw et al., 1994). However, the exceptions to this overall positive linear relationship have been reported in many studies (Zhuang et al., 2003; Nguyen-the & Carlin, 1994). Sapers, Miller, Jantschke et al. (2001) did not observe a consistent difference in total APC between unspoiled and spoiled cut cantaloupe during refrigerated storage. However, in the same report, a lower APC was consistently associated with unspoiled products. These phenomena suggest the following:

1. Most, if not all, of the analyses that are currently conducted to determine microorganism populations of cut produce during storage are not specific enough to be associated with the shelf life or spoilage.
2. Low microbial counts are necessary to avoid or reduce spoilage of cut produce; however, high total microbial populations do not always correlate with spoilage.

3. It is not practical to use a microbiological specification based only on nonspecific microbial test results to reject fresh-cut products on a commercial level. APC and yeast, or lactic acid bacterial counts cannot be used solely to judge or predict shelf life or spoilage of lot of products, although there is an overall linear relationship between microbial load and quality of fresh-cut produce.

Formation of organic acids such as lactic acid and acetic acid associated with decreased pH values and generation of volatile compounds such as ethanol from fermentation of sugar by yeasts are additional mechanisms that result in aroma and flavor defects of fresh-cut products packaged under MAP. Carlin et al. (1989, 1990) determined that storage of shredded carrots under MAP at 10°C resulted in a pH decrease caused by the growth of lactic acid bacteria. Jacxsens et al. (2003) concluded that the production of detectable organic acids and reduction of pH caused by the growth of lactic acid bacteria and yeasts in mixed cut bell peppers and grated celery stored under MAP at 7°C resulted in unacceptable organoleptic properties, including off-flavor, odor, and taste. Lopez-Galvez, Peiser, and Nie (1997) reported the off-odors produced in salad mixes sensorially correlated with ethanol and acetaldehyde concentrations. Babic et al. (1992) and Fleet (1992) reported that large cell numbers of yeasts ($>10^5$ cfu/g) produce off-flavors in fresh-cut produce from the production of ethanol, organic acids, and volatile esters.

Bacterial soft rot, which is characterized by water-soaking and formation of a slimy surface on plant tissues, has been identified as the leading cause of storage disorders in many types of whole produce (Lund, 1983), and is frequently observed in fresh-cut fruits (Ukuku & Fett, 2002; O'Connor-Shaw et al., 1994) and vegetables (Artes & Martinez, 1996; Carlin et al., 1989; Robbs et al., 1996a, 1996b, Hakim, Austin, Batal, Gullo, & Khatoon, 2004) during storage. Bacterial soft rot results from degradation of plant cell walls by pectolytic enzymes produced by a variety of microorganisms, including *E. carotovora*, *P. marginalis* (Lund, 1983), *Botrytis, Clostridium, Alternaria*, *Geotrichum,* and *Fusarium* (Bulgarelli & Brackett, 1991). Many microbes use pectolytic enzymes to overcome plant defense mechanisms and access plant nutrients. The pectolytic enzymes, including pectin methyl esterase (PME), polygalacturonase (PG), pectin lyase (PNL), and pectate lyase (PL), can degrade pectins in the middle lamella of the cell, thereby resulting in liquification of the plant tissue leading to conditions such as soft rots. Other enzymes such as hemicellulase, cellulases, and proteases are also involved in the spoilage process but are usually secondary to pectinases (Liao, 2005). In the last two decades, molecular genetic research has revealed that multiple isozymic forms of the pectolytic enzymes exist. These are inducible and are not equally involved in the degradation of plant tissues. Their importance in soft-rot formation is dependent on bacterial genus. For example, there are three to five isozymes of PL in *E. chrysanthemi*, and at least one is inducible by plant constituents. The biological function and pathological effect of each PL isozyme and the specificity of its genetic expression are not yet clear. No single pectolytic enzyme in *E. chrysanthemi* has been shown to be essential to initiate a spoilage defect (Py, Barras, Harris, Robson, & Salmond, 1998). In vitro, the alkaline isozyme usually displays the highest degree of tissue-degrading

ability (Payne, Schoedel, Keen, & Collmer, 1987; Py, et al., 1998). In contrast, in *P. viridiflava*, PL is the principal or sole pathogenicity factor causing soft rot (Liao, et al., 1988; Liao, Sullivan, Gardy, & Wong, 1997). Mutants that were defective in the production or secretion of PL were unable to induce soft rot on potato tuber slices. When cloned PL gene was mobilized into the mutants, the PL-producing and soft-rotting ability of monopectolytic mutants was restored. In vitro, purified PL from *Pseudomonas* can induce soft rot of potato tuber slices. Production of *Pseudomonas* PL in vivois mediated by the product of the genes *gac*S/*gac*A, which encodes a sensory and a regulator protein in a two-component regulatory protein family (Liao, Mc Callus, Wells, Tzean, & Kang, 1996). Some *P. fluorescens* strains can be induced by calcium ions, although most are induced by pectic substrates (Liao, Mc Callus, & Wells, 1993). In vitro, purified PG was able to cause soft rot of potato tuber slices, whereas purified PME and PNL were not.

In summary, microbial spoilage of fresh-cut produce can be caused by microbial growth and/or by microbial metabolic processes. There is a strong relationship between microbial populations and shelf life if the cause of quality failure is visible growth of microflora on the surface. However, there is a relationship between microbial cell numbers and shelf life if the cause of quality failure requires specific metabolic activity, particularly under conditions of temperature abuse.

Prevention and Control of Microbiological Spoilage

Many thermal and nonthermal technologies have been developed to control microorganisms on fresh-cut produce. These have been summarized by Farber et al. (2003) and Sapers, Gourney, and Yousef (2005). Types of thermal processing used to treat fresh-cut produce include hot water, hot steam, and hot sanitizing solution. Thermal processing is a relatively new technology to the fresh-cut produce industry. Biosteam® Technologies trademarked ThermoSafe system is an example of thermal technology developed for fresh-cut processing. Biosteam's equipment is the size of a commercial dishwasher and uses hot steam to surface sanitize raw fruits and vegetables before peeling. Laboratory studies with inoculated whole melons revealed that the ThermoSafe process can effectively reduce microbial cell numbers on the surface of produce by 5 log units. The process time (from seconds to minutes) and temperature (from 60 to 100°C) are dependent upon the commodities being treated. There are a number of difficulties associated with the application of thermal processes to fresh-cut fruits and vegetables. For example, thermal processing cannot be used for fresh-cut commodities such as leafy vegetables and berries because of deterioration of quality characteristics. Also, can other processed products be called fresh-cut after a thermal process treatment is applied? Another limitation is that thermal processes are generally an inefficient use of energy and create a challenge for cold chain management which is needed by fresh-cut processors for product distribution. Further research and development is needed to validate thermal processes that can achieve a 5-log microbial reduction without affecting the quality of processed produce.

Nonthermal processing technologies can be classified as either physical or chemical. Physical technologies include high pressure, irradiation, pulsed electric fields, pulsed white light, ultrasound, and ultraviolet radiation. Some of these methods are generally not applicable commercially because they are too expensive (high pressure and pulsed electric fields), do not have consumer acceptance (irradiation), or require process validation of efficacy (UV and pulsed white light). The mechanisms and application of these methods have been well reviewed by Lund et al. (2000) and Ohlsson and Bengtsson (2002).

Chemical technologies can be divided into gas-phase sanitation and liquid-phase sanitation based on the physical state of the chemical used. Examples of gas-phase sanitation include ozone and chlorine dioxide. One of the difficulties in the application of gas-phase technologies is that a special in-line closed system is needed for the treatment of produce. These applications could also pose an employee safety issue. The most widely used chemical treatment in the fresh-cut produce industry is chlorinated water.

In addition to these active control measures, other factors important in the prevention of microbial spoilage include raw material quality, processing technologies, good manufacturing practices (GMP), packaging, and temperature management. High-quality raw materials can both reduce the potential for surface contamination and maximize the plant self-defense system. Diseased or damaged products are difficult to decontaminate using current methods of prevention and treatment and can contaminate products with low levels of microbes (Sapers, Miller, Jantschke, et al., 2001; Sapers, Miller, Pilizota, et al., 2001; Wiley, 1994). Zhuang et al. (2003) found that during storage yeast populations were significantly higher on cut honeydew melons having soft tissue than from melons that were firm. It is commonly known that in climacteric fruits, the increase in respiration just prior to full ripening generally coincides with a major reduction in fruit resistance to pathogens. Damaged cells have greater rates of respiration subsequently leading to cellular senescence or death and increased susceptibility to fungal colonization.

Processing techniques, including peeling, cutting, washing, and dewatering, also influence the vulnerability of fresh-cut fruits and vegetables to microbiological spoilage. Fresh-cut processing by peeling or abrasion or exposing inner nutritious fruit cells by cutting removes natural epidermal barriers to microbial attack. These processing steps result in accumulation of surface moisture and exposure of tissue to microbial contaminants. Barry-Ryan and O'Beirne (2000) determined with carrot slices that an abrasive peeling method resulted in significantly more lactic acid bacteria compared to hand peeling. Coarse abrasion resulted in significantly more total mesophilic aerobic bacteria during storage compared to hand peeling and fine abrasion peeling of product. Wash water with sanitizers can affect microbial contamination of fresh-cut produce (Zhuang et al., 2003), especially when the microbial load is low on the cut surface. Francis and O'Beirne (1997) found that dipping cut lettuce into 100 ppm chlorinated water significantly increased *Listeria innocua* levels compared with undipped samples. Bolin, Stafford, King, and Huxsoll (1977) determined that dewatering with centrifugation extended shelf life of cut lettuce by at least 5 days and concluded that the presence of any free moisture or cellular fluids

on the lettuce surface reduced shelf life. Although Bolin et al. (1977) did not determine microbial populations fresh-cut processors have observed for a long time that wet fresh-cut products decay considerably more rapidly compared to those that are well dewatered such as peeled carrots. Fresh fruits and vegetables have a high water activity (>0.90), hence reducing water activity is not an option for the prevention of microbial spoilage in fresh-cut products. Therefore, it is important to minimize the amount of residual surface moisture after processing.

GMPs also influence the microbial spoilage and subsequent shelf life of fresh-cut produce. It is important to train all employees in GMPs before they can work in processing operations. Fresh-cut fruits and vegetables are minimally processed and usually consumed raw. To date, intervention strategies that have been developed cannot completely eliminate microbial growth due to the fragility of raw plant tissues, particularly leafy vegetables. Therefore, preventing contamination, especially through effective implementation of GMPs, is the most effective strategy to minimize microbial contamination during processing. It is well known in the fresh-cut industry that fresh-cut samples prepared in the laboratory often have substantially longer shelf lives than samples produced by the commercial process. This is largely due to special precautions taken in the laboratory in handling the produce which reduces both the damage and the opportunity for contamination. Hence, this reinforces the importance of GMPs. Studies have revealed that during commercial processing both microbial populations and the composition of the microbial flora change dramatically on fresh-cut product (Garg et al., 1990; Zhuang et al., 2003, Fig. 2). The contaminating microorganisms can be transferred from gloves and utensils (Cantwell & Suslow, 2002). Beginning with proper training and employing good hygienic practices will have a positive influence on the microbiological shelf life of fresh-cut produce at the commercial level.

Packaging affords several means to minimize microbial spoilage. As mentioned previously, MAP is one of the most important extrinsic factors that affect microbial spoilage of fresh-cut produce. In recent years, several new modified atmosphere packaging treatments have emerged aimed at preventing microbial growth by actively introducing antimicrobial agents into the packages (Han, 2003; Farber et al., 2003). One method is the use of large concentrations of oxygen or super-atmospheric oxygen packaging (>70% O_2). The key to successful implementation of MAP of fresh-cut produce in commercial practice is establishing equilibrium-modified atmospheres (usually 2–5% O_2 and 5–15% CO_2) that can slow down the metabolism of processed produce and inhibit microbial growth by the selection of correct packaging material permeability for O_2 and CO_2 and the correct ratio of product weight and surface area of packages. However, due to the high-level respiratory activity of fresh-cut produce and the potential variation in storage temperature due to placement of packaged products on display shelves in retail stores, O_2 levels in MAP products can decrease to create anaerobic conditions (<2% of O_2 and >20% of CO_2). Anaerobic conditions are favorable for growth of anaerobic bacteria and can result in undesirable fermentation reactions and spoilage. Using high O_2 concentrations can overcome the disadvantage of anaerobic atmosphere packaging. Several studies have revealed that high O_2 concentrations have been

effective in reducing microbial growth, preventing anaerobic fermentation, and also inhibiting enzymatic discoloration (Day, 1996, 2000; Jacxsens et al., 2001; Allende et al., 2002). Kader and Ben-Yehoshua (2000) suggested that high O_2 levels (> 40%) could generate reactive oxygen species (O_2^-, H_2O_2, $OH^\bullet$) in plant cells, damage vital cell components, and thereby reduce cell viability. A concern with this technology is potential worker safety implications during packaging in the production environment. Oxygen concentrations higher than 25% are explosive (BCGA, 1998). Another concern associated with antimicrobial packaging is how these treatments will change or modify the normal microbial flora on fresh-cut produce during storage, and whether this change is advantageous remains to be determined. Jacxsens et al. (2001) observed that growth of the plant pathogen *E. carotovora* at 4°C was stimulated by increased O_2 levels, although yeast growth was reduced. Other new packaging developments include the use of microperforated films and Intelimer packaging (Landac film).

Another important technology to minimize microbial spoilage is adequate cold chain temperature management. Effective cold chain management begins with raw product cooling in the field through processing and retailing and ends at the restaurant or consumer's table. Unfortunately, there are often breaks in the cold chain, which have an additive effect on reducing the optimum shelf life of a product. Sales representatives in the fresh-cut industry have correlated poor performance of fresh-cut produce in retail stores with breaks in cold chain temperature management. Regardless of raw material quality, GMPs, processing conditions, antimicrobial treatments, types of antibacterial packaging, and abusive temperatures shorten the shelf life of fresh-cut produce.

Methods for Detection and Isolation of Spoilage Microorganisms

The methods used to detect and isolate spoilage microorganisms are mainly based on cultural procedures. For example, for fresh-cut fruit O'Connor-Shaw et al. (1994) extracted microorganisms from the fruits (1:5 dilution) using sterile 0.1% peptone water and 0.5% sodium chloride, macerated this preparation by stomaching for 1 min and using the following methods for enumerating different microorganisms: standard methods agar (SMA) with incubation at 25°C for 3 days for aerobic plate counts; dichloran rose bengal chloramphenicol agar with incubation at 25°C for 5 days for yeasts and molds; Man, Rogosa and Sharpe (MRS) agar with anaerobic incubation at 30°C for 6 days for lactic acid bacteria. Ukuku and Fett (2002) enumerated microbes on cut melons (20 g), using plate count agar (PCA) and incubation at 30°C for 3 days for mesophilic aerobic bacteria; PCA + crystal violet at 30°C for 3 days for Gram-negative bacteria; *Pseudomonas* isolation agar and incubation at 27°C for 3 days for pseudomonads; MRS agar with 0.08% sorbic acid and incubation at 30°C for 3 days for lactic acid bacteria; and Czapek malt agar (CMA) for yeasts and molds. Allende et al. (2002) enumerated microbes on mixed vegetable salads (30 g) by plating homogenates in peptone saline on:

PCA and incubating at 22°C for 3 days for total psychrotrophic bacteria; MRS agar and incubating at 30°C for 3 days for lactic acid bacteria; yeast glucose chloramphenicol agar and incubating at 30°C for 3 days for yeasts; violet red bile glucose agar and incubating at 37°C for 2 days for Enterobacteriaceae. Kakiomenow, Tassou, and Nychas (1996) enumerated pseudomonads on shredded carrots (25 g) by plating homogenates in sterile ¼ strength Ringer's solution on cetrimide–fucidin–cephaloridine agar and incubating at 25°C for 48 h. Garg et al. (1990) homogenized various fresh-cut vegetables (20 g) with sterile water and enumerated mesophilic bacteria on PCA held at 30°C for 2 days, psychrotrophic bacteria held at 3.3°C for 10 days, and molds and yeasts on potato dextrose agar adjusted to pH 3.5 with tartaric acid held at 20°C for 5 days. Gimenez et al. (2003) macerated artichokes with 0.1% peptone plus 0.5% sodium chloride and enumerated mesophilic aerobic bacteria on PCA held at 30°C for 3 days and mesophilic anaerobic bacteria using the same methods but under anaerobic conditions, and psychrotrophic bacteria on PCA held at 7°C for 10 days. Liao and Wells (1987) enumerated pectolytic bacterial populations on crystal violet pectate (CVP) agar. In the fresh-cut produce industry, 3 M Petrifilm methods are widely used to enumerate total plate counts, coliforms, lactic acid bacteria, and yeasts and molds because of convenience and minimal need for incubator and operating space. Specificities and detailed operating procedures of many of these methods are described by Downes and Ito (2001) and Sapers et al. (2005).

Microbiological Spoilage of Fermented and Acidified Vegetable Products

Introduction

Spoilage of fermented and acidified vegetable products is prevented by low pH (typically between pH 3 and 4) and the presence of organic acids, including lactic and acetic acids. Several excellent reviews of microbial metabolic end products of lactic acid bacteria (LAB) from vegetable fermentations have been published (Adams & Nicolaides, 1997; DeVuyst & Vandamme, 1994; Holzapfel, Geisen, & Shilling, 1995). Fermented vegetables are considered in the United States to be acid foods, having a pH below 4.6. Examples include cucumber pickles and sauerkraut. Acidified vegetables are foods to which acids or acidic food ingredients have been added to lower the pH below 4.6 (CFR, 1979). The acidified foods regulation was promulgated to prevent botulism in improperly acidified foods. Research had shown that *Clostridium botulinum* cannot grow and produce toxin at or below pH 4.6 (Ito et al., 1968). The market for acid and acidified vegetable products is currently dominated by acidified, not fermented, cucumber pickles (Fleming, Kyung, & Breidt, 1995). The largest sales of fermented products in the US market are hamburger dill chip pickles. Other fermented and acidified vegetable products include olives, sauerkraut, pickled peppers, and assorted pickled vegetables. Acidified cucumber

pickles are typically produced with a pH value of ca. 3.7 and have acetic acid as the primary acidulent. These pickles are typically heated (pasteurized) or refrigerated to prevent spoilage. Other acidified products, including pickled peppers, can be preserved primarily by acetic acid if the pH values are below 3.3 and acid concentrations are above 2.5%. These products may be shelf stable without pasteurization.

Organic acids have widespread application for preventing food spoilage in fermented and acidified foods (Brul & Coote, 1999; Shelef, 1994; Sofos, 1993). The bactericidal activity of weak organic acids is presumably due to acidification of the cell cytoplasm, facilitated by the diffusion of uncharged protonated acid across bacterial membranes, as well as the accumulation of acid anions in the interior of the cell. The acid anion, which does not readily cross bacterial membranes, may accumulate to molar concentrations in bacterial cells, depending on the difference between intracellular and extracellular pH (Diez-Gonzalez & Russell, 1997a, 1997b). The type, concentration, and pH of organic acid solutions help to determine the effectiveness of acids in preventing spoilage. Types of organic acids added to foods include salts of benzoic acid and sorbic acid, as well as acetic, lactic, and propionic acids. A given acid concentration and pH may independently affect the growth and death of bacterial cells, or these factors may interact (Passos, Ollis, Fleming, Hassan, & Felder, 1993). Reported pH values for the inhibition of growth of *E. coli* O157:H7 varied by 1.0 pH unit, depending on whether acetic acid (minimum inhibitory concentration [MIC] = pH 5.5) or HCl (MIC = pH 4.5) was used to lower pH (McKellar & Knight, 1999). In addition, the effectiveness of weak organic acids in inhibiting the growth of bacteria may be modulated by factors other than pH. Temperature is a primary factor influencing antimicrobial activity, with increasing temperature resulting in increasing effectiveness (Breidt, Hayes, & McFeeters, 2004; Brudzinski & Harrison, 1998; Hsin-Yi & Chou, 2001; Presser, Ross, & Ratkowshy, 1998; Uljas & Ingham, 1998).

An important quality factor for cucumber pickles is a solid and crisp texture for the cucumbers. Gas pockets that can form within cucumbers during fermentation result in bloating of cucumbers, a form of spoilage making them undesirable for commercial sale. Yeasts and heterofermentative lactic acid bacteria are largely the source of this gas (Bell & Etchells, 1956; Etchells, Borg, & Bell, 1968). In addition, homofermentative lactics have been implicated in this problem (McFeeters, Fleming, & Daeschel, 1984). *Lactobacillus plantarum* and other lactic acid bacteria decarboxylate malic acid (present in cucumbers), thereby producing lactic acid and carbon dioxide via a malolactic enzyme. This one-step reaction results in the net loss of one proton from solution, so it raises the pH, possibly conferring a selective advantage for microorganisms in acidic environments. To prevent bloater damage to cucumbers from internal gas pockets of carbon dioxide, a gas purging technology was developed in the 1970s (Costilow, Bedford, Mingus, & Black, 1977; Fleming, Etchells, Thompson, & Bell, 1975; Fleming, Thompson, Etchells, Kelling, & Bell, 1973). Prior to this development, the formation of hollow centers or bloated cucumbers was a significant economic problem for the cucumber fermentation industry.

Microbiological and Nonmicrobial Spoilage

Spoilage of fermented or acidified vegetables due to softening can result from enzymatic or nonenzymatic activities. The softening of cucumber pickles has been extensively studied. Cucumbers naturally contain polygalacturonases which are involved in the natural maturation process of the fruit (Saltveit & McFeeters, 1980). Another source of polygalacturonase enzyme is fungi associated with the cucumber fruit flowers (particularly on small fruit), which can cause softening problems (Bell, Etchells, & Costilow, 1958; Etchells, Bell, Monroe, Masley, & Demain, 1958). However, this problem can be minimized by simply removing the flowers prior to brining. Storing fermented cucumbers in high concentrations of NaCl (greater than 10%) can reduce polygalacturonidase activity to prevent softening (Etchells, Borg, & Bell, 1961; McFeeters & Fleming, 1989), but this can lead to problems with salt waste disposal. Nonenzymatic softening can also occur with cucumber pickles (McFeeters & Fleming, 1989, 1990). The use of low concentrations (less than 0.5%) of calcium chloride can help retain firmness of brined vegetables (Doesburg, 1965; Fleming, McFeeters, & Thompson, 1987; Van Buren, 1986). Calcium is widely used in acidified and fermented vegetable products to prevent softening, although the mechanism of how calcium works to retain firmness remains unclear (McFeeters & Fleming, 1990).

The growth of film yeasts on the surface of cucumber brine fermentation tanks can also be a source of enzymatic softening. Commercial cucumber fermentation tanks (fiberglass tanks, ca. 40,000 L or 10,000 gallons) are usually situated in outdoor "brine yards," so that the brine surface is exposed to the UV rays of the sun, hence preventing yeast growth. The cucumbers are held below the brine surface by wooden headboards. While some yeasts may produce polygalacturonidase (Bell & Etchells, 1956), softening may also result from yeasts consuming lactic acid, resulting in an increase of pH. This can result in the growth of other microorganisms, including propionibacteria and clostridial species (Fleming, Daeschel, McFeeters, & Pierson, 1989) which produce degradative enzymes. In this secondary fermentation, lactic acid is converted anaerobically to propionic and butyric acids with a concomitant increase in pH, although the details of the microbial ecology and biochemical changes occurring remain to be elucidated. This type of secondary fermentation can also occur if the salt concentration in the fermentation brine is too low. Similar spoilage fermentations are responsible for malodorous butyric acid production in olive fermentation. Known as "zapatera" spoilage, this can occur when the pH of the fermentation remains about ca. 4.5, possibly due to residual sodium hydroxide from the initial lye treatment used for olive fermentation (Delmouzos, Stadtman, & Vaughn, 1953; Fernandez, Garcia, & Balbuena, 1995; Plastourgos & Vaughn, 1957).

Fermented cabbage, including sauerkraut and Korean kimchi, is also subject to spoilage due to softening. Yeasts, propionibacteria, and clostridial species may be involved. Sauerkraut is typically fermented in the United States in indoor cement fermentation tanks holding up to 90 tons of cabbage. The cabbage is spread to form a concave surface which is covered by a plastic film that is flooded with water to

maintain anaerobic conditions under the film. If the temperature (ca. 18°C) and salt concentration (equilibrated around ca. 2%) are correct, and anaerobic conditions are maintained, high-quality sauerkraut is produced by a natural lactic acid bacteria fermentation (Pederson & Albury, 1969). Softening problems can occur with commercial sauerkraut when the dry salting process used for preparing the fermentation tanks is at too low a salt concentration or the sale is unevenly distributed. Exposure of the fermenting cabbage to air can allow the growth of a variety of spoilage microorganisms, including molds. Formation of pink color in the sauerkraut is also a frequent spoilage problem. The pink color has been studied since the 1920s and is attributed to yeasts, including *Rhodotorula* species (Fred & Peterson, 1922; Pederson & Kelly, 1938), and possibly lactic acid bacteria (Stamer, Hrazdina, & Stoyla, 1973).

A spoilage problem for both sauerkraut and kimchi is an excess accumulation of lactic acid. Cabbage fermentations, including both sauerkraut and kimchi, typically occur in two stages: an initial heterolactic fermentation, dominated by *L. mesenteroides* and related species, as well as *Weissella* species (Plengvidhya, 2003), followed by a homolactic phase dominated by *Lactobacillus* species, typically *L. plantarum* (Fleming et al., 1995; Pederson & Albury, 1969). A homolactic phase is needed to produce good-quality sauerkraut; however, kimchi is commonly preferred as a lightly fermented, somewhat carbonated, product resulting from the heterolactic phase of fermentation. Accumulation of excess lactic acid is considered a spoilage problem with both sauerkraut and kimchi fermentations, although some people prefer a more acidic product. Unlike cucumbers which typically contain ca. 2% sugar, commercial cabbage used for sauerkraut may have 5% or more sugar (Fleming et al., 1995), primarily glucose and fructose, and the homolactic stage of fermentation may continue for an extended period, resulting in acid concentrations of 3% or greater.

Prevention and Control of Spoilage

The spoilage microflora for acidified and fermented vegetable products may consist of yeasts, molds, and lactic acid bacteria. Yeasts and molds that can tolerate low water activity are the primary spoilage agents of fermented products, including sweet pickles (Bell & Etchells, 1952; Etchells, 1950). A preservation prediction chart having the combined concentrations of acetic acid (1–4%) and sugar (10–50%) needed to preserve cucumber pickles is used to prevent spoilage of sweet pickles (Bell & Etchells, 1952). In addition, sorbic acid can be added to inhibit yeasts (Etchells et al., 1961). Most acidified vegetable products are pasteurized to prevent growth of acid-tolerant microorganisms. Acidified cucumber products by definition are not fermented, and acid-tolerant lactic acid bacteria may grow by fermenting the sugars naturally present. Recommended pasteurization treatments to prevent spoilage and ensure safety have been determined (Breidt, Hayes, Osborne, & McFeeters, 2005; Etchells & Jones, 1942). The temperatures and times typically used in commercial pasteurization processes today (5–15 min at 70–80°C) kill the

lactic acid bacteria and yeasts. However, if the necessary time–temperature conditions are not obtained, growth of lactic acid bacteria may occur resulting in turbidity, gas production, pressure formation, and leakage of brine from jars. One particularly hazardous type of spoilage that can occur is due to improper sealing of jars or containers, thereby allowing entry of oxygen. This can result in the growth of oxidative molds that can aerobically metabolize lactic acid or acetic acid. As a result, the pH can increase above 4.6, and if aerobic conditions are maintained in the bottom of the container, clostridial spores may germinate, potentially leading to the production of botulinum toxin.

Future Needs

There are several needs for further investigation to improve our overall understanding of microbiological spoilage of produce. Some examples are given here:

1. Identification of the specific spoilage microorganisms for different types of pre- and fresh-cut products stored under unique packaging conditions. Currently, mesophilic aerobic bacteria are widely used as indicators of both general quality and microbiological quality of fresh-cut produce products. This is insufficient for knowing if pathogen contamination occurs or for predicting sensory quality changes. Additionally, storage temperature and packaging methods have a significant impact on microbiological spoilage patterns. There have been many studies on microbial spoilage patterns on specific types of fresh-cut produce packaged in specific types of packaging films and stored at a specific refrigerated temperature, such as fresh-cut carrots (Nguyen-the and Carlin, 1994) held at 10°C and fresh-cut celery (Robbs et al., 1996a, 1996b) held at 5°C. However, there are few research reports on spoilage patterns of fresh-cut produce products stored under the MAP conditions and at different temperatures such as 2 vs. 10°C. More information on the relationship of microbial spoilage to product preservation will be valuable, especially considering the direction being taken to improve cold chain management in retail and foodservice environments and distribution that can lead to extending the shelf life of products.
2. There is a pressing need to develop simple and rapid assays to measure specific spoilage microorganisms and to better establish relationships between spoilage microbial populations and spoilage of whole and fresh-cut produce products, especially fresh-cut fruits. For example, current methods to enumerate psychrotrophic bacteria require 3–10 days to complete which encompasses the entire shelf life of most whole and fresh-cut products. The shelf life of most whole and fresh-cut products is approximately 8–14 days. Hence, it is difficult to use existing methods as a basis for making corrective actions in the processing facility not only at the time of production but also to remove contaminated material at retail. In addition, quality limit specifications need to be better defined for fresh processed and end-of-shelf life products relative to spoilage microorganisms.

3. There is a need for investigation of spoilage patterns and microflora of fresh-cut products packaged with new, emerging MAP technologies, including antimicrobial packaging, microperforated packaging, Intelimer® packaging, and high O_2 backflush, in commercial practice. With the continuing development of packaging technologies and changes in marketing fresh and fresh-cut produce, the spoilage microflora of packaged produce in the future may be completely different from today. For example, the headspace profile (ratio of CO_2/O_2 at equilibrium) of fresh-cut produce in microperforated packages is significantly different from that packaged with conventional films. There is very limited information regarding how changes in atmospheric composition affect spoilage microflora profile during refrigerated storage.
4. There is a need for a better understanding of the plant defense systems of fresh-cut produce and their role in controlling the microflora and spoilage patterns under refrigerated conditions. Plant defense responses appear to influence the spoilage pattern of fresh-cut produce. For example, the mesophilic aerobic bacterial population of cut pineapple treated with methyl jasmonate (both by vapor and dipping) decreased by 3 log CFU/g after 12 days of storage (Martinez-Ferrer and Harper, 2005). A purified ethanolic extract of peeled and shredded carrots had an antimicrobial effect against a wide range of microorganisms, including *L. mesenteroides, P. fluorescens, Candida lambica,* and *E. coli*. C_6-aldehyde, a common volatile product produced by plant tissue through enzymatic lipid peroxidation, inhibited spoilage microbes on fresh-cut produce and has been hypothesized as a factor in the plant defense mechanism (Zhuang, Barth, & Hildebrand, 2002). There are few studies addressing the understanding of plant defense systems after wounding fruit or vegetable tissue under refrigerated conditions and their impact on microbial spoilage of different commodities and during a variety of seasons.

Temperature is one of the most impactful factors affecting the quality and microbiological characteristics of produce, hence there is need to further investigate the effect of breaks in the cold chain on microbial flora and shelf life of both intact and fresh-cut produce, especially fresh-cut fruits.

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