

## **Multistate Research Project S-294**

**TITLE:** Postharvest Quality and Safety in Fresh-cut Vegetables and Fruits

**DURATION:** October 1, 2005-September 30, 2011

### **STATEMENT OF THE ISSUES AND JUSTIFICATION:**

Consumption of fresh-cut produce has increased at an annual rate of approximately 10% since 1995 and the market for fresh-cut fruits and vegetables is estimated at \$10-12 billion annually (IFPA, 2004). The International Fresh-cut Produce Association (IFPA) estimates that fresh-cut products currently make up more than 15% of all fresh produce marketed in the U.S. Postharvest losses of fresh-cut produce are difficult to estimate but given the highly perishable nature of fresh-cuts compared to intact produce, the retail value of fresh-cut produce losses may exceed \$1 billion annually.

The appearance, convenience, and generally high nutritive value of fresh-cut vegetables and fruits are bringing about increased sales of fresh produce, but repeat sales of the fresh-cuts is dependent upon assurance of its safety and the products having pleasing texture and flavor. To date, the industry has relied on established technologies derived mainly from practical experience to maintain visual quality and shelf-life with less consideration of the quality characteristics that drive repeat sales such as good flavor retention, maintenance of an appealing texture (crispness, crunchiness), package labeling underscoring the high nutritive value of the product, and increased microbial quality leading to extended shelf stability and food safety. Through interaction with the IFPA we know that current technologies, especially for fresh-cut fruits, do not provide the shelf stability needed to supply long distance domestic markets.

Unfortunately, as produce consumption has increased in the U.S. in recent years, so has the number of produce-related outbreaks of foodborne illness (Beuchat, 2002; National Advisory Committee on Microbiological Criteria for Foods, 1999; Nguyen-the and Carlin, 2000). Produce-related outbreaks accounted for 6% of all reported foodborne outbreaks in the 1990s compared to only 0.7% in the 1970s (FDA, 2004, Sivapalasingam et al., 2004). The CDC reported that foodborne outbreaks associated with fresh produce doubled between the period 1973-87 and 1988-92 (Buck et al., 2003). The conditions on the cut surface of fresh-cut products, with the presence of water and compounds that microbes can use for nutrition, provide ideal conditions for growth, however, it is difficult to compare the results of studies on survival and growth of pathogens done in different laboratories because substantial variations exist in methods for inoculation, treatment, or storage, and in procedures used to detect, recover, or enumerate pathogens on raw produce. The continuing nature of such produce-related outbreaks represents a threat to further increases in per capita consumption due to lowered confidence in the microbial safety of the product by the consuming public. Such outbreaks can also be very costly to growers, processors, shippers and restaurants.

Integration of physiological, pathological, food safety, and instrumental and sensory quality measurement concepts is essential for developing the most effective handling

procedures and innovative, new technologies for maintaining quality and shelf stability of fresh-cut products. Much experimental work will be needed to optimize and integrate new and emerging treatments in diverse fresh-cut products. This fact supports the proposed integrated approach of having parallel projects in different states and of focusing the research into specific areas of importance. Alternative and emerging technologies for maintaining the quality and shelf stability of fresh-cut produce are being introduced at a rate that often precludes thorough evaluation of instrumental and sensory quality attributes, and their impact on product nutritional value, microbial quality and food safety. To do so, a multidisciplinary approach as proposed herein also will be needed to optimize the new and emerging treatments.

### **RELATED CURRENT AND PREVIOUS WORK:**

As a result of physiological and microbial deterioration occurring during storage and marketing of fresh produce, and especially fresh-cut produce, there is an urgent need to develop effective, non-damaging treatments for maintaining the quality (appearance, flavor, texture, nutritional value) and food safety of fresh harvested produce (How, 1990). Most of the sales of fresh-cut produce have been in the vegetable (salad, carrot slice) area (Garrett, 2002) and current handling practices for fresh-cut vegetables have been described (Barth et al., 2002). More recent research and commercial interest is focusing on fresh-cut fruits and melons (Bai et al., 2003; 2004; Beaulieu et al., 2004; Beaulieu and Gorny, 2002; Bett-Garber et al., 2003; Saftner et al., 2003a,b; Soliva-Fortuny and Martin-Belloso, 2003). With over 200 different vegetable and fruit crops with potential for development as fresh-cut products, each with unique physiology and handling requirements, an integrated, scientific approach to research and development including microbiological interactions with these products is critically needed.

A search of the CRIS database revealed no other multistate projects or coordinating committees dealing with fresh-cut vegetables and fruits, and also none with plant physiologists, food scientists and microbiologists working together. The proposed multistate project is needed in order to provide coordination and collaboration among the scientists working in this field if duplication of effort is to be avoided and the available time and resources are to be effectively applied. In this way, more effective approaches that are more widely applicable to different fresh-cut products may be more quickly developed. This research broadly supports the goals of the Food Safety and Quality National Initiative. It also is in line with the SAAESD Programmatic Plan (2000) in the areas of food safety, plant food processing systems, and functional foods.

### **Critical Review of Previous Project Accomplishments**

This proposal is for a replacement project to S-294, Postharvest Quality and Safety in Fresh-cut Vegetables and Fruits. S-294 resulted in numerous collaborative activities including NRI, IFAFS and NIFSI grants with multistate collaboration. The project members developed information on preharvest and postharvest treatment and storage effects on the nutritional value of fresh-cut products; developed or evaluated new tools, treatments and cultivars to improve the quality and safety of fresh-cut vegetables and fruits; developed new information on the contamination and attachment of microbes to fresh-cut product as well as developing novel approaches to microbial control; and

elucidated the physiological processes underlying both positive and negative quality changes associated with fresh-cut processing.

With this new project, we plan to move more strongly in the direction of standardization of methods for sensory quality analysis and microbiological procedures among the members. We have identified the newly emerging treatments and techniques for assuring fresh-cut quality that need to be tested and evaluated and applied to new fresh-cut products as replacements or supplements to existing procedures; similarly, we have identified physiological processes that may control many of the observed quality changes during storage of fresh-cut products. We see a need to develop standard protocols for evaluation of the efficacy of sanitizers and the appropriateness of experimental protocols for microbiological challenge studies with fresh-cut produce. Surrogate organisms for human pathogens are needed to allow larger scale evaluation of intervention treatments. New sanitizers and natural product antimicrobials and also physical treatments to control microbes have been identified for testing. Because of the potential for treatment interactions between vegetable and fruit tissues and microbes, we plan on close coordination between microbiologists and plant/food scientists in all of the above activities.

### **Fresh-cut Product Quality**

Any pre-harvest condition that stresses the plant also will affect the quality and shelf-life of the associated postharvest crop (Monselise and Goren, 1987; Nigh, 1990). Knowledge of these conditions is important for assessing the postharvest potential of fresh produce (Blacharski et al., 2001; Borve and Sekse, 2000; Gorny et al., 1998; Kim et al., 1993), especially those that will be further stressed by fresh cutting. The maturity of fruits and vegetables intended for fresh-cut processing is a critical factor determining the potential quality and shelf life of the product (Beaulieu, 2005; Beaulieu et al., 2004; Soliva-Fortuny et al., 2002, 2004). The suitability of genotypes for fresh-cut processing has rarely been considered. Integration of cultivar selection, pre-harvest and postharvest conditions and treatments is needed to obtain the best possible quality of the marketed fresh-cut product.

Quality of fresh-cut products is highly dependent on minimizing injury to the product. It has been clearly shown that the degree of injury incurred during the cutting process has a tremendous influence on slice quality and shelf-life (Portela and Cantwell, 2001; Toivonen et al., 2005). Since no literature exists to guide equipment manufacturers on improving the cutting process, there is a strong need to conduct research to provide the necessary guidance. This requires studies to identify the extent of injury (i.e. bruising, tearing) and to measure effects in a direct manner (i.e. tissue leakage).

Understanding the components of eating quality involves comparison of instrumental with sensory analysis. A large portion of the flavor of fruits is ascribed to their contents of sugars and organic acids, complemented by volatile aromatic compounds (Baldwin et al., 1998). Non-chemical means for estimating the contents of sugars and acids in fresh-cut fruits such as visible/near infrared (Vis/NIR) spectrophotometry would be beneficial for both research and quality control. Phenolic compounds may contribute to astringency

or bitterness (Mazza and Miniati, 1993). Gas chromatography combined with olfactometry is widely used in aroma research to identify volatile compounds responsible for product flavor (Beaulieu and Baldwin, 2002; Jordán et al., 2001a; 2001b; Plotto et al., 2000; Schieberle and Hofmann, 1997). In addition, the electronic nose (e-nose) discriminates samples based on pattern response of an array of sensors that respond to volatile compounds (Goodner et al., 2000; Shaw et al., 2000; Springett, 1991). Much more detailed sensory work needs to be done on fresh-cut produce, especially when treated with natural products, heat, 1-MCP etc. that might affect flavor and textural attributes of the fresh-cut products and impact overall eating quality.

Many attempts at measuring texture have used sensory analysis coupled with instrumental measurements (Abbott et al., 1984; DeBelie et al., 2002; Drake, 1962; Harker et al., 2002; Mohamed et al., 1982; Szczesniak, 1963; Vickers, 1981; Vickers and Bourne, 1976; Vincent, 1998). To date there are not generally accepted definitions of textural attributes applicable to fresh-cut products, sensory scale anchors, or methods for their measurement (Fillion and Kilcast, 2002; Harker et al., 1997). The proposed project will include study of practical methods for measuring flavor and texture of fresh-cut fruits and vegetables, and relating to sensory measurements using modern multivariate statistical techniques.

#### **Technologies for Maintaining Quality and Shelf Stability.**

Modified atmospheres (MA) help maintain the freshness and quality of fresh-cut fruits and vegetables by inhibiting metabolic activity, ethylene sensitivity and production, and/or physiological and pathological deterioration during storage (Gorris and Tauscher, 1999; Saltveit, 1997). The optimal atmosphere for intact and cut produce may vary greatly because of the removal of barriers to gas permeation (Beaudry, 2000; Watkins, 2000). Only limited information is available on optimal MA storage of fresh-cut produce (Barth et al., 2002; Beaulieu and Gorny, 2002). Semi permeable plastic films are used for modifying the atmosphere inside packages (Church and Parsons, 1995). Improper modified atmosphere packaging (MAP) of fresh-cuts can lead to anaerobic respiration, off-flavor, discoloration, CO<sub>2</sub> injury, and decay leading to reduced shelf-life (Gonzalez et al., 2004; Mateos et al., 1993). Due to the perishability of fresh-cut produce, the package atmosphere is often actively established.

Natural products and generally regarded as safe (GRAS) substances that often have antimicrobial/antioxidative activities or otherwise maintain the freshness and quality of the fresh-cut produce are of interest. Both chlorination and natural product/GRAS treatments have seldom been applied to fresh-cut fruit that have little or no browning (Beaulieu and Baldwin, 2002), e.g., fresh-cut melon products. This is unfortunate since intact and fresh-cut melons (particularly cantaloupes) are susceptible to microbial contamination (Bai et al., 2003; Beuchat, 1996; Center for Disease Control and Prevention, 2002). Chlorinated processing solutions supplemented with relatively low concentrations of calcium supplied either as a salt or chelate can more than double shelf stability and to delay quality deterioration without affecting fresh-cut melon sensory quality (Luna-Guzmán and Barrett, 2000; Saftner et al., 2003b).

For fresh-cut produce susceptible to browning, several natural products/GRAS substances have been evaluated for their ability to control discoloration, softening and decay (Garcia and Barrett, 2002). Tissue breakdown is a major limiting quality factor for fresh-cut fruits, and the use of commercially approved enzyme preparations (primarily pectinesterases) along with calcium application, as well as pH adjustment to neutralize fruit acids, may aid in maintaining or improving their firmness and overall stability. Other preservative treatments, several of which have antimicrobial properties, have been employed alone or as combined treatments to maintain the fresh-like characteristics of fresh-cut produce during storage (Buta et al., 1999; Garcia and Barrett, 2002; Brecht, 1995; Sapers et al., 1989; Sapers and Miller, 1992). The penetration of ascorbic acid-based antibrowning formulations can be improved by vacuum or pressure infiltration instead of dipping or spraying (Sapers et al., 1990). However, excessive sorption of antibrowning solution can result in tissue translucency and reduced shelf stability.

Sanitizers used in the fresh-cut industry include chlorine and chlorine derivatives, acid-anionic sanitizers, hydrogen peroxide, peroxyacetic acid and acidified sodium chlorite (21 CFR 178.1010). Hydrogen peroxide treatments of intact cantaloupes reduced microbial load on the fresh-cut product prepared from the sanitized fruit (Ukuku et al., 2004; Ukuku and Fett, 2004), and both quality maintenance and shelf-life extension were improved when microbial loads are reduced on fresh-cut melon products (O'Conner-Shaw et al., 1996). Another relatively new acidified sodium chlorite-based sanitizer has been shown to effectively maintain quality and food safety of fresh-cut carrots (Gonzalez et al., 2004). Many of these new sanitizers inhibit microbial proliferation on fresh-cut surfaces by oxidatively stressing plant (and human) pathogens, though they can cause bleaching or discoloration of some fresh-cuts such as melon chunks and apple slices. However, treatment with oxidative sanitizers followed by treatment with an antioxidant appears to effectively maintain microbial quality of fresh-cut apple slices (Robert Saftner, unpublished data). Additionally, for the sanitizers and food-grade antimicrobial food additives and preservatives to be effective, they must be able to penetrate to the sites within the fresh-cut produce where the plant and human pathogens are located. Several classes of surface active agents approved for food use can aid penetration of substances within apples (Saftner et al., 1997) and other produce, but their compatibility with fresh-cuts and their efficacy when combined with sanitizers in fresh-cuts have not been tested.

1-Methylcyclopropene (1-MCP) suppresses ethylene-mediated ripening and senescence of most climacteric fruit on which it has been tested (Blankenship and Dole, 2003). In fresh-cut apple products, 1-MCP treatment maintained quality by inhibiting textural and flavor-associated acidity changes (Bai et al., 2004; Jiang and Joyce, 2002). However, a potential disadvantage of 1-MCP is that it might adversely affect ethylene-induced disease resistance (Bent et al., 1992). Although reports to date have been contradictory (Bai et al., 2004; Janisiewicz et al., 2003; Jiang and Joyce, 2002; Leverentz et al., 2003; Saftner et al., 2003a). While 1-MCP reduces volatile formation in ripening whole fruit (Blankenship and Dole, 2003), the same has not been consistently observed for fresh-cut fruit (Bai et al., 2004), possibly due to the advanced ripeness stages used for fresh-cut. Despite the potential benefits of 1-MCP use to the fresh-cut industry, the

logistics of 1-MCP application(s), dosage considerations, and interactions with MAP and alternative treatments have yet to be adequately explored.

The need to kill plant pathogens and maintain microbial quality of fresh-cut produce is counterbalanced by the demand for “clean” labels, meaning minimal, if any, use of chemical additives and preservatives (Mermelstein, 2001). Thermal processing is the major technique for non-chemical food preservation. Pre-storage heat treatment appears to be one of the most promising new means for postharvest quality maintenance and control of decay (Lurie, 1998). Preprocessing heat treatment of intact fruit has not been adequately tested for its effect on the quality and shelf stability of resulting fresh-cut products (Abreu et al., 2003; Bai et al., 2004; Kang and Saltveit, 2003; Lamikanra et al., 2005; Loaiza-Velarde, et al., 2003).

Relatively new food processing technologies have been developed as alternatives to thermal treatments, such as ultraviolet irradiation, high hydrostatic pressure, gamma irradiation, and pulsed microwave irradiation. Some of these may have applicability to maintaining the quality of fresh-cut produce and have been studied as potential alternatives or adjuncts to MAP (Chervin and Boisseau, 1994; Hagenmaier and Baker, 1997; Hoover, 1997; Shin and Pyun, 1997).

Intact and fresh-cut fruits and vegetables are important dietary sources of vitamin A, C and E, minerals, carotenoids, polyphenols (flavonoids), and other antioxidant phytochemicals. Mounting evidence suggests that consumption of fresh produce has long-term health benefits, and may prevent or reduce the risk of several chronic diseases (Anon., 2003; Cooper, 2004). Despite the many claimed health benefits of consuming fresh produce, little is known of the effects of fresh-cut processing technologies, handling, storage and treatments such as MAP, natural products, sanitizers, heat, etc. on the nutritional quality of fresh-cut products. Certainly, the increased exposure of fresh produce to air and light by fresh cutting would be expected to adversely impact their nutritional value (McCarthy and Matthews, 1994; Park and Lee, 1995; Wright and Kader, 1997a,b). Levels of phytonutrients as affected by fresh-cut processing and associated treatments and during handling and storage are known for only a few fresh-cut products. Cutting increased the phenolic content and antioxidant capacity of fresh-cut lettuce and other vegetables (Cisneros-Zevallos and Heredia, 2004). This suggests that stressful treatments such as fresh cutting can increase nutrient levels in some commodities under certain circumstances. More information is needed related to phytonutrient levels in fresh-cut produce and how emerging fresh cutting technologies are impacting phytonutrients.

### **Physiology of Fresh-cut Products**

The physiology of fresh-cut vegetables and fruits is typical of that observed in plant tissues that have been wounded or exposed to stress conditions (Brecht et al., 2004). This includes increased respiration and ethylene production, and, in some cases, induction of wound-healing processes. Other consequences of wounding are chemical or physical in nature, such as oxidative browning reactions and lipid oxidation, or enhanced water loss. Appearance of new RNA and protein species in wounded tissues provides evidence for

genomic control of the response. Minimizing the negative consequences of wounding in fresh-cut vegetables and fruits can result in increased shelf-life and greater maintenance of nutritional, appearance, and taste quality in these products.

*Consequences of wounding.* Wounding of plant tissues induces elevated ethylene production rates (Abeles et al., 1992), which may accelerate deterioration and senescence in vegetative and nonclimacteric tissues and promote ripening of climacteric fruits. Ethylene produced by the physical action of cutting was sufficient to accelerate softening of banana and kiwifruit, and chlorophyll loss in spinach but not broccoli (Abe and Watada, 1991). The level of ethylene has been shown in several vegetables and fruits to increase in proportion to the amount of wounding. The increase in respiration seen in wounded plant tissues is thought to be a consequence of elevated ethylene, which stimulates respiration.

Wounding of plant tissues in the course of preparation of fresh-cut products may cause membrane lipid degradation (Deschene et al., 1991; Picchioni et al., 1994; Rolle and Chism, 1987; Zhuang et al., 1997). Extensive enzymatic degradation occurs in damaged membrane systems, causing loss of lipid components and loss of compartmentation of enzymes and substrates (Marangoni et al., 1996). The ethylene produced upon wounding may play a role in this process by increasing the permeability of membranes and reducing phospholipid biosynthesis (Watada et al., 1990). The enzymatic reactions catalyzed by lipid acyl hydrolases and phospholipase D produce free fatty acids from the membrane lipids (Picchioni et al., 1994). These free fatty acids are toxic to many cellular processes and are capable of causing organelle lysis, and binding to and inactivating proteins. Lipoxygenase catalyzes the peroxidation of di- and tri-enoic fatty acids to form conjugated hydroperoxides, resulting in the generation of free radicals, which can attack intact membranes, causing further membrane disruption. Lipoxygenase activity is also involved in the production of desirable and undesirable aroma volatiles (Mazliak, 1983).

Discoloration due to browning and yellowing due to loss of chlorophyll occur in fresh-cut vegetables and fruits as a result of the disruption of compartmentation that occurs when cells are broken, allowing substrates and oxidases to come in contact (Heaton and Marangoni, 1996; Martinez and Whitaker, 1995). Wounding also induces synthesis of a number of enzymes involved in the browning reactions or substrate biosynthesis (Rolle and Chism, 1987). Phenylalanine ammonia-lyase (PAL) catalyzes the rate-limiting step in phenylpropanoid metabolism (Ke and Saltveit, 1989). Both ethylene and wounding induce PAL activity in many plant tissues (Abeles et al., 1992; Lopez-Galvez et al., 1996b), but apparently by separate mechanisms. Browning occurs when the products of phenylpropanoid metabolism, such as various phenolic and possibly other substrates (e.g. anthocyanins) are oxidized in reactions catalyzed by phenolases such as polyphenoloxidase (PPO) or peroxidases (Martinez and Whitaker, 1995). Thus, both the relative activities of the oxidases and the concentrations of the substrates (Hansche and Boynton, 1987) can affect the intensity of browning in different tissues and crops. Oxidative browning at the cut surface may be the limiting factor in storage of many fresh-cut vegetables and fruits.

*The wound signal.* A signal that transduces the physical wound in lettuce into a physiological response has been shown to be produced at the site of injury and to migrate or propagate into adjacent, non-injured tissue where it induces increased phenolic production (Choi et al., 2005; Ke and Saltveit 1989). A kinetic analysis of wound induction of ethylene and of PAL in lettuce showed that increased PAL activity did not proceed through the induced synthesis and action of ethylene (Ke and Saltveit, 1989). While high concentrations of a number of putative wound signal chemicals (i.e., abscisic acid, jasmonic acid, methyl jasmonate, salicylic acid) slightly increased PAL activity, none of them produced the many-fold increase seen in wounded leaf tissue (Campos-Vargas and Saltveit, 2002).

Products of the oxylipin biosynthetic pathway [e.g., jasmonic acid (JA)] are part of the wound signal complex in a number of plants (Creelman and Mullet, 1997; Peña-Cortés and Willmitzer, 1995; Turner et al., 2002). Mechanical injuries promote increased levels of JA (Creelman et al., 1992; Peña-Cortés et al., 1993; Peña-Cortés et al., 1995). The release of phosphatidic acid (PA) from membrane phospholipids by phospholipase D (PLD) is thought to be one of the first reactions in this pathway (Creelman and Mullet, 1997; Leon et al., 2001). Subsequent steps produce a myriad of phytoactive compounds that participate in tissue responses to biotic and abiotic stresses (Farmer and Ryan, 1992; Farmer et al., 1998; Munnik, 2001; Schaller, 2001).

Salicylic acid increases in plants in response to a number of stresses and its application can elicit similar stress responses (Klessig and Malamy, 1994). However, SA can also inhibit certain stress responses by blocking JA biosynthesis (Doares et al., 1995; Peña-Cortés et al., 1993).

*Water loss.* Cutting or peeling fruits and vegetables exposes the interior tissues and drastically increases the rate of evaporation of water (Burton, 1982). Thus, avoiding desiccation at the cut surface of fresh-cut products is critical for maintaining acceptable visual appearance. Desiccation can also induce stress ethylene production in detached vegetables and fruits (Yang, 1985). Water loss during storage has been shown to enhance the loss of both ascorbic acid and total carotene (Barth and Zhuang, 1996; Barth et al., 1990; Nunes et al., 1998) either directly or by induction of stress ethylene.

*Wound-generated protective antioxidant and reactive oxygen species.* Fruits and vegetables are good sources of bioactive components such as carotenoids, polyphenolics, and other organic components with potential anticarcinogenic properties (Homnava et al., 1990; Simonne et al., 1997; Steinmetz and Potter, 1996; Waladkhani and Clemens, 2001). Phytochemicals present in plants can act as reducing agents, free radical terminators, metal chelators, and singlet oxygen quenchers and mediate the activity of various oxidizing enzymes (Ho, 1992; Okuda, 1993).

When vegetables and fruits are peeled, sliced, diced, shredded, etc. to create fresh-cut products, it has been assumed that the tissue wounding that occurs causes accelerated loss

of nutritional value (Klein 1987; Matthews and McCarthy 1994), just as other physiological and biochemical reactions leading to undesirable quality changes such as discoloration and negative textural changes are accelerated. This assumption has never been rigorously tested and, in fact, plants respond to numerous abiotic and biotic stresses by up-regulation of antioxidant compounds (Baker and Orlandi, 1995; Bray et al., 2000; Wang et al., 2003). It has been proposed (Cisneros-Zevallos, 2003) and preliminary work by project members in Texas and Florida (Cisneros-Zevallos and Heredia, 2004; Simonne et al., unpublished) supports the idea that wounding of fruits and vegetables can enhance tissue antioxidant levels.

Degradative oxidative processes are also potentially initiated with cutting and post-cutting operations in fresh-cut product. Evidence for this exists in the literature in regard to browning, lipid peroxidation, and flavor changes (Toivonen, 2003; Hodges et al., 2004). While a comprehensive model to explain these phenomena has been developed (Toivonen, 2004), much of the model needs verification. Results of this research is expected to provide some fundamental knowledge that will lead to improved processing and handling procedures and consequently to improved fresh-cut quality at the consumer level.

### **Microbiology and Food Safety of Fresh-cut Products**

An evaluation of the efficacy of sanitizers or appropriateness of experimental protocols for challenge studies must be preceded by the development and validation of a standard method for inoculation, detection, and enumeration of bacterial pathogens on both whole (intact) and fresh-cut produce. Procedures for inoculating produce with pathogens include dipping in a suspension of cells or applying a known volume of cell suspension (spot inoculation). Optimum conditions for culturing pathogens that may be present on or in produce differ, thus requiring different methods for preparing cells to be used as inocula. The selection of the strain or strains of a particular pathogen to be used in studies designed to determine the number of cells present or the efficacy of a decontamination treatment is also important. Because stationary phase bacterial cells are generally more tolerant than are logarithmic growth phase cells to environmental stresses, the former should be used for studies to develop optimum procedures for maximum recovery from inoculated produce. A marker may be desirable to facilitate the recovery of cells. Adaptation of Gram-negative pathogens to nalidixic acid (50 µg/ml), for example, has been successfully used in achieving these objectives (Beuchat et al., 2001; Harris et al., 2001). The vehicle in which pathogens are entrapped is likely to be an organic material. For this reason, to simulate practical conditions, the carrier for the inoculum should contain organic material. A 5% solution of horse serum albumen has been used as a carrier in studies to determine the efficacy of sanitizers (Beuchat et al., 2001; Harris et al., 2001), although an aqueous peptone solution or nutrient broth may also be appropriate. Decontamination studies require the use of high numbers of cells in the inoculum to enable measurement of several log reductions. Challenge studies, on the other hand, require inocula containing relatively low numbers of cells to enable measurement of growth during subsequent storage.

Establishing a protocol(s) for efficient recovery of pathogens from fruits, vegetables, and tree nuts is paramount before proceeding to experiments designed to determine the efficacy of treatment with sanitizers. Procedures for separating produce from the chemical treatment solution, washing with a specific neutralizer, and subsequent homogenization or washing in a specific volume of a given diluent should likewise be standardized. In the case of physical decontamination treatments, standardization of conditions used to apply the potentially lethal force, i.e., temperature, irradiation, or pressure, would also facilitate comparison of observations across laboratories.

The presence of stressed or injured microbial cells on produce should be recognized, and resuscitation techniques may be necessary. Protection of cells on the surface of produce that, for example, may be debilitated by desiccation or as a result of exposure to a harsh acidic environment that may occur if samples were processed by methods other than simple washing is important if these injured cells are to be detected. Incorporation of a resuscitation step into the detection or enumeration protocol should be considered.

Of all the agents used to sanitize the surface of foods, water is probably the most readily acceptable to the public. Unlike chemical sanitizers that only affect the surface of produce, hot water (heated potable city water) washing can inactivate bacteria below the produce surface (Breidt et al. 2000), and thus, is potentially more effective than chemical washes (Annous et al. 2004, Breidt et al. 2000, Lichter et al. 2000). Hot water immersion provides excellent heat transfer between the produce and the heating medium (Couey 1989) and can quickly establish a uniform temperature profile on the surface of produce (Annous et al. 2004, Couey 1989).

Our initial results indicate that  $\geq 5$  log CFU/cm<sup>2</sup> reductions in *Salmonella* Poona populations on cantaloupe surfaces can be achieved following commercial-scale hot water immersion of whole melons at 76°C for 3 min (Annous et al. 2004). Also, this hot water commercial-scale treatment of whole melons was not detrimental to either the quality or shelf-life of fresh-cut pieces prepared from the treated melons. Hot water immersion (70°C for 2 min or 80°C for 1 min) was also shown to be effective in reducing populations of *E. coli* O157:H7 on the surface of oranges (Pao and Davis 1999). Since individual commodities have different thermal tolerances, the hot water immersion treatment needs to be tailored to each commodity. While the rind of a cantaloupe (Annous et al. 2004) and the peel of an orange (Pao and Davis 1999) effectively insulate the flesh from thermal damage at temperatures above 70°C, the peel of an apple does not protect the flesh from thermal damage at temperatures above 60°C (Lurie et al. 1998, Sapers et al. 2002).

Chlorine dioxide (ClO<sub>2</sub>) exists as a gas at STP. It can be produced by acidification of sodium chlorite solution or by mixing a dry activator (ferrous salt) and sodium chlorite powder at relative humidity  $\geq 30\%$ . Since ClO<sub>2</sub> gas is readily soluble in water, it tends to dissolve in the free water at wound sites on fruits and vegetables and microbes in those areas are likely to be inactivated. This makes ClO<sub>2</sub> attractive for fresh-cut applications. The application of gaseous ClO<sub>2</sub> to inoculated apples (Du et al. 2003, Sapers et al. 2003), lettuce leaves (Lee et al., 2004), green peppers (Han et al. 2001), and strawberries (Han et

al. 2004) resulted in population reductions of  $\geq 5$  log CFU without adversely affecting the color of the product.

Since the use of synthetic fungicides is becoming increasingly limited and regulated, alternative methods of controlling postharvest pathogens are constantly being sought. Several natural products have shown promise in reducing postharvest diseases and disorders in horticultural crops and thus in maintaining shelf stability. Methyl jasmonate has been shown to reduce microbial contamination and/or decay of several fruits and vegetables (Buta and Moline, 1998; Droby et al., 1999; Gonzalez-Aguilar et al., 2001; Meir et al., 1996; Moline et al., 1997). Ethanol has also been found to have antimicrobial properties (Lichter et al., 2002) and is also an ethylene biosynthesis inhibitor (Kelly and Saltveit, 1988; Saltveit and Mencarelli, 1988). Essential oils are known to have a broad spectrum of antimicrobial and decay control activity (Arras and Usai, 2001; Janssen et al., 1987; Plotto et al., 2003; Ponce et al., 2003; Suppakul et al., 2003; Tsao and Zhou, 2000a; Wang et al., 2001). Depending on their mode of application and dose applied, essential oils or analytes thereof, can be phytotoxic to the commodities (Plotto et al., 2003; Tsao and Zhou, 2000a) or be ineffective (Tsao and Zhou, 2000b). Acetic acid or vinegar vapor was effective in reducing plant pathogen-induced decay of several fruits (Liu et al., 2002; Sholberg et al., 2000), thus maintaining their quality and shelf-life. Exposure of avocado fruit to acetaldehyde vapor delayed fruit softening and inhibited ethylene production (Pesis et al., 1998).

The predominant problem which currently impacts on the majority of fresh-cut apple slice processors is the growth of fungi on the cut surfaces, resulting in a phenomenon coined “secondary browning”. While some work has shown that addition of a natural antimicrobial, vanillic acid, can reduce the problem, there needs to be more extensive work done to determine if the problem can be controlled by various other means. It is possible that packaging atmosphere, previously untested natural source antifungal agents or organisms, improved cultivars or improved post-cutting processes could alleviate this serious quality problem.

Application of edible coatings to fresh-cut produce helps to maintain produce quality and shelf stability, act as barriers to gas and vapor permeations, and can serve as reservoirs for the controlled release of natural products/GRAS substances (Baldwin et al., 1995a; 1995b; 1996). While many natural products/GRAS substances have broad-spectrum antimicrobial activity and otherwise maintain quality in diverse plant tissues, such technologies have not been accepted commercially since they may damage or impart undesirable sensory attributes to foods, oftentimes due to the high concentration of the applied additives and/or preservatives.

#### **OBJECTIVES:**

**Objective 1.** Develop, evaluate, and standardize subjective and objective quality evaluation methods in intact and fresh-cut fruits and vegetables.

**Objective 2.** Develop new strategies to maintain fresh-cut product quality

**Objective 3.** Improve understanding of biochemical, physiological and molecular mechanisms that affect fresh-cut product quality.

**Objective 4.** Standardize methods for recovering pathogenic and spoilage microorganisms from intact and fresh-cut produce including tree nuts.

**Objective 5.** Evaluate and control unintentional and intentional microbial contamination of intact and fresh-cut produce.

**PROCEDURES:**

All produce will be appropriately prepared and cut under highly sanitary conditions at refrigerated temperatures where the processing area, tools, and gloved hands are appropriately sanitized and personnel wear proper clothing to protect the cut produce from contamination. Any post-cutting treatments and packaging will also be performed using good manufacturing practices. After treatment, the produce would be stored at appropriate refrigerated temperatures and durations depending on the commodity, stage of maturity at harvest or treatment, and storage temperature prior to processing.

**Objective 1:** We propose to 1) compile existing methods used for sensory analysis and write guidelines for testing horticultural crops, 2) compare techniques for product profiling and difference testing, and 3) continue relating instrumental with sensory measurements by using newly developed instruments and statistical techniques. Quality rating scales were compiled as a first-step towards standardization of quality evaluation procedures (Kader and Cantwell, 2005). All S-294 participants will review the rating scales. Sensory evaluation methods and objective methods of measuring color, firmness, and composition will be proposed. These will be discussed, agreed-upon revisions made, and the procedures finalized and distributed within and outside the multistate project. The updated sensory analysis guide will be a collaborative effort between, and not exclusive to, participants from CA, FL, ARS-FL, ARS-LA, ARS-MD, and BC.

For comparison of techniques between laboratories, fresh-cut carrots will be shipped from CA to the labs at BC, ARS-FL, and ARS-LA, and quantitative descriptive analysis, ranking, or discrimination analysis will be used, depending on respective resources. Additionally, different melon cultivars from ARS-LA will be shipped to CA and ARS-FL. The same protocol for cutting the melons will be used, and comparison between laboratories made for descriptive analyses. The FL and ARS-FL laboratories will compare sensory methods using the same raw materials.

Quality evaluation comparing sensory data with physicochemical analysis will continue with novel instrumentation such as the SBSE (stir bar sorptive extraction) technology combined with olfactometry (ARS-FL), electronic nose technology (FL, ARS-FL); also, interactions between volatile and non-volatile components of flavor (ARS-FL). The chemical data for flavor compounds will be combined with sensory data to determine what type of aroma profile and sugar/acid ratios give the highest flavor quality (preference) or off-flavor (low preference) ratings for orange juice, fresh-cut tomato, or other tropical fruits/fruit products (ARS-FL).

**Objective 2:** We will evaluate MAP and new/emerging MAP-alternatives and the means to integrate these studies into a hurdle strategy to maintain the appearance, texture, flavor and nutritional quality of diverse fresh-cut products. Treatments also will be evaluated for their ability to maintain microbial quality and food safety. Proposed treatments may be done before and/or after fresh-cut processing. Fresh-cut apples, melons and other fruits will receive the most emphasis, but fresh-cut vegetable products, especially those related to or potentially related to the salad bar industry will also be tested. Fresh-cut produce that will be studied include cut apples/pears (AL, BC, ARS-FL, IA, ARS-MD, MI, NY, OR, ARS-PA), melons (AL, CA, FL, IA, ARS-LA, ARS-MD, ARS-PA), subtropical and tropical fruits (CA, ARS-FL, FL), cut lettuce and other leafy vegetables (AL, CA, IA, ARS-MD), tomatoes (CA, -FL, ARS-MD, ARS-PA, Spain), broccoli and cauliflower florets, carrots and other salad vegetables (CA, ARS-MD, MI, ARS-PA) and sweetcorn kernels (FL).

Selection of varieties with enhanced shelf stability and initial product quality will be an important component of this project; genotype selections will be based on reduced or delayed ripening (physiological, genetic) characteristics, greater resistance to plant pathogens and microbial contamination, better appearance and flavor, enhanced texture retention, and higher nutritional value. Work in this area will be conducted in BC, ARS-FL, ARS-MD, MI, and Spain. Selection of optimal quality produce for fresh-cut processing is another important consideration. Since fresh-cut products are intended for immediate consumption, fresh-cut fruit should be ripe or nearly ripe and vegetables should be fresh and showing no signs of senescence. Immature crops may never develop the quality or shelf stability needed for fresh-cutting. Studies on initial product quality including optimal fruit maturity, preconditioning, trait targeting, and microbial quality will be emphasized. Non-destructive instrumental measurements of fruit and vegetable quality will also be evaluated. Work in this area will be conducted in CA, ARS-FL, FL, ARS-LA, MI, OK, and Spain.

To develop basic information on the effects of cutting apparatus and blade designs of apple/pear corer/wedgers, the influence of cutting surface angles, forces required to make the cut, and blade edge characteristics will be evaluated (BC). Evaluations will be benchmarked relative to quality and rates of deterioration after cutting. The concept behind pre-cutting treatments of intact produce destined for fresh-cut products is to reduce ethylene synthesis or action, undesirable enzyme activities, and/or microbial loads in the fresh-cut products. Preharvest and pre-cutting 1-MCP treatments and pre-cutting heat treatments will receive the most attention, but other pre-cutting treatments may include ethanol, reduced O<sub>2</sub> (0-2 kPa) and elevated CO<sub>2</sub> (10-30 kPa) or O<sub>2</sub> (40-100 kPa) atmospheres, hot water brushing, and new and emerging sanitizers.

Proposed post-cutting treatments are intended to slow physiological and pathologically induced deterioration of the fresh-cut products. A broad range of post-cutting treatments are envisioned to include MA storage and MAP; and treatments with 1-MCP, natural products (emphasis on broad-spectrum antimicrobial, antioxidants, Ca-containing and GRAS substances), enhanced post-cutting washing/sanitizing steps, newer

sanitizers such as ozone, electrolyzed water and organic acids, edible coatings with or without food additives and preservatives, novel microbial antagonists that out compete pathogens and have a long history of being consumed by humans, and non-chemical treatments such as short-term, minimal exposure to high hydrostatic pressure, UV-C and ionizing irradiation. Pectinesterase application with and without calcium in order to firm the tissue by creating pectin crosslinking will be evaluated for its effect on fresh-cut fruit tissue softening and watersoaking development during storage. Rinsing the cut fruit with buffered alkaline solution will also be tested to determine if hyper-acidification of the cut surface due to vacuole rupture might play a role in the development of watersoaking and softening due to activation of hydrolases in the cell wall and membrane.

Novel packaging technologies also will be studied to avoid tissue injury during storage and to maintain fresh-like quality for an extended duration. Some of the post-cutting treatments will be performed in-package, and many will integrate MAP with other post-cutting treatments. Integration of chemical and physical interventions with antibrowning agents, MAP, and other industrial practices will facilitate the transfer of developed technologies to the fresh and fresh-cut produce industry. Furthermore, when adverse effects on quality and nutrients are induced by an intervention technology, means that can reduce or minimize the effects will be investigated. For example, use of other preservatives, antioxidants and MAP can be tested.

The effects of antimicrobial and safety interventions on microbe levels and quality attributes of fresh-cut fruits and vegetables is an area that will involve interaction between the physiologists and microbiologists in the project. Work in the areas described above will be conducted in AL, BC, CA, ARS-FL, FL, GA, IA, ARS-LA, MI, ARS-MD, OR, and PA.

Quality evaluations are needed to access the effects of product selection, pre- and post-cutting treatments, packaging technologies, and changes during storage. These techniques can be separated into physicochemical analyses and sensory evaluations. The strategy will be to first evaluate the effects of chemical and physical treatments on visual quality to determine whether the treatments are worth further study in terms of more complex physicochemical analyses and sensory evaluations during post-treatment storage periods at 1 to 10°C depending on commodity and commercial practices. Physicochemical techniques will include mostly instrumental measurements of surface pH, phytonutrient levels, surface color, firmness, sugar and acid levels, aromatic volatile abundance, microbial loads, and dissolved solid/electrolyte contents. At first, all participants will use a broad range of instrumental methods to measure quality, but later standardized methods are anticipated pursuant Objective 1. Sensory evaluations will be conducted by untrained panelists and by trained judges depending on the nature of the evaluation. Both intensity and overall acceptability characteristics of the fresh-cut products will be evaluated and later standardized per Quality Objective 1. Sensory evaluations will be conducted in CA, ARS-FL, FL, ARS-LA, LA, ARS-MD, and ARS-PA.

**Objective 3:** Although temperature management will be addressed primarily in Objective 2 activities, there will be some work in this Objective regarding the physiological

underpinnings of fresh-cut temperature responses. The respiratory response and recovery or re-equilibration of fresh-cut vegetables and fruits to wounding and to fluctuating temperatures will be investigated in FL and TX. Aerobic and anaerobic respiratory metabolism in fresh-cut tissues, including the basis for tolerances of fresh-cut vegetables and fruits to low O<sub>2</sub> and elevated CO<sub>2</sub> levels will be studied in MD and MI. The apparent lack of chilling injury symptom development in fresh-cut tropical and subtropical species in terms of more basic physiological responses of the tissues to chilling stress such as textural alterations and aroma volatile production will be investigated in more detail in AL, CA, FL, ARS-FL, and ARS-LA. Respiration (CO<sub>2</sub> and O<sub>2</sub>) and fermentative volatiles (ethanol and acetaldehyde) will be measured using standard gas chromatographic (GC) techniques and respiratory metabolism by standard enzymological methods. Textural changes will be evaluated using mechanical measurements of tissue firmness as well as enzyme assay and compositional analysis of cell wall polymers according to Huber and O'Donoghue (1993).

Ethylene-dependent and ethylene-independent wound responses in fresh-cut lettuce and fruits will be investigated in CA and FL by utilizing inhibitors of ethylene binding such as 1-MCP. The role of membrane deterioration in terms of electrolyte efflux (FL) and analysis of lipoxygenase and phospholipase action (FL and ARS-MD) will also be investigated. The role of free radicals in fresh-cut product deterioration will be investigated in BC. Ethylene will be measured by standard GC technique.

Several participants will investigate the physiological and biochemical causes of quality changes, especially undesirable color (browning) and textural changes. Activation and inactivation of key enzymes involved in color, texture, nutritional and flavor changes in fresh-cut products will be the focus of work in CA and ARS-LA. Initial maturity and quality effects on subsequent quality changes in fresh-cut vegetables and fruits will be evaluated in CA, FL, and ARS-LA. Other participants will focus on understanding the biochemical pathways involved in softening (AL, CA, FL, ARS-MD, and OK) and browning (CA). The role of phospholipase activation upon wounding in limiting the shelf-life of fresh-cut fruits will be investigated in ARS-MD. Sensory and instrumental measures of flavor quality will be correlated by project members in CA, ARS-FL, and ARS-LA. Visual quality will be evaluated by applying standard hedonic scoring systems (e.g. Gorny et al., 1998; Lopez-Galvez et al., 1996a), reflectance color measurements, and spectrophotometric analysis of chlorophyll, anthocyanin, carotenoid, and phenolic pigments (Tomás-Barberan et al., 1997). Textural alterations will be analyzed as described previously. Lipid class analysis will be conducted as previously described by Picchioni et al. (1996), and lipid degrading enzyme activity as described by Todd et al. (1992). Since apparent responses to temperature, ethylene, etc. can be strongly affected by different fresh-cut preparation procedures, certain basic preparation procedures such as slicing procedures, slice or chunk sizes, and sanitation methods will need to be agreed upon, especially by those participants working with the same or similar types of products. Similarly, standard hedonic scoring systems and physical measurement methods for color and texture for each common product will be used as much as possible.

**Objective 4:** The overall objective of this project will be to develop, using an inter-laboratory collaborative approach, a basic protocol for inoculation and recovery that could be modified according to various groups of fruits, vegetables, and tree nuts, but used in a standard way to test for the presence and/or populations of foodborne pathogens and for the effectiveness of sanitizers in killing these microorganisms. The method would be recommended as a standard protocol for use by USDA, FDA, EPA, and researchers to determine the efficacy of sanitizers in killing pathogens such as *Escherichia coli* O157:H7, *Salmonella*, and *Listeria monocytogenes*. Adoption of the method would facilitate comparison of the relative effectiveness of sanitizers for raw fruits and vegetables, regardless of the laboratory conducting the test. The basic protocol could also be used in challenge studies to determine survival and growth characteristics of pathogens on raw produce and tree nuts subjected to various processing and storage conditions. This work will be conducted by project members in CA, FL, GA, IA, ARS-MD, and ARS-PA.

The goals of the research to be performed under this objective are:

1. To develop a standard method(s) for inoculating the surface of fresh and fresh-cut fruits and vegetables, and tree nuts with bacteria capable of causing illness.
2. To determine the effects of time between inoculation and retrieval on viability and recoverability of foodborne pathogens.
3. To develop a standard method(s) for evaluating the effectiveness of sanitizers to remove or kill foodborne pathogenic bacteria on the surface of fresh and fresh-cut fruits and vegetables, and tree nuts.
4. To validate these methods in independent laboratories.

It is proposed that two inoculation procedures (dipping and spot inoculation) and two inoculum drying procedures be evaluated. The performance of methods under practical conditions will be evaluated. Suggested factors to be investigated are summarized as followed:

1. Pathogens (*Salmonella*, *E. coli* O157:H7, *L. monocytogenes*)
2. Fresh and fresh-cut produce (cabbage, carrot, alfalfa sprouts, green onions, peppers, cilantro, avocado, pear, peach, cherry, grapes, blueberry)
3. Tree nuts (almond, pecan, walnut, hazelnut)
4. Carrier for inoculum (deionized water and 5% horse serum)
5. Inoculation method (dip and spot)
6. Drying times (1 h and 24±1 h, at 4°C or 22°C, depending on type of produce or tree nut)
7. Treatment (water and 200 µg/ml chlorine)

Inoculum for each pathogen will consist of a five-strain mixture. Strains isolated from patients with infections associated with eating produce or tree nuts, or strains isolated from produce or tree nuts will be used. Pathogens will be cultured and inocula will be prepared according to procedures developed during the first phase of the project (Lang et

al., 2004a,b). Procedures recommended in these reports for inoculation and drying of inoculum will be used. Details of procedures for inoculating fresh-cut produce and tree nuts will require that preliminary studies be done.

Procedures for treatment and recovering pathogens from treated and untreated produce will follow those reported by Lang et al. (2004a,b). Modifications of these procedures will be needed for fresh-cut produce and tree nuts. Likewise, microbiological analysis of produce and tree nuts will follow or be adapted from recommended procedures evolving from the first phase of the S-294 project, with modifications as necessary.

All experiments will be replicated three times and at least four samples will be analyzed in each replicate trial. Data will be subjected to appropriate statistical to determine differences between and among treatments.

**Objective 5:** Types of produce that will be evaluated initially include apples, lemons, lettuce, melons, mangoes, oranges, peppers, sprout seed, and tomatoes. A variety of intervention technologies will be tested alone and in combination. Common microbiological methods will be used for enumeration of native microbes and pathogens that survive the intervention treatments. Appropriate controls will be included in each experiment and experiments will be replicated at least two times.

The attachment and survival traits of possible surrogates on produce will be compared to their respective pathogens in order to allow larger scale evaluation of intervention on a larger scale and in a safer environment (GA). The likelihood of unintentional or intentional contamination of produce via ice will be investigated by preparing crushed ice from inoculated water and applying to fresh-cut products (GA). The efficacy of treating produce with sanitizers in killing foodborne pathogens and/or their respective surrogates attached to the surfaces and within tissues of produce will also be evaluated (GA). Possible intervention steps to reduce hazards posed by foodborne pathogens, surrogates, or microbial toxins would have emphasis on simple, economical methods (e.g., increased or modified washes and treatments with chlorine, chlorine dioxide, and/or ozone water treatment). Comparing the initial population levels to those that survive on the product after treatment will give a level of success.

The fate of *Clostridium botulinum* neurotoxin on fresh and fresh-cut produce will be investigated (GA). Contamination of produce with botulinum toxin by terrorists is possible. Stability of the toxin on fresh foods has not been fully investigated. The stability of the toxin will be studied on a limited number of types of fresh and fresh-cut produce items. Produce will be spiked with the toxin and then levels over time determined using an AOAC approved ELISA method.

Commodity tolerance at different maturity stages to hot water immersion over a range of temperatures and effects on pathogen populations will be determined (FL, ARS-PA). Whole produce (initially melons and tomatoes) will be inoculated with the appropriate pathogen of interest or its surrogate to give an initial microbial load of  $10^6$  CFU/gm or

cm<sup>2</sup> as previously described (Annous et al. 2004). The potential for reducing the transfer of microorganisms of interest from the peel to the flesh of produce during fresh-cut preparation will be evaluated by testing of fresh-cut pieces for pathogen contamination. The potential for injured cells of interest to grow following storage will be also evaluated after 1-21 days in storage by plating to selective and nonselective media. Subtracting populations enumerated on selective media from those on nonselective media gives an estimate of the number of injured cells present. The impact of hot water immersion on the produce quality will be evaluated using sensory evaluation such as taste panels and/or sophisticated chemical and flavor analyses.

For sanitizing produce that cannot withstand hot water treatments, application of gaseous chlorine dioxide (ClO<sub>2</sub>) is an alternative. Recently, in initial experiments we demonstrated that reductions of  $\geq 5 \log \text{CFU/cm}^2$  in *Salmonella* Poona populations on cantaloupe surfaces following ClO<sub>2</sub> gas treatment (Annous and Fett, unpublished). Further studies will be conducted with whole cantaloupe melons, tomatoes, grapes, and other commodities including sprout seed (FL, ARS-PA). Whole produce (initially melons and tomatoes) will be inoculated as described above. Gas treatment will be conducted in specially designed fumigation chambers (recently designed and constructed at ARS-PA) for produce or sprout seeds. Various times of exposure and ClO<sub>2</sub> gas concentrations will first be tested for the effect on produce sensory qualities and shelf-life. Exposure times and concentrations will be optimized at 4 or 20°C at varying relative humidities for reduction or elimination of pathogens on inoculated fresh produce. Chlorine dioxide gas will be generated on-site using different available generation technologies. Residual microbial populations will be enumerated as described above. The effect of ClO<sub>2</sub> gas treatment on product quality will be evaluated by methods as described below.

Organic acids, including acetic, lactic, propionic, citric, malic, benzoic, and ascorbic acids, will be tested in IA and ARS-PA. Fresh-cut apple or bell pepper prepared as previously described (Liao and Sapers 2000, Liao and Cooke 2001) will be inoculated with a *Salmonella* cocktail mixture and washed once with pure water to remove the unattached bacteria. After varying lengths of storage, apple or bell pepper disks will be prepared and washed with varying concentrations of one or a combination of the aforementioned organic acids to determine efficacy. The organic acid(s) most effective in removing attached bacteria from fresh-cut pepper or apple disks will be applied in combination with another sanitizer (chlorine, ozone, hydrogen peroxide, or heat). Acid injured cells that survive the initial treatment with organic acid may be more susceptible to the secondary treatments leading to synergistic activity. The total number of viable and injured bacteria will be determined by plating the samples on non-selective media such as brain heart infusion agar and selective media such as XLT-4 agar. Antimicrobial activity of each organic acid at various concentrations against *Salmonella* cells in suspension or on apple or pepper disks will be determined by a method we previously described (Liao and Shollenberger 2003).

*Salmonella* mutants resistant to acetic acid will be isolated using transposon mutagenesis. The effect of acid-tolerance on the attachment and survival of these mutants to apple or bell pepper disks will be determined. The disks containing acid-tolerant

bacteria will be treated with acetic acid or other organic acid at the concentration lethal to the wild-type of *Salmonella*. The number of viable *Salmonella* remaining on the disk and the number of bacteria washed off in the acid solution will be enumerated to determine the efficiency of organic acid wash for removing *Salmonella* from the surfaces of fresh-cut apple or bell pepper slices

A number of natural products with broad-spectrum antimicrobial activities, newer sanitizers such as ozone, electrolyzed water and organic acids, edible coatings with or without food additives and preservatives, novel microbial antagonists, and non-chemical treatments such as short-term, minimal exposure to high hydrostatic pressure, UV-C and ionizing irradiation will be tested for antimicrobial effects in conjunction with Objective 2 activities with interaction between the physiologists and microbiologists in the project (see above for participating locations).

Application of antimicrobial compounds in conjunction with edible coatings will be done in ARS-FL. This will be done in a two-part study. First essential oils or phytoalexins with antimicrobial activity will be screened *in vitro* for effectiveness against common pathogens of citrus and tropical fruits. To test spoilage organism sensitivity to experimental treatments with essential oils or phytoalexins, methods traditionally employed will be initially used. Essential oil components such as thymol, carvacrol, trans-2-hexenal, citral, etc., known to have a broad spectrum of antimicrobial activity and also resveratrol and stilbene or their derivatives will be first tested on the target microorganisms. The specific organisms used for study will be those (primarily fungal) that are problematic on harvested subtropical/ tropical fruit. *Diplodia natalensis*, *Colletotrichum gloeosporioides* and *Penicillium digitatum*, have been recently isolated from diseased fruit to ensure virulence of the organisms as sub-culturing of fungi can cause a reduction in their pathogenic abilities. Methods to assist determining efficacy of the treatments are the agar diffusion method and the microdilution plate method. To evaluate the inhibitory effects of experimental solutions on organisms, the minimum inhibitory concentration (minimum level of experimental solution that produces an approximate 90% reduction in growth) and the minimum level of solution concentration which produces an approximate 99% reduction in populations will be measured (Ponce et al., 2003; Sparado et al., 2002; Wilson, 1997).

Once the *in vitro* evaluation has been done, the second *in vivo* phase of the study with fruit will commence. Fresh-cut fruit will be treated by either spraying or dipping in a solution containing the natural compounds described above that were effective in inhibiting pathogen growth *in vitro*, or by incorporating the compound(s) into fruit coatings. The active material must be made available to the target organism by choosing the right solvent or carrier, and, at the same time, making sure it does not impart phytotoxicity to the commodity. To this end, the antimicrobial compounds will be tested without coatings on fresh-cut fruits for toxicity and then incorporated into different coating formulations to find optimum conditions wherein the coating is compatible with the essential oil or phytoalexin, but does not bind it to the extent it is no longer available to the fruit or the pathogen. Coatings will be initially chosen based on the solubility characteristics of the antimicrobial compound. In some cases it may be necessary to first

dip or spray with the antimicrobial substance, then coat the fruit. It is to be expected that any successful antimicrobial action will be fairly specific for types of microorganisms inhibited. Therefore, much experimental work will be needed to optimize treatments for different microorganisms. Fresh-cuts will be assessed for % decay and size of lesions as well as % damage due to phytotoxicity.

#### **EXPECTED OUTCOMES:**

Results from this proposal will be available for use by fresh-cut growers and processors through the relationship between the S-294 project and the IFPA. The S-294 annual meeting has been held in conjunction with the IFPA convention since 1999. The results of this research will also contribute detailed scientific information relating physiology in diverse plant tissues to their quality and shelf stability and provide alternative strategies to the U.S. fresh-cut industry to control deterioration. These will be reported in refereed and other scientific journals. Improved appearance, taste and other quality characteristics combined with increased shelf-life will likely result in improved nutritional benefits to consumers and decreased postharvest losses to the U.S. fresh-cut industry.

A compilation of quality rating scales (an update of the one by Kader and Cantwell, 2005), recommended compositional analysis methods, and a practical guide of sensory analysis for horticulturists will be completed and distributed to interested S-294 participants and fresh-cut produce industry's R&D and QA personnel. Other research projects will be more effective through the use of these new objective methods of quality assessment. Comparison of methods between laboratories will allow standardization of protocols to answer specific questions in future research, such as sample size and preparation for a sensory panel, and develop lexicons for descriptive analysis of fresh-cut products that may be used in future research. This project will contribute to a better understanding of the physico- and chemical properties of fresh-cut products. Outcomes will include procedures for new fresh-cut products and extended shelf life with enhanced quality for existing products.

Microbiology work in this project will result in the development of standard methods to determine survival and growth characteristics and test the efficacy of sanitizers to remove or kill bacterial pathogens on fresh and fresh-cut fruits and vegetables, and tree nuts. These methods, with minor variations, can then be widely used by researchers and regulatory agencies to determine the presence and numbers of *Salmonella*, *E. coli* O157:H7, and *L. monocytogenes* on produce and to demonstrate the effectiveness of sanitizers. The process for authorizing the use of safe, highly efficacious treatments to reduce the risk of infections associated with consuming potentially contaminated fresh-cut fruits and vegetables, and tree nuts can then more expeditiously proceed. Outcomes will include safer chemical and sustainable non-chemical strategies to reduce postharvest use of potentially hazardous chemicals to inhibit spoilage and decay that will benefit the environment and the consumer.

#### **ORGANIZATION AND GOVERNANCE:**

The offices of the Technical Committee will be the chair, vice-chair, secretary, and past-chair and will serve as the Executive Committee. The first three officers will be elected

for two-year terms at the organizational meeting for the Technical Committee. Thereafter, a secretary will be elected biennially at the annual Technical Committee meeting. All voting members of the Technical Committee will be eligible for office. The officers will be promoted biennially in the following sequence: secretary to vice-chair, vice-chair to chair, chair to past-chair. These four officers will then constitute the Executive Committee to provide leadership and continuity and/or immediate action. The duties of the chair, vice-chair, and secretary will be as prescribed in the Guidelines for Multistate Research Activities; that of the past-chair will be to serve as a resource for the other committee members in carrying out their duties.

The project will have two subcommittees with chairs appointed by the Executive Committee chair. These will be the Quality and Physiology Subcommittee and the Microbiology Subcommittee. Members of each subcommittee will be those Technical Committee members whose research falls under the subcommittee area. The purpose of the subcommittees is to coordinate research activities within each research area, foster grant-writing activities, and create reports as directed by the Executive Committee.

## APPENDIX E

### Format for Reporting Projected Participation

For each participant in this activity, include his/her name and e-mail address, employing institution/agency, and department; plus, as applicable:

1. For research commitment, indicate the CRIS classifications [Research Problem Area(s) (RPA), Subject(s) of Investigation (SOI), and Field(s) of Science (FOS)], and estimates of time commitment by Scientists Years (SY) (not less than 0.1 SY), Professional Years (PY), and Technical Years (TY);
2. For extension commitment, indicate FTE and one or more of the seven extension programs (See <http://www.reeusda.gov/1700/programs/baseprog.htm>); and,
3. Objective(s) under which the each participant will conduct their studies.

**Project or Activity Designation and Number (if applicable):** S-294

**Project or Activity Title:** Postharvest Quality and Safety in Fresh-cut Vegetables and Fruits

**Administrative Advisor:** William F. Brown

Participant Name and E-mail address	Institution and Department	Research						Extension		Objectives				
		CRIS Codes			Personnel			FTE	Program	1	2	3	4	5
RPA	SOI	FOS	SY	PY	TY									
<b>Total SY, PY, TY and FTE</b>														

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