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Development of an Imaging System to Detect Fecal Material and Signs of Animal Intrusion in Produce Fields prior to Harvest

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Imaging systems have great potential for improving food safety by reducing risks associated with pathogens of fecal origin. For example, an accepted practice is for produce fields to be surveyed just prior to harvest for signs of fecal material and animal intrusion. Problematic areas are flagged for exclusion from harvest. To enhance this survey process, we are developing an automated, tractor mounted, imaging system that can direct attention to sites with evidence of fecal material or damaged vegetation. We have demonstrated that small quantities of fecal material from animals and birds can be detected by measuring fluorescence responses at selected wavelengths following excitation with an ultraviolet or a violet light source. Similarly, the ability to detect damaged plants using fluorescence responses is well document in prior literature. However, transferring laboratory findings to a commercially-usable device is not a trivial task. One goal of this presentation is to outline some of the issues, and related time delays, that have to be addressed for the laboratory-based technology to be successfully transferred. For example, a major problem with using fluorescence as a detection method in produce fields is that the intensities of fluorescence responses are generally much lower than the intensity of natural ambient light reflected from measured surfaces. One method of circumventing this dilemma is to use a super-bright illumination source such as a laser. Using a laser for illumination raises new problems, including finding a laser that will work reliably when mounted on a tractor, cost, and eye safety. This presentation will consider the process used to identify the design goals of a device to detect fecal material in fields, experimental results that provide the scientific basis of the device, and identification of components, and industrial sources of components, needed to build a commercially-usable device.

Chlorine Dioxide Gas as a Promising Antimicrobial Agent for Fresh Fruit and Vegetables

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Foodborne outbreaks, originating from raw produce, have been on the rise. Lettuce, spinach, onions, tomatoes, melons, sprouts, berries and fruit juices have been associated with contamination and illnesses from bacterial, viral, and parasitic organisms. In response to these outbreaks, Good Agricultural Practices are being encouraged for on-farm production and distribution, and, the FDA is seeking more effective microbial reduction strategies (5 log reduction) during food processing and in retail food establishments. Chlorine dioxide ($Cl0_2$), in the aqueous and gaseous form, has emerged as a strong antimicrobial agent with various applications for reducing foodborne pathogen levels in produce. This presentation will focus on the use of gaseous $Cl0_2$ on different produce surfaces for reduction of *Salmonella* spp., *E. coli* O157:H7, and *Listeria monocytogenes.* $Cl0_2$ gas was used successfully, and led to a 5 log reduction or higher in these pathogens, for apples (4.0 mg/l, 30 minutes), strawberries (0.6 mg/l, 15 minutes), green peppers (0.6 mg/l, 30 minutes), oranges (0.24 mg/l, 10 minutes), and tomatoes (0.1 mg/l, 15 minutes; 8 mg/l, 90 seconds). A 4 log reduction was obtained on melon surfaces (6.0 mg/l, 10 minutes). The antimicrobial effectiveness on lettuce and other leafy greens was far less effective (1.3 log reduction), and $Cl0_2$ gas treatment led to poor quality (leaf color). D-values and z-values for these pathogens have been determined on produce surfaces so that efficient processes can be designed. Quality parameters, including product color, shelf-life, and residuals will also be reported. To study potential industry applications, both batch and continuous tunnel systems have been developed and are being validated for processing efficacy using a surrogate organism. $Cl0_2$ gas is a powerful antimicrobial technology that warrants further study to identify effective uses for the food industry.

Measuring Supply Chain Trends in the Packaging of Stem-free Fresh Sweet Cherries: a Sustainable Approach in Package Design, Product Characteristics and Expectations

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This study focuses on developing a survey tool to determine and evaluate the current supply chain trends in the packaging of a new fresh product, stem-free fresh sweet cherries. This novel approach is attempted, in order to create a sustainable packaging system for the launch of the cherries. Methods developed during the course of study will likely aid in the development of packaging systems for other produce. The comprehensive, in-depth survey is planned to collect critical information from cherry growers, packers, retailers and consumers. Response variations between genders, ethnicities and age, are taken into consideration. The acceptance of plastics made from natural resources as alternative packaging materials to the petroleum-based ones is explored. Key characteristics such as package design and optimum size, as well as convenience features, like easy-open and re-closability, are examined. Shelf-life and safety expectations versus preferences in packaging technologies, like modified atmosphere packaging, are also points that this study covers.

Comparative Shelf Life Study of Blackberry Fruit in Bio-based and Petroleum-based Containers under Retail Storage Conditions

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The shelf life of blackberries is relatively short, 2-3 days at 0°C, due to its fragile skin and high respiration rate. Different marketing strategies can be used to retain blackberry quality and to extend its shelf life during postharvest. Packaging is one of these strategies. Blackberries are currently packaged in petroleum-based vented clamshell containers. Other packaging alternatives like a different container design or packaging material could be used to extend the blackberry shelf life. This study investigates the effect of a different packaging design, incorporated using different packaging materials including bio-based and petroleum-based ones, on the blackberry postharvest shelf life under retail storage conditions. 'Cancaska' and 'Chester', two major blackberry varieties grown in Michigan, USA, were packaged in close containers made from poly(lactic acid), PLA, and poly(styrene), PS, and stored at 2 ºC and 85 % RH for three weeks. Physico-chemical and microbiological properties of blackberries including weight loss, headspace evolution, pH, solid soluble content (SSC), titratable acidity (TA), SSC to TA ratio, texture and fungal presence were monitored during storage. Barrier properties to water and gases (CO_2 and O_2) of both packaging materials were measured for discussion purposes.

Aroma Compounds in Fresh Cut Pomegranate Arils

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Little published information exists regarding flavor and aroma compounds in pomegranate (*Punica granatum*). Although arils have fruity and sweet characteristics, we found no publications describing actual compounds responsible for their typical flavor. Since most commercial usage of pomegranates involves juices, we too investigated the oxidative changes in volatiles with an inhibitor. Our goal is to determine compounds in 'Wonderful' fresh cut arils and juices that have "flavor" importance. Hand-pressed (Miracloth) aril juice was analyzed, and compared with blended arils and arils blended with a lipoxygenase inhibitor, n-propyl gallate (n-PG) under low oxygen, which restricts secondary volatile generation arising from oxidation. A divinylbenzene carboxen polydimethylsiloxane solid phase microextraction (SPME) was performed with 10-ml juice in the headspace of 20-ml vials for 12.5 minute exposure at 40°C, prior to injection into a HP6890/5973 GC-MS with a DB-5 column. Compounds were identified in triplicate runs with our in-house index and/or HP ChemStation software searched against the NIST and Wiley libraries. Miracloth expressed arils contained several low molecular weight esters, alcohols, aldehydes and terpenoids. The predominant compounds were ethanol, hexanal, hexanol, a-pinene, b-pinene, limonene, 4-terpineol and bergamotene. Also recovered were ethyl 2-methylbutanoate, (*E*)-2-hexenal, 2-methylbutyl acetate, ethyl hexanoate, and eucalyptol. Several of these compounds are known to be flavor/aroma-important in fruits. The predominant compounds in arils blended in the presence of n-PG in low O_2 were ethanol, ethyl acetate, a-pinene, b-pinene, limonene and bergamotene. We tentatively believe that 3-methylbutanal, hexanal, (*Z*) 3-hexanol, hexanol, (*E,E*) 2,4 hexadienal, 4-terpineol, a-terpineol and p-cymene were generated oxidatively, and their flavor contribution during mastication and juicing operations remains to be evaluated.

Does Chilling Injury Play a Role in Determining the Shelf-life of Fresh-cut Tropical or Subtropical Products? A Case Study with Fresh-cut 'Kent' Mango.

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Fruit and vegetable species that evolved in subtropical and tropical regions of the world are known to be sensitive to low, non-freezing temperatures between 32 to 60 F, which can cause disruption of normal metabolism and result in various undesirable *chilling injury* symptoms, including discoloration, ripening disruption, and lack of normal aroma development. For example, mango fruit are known to be injured by temperatures below the range of 46 to 54 F, depending on the ripeness stage, with riper fruit being tolerant of lower temperatures. In handling fresh-cut products, however, temperatures from 32 to 41 F are the norm, chosen for both food safety and product quality considerations. In order to determine if chilling injury plays a role in determining the shelf-life of fresh-cut tropical and subtropical products, we held whole 'Kent' mango fruit and fresh-cut slices for 10 days at 41 F or 54 F and monitored quality changes and the appearance of chilling injury symptoms, including synthesis of aroma volatiles. The results indicate that exposure of fresh-cut mango to 41 F did cause some chilling stress that resulted in lower vitamin C content, less aroma, and greater softening, however, there were no visible symptoms of chilling injury in either the whole or fresh-cut mango. On the other hand, handling fresh-cut mango slices at 54 F led to very rapid and severe quality loss. We conclude that the short shelf life of fresh-cut mango, and presumably other chilling sensitive fruits and vegetables, precludes the development of significant chilling injury symptoms prior to the end of shelf life due to other, normal losses in quality. Thus, for all practical purposes, the shelf life of fresh-cut products, including those that are subject to chilling injury, is probably best maintained at the lowest possible, non-freezing temperature.

Successful Control of Watersoaking in Fresh-cut Tomato and Cantaloupe by 1-MCP (SmartFresh™) Depends on Fruit Ripeness Stage when Cut.

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Watersoaking or flesh translucency is a disorder that commonly develops in several fresh-cut products including tomato and melons, limiting their shelf life. Although tomato and melons are chilling sensitive crops and are commonly handled as fresh-cut products at temperatures of 41 F or lower (i.e., chilling temperatures), watersoaking is not a chilling injury symptom. Rather, watersoaking is a physiological disorder that is caused by the combination of wounding and stress- and ripening-associated ethylene. Development of watersoaking is delayed at lower temperatures and accelerated at higher temperatures. In our research with fresh-cut tomato and cantaloupe, we have found that 1-methylcyclopropene (1-MCP; SmartFresh™), which inhibits ethylene action, can reduce the development of watersoaking. SmartFresh is more effective when applied to partially ripe fruit than when it is applied to ripe fruit, more effective in reducing watersoaking in the less advanced maturity tissues within each fruit, and is also more effective when applied at lower temperatures. Overall, however, the effect of SmartFresh on watersoaking is minimal when it is applied to fruit that are cut at the more advanced, optimal ripeness stages that result in the best flavor quality. Thus, new approaches involving SmartFresh or other technologies still need to be developed to better overcome the effects of ethylene on fresh-cut tomato and melons.

Sugar Snap Pea Quality Changes in Relation to Storage Temperature and Atmospheres

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Sugar snap peas are increasing in importance as components of vegetable trays. Sugar snap peas (cv Sugar Daddy and Sugar Snap) were stored at 5°C (41°F) in air or air or a range of controlled atmospheres (3%O2 alone or with 6, 12 or 18%CO2, and 1 or 10%O2 with 12%CO2). After 9 days storage, peas in air and all atmospheres were of marketable quality. By 12 days, peas of cv Sugar Snap were higher quality than peas of cv. Sugar Daddy. By 15 days, both cultivars were injured by the 3%O2+18%CO2 and 1%O2+12%CO2 atmospheres. The best storage atmospheres for 18 days were normal air, 3%O2, 3%O2+6%CO2, and 10%O2+12%CO2. Sugar and Vitamin C concentrations declined with time under all storage conditions, but were not notably affected by the atmospheres. Very high concentrations of acetaldehyde (toxic to plant cells) and ethanol (relatively nontoxic) were found in peas stored in 1%O2+12%CO2, with significant increases after 6 days of storage. Increases in fermentative volatiles were also observed in the 3%O2 +12% or 18%CO2 atmospheres. The same 3 atmospheres also resulted in increased electrolyte leakage (an estimate of membrane integrity) and ammonia concentrations (toxic product of protein breakdown). In another experiment the same two cultivars were stored at 0°C (32°F) in air and at 5°C (41°F) in air or CA (3%O2, 3%O2 + 6%CO2, 3%O2 + 12%CO2, or 10%O2 +12%CO2) for up to 24 days. Visual quality evaluations indicated that all peas were marketable after 24 days under all conditions. Sugar and Vitamin C concentrations declined under all storage conditions, but concentrations were not affected by the atmospheres used. Other indicators of stress (acetaldehyde and ethanol, ammonia, and electrolyte leakage) also showed that the peas tolerated all storage conditions. Respiration rates were similar between the 2 cultivars and averaged 11.5, 16.5, and 32 µL CO2/mg-h at 0, 5 and 10°C, respectively. The most beneficial storage condition for sugar snap peas is air at or very close to 0°C. At 5°C, some of the atmospheres offer some benefit over air storage at that temperature.

Quality Considerations for Grape Tomatoes in Vegetable Trays

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Grape and cherry tomatoes now constitute about 24% of the value of all tomatoes sold in U.S. supermarkets. New marketing opportunities, such as grape tomatoes in trays of fresh-cut vegetables, expose fruit to temperatures of 5C (41F) or below, often in combination with modified atmospheres. These conditions are at odds with usual storage and handling recommendations for good tomato quality. Several storage studies were conducted on different varieties of grape tomatoes. At 5C grape tomato fruit (orange-red) could be stored up to 18 days and still be of marketable quality if kept cold. Continuous storage at 5C resulted in minimal weight loss (a significant cause of quality loss in grape tomato), no lycopene synthesis, retention of Vitamin C, but decreases in sugar concentrations. However if fruit were transferred from 5C (41F) and 10C (50F) to warmer temperatures, typical chilling injury symptoms (decay, poor color formation) occur as expected on the fruit stored at 5C but not on those stored at 10C. Controlled atmospheres of 3 or 10% oxygen with 0, 7, 12 or 18% carbon dioxide provided little benefit but were tolerated by grape tomatoes for up to 3 weeks at 5C (based on visual appearance, discoloration, decay, off-odors, and changes in sugars, Vitamin C, and ethanol and acetaldehyde concentrations). Near ripe high quality grape tomatoes perform well as components of fresh-cut vegetable trays under temperatures and atmospheres not recommended for tomatoes. Future work should focus on the eating (sensory) quality of the grape tomatoes under these conditions. For performance in vegetable trays, the differences among grape tomato varieties were not as important as overall freedom from defects (shrivel, mechanical damage) and the stage of ripeness.

Chitosan Effects on the Infectivity of Human Enteric Virus Surrogates for Future Application in Produce

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Chitosan, an in-expensive, non-toxic, ecologically friendly, and biodegradable compound is known to inhibit many bacteria, fungi, and yeasts. Chitosan's very recent approval by the EPA for use as a biopesticide on various field and horticultural crops [EPA-HQ-OPP-2007-0566-0019] provides a novel approach to enhance food safety. The efficiency of chitosan to inhibit viral infections depends on its molecular weight, degree of acetylation, concentration, and application method. Therefore chitosan effects on human enteric viruses that have not been reported, need investigation. In the absence of cell culture based infectivity assays for human noroviruses, surrogates such as murine norovirus (MNV-1) and feline calicivirus (FCV-F9), and bacteriophage MS2 (ssRNA) are used. The purpose of this study was to determine the effect of chitosan on human enteric viral surrogates, MNV-1, FCV-F9, and MS2 bacteriophage. Chitosan oligosaccharide lactate (MW <5kDa) and water-soluble chitosan (MW 53kDa) at concentrations of 1.4%, 0.7%, and 0.35% were incubated at 37ºC for 2 to 3 h with equal volume of each virus. Chitosan effects on each duplicate treatment were evaluated using standardized plaque assays and compared to untreated virus controls. The water-soluble chitosan at 0.7% decreased FCV titer by ~2.83 log10 PFU/ml, with lesser effects at lower concentrations and also decreased MS2 by ~1.18 to 1.41-log10 PFU/ml regardless of the concentration used. Chitosan oligosaccharide was shown to be less effective than water-soluble chitosan on all three tested viruses. Further studies on chitosan effects using lower incubation times and temperatures are on-going to determine its suitability as treatments to improve produce safety.

Biopreservation of Fresh-cut Salads using Bacteriocin Producing Lactic Acid Bacteria Isolated from Commercial Produce

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Lactic acid bacteria (LAB) produce an array of antimicrobial substances such as organic acids, H_2O_2, ethanol and bacteriocins. LAB have generally recognized as safe status and may be safely used in food preservation. However, most bacteriocins have been extracted from and applied to meat and dairy products with little emphasis on fresh produce. The objectives of this study are to isolate and identify bacteriocin producing LAB from plant origin, and evaluate antimicrobial applications for fresh-cut vegetable products. Fourteen different vegetable products were investigated for LAB using de man, Rogosa and Sharpe (MRS) and Nitrite Actidione Polymyxin (NAP) media. From 218 potential LAB isolates, 92 were confirmed catalase negative and gram positive. The cell-free supernatants of 92 LAB isolates were screened for antimicrobial properties using the agar diffusion bioassay with *Lactobacillus sakei* and *L. innocua* as indicator organisms. Eight LAB strains isolated from mung bean sprouts, swiss chard and mini-seedless cucumbers inhibited both indicator organisms. The presence of bacteriocins was indicated by inhibition zones following the neutralization of pH and elimination of H_2O_2. These zones were disappeared following the addition of proteolytic enzymes. The eight LAB isolates were identified using 16S rRNA gene sequencing. Antimicrobial effectiveness was tested against common fresh-cut produce pathogens and spoilage organisms at 5 and 20 $^{\circ}$C. Bacteriocins produced by all isolates showed significant antimicrobial effects against *L. innocua,* with inhibition zones ranging from 3.7 to 7.0 mm at 5 $^{\circ}$C and 4.3 to 8.1 mm in diameter at 20 $^{\circ}$C. The organic acids and H_2O_2 produced by LAB showed strong antimicrobial effects against *Pseudomonas fluorescens, Erwinia carotovora, Bacillus cereus* and weak effects against *Leuconostoc mesenteroides.* The results also suggest that seleted LAB isolates were effective at inhibiting the growth of *L. innocua* when applied in fresh-cut salads during cold storage.

Influence of Packaging on the Flavor of Fresh-Cut Apple Slices

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Maintaining good flavor of fresh-cut produce is imperative for consumer satisfaction. Packaging can influence fresh-cut product flavor and can determine whether acceptable flavor reaches the consumer. To determine the effects of packaging on the flavor of fresh-cut apple slices, 'Gala' apples were washed, sliced and dipped in an anti-browning solution prior to packaging in two packaging systems having contrasting steady-state O_2 atmospheres. The first package was comprised of a solid multi-layered polyolefin film (SF) that established a steady-state headspace atmosphere of high CO_2 (5 to 7%) and low O_2 (2 to 5%) concentrations. The second package was comprised of an ultra micro-perforated (UP) film which produced a headspace atmosphere consisting of high CO_2 (5 to 7%) and high O_2 (15 to 17%) concentrations. Fruit were stored at 5 °C for up to 21 d. Package headspace compositions were monitored for ethylene, CO_2, and O_2 concentration. Headspace volatile analysis (straight chain esters, branched esters, terpenes, alcohols, estragole, other) was conducted on the slices using gas chromatography after 1, 14 and 21 d. Quality attributes (soluble solids concentration, titratable acidity, color, and relative juice loss) were assessed at 0, 4, 7, 14, and 21 d. Sensory analysis was conducted on day 14, by trained judges (n=20) for 5 sensory attributes (fruit aroma, fruity flavor, sweetness sourness, hardness), one visual attribute (degree-of-browning) and overall preference. Apple slices in the UP packages had less juice loss and higher concentrations of estragole, straight chain esters, total and other volatiles. The UP apple slices had significantly higher fruity aroma, fruity flavor, perceived sweetness and better textural characteristics, as perceived by the sensory panel, than SF packaged apple slices. These superior fruit quality characteristics were associated with lower ethylene and higher O_2 concentrations in UP packages compared to the SF packages.

Fate of *Salmonella enterica* and *Escherichia coli* O157:H7 on Romaine Lettuce Following Immersion in PRO-SAN – a Biodegradable Foodgrade Sanitizer

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Romaine lettuce, a popular vegetable salad item, has been linked to recent recalls due to contamination with *Salmonella*. In this study, PRO-SAN (a commercial vegetable wash) and its derivatives were tested for killing *Salmonella enterica* and *Escherichia coli* O157:H7 on Romaine lettuce without negatively affecting sensory properties. Lettuce leaves inoculated with *Salmonella* or *E. coli* O157:H7 (~7.10 $\log_{10}$ CFU/leaf) were immersed (for 2.0 or 4.0 minutes) in water (control), chlorine (CHL; 150 ppm; pH 6.4), PRO-SAN, PRO-SAN-LC, PRO-SAN-soft, or PRO-SAN-PF at concentrations (0.1 or 1.0%) stated by the manufacturer. Immersion in water alone resulted in 1.07 and 1.28 log reductions respectively, for *Salmonella* and *E. coli* O157:H7. Log reductions of the pathogens on lettuce treated with CHL and PRO-SAN-based solutions ranged from 2.47 to 3.69. The largest reductions were observed with PRO-SAN or PRO-SAN-soft which resulted in ~3.69 and 3.54 log reductions of *Salmonella* and *E. coli* O157:H7, respectively. Increasing the treatment time for lettuce from 2.0 to 4.0 minutes did not significantly increase destruction of the pathogens ($P>0.05$). Also, PRO-SAN treatments did not alter the appearance of the lettuce during 14 days of storage at 4 ºC. In conclusion, PRO-SAN-based vegetable washes are effective in initially reducing viable populations *Salmonella* and *E. coli* O157:H7 on Romaine lettuce and maintaining good product quality during refrigerated storage.

Antilisterial Effects of Gravinol®-S Grape Seed Extract at Low Levels in Aqueous Media and its Potential Application as a Produce Wash

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Grape seed extract (GSE) is a rich source of proanthocyanidins, a class of natural antioxidants reported to have wide-ranging bioactivities as anti-inflammatory, anti-carcinogenic and antimicrobial agents. GSE's ability to rapidly inactivate *L. monocytogenes in vitro*, combined with its Generally Recognized as Safe (GRAS) status, makes it an attractive candidate for control of *Listeria* in or on foods. Previously, GSE has been used at relatively high levels (1%), in complex food matrices, and in combination with other antimicrobials. We sought to characterize the antilisterial effects of a commercial GSE preparation (Gravinol®-S) alone at much lower levels (0.00015% - 0.125%) in aqueous solution and to test its possible use as an antimicrobial wash for fresh produce surfaces. Based on broth microdilution tests, the minimum inhibitory concentrations (MICs) of GSE against *L. monocytogenes* Scott A and *L. innocua* ATCC 33090 were as low as 50 and 78µg ml^{-1}, respectively. GSE was evaluated in 0.85% saline against live cells of *L. innocua* via flow cytometry, using propidium iodide as a probe for membrane integrity. At sub-MIC levels and after only 2 min exposure, treatment with GSE caused rapid permeabilization and clumping of *L. innocua*, results that we confirmed for *L. monocytogenes* using fluorescence microscopy and Live/Dead staining. At higher levels (0.125%), GSE reduced viable cell counts for *L. monocytogenes* by approximately 2 logs within 2 min on tomato surfaces. These results suggest the potential for GSE as a natural means for control of *Listeria* spp. on low-complexity foods such as tomatoes.

Laser Labels: A New Bio-secure Method to Identify Florida Citrus

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Laser labels provide required information on produce surfaces by using a low-energy CO_2 beam to etch the surface and expose the contrasting underlying layer. These labels can be any size or arrangement of alphanumerical characters. Because the outer layer of the cuticle is compromised there is the concern of increased water loss, peel deformity and enhanced decay. Studies on the effect of laser labeling on postharvest storage were conducted with Florida grapefruit and tangerines. For the grapefruit, when carnauba wax was applied directly after etching, there was minimal water loss and no increase in decay even when the fruit were inoculated with spores of *Penicillium digitatum*.
For tangerines, application of wax after etching minimized water loss to levels of non-etched fruit and there was no increase in decay even when the fruit were inoculated with *Penicillium* spores. Peel deterioration was not a problem when the label was waxed and the fruit were stored at high relative humidity.

Factors Affecting the Levels of Wound-induced Oxy-radicals in Romaine Lettuce

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Wound response is a well known phenomenon and it's implications on quality in fresh-cut leafy vegetables has been well studied. One of the events which occur in response to wounding is the release of oxygen radicals (superoxide anion, hydrogen peroxide, phenolic acid quinones, etc). These radicals are thought to have a role in quality decline, but micro-organisms are also sensitive to oxygen radical injury. Therefore we have conducted several experiments to determine if oxygen radical levels in wounded tissue of Romaine lettuce vary in response to factors such as age of lettuce after harvest, heat treatments and part of the leaf wounded. A method was developed to allow real-time capture of superoxide anions and hydrogen peroxide generated at the wound site. The results to date show that leaves stored for more than one week produce significantly less oxygen radicals than those cut immediately after harvest. After two weeks storage, oxy-radical drops to nearly zero. Heat treatment halves the production of oxygen radicals and lower leaf portions produce less than mid and upper leaf portions. The implications of these findings on human pathogen growth on cut surfaces are being assessed at this time.

The National Institute of Food and Agriculture (NIFA): Grants Opportunities Related to Fresh and Fresh-cut Produce

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The National Institute of Food and Agriculture (NIFA) was established by the 2008 Farm Bill to serve the nation's needs by supporting exemplary research, education, and extension that addresses many challenges facing the nation. NIFA works with the best and brightest scientists at universities and colleges and other institutions throughout the United States and around the world to find innovative solutions to global problems. With a timely, integrated approach and collaboration with other federal science agencies, NIFA will also serve as a vital contributor in science policy decision-making. Current priority science areas are: 1) Global Food Security and Hunger, 2) Climate Change, 3) Sustainable Energy (Biofuels), 4) Childhood Obesity and 5) Food Safety. NIFA's principal competitive funding streams of interest to the fresh and fresh-cut produce industry are Agriculture and Food Research Initiative (AFRI), Specialty Crops Research Initiative (SCRI), National Integrated Food Safety Initiative (NIFSI). Other sources include, Small Business Innovation Research (SBIR), Nanoscale Science and Engineering (Nanotechnology), Higher Education Grants, Pest Management, Organic Transition, and Organic Agriculture Research and Extension Initiative (OAREI). In addition, portions of formula funds are utilized by the Land Grant University partners in conducting research and extension activities. Starting fiscal year 2010, AFRI program will be making highly focused (see priority science areas above), and outcome/impact oriented grants of up to $ 25 million for a period of up to 5 years with opportunities for renewal in some cases. Details on the nature and types of funding opportunities and links to current Request for Applications (RFA) will be provided in the poster presentation.

Integrative Approach for the Assessment of Food Safety in Spinach Cultivation

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Current fresh produce supply chain management emphasizes continued progress to integrate product quality, safety, and environmental responsibility. According to the World Food Programme, consumers require that food be safe, of good sensory quality, available in adequate amounts and affordable to ensure acceptable nutritional and health status of the population. Produce safety should not only include the control of chemical hazards and human pathogens but also consider the nutritional quality for consumers and the impact of cultivation practices on human health concerns from environmental toxicants. Beyond safety and food quality, co-management principles emphasize the sustainability of these practices and protection of water and environmental quality. Current agronomic practices for economical spinach cultivation and marketing in California depend on consistent pest management practices together with optimal water and plant nutrient supply. High rates of nitrogen fertilization, relative to UCCE Recommendations, and ample irrigation are common, especially during the summer months. The impact of these practices on *E. coli* O157:H7 colonization and internalization of spinach leaves as well as the human nutritional quality of this leafy green in relation to nitrogen applications were characterized. Field and greenhouse grown spinach (cv Whale, Bolero, Shasta, Avenger, Blackhawk, Emilia, Barbosa) were cultivated with different nitrogen regimes ranging from 100 and 340 kg/ha total N, from sufficiency to excess, and harvested before dawn after 30-35 days of cultivation. The overall N rate affected the concentration of nitrate and oxalate in the leaves, resulting in levels that substantially exceed Acceptable Daily Intake Standards (ADI), at the highest N rates. Nitrate and oxalate concentrations were as high as 12,000 ppm and 40 mg/g DW, respectively, following a positive relationship between N-dose and accumulation. Nitrate concentration in spinach was between 1.4 and 5 fold higher than the allowed 2,500ppm concentration by the European Union. Leaves from low nitrogen (sufficiency doses) were less bruise/fracture susceptible, had a higher cell wall and sugar content, and a more compact tissue structure with fewer intercellular spaces when compared to the highest nitrogen regime. Lower N-doses and the resultant leaf traits were positively correlated with longer shelf life and ADI-conforming nutritional quality during storage at 7.5C. In lab studies, *E. coli* O157:H7 survival and internalization of postharvest inoculated leaves at log 2 CFU/ml was greatest on plants cultivated with nitrogen concentrations greater than 100kg/ha. Although populations from leaves disinfected with 1% silver nitrate were similar between all N treatments and leaf maturity; the number of leaves internalized by *E. coli* O157:H7 was on average 70% greater with high N fertilization rates. Initial estimation of the amount of N left in the field after the application of 260kg/ha over 50 days of cultivation indicate that 65-80% of the N input is left in the soil, including 10 to 15% from plant residues, and is susceptible to soil leaching and runoff to watersheds. Overall, our data indicate that an integrative approach is warranted which balances goals in yield, product quality, nutritional and microbial quality with goals in sustainable cultivation practices, consumers protection, and the environment quality.

Field Assessment of Surface Contamination and Systemic Transference of an Attenuated *Salmonella* Typhimurium to Melon Fruit from Controlled Contamination of Irrigation Water

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Establishing microbiological quality standards for irrigation water, within food safety management programs for fresh produce is a contentious topic. The California melon industry requested the initiation of a study on the potential for systemic transfer of *Salmonella* to mature fruit in a field environment. We evaluated the survival and dispersal of an applied avirulent (non-pathogenic) isolate of *Salmonella* Typhimurium following controlled irrigation-source contamination events in an open field environment at the UC Davis Plant Sciences Research Farm Facility. Cantaloupe ('Oro Rico') and Honeydew ('Summer Dew') were transplanted in replicated plots into Reiff Coarse Loam and were irrigated, by furrow or sub-surface emitter tape, containing a rifampicin resistant (100mg/L) isolate of virulence-attenuated *Salmonella*. "Contamination" levels were targeted at Log 4 and 6 CFU/ml of water applied. At several periods, samples of water, furrow and seed-bed soil, rhizosphere soil, soil surrounding the emitter tape, and environmental samples were processed by standard protocols including selective enrichment and molecular confirmation. Mature fruit in the central bed (crown) as well as fruit developing in contact with furrow slopes were processed for enumeration or PCR detection, with and without disinfection with 0.1% mercuric chloride (10 min + 1 min sterile water rinse), as above. Applied *Salmonella* Typhimurium was readily recovered from irrigation inputs, soil, and environmental samples throughout the trial. The applied strain was regularly recovered from cantaloupe but not honeydew developing in "contaminated" furrows. No *Salmonella* was recovered from the rind or in the sub-rind tissue taken below the abscission zone in over 200 crown fruit. The applied *Salmonella* survived in the rhizosphere of melons irrigated by sub-surface drip injection but no evidence of transfer to fruit was detected. These results support greenhouse outcomes in our lab that strongly suggest a very high threshold of contamination from irrigation water would be required to make the risk of root uptake and transfer to fruit a significant concern. External contamination of fruit remains a plausible direct or indirect route from irrigation water and sufficient justification for establishing water quality protection and contamination risk awareness programs.

Assessing the Performance of Modified Moore Swabs as a Low-Cost Method of Large Volume Irrigation Source Surveys

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Widely-adopted irrigation water quality standards for fresh produce are based on the assumption that a low cost fecal indicator, generic *E. coli*, provides practical and accurate predictive information on the risk potential for human pathogen presence in the water source or conveyance and distribution system. To both challenge this assumption and to improve the predictive value of irrigation source analysis in both research and commercial practice, we utilized a capture-swab similar to the Moore Swab approach used by researchers monitoring sewer effluent, manure run-off, and pathogen-impacted irrigation water. Modified Moore Swabs (MMS) were developed with the intent to analyze for the presence of *E. coli* O157:H7 and *Salmonella* using nonselective enrichment and immunomagnetic separation system (IMS) or membrane filtration coupled with PCR and confirmation plating on selective media. This sampling strategy was evaluated to re-assess regional irrigation sources and modes of delivery (such as overhead irrigation) to trap the bacteria, either free or attached to suspended solids, by surveying larger volumes of source water than typical grab-samples used for indicator organism (IO) evaluations. After filtration of reservoir or irrigation district canal water with MMS, pathogen recovery was accomplished by extracting the MMS from the cassette and placing the saturated swab in a sterile Whirl-pak® bag holding 200ml double strength (2X) Universal Pre-enrichment Broth (UPB) followed by an overnight incubation (O.N.) at 37ºC. For *E. coli* O157:H7 recovery, 10 ml of enrichment was transferred to 90 ml of mEHEC and incubated at 42ºC for 24 h. Biocontrol GDS®-O157 was used for detection. For *Salmonella* detection, 10 ml of 2X UPB enrichment was transferred to 90 ml Tetrathionate Broth Base (TBB) followed by 6 h incubation at 42ºC. After incubation 20 ml of sample was transferred into 180 ml of M Broth for a final O.N. incubation at 37ºC. BAX®-Salmonella and GDS®-Salmonella were used for detection. An additional real-time PCR to Salmonella virulence markers was also used for molecular confirmations. Prior conventional surveys, based on 100 ml samples, were always negative for pathogen presence. With MMS, *E. coli* O157:H7 and *Salmonella* were detectable in irrigation source water samples, at low frequencies, but not consistently correlated with indicator *E. coli* determined from standard 100 ml sample volumes. To date we have shown that MMS are an effective low-cost method for filtration capture of large water volumes for pathogen screening of sources and conveyances.