

2006 ANNUAL REPORT OF COOPERATIVE REGIONAL RESEARCH PROJECT S-294

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Project: S-294, Postharvest Quality and Safety in Fresh-Cut Vegetables and Fruits

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University of California, Davis:

University of Florida:

University of Georgia, Athens:

Iowa State University:

Michigan State University:

Louisianan State University:

Cornell University, Geneva:

Oregon State University:

Texas A&M University:

University of Illinois:

University of Tennessee:

USDA-ARS, Florida:

USDA-ARS, Louisiana:

USDA-ARS, Pennsylvania:

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Introduction: The primary focus of our research is on factors affecting flavor and nutritional quality of fresh-cut fruit products and the reasons why their flavor-life is shorter than appearance-life.

Activities

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

2.1. Combined Effects of 1-Methylcyclopropene, Calcium Chloride Dip, and/or Atmospheric modification on quality Changes in fresh-cut Strawberries (Encarna Aguayo, Rattanawan Jansasithorn, and Adel A. Kader)

The aim of this study was to determine the effects of 1-methylcyclopropene, 1-MCP ($1 \mu\text{L L}^{-1}$ for 24 h at 5°C) on quality attributes and shelf life of fresh-cut strawberries. The 1-MCP was applied before (whole product) and/or after cutting (wedges), followed by storage in a continuous flow of air or air + $1 \mu\text{L L}^{-1}$ C_2H_4 . The combined effects of 1-MCP and CaCl_2 dips (1% for 2 min) and/or CA ($3 \text{ kPa O}_2 + 10 \text{ kPa CO}_2$) were also examined. The application of only 1-MCP before and/or after cutting did not have a significant effect on firmness and appearance quality during storage for up to 12 days at 5°C . The exposure to a continuous flow of $1 \mu\text{L L}^{-1}$ C_2H_4 in air during storage did not increase softening rate. 1-MCP applied before cutting or both before and after cutting of the strawberries increased respiration rate but reduced C_2H_4 production rate. Exposure to 1-MCP had a synergistic effect when combined with CaCl_2 plus CA. The combined treatment of 1-MCP + CaCl_2 + CA slowed down softening, deterioration rate, TA and microbial growth. Compared to control, which had a 6-day shelf -life, the shelf life of fresh-cut strawberries subjected to the combination treatment was extended to 9 days at 5°C . (accepted for publication in Postharv. Biol. Technol., 2006)

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

3.1. Nutrient Retention in Fresh-cut vs. Whole Fruits (Maria I. Gil, Encarna Aguayo, and Adel A. Kader)

The influences of processing and storage on the quality indices and nutritional content of fresh-cut fruits were evaluated in comparison to whole fruits stored for the same duration, but prepared on the day of sampling. Fresh-cut pineapples, mangoes, cantaloupes, watermelons, strawberries and kiwifruits and whole fruits were stored for up to 9 days in air at 5°C . The postcutting life based on visual appearance was shorter than 6 days for fresh-cut kiwifruit and shorter than 9 days for fresh-cut pineapple, cantaloupe and strawberry. On the other hand, fresh-cut watermelon and mango pieces were still marketable after 9 days at 5°C . Losses in vitamin C after 6 days at 5°C were 5% or less in mango, strawberry, and watermelon pieces, 10% in pineapple pieces, 12% in kiwifruit slices, and 25% in cantaloupe cubes. No losses in carotenoids were found in kiwifruit slices and watermelon cubes, while losses in pineapples were the highest at

25% followed by 10 to 15% in cantaloupe, mango, and strawberry pieces after 6 days at 5 °C. No significant losses in total phenolics were found in any of the fresh-cut fruit products tested after 6 days at 5 °C. Light exposure promoted browning in pineapple pieces and decreased vitamin C content in kiwifruit slices. Total carotenoids contents decreased in cantaloupe cubes and kiwifruit slices, but increased in mango and watermelon cubes in response to light exposure during storage at 5 °C for up to 9 days. There was no effect of exposure to light on the content of phenolics. In general, fresh-cut fruits visually spoil before any significant nutrient loss occurs. (accepted for publication in *J. Agric. Food Chem.*, 2006)

3.2. Quality retention and potential shelf life of fresh-cut lemons as affected by cut type and temperature (Francisco Artés-Hernández, Fernando Rivera-Cabrera and Adel A. Kader)

Kader)

The effects of four cut types of ‘Lisbon’ lemons (wedges, slices, ½ and ¼ slices) and storage at four temperatures (0, 2, 5 and 10 °C) on post-cutting life were studied. Respiration rates of all cut types that were stored at 0, 2 and 5 °C up to 8 days were 2 to 5 times higher than those of the whole lemons, while the increase was up to 12-fold at 10 °C. Small differences among treatments were observed in the post-cutting changes of color parameters and chemical composition. Based on the sensory analysis, the four cut types remained marketable for up to 7 days at all tested temperatures, but only the wedges, slices, and ½ slices stored at 0, 2 and 5 °C preserved their sensory attributes for up to 10 days.. Good retention of vitamin C (about 85% ascorbic acid and 15% dehydroascorbic acid) and antioxidant capacity were found after 10 days at 0, 2, and 5 °C. Ethanol was the main fermentative metabolite found (88% of the total) and its concentration increased by up to 3-fold in slices, ½ and ¼ slices after 10 days at 10 °C. Acetaldehyde concentrations did not exhibit any significant changes. Total phenolics concentrations decreased gradually throughout the storage period in all cases. (submitted for publication in *Postharv. Biol. Technol.*, 2006)

Publications

Vilas-Boas, E. V. and A. A. Kader. 2006. Effect of atmospheric modification, 1-MCP and chemicals on quality of fresh-cut banana. *Postharv. Biol. Technol.* 39:155-162.

Marrero, A. and A.A. Kader. 2006. 2006. Optimal temperature and modified atmosphere for keeping quality of fresh-cut pineapples. *Postharv. Biol. Technol.* 39:163-168.

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Introduction: This past year, the research emphasis in my lab shifted to a more basic study of the perception, synthesis, and propagation of the signal(s) that produce physiological changes in fresh-cut fruits and vegetables. In the first of a series of four papers we presented data suggesting that phospholipase D (PLD) and its products linolenic acid (LA) and phosphatidic acid (PA) are involved in producing the wound signal responsible for increased wound-induced PAL activity, phenolic accumulation and browning in fresh-cut lettuce leaf tissue (1). In the second paper (2) we used specific inhibitors to further implicate the phospholipid signaling pathway in the generation of a wound signal that induces phenolic metabolism in wounded leaf tissue. We diverged slightly from this line of research in the third paper (3) to study the effect of

mono-carboxylates on the wound signal and tissue browning. Subsequent research explored the effectiveness of di-carboxylates and aromatic carboxylates in reducing phenolic metabolism and tissue browning (4).

Activities

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

3.1 Wound-induced phenolic accumulation and browning in lettuce (*Lactuca sativa* L.) leaf tissue is reduced by exposure to n-alcohols. Choi, Y-J, F.A. Tomás-Barberán, and M.E. Saltveit

A wound signal originates at the site of injury in lettuce (*Lactuca sativa* L.) leaf tissue and propagates into adjacent tissue where it induces a number of physiological responses which include increased phenolic metabolism with the de novo synthesis of phenylalanine ammonia lyase (PAL, EC 4.3.1.5), the synthesis and accumulation of soluble phenolic compounds (e.g., chlorogenic acid), and subsequent tissue browning. Exposing excised mid-rib leaf tissue to vapors (20 $\mu\text{mol/g}$ FW) or aqueous solutions (100 mM) of n-alcohols inhibited this wound-induced tissue browning by 40% and 60%, respectively. Effectiveness of the alcohol increased linearly from ethanol to the seven-carbon heptanol, and then was lost for the longer n-alcohols 1-octanol and 1-nonanol (Fig. 1). The 2- and 3-isomers of the effective alcohols did not significantly reduce wound-induced phenolic accumulation at optimal 1-alcohol concentrations, but significant reductions did occur at much higher concentrations (100 $\mu\text{mol/g}$ FW) of the 2-, and 3-isomers. The active n-alcohols were maximally effective when applied during the first 2 h after excision, and were ineffective if applied 12 h after excision. Phospholipase D (PLD) and its products linolenic acid (LA) and phosphatidic acid (PA) are thought to initiate the oxylipin pathway that culminates in the production of jasmonic acid, and PLD is specifically inhibited by 1-butanol, but not by 2-, or 3-butanol.

These results suggest that PLD, LA, PA, and the phospholipid signaling pathway may be involved in producing the wound signal responsible for increased wound-induced PAL activity, phenolic accumulation and browning in fresh-cut lettuce leaf tissue.

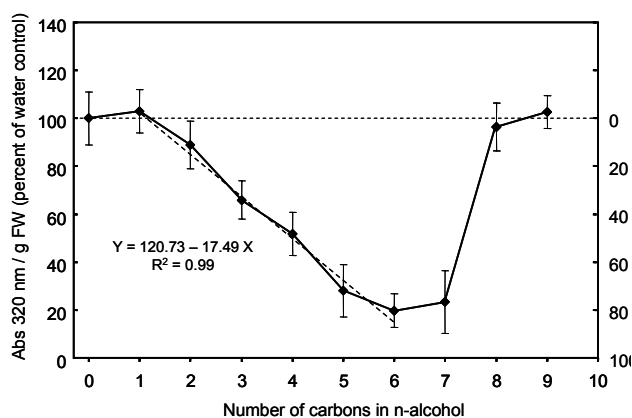


Fig. 1. Phenolic content of excised 5-mm Romaine mid-rib segments exposed to vapors from n-alcohols (from 1-carbon methanol to 9-carbon nonanol). Tissue was exposed to 20 μmol of the alcohol per g FW for 12 h at 10 °C. The regression line and linear equation for the normal (1-alcohol) alcohols from 1 to 6 carbons is shown.

3.2. Involvement of components of the phospholipid signaling pathway in wound-induced phenylpropanoid metabolism in lettuce (*Lactuca sativa* L.) leaf tissue. Saltveit, M.E., Y-J Choi, and F.A. Tomás-Barberán

Exposure to 1-butanol vapors or aqueous solutions inhibited wound-induced increase in PAL activity and phenolic metabolism. Phospholipases D (PLD, EC 3.1.4.4), an enzyme involved in the phospholipid signaling pathway is specifically inhibited by 1-butanol. Re-wounding tissue in which an effective 1-butanol concentration had declined below active levels by evaporation, did not elicit the normal wound response. It appears the 1-butanol treated tissue developed resistance to wound-induced increases in phenylpropanoid metabolism that persisted even when active levels of 1-butanol were no longer present. However, a metabolic product of 1-butanol, rather than 1-butanol itself, may be the active compound eliciting persistence resistance. Inhibiting a subsequent enzyme in the phospholipid signaling pathway, lipoxygenase (LOX; EC 1.13.11.12) with 1-phenyl-3-pyrazolidinone (1P3P) or reducing the product of LOX activity with diethyldithio-carbamic acid (DIECA) also inhibited wound-induced PAL activity and phenolic accumulation.

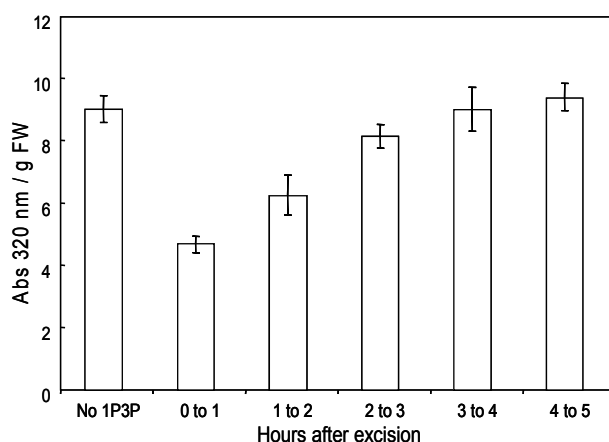


Fig. 2. Phenolic content of mid-rib segments treated with 0.5 mM 1-phenyl-3-pyrazolidinone (1P3P) at different times after excision. Phenolic content was determined by measuring the absorbance of a clarified methanol extract at 320 nm after holding the tissue for a total of 48 h after the initial excision.

The effectiveness of 1-butanol, DIECA, and 1P3P declined as the beginning of the 1 h immersion period was delayed from 0 to 4 h after excision (Fig. 2). This decline is consistent with involvement of the inhibitors in the production or propagation of a wound signal. The wound signal moves into adjacent tissue at ca. 0.5 cm h^{-1} , so delaying application would allow the signal to move into and induce the wound response in adjacent tissue before the delayed application inhibited synthesis of the signal. Salicylic acid (SA) inhibits allene oxide synthase (AOS, EC 4.2.1.92), another enzyme in the phospholipid signaling pathway. Exposure to 1 or 10 mM SA for 60 min reduced WIPA by 26% or 56%, respectively. However, 1 mM SA lost its effectiveness if applied 3 h after excision, while 10 mM SA remained effective even when applied 4 h after excision. At 1 mM, SA may be perturbing the wound signal through inhibition of AOS, while at 10 mM it appears to have some generally inhibitory effect on subsequent phenolic metabolism.

These data further implicate the phospholipid signaling pathway in the generation of a wound signal that induces phenolic metabolism in wounded leaf tissue (Fig. 3).

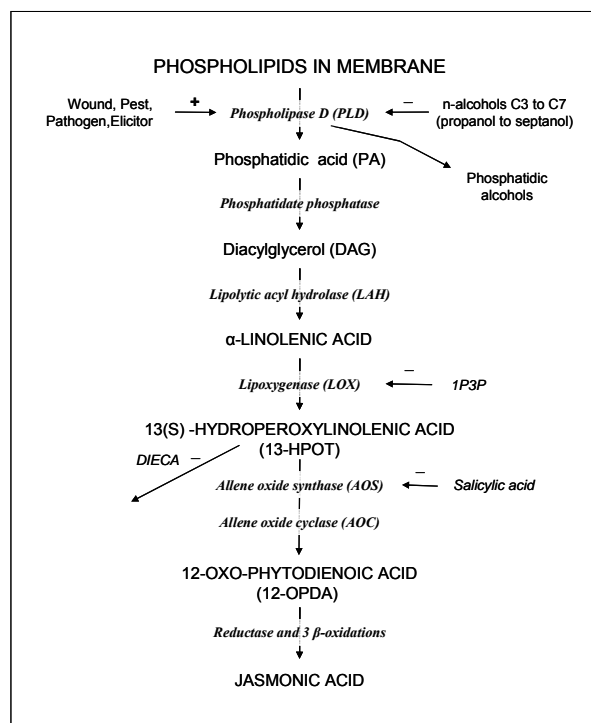


Fig. 3. Hypothetical model for the phospholipid signaling pathway with enzymes, products and inhibitors.

3.3. Mono-carboxylic acids and their salts inhibit wound-induced phenolic accumulation in excised lettuce (*Lactuca sativa* L.) leaf tissue. Saltveit, M.E., Y-J Choi, and F.A. Tomás-Barberán

Exposure of excised 5-mm mid-rib segments of romaine lettuce leaf tissue to vapors of mono-carboxylic acids or aqueous solutions of mono-carboxylic acids or their salts inhibited wound-induced phenolic accumulation (WIPA) and subsequent tissue browning. The decline in phenolic content followed a quadratic curve with increasing concentration, reaching a maximum inhibition after 60 min of $74 \pm 8\%$ for 50 mM sodium acetate (2 carbons, C2), and $91 \pm 4\%$ for 20 mM sodium decanoate (capric acid, C10). Respiration (i.e., carbon dioxide production) was unaffected by concentrations of formic, acetic, or propionic acids that reduced wound-induced phenolic content or that increase ion leakage from the tissue into an isotonic mannitol solution. However, WIPA was suppressed up to 70% at concentrations (20 mM acetate) that did not increase ion leakage over that of water controls. Various acetate salts (i.e., ammonium, calcium, magnesium, sodium) all produced the same level of inhibition. The effectiveness of the compounds increased with increasing number of carbons in the molecule from 1 to 10, but was unaffected by whether the carbons were a straight chain or branched, or whether the treatment was delayed by up to 6 h.

An unexpected and difficult to explain result is that the effectiveness of butyrate (C4) in reducing WIPA (27% reduction at 20 mM) was significantly less than that predicted from the response of the two adjacent mono-carboxylates similarly applied (Fig. 4); propionate (C3) (62%) and valerate (C5) (73%). A similar deviation in effectiveness did not occur with n-butanol in the n-alcohol series from 1 to 7 carbons (Choi et al., 2005). The physiological cause for the anomalous behavior of butyrate is under investigation.

It appears that, unlike the n-alcohols, mono-carboxylates are not interfering with the synthesis or propagation of a wound signal, but are interfering with subsequent steps in the production and accumulation of wound-induced phenolic compounds.

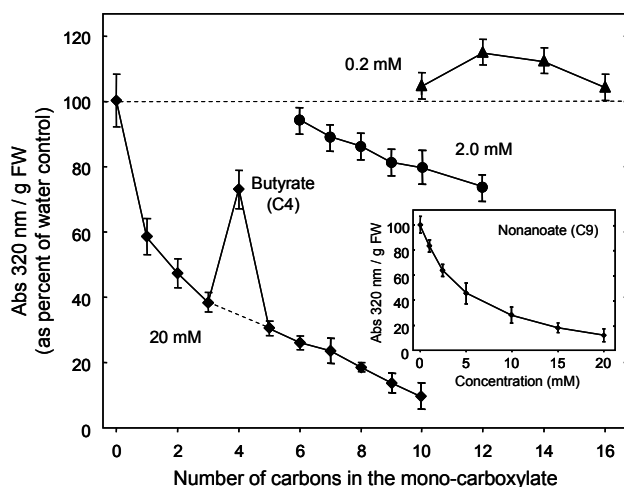


Fig. 4. Phenolic content of excised 5-mm Romaine mid-rib segments exposed to 20, 2.0, or 0.2 mM aqueous solutions of various mono-carboxylates. Phenolic content was determined by measuring the absorbance of a clarified methanol extract at 320 nm after holding the tissue for 48 h at 10 °C. Vertical lines associated with each bar represent the standard deviation about that mean.

Publications

1. Choi, Y.J., F. A. Tomás-Barberán, and M.E. Saltveit 2005. Wound-induced browning in lettuce (*Lactuca sativa* L.) leaf tissue is reduced by exposure to n-alcohols. *Postharvest Biology and Technology*. 37: 47-55.
2. Saltveit, M.E., Y.J. Choi, and F. A. Tomás-Barberán. 2005. Involvement of components of the phospholipid signaling pathway in wound-induced phenylpropanoid metabolism in lettuce (*Lactuca sativa* L.) leaf tissue. *Physiologia Plantarum* 125: 345-355.
3. Saltveit, M.E., Y.J. Choi, and F. A. Tomás-Barberán. 2005. Mono-carboxylic acids and their salts inhibit wound-induced phenolic accumulation in excised lettuce (*Lactuca sativa* L.) leaf tissue. *Physiologia Plantarum* 125: 454-463.
4. Saltveit, M.E. and Y-J. Choi, Y-J. 2006. Aromatic- and di-carboxylates inhibit wound-induced phenolic accumulation in excised lettuce (*Lactuca sativa* L.) leaf tissue. *Postharvest Biology and Technology* (In Review).

Institution: University of Florida

Participants: Food Science & Human Nutrition: Schneider, K. R.; Sims, C. A.; Talcott, S. T.
Horticultural Sciences: Brecht, J. K.; Huber, D. J.; Sargent, S. A., Plant Pathology: Bartz, J. A.

Activities

Food Science & Human Nutrition

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

APPROACH: Methods to inhibit degradation or to promote biosynthesis of phytochemical components of fruits and vegetables will be investigated. This will include the utilization of compounds isolated from natural sources as antioxidants and enzyme inhibitors and evaluating postharvest storage methods that enhance phytochemical production and retention. Emphasis will be placed on polyphenolic and carotenoid retention as a means to improve antioxidant capacity and overall quality. Commodities for investigation may include: tomatoes, carrots, potatoes, grapes, grape seeds, blueberries, and peppers.

PROGRESS: 2004/10 to 2005/09

Dense phase-CO₂ processing (DP-CO₂) is a promising pasteurization technology and may lessen detrimental effects to thermolabile phytochemicals such as anthocyanins. Similarly, the formation of intermolecular copigments by the addition of purified polyphenolics will assert a protective effect against anthocyanin and ascorbic acid (AA) degradation. To enhance anthocyanin stability, this study evaluated exogenously added polyphenolic cofactors from thyme (*Thymus vulgaris*) on the phytochemical and sensory attributes of AA-fortified muscadine grape juice model system following thermal and DP-CO₂ processing. Copigmentation along with DP-CO₂ processing was demonstrated to be effective in preventing phytochemical and quality deterioration in muscadine grape juice during processing and storage. Dense phase-CO₂ processing can also reduce microbial loads without adverse effects on phytochemicals or sensory characteristics. Micrographic observation aided understanding of microbial inactivation and similar microbial stability was observed between DP-CO₂ and thermally pasteurized juices during storage. DP-CO₂ served to protect phytochemical and antioxidant levels throughout processing and storage without comprising microbial stability or sensory attributes of the juices. Glucosidase and tannin acyl hydrolase are also commonly used as a processing aid for foods and beverages and is often associated with quality improvement such as releasing aroma components or removing undesirable compounds. Polyphenolic isolates from muscadine grape juice were prepared and evaluated for changes in the presence of these enzymes. Incubation with tannase revealed no specific activity for the ellagic acid glycosides or HHDP units of ellagitannins. However, free gallic acid significantly increased in the presence of tannase (17.8-fold increase) indicating the presence of gallotannins or gallic acid esterified to ellagitannins. A book chapter is in progress to highlight the nutrient and non-nutrient losses associated with fresh-cut fruits and vegetables as an ending to this research project.

IMPACT - 2004/10 to 2005/09: Information on phytochemical content, stability, and antioxidant capacity during processing and storage were evaluated. By monitoring these factors under actual food processing and storage conditions, a better understanding of food quality and potential health promoting properties were assessed.

PUBLICATIONS - 2004/10 TO 2005/09:

- Allen, R.L., B.R. Warren, D.L. Archer, S.A. Sargent, and K.R. Schneider. 2005. Survival of *Salmonella* spp. on the surfaces of fresh tomatoes and selected packing line materials. *HortTechnology* 15:831-836.
- Brenes, C.H., Del Pozo-Insfran, D., Talcott, S.T. 2005. Stability of copigmented anthocyanins and ascorbic acid in a grape juice model system. *Journal of Agricultural and Food Chemistry*. 53, 49-56.

- Del Pozo-Insfran, D. Percival, S.S., Talcott, S.T. 2006. Acai (*Euterpe oleracea*) polyphenolics in their glycoside and aglycone forms induce apoptosis of HL-60 leukemia cells. *Journal of Agricultural and Food Chemistry* 54(4),1222-1229.
- Del Pozo-Insfran, D. Duncan, C.E., Yu, K.C. Chandler, C.K. Talcott. S.T. 2006. Polyphenolics, ascorbic acid, and soluble solids concentrations of strawberry cultivars and selections grown in a winter annual hill production system. *J. Amer. Soc. Hort. Sci.* 131:89-96.
- Lee, J.H., Johnson, J.V., Talcott, S.T. 2005. Identification of Ellagic Acid Conjugates and Other Polyphenolics in Muscadine Grapes by HPLC-ESI-MS. *Journal of Agricultural and Food Chemistry*, 53, 6003-6010.
- Fox, A.J., Del Pozo-Insfran, D., Lee, J.H., Sargent, S.A., Talcott, S.T. 2005. Ripening Induced Chemical and Antioxidant Changes in Red Bell Peppers Affected by Ethylene Degreening and Time of Harvest. *Hortscience*. 2005. 40(3), 732-736.
- Mertens-Talcott, S.U., Bomser, J.A., Romero, C., Talcott, S.T., Percival, S.S. 2005. Ellagic acid potentiates the effect of quercetin on p21waf1/cip1, p53, and MAP-kinases without affecting intracellular generation of reactive oxygen species in Vitro. *Journal of Nutrition*, 135, 609-614.
- Talcott, S.T., Peele, J.E., Brenes, C.H. 2005. Red Clover Isoflavonoids as Anthocyanin Color Enhancing Agents in Muscadine Wine and Juice. *Food Research International*, 10, 1205-1212.
- Warren, B.R., M.E. Parish and K.R. Schneider. 2005. Comparison of chromogenic *Shigella* spp. plating medium with standard media for the recovery of *Shigelia boydii* and *Shigelia sonnei* from tomato surfaces. *J. Food Protection* 68:621-624.
- Yuk, H.-G., Bartz, J. A., and Schneider, K. R. 2005. The effectiveness of individual or combined sanitizer treatments for inactivating *Salmonella* spp. on smooth surface, stem scar, and wounds of tomatoes. *J. Food Sci.* 70:M409-M414.

Horticultural Sciences

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

APPROACH: This project will focus on the effects of fresh-cut preparation and handling, including techniques and technology, on the physiology, biochemistry, and quality of fresh-cut vegetables and fruits. Technology to be evaluated will include modified atmosphere packaging, application of novel food grade GRAS chemicals, and use of temperature-control regimes as alternatives to application of chemical compounds. We will also investigate the physiological and biochemical causes of quality changes, especially aroma, color (including browning), and textural changes, in fresh-cut vegetables and fruits. These studies will include the role of ethylene and the respiratory response and recovery or re-equilibration of fresh-cut vegetables and fruits following wounding and fluctuating temperatures. As one means of assessing the role of ethylene, commodities will be treated with the ethylene-action inhibitor 1-methylcyclopropene prior to processing into fresh cut. We will investigate textural changes associated with wounding during preparation and storage of fresh-cut vegetables and fruits. We will investigate the physiology of fresh-cut tropical and subtropical species when exposed to low temperatures in terms of the tissue response to chilling stress such as textural alterations, and aroma volatile production. Fresh-cut vegetables and fruits that will be studied include sweet corn kernels, lettuce, mango, papaya, peaches, and watermelon. Selection of intact items for cutting will include studies on initial product quality, identification of appropriate cultivars, and optimum maturity at harvest.

PROGRESS - 2004/10 to 2005/09: Methods of pretreating whole fruit for improvement of fresh-cut quality and shelf life were developed. Whole apples and mangoes were pretreated with 1-MCP, heat or ethanol. Heat and ethanol pretreatments resulted in extended shelf life for cut apple, and ethanol treatment enhanced appearance and reduced decay in mango, although some off flavor was associated with the higher level ethanol treatments. Correlation of total sensory (appearance, texture, flavor, odor), compositional, and microbiological quality changes in fresh-cut zucchini in MAP with machine vision and electronic nose measurements were done. Coatings on apple, peaches and mango showed some promising results for inhibition of browning and maintenance of flavor volatiles (mango) that warrant further research. 1-MCP treatment can help to reduce chilling injury of papaya fruit and the shelf life extended from 2 to 6 days. Slices from whole light red or red tomatoes pretreated with 1-MCP maintained greater pericarp firmness and developed less watersoaking (senescence) symptoms. Nonmelting-flesh (NMF) peach cultivars are better suited for fresh-cut processing than melting-flesh (MF) cultivars because their firmer texture allows the use of riper fruit with better flavor than the less ripe fruit that must be used for fresh-cut MF peaches. Fresh-cut sweetcorn kernels shelf life is limited by flavor loss and after-cooking browning. The latter increases with advanced maturity and higher storage temperature. Reduced O₂ plus elevated CO₂ (2% O₂ + 10% CO₂) is very beneficial in maintaining visual quality (preventing after-cooking browning) and reduced sugar and flavor losses during 10 days storage at 5C compared to storage in air. The effects of cutting on the firmness, activities of cell wall and membrane hydrolases, and ethylene biosynthetic enzymes in fresh-cut versus intact papaya fruit during storage at 5C were studied. The data suggest that the increase in the activities of enzymes targeting cell walls and membranes, and changes in the apoplastic environment of fresh-cut tissue as a result of membrane damage, contribute to the rapid deterioration of fresh-cut tissue. The comparison of mRNA transcripts between intact and fresh-cut papaya fruit revealed that 12 genes were differentially expressed in response to cutting.

IMPACT - 2004/10 to 2005/09: Fresh-cut produce can help increase the consumption of fresh produce due to its convenience and attractive appearance and flavor. Development of novel approaches for assuring the quality and safety of fresh-cut produce depends on a better understanding of fresh-cut vegetable and fruit physiology, including nutrients and other functional components as affected by storage and handling.

PUBLICATIONS - 2004/10 to 2005/09:

- Boynton, B.B., B.A. Welt, C.A. Sims, M.O. Balaban, J.K. Brecht, and M.R. Marshall. 2006. Effects of low-dose electron beam irradiation on respiration, microbiology, texture, color, and sensory characteristics of fresh-cut cantaloupe stored in modified atmosphere packages. *J. Food Sci.* 71:S149-S155.
- Boynton, B.B., B.A. Welt, C.A. Sims, J.K. Brecht, M.O. Balaban, and M.R. Marshall. 2005. Effects of low dose electron beam irradiation on respiration, microbiology, color and texture of fresh-cut cantaloupe. *HortTechnology* 15:802-807.
- Fonseca, S.C., F.A.R. Oliveira, J.K. Brecht, and K.V. Chau. 2005. Influence of low oxygen and high carbon dioxide on shredded Galega kale quality for development of modified atmosphere packages. *Postharvest Biol. Technol.* 35:279-292.
- Ergun, M., D.J. Huber, J. Jeong, and J.A. Bartz. 2006. Extended shelf life and quality of fresh-cut papaya derived from ripe fruit treated with the ethylene antagonist 1-methylcyclopropene. *J. Amer. Soc. Hort. Sci.* 131:97-103.

- Nunes, M.C.N., J.K. Brecht, A.M.M.B. Morais, and S.A. Sargent. 2005. Possible influences of water loss and polyphenol oxidase activity on anthocyanin content and discoloration in fresh ripe strawberry (cv. Oso Grande) during storage at 1 °C. *J. Food Sci.* 70:79-84.
- Plotto, A., J. Bai, J.A. Narciso, J.K. Brecht, and E.A. Baldwin. 2006. Ethanol vapor prior to processing extends fresh-cut mango shelf-life by decreasing spoilage, but does not always delay ripening. *Postharvest Biol. Technol.* 39:134-145.

Plant Pathology

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

APPROACH: Compare standard chlorine solutions to other oxidative solutions for ability to inactivate microbes on the cut surfaces of various products. Attention will be paid to recovery of microbes from oxidative damage. Recovery of microbes on growth media will be compared to recovery of microbes on plant tissues to determine how well the media models plant tissues. Common chemicals such as ethanol will be added to heated water at low concentrations to determine if efficacy can be improved without increasing the phytotoxicity.

PROGRESS - 2004/10 to 2005/09: Chlorine dioxide gas generated from either a mixture of dry ingredients or by off-gassing from a solution was used to inactivate soft rot bacteria in wounds on tomato fruit. At a ratio of approximately 2.0 mg chlorine dioxide to 1 kg of tomato fruit, soft rot was prevented or greatly reduced at 6 x 1 sq cm wounds on green tomato fruit. Tomato box material reacts with chlorine dioxide and, is an irreversible sink. Free moisture absorbs chlorine dioxide but is a reversible sink since the gas comes back out of the solution as the headspace concentrations decrease. Water channels in the surface of tomato fruit enable water soluble dyes or bacterial suspensions to readily internalize.

IMPACT - 2004/10 to 2005/09: Chlorine dioxide gas has potential to eliminate gram negative bacteria from fresh wounds on tomato fruit. Processes that lead to water congestion of openings in a tomato fruit surface enable ready internalization of particular matter including bacteria.

PUBLICATIONS - 2004/10 to 2005/09:

- Bartz, J.A. and J.W. Scott. 2005. Water infiltration and postharvest problems in tomato fruit. Florida Tomato Institute Proceedings pp. 20-23. (Gilreath and W.M. Stall, eds.) PRO 522. University of Florida/IFAS Extension and Citrus & Vegetable Magazine.
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Institution: University of Georgia, Athens and Griffin Campuses.

Participants: FST & EFS Athens, William C. Hurst - bhurst@uga.edu, Mark A. Harrison - mahfst@uga.edu; Center for Food Safety, Griffin, Larry Beuchat - lbeuchat@uga.edu

Activities

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

Food Science & Technology, University of Georgia, Athens Campus

RESEARCH OVERVIEW

PLEASE NOTE: Fresh produce research projects at UGA that may have a potential impact on fresh-cut processing have also been included in this listing.

- Microflora levels on cantaloupes
- *E. coli* O157:H7 attachment on cut carrots and lettuce
- *Enterobacter sakazakii* survival, growth and control methods
- *Enterobacter sakazakii* growth/temperature ranges on fresh-cut fruits, vegetables, and in unpasteurized juice
- Effectiveness of sanitizers to control *Bacillus* sp. spores on various surfaces
- Thermal tolerance of acid-adapted *Salmonella*, *E. coli* O157:H7, and *Listeria monocytogenes* in cantaloupe and watermelon juices and pasteurization heat requirements.
- Role of *Diploscapter* sp. nematode as potential vehicle for pathogen contamination of pre-harvest fruits and vegetables
- Attachment or infiltration rates of *Salmonella* Poona into cantaloupe rind and stem scar tissues

5.1. Microflora on Georgia-grown Cantaloupes Related to Packaging and Handling Practices. E. Deann Akins, Mark A. Harrison, and William C. Hurst, University of Georgia, Dept. of Food Science and Tech. Athens, GA 30602, USA

A study at four Georgia grower/packinghouses enumerated aerobic bacteria populations on cantaloupes as they were brought in from the field, after washing, and after packaging. Two facilities used chlorinated water, one used heated water, one used combination of heat and chlorinated water to wash the fruit. Water temperatures 41 to 50°C for 5 to 10 min did not reduce microbial population significantly. Microbial populations after chlorinated wash were < 0.5 log lower than on non-chlorinated wash. However, after packing, the number of aerobic populations on the surfaces were approximately the same as prewashed cantaloupes! Further study should look at sanitary conditions, worker hygiene and equipment sanitation to understand why microbial populations increased after leaving the dump tanks.

5.2. Attachment of *Escherichia coli* O157:H7 to Lettuce and Carrot Surfaces and Possible Internalization. Jinkyung Kim and Mark A. Harrison, University of Georgia, Dept. of Food Science and Tech., Athens, GA 30602, USA

Escherichia coli O157:H7 cells on cut carrots and lettuce examined with scanning electron microscopy (SEM) indicate that greater numbers attached at ambient (20°C) rather than at refrigeration temperatures (4°C). *E. coli* cells attached themselves to cut surfaces at various different angles, which may influence the intensity of attachment. Also, *E. coli* O157:H7 could

be internalized through the stomata in lettuce; stomata were missing on baby carrots due to processing. Visible mechanical damage from processing to surface of baby carrots played a role in bacteria internalization. Internalization may result in an underestimation of bacterial contamination and reduce sanitizing efficacy due to limited contact with sanitizing agents.

Center for Food Safety at the University of Georgia, Griffin Campus

5.3. Survival and growth of Enterobacter sakazakii on fresh produce as affected by temperature, and effectiveness of sanitizers for its elimination. H. Kim and L. R. Beuchat, Center for Food Safety, University of Georgia, Griffin, GA

Enterobacter sakazakii is an emerging foodborne pathogen known to cause meningitis, sepsis, bacteremia, and necrotizing enterocolitis in preterm neonates and immuno-compromised adults. *E. sakazakii* has not been linked to the consumption of fresh produce, but it has been isolated from lettuce and other vegetables, thereby representing a potential risk to produce safety. Studying the survival and growth of *E. sakazakii* on the surface of apples, cantaloupes, strawberries, lettuce, and tomatoes stored at 4, 12, and 25°C for up to 28 days determined that populations significantly decreased ($p \leq 0.05$) on all test produce at all storage temperatures. A second objective was to determine the effectiveness of chlorine, aqueous chlorine dioxide, and a peroxyacetic acid-based sanitizer (Tsunami 200®) in killing *E. sakazakii* inoculated in an organic carrier onto the surface of apples, tomatoes, and lettuce. Chlorine was less effective in killing *E. sakazakii* on lettuce than on apples or tomatoes. Treatment of lettuce with Tsunami 200® (40 and 80 µg/ml) for 5 min caused a reduction of ≥ 5.31 log CFU/sample. Results provide insights to predicting survival characteristics of *E. sakazakii* on produce and the efficacy of sanitizers in killing the bacterium.

5.4. Survival and Growth of Enterobacter sakazakii on Fresh-cut Fruits and Vegetables and in Unpasteurized Juice as Affected by Storage Temperature. H. Kim and L. R. Beuchat, Center for Food Safety, University of Georgia, Griffin, GA

Outbreaks of *E. sakazakii* infections associated with fresh produce have not been documented, although its ability to grow at temperatures as low as 5.5°C raises concern about survival and growth on fresh-cut produce and in unpasteurized juice in retail, food service and home storage. The survival and growth characteristics of *E. sakazakii* on fresh-cut apple, cantaloupe, strawberry, watermelon, cabbage, carrot, cucumber, lettuce, and tomato and in juice prepared from these fruits and vegetables was studied. Populations did not change or gradually decreased in fresh-cut produce and juice stored at 4°C, but grew on fresh-cut apple, cantaloupe, watermelon, cucumber, and tomato and in all juices except apple, strawberry, cabbage, and tomato juice at 12°C. All fresh-cut fruits and vegetables except strawberry supported growth of *E. sakazakii* at 25°C. Further characterization of the behavior of *E. sakazakii* on fresh produce and in unpasteurized juice as affected by commercial packaging and handling practices is warranted.

5.5. Evaluation of Chlorine, Chlorine Dioxide, and a Peroxyacetic Acid-based Sanitizer for Effectiveness in Killing Bacillus cereus enterica and Bacillus thuringiensis Spores in Suspensions, on the Surface of Stainless Steel, and on Apples. A. C. Kreske, J.-H. Ryu, and L. R. Beuchat, Center for Food Safety, University of Georgia, Griffin, GA

With increased international attention focused on the threat of bioterrorism, produce and other ready-to-eat foods may be considered as potential vehicles for intentional contamination with disease-causing microorganisms. The resistance of *Bacillus anthracis* spores and spores of other *Bacillus* species to sanitizers used to decontaminate produce has been given only meager

research attention. *B. cereus* and *B. thuringiensis* spores were used as surrogates for *B. anthracis* spores to study the efficacy of chlorine (10 - 200 µg/ml), chlorine dioxide (10 - 200 µg/ml), and Tsunami 200®, a peroxyacetic acid-based sanitizer (40 - 80 µg/ml), in killing these spores in suspension, on the surface of stainless steel, and on apples.

Planktonic spores of *B. cereus* and *B. thuringiensis* were more sensitive to sanitizers than were spores on the surface of stainless steel or apples. At the same concentrations, chlorine was more effective than chlorine dioxide in killing spores in suspension and on stainless steel. Chlorine and chlorine dioxide, at concentrations of 10 - 100 µg/ml, were equally effective in killing spores on apples. Significant reductions of $\geq 3.8 - 4.5$ log CFU/apple were achieved by treatment with 100 µg/ml of either sanitizer. The peroxyacetic acid sanitizer (40 and 80 µg/ml) was not effective in killing *Bacillus* spores in the test systems investigated.

5.6. Thermal Tolerance of Acid-adapted and Unadapted *Salmonella*, *Escherichia coli* O157:H7, and *Listeria monocytogenes* in Cantaloupe Juice and Watermelon Juice.

M. Sharma, B. B. Adler, M. D. Harrison, and L. R. Beuchat, Center for Food Safety, University of Georgia, Griffin, GA

Decimal reduction times (*D* values) of *Salmonella*, *E. coli* O157:H7, and *L. monocytogenes* cells suspended in unpasteurized cantaloupe juice and watermelon juice as affected by acid adaptation preceding exposure to heat were determined. Acid-adapted cells of *Salmonella* and *E. coli* O157:H7 had increased thermal tolerance; *L. monocytogenes* cells did not. Pasteurization conditions necessary to achieve elimination of pathogens from these juices must be more severe if cells are habituated to acidic environments. Insights from this study provide guidance to developing pasteurization processes to eliminate *Salmonella*, *E. coli* O157:H7, and *L. monocytogenes* in cantaloupe juice and watermelon juice.

5.7. Potential Role of *Diploscapter* sp. Strain LKC25, a Bacterivorous Nematode from Soil, as a Vector of Food-borne Pathogenic Bacteria to Preharvest Fruits and Vegetables. D. S. Gibbs, G. L. Anderson, L. R. Beuchat, L. K. Carta, and P. L. Williams, Center for Food Safety, University of Georgia, Griffin, GA

A thermo-tolerant, free-living, soil bacterial-feeding nematode, *Diploscapter* sp. strain LKC25, commonly found in compost, sewage, and agricultural soil in the United States was studied to determine its potential role as a vehicle of *Salmonella enterica* Poona, enterohemorrhagic *Escherichia coli* O157:H7, and *Listeria monocytogenes* in contaminating preharvest fruits and vegetables. Attraction of *Diploscapter* sp. strain LKC25 to colonies of pathogenic bacteria on tryptic soy agar within 10, 20, 30, and 60 min and 24 h revealed that within 24 h, $\geq 90\%$ of the worms were embedded in colonies. The potential of *Diploscapter* sp. after exposure to bacteria inoculated into soil or a mixture of soil and composted turkey manure indicate that pathogenic bacteria can be shed from this nematode. Results also demonstrate its potential to serve as a vector of foodborne pathogenic bacteria in soil, with or without amendment with compost, to the surface of preharvest fruits and vegetables in contact with soil.

5.8. Attachment of *Salmonella* Poona to Cantaloupe Rind and Stem Scar Tissues as Affected by Temperature of Fruit and Inoculum. G. M. Richards, and L.R. Beuchat, Center for Food Safety, University of Georgia, Griffin, GA

The effect of temperature differentials (i.e., when the temperature of the fruit is higher than the temperature of the water in which it is immersed) on infiltration of *Salmonella* Poona into cantaloupe rind at 4 and 30°C showed changes in fruit weight and populations of the pathogen recovered from rinds and stem scar tissues of Eastern and Western cantaloupes. Western

cantaloupes' percent weight increase was significantly greater ($P \leq 0.05$) than that in Eastern cantaloupes for all cantaloupe and inoculum temperature combinations. *Salmonella* Poona attachment to or infiltration of Eastern, but not Western, cantaloupe rind is enhanced when the fruit is at 4°C, compared to 30°C immersed suspension. *Salmonella* Poona in immersion water can adhere to or infiltrate surface tissues of cantaloupes. The populations of *Salmonella* Poona recovered from stem scar tissues of Eastern and Western types of cantaloupes were not significantly ($P > 0.05$) affected by cantaloupe and inoculum temperature combinations. Populations of cells adhering to or infiltrating various cantaloupe tissues is not dictated entirely by temperature differentials between fruits and immersion suspensions; rather, it also apparently is influenced by structures unique to surface tissues.

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Institution: Iowa State University

Participants: Aubrey Mendonca, Terri Boylston, Byron Brehm-Stecher, Cheryl Reitmeier, Lester Wilson

Introduction: Research is being conducted to improve the microbial quality and safety of fresh produce. Efforts are being made to increase the antimicrobial effectiveness of foodgrade chemical sanitizers for killing pathogens on the skin surface of cantaloupes and apples with the aim of reducing contamination of the fresh-cut fruit. Hydrogen peroxide, selected surfactants, and organic acids alone or in combination are being tested for improving the microbial quality of cantaloupe, apples, lettuce and almonds. Also, the effectiveness of low dose irradiation is being evaluated for eliminating microbial contamination on the outer rind surface of cantaloupe. The main objective is to develop and evaluate novel approaches for assuring the quality and safety of fresh-cut vegetables.

Activities

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

- 5.1. Determine efficacy of selected chemicals for destroying *Salmonella* and *Escherichia coli* O157:H7 on cantaloupe skin and whole apples
- 5.2. Determine the fate of pathogens on the outer rind surface of cantaloupe and whole apples following sequential application of chemical washes and low dose irradiation

- 5.3. Evaluate the impact of selected antimicrobial treatments on quality characteristics of cantaloupe and apples

Work in Progress

Experiments are being conducted to enhance the antimicrobial activity of selected organic acids, hydrogen peroxide and sodium bicarbonate buffer solutions against human enteric pathogens on fresh produce including whole cantaloupe and raw almonds. This approach involves evaluating the antimicrobial efficacy of these antimicrobials in combination with various concentrations of chelating agents. The effectiveness of applying antimicrobial wash solutions followed by ozonation or low dose irradiation to destroy pathogens on fresh produce is being investigated along with the influence of antimicrobial interventions on microbial shelf-life and selected quality attributes (including color, texture, etc) of the fresh produce.

Publications

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Orozaliev, A. Efficacy of selected chemicals and electron beam irradiation for destroying *Escherichia coli* O157:H7 and *Salmonella* on cantaloupe skin. M.S. Thesis, Iowa State University, 2005.

Mendonca A., and A. Orozaliev. Combined effectiveness of hydrogen peroxide and a food-grade surfactant for destroying *Salmonella* and *Escherichia coli* O157:H7 on whole cantaloupe. Abstr. 016-06. IFPA Annual Meeting on April 26-29, 2006.

Institution: University of Illinois

Participants: Hao Feng, email: haofeng@uiuc.edu; Mosbah M. Kushad, email: kushad@uiuc.edu

Activities

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

3.1. Changes in glucosinolate levels in crushed horseradish roots and broccoli (Mosbah Kushad)

Glucosinolates are thioglucosides found primarily in cruciferous vegetables like broccoli, horseradish, kale, Brussels sprouts, and cabbage. The health benefits of cruciferous vegetables have been linked to these compounds. So far about 150 of these compounds have been identified, however only about 15 have been detected in commonly consumed vegetables like broccoli and cabbage. For example, broccoli is rich in glucoraphanin, horseradish and cabbage are rich in sinigrin, while kale varieties contain a combination of both of these glucosinolates. Glucosinolates as they exist in intact plants have no biological activity against human diseases,

however, when vegetables containing these compounds are cut, crushed or chewed, they become hydrolyzed by an enzyme called myrosinase. Glucosinolates and myrosinase are sequestered in different organelles in the intact cells. Disruption of these organelles results in myrosinase mediated deglucosylation of glucosinolates into isothiocyanates. Glucoraphanin in broccoli converts into sulforaphane, while sinigrin converts into allylisothiocyanate. Both sulforaphane and allylisothiocyanates have been shown to reduce several types of gastrointestinal cancers in laboratory animals.

During the last few years we have been evaluating the molecular and biochemical characteristics of the myrosinase enzyme in cruciferous vegetables, mainly horseradish. Using available methods, horseradish contains significantly higher myrosinase activity compared to broccoli and kale. For this reason, we have purified myrosinase from horseradish roots. The purified enzyme from horseradish has two identical subunits of a total molecular mass of about 130. The enzyme exhibited a very broad pH and temperature optimum. The enzyme reached its highest activity at pH 5.8 and maintained nearly 50% of its activity at pH 3.4 and 9. The optimum temperature for the purified enzyme was between 37°C and 50°C but maintained nearly 60% of its activity at 59 to 62°C. The broad pH and temperature range suggest that the enzyme is capable of surviving the environment of the stomach. It also suggests that the conversion of glucosinolates into bioactive isothiocyanates continues after the cruciferous vegetable are consumed.

In addition, we have found that horseradish myrosinase is capable of breaking down different types of glucosinolates in different vegetables and that the breakdown is very rapid. We have also been successful in cloning this enzyme in *Spodoptera frugiperda* insect cells.

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

Dr. Hao Feng, Department of Food Science and Human Nutrition

5.1. Inactivation of *Escherichia coli* O157:H7 with Peroxyacetic Acid, Acidic Electrolyzed Water, and Chlorine on Cantaloupes and Fresh-cut Apples

The necessity of conserving freshness and nutrition in fresh and fresh-cut produce precludes the use of high temperature sterilization or preservatives to control food borne pathogens. Therefore, an improved wash step for the removal of human pathogens and spoilage microorganisms is highly desirable. The main objective of this study was to investigate the inactivation of *E. coli* O157:H7 with peroxyacetic acid (POAA), acidified electrolyzed water (AEW), and chlorine on cantaloupes and fresh-cut apples. These two types of produce were chosen for their rough and porous surface characteristics so that limitations of the sanitizers for the inactivation of *E. coli* O157:H7 could be evaluated. Apple cylinders were dip inoculated with a cell suspension of *E. coli* O157:H7 and then treated with sterilized water (control), chlorine, AEW or POAA. Cantaloupe cylinders with skin were spot inoculated with *E. coli* O157:H7 before treatment with sterilized water (control), AEW or POAA. All sanitizer treatments showed a significantly ($P < 0.05$) higher inactivation than the control. The POAA treatment was more effective in the inactivation of *E. coli* O157:H7 compared to other sanitizers used in this study. The residual counts of *E. coli* O157:H7 on the surfaces of fresh-cut apples or cantaloupes exhibited a dual-phasic reduction behavior, with a fast inactivation (D-values of 0.8-5.0 min) in the first min (phase I) of a treatment followed by a much slower inactivation (D-values of 14.6-59.8 min) in the remaining time (phase II). The dual-phasic inactivation seems to be related to the fruit

surface topography that determines the bacterial distribution and may be used to ascertain the optimal washing time in a sanitation treatment.

5.2. Washing Conditions Affect the Inactivation of *Escherichia coli* O157:H7 on Fruit Surfaces

Washing with sanitizers is an important step to reduce microbial populations during produce postharvest handling. The washing efficacy of sanitizers has been extensively studied in the contexts of produce-to-sanitizer ratio, washing time, and washing temperature. Little attention has been paid to the effect of flow conditions on the efficacy of a washing treatment. This study was undertaken to investigate effects of washing conditions such as flow rate, agitation rate, and treatment time on bacterial reduction on rough fruit surfaces. Cantaloupe and apples cylinders were prepared with and without skins respectively. The top surfaces of the cylinders were spot inoculated with *E. coli* O157:H7 before treatment with a sanitizer (Tsunami). Models were developed to determine correlation between *E. coli* O157:H7 inactivation and the wash conditions. The results showed that the residual bacterial population decreased with increasing agitation rate, flow velocity, and treatment time. A quadratic regression function was found to best fit the flow velocity/agitation rate to normalized log reductions (N/N_0 , with N the final pathogen population and N_0 the initial pathogen population). A logarithmic relationship was found to best correlate the treatment times to $\log N/N_0$ at each flow rate. It is therefore important to maintain the optimum flow velocity, agitation rate, and washing time to achieve the maximum reduction of bacterial populations and ensure food quality and safety.

5.3 Examination of Cell Morphological Changes of *Escherichia coli* Treated with Acidic Electrolyzed Water, Peroxyacetic Acid and Chlorine Using a MFP-3DTM Atomic Force Microscope

The MFP-3DTM atomic force microscopy (AFM) is a newly developed AFM with high precision, accuracy and flexibility in acquiring and analyzing images and conducting specific measurements. The aim of this study was to investigate the bactericidal mechanism of acidic electrolyzed water (AEW), peroxyacetic acid (POAA) and chlorine on *Escherichia coli* K12 cells by examining the changes in the cell morphology of the bacterium with a MFP-3DTM AFM. One ml *E. coli* K12 cell suspension was treated with 9 ml AEW, chlorine or POAA for 30, 60, or 120 s. At the end of each contacting time, 0.1 ml treated mixture was diluted with sterilized water, from which 5 ml was taken and concentrated on an isopore filter. The filter was fixed to the center of a glass slide for AFM imaging in a tapping mode with a silicon tip. The force was measured in a contact mode with a silicon nitride tip. Viable bacteria were enumerated by the culturing method using MacConkey agar media. Cell morphology changes were observed on *E. coli* after a 30 s treatment. Extending contacting time to 120 s did not cause any additional morphological changes. POAA treatment caused separation of cell membrane from cell cytoplasm while AEW and chlorine treatments damaged cell surfaces as evidenced by significant changes in surface topography and morphology. Adhesion force between AFM tip and cell surface was sharply decreased when morphological changes were observed. The differences in cell morphological changes of *E. coli* cells treated with three sanitizers indicate that different bactericidal mechanism among the sanitizers may have existed. The similarity in cell morphological changes of *E. coli* treated with AEW and chlorine suggests a similar inactivation mechanism. MFP-3DTM AFM can be a useful tool to elucidate the bactericidal mechanism of sanitizers by examining the cell morphological changes.

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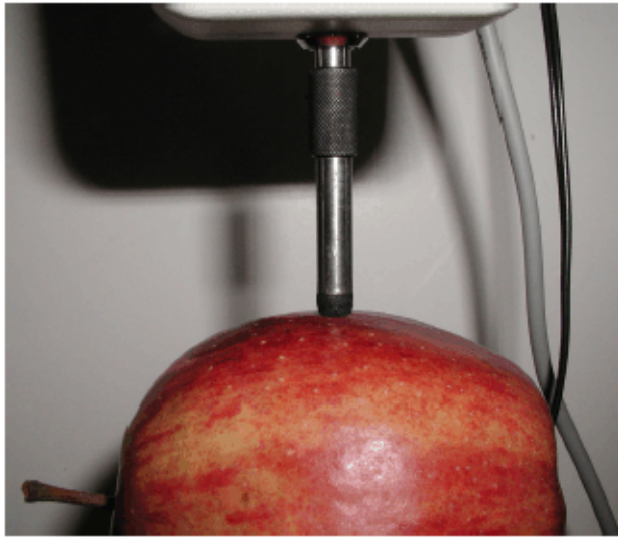
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Introduction: Two projects were completed this past year. One project was to develop a prototype non-destructive firmness tester for apple. Working with the USDA Ag. Engineering group and the Biosystems and Ag. Engineering Department at MSU, a prototype was developed that accurately assesses fruit textural properties while bringing about minimal damage to the fruit. An on-line unit should be able to accurately identify and cull soft apple fruit and avoid their use in fresh-cut fruit preparations. A second project was to evaluate the impact of non-target materials on the sorption of 1-MCP, which might be included in packaged produce or used to treat produce in packages. We found that cellulose-containing materials were not inert and could remove a significant portion of the 1-MCP.

Activities

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



Development of a bioyield tester for intact apple (Tipper, Lu, Beaudry, Srivastava). Firmness is an important quality attribute for intact and fresh-cut apple fruit and directly influences consumer purchase decisions. Currently, several fruit growing states in the United States require that fresh apples meet a specific firmness standard before they can be shipped to the market. The Magness-Taylor (MT) fruit firmness tester is the standard device used by the industry to measure fruit firmness. MT firmness measurements involve the penetration of a round steel probe (11 mm

in diameter for apples) into fruit flesh to a specified depth (normally 9 mm). The maximum force recorded is considered to be a measure of fruit firmness. The penetration process takes place in the form of compression, shearing, and tension, which is difficult to quantify. The technique is destructive and prone to operational error. Many nondestructive or minimally destructive devices or methods have been developed for measuring fruit firmness. Most nondestructive mechanical methods measure elastic properties, such as elastic modulus, as an index of fruit firmness.

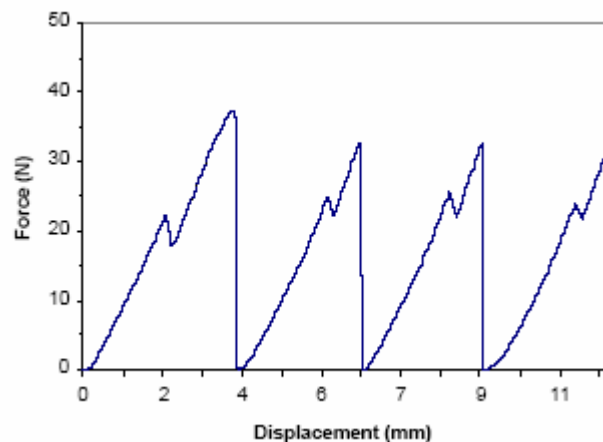
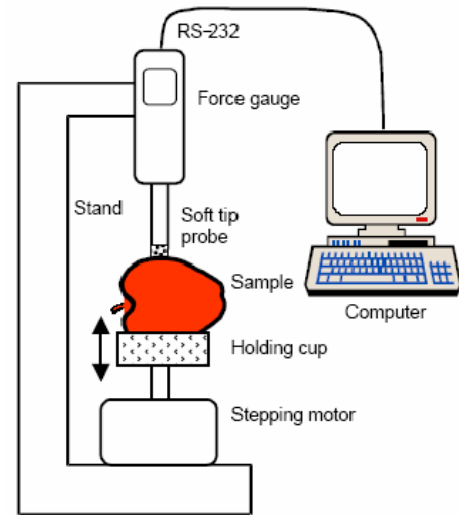
The concept of using bioyield force as an indicator of fruit firmness goes beyond elastic deformation but has not resulted in a complete rupture of fruit tissue. According to Hertz contact theory, when a rigid probe is compressed against a fruit, the stress distribution is not uniform within the contact area and stress concentration occurs. This non-uniform stress distribution causes the tissue within the contact region to fail gradually, making it more difficult to detect the bioyield point from the force-deformation curve. We studied several sizes of rigid probes with different tip designs, i.e., the elasticity and thickness of the tip material. A soft rubber tip allows for large elastic deformation, producing a uniform stress distribution within the contact area. This uniform stress distribution should improve the detection of the bioyield point because the fruit tissue within the contact region tends to fail simultaneously, creating a sudden drop in force that is easy to detect. Experiments of Ababneh (2002) confirmed that a soft tip probe usually produced a smooth force-deformation curve with a sharp bioyield point, whereas with a rigid probe, the force-deformation curve was less smooth, characterized with a series of micro failure taking place over a range of deformation and the bioyield point was less evident and sometimes could not be identified. A correlation of 0.83 between bioyield force and MT firmness was obtained for the pooled data of four apple varieties.

The objective of this research was to further evaluate the bioyield probe designed by Ababneh by coupling it to a portable digital force gauge for measuring the firmness of apple fruit with the ultimate goal of developing a portable bioyield tester for laboratory and field uses. Specific objectives were to:

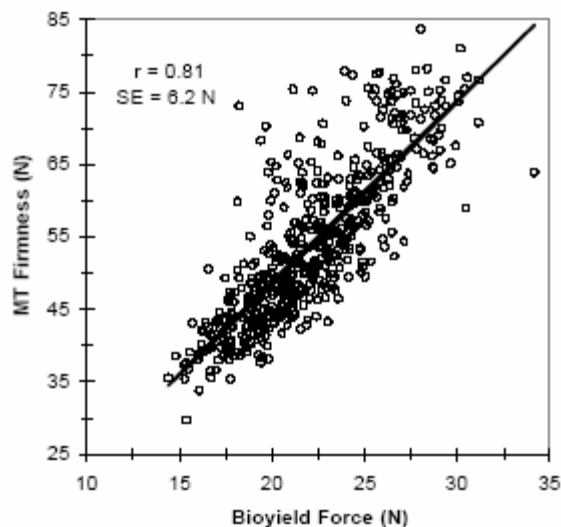
1. Determine correlations between MT firmness and bioyield force for two cultivars of apples;

2. Compare the changes of bioyield force and MT firmness over time under different postharvest storage conditions/treatments; and
3. Evaluate the variability of bioyield force and MT firmness measurements within individual apple fruit. In this article, unless otherwise specified, the term 'bioyield force' is referred to the force to cause bioyield by the soft tip probe, designated as F_{byst} , which is different from the bioyield force for the rigid probe.

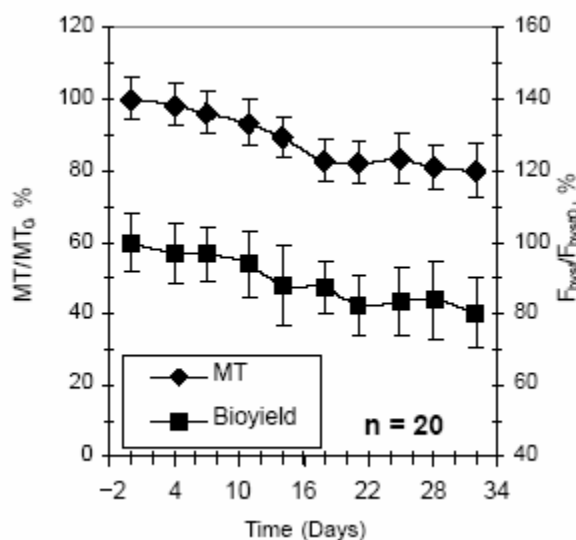
The specially designed soft tip bioyield probe was coupled to a handheld digital force gauge that was mounted on a tabletop motor-driven stand. Bioyield force (or F_{byst} = force to cause bioyield by the soft tip probe) and Magness-Taylor (MT) firmness were measured on Delicious and Golden Delicious apples under different storage conditions and/or treatments. The correlation between MT firmness and F_{byst} ranged from 0.62 to 0.81 with a standard error between 5.2 N and 6.7 N. Bioyield force decreased at about the same rate as that for MT firmness when apples were stored in refrigerated air at 5°C. When fruits were kept at room temperature (24°C), the percent decline in F_{byst} over the test time period was less than that in MT firmness. Bioyield force measurements within individual apples were 42.6% to 46.8% more variable than MT firmness, as measured by the coefficient of variation. Since the bioyield tester does not degrade apple fruit, it will be useful for measuring and monitoring fruit firmness during growth, harvest, and postharvest operations.



Force-deformation curves acquired from an apple fruit with the specially-designed 6.4-mm diameter bioyield tester at a loading rate of 0.37 mm/s



Correlation between Magness-Taylor firmness and bioyield force for the pooled data from three groups of Delicious apples (from cold and room temperature storage). Each data point represents the average of four measurements on each fruit.



Changes in MT firmness and bioyield force (F_{byst}) for Delicious apples over time, expressed as a percentage of the respective values for the first test date (day 0) for three groups of apples.

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

Sorption of 1-MCP by non-target materials (Vallejo, Beaudry). The sorption of 1-methylcyclopropene (1-MCP) by a number of 'non-target' materials found in apple (*Malus x domestica* Borkh.) and pear (*Pyrus communis* L.) fruit storages was measured as a function of time, temperature, and moisture content. Materials included bin construction materials [high density polyethylene (HDPE), polypropylene (PP), extensively- and slightly-weathered oak (*Quercus* sp.), weathered fir (*Abies* sp.) plywood, and cardboard] and wall surface materials (polyurethane foam and cellulose-based fire retardant). Bin construction material test pieces had an external surface area of 180 cm². We placed 'non-target' materials in 1-l glass jars and added 1-MCP gas to the headspace at an initial concentration of approximately 30 $\mu\text{l l}^{-1}$. Gas concentrations were measured after 2, 4, 6, 8, 10, and 24 h. The concentration of 1-MCP in

empty jars was stable for the 24-h holding period. There was little to no sorption by HDPE, PP, polyurethane foam, or fire retardant. However, plywood, cardboard, slightly-weathered oak, and extensively-weathered oak absorbed 16, 18, 55, and 75 percent of the 1-MCP after 24 h. Moistening the test material increased the rate of sorption of 1-MCP for cardboard, plywood, weathered oak, and non-weathered oak, resulting in a depletion of approximately 98, 70, 98, and 98 percent, respectively, in 24 h. For oak bin material, the rate of sorption was not impacted by temperature and increasing the surface area by approximately 100% only marginally increased the rate of sorption. When moistened oak bin material was included with apple fruit in a proportion similar to that found in fruit storage, 90% depletion occurred in 6 h compared to approximately 80% in 24 h for fruit alone. The data suggest that 1-MCP levels can be compromised by wooden and cardboard bin and bin liner materials, but not by plastic bin materials or wall surface materials commonly used in Michigan.

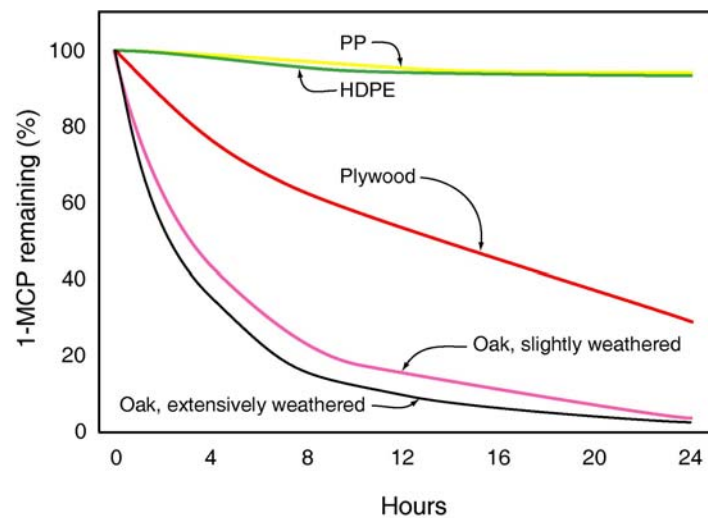


Figure 1. Effect of material on the sorption of 1-MCP at 20 °C for wetted test samples of ‘non-target’ bin materials found in apple and pear fruit storages as a function of time. The initial 1-MCP concentration was approximately 30 ppm.

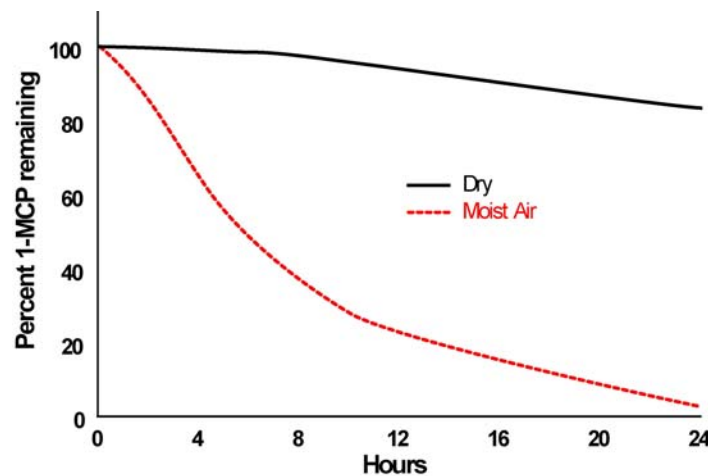


Figure 2. Effect of moistening of cardboard packaging material on time-dependent depletion of 1-MCP at 20 °C.

Bulleted brief synopsis of each major project underway in 2005-2006.

- Evaluated 1-MCP sorption by non-target materials and found that those containing cellulose absorbed significant quantities of 1-MCP (especially when dampened), which might compromise efficacy.
- Participated in a project to develop a prototype that is useful for non-destructive evaluation of apple fruit texture.
- Evaluated the impact of oxygen on the rate and extent of browning of cut leaf lettuce in modified atmosphere packages.

Bulleted brief synopsis of additional major project(s)

- Wrote a review article on modified atmosphere packaging
- Project underway to determine if wood or packing materials scalp sufficient 1-MCP to reduce its efficacy; determining the impact on the lowest effective concentration.
- Evaluated a microarray we constructed for determining the impact of ripening on gene expression in apple with special attention given to changes in genes associated with volatile synthesis and texture.

Publications

- Ben-Yehoshua, S., R.M. Beaudry, S. Fishman, S. Jayanty, and N. Mir. 2005 Modified atmosphere packaging and controlled atmosphere storage In: *Environmentally Friendly Technologies for Produce Quality*, S. Ben-Yehoshua, ed., CRC Press, Boca Raton, FL, pp. 61-112.
- Watkins, C.B., M. Erkan, J.F. Nock, K.A. Iungerman, R.M. Beaudry, and R.E. Moran 2005. Harvest date effects on maturity, quality and storage disorders of 'Honeycrisp' apples. *HortScience*, 40:164-169.
- Gao, Zhifang, S. Jayanty, R. Beaudry, and W. Loescher 2005. Sorbitol transporter expression in apple sink tissues: Implications for fruit sugar accumulation and watercore development. *J. Amer. Soc. Hortic. Sci.* 130(2):261-268.
- Lu, R., A. Srivastava, and R. Beaudry. 2005. A new bioyield tester for measuring apple fruit firmness. *Amer. Soc. Ag. Eng.* 21(5):893-900.
- Carrasco, B., J.F. Hancock, R.M. Beaudry, and J.B. Retamales. 2005. Chemical composition and inheritance patterns of aroma in *Fragaria hians* and *Fragaria virginiana* progenies. *HortScience*, 40:1649-1650.

Institution: Oregon State University

Participant: Jinhe Bai, Telephone 541-386-2030; Email: Jinhe.bai@oregonstate.edu

Activities

Objective 2: To develop new strategies to maintain fresh-cut product quality

- Effect of harvest maturity on quality of pear salad: traditional harvest maturity of pears is not suitable to process salad because the taste is flat and the texture is coarse at an unripe stage. In this research, 'Anjou' pears were harvested at commercial maturity and one month after the commercial harvest (delayed harvest). After two and five months of cold storage, fruit were sliced and stored at 1°C for up to 3 weeks. Fruit were larger in size,

less sour, sweeter (higher SS/TA ratio), richer in aroma, had less browning potential, and had a juicier and crispier texture by delaying harvest. A significant improvement of salad quality was achieved by delaying harvest when the raw fruit were stored for two months. However, both commercial and delayed harvested pears had poor quality with little difference between treatments when stored for five months.

- Effect of ethanol release pad on cored cherries: Ethanol-release powder (Antimold Mild®, Freund Industrial, Japan) allows ethanol vapor to diffuse gradually. It is made from ethanol absorbed onto silica gel that is packed in a special film, laminated with ethylene-vinylacetate and Japanese paper, which regulates ethanol diffusion. The ethanol pads reduced deterioration of wound surface and increased shelf-life of cored cherries at 1, 10 and 20°C.

Publications

Plotto, A., J. Bai, J.A. Narciso, J.K. Brecht, and E.A. Baldwin. 2006. Ethanol vapor prior to processing extends fresh-cut mango shelf-life by decreasing spoilage, but does not always delay ripening. *Postharvest Biol. Technol.* 39: 134-145.

Institution: USDA/ARS Citrus & Subtropical Products Laboratory

Participants: Elizabeth A. Baldwin (ebaldwin@citrus.usda.gov), Jan Narciso (Jan@citrus.usda.gov), and Anne Plotto (Plotto@citrus.usda.gov)

Introduction

The ARS Citrus & Subtropical Products Laboratory group has expertise in food science, postharvest physiology, postharvest pathology, edible coatings, sensory and flavor chemistry that can be applied to fresh-cut fruit and vegetable research. Cooperative relationship exists with the Horticultural Sciences Department at the University of Florida. For this reporting period, work on pretreatment of whole mango for fresh-cut benefit and post-cutting treatment of mango slices was completed and resulted in two publications, while another manuscript is in preparation. Work on strawberries both pre- and post harvest has been conducted for two seasons and a manuscript is in preparation. Treatment of mangoes with 1-MCP was beneficial for fresh-cut shelf life, but the levels of 1-MCP needed are above what is allowed. Treatment of mangoes with ethanol vapor reduced spoilage and maintained appearance, but resulted in off-flavor at the higher treatment levels. Treatment of intact mangoes with peroxyacetic acid reduced microbial counts in the subsequent cut slices compared to treatment with chlorine, which has been published. Pre- and post-harvest treatment of strawberries with peroxyacetic acid reduced decay and extended postharvest shelf life. Postharvest treatment of strawberries, whole or topped, with chitosan coatings or pectin in acetate buffers also reduced decay. Treatments were developed for enzyme-peeled oranges to improve appearance, texture and microbial stability, some of which has been published.

Activities

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

- Pre-cutting treatments to optimize post-cutting shelf life and reduce microbial contamination from the fruit surface were explored on mango and topped strawberries. Flavor, decay and shelf-life analysis of 1-MCP treated tomatoes was also done.
- Flavor was evaluated on fruit homogenate by headspace GC using standard curves where authentic standards were injected into deodorized fruit homogenate at 5-7 levels with any volatiles left in the deodorized background subtracted. Some samples were run on GC-MS equipped with the same column(s) for peak retention time identification. Both polar and non-polar columns were used (Stabilwax and DB-5).
- Sensory ranking of samples for overall preference, firmness, tartness, (mango) flavor and off-flavor was performed using experienced consumer panels of 15+ members, repeated were compared to the instrumental results.
- Methods for testing and comparing sensory and analytical measurements for flavor research are ongoing for tomato flavor and orange juice aroma thresholds in the juice matrix. Perception of β -ionone was found to be bi-modal in that roughly half the panelists were super perceivers while the other half were not. This was not based on age or sex, but appeared to be a genetically-based.
- Trained panel rating of generated flavor descriptors for aroma and flavor of fresh tomato was also performed. Panelists (8-10) were trained using the Meilgaard method
- Texture was measured using a texture analyzer TX2i equipped with a 1-cm diameter probe inserted 5 mm for two measurements on each fruit (mango) slice, or 2 mm on radial arms of a tomato cross-section with a probe (method developed previously by Judy Abbott).
- Cut fruit color was measured with a Minolta CR-300 Chroma Meter calibrated to a white plate using the CIE L*, a*, b* system. Usually Hue angle was used.

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

- Methods to extend shelf life of fresh cut produce were and will continue to be tested, and sensory and chemical analysis of flavor descriptors, acceptability, and concentration of chemical components will be evaluated. Relationships between treatments, chemical flavor components and flavor descriptors will be explored.
- Color, firmness and physiological parameters such as ethylene production and respiration rate will also be monitored for effect of pre- and post-cutting treatments and storage conditions.
- Mango fruit were cut and treated with edible polysaccharide coatings with or without additives at applied to cut mango pieces. Weight loss, respiration, ethylene, color, firmness, sugar, acids and aroma measurements were taken.

- Strawberries were treated pre- and/or postharvest with antimicrobial compounds including peroxyacetic acid and plant extracts, then evaluated for postharvest decay. Some compounds were also evaluated in vitro by placing compounds on paper disks in agar inoculated with fruit pathogens and measuring zones of inhibition. Promising compounds were also evaluated on fruit peel pieces in vitro, and ultimately on whole or cut fruit.
- Treatments were developed for enzyme-peeled oranges to improve appearance, texture and microbial stability of the peeled slices.

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

- Strawberries were treated with pectin and chitin/chitosan fragments and pectin and chitosan coatings. Fruit were evaluated for ethylene production and incidence of decay. Frozen samples will be analyzed for induced plant defense proteins.

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

- Routinely recover non-pathogenic microorganisms from fruit surfaces to screen for antagonist activity and pathogenic organisms for culture collection.

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

- Microbial assays were conducted on the cut fruit where 3-4 representative fruit pieces were taken and placed in sterile sampling bags with sterile phosphate buffer, agitated for 2 min and aliquots taken in triplicates and analyzed on a Whitley Automatic Spiral Plater. Isolations of microorganisms from fruit pieces were made using plate count agar and potato dextrose agar for bacteria, yeasts and molds.
- Pre-treatment of whole mango fruit with peroxyacetic acid was found to be more effective than chlorine in reducing microbial counts of the cut slices in combination with post-cutting sanitizing treatments with no adverse effects on quality.

Publications

Bai, J., E.A. Baldwin, K.L. Goodner, J.P. Mattheis, and J.K. Brecht, J.K. 2005. Response of four apple cultivars to 1-methylcyclopropene treatment and controlled atmosphere storage. *HortScience* 40:1534-1538.

Baldwin, E.A. and B. Wood. 2006. Use of edible coatings to preserve pecans (*Carya Illinoensis*) at room temperature. *HortScience* 41:188-192. 2006

Cameron, R., G. Luzio, E. Baldwin, J. Narciso and A. Plotto. 2005. Production of narrow-range size-classes of polygalacturonic acid oligomers. *Proc. Fla. State Hort. Soc.* 118:406-409.

Georgelis, N., J.W. Scott, and E.A. Baldwin. 2006. Inheritance of high sugars from tomato accession PI270248 and environmental variation between seasons. *J. Amer. Soc. Hort Sci.* 131: 000-000 (in press).

Ismail, M.A., H. Chen, E.A. Baldwin, and A. Plotto. 2005. Changes in enzyme-assisted peeling efficiency and quality of fresh ‘Valencia’ orange and of stored ‘Valencia’ orange and ‘Rub Red’ grapefruit. *Proc. Fla. State Hort. Soc.* 118:403-405.

Ismail, M.A., H. Chen, E.A. Baldwin, and A. Plotto. 2005. Optimizing the use of hydrolytic enzymes to facilitate peeling of citrus fruit. *Proc. Fla. State Hort. Soc.* 118:400-402..

Mahattanatawee, K., K.L. Goodner, and E.A. Baldwin. 2005. Volatile constituents and character impact compound of Florida’s tropical fruit. *Proc. Florida State Hort. Soc.* 118:414-418.

Narciso, J. and A. Plotto. 2005. A comparison of sanitation systems for fresh-cut mango. *Hort. Tech.* 15(4): 837-842.

Narciso, J. 2005. An assessment of methods to clean citrus fruit surfaces. *Proc. Fla. St. Hort. Soc.* 118:437-440.

Narciso, J. and M.E. Parish. 2005. A method for detecting fungal contamination in paperboard cartons. EDIS: IFAS Extension. FSHN04-07. 5 pg.

Plotto, A., J. Bai, J.A. Narciso, J.K. Brecht, and E.A. Baldwin. 2005. Ethanol vapor prior to processing extends fresh-cut mango storage by decreasing spoilage, but does always delay ripening. *Postharv. Biol. and Technology* 39: 134-145.

Plotto, A. and J. Narciso. 2006. Guidelines and acceptable postharvest practices for organically grown produce. *HortScience* 41(2): 287-291.

Plotto, A., K.W. Barnes, and K.L. Goodner. 2006. Specific Anosmia observed for β -ionone, but not for α -ionone. *J. Food Sci.* In press.

Institution: Produce Quality and Safety Laboratory, Beltsville Agricultural Research Center, Agricultural Research Service, United States Department of Agriculture, Beltsville, MD 20705.

Participants: William Conway (Conwayw@ba.ars.usda.gov)
 Kenneth C. Gross (Grossk@ba.ars.usda.gov)
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 James L. McEvoy (McEvoyj@ba.ars.usda.gov)
 Robert A. Saftner (Saftnerr@ba.ars.usda.gov)
 Chien Yi Wang (Wangc@ba.ars.usda.gov)

Introduction

The Produce Quality and Safety Laboratory (PQSL) group is integrating its multidisciplinary expertise in postharvest physiology, plant physiology, plant pathology, microbiology, horticulture and food science to conduct fundamental and applied research on fresh produce. Our goal is to develop technology to maintain and enhance food safety and quality of fresh and fresh-cut fruits and vegetables. Specific goals are: 1) to understand changes of plant metabolic process, food-borne microbial attachment, growth and survival, and host-pathogen interactions as influenced by post-harvest handling, agricultural practice, and fresh-cut preparation conditions; 2) to devise intervention strategies to eliminate or control the presence and/or growth of foodborne pathogens using biocontrol agents, ozone, electrolyzed water, and other physical/chemical approaches; 3) to develop integrated strategies to maintain quality of fresh and fresh-cut product using CA and MAP, varietal selection, preharvest growing strategies, and other emerging technologies such as 1-MCP; and 4) to advance quality evaluation technology by integrating sensory and instrumental analysis of texture in fresh and fresh-cut produce.

Collaborative Project Titles with Other S-294 Members:

Improving food safety and quality of fresh-cut produce (Drs. Yaguang Luo and Hao Feng)

Publications

1. Allende, A., McEvoy, J., Luo, Y. and Wang, C.Y. 2005. Effectiveness of two-sided UV-C treatments in inhibiting natural microflora and extending the shelf-life of minimally processed 'Red Oak Leaf' lettuce. *J. Food Microbiology*. In press.
2. Leverentz, B., Conway, W. S., Janisiewicz, W. J., Abadias, M., Kurtzman, C. P., and Camp, M. J. 2005. Biocontrol of the food-borne pathogens *L. monocytogenes* and *Salmonella enterica* Serovar Poona on fresh-cut apples with naturally occurring bacterial and yeast antagonists. *Appl. Environ. Microbiol.* 72:1134-1140.
3. Ruiz-Cruz, S., Luo, Y., Gonzalez, R.J., Tao, Y., Gonzalez, G. 2006. Effect of Acidified Sodium Chlorite Applications on Microbial Growth and the Quality of Shredded Carrots. *J. Food Ag.* In press.
4. Saftner, R. A., Abbott, J. A., Lester, G., Vinyard, B. T. 2006. Sensory and analytical comparison of orange-fleshed honeydew to cantaloupe and green-fleshed honeydew for fresh-cut chunks. *Postharvest Biology Technol.* In press.
5. Saftner, R. A., Abbott, J. A., Bhagwat, A. A., Vinyard B. T. 2005. Quality measurement of intact and fresh-cut slices of Fuji, Granny Smith, Pink Lady, and GoldRush apples. *J. Food Sci.* 70:S317-S324.
6. Vargas, A., Kim, M., Tao, Y., Lefcourt, A., Chen, Y.R., Luo, Y., Song, Y. 2005. Fecal contamination detection and classification on cantaloupes using hyperspectral fluorescence imagery. *J. Food Sci.* 70 (8): 471-0476.

Funded Proposals & Grants pertaining to fresh-cut produce

- Luo, Y. Evaluate the effect of SmartFresh™ treatment concentration and application time on the quality and shelf-life of fresh-cut watermelons (A trust fund with AgroFresh, Inc. Springhouse, PA).
- Luo, Y. and McEvoy, J. 2006. Investigate “Bacterial Pathogen Transfer Rates through Perforated Films”; A Trust Fund with Amcor Flexibles Corp. (United Kingdom).

Work in Progress:

William Conway. In collaboration with Drs. Maribel Abadias, Britta Leverentz, and Wojciech J. Janisiewicz, fresh - cut apples contaminated with either *L. monocytogenes* or *S. Poona*, both foodborne pathogen strains from outbreaks on produce, were treated with one of seventeen

antagonists originally selected for their ability to inhibit fungal postharvest decays on fruit. While most of the antagonists allowed for increasing growth of the foodborne pathogens on fresh-cut apple tissue, four antagonists, including *Gluconobacter asaii* (T1-D1), a *Candida spp.*, *Discosphaerina fagi* and *Metschnikowia pulcherrima* prevented their growth or survival. The contaminated apple tissue plugs were stored for up to seven days at two different temperatures. The four antagonists survived or grew on the apple tissue at 10 or 25°C. These four antagonists reduced the *L. monocytogenes* populations and except for the *Candida spp.* (T4-E4), also reduced *S. Poona* populations. The reduction was higher at 25°C than at 10°C, and the growth of the antagonists, as well as pathogens, increased at the higher temperature.

Yaguang Luo. The major research activities in Dr. Luo's research program include the evaluation of the effects of the SmartFresh application on the quality and shelf life of fresh-cut watermelons, broccoli, and tomatoes, as well as the influence of fresh-cut preparation conditions on microbial growth. In collaboration with Drs. R.A. Saftner, J. Abbott, J.L. McEvoy and H. Feng and AgroFresh Inc., Dr. Luo evaluated the effect of 1-MCP application time and concentration on quality retention of fresh-cut watermelons. Dr. Luo collaborated with Dr. H. Feng (UIUC) on the investigation of the surface topography of fresh-cut fruits, as well as washing conditions on the removal of pathogenic bacteria. Dr. Luo also developed a novel application of a chemical substance with dual effectiveness in browning inhibition and pathogen inactivation and filed a patent application on this. Dr. Luo also collaborated with Dr. Jim McEvoy to evaluate the effect of micro perforation of packaging film on the potential of pathogen transfer. In collaboration with Dr. W. Conway and AirOcare Inc. (Rockville, MD), Dr. Luo investigated the effect of ozone and active oxygen on ripening delay and shelf life extension of bananas, strawberries and grapes.

Robert Saftner. In collaboration with Dr. Bhagwat, have shown that an in-house processing aid/preservative formulation maintains the analytical, sensory and microbial quality and food safety of fresh-cut apple products: the formulation prevented contamination and growth of *Lysteria monocytogenes* and *Salmonella spp.* on packaged apple slices contaminated with low concentrations (<20 cfu g⁻¹) of the human pathogens and inhibited contamination and growth at higher pathogen concentrations.

In collaboration with Gene Lester, have evaluated the analytical, microbial and sensory quality attributes of a netted, orange-fleshed hybrid melon bred specifically for the fresh-cut industry in the winter season. Consumers liked the flavor, texture, sweetness and overall eating quality of the hybrid chunks better than those of its inbred parents and winter-available honeydew and as well or better than that of winter available cantaloupe. Hybrid fruit stored 5 weeks under refrigeration and MA conditions, then fresh-cut and stored 14 d in air at 5 °C maintained good quality ($F_{\max} = 51\text{N}$, $\text{SSC} > 12\%$, surface pH 6.0, β -carotene and ascorbic acid concentrations = 14 and 182 mg kg⁻¹, respectively) and showed no signs of tissue translucency or surface pitting despite microbial populations approaching 8 log cfu g⁻¹.

In collaboration with Drs. Luo and McEvoy, have evaluated the efficacy of 1-MCP treatment of whole watermelon at maintaining the analytical quality of whole and fresh-cut watermelon exposed to ethylene. Results indicated that low (0.5 and 1.0 $\mu\text{L L}^{-1}$ for 18 h at 20°C) dosage 1-MCP treatments of whole watermelons prevented ethylene-mediated quality deterioration in fresh-cut slices but did not otherwise maintain the quality or shelf stability of watermelon slices stored under modified atmospheric conditions at 5 °C.

Institution: USDA, ARS, Eastern Regional Research Center, 600 E. Mermaid Lane, Wyndmoor, PA 19038

Participant: Xuotong Fan (email:xfan@errc.ars.usda.gov), Bassam Annous

Introduction: Fresh produce is an important part of a healthy diet as it provides needed nutrients, fiber and antioxidants and increasing the consumption of produce by the U.S. consumer is desirable. Unfortunately, during the last three decades there has been an increase in the number of foodborne outbreaks due to consumption of fresh and fresh-cut fruits and vegetables contaminated with a variety of human bacterial pathogens such as *Salmonella* spp., *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Shigella* spp. There is a need for new or improved cost-effective intervention technologies which when implemented can reduce the risk of produce-related outbreaks due to naturally occurring contamination or deliberate contamination. In order for effective interventions to be commercialized, product quality after treatment must be maintained. The effects of single or combined effective interventions on visual quality (e.g., color and appearance), odor, texture, vitamin content, antioxidant levels and shelf-life needed to be assessed.

Activities

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

2.1. Combination of Hot Water Surface Pasteurization of Whole Fruit and Low Dose Gamma Irradiation of Fresh-cut Cantaloupe (X. Fan, B. Annous, K. Sokorai, A. Burke, J. Mattheis)

Many antimicrobial sanitizers often have limited effectiveness for reducing the microbiological population on the surface of cantaloupes, partially due to the rough surface (netting). It has been shown by ERRC scientists that surface pasteurization with hot water resulted in more than 5 log CFU/cm² reduction in *Salmonella*. Ionizing radiation is an effective non-thermal technology that inactivates a number of foodborne pathogens on fresh fruits and vegetables. The objective of this study was to investigate the effect of hot water treatment of whole cantaloupe prior to cutting followed by the low dose irradiation of fresh-cut cantaloupe prepared from the treated melons on indigenous microbial population and quality of fresh-cut melon during storage at 4°C. Whole cantaloupes were washed in tap water at 20°C or 76°C for 3 min. Fresh-cut cantaloupe cubes, prepared from the washed fruit, were then packaged in clamshell containers, and half the samples were exposed to 0.5 kGy of gamma radiation. Native populations of microflora and sensory qualities were determined during subsequent storage at 4°C over a period of 7 days. Results showed that hot water surface pasteurization reduced the microflora population by 3.3-logs on the surface of whole fruits, resulting in a lower microbial load on fresh-cut cubes, compared to those from cold water treated fruit. Irradiation of cubes prepared from untreated fruit to an absorbed dose of 0.5 kGy achieved similar low microbial load of the cubes as those prepared from hot water treated fruit. The combination of the two treatments was able to further reduce the microflora population. During storage, the headspace atmosphere of the packages was not significantly influenced by any of the treatments. Color, titratable acidity, pH, ascorbic acid, firmness, and drip loss were not consistently affected by treatment with irradiation, hot water or the combination of the two. Cubes prepared from hot water treated whole fruit had slightly lower soluble solids content. Our results showed that the combination of hot water pasteurization

of whole cantaloupe and low dose irradiation of packaged fresh-cut melon can reduce the population of native microflora while maintaining quality of this product.

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

5.1. Effects of Negative Air Ions on *E. coli* ATCC 25922 Inoculated on Mung Bean Seed and Apple Fruit (X. Fan, W. Fett, B. Mitchell)

The effect of negative air ions on the reduction of *E. coli* ATCC 25922 inoculated on mung bean sprout seed and whole or fresh-cut apple fruit was studied. Mung bean seeds, whole “Gala” apples, and apple slices were inoculated with *E. coli* ATCC 25922 before being exposed to negative air ions for up to 18 h at room temperature (~23°C). Results showed a less than 0.5 log₁₀ reduction of *E. coli* on mung bean seed even after 18 h of exposure. The reduction of *E. coli* on the surface of whole apples increased with increasing exposure time from 0.5 to 3 h, but the maximum reduction was less than 1 log₁₀. Increasing exposure time from 3 h to 18 h did not lead to increased treatment efficacy. No reduction of *E. coli* was observed on apple slices after 3 h treatments. When the negative air ion system was applied together with acetic acid vapor, no additive or synergistic effect of negative ions on the reduction of *E. coli* was found. The results suggested that negative air ions had a very limited effect on the population of *E. coli* on mung bean seed and apples.

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Activities

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

A patent was filed regarding a multi-functional technology to preserve quality in all fresh-cut products, however the details cannot be disclosed until publication. First disclosure is possible by July 2006.

Work with Dr. Kwang Deog Moon (Korea) provided early indication that micro-perforated films resulting in ~ 8% O₂ and ~10% CO₂ atmospheres can tremendously improve flavor and aroma in apple slices. Only preliminary work has been done, however the results are quite dramatic. Further work will require collaboration with a lab capable of measuring aroma volatiles to provide instrumental confirmation of this effect.

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

Ongoing work has continued with Dr. Jennifer DeEll (Ontario Ministry Agriculture, Food and Rural Affairs) in regards to comparative suitability of cultivars for fresh-cut apple slices. A variety which was consistently “non-browning” from the AAFC breeding program at St-Jean-sur-Richelieu, Quebec has been named ‘Eden’. This cultivar has low phenolic content, however, it is not known if this is the only factor in its apparent resistance to cut-edge browning. While it is classed as ‘non-browning’ it can still brown if stored in air too long, or in improper CA storage conditions. Further work is required to better understand optimal storage conditions.

Other cultivars which appear to be “low-browning” are ‘Ambrosia’ and a new cultivar from the AAFC breeding program in Summerland named ‘Sabina’. The mechanism for these two cultivars low browning character appears to be largely due to the resistance of their membranes to deterioration after cutting.

There is ongoing work with Dr. Jennifer DeEll (Ontario Ministry Agriculture, Food and Rural Affairs), Pride Pak (Mississauga, ON) and the Ontario Apple Growers to develop strategies to

identify when apple lots from storage are likely to have quality problems. This work is not analyzed or summarized at this point in time.

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

A research project funded by the Okanagan-Kootenay Cherry Growers' Association found that most hydro-cooler systems in the industry were performing well. Packing houses which charged their chlorine once only, at the beginning of days run, were able to maintain effective chlorination for the whole day. Coliforms, *E. coli* and aerobic mesophile bacteria were not found in appreciable levels in the hydro-cooling water during the harvest/packing season. This indicates that the packing house practices are sufficient. On the other hand, harvested cherries were coming in the field with high contamination levels. While spot tests were done on hands of pickers, no clear indication was found as to the source of the levels of contamination intercepted at the packing house. Next year will focus on environmental sampling to get a better handle on how and where microbial contamination is occurring on the cherries.

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