

Institution: University of Georgia, Athens and Griffin Campuses.

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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 immunocompromised 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 ≥ 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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