

## PRODUCE

### **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)

In recent years, the incidence of foodborne diseases associated with fresh produce has increased. During the decade preceding 1999, approximately 12% of foodborne illnesses in the U. S. have been linked to fresh fruits and vegetables. Bacteria belonging to the family Enterobacteriaceae have caused or been associated with outbreaks of foodborne illnesses implicating unpasteurized juice and fresh fruits and vegetables. Examples of these outbreaks include *Escherichia coli* O157:H7 infection linked to the consumption of lettuce and apple cider, salmonellosis linked to tomatoes and cantaloupe, and shigellosis linked to parsley. Outbreaks of *E. sakazakii* infections associated with fresh produce have not been documented. However, isolated *E. sakazakii* has been isolated from 8 out of 9 food factories and from 5 out of 16 households, and the organism has been isolated from lettuce and other vegetables. Because of its presence in the environment, there is a risk of contamination of fresh produce with *E. sakazakii*. 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 at storage temperatures used at retail and in food service and home environments.

We did a study to determine 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. Produce and juice were inoculated with *E. sakazakii* at populations of 2 - 3 log<sub>10</sub> CFU/g and 1 - 2 log<sub>10</sub> CFU/ml, respectively, and stored at 4, 12, or 25°C. 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. Growth occurred in all juices except apple, strawberry, and cabbage juice, followed by decreases in population to < 1 CFU/ml after 48 - 72 h, which coincided with decreases in pH and an increase in population of lactic acid bacteria. Increases in total counts occurred in all juices except strawberry juice stored at 25°C and apple and strawberry juice stored at 12°C. Total counts increased in cantaloupe, carrot, cucumber, and lettuce juice stored at 4°C. Populations of molds and yeasts increased in apple and tomato juice stored at 25°C but decreased to < 1 CFU/ml in cabbage, lettuce, and cucumber juice. 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.

### **SURVIVAL AND GROWTH OF *ENTEROBACTER SAKAZAKII* IN INFANT RICE CEREAL RECONSTITUTED WITH WATER, MILK, LIQUID INFANT FORMULA OR APPLE JUICE**

(G. M. Richards, J. B. Gurtler, and L. R. Beuchat)

Documented cases of infection caused by *Enterobacter sakazakii* are rare, although they have been more frequent during the past two decades. The bacterium has been implicated most often in causing illness in preterm neonates, infants and children from 3 to 4 years of age, with at least 76 cases and 19 deaths of infants being reported. Cases of *E. sakazakii* infections in adults also have been reported. Conditions affecting survival and growth of *E. sakazakii* in reconstituted infant formulae have been described. Minimum growth temperatures were reported to be 5.5 - 8.0°C. Guidance and recommendations concerning control and elimination of *E. sakazakii* in powdered infant formulae and reconstituted formulae have been issued in a joint report by the Food and Agriculture Organization/World Health Organization. Reports that *E. sakazakii* has been isolated from rice and rice products and that infants and young children have been diagnosed with infection caused by the bacterium raises concern about its behavior in reconstituted infant cereals.

We undertook a study to determine the survival and growth characteristics of *E. sakazakii* in infant rice cereal reconstituted with various liquids. The influence of storage temperature on survival and growth was determined. A commercially manufactured dry infant rice cereal was reconstituted with water, apple juice, milk, or liquid infant formula, inoculated with a 10-strain mixture of *E. sakazakii* at populations at 0.27, 0.93, and 9.3 CFU/ml, and incubated at 4, 12, 21, or 30°C for up to 72 h. Growth did not occur in cereal reconstituted with apple juice, regardless of storage temperature, or in cereal reconstituted with water, milk, or formula and stored at 4°C. The lag time for growth in cereal reconstituted with water, milk, or formula decreased as the incubation temperature (12, 21 and 30°C) was increased. Upon reaching maximum populations of 7 - 8 log<sub>10</sub> CFU/ml, in some instances populations decreased to nondetectable levels during subsequent storage which was concurrent with decreases in pH. *Enterobacter sakazakii*, initially at very low populations, can rapidly grow in infant rice cereal reconstituted with water, milk, or infant formula. Reconstituted infant rice cereal can support luxuriant growth of *E. sakazakii*. Reconstituted cereal that is not immediately consumed should be discarded or stored at a temperature at which *E. sakazakii* and other food-borne pathogens cannot grow.

**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)**

Surveys conducted by the U.S. Food and Drug Administration revealed that rinds of 7.3% of imported cantaloupes and 4.3% of domestically grown cantaloupes were positive for *Salmonella* or *Shigella*. Numerous national and international outbreaks of salmonellosis have been epidemiologically linked to fresh cantaloupes. *Salmonella* Poona was the predominant serotype responsible for these outbreaks. Removal of field heat from cantaloupes is often accomplished by forced-air cooling; however, hydrocooling and top icing are methods also currently used in the industry to rapidly attain temperatures of 2 to 4°C. The extent of infiltration of water into fruits and vegetables is generally dependent on factors such as length of exposure time, magnitude of temperature differential, immersion depth, agitation, viscosity of the external environment, and size and number of portals leading to internal airspaces. A negative temperature differential (i.e., when the temperature of the fruit is higher than the temperature of the water in which it is immersed) theoretically enhances infiltration of water and any microorganisms it might contain. Infiltration of water and plant pathogens into tomatoes has also been shown to be influenced by time- and temperature-independent hydrostatic forces in addition to time-dependent temperature differential phenomena.

The effect of temperature differentials on infiltration of *Salmonella* into cantaloupe rind has been described. The objective of this study was to assess the effects of temperature differentials between cantaloupes and suspensions (both at 4 and 30°C) of *Salmonella* Poona on changes in fruit weight and populations of the pathogen recovered from rinds and stem scar tissues of Eastern and Western cantaloupes. The percent weight increase in Western cantaloupes 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. The number of *Salmonella* Poona cells removed from rind tissue of Western cantaloupes at 30°C was significantly less ( $P \leq 0.05$ ) than that recovered from rind tissues of cantaloupes at 4 or 30°C that were immersed in inoculum at 4°C. *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 are not dictated entirely by temperature differentials between fruits and immersion suspensions; rather, they apparently are also influenced by structures unique to surface tissues.

**EVALUATION OF CHLORINE DIOXIDE AND A PEROXYACETIC ACID-BASED SANITIZER  
FOR EFFECTIVENESS IN KILLING *BACILLUS CEREUS* 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)

Pathogenic microorganisms can contaminate produce through contact with feces, sewage, water, soil, animals, foods, and other sources. 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 use of *Bacillus anthracis* as a weapon of terrorism was apparent in the fall of 2001 when letters laden with spores of the bacterium and sent through the U.S. postal system killed five people. The use of produce as a vehicle to transmit *B. anthracis* spores for the intended purpose of causing illness and death is an issue that deserves attention. While chlorine and chlorine dioxide inactivation of *B. anthracis* and other bacterial bioterrorism agents has been studied, the resistance of *B. anthracis* spores and spores of other *Bacillus* species to sanitizers used to decontaminate produce has been given only meager research attention.

Spores of *B. anthracis*, *B. cereus*, and insecticidal, crystal toxin-producing strains of *Bacillus thuringiensis* can be found in the soil. All three species are similar in physiological and morphological characteristics and all can cause human diseases. In the study summarized here, *B. cereus* and *B. thuringiensis* spores were used as surrogates for *B. anthracis* spores. The sensitivity of spores of these species to treatment with sanitizers would give insights to the behavior of *B. anthracis* spores. The objective of the study was to evaluate the efficacy of chlorine (10 - 200 µg/ml), chlorine dioxide (10 - 200 µg/ml), and Tsunami 200<sup>®</sup>, a peroxyacetic acid-based sanitizer (40 - 80 µg/ml), in killing spores of *B. cereus* and *B. thuringiensis* in suspension, on the surface of stainless steel, and on apples.

Water and 5% horse serum were used as carriers for spore inoculum applied to the surface of stainless steel coupons and 5% horse serum was used as a carrier for inoculum applied to apples. Inocula were dried on stainless steel for 5 h and on apples for 22 - 24 h before treating with sanitizers. At the concentrations of sanitizers tested, sensitivities of planktonic *B. cereus* and *B. thuringiensis* spores were similar. A portion of the spores surviving treatment with chlorine and, more markedly, chlorine dioxide had decreased tolerance to heat. Planktonic spores of both species 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. The lethality of chlorine dioxide was markedly reduced when inoculum on stainless steel coupons was suspended in 5% horse serum as a carrier rather than water. Chlorine and chlorine dioxide, at concentrations of 10 - 100 µg/ml, were equally effective in killing spores on apples. Significant reductions of  $\geq 3.8 - 4.5 \log_{10}$  CFU/apple were achieved by treatment with 100 µg/ml of either of the two sanitizers. The peroxyacetic acid sanitizer (40 and 80 µg/ml) was ineffective in killing *Bacillus* spores in the test systems investigated. Results provide information on the effectiveness of sanitizers commonly used in the food processing industry in killing *Bacillus* spores in suspension, on a food-contact surface, and on a ready-to-eat food. These insights will be useful when developing sanitization strategies focused on reducing spoilage of foods and risks of foodborne diseases associated with *Bacillus* species.

**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)

*Enterobacter sakazakii* is an emerging foodborne pathogen known to cause meningitis, sepsis, bacteremia, and necrotizing enterocolitis in preterm neonates and immunocompromised adults. This bacterium has been found in several types of foods, food processing plants, and the environment, although outbreaks of infection have been associated primarily with reconstituted, temperature-abused infant formula. While *E. sakazakii* has not been reported to cause illnesses linked to the consumption of fresh

produce, it has been isolated from lettuce and other vegetables, thereby representing a potential risk to produce safety. We have observed that *E. sakazakii* can grow on several types of fresh-cut produce and in fruit and vegetable juices. Chlorinated water, chlorine dioxide (gaseous and aqueous), and peracetic acid-based sanitizers are among the chemical treatments used to reduce populations of microorganisms on fresh fruits and vegetables. An objective of this study was to determine the survival and growth characteristics 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. A second objective was to determine the effectiveness of chlorine, aqueous chlorine dioxide, and a peroxyacetic acid-based sanitizer in killing *E. sakazakii* inoculated in an organic carrier onto the surface of apples, tomatoes, and lettuce.

Populations significantly decreased ( $p \leq 0.05$ ) on all test produce at all storage temperatures. The efficacy of chlorine, chlorine dioxide, and a peroxyacetic acid-based sanitizer (Tsunami 200®) in killing the bacterium on apples, tomatoes, and lettuce was determined. Chlorine and chlorine dioxide, at  $\geq 50$   $\mu\text{g/ml}$ , were equivalent in killing *E. sakazakii* on apples. Populations of *E. sakazakii* on apples treated with 10  $\mu\text{g/ml}$  chlorine dioxide for 1 or 5 min were significantly reduced ( $p \leq 0.05$ ) by 3.38 and 3.77  $\log_{10}$  CFU/apple, respectively, compared to the number remaining on apples after washing with water. Treatment with Tsunami 200 at 40  $\mu\text{g/ml}$  for 1 min caused reductions of  $\geq 4.00$   $\log_{10}$  CFU/apple. Reductions of  $\geq 3.70$   $\log_{10}$  CFU/tomato were achieved by treatment with 10  $\mu\text{g/ml}$  chlorine or chlorine dioxide or 40  $\mu\text{g/ml}$  Tsunami 200 for 5 min. Reductions in populations of *E. sakazakii* on lettuce treated with chlorine at 10, 50, and 100  $\mu\text{g/ml}$  for 1 min ranged from 1.61 to 2.50  $\log_{10}$  CFU/sample ( $26 \pm 4$  g), compared to populations remaining on lettuce washed with water. Chlorine was less effective in killing *E. sakazakii* on lettuce than on apples or tomatoes. Treatment of lettuce with Tsunami 200 (40 and 80  $\mu\text{g/ml}$ ) for 5 min caused a reduction of  $\geq 5.31$   $\log_{10}$  CFU/sample. Results provide insights to predicting survival characteristics of *E. sakazakii* on produce and the efficacy of sanitizers in killing the bacterium.

**EVALUATION OF GASEOUS CHLORINE DIOXIDE AS A SANITIZER FOR KILLING SALMONELLA, ESCHERICHIA COLI O157:H7, LISTERIA MONOCYTOGENES, AND YEASTS AND MOLDS ON FRESH AND FRESH-CUT PRODUCE (K. V. Sy, M. B. Murray, M. D. Harrison, and L. R. Beuchat)**

Treatment of fruits and vegetables with sanitizers often results in reductions in populations of pathogens not exceeding 2 to 3  $\log_{10}$  CFU/g and cannot be relied upon to eliminate safety risks. The lack of effectiveness of sanitizers for killing high numbers of pathogens on produce can be attributed in part to difficulties in delivering aqueous chemical sanitizers to surface or subsurface areas where pathogens may be lodged. Treatment with aqueous chemical solutions can result in residual moisture on the surface of fruits and vegetables, which can promote the growth of yeasts and molds, thus reducing fresh-market shelf life. Growth of molds can in turn increase the pH of produce tissues and enhance the growth of infectious toxigenic foodborne pathogens thereby increasing safety risks.

We undertook a study to evaluate  $\text{ClO}_2$  gas for its effectiveness in killing *Salmonella enterica*, *E. coli* O157:H7 and *L. monocytogenes* inoculated onto the surfaces of fresh-cut cabbage, carrot, and lettuce and its effectiveness in killing *Salmonella*, yeasts, and molds on the surfaces of fresh apples, tomatoes, onions, and peaches. Inoculum (100  $\mu\text{l}$ , ca. 6.8  $\log_{10}$  CFU) containing five serotypes of *Salmonella enterica*, five strains of *E. coli* O157:H7, or five strains of *L. monocytogenes* was deposited on the skin and cut surfaces of fresh-cut vegetables, dried for 30 min at 22°C, held for 20 h at 4°C, and then incubated for 30 min at 22°C before treatment. The skin surfaces of apples, peaches, tomatoes, and onions were inoculated with 100  $\mu\text{l}$  of a cell suspension (ca. 8.0  $\log_{10}$  CFU) containing five serotypes of *Salmonella*, and inoculated produce was allowed to dry for 20 to 22 h at 22°C before treatment. Treatment with  $\text{ClO}_2$  at 4.1 mg/liter significantly ( $\alpha = 0.05$ ) reduced the population of foodborne pathogens on all produce. Reductions resulting from this treatment were 3.13 to 4.42  $\log_{10}$  CFU/g for fresh-cut cabbage, 5.15 to 5.88  $\log_{10}$  CFU/g for fresh-cut carrots, 1.53 to 1.58  $\log_{10}$  CFU/g for fresh-cut lettuce, 4.21  $\log_{10}$  CFU per apple, 4.33  $\log_{10}$  CFU per tomato, 1.94  $\log_{10}$  CFU per onion, and 3.23  $\log_{10}$  CFU per peach. The highest reductions

in yeast and mold populations resulting from the same treatment were 1.68 log<sub>10</sub> CFU per apple and 2.65 log<sub>10</sub> CFU per peach. Populations of yeasts and molds on tomatoes and onions were not significantly reduced by treatment with 4.1 mg/liter ClO<sub>2</sub>. Substantial reductions in populations of pathogens on apples, tomatoes, and onions but not peaches or fresh-cut cabbage, carrot, and lettuce were achieved by treatment with gaseous ClO<sub>2</sub> without markedly adverse effects on sensory qualities.

**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)

Outbreaks of foodborne infections associated with the consumption of fresh fruits and vegetables as well as unpasteurized juices contaminated with pathogenic bacteria have been documented. Outbreaks of salmonellosis and *Escherichia coli* O157:H7 infections have been linked to the consumption of cantaloupes. Watermelons have been implicated in outbreaks of salmonellosis and shigellosis. Pathogens known to be contaminants on the surface of melon rinds can be translocated to the edible tissues and juices when melons are cut to prepare for consumption. *Salmonella* can rapidly grow on sliced cantaloupe, watermelon, and honeydew melon, and in cantaloupe juice and watermelon juice. *Escherichia coli* O157:H7 has been reported to grow on cantaloupe and watermelon cubes and *Listeria monocytogenes* can grow in cantaloupe and watermelon pulp. The U.S. Food and Drug Administration has implemented a HACCP program that focuses on minimizing microbiological safety risks that may be associated with fruit and vegetable juices. One of the interventions to eliminate foodborne pathogens is heat treatment. The use of melon juice in blends of non-pasteurized and pasteurized fruit juices offered for sale to the consumer has increased in recent years. To date, research efforts on the microbiological safety of pasteurization processes for fruit juices have concentrated largely on determining *D* values (decimal reduction times) for *Salmonella*, *E. coli* O157:H7, and *L. monocytogenes* in apple juice. We undertook a study to determine the *D* values of these pathogens in cantaloupe juice and watermelon juice as affected by acid adaptation preceding exposure to heat.

*Salmonella enterica* serotype Poona, *Salmonella enterica* serotype Saphra, two strains of *E. coli* O157:H7, and two strains of *L. monocytogenes* were grown in tryptic soy broth (TSB) and TSB supplemented with 1% glucose for 24 h at 37°C. Decimal reduction times (*D* values) of cells suspended in unpasteurized cantaloupe juice and watermelon juice were determined. Acid-adapted cells of *Salmonella* and *E. coli* O157:H7, but not *L. monocytogenes*, had increased thermal tolerance compared to cells that were not acid-adapted. There was no correlation between soluble solids content of the two types of juice and thermal resistance. Growth of *Salmonella* and *E. coli* O157:H7 in cantaloupe juice, watermelon juice, or other acidic milieu, either in preharvest or postharvest environments, may result in cross protection to heat. The pasteurization conditions necessary to achieve elimination of pathogens from these juices would consequently have to 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.