

CHEMICAL TREATMENTS FOR INACTIVATION OF PATHOGENS

INHIBITORY EFFECT OF OXALIC ACID ON BACTERIAL SPOILAGE OF RAW, CHILLED CHICKEN

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Processing of poultry consists of several steps, some of which can result in increased microbial counts, leading to a reduction in shelf life and increased safety risk. Opportunities for microbial cross-contamination exist during transport of birds from the farm to the processing plant and during processing and packaging. An increase in the number of microorganisms on the surface of carcasses can occur during removal of feathers, evisceration, washing, and chilling. Contamination from aerosols generated in the processing environment and from process water, ice, equipment, and the hands of workers can also occur. The use of organic acids and their salts for surface decontamination and extension of shelf life of poultry and beef has been investigated. Lactic acid and acetic acid also have been successfully used to reduce microbial populations on poultry. Other sanitizers evaluated have included lauric, palmitic, myristic, palmitoleic, stearic, oleic, linoleic, and linolenic acids, trisodium phosphate, potassium sorbate, and electrolyzed water but the use of oxalic acid to reduce populations of microorganisms on raw poultry has not been described. Oxalic acid occurs naturally in many fruits and vegetables and the human body synthesizes oxalic acid from ascorbic acid. Vegetables and herbs containing highest amounts of oxalic acid include parsley (1.70%, dry weight basis), chives (1.48%), spinach (0.48%), beet leaves (0.61%), carrots (0.50%), and radish (0.48%). Commercial soy foods contain up to 2.06 mg of oxalate/g. Eaten in large amounts, oxalic acid may combine with calcium and other minerals to form less soluble oxalates. The predicted LD₅₀ in rats is 375 mg/kg. Extrapolating from this dose, for a person weighing 150 lb (68.1 kg), consumption of 25.5 g of oxalic acid would be required for an LD₅₀, although smaller amounts may cause illness.

We did a study to evaluate oxalic acid for its effectiveness in killing microflora on the surface of raw chicken breasts, to identify the predominant bacteria that survive on chicken treated with oxalic acid, to evaluate the inhibitory effects of oxalic acid on growth of predominant spoilage microorganisms on treated breasts, and to determine the effects of treatment with oxalic acid on the color of breasts during subsequent storage at 4°C. Raw chicken breasts were dipped in solutions of oxalic acid (0, 0.5, 1.0, 1.5 and 2.0% [w/v]) for 10, 20, and 30 min, individually packed in oxygen-permeable polyethylene bags, and stored at 4°C. Total plate counts (TPC) and populations of *Pseudomonas* spp. and Enterobacteriaceae on breasts were determined before treatment and after storage for 1, 3, 7, 10, and 14 days. pH and Hunter L, a, and b values of the breast surface were measured. The TPC were ca. 1.5 and 4.0 log₁₀ CFU/g higher on untreated chicken breasts after storage for 7 and 14 days, respectively, compared to TPC on breasts treated with 0.5% oxalic acid, regardless of dip time. Differences in counts on chicken breasts treated with water and 1.0 - 2.0% of oxalic acid were greater. Populations of *Pseudomonas* spp. on chicken breasts treated with 0.5 - 2.0% oxalic acid and stored at 4°C for 1 day were less than 2 log₁₀ CFU/g (detection limit), compared to 5.14 log₁₀ CFU/g of untreated breasts. *Pseudomonas* grew on chicken breasts treated with 0.5% oxalic acid to reach counts not exceeding 3.88 log₁₀ CFU/g after storage for 14 days. Counts on untreated chicken exceeded 8.83 log₁₀ CFU/g at 14 days. Treatment with oxalic acid caused similar reductions in Enterobacteriaceae counts. *Kocuria rhizophila* was the predominant bacterium isolated from treated chicken. Other prominent bacteria included *Escherichia coli* and *Empedobacter brevis*. Treatment with oxalic acid caused a slight darkening in color (decreased Hunter L value), retention of redness (increased Hunter a value), and increase in yellowness (increased Hunter b value). Results show that oxalic acid has potential for use as a sanitizer to reduce populations of spoilage microorganisms naturally occurring on raw chicken, thereby extending the shelf life.

THERMAL TOLERANCE OF ACID-ADAPTED AND UNADAPTED *SALMONELLA*, *ESCHERICHIA COLI* O157:H7, AND *LISTERIA MONOCYTOGENES* IN CANTALOUPE JUICE AND WATERMELON JUICE

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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.

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 log₁₀ 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 ClO₂ 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 µl, ca. 6.8 log₁₀ 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 µl of a cell suspension (ca. 8.0 log₁₀ 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 ClO₂ 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 log₁₀ CFU/g for fresh-cut cabbage, 5.15 to 5.88 log₁₀ CFU/g for fresh-cut carrots, 1.53 to 1.58 log₁₀ CFU/g for fresh-cut lettuce, 4.21 log₁₀ CFU per apple, 4.33 log₁₀ CFU per tomato, 1.94 log₁₀ CFU per onion, and 3.23 log₁₀ CFU per peach. The highest reductions in yeast and mold populations resulting from the same treatment were 1.68 log₁₀ CFU per apple and 2.65 log₁₀ CFU per peach. Populations of yeasts and molds on tomatoes and onions were not significantly reduced by treatment with 4.1 mg/liter ClO₂. 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 ClO₂ without markedly adverse effects on sensory qualities.

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 ≥ 50 µg/ml, were equivalent in killing *E. sakazakii* on apples. Populations of *E. sakazakii* on apples treated with 10 µg/ml chlorine dioxide for 1 or 5 min were significantly reduced ($p \leq 0.05$) by 3.38 and 3.77 log₁₀ CFU/apple, respectively, compared to the number remaining on apples after washing with water. Treatment with Tsunami 200 at 40 µg/ml for 1 min caused reductions of ≥ 4.00 log₁₀ CFU/apple. Reductions of ≥ 3.70 log₁₀ CFU/tomato were achieved by treatment with 10 µg/ml chlorine or chlorine dioxide or 40 µg/ml Tsunami 200 for 5 min. Reductions in populations of *E. sakazakii* on lettuce treated with chlorine at 10, 50, and 100 µg/ml for 1 min ranged from 1.61 to 2.50 log₁₀ CFU/sample (26 ± 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 µg/ml) for 5 min caused a reduction of ≥ 5.31 log₁₀ 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 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

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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[®], 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.