

***ESCHERICHIA COLI* O157:H7**

DEATH OF *SALMONELLA*, *ESCHERICHIA COLI* O157:H7, AND *LISTERIA MONOCYTOGENES* IN SHELF-STABLE, DAIRY-BASED, POURABLE SALAD DRESSINGS

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Commercial sterilization of salad dressings by treatment at high temperatures is not an option for eliminating microorganisms because it would destroy the physical integrity and result in products with substantially different sensory qualities. Commercial processing and preservation of salad dressings instead depends on a combination of intrinsic factors, and possibly mild heat treatments, to reduce, control, or eliminate microorganisms. Commercial salad dressings are also manufactured under strict quality controls, as manufacturers adhere to good manufacturing practices. Storage temperature can affect the physical stability and sensory quality of salad dressings, as well as the rate of growth of spoilage microorganisms. The lethality of the harsh environment imposed by intrinsic factors characteristic of salad dressings to foodborne pathogens that may become contaminants during postprocess handling would be anticipated to act synergistically or additively with non-refrigerated temperatures to cause death of these pathogens at a more rapid rate. The amounts and types of pourable salad dressings available for purchase in large containers for use in food service and home settings have increased in recent years. This presents an increased possibility of postprocess contamination, e.g., at salad bars where portions are removed from the same container by several different people over an extended period of time. The behavior of *Salmonella*, *E. coli* O157:H7, and *L. monocytogenes* that may contaminate salad dressings at some point after opening containers in foodservice or home settings has not been critically evaluated.

The objectives of this study were to determine the death rates of *Salmonella*, *Escherichia coli* O157:H7, and *Listeria monocytogenes* in three commercially manufactured full-fat ranch salad dressings, three reduced-fat ranch salad dressings, two full-fat blue cheese salad dressings, and two reduced-fat blue cheese salad dressings and to affirm the expectation that these dressings do not support the growth of these pathogens. The respective initial pH values of the four types of shelf-stable, dairy-based, pourable dressings were 2.87 - 3.72, 2.82 - 3.19, 3.08 - 3.87, and 2.83 - 3.49. Dressings were inoculated with low (2.4 - 2.5 log₁₀ CFU/g) and high (5.3 - 5.9 log₁₀ CFU/g) populations of separate five-strain mixtures of each pathogen and stored at 25°C for up to 15 days. Regardless of the initial inoculum population, all test pathogens rapidly died in all salad dressings. *Salmonella* was undetectable by enrichment (<1 CFU/25-ml sample in three replicate trials) in all salad dressings within 1 day, and *E. coli* O157:H7 and *L. monocytogenes* were reduced to undetectable levels by enrichment between 1 and 8 days and 2 and 8 days, respectively. *E. coli* O157:H7 was not detected in four of the ten salad dressings stored for 2 or more days and nine of the ten dressings stored for 6 or more days after inoculation. *L. monocytogenes* was detected in nine of the ten salad dressings stored for 3 days but in only one dressing, by enrichment, at 6 days, indicating that it had the highest tolerance among the three pathogens to the acidic environment imposed by the dressings. Overall, the type of dressing (i.e., ranch vs. blue cheese) and level of fat in the dressings did not have a marked affect on the rate of inactivation of pathogens. Total counts and populations of lactic acid bacteria and yeasts and molds remained low or undetectable (< 1.0 log₁₀ CFU/ml) throughout the 15-day storage period. Based on these observations, shelf-stable, dairy-based, pourable ranch and blue cheese salad dressings manufactured by three companies and stored at 25°C do not support the growth of *Salmonella*, *E. coli* O157:H7, and *L. monocytogenes* and should not be considered as potentially hazardous foods (time/temperature control for safety foods) as defined by the U.S. Food and Drug Administration Food Code.

DEVELOPMENT OF RAPID DETECTION TECHNIQUE FOR PATHOGENS USING NANOROD-BASED SENSOR

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The biosecurity and safety of the food and water supply are a serious concern. Novel solutions are required for the development of rapid, reliable, and highly sensitive biosensors for the detection of low level of pathogens in food and water. Development of both the quartz crystal microbalance (QCM) and a nanoparticle-based bioassay technique for detection of *E. coli* O157:H7 and the bio-functional Au/Si nanorods for detection of Respiratory Syncytial Virus (RSV) were investigated. For *E. coli* O157:H7, the antibodies were immobilized onto gold electrodes of a QCM using a self-assembled monolayer method. Binding of *E. coli* O157:H7 cells onto the immobilized antibodies decreased the crystal resonant frequency. The difference in frequency between the phosphate buffered saline baseline and the frequency of bound *E. coli* O157:H7 demonstrated that QCM might have the potential for detecting a low level of *E. coli* O157:H7. In the nanoparticle bioassay technique, *E. coli* cells were first treated with lysozyme, then incubated with Hoechst 33258, and finally incubated with antibody-conjugated nanoparticles. A high intensity of fluorescence light produced by the nanoparticles could be observed using a fluorescence microscope. For RSV infected cells study, the Si nanorods were first fabricated by a glancing angle deposition method where the Au was sputtered onto Si nanorods. Dye was then immobilized onto the annealed Si nanorods and antibody of RSV was annealed to Au. An enhanced fluorescence signal produced by the attached dye molecules ensured a potential technique for detection of virus.

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

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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.

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.