SALMONELLA

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

(L. R. Beuchat, J.-H. Ryu, B. B. Adler, and M. D. Harrison)

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 $\log_{10}$ CFU/g) and high (5.3 - 5.9 $\log_{10}$ 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 $\log_{10}$ 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.

GREEN FLUORESCENT PROTEIN LABELING OF *LISTERIA* AND *SALMONELLA* FOR FOOD SAFETY-RELATED STUDIES

(G. Zhang, L. Ma, and M. P. Doyle)

Many studies with foodborne bacterial pathogens require tracking of the introduced bacterial strain in order to monitor its fate in complex environments. With an easily detectable phenotype, the green fluorescent protein (GFP) gene (*gfp)*, has been used to label many microorganisms for localization and gene expression studies. The objectives of this study were to label *Listeria monocytogenes, Listeria innocua* and *Salmonella* strains with GFP and characterize the *gfp*-labeled strains in terms of stability of label and the effect of the label on bacterial growth, two important characteristics pertinent to their intended application.

Plasmids containing the *gfp* gene were introduced into *Salmonella* and *Listeria* strains by conjugation and electroporation. Expression of GFP in labeled strains was determined by epifluorescence microscopy of colonies. Stability of the label was investigated through sequential propagations of labeled strains in the absence of antibiotic selection, and rates of plasmid-loss were calculated. Growth curves were determined comparing the parent strain with its corresponding labeled derivatives to determine the effect of *gfp*-labeling on bacterial growth. *Salmonella* strains were labeled by the calcium chloride method. *gfp*-labeling of *Listeria* by conjugation was easily performed but not universally effective for all strains, whereas electroporation was an effective method for labeling all *Listeria* strains. Plasmid stability varied among the labeled strains. When grown in non-selective media for two consecutive subcultures (ca. 40 generations), the rates of plasmid-loss among labeled *Salmonella* and *Listeria* strains ranged from 15.8%-99.9% and 8.1% -93.4%, respectively. Complete loss (>99.9%) of the plasmid was observed in some labeled strains when grown for five consecutive subcultures in the absence of selective pressure, whereas it remained stable in other cultures. Maintaining the *gfp*-plasmid had an insignificant effect on growth of most labeled strains. In conclusion, *Salmonella* and *Listeria* strains can be effectively labelled with the plasmid-borne *gfp* gene and in several isolates will be stable for many generations without adversely affecting growth rates.

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 4ºC. 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.

INACTIVATION OF *SALMONELLA* IN MANURE-BASED COMPOSTS WITH VARYING C:N RATIOS

(M. C. Erickson, J. Liao, L. Ma, X. Jiang, and M. P. Doyle)

Composting is a process whereby organic matter is decomposed by microorganisms to generate a stable amendment that improves soil quality and fertility. To date, the primary criteria for ensuring the microbiological safety of composts have been adherence to narrowly defined time-temperature conditions. To expand the guidelines whereby inactivation of pathogens could be assured, this study sought to determine if the carbon:nitrogen (C:N) ratio or the presence of ammonium sulfate affects the inactivation of *Salmonella* spp. in cow manure-based compost mixtures. Evaluation of compost conditions on pathogen inactivation was conducted using a bioreactor system. The days to achieve non-detection of *Salmonella* spp. by enrichment culture was used as the endpoint. In addition to pathogen levels, pH and temperature were monitored at 4 locations within the bioreactor. Location within the bioreactor was not a significant variable affecting pathogen inactivation. Compost preparations with an initial C:N ratio of 20:1 required a maximum of 4 days of storage before *Salmonella* was not detected whereas 30:1 and 40:1 C:N preparations required up to 7 days of storage. Both 20:1 and 30:1 C:N preparations were characterized by a decrease in pH to 5.5-5.7 before pH values increased to > 8. In contrast, pH values of 40:1 C:N preparations increased immediately to > 8, generally within the first day of storage. Maximum temperatures encountered in 20:1 C:N preparations for inactivation of pathogens were less than 50°C. Consequently, the cumulative heat exposure required for pathogen inactivation in 20:1 C:N preparations was five-fold less than in 40:1 C:N preparations. Temperatures within preparations supplemented with 0.08% ammonium sulfate were higher than unsupplemented preparations during the first 2 days of storage, however; these higher temperatures did not consistently translate into more rapid rates of pathogen inactivation.

CONTAMINATION OF COMMERCIAL BROILER BREEDER ROOSTERS BY *CAMPYLOBACTER*, *SALMONELLA*, AND *CLOSTRIDIUM PERFRINGENS*

(N. A. Cox, C. L. Hofacre, R. J. Buhr, J. L. Wilson, J. S. Bailey, L. J. Richardson, D. E. Cosby, M. T. Musgrove, K. L. Hiett, and S. M. Russell)

The present study was conducted to determine if several foodborne pathogens (*Campylobacter*, *Salmonella*, and *Clostridium perfringens*) could be isolated from the ductus deferens, testes, and ceca of 45- to 65-wk old commercial broiler breeder roosters. Aseptic necropsy was performed on 15 roosters (five roosters from 3 separate commercial breeder farms) to remove the ductus deferens, testes, and ceca without surface contamination from blood and other tissues. None of the foodborne pathogens were isolated from the testes of the roosters. In the ductus deferens, *C. perfringens* was isolated from 1 of the 15 roosters, whereas no *Campylobacter* or *Salmonella* was isolated from this tissue. *Campylobacter* was cultured from the ceca of all 15 roosters, *C. perfringens* was isolated from 14 of 15 roosters, and *Salmonella* from 2 of 15 roosters. These data suggest that the contamination of semen by these foodborne

pathogens is via fecal or cecal contamination as the semen passes through the cloaca and not from bacterial colonization of the testes and ductus deferens.

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 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 µl, 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 µl 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 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 $\log_{10}$ CFU per apple and 2.65 $\log_{10}$ CFU per peach. Populations of yeasts and molds on tomatoes and onions were not significantly reduced by treatment with 4.1 mg/liter ClO_2. 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_2 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.