

## CHEMICAL INACTIVATION OF PATHOGENS

### EFFICACY OF CHLORINE AND A PEROXYACETIC ACID SANITIZER IN KILLING *LISTERIA MONOCYTOGENES* ON ICEBERG AND ROMAINE LETTUCE USING CONDITIONS SIMULATING THOSE USED IN A COMMERCIAL PROCESS (L. R. Beuchat, B. B. Adler, and M. M. Lang)

Several studies have described the efficacy of chlorine and other sanitizers in killing *Listeria monocytogenes* on inoculated, cut lettuce. Various ratios of weight of lettuce and volume of treatment solution, as well as different treatment times and temperatures, have been used in these studies, making comparisons across laboratories difficult. In some instances, treatment conditions did not mimic those used in commercial fresh-cut lettuce operations. A recent study, done in collaboration with a commercial fresh-cut iceberg lettuce processor in Australia, evaluated the effectiveness of chlorine and a mixture of hydrogen peroxide and peroxyacetic acid in killing *L. monocytogenes* on shredded lettuce. The ratio of lettuce weight to treatment solution volume was 1:19. Results of the experiment were used to demonstrate how the manufacturer could meet a food safety objective of < 100 cfu of *L. monocytogenes*/g of lettuce.

We undertook a study to determine the efficacy of chlorine (100 µg/ml) and a peroxyacetic acid sanitizer (Tsunami 100) (80 µg/ml) in killing *L. monocytogenes* inoculated at populations of 1 – 2, 2 – 3, and 4 – 5 log<sub>10</sub> cfu/g of iceberg lettuce pieces, shredded iceberg lettuce, and Romaine lettuce pieces. Treatment conditions simulated those used by a commercial fresh-cut lettuce processor. The ratio of lettuce:treatment solution was 1:100 (wt:vol), treatment temperature was 4°C, and total treatment time was 30 sec. Compared to washing with water, treatment of iceberg lettuce pieces containing all levels of inoculum and shredded iceberg lettuce containing 2 – 3 or 4 – 5 log<sub>10</sub> cfu/g with chlorine or Tsunami resulted in significant reductions ( $P \leq 0.05$ ) in populations of the pathogen. Populations recovered from Romaine lettuce pieces treated with chlorine or Tsunami were not significantly different than populations recovered from pieces washed with water, regardless of the inoculum level. Within lettuce type and inoculum level, in no instance was the number of *L. monocytogenes* recovered from lettuce treated with chlorine or Tsunami significantly different. The rate of decrease in free chlorine concentration in treatment solution as affected by the wt:vol ratio (1:100, 1:10, 2:10, and 4:10) of lettuce:solution was determined. The rate of reduction increased as the ratio decreased, with an overall order of magnitude of reduction being shredded iceberg lettuce > iceberg pieces > Romaine pieces. Highest reductions in free chlorine concentration in solutions used to treat shredded lettuce are attributed to the release of tissue juices, which increases the concentration of soluble organic materials available for reaction with chlorine.

### STUDIES ON THE PREVENTION OF BOTULINUM TOXIN PRODUCTION IN HOME-CANNED VEGETABLES TO PROVIDE A PRACTICAL TREATMENT TO REDUCE BOTULISM IN THE REPUBLIC OF GEORGIA (ROG) (C.-M. Lin, L. Thurber, and M. P. Doyle)

Due to lack of equipment and facilities, people in the Republic of Georgia (ROG) use boiling water instead of a pressure cooker to prepare home-canned vegetables. These boiling conditions for a short period of time will not inactivate spores of *Clostridium botulinum*, hence botulinum toxin frequently forms in canned foods resulting in the ROG having the highest incidence of botulism in the world. Based on epidemiologic data, the occurrence of botulism in the ROG is lower in areas where acetyl salicylic acid (ASA, aspirin) is added to home-canned vegetables. In addition, acidified calcium sulfate (ACS, Safe<sub>2</sub>O) produced by Mionix Corp. has been shown to inhibit the growth of pathogenic bacteria in foods. The objective of this study was to evaluate the efficacy of ASA and ACS in preventing botulinum toxin production in home-canned vegetables prepared with utensils and according to methods used in the ROG. Because of difficulties in importing vegetables from the ROG, domestic cucumbers and tomatoes were used and canned based on methods demonstrated by two ROG scientists. Due to differences in the composition of domestic and ROG vegetables, home-canned cucumbers and tomatoes prepared in the laboratory did not support the growth of *C. botulinum* independent of the addition of ASA or ACS. However, sensory characteristics of the vegetables were better when ACS or ASA, especially ACS, was added. Based on a small number of cucumbers and tomatoes imported from the ROG, it was determined that domestic cucumbers and

tomatoes have higher acidity (lower pH) than those from the ROG. In addition, ROG water contained high concentration of CaCO<sub>3</sub> compared to Griffin tap water. Hence, the home-canned vegetables prepared in the laboratory using produce from the U.S. did not adequately simulate canned vegetables as they are prepared in the ROG. Recently, CDC epidemiologists have collected more information regarding vegetables canned in the ROG, including acidity of fresh and canned vegetables, pickling recipes, and water quality. The project will be resumed during the next vegetable-growing season in the ROG when produce from the ROG can be used.

**FATE OF *SALMONELLA* IN NUTRIENT BROTH AND ON THE SURFACE OF RAW OYSTERS  
AS AFFECTED BY CHITOSAN  
(W. Klypradit, P. Chhabra, and Y. W. Huang)**

This study was initiated to determine the effect of chitosan, a modified carbohydrate polymer derived from chitin, on growth inhibition of *Salmonella* spp. as tested in nutrient broth and in shelled oysters. Three levels of chitosan, 1, 2, and 3%, were prepared in 0.5% acetic acid and the pH adjusted to 6.5 by adding NaOH solution. Pure cultures of *Salmonella* were inoculated into nutrient broth containing the chitosan in acetic acid or applied to oysters coated with the chitosan/acetic acid material. All nutrient broth samples were kept at 7°C for a period of 48 h while oysters were kept at 4°C for 15 d. Stored inoculated broths and oysters were sampled at 12-h and 5-d intervals, respectively. Initial counts of all broth samples averaged  $6.0 \times 10^8$  cfu/ml. A 7-log<sub>10</sub> reduction occurred in the presence of chitosan/acetic acid after 48 h incubation at 7°C while samples containing 0.5% acetic acid alone exhibited a 2-3 log<sub>10</sub> reduction. The initial counts in oysters averaged  $1.8 \times 10^5$  cfu/g. After a 5-d storage period, *Salmonella* populations started to decline. After a 15-d storage period, *Salmonella* populations declined 1, 3, and 2-log<sub>10</sub> in oysters exposed to 1, 2, and 3% chitosan, respectively. No reduction in *Salmonella* population was observed in oysters coated with an acetic acid solution. Overall, 2% chitosan was considered the best treatment level for inactivation of *Salmonella* on raw oysters.

**VIABILITY OF ACID-ADAPTED *ESCHERICHIA COLI* O157:H7 IN GROUND BEEF  
TREATED WITH ACIDIC CALCIUM SULFATE  
(L. R. Beuchat and A. J. Scouten)**

Exposure of microorganisms to sublethal stress can result in greater resistance of cells upon subsequent exposure to the same or unrelated stress. Acid adapted or acid shocked *Escherichia coli* O157:H7 has been shown to have increased resistance to heat, salt, and acidic pH. The type of acidulant used to achieve a given pH can influence the rate of inactivation of *E. coli* O157:H7 as well as its sensitivity upon subsequent exposure to acid or other stress environments. The effectiveness of treatment of beef carcasses with organic acids for the purpose of reducing or eliminating surface microflora, including *E. coli* O157:H7 and other pathogens, varies with type, concentration and temperature of acid, the presence of surfactants, contact time, application pressure, tissue type, and sensitivity of the microorganism. The potential for *E. coli* O157:H7 surviving acid rinse treatment of beef carcasses to exhibit increased tolerance to acidic environments or refrigeration temperatures to which processed beef may be exposed raises a concern about the impact of organic acid sanitizers on safety risks. Supplementation of ground beef with acidic calcium sulfate (ACS), the basis for a commercial food additive called Safe<sub>2</sub>O™, which contains calcium hydroxide, sulfuric acid, and calcium sulfate, has been suggested as an intervention to control the growth of spoilage and pathogenic bacteria. Claims are that ACS kills bacteria at levels nearly equal to irradiation and continues to inhibit microbial growth and recontamination long after treatment. Bacterial kill in excess of 5 log<sub>10</sub> is claimed. The effects of ACS on survival of acid-adapted *E. coli* O157:H7 in refrigerated ground beef have not been reported.

The objectives of this study were to determine if treatment of three strains of *E. coli* O157:H7 in broth acidified with lactic acid, acetic acid, or ACS results in a change in tolerance of cells upon exposure to a second acidic environment and to determine if *E. coli* O157:H7 grown on an agar medium at pH 4.5 achieved using ACS as an acidulant changes in its ability to survive in ACS-treated ground beef. Differences in tolerance to acidic environments were observed among strains but the level of tolerance was not affected by the acidulant to which cells had been exposed. Cells of *E. coli* O157:H7 adapted to grow on tryptic soy agar acidified to pH 4.5 with ACS were compared to cells grown at pH 7.2 in the absence of ACS for their ability to survive after inoculation into ground beef treated with ACS, as well as untreated beef. The number of ACS-adapted cells recovered from ACS-treated beef was significantly ( $\alpha = 0.05$ ) higher than the number of control cells recovered from ACS-treated beef

during the first 3 days of a 10-day storage period at 4°C, suggesting that ACS-adapted cells are initially more tolerant than unadapted cells to reduced pH in ACS-treated beef. Regardless of treatment of ground beef with ACS or adaptation of *E. coli* O157:H7 to ACS before inoculating ground beef, the pathogen survived in high numbers.

**SURFACE MATERIAL, TEMPERATURE AND SOIL EFFECTS ON THE SURVIVAL  
OF SELECTED FOODBORNE PATHOGENS IN THE PRESENCE OF CONDENSATE  
(J. Allan, J. L. Kornacki, and Z. Yan)**

Survival of foodborne pathogens in food processing facilities is affected by factors including surface materials, nutrients, moisture and temperatures. The effects of surface-type [stainless steel, Delrin® (DuPont) acetal resin, and fiberglass reinforced plastic wall paneling (FRP), and mortar surfaces], soil, and temperature on the survival of *Listeria monocytogenes*, *Salmonella* spp., and *Yersinia enterocolitica* were evaluated in the presence of condensation. Surface coupons soiled and non-soiled with sterile porcine serum were exposed to cell suspensions made from five-strain cocktails of each organism ( $10^7$  cfu/ml) in Butterfield's phosphate buffer (BPB) and incubated for 2 h at 25°C allowing attachment of cells. Three surfaces, stainless steel, Delrin and FRP, were included in the same experiment. The coupons were rinsed to remove unattached cells, incubated at either 4°C or 10°C under condensate-forming conditions, and sampled at six time intervals over a 15-day period. For enumeration, cells were removed from the coupons by vigorous shaking in 100 ml BPB with 3 g of glass beads and the BPB was plated on tryptic soy agar with 0.6 % yeast extract. The results showed that stainless steel did not support the survival of *Listeria* as well as acetal resin or FRP. Acetal resin and stainless steel were less supportive of *Salmonella* than FRP. All three surfaces supported the survival of *Yersinia* over the 15-day trial equally at 10°C. Temperature had little effect on survival of *Listeria* or *Salmonella* across all three surfaces. However, *Yersinia* displayed growth on FRP at 10°C, but death at 4°C. Serum had a protective effect on *L. monocytogenes* on all surfaces, but did not affect survival of *Salmonella* or *Yersinia* on stainless steel, acetal resin, or FRP.

Since mortar surface is very different from the three surfaces described above, it was tested separately. The method to enumerate bacterial cells on the mortar surface involved applying sonication to remove bacterial cells and determining the cfu/coupon at 9 to 10 sampling periods over a total of 120 h. In general, the mortar surface had a significant inhibitory effect against all the bacteria tested compared to the three surfaces described above because of alkaline pH (increased to pH 11 within 6 h) when submerged in BPB. *Listeria* and *Salmonella* survived better on mortar than *Yersinia* throughout the 120-h incubation period, partially due to the alkaline resistance of *L. monocytogenes* and *Salmonella* spp. Serum had a protective effect on the survival of all three organisms. Differences in temperature did not affect the survival of *Salmonella* or *Yersinia*, whereas populations of *L. monocytogenes* declined more rapidly at 10°C than at 4°C after 24 h.