

CHEMICAL INACTIVATION

REDUCTION OF SALMONELLAE ON PRODUCE AND POULTRY BY ORGANIC ACID PLUS DETERGENT (M.P. Doyle)

A group of organic acids, including lactic acid, acetic acid, and levulinic acid, and sodium dodecyl sulfate (SDS), were evaluated individually or in combination for their ability to inactivate *Salmonella* and *E. coli* O157:H7. Results from pure culture assays in water with the treatment chemical reveal that 0.5% organic acid and 0.05-1% SDS, when used individually, reduced pathogen cell numbers by <2 log CFU/ml within 20 min at 21°C. Combining any of these organic acids at 0.5% with 0.05% SDS resulted in >7 log CFU/ml inactivation of *Salmonella* and *E. coli* O157:H7 within 10 sec at 21°C. A combination of levulinic acid and SDS was evaluated at different concentrations for pathogen reduction on lettuce at 21°C, on poultry (wings and skin) at 8°C, and in water containing chicken feces or feathers at 21°C. Results revealed that treatment of lettuce with a combination of 3% levulinic acid plus 1% SDS, for < 20 sec reduced both *Salmonella* and *E. coli* O157:H7 populations by > 6.7 log CFU/g on lettuce. *Salmonella* and aerobic bacteria populations on chicken wings were reduced by > 5 log CFU/g by treatment with 3% levulinic acid plus 2% SDS, for 1 min. Treating water heavily contaminated with chicken feces with 3% levulinic acid plus 2% SDS, reduced *Salmonella* populations by >7 log CFU/ml within 20 sec. The application of levulinic acid plus SDS as a wash solution may have practical application for killing foodborne enteric pathogens on fresh produce and uncooked poultry.

COMBINATIONS OF ANTIMYCOTICS TO INHIBIT THE GROWTH OF MOLDS CAPABLE OF PRODUCING 1,3-PENTADIENE (D. A. Mann and L. R. Beuchat)

Raw and pasteurized foods and beverages as well as products subjected to more rigorous thermal processes, e.g., hot-fill beverages, can contain a wide range of molds. These molds may grow during the expected shelf life of these products to cause visual spoilage or result in the production of volatile compounds that are offensive to the consumer. Some of the molds known to grow in the presence of potassium sorbate or survive thermal processes commercially applied to foods and beverages can also produce mycotoxins, thereby posing a public health concern. Degradation of sorbate through decarboxylation by some strains of penicillia can result in the accumulation of 1,3-pentadiene, a volatile compound having an odor described as being similar to that of kerosene, acrylic paint, or petroleum products. Other molds that may also degrade sorbate include *Aspergillus*, *Fusarium*, *Mucor*, *Geotrichum*, and *Trichoderma* species. Strains of yeasts belonging to *Zygosaccharomyces rouxii* and *Debaryomyces hansenii* are also capable of spoiling sorbate-containing high-sugar foods by producing 1,3-pentadiene. While the control of sorbate-resistant molds and yeasts in some types of foods and beverages may be achievable through the addition of high concentrations of the preservative, the adverse effect of off aromas and off flavors that may result make this approach impractical. Instead, the use of antimycotics other than sorbate, e.g., natamycin, ethylenediaminetetraacetic acid, and propionate, or a low concentration of sorbate in combination with other antimycotics may be an alternative to prevent or retard the growth of 1,3-pentadiene-producing molds. We did a study to evaluate potassium sorbate, sodium benzoate, calcium propionate, disodium ethylenediaminetetraacetic acid (EDTA), and natamycin, alone and in combination, for their effectiveness in preventing the growth of five molds isolated from Parmesan cheese and a lemon-flavored drink subjectively judged to contain 1,3-pentadiene. Growth of *Penicillium brevicompactum*, *Penicillium roqueforti*, *Paecilomyces variotii*, *Aspergillus niger*, and *Cephalosporium fragrans* on model agar media containing Parmesan cheese (PRM agar) (pH 5.5) and lemon-flavored drink (LD agar) (pH 2.6) supplemented with antimycotics was studied. All molds grew well at 21°C on PRM agar containing potassium sorbate (3,500 µg/ml), calcium propionate (3,000 µg/ml), or natamycin (20 µg/ml). Combinations of potassium sorbate (250 - 1,000 µg/ml), calcium propionate (250 - 1,000 µg/ml), and/or natamycin (10 - 18 µg/ml) greatly inhibited or prevented growth of molds on PRM agar, indicating their potential as preservative systems at pH values resulting in large percentages of the acids in dissociated forms. Three of the five molds grew on LD agar containing potassium sorbate or sodium benzoate at a concentration of 200 µg/ml. Growth did not occur within 70 days on LD agar containing EDTA (30 µg/ml) in combination with potassium sorbate and sodium benzoate at 50 and 175 µg/ml, respectively, or 175 and 50 µg/ml,

respectively. Results of this study show that preservative systems containing a reduced concentration of potassium sorbate, in combination with other antimycotics, particularly natamycin, have potential for controlling the growth of molds thought to be capable of producing 1,3-pentadiene.

PRODUCTION AND STABILITY OF CHLORINE DIOXIDE IN ORGANIC ACID SOLUTIONS AS AFFECTED BY pH, TYPE OF ACID, AND CONCENTRATION OF SODIUM CHLORITE, AND ITS EFFECTIVENESS IN INACTIVATING *BACILLUS CEREUS* SPORES
(H. Kim, Y. Kang, L. R. Beuchat, and J.-H. Ryu)

Aqueous chlorine dioxide (ClO₂) has been approved as a disinfectant in beverage bottling plants and food processing, handling, and storage plants and as a sanitizer for food processing equipment. Because of its bactericidal activity over a wide range of pH, rapid action, and limited reaction with organic materials, ClO₂ has been effectively used to kill *Escherichia coli* O157:H7, *Enterobacter sakazakii*, *Listeria monocytogenes*, *Bacillus cereus*, and *Bacillus thuringiensis*. One of the disadvantages of ClO₂ as a sanitizer, however, is its instability during production and storage. Because of its low stability, ClO₂ should be prepared on site and cannot be stored for long periods of time. We studied the production and stability of chlorine dioxide (ClO₂) in organic acid solutions and its effectiveness in killing *B. cereus* spores. Sodium chlorite (5,000, 10,000, or 50,000 µg/ml) was added to 5% acetic, citric, or lactic acid solution, adjusted to pH 3.0, 4.0, 5.0, or 6.0, and held at 21°C for up to 14 days. The amount of ClO₂ produced was higher as the concentration of sodium chlorite was increased and as the pH of the acid solutions was decreased. However, the stability of ClO₂ was enhanced by increasing the pH of the organic acid solutions. To evaluate the lethal activity of ClO₂ produced in various acid solutions as affected by acidulant and pH, suspensions of *B. cereus* spores were treated at 21°C for 1, 3, 5, or 10 min in hydrochloric acid or organic acid solutions (pH 3.0, 4.0, 5.0, or 6.0) containing ClO₂ at concentrations of 100, 50, or 25 µg/ml. Populations of viable spores treated with ClO₂ at concentrations of 100 or 50 µg/ml in organic acid solutions decreased more rapidly than populations treated with the same concentrations of ClO₂ in HCl. Rates of inactivation tended to increase with higher pH of ClO₂ solutions. Results show that ClO₂ formed in organic acid solutions has higher stability and is more lethal to *B. cereus* spores than ClO₂ formed at the same concentration in HCl solution. This finding emphasizes the benefits of using organic acid solutions to prepare ClO₂ intended to be used as an antimicrobial.

INACTIVATION OF *SALMONELLA* TYPHIMURIUM AND *ESCHERICHIA COLI* O157:H7 IN ALFALFA SEEDS BY LEVULINIC ACID AND SODIUM DODECYL SULFATE WASHING TREATMENT
(T. Zhao, P. Zhao, and M.P. Doyle)

Since 1995, raw sprouts have been increasingly implicated as the vehicles of outbreaks of *E. coli* O157:H7 and *Salmonella* nationally and internationally. Most involved alfalfa sprouts, but cress, mung bean, and clover sprouts were also implicated. Thirteen *Salmonella* serotypes were isolated from the clinical cases. Various intervention approaches, including heating and chemical treatments (NaOCl, Ca(OCl)₂, acidified NaClO₂, acidified ClO₂, Na₃PO₄, Vegi-Clean, Tsunami, Vortexx, and H₂O₂) have been evaluated for their efficacy for reduction of *E. coli* O157:H7 contamination in alfalfa seeds. Results revealed that none of the above chemical treatments was able to eliminate or satisfactorily reduce *E. coli* O157:H7 on alfalfa seeds and sprouts. The focus of this research project was to determine the optimum concentration and exposure time of our newly developed chemical solution (levulinic acids plus sodium dodecyl sulfate, SDS) for treatment of alfalfa seeds for reduction of *E. coli* O157:H7 and *Salmonella* and to determine whether this treatment would affect seed germination characteristics. A 5-strain mixture of *E. coli* O157:H7 or *S. Typhimurium* at 10⁸ CFU/g was inoculated onto the alfalfa seeds. The seeds were dried at 21°C in a laminar flow hood for up to 72 h. *E. coli* O157:H7 counts at 4, 24, 48, and 72 h were 8.1, 4.8, 4.0 and 4.0 log CFU/g, respectively; and of *S. Typhimurium* at 4, 24, 48, and 72 h were 6.6, 4.4, 4.3, and 4.1 log CFU/g, respectively. The 0.5% levulinic acid and 0.05% SDS treatment for 5 min at 21°C reduced *E. coli* O157:H7 and *S. Typhimurium* populations by 5.6, >3.3, >2.4, >2.3; and 6.4, >2.7, 4.3, and 2.4 log CFU/g on seeds contaminated and dried for 4, 24, 48, and 72 h, respectively. However, some samples treated were positive by selective enrichment only. Seeds contaminated with 10⁴ *E. coli* O157:H7 cells/g and dried for 2 h at 21°C in a laminar flow hood were tested with 0.5% levulinic acid plus 0.05% SDS at 40°C for up to 5 min. *E. coli* O157:H7 in samples (25 g) tested at 1, 2, 3, and 5 min of exposure were negative by a direct

plating method, but were positive by selective enrichment (<0.7 log CFU/g). All the levulinic acid + SDS used for treatment at 1, 2, 3, and 5 min were negative for *E. coli* O157:H7 by selective enrichment culture.

REDUCTION OF *ESCHERICHIA COLI* O157:H7 ON PRODUCE USING ELECTROLYZED WATER UNDER SIMULATED FOOD SERVICE OPERATION CONDITIONS

(P. Pangloli, Y.-C. Hung, L. R. Beuchat, C. H. King, and Z.-H. Zhao)

Bacterial pathogens most often involved in produce-related outbreaks of infections are *Salmonella* and *Escherichia coli* O157:H7, accounting for 50 and 20%, respectively, between 1998 and 2002. In 2005 – 2006, four multistate outbreaks of salmonellosis associated with eating tomatoes in restaurants sickened at least 450 people in 21 states. In 2006, outbreaks of *E. coli* O157:H7 infections linked to bagged spinach affected at least 183 people in 26 states and outbreaks associated with consumption of lettuce in fast-food restaurants sickened 81 individuals in three states. In 2008, an outbreak of salmonellosis implicating consumption of jalapeno peppers contaminated with *Salmonella* Saintpaul involved more than 1,400 infected people in 43 states, the District of Columbia, and Canada. Contamination of produce with pathogens can occur during production, harvesting, processing, storage, and handling or during preparation in food service kitchens or at home. Vegetables and fruits such as lettuce, cabbage, tomatoes, lemons, and oranges used to make salads and fresh-squeezed juices or in sandwiches in restaurant kitchens often require washing with water before serving. However, this washing step may be ineffective in completely removing all pathogenic microorganisms from produce. Electrolyzed water (EW) is produced through electrolysis of a mild salt (NaCl) solution in a chamber with cathode and anode electrodes. Acidic EW (AcEW), generated from the anode side, is lethal to most foodborne bacterial pathogens due to its low pH, high oxidation-reduction potential (ORP), and the presence of hypochlorous acid. Alkaline EW (AlkEW), generated from the cathode side, has a strong cleaning effect. Most studies examining the efficacy of EW as a produce sanitizer have been conducted under conditions not necessarily mimicking food service practices. The objective of our study was to evaluate the efficacy of EW in killing or removing *E. coli* O157:H7 attached to the surface of produce under simulated food service operation conditions. The efficacy of EW in killing the pathogen during washing and/or chilling iceberg lettuce, and washing cabbage, tomatoes, and lemons was determined. We evaluated the efficacy of EW in killing *Escherichia coli* O157:H7 using washing and/or chilling treatments simulating those followed in some in food service kitchens. Greatest reduction on lettuce were achieved by sequentially washing with 14 A acidic EW (AcEW) for 15 or 30 s followed by chilling in 16 A AcEW for 15 min. This procedure reduced the pathogen by 2.8 and 3.0 log CFU/leaf, respectively, whereas washing and chilling with tap water reduced the pathogen by 1.9 and 2.4 log CFU/leaf. Washing cabbage leaves for 15 or 30 s with tap water or 14 A AcEW reduced the pathogen by 2.0 and 3.0 log CFU/leaf and 2.5 to 3.0 log CFU/leaf, respectively. The pathogen was reduced by 4.7 log CFU/lemon by washing with 14 A AcEW and 4.1 and 4.5 log CFU/lemon by washing with tap water for 15 or 30 s. A reduction of 5.3 log CFU/lemon was achieved by washing with 14 A alkaline EW for 15 s prior to washing with 14 A AcEW for 15 s. Washing tomatoes with tap water or 14 A AcEW for 15 s reduced the pathogen by 6.4 and 7.9 log CFU/tomato, respectively. Application of EW using procedures mimicking food service operations may minimize cross-contamination and reduce the risk of *E. coli* O157:H7 being present on produce at the time of consumption.

INACTIVATION OF *SALMONELLA* SPP. AND *E. COLI* O157:H7 ON TOMATOES BY ALLYL ISOTHIOCYANATE, CARVACROL AND CINNAMALDEHYDE IN VAPOR-STATE

(M.M. Obaidat and J.F. Frank)

The activity of various volatile antimicrobials in vapor state was determined. *Salmonella* spp. and *E. coli* O157:H7 on sliced and whole tomatoes were treated in a 120 ml sealed container with various concentrations of allyl isothiocyanate (AIT), cinnamaldehyde and carvacrol in vapor-state, with incubation at 4 and 10°C for up to 10 days and at 25°C for up to 10 h. AIT exhibited the greatest inactivation against the pathogens on sliced and whole tomatoes followed by cinnamaldehyde. The lowest level of AIT (1µl/120 ml volume) inactivated *Salmonellae* on sliced tomatoes by 1.0 and 3.5 log CFU at 4 and 10°C, respectively, in 10 days and by 2.8 log CFU at 25°C in 10 h. This level of AIT inactivated *Salmonellae* on whole tomatoes by 1.5 and 2.2 log CFU at 4 and 10°C, respectively, in 10 days and by 2.8 log CFU at 25°C in 10 h. AIT also inactivated *E. coli* O157:H7 on sliced tomatoes by 4.0 and 3.0 log at 4 and 10°C, respectively, in 10 days with no inactivation at 25°C in 10 h. AIT reduced *E. coli* O157:H7 on whole tomatoes surface by 3.0 and 1.0 log CFU at 4 and 10°C, respectively, in

10 days and by 2.0 log CFU at 25°C in 10 h. Greater inactivation occurred for all treatments at 10 than at 4°C. Pathogens on sliced tomato were not inactivated at 25°C. Antimicrobials in vapor form may be useful for controlling pathogens on fresh tomatoes marketed in packages containing head space.

TRANSFER OF *ESCHERICHIA COLI* O157:H7 TO ICEBERG LETTUCE VIA SIMULATED FIELD CORING
(P. J. Taormina, L. R. Beuchat, M. C. Erickson, L. Ma, G. Zhang, and M. P. Doyle)

Between 1995 and 2006, nine outbreaks of *E. coli* O157 infections were attributed to consumption of lettuce or spinach grown in or near the Salinas Valley in California. Investigation of this region led to the conclusion that *E. coli* O157 contamination of growing fields and produce is a dynamic and interrelated process involving transport and distribution of the pathogen via the watershed or possibly other non-water mechanisms. Epidemiologic evidence and traceback investigations indicated that STEC within the Salinas Valley growing region may be transferred in some way to leafy greens during cultivation and/or harvesting. Questions remain as to the mechanism(s) whereby cells of STEC become associated with lettuce in ways that they survive processing and distribution and reach the consumer. A major portion of commercially grown iceberg lettuce is cut and cored in the field. It is thought that soil might be transferred to edible tissues during this practice. Referred to in the industry as “field-coring” or “cut and core,” the process involves field workers using hand-held devices consisting of a stainless steel blade, shaft, and cylindrical coring ring to sever lettuce heads from roots and remove the core. Initial stem cuts are made near the soil surface using a wedge-shaped metal blade. The coring ring is then inserted around the stem of the lettuce head to remove the core. Depending on worker accuracy with each event, as well as soil conditions, blades of field-coring devices may contact the contaminated soil and transfer it to lettuce tissues, resulting in cross contamination. Transfer of foodborne pathogens from stainless steel to lettuce can occur to various extents, depending on the amount of water on the leaf surface. The potential for cut tissues of lettuce to become contaminated with the pathogen as a result of physical damage incurred during harvesting merits further study. Specifically, the potential and degree to which *E. coli* O157:H7 can become transferred to cored lettuce during field-coring needs to be determined. We undertook a study to determine the extent of contamination of iceberg lettuce via soil inoculated with *E. coli* O157:H7 by simulating mechanical damage of lettuce tissue resulting from cut-and-core practices used in commercial harvesting operations. The efficacy of chlorinated water treatment for removal or inactivation of *E. coli* O157:H7 on coring blades and cored lettuce was determined. The goal of the study was to provide information identifying various factors associated with contamination and elimination of *E. coli* O157:H7 on field-cored iceberg lettuce. Chlorinated water treatment was evaluated for its efficacy in removing or inactivating *E. coli* O157:H7 on the blade portion of the field-coring device (FCD) and on cored lettuce. FCD inoculated by immersing blades in soil containing *E. coli* O157:H7 at 3.74 or 6.57 log CFU/g contained 3.13 and 4.97 log CFU/blade, respectively. Treatment of inoculated FCD blades by immersing in chlorinated water (200 µg/ml, total chlorine) for 10 s resulted in a reduction of 1.56 log CFU/blade, which was 1.42 log CFU/blade greater than achieved using water, but insufficient to eliminate the pathogen on blades. FCD inoculated by contacting soil containing *E. coli* O157:H7 at 2.72 and 1.67 log CFU/g, then repeatedly used to cut and core ten lettuce heads, transferred the pathogen to ten and five consecutively processed heads, respectively. Lettuce cores remained positive for the pathogen after spraying with 100 µg/ml free chlorine for 120 s at 2.81 kg/cm² (40 psi), regardless of the inoculum level. The number of *E. coli* O157:H7 recovered from inoculated lettuce cores treated for 10 s with chlorine was significantly ($P \leq 0.05$) different than the number recovered from tissues treated with water. Dipping contaminated FCD in chlorinated water may not be effective in killing the pathogen and controlling cross-contamination from head to head. Spraying contaminated lettuce with chlorinated or untreated water reduces but does not eliminate *E. coli* O157:H7.