

## CHEMICAL TREATMENTS FOR ELIMINATION OF PATHOGENS

### COMPARISON OF TREATMENT OF FRESH-CUT LETTUCE AND DICED TOMATOES WITH SODIUM HYPOCHLORITE AND CALCIUM HYPOCHLORITE FOR EFFECTS ON MICROBIOLOGICAL AND SENSORY QUALITIES

(J. L. Simmons J.-H. Ryu, and L. R. Beuchat)

Outbreaks of salmonellosis, shigellosis, and *Escherichia coli* O157:H7 and viral infections have been associated with consumption of fresh produce. In some countries, minimally processed produce is routinely washed with chlorinated water to reduce or eliminate pathogenic and spoilage microorganisms. Sodium hypochlorite (NaOCl) traditionally has been used to prepare treatment solutions containing desired concentrations of free (available) chlorine. It is known that treatment of some types of fresh-cut produce with calcium can result in a firming of tissue texture, thereby reducing the amount of fluid released, with a consequent preservation of sensory quality and extension of shelf life. The role of calcium in prolonging shelf life is attributed in part to stabilization of cell membranes by reacting with negatively charged phospholipids and proteins to prevent solute leakage. Theoretically, treatment solutions containing a given concentration of free chlorine at a particular pH and temperature should be equally effective in killing microorganisms on produce, regardless of the type of hypochlorite salt, i.e., NaOCl or calcium hypochlorite [Ca(OCl)<sub>2</sub>], used to achieve that concentration. A potential added benefit of using Ca(OCl)<sub>2</sub> may be the retention of sensory quality resulting from the presence of calcium in the treatment solution that may not be achieved using NaOCl.

We undertook a study to determine if the type of hypochlorite salt [NaOCl and Ca(OCl)<sub>2</sub>] used to prepare chlorinated water for sanitizing fresh-cut iceberg, Romaine, and mesclun lettuce, and diced tomatoes affects lethality to microflora naturally occurring on the produce, as well as sensory quality during subsequent storage at 4°C for up to 13 days. The type of hypochlorite salt did not have a significant effect ( $P > 0.05$ ) on reductions in mesophilic aerobic microorganisms (total counts) or yeasts and molds, regardless of the concentration of free chlorine (50 - 200 µg/ml) in solutions used to treat produce. Appearance, color, aroma, texture, and overall quality of treated fresh-cut lettuce and diced tomatoes were likewise unaffected by the type of hypochlorite salt used to prepare chlorine treatment solutions. While NaOCl and Ca(OCl)<sub>2</sub>, at concentrations evaluated in this study, were indistinguishable in terms of effects on microbiological and sensory quality of produce, the choice of hypochlorite salt used to prepare chlorinated water should also consider factors such as cost of the salt, ease of control of desired concentration in the water, worker safety, and problems associated with disposal of treatment water after it has been used. The advantages of selecting one hypochlorite salt over the other must be decided and evaluated by informed personnel in each fresh-cut produce operation.

### EFFICACY OF SANITIZERS AND DISINFECTANTS ON PATHOGENIC PARASITES

(Y.R. Ortega, M.P. Torres, S. Van Exel, L. Moss, and V. Cama)

Protozoan parasites have been associated with foodborne outbreaks. Parasite contamination has been of particular interest in produce and fruits that are consumed raw. The objective of this study is to identify disinfectants and sanitizers that can inactivate parasites. Hydrogen peroxide, ammonium hydroxide, N-alkyl dimethyl benzyl ammonium chloride and a peroxyacetic acid based sanitizers at various concentrations and treatment times were examined for its ability to inactivate *Encephalitozoon intestinalis* spores. Microsporidia spores could be efficiently inactivated at a 5% ammonium hydroxide concentration for 1 min. Prolonged exposure to lower dilutions did not significantly reduce spore counts as determined by *in vitro* cultivation. Tsunami at 40µg/ml partially inactivated microsporidial spores when incubated at 1 min but was not statistically significant. Complete inactivation was achieved with at least 5 min exposure, whereas at 20µg/ml, complete inactivation was achieved after 15 min incubation. In this study, hydrogen peroxide at all concentrations and ammonium hydroxide at 5% effectively inactivated microsporidial spores. Therefore, *E. intestinalis* spores are more susceptible to inactivation than *Cryptosporidium* oocysts when using these two chemicals. *Cryptosporidium parvum*, *Encephalitozoon intestinalis*, and *Cyclospora cayetanensis* were experimentally inoculated into lettuce and basil leaves and treated with gaseous chlorine dioxide at 4.1 mg/ml. After treatment, *Cryptosporidium* and microsporidia viability was determined by *in vitro* cultivation using HCT-8 and RK-13 cell lines respectively. *Cyclospora* viability was determined by sporulation for 2 week period at 23C. *C. parvum* log reduction of 2.75 to 3.26 was

achieved. Microsporidia reduction was 3.6 and 3.58. No reduction was observed in Cyclospora sporulation, whereas a 2.6 to 3.2 log reduction of *E. coli* was achieved.

**CONTROL OF *ENTEROBACTER SAKAZAKII* IN RECONSTITUTED INFANT FORMULA  
USING THE LACTOPEROXIDASE SYSTEM**  
(J.B. Gurtler and L.R. Beuchat)

Neonatal bacteremia and meningitis caused by *Enterobacter sakazakii* have been associated with consumption of reconstituted powdered infant formula. Reconstituted formula is an excellent substrate for growth of *E. sakazakii*, thus raising a need for elimination or control interventions. The lactoperoxidase system (LPOS) has been shown to prevent the growth of *Escherichia coli*, *Campylobacter jejuni*, *Salmonella*, *Shigella*, and other foodborne pathogens in various types of foods. We undertook a study to determine if LPOS can be used to control the growth of *E. sakazakii* in a commercially manufactured milk-based powdered infant formula upon reconstitution with water. The effect of temperature on inhibitory or lethal activity was examined. Initially at 0.04 CFU/ml, *E. sakazakii* grew to 2.40 - 2.74 log CFU/ml in reconstituted infant formula held at 30 or 37°C for 8 h and to 0.60 log CFU/ml in formula held for 12 h at 21°C. The pathogen was not detected (less than 1 CFU/227 ml) by enrichment of formula treated with 10 - 30 µg/ml lipoperoxidase (LPO) and stored for 24 h at 37°C or 30 µg/ml LPO and stored for 24 h at 30°C. Populations of *E. sakazakii*, initially at 4.40 log CFU/ml of reconstituted infant formula containing 5 µg/ml LPO, did not change significantly ( $p > 0.05$ ) for up to 12 h at 21 and 30°C. Populations either decreased significantly ( $p \leq 0.05$ ) or were unchanged in formula supplemented with 10 µg/ml LPO and stored at 21, 30, or 37°C for up to 24, 8, and 8 h, respectively. Results indicate that LPOS can be used to control the growth of *E. sakazakii* in reconstituted infant formula, thereby potentially reducing the risk of neonatal infections resulting from consumption of formula that may be contaminated with the pathogen.

**EVALUATION OF PRESERVATIVES TO CONTROL THE GROWTH  
OF MOLDS CAPABLE OF PRODUCING 1,3-PENTADIENE**  
(D.A. Mann and L.R. Beuchat)

It was discovered in the mid 1960s that some *Penicillium* species are capable of growing in cheese containing potassium sorbate at concentrations as high as 7,100 µg/g. These penicillia degrade sorbic acid and produce 1,3-pentadiene, a volatile compound with an unpleasant hydrocarbon-like odor. More recently, *Aspergillus* and other molds have been reported to be capable of degrading sorbic acid and producing 1,3-pentadiene in a variety of foods and beverages. The use of preservatives other than sorbate to control the growth of molds in foods and beverages would eliminate problems associated with the production of 1,3-pentadiene. Reduced levels of sorbate in combination with propionate, benzoate, natamycin, and/or ethylenediaminetetraacetic acid (EDTA) offer an approach toward achieving that goal. While the concentration of sorbate effective in preventing the growth of some species of 1,3-pentadiene-producing molds in specific types of foods and beverages stored under certain time-temperature conditions may be known, the effectiveness of reduced concentrations of sorbate, in combination with other antimicrobials, has not been defined. We did a study to evaluate potassium sorbate, sodium benzoate, calcium propionate, disodium 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. These products were subjectively judged to contain 1,3-pentadiene. Growth of *Penicillium brevicompactum*, *Penicillium roqueforti*, *Paecilomyces variotii*, *Aspergillus niger*, and *Cephalosporium fragrans* on agar media containing Parmesan cheese (20% [w:v] plus 1.5% agar) and lemon-flavored drink (LD) agar (2.0%) supplemented with antimicrobials was studied. All molds grew well at 21°C on Parmesan cheese agar (PRM, pH 5.5) 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 the growth of test molds in PRM agar. Molds grew on LD agar (pH 3.5) not containing preservatives. 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 30 µg/ml EDTA in combination with potassium sorbate and sodium benzoate at 50 and 175 µg/ml, respectively, or 175 and 50 µg/ml, respectively. These preliminary studies using Parmesan cheese- and lemon drink-based agar model systems reveal that preservative systems containing a reduced concentration of potassium sorbate, in combination with other antimicrobials, have potential for controlling the growth of molds capable of producing 1,3-pentadiene in foods and beverages.

