

## PRODUCE

**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.

**RADIO-FREQUENCY HEATING OF ALFALFA SEED FOR REDUCING HUMAN PATHOGENS  
(S. O. Nelson, C.-Y. Lu, L. R. Beuchat, and M. A. Harrison)**

The production of sprouts from alfalfa and other seeds for human consumption is a substantial industry; however, there have been several outbreaks of illness associated with sprouts, and contamination by *Salmonella* and *Escherichia coli* O157:H7 has been identified as the cause. Contaminated seed used for sprouting is considered the most likely source of these human pathogens. No sprout-related illness attributable to *Listeria monocytogenes* has yet been documented, but this pathogen also poses a potential threat. Because most of the outbreaks of infections have been attributed to contaminated sprouting seed, several methods have been studied for decontaminating seed. Treatment of alfalfa seed in hot water at 54°C significantly reduced seed viability. Several aqueous solutions of chemicals, including chlorine, chlorine dioxide, hydrogen peroxide, trisodium phosphate, ethanol, peracetic acid, and some commercial fruit and vegetable produce wash solutions have been studied for decontaminating alfalfa seed. None of these treatments eliminated *E. coli* O157:H7 or *Salmonella* from alfalfa seed intended for sprouting.

Earlier research has shown that radio-frequency (RF) and microwave dielectric heating treatments are effective for increasing the germination percentage of alfalfa seed lots containing high percentages of hard seed. Hard seeds occur naturally and are viable seeds with seed coats that are impermeable to water. Therefore, they will not germinate promptly when planted, but they may germinate several weeks, months, or years later when the seed coat becomes permeable through natural processes. Mechanical scarification of such seed lots to increase germination is

common practice for alfalfa, but the abrasive process scratches the seed coat thus providing a favorable environment for bacterial attachment, which may make sanitization with liquids more difficult. Thus, it appeared reasonable to explore the possible use of dielectric heating for reduction of bacterial populations on alfalfa seed, especially since the improvement of germination and subsequent sprout yield can be achieved without mechanical abrasion of the seed coat. Similar consistent increases in alfalfa seed germination through hard seed reduction have been achieved by dielectric heating at frequencies of 5, 10, 39, or 2,450 MHz. Treatment at 39 MHz was selected for this study because of equipment availability and because it provides a more uniform electric field for exposure of the samples than is commonly available in microwave ovens.

The potential for controlling human bacterial pathogens on alfalfa seed used in the production of sprouts by dielectric heating was studied by experimental exposure of alfalfa seed artificially contaminated with *Salmonella*, *E. coli* O157:H7, and *L. monocytogenes* to RF dielectric heating treatments at 39 MHz and different electric field intensities for varying time of exposure. Moisture content of alfalfa seed and final temperatures produced by the RF exposures were determined, and control and treated seed samples were analyzed in the laboratory for reduction of bacterial populations and effects on seed germination. Significant reductions in populations of all three pathogens were achieved without reductions in seed germination. However, exposures that provided substantial reductions in pathogen levels were not achieved without significant damage to seed germination. Treatments providing moderate reductions in bacterial pathogen populations also increased alfalfa seed germination through reductions in hard seed percentages, so the combined benefits need to be considered in evaluating dielectric heating treatments for practical use.

**ATTRACTION OF A FREE-LIVING NEMATODE, *CAENORHABDITIS ELEGANS*,  
TO FOODBORNE PATHOGENIC BACTERIA, AND ITS POTENTIAL AS A VECTOR OF *SALMONELLA* POONA  
FOR PREHARVEST CONTAMINATION OF CANTALOUPE  
(K. N. Caldwell, G. L. Anderson, P. L. Williams, and L. R. Beuchat)**

Soil is a source of microbial contamination of fruits and vegetables, as evidenced by the isolation of soil-residing pathogenic bacteria from produce. Free-living, microbivorous nematodes are among the primary grazers of bacteria in soils and also have potential to serve as vectors of microorganisms, including enteric pathogens, to the surface of fruits and vegetables. Most nematologists do not attach particular importance to free-living nematodes as vectors of plant pathogens. However, a critical examination of the role nematodes may play in plant and perhaps human diseases has been suggested. *Caenorhabditis elegans*, a microbivorous, free-living nematode, has been used extensively in biological studies. Feeding primarily on bacteria, the adult worm lives approximately 2 weeks under optimal environmental conditions. The worm is routinely cultured in the laboratory on *Escherichia coli* OP50, a uracil-deficient non-pathogenic strain that grows slowly on K agar but serves as a nutrient source for multiplication and reproduction. The objectives of this study were to determine the propensity of *C. elegans* to migrate toward three human enteric pathogens and cantaloupe juice, as well as its survival and reproductive behavior in the presence of these pathogens. The potential of *C. elegans* as a vector to transport *Salmonella* in soil to the surface of cantaloupe rind was also investigated.

The propensity of *C. elegans* to be attracted to seven strains of *Escherichia coli* O157:H7, eight serotypes of *Salmonella*, six strains of *Listeria monocytogenes*, and cantaloupe juice was investigated. Adult worms (20 - 30) were placed on the surface of K agar midway between a 24-h bacterial colony and 10 µl of uninoculated tryptic soy broth (TSB) or cantaloupe juice positioned 1.5 cm apart. The number of nematodes that migrated to the colony, TSB, or cantaloupe juice within 5, 10, 15, and 20 min at 21°C was determined, followed by incubating plates at 37°C for up to 7 days to determine the ability of *C. elegans* to survive and reproduce in bacterial colonies. The nematode was attracted to colonies of all test pathogens, and survived and reproduced within colonies for up to 7 days. *C. elegans* was not attracted to cantaloupe juice. The potential of *C. elegans* to serve as a vector to transport *Salmonella* Poona to cantaloupe rind was investigated. Adult worms that had been immersed in a suspension of *S. Poona* were deposited 1 or 3 cm below the surface of soil on which a piece of cantaloupe rind was placed. The rind was analyzed for the presence of *S. Poona* after 1, 3, 7, and 10 days at 21°C. The presence of *S. Poona* was evident more quickly on rind positioned on soil beneath which *C. elegans* inoculated with *S. Poona* was initially deposited compared to rind on soil beneath which *S. Poona* alone was deposited. The time required to detect *S. Poona* on rind was longer when the rind was placed 3 cm above the inoculum, compared to 1 cm. Free-living nematodes may play a role in the preharvest dispersal of incidental human pathogens in soil to the surface of raw fruits and

vegetables in contact with soil during development and maturation, as evidenced by the behavior of *C. elegans* as a test model.

**PROTEOLYTIC YEASTS ISOLATED FROM RAW, RIPE TOMATOES  
AND METABIOTIC ASSOCIATION OF *GEOTRICHUM CANDIDUM* WITH *SALMONELLA*  
(W. N. Wade, R. Vasinnyi, T. Deak, and L. R. Beuchat)**

Post-harvest decay of tomatoes can be caused by several molds, but the *Alternaria*, *Botrytis*, *Cladosporium*, *Fusarium*, *Rhizoctonia*, and *Rhizopus* species are most commonly involved. Yeasts and yeast-like organisms such as *Geotrichum candidum*, which causes sour rot of tomatoes, also contribute to post-harvest losses. Metabiotic associations between molds and bacteria capable of causing human diseases are a public health concern. Growth of *Alternaria* and *Fusarium* in fresh tomatoes has been reported to increase the pH of tissues to values as high as 8, allowing *Clostridium botulinum* to grow and produce toxin. The pH of tomato juice has been shown to increase from 4.1 to greater than 9.0 when inoculated with molds. *Aspergillus gracilis* and species of *Penicillium* and *Cladosporium* have been reported to grow in tomato juice and increase the pH to levels supporting toxin production by *C. botulinum*. We have observed that *Alternaria alternata* and *Cladosporium* species co-inoculated with *Salmonella* into raw ripe tomatoes increase the pH of pulp, resulting in enhancement of the rate of growth of the pathogen. Food spoilage yeasts are infrequently examined for proteolytic activity, although some species known to grow in a wide range of foods can cause significant proteolysis. Several genera, including *Aureobasidium*, *Candida*, *Endomycopsis*, *Kluyveromyces*, and numerous sporobolomycetes exhibit proteolytic activity. Highly proteolytic *Candida* species have been isolated from ripe amapa fruit and yeasts known to have proteolytic activity grow well in guava and tomato fruits. In results from investigations of the extracellular enzymatic activity profiles of yeast and yeast-like strains isolated from tropical environments, 7 of 196 (3.6%) strains of ascomycetes and 48 of 155 (31%) strains of basidiomycetes exhibited protease activity.

Several outbreaks of salmonellosis associated with consuming raw tomatoes have been documented. Environmental and ecological factors that may affect the survival and growth of *Salmonella* in uncooked tomatoes have not been clearly defined, although pre- and post-harvest infection with yeasts and molds may play a role. Metabiotic associations between yeasts and foodborne bacterial pathogens that may occasionally occur as incidental contaminants on raw tomatoes have not been described. We did a survey of raw, ripe, decayed, or damaged tomatoes to determine the presence of proteolytic yeasts. We then studied the survival and growth of *Salmonella* co-inoculated with a proteolytic strain of *G. candidum* into raw ripe tomatoes. Sixty-two of the 371 (16.7%) fungi isolated from 215 decayed or damaged tomatoes and 12 of the 62 (19.4%) yeasts showed proteolytic activity on gelatin agar and/or standard methods caseinate agar. The pH of tomato pericarp (pulp) tissue from which 9 or the 12 yeasts were isolated ranged from 4.3 to 7.5 (mean = 5.3) compared to 4.2 - 5.1 (mean 4.8) for sound pulp tissue in the same tomatoes. The 12 proteolytic yeasts consisted of four strains of *Cryptococcus albidus*, two strains each of *Debaryomyces hansenii* and *Trichosporon pullulans*, and one strain each of *Cryptococcus humicolus*, *Cryptococcus laurentii*, *Geotrichum candidum*, and *Sporidiobolus pararoseus*. Survival and growth characteristics of a five-serotype mixture of *Salmonella* co-inoculated with *G. candidum* into sound (not chill injured) and chill-injured tomatoes were studied. Storage of sound tomatoes at 15°C for 10 days resulted in an increase in population of 7.6 log<sub>10</sub> cfu of *Salmonella*/g of a 2-g sample of co-infected pulp tissue. Increases were less in tissue inoculated with *Salmonella* only, *Salmonella* on day 0 followed by *G. candidum* on day 3, or *G. candidum* on day 3, or *G. candidum* on day 0 followed by *Salmonella* on day 3. Trends were similar in sound inoculated tomatoes stored at 25°C. Growth of *Salmonella* was enhanced in chill-injured tomatoes compared to sound tomatoes; a population of 10 log<sub>10</sub> cfu/g of chill-injured pulp tissue was reached within 10 days at 25°C. Results clearly show that growth of a proteolytic, alkalinizing yeast such as *G. candidum* in raw tomatoes enhances conditions for growth of *Salmonella*. The removal of tomatoes infected with proteolytic yeasts and other fungi from lots intended for minimally processed tomato products is an essential step in reducing the risk of human diseases caused by pathogenic bacteria favored by increased pH of decayed pulp tissue.

**ATTACHMENT OF *ESCHERICHIA COLI* O157:H7 GROWN IN TRYPTIC SOY BROTH  
AND NUTRIENT BROTH TO APPLE AND LETTUCE AS RELATED  
TO CELL HYDROPHOBICITY, SURFACE CHARGE, AND CAPSULE PRODUCTION  
(A. N. Hassan and J. F. Frank)**

This study investigated the effect of growth in tryptic soy broth (TSB) and nutrient broth (NB) on the ability of *E. coli* O157:H7 to attach to lettuce and apple. In addition, surface hydrophobicity, charge and capsule production were determined by cells grown in these media. Cells grown in NB attached less to lettuce and apple surfaces than did those grown in TSB. TSB, but not NB, supported capsule production by *E. coli* O157:H7. Cells grown in TSB were more hydrophilic than those grown in NB. No difference was found in the electrokinetic properties of cells grown in these media. Electrostatic and hydrophobic interactions and surface proteins did not appear to play an important role in the attachment of *E. coli* O157:H7 to these surfaces. Of the factors studied, only capsule production was associated with attachment ability.

**EFFECT OF INOCULUM SIZE, RELATIVE HUMIDITY, STORAGE TEMPERATURE, AND RIPENING STAGE  
ON THE ATTACHMENT OF *SALMONELLA* MONTEVIDEO TO TOMATOES AND TOMATILLOS  
(M. H. Iturriaga, E. F. Escartin, L. R. Beuchat, and R. Martinez-Peniche)**

Bacterial survival and growth on and in fruits and vegetables depends, in part, on their ability to attach to surfaces. Sessile microorganisms have advantages in that they are more difficult to mechanically remove from surfaces and are more resistant to disinfectants compared with planktonic cells. It has been observed that attachment of bacterial cells is affected by several factors, including the medium in which they are grown, motility, temperature, length of contact time, and production of extracellular polysaccharides. Information describing the attachment of human pathogens on fruits and vegetables is limited; however, the process appears to be similar to that of adhesion of plant pathogenic and non-pathogenic bacteria on leaves. One possible intervention to minimize the risk of human infections associated with the consumption of raw fruit and vegetables contaminated with foodborne pathogens is the application of chemical treatments. The increased resistance of attached microorganisms to sanitizing agents may partially explain the observed limited effect of disinfection treatments. A clearer understanding of the process of bacterial adhesion to fruit surfaces is essential to devising more effective methods for removal. The objective of this research was to evaluate the effects of inoculum size, relative humidity, ripening stage, and storage temperature on the attachment of *S. Montevideo* to the surface of tomatoes and tomatillos.

The influence of inoculum populations and environmental factors on attachment of *Salmonella* Montevideo to the surface of tomatoes and tomatillos was evaluated. To study the effect of inoculum size, red, ripe tomatoes were spot inoculated with bacterial suspensions ( $10^5$  and  $10^8$  cfu/fruit) and stored at 22°C under 100% relative humidity. The effects of temperature (12, 22, and 30°C) and relative humidity (75, 85, and 97%) on attachment of the pathogen ( $10^7$  cfu/fruit) on tomatoes (red and green) and ripe tomatillos were also evaluated. Inoculated fruit were stored for 90 min at all combinations of temperature and relative humidity, and after washing with water the number of cells attached to the surface was determined. *S. Montevideo* attached to the surface of tomatoes within 90 min. A direct correlation between the number of attached cells and the population in the inoculum was observed. The percentage of cells that attached immediately after inoculum was approximately 0.3% for the test products. After storage for 90 min at various temperature/relative humidity conditions, the number of adhering cells ranged from 4.0 to 5.4  $\log_{10}$  cfu/fruit (1.2% of inoculum). Both the type of product and the temperature/relative humidity condition had a significant ( $P \leq 0.05$ ) effect on attachment of *S. Montevideo* to the surfaces of tomatoes and tomatillos. Scanning electron micrographs of the cuticles of inoculated washed tomatoes and tomatillos revealed typical skin cell patterns, and only a few randomly dispersed *S. Montevideo* were observed. Deposition of *S. Montevideo* on the surface of tomatoes and tomatillos may result in attachment and subsequent colonization under suitable conditions.

**FACTORS AFFECTING SURVIVAL, GROWTH, AND RETRIEVAL OF *SALMONELLA* POONA  
ON INTACT AND WOUNDED CANTALOUPE RIND AND IN STEM SCAR TISSUE  
(L. R. Beuchat and A. J. Scouten)**

The first documented outbreak of salmonellosis in the U.S. that was associated with consuming cantaloupes was reported in 1990. Consumption of cantaloupes imported from Mexico and Central America was linked to 295 cases of *Salmonella enterica* serotype Chester infections in 30 states. In the summer of 1991, more than 400 cases of *Salmonella enterica* serotype Poona infections in the U.S. and Canada were associated with the consumption of cantaloupe. Twenty-five cases of *Salmonella enterica* serotype Saphra infections in the U.S. were linked to cantaloupes imported from Mexico in 1997 and 22 cases of *Salmonella enterica* serotype Oranienburg infections in

Canada in 1998 were attributed to consuming cantaloupes. Subsequent outbreaks of *S. Poona* infections in the U.S. and Canada have been associated with eating cantaloupes grown in Mexico. A survey to determine the presence of foodborne pathogens on fresh produce imported to the U.S. revealed that 11 of 151 (7.3%) cantaloupes were contaminated with *Salmonella* or *Shigella*. A survey of domestically grown fruits and vegetables reported the presence of *Salmonella* on 3 of 92 (3.3%) cantaloupes. Clearly, the pathogen can be found on the surface of cantaloupes at various points postharvest and is somehow transferred to the edible portion before consumption, resulting in human infections. As few as 150 *Salmonella*/cm<sup>2</sup> of netted rind surface of cantaloupes have been shown to contaminate the edible portion upon cutting.

Several researchers have evaluated sanitizers for their efficacy in killing or removing foodborne pathogens and spoilage microorganisms on the surface of cantaloupe rind and the interior flesh. It is difficult to compare the effectiveness of various sanitizers across, and sometimes within, laboratories because of variations in methodology. Methods used to analyze cantaloupes not intentionally inoculated with *Salmonella* also vary among research and testing laboratories. While the development of a standard method to analyze raw fruits and vegetables for *Salmonella* and other pathogens has been promoted, still there are many factors that could influence the efficiency of retrieval of *Salmonella* from cantaloupes that need to be investigated before an optimum method can be recommended. We conducted a study to determine the survival and recovery of *Salmonella enterica* serotype Poona from cantaloupe rind as affected by environmental conditions between the time of contamination and analysis. Detection and enumeration of the pathogen as influenced by analytical methods were also investigated. Combinations of preenrichment broth (lactose broth or universal preenrichment broth), enrichment broth (Rappaport-Vassiliadis broth or tetrathionate broth), and selective agar medium (bismuth sulfite agar or xyline lysine desoxycholate agar) for detecting *S. Poona* on inoculated cantaloupes stored at 4°C for 7 days or 21°C for 3 days were equivalent in performance. The use of nalidixic acid as a marker in *S. Poona* and in media used to enhance detection or enumeration of the pathogen by inhibiting background microflora in sanitizer efficacy studies, for example, would not adversely affect its survival on or recovery from cantaloupes. Overall, the composition of the carrier (water or 5% horse serum, a high organic matrix) used to prepare inocula did not influence the number of *S. Poona* recovered from the intact rind surface, wounds in the surface, or the stem scar tissue. Regardless of inoculation site or composition of the carrier, populations on spot inoculated melons stored at 4°C remained constant between 2 and 24 h after inoculation. The pathogen grew within 24 h in wounds of spot- and dip-inoculated cantaloupes stored at 21 and 37°C. The addition of up to 1.0% Tween 80 to 0.1% peptone used to remove *S. Poona* from the rind surface did not adversely affect viability and may have enhanced detachment. Consideration of these observations is recommended when developing a method to test the efficacy of sanitizers in killing salmonellae on the rind surface of inoculated cantaloupes and to detect or enumerate salmonellae that may be natural contaminants.

#### **INFLUENCE OF VARIATIONS IN METHODOLOGY ON POPULATIONS OF *LISTERIA MONOCYTOGENES* RECOVERED FROM LETTUCE TREATED WITH SANITIZERS**

**(A. B. Burnett, M. H. Iturriaga, E. F. Escartin, C. A. Pettigrew, and L. R. Beuchat)**

The lack of a standard method(s) to quantitate pathogens on raw fruits and vegetables has resulted in great variations in methodology used by researchers and in commercial testing laboratories. While some of these variations may not affect the efficiency of enumerating pathogens on a given produce item, most have not been properly evaluated or validated. The importance of optimum procedures for recovering pathogens that may be stressed or injured as a result of desiccation or exposure to chemical sanitizers, for example, is increased when the efficacy of decontamination treatments is being assessed. If the enumeration method does not recover all viable cells from treated produce, an underestimation of populations will result.

Progress is being made in developing and validating a standard method to evaluate the effectiveness of produce sanitizers, although modifications of a basic method will likely be necessary to achieve maximum recovery of pathogens surviving treatment. Major variations in sanitizer efficacy methodology currently used by researchers include inoculation of produce by dipping in a cell suspension versus spot inoculation of the test pathogen, drying inoculum for times ranging from a few seconds to 24 h, failure to use a neutralizing agent to terminate the activity of the lethal component, and rinsing, stomaching, or blending to process treated samples for enrichment or direct plating. In only a few studies have comparisons of variations in specific steps in produce sanitizer efficacy methods been made. Survival of *Escherichia coli* O157:H7, *Salmonella*, and *Listeria monocytogenes* on tomatoes, as affected by drying time after application of inoculum, and subsequent effectiveness of point-of-use sanitizers in

reducing populations have been investigated. We have examined sample processing methods for their influence on populations of *Salmonella* recovered from twenty-six types of raw fruits, vegetables, and herbs. Overall, processing samples by washing in 0.1% peptone, stomaching, or homogenizing did not substantially affect the number of *Salmonella* recovered.

The study reported here was done to determine if variations in methodology influence the efficacy of chlorinated (200 µg/ml free chlorine) water and FIT® Professional Line Produce Cleaner (FIT®) in killing *L. monocytogenes* spot inoculated onto iceberg lettuce. Variations in methodology included composition of broth media used to grow cells to prepare inocula, number of strains present in the inoculum, time and temperature between inoculation and treatment with sanitizers, sample processing method, and composition of direct plating media used to enumerate the pathogen. The efficacy of the two sanitizers was not influenced by the composition of the medium used to culture *L. monocytogenes* used in inocula, the number of strains in the inoculum, or the recovery medium used to enumerate the pathogen on lettuce after treatment. Drying inoculum on lettuce for 45 min at 37°C caused more cells to die or not be retrieved compared to drying inoculum for 30 min at 25°C. However, the percentage of cells in the inoculum recovered from lettuce treated with chlorine or FIT® was not significantly different, regardless of the drying method. Stomaching, homogenizing, or stomaching followed by homogenizing lettuce treated with sanitizers resulted in recovery of similar numbers of *L. monocytogenes*, indicating that stomaching and homogenizing are equivalent in extracting cells; the sequential use of both processing methods did not substantially increase the efficiency of recovery. Washing lettuce with water or treating lettuce with 200 µg/ml chlorine or FIT® resulted in decreases in populations of 0.60, 1.76, and 1.51 log<sub>10</sub> cfu/lettuce, respectively, regardless of variations in test parameters. Reductions caused by sanitizers were significantly greater ( $\alpha = 0.05$ ) than that observed for water but not significantly different from each other. It is concluded that evaluation of sanitizers for their efficacy in killing *L. monocytogenes* on lettuce can be determined by spot inoculating 50 µl of a five-strain mixture of cells from 24-h cultures suspended in 5% horse serum albumen, followed by drying the inoculum for 45 min at 37°C, treatment by submerging in 50 ml of sanitizer for 5 min, stomaching samples in 50 ml of Dey-Engley neutralizing broth for 2 min, and enumerating survivors on modified Oxford medium.

**EVALUATION OF INOCULATION METHOD AND INOCULUM DRYING TIME FOR THEIR EFFECTS ON SURVIVAL AND EFFICIENCY OF RECOVERY OF *ESCHERICHIA COLI* O157:H7, *SALMONELLA*, AND *LISTERIA MONOCYTOGENES* INOCULATED ON THE SURFACE OF TOMATOES**  
(M. M. Lang, L. J. Harris, and L. R. Beuchat)

Contamination of raw produce with pathogenic microorganisms can occur at any of several points from the field through the time of consumption. Given sufficient time and appropriate environmental conditions, pathogens can grow to populations exceeding 10<sup>7</sup> cfu/g of tomato. Work has been done to define conditions that result in contamination of produce and subsequent growth of pathogens during storage. Researchers have also evaluated the effectiveness of a wide range of chemical sanitizers and physical treatments to decontaminate fresh produce. Results of studies done in different laboratories are difficult to compare, however, because of numerous variations in methodologies employed and incompleteness in describing results. A study was undertaken with the objective to evaluate procedures for inoculating *Escherichia coli* O157:H7, *Salmonella*, and *Listeria monocytogenes* onto the surface of tomatoes with the goal of selecting an inoculation procedure to be used in a standard method. Dip, spot, and spray inoculation were evaluated. A second objective was to examine the effect of time between application of inoculum and analysis of tomatoes on the viability and retrievability of pathogens. Inocula applied to tomatoes were subjected to two drying times followed by either no treatment or treatment with water (control) or chlorine (200 µg/ml), then analyzed for the presence of surviving cells.

Five-strain mixtures of *Escherichia coli* O157:H7, *Salmonella*, or *Listeria monocytogenes* were applied to tomatoes by dip, spot, or spray inoculation methods. Inocula were dried for 1 or 24 h at 22°C before tomatoes were treated with water (control) or chlorine (200 µg/ml). Significantly ( $\alpha = 0.05$ ) higher populations (cfu/tomato) of *E. coli* O157:H7 and *Salmonella* were recovered from dip-inoculated tomatoes compared to spot- or spray-inoculated tomatoes. This is attributed to larger numbers of cells adhering to tomatoes subjected to dip inoculation. Populations of *E. coli* O157:H7 and *Salmonella* recovered from spot- and spray-inoculated tomatoes containing the same initial number of cells were not significantly different. Significantly different populations of *L. monocytogenes* were recovered from inoculated tomatoes (dip > spot > spray). Populations of pathogens recovered from tomatoes were significantly higher when inocula were dried for 1 h compared to 24 h. Significant differences (water > chlorine) were observed in populations of all pathogens recovered from tomatoes treated with chlorine,

regardless of inoculation method or drying time. Results indicate that inoculation method, drying time, and treatment affect survival and/or recovery of foodborne pathogens inoculated onto the surface of tomatoes. It is recommended that spot inoculation with a drying time of 24 h at 22°C be used in a standard method to determine the efficacy of chlorine and other sanitizers in killing foodborne pathogens on tomatoes.

**SURVIVAL AND RECOVERY OF *ESCHERICHIA COLI* O157:H7, *SALMONELLA*, AND *LISTERIA MONOCYTOGENES* ON LETTUCE AND PARSLEY AS AFFECTED BY METHOD OF INOCULATION, TIME BETWEEN INOCULATION AND ANALYSIS, AND TREATMENT WITH CHLORINATED WATER  
(M. M. Lang, L. J. Harris, and L. R. Beuchat)**

Given sufficient time and appropriate environmental conditions after contamination, pathogens can grow to populations exceeding  $10^7$  cfu/g of lettuce and  $10^6$  cfu/g of parsley. Conditions that result in contamination of produce with pathogens and subsequent growth during storage have been described. A wide range of chemical sanitizers and physical treatments for decontamination of fresh produce has been evaluated. Results are difficult to compare, however, because of the numerous variations in methodologies. The lack of uniformity of methods used to treat produce with sanitizers and enumerate microorganisms surviving treatments makes it difficult to assess their effectiveness and establish industry recommendations and guidelines for their use. The development of a standard method would minimize or eliminate variations in methodologies used in various laboratories, thereby enabling a comparison of pathogen reductions resulting from treatment with various sanitizers. A single method may not be applicable for all fruits and vegetables but a basic test method that could be modified as necessary to accommodate natural variations in fresh and fresh-cut produce would be the goal.

One of the objectives of the study reported here was to evaluate three methods (dip, spot, and spray) for inoculating *E. coli* O157:H7, *Salmonella*, and *L. monocytogenes* onto the surface of two types of leafy salad vegetables, lettuce and parsley, with the goal of establishing a procedure for use in a standard method to test the efficacy of sanitizers. A second objective was to examine the effect of time between application of inoculum and analysis of lettuce and parsley on the viability and recovery of pathogens. Inocula applied to lettuce and parsley were subjected to two drying times, followed by either no treatment or treatment with water or chlorine then analyzed for the number of surviving cells. Inocula were dried for 2 h at 22°C or for 2 h at 22°C followed by 22 h at 4°C before treating with water (control) or chlorine (200 µg/ml). Significantly ( $\alpha = 0.05$ ) higher populations (cfu/lettuce or parsley sample) of *E. coli* O157:H7 and *Salmonella* were recovered from dip-inoculated produce compared to spot- or spray-inoculated produce. This is attributed to larger numbers of cells adhering to lettuce and parsley subjected to dip inoculation. Populations of *E. coli* O157:H7 and *Salmonella* recovered from lettuce inoculated by spot and spray methods were not significantly different but populations recovered from spot-inoculated parsley were significantly higher than those recovered from spray-inoculated parsley, even though the number of cells applied was the same. Significantly different populations of *L. monocytogenes* were recovered from inoculated lettuce (dip > spray > spot); populations recovered from dip-inoculated parsley were significantly higher than those recovered from spot- or spray-inoculated parsley, which were not significantly different from each other. Populations of pathogens recovered from lettuce and parsley after drying inoculum for 2 h at 22°C were significantly higher than or equal to populations recovered after drying for 2 h at 22°C followed by 22 h at 4°C. Significant differences (water > chlorine) were observed in populations of all pathogens recovered from treated lettuce and parsley, regardless of inoculation method and drying time. It is recommended that spot inoculation with a drying time of 2 h at 22°C followed by 22 h at 4°C be used to determine the efficacy of chlorine and other sanitizers in killing foodborne pathogens on lettuce and parsley.

**INOCULATION STRATEGIES AND PARASITE RECOVERIES FROM EXPERIMENTALLY SPIKED PRODUCE  
(C. Tatum and Y. R. Ortega).**

Protozoan parasites have long been associated with water and foodborne outbreaks. Those parasites that most commonly have been associated with foodborne outbreaks, causing prolonged diarrheal illness, include *Cryptosporidium parvum*, *Cyclospora cayetanensis*, and *Giardia lamblia*. Most parasites need a host to multiply, therefore conventional propagation used with bacterial pathogens cannot be applied. Due to this limitation, recovery procedures are very crucial for parasite identification.

To evaluate recovery procedures, experimental spiking of basil, lettuce, and raspberries were done using three inoculation methods (spot, spray, and dip) and various concentrations of the three parasites ( $10^2 - 10^5$

ooocysts/produce). The greatest recoveries were obtained through spot inoculation followed by spray and dip inoculations. In all three food matrices *Giardia* cysts (20 – 90%) were recovered to a greater extent than *Cryptosporidium* while *Cyclospora* had the lowest recoveries. Recoveries of *Cryptosporidium* and *Giardia* were greatest on basil and lettuce than raspberries.

