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Survival and Growth of *Listeria monocytogenes* and *Escherichia coli* O157:H7 in Ready-to-Eat Iceberg Lettuce Washed in Warm Chlorinated Water

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ABSTRACT

Cut iceberg lettuce inoculated with *Escherichia coli* O157:H7 and *Listeria monocytogenes* before and after washing for 3 min in cold (4°C) and warm (47°C) water containing 100 mg/liter total chlorine was stored at 1 and 10°C in oxygen-permeable film packages (6,000 to 8,000 cc/m^2/24 h). Cold chlorinated water was detrimental to the survival of *E. coli* O157:H7 and *L. monocytogenes* at both storage temperatures. In contrast, washing in warm chlorinated water favored the growth of both pathogens in lettuce stored at 10°C. There was no evidence of a relationship between the magnitude of spoilage microflora and the fate of either bacterium.

Physiological changes or injuries incurred during processing and storage lead to the appearance of defects that limit the shelf life of packaged, ready-to-eat lettuce. Objectionable quality attributes can include tissue discoloration ranging from yellow to brown, texture loss, softening, exudation, and the development of off-odors and -flavors. Current processing schemes for ready-to-eat lettuce normally include a washing step in cold chlorinated water, water removal by centrifugation, and packaging. Low oxygen levels are considered desirable for the reduction of enzymatic browning and can be achieved passively, by tissue respiration, or by the injection of gas mixtures into impermeable film bags. Despite these measures, the shelf life of ready-to-eat lettuce is limited, and processing schemes that reduce or delay physiological damage are under investigation. One approach that has shown some promise is the application of mild heat during washing. Delaquis et al. *(11)* showed that a 3-min dip in chlorinated (100 mg/liter total Cl) water at 47°C provided optimum retention of appearance in stored, packaged iceberg lettuce. Sensory evaluation of lettuce processed in this manner confirmed that the treatment delays the onset of discoloration, improves the retention of texture, and reduces the development of bitterness *(10).* Similar improvements in quality have been reported for persimmons *(9),* apples *(25),* and broccoli *(29)* treated at elevated temperatures prior to storage under refrigeration.

Physiological changes triggered by processing are accompanied by alterations in the microbial ecology of lettuce that can contribute to quality defects. The microflora of unprocessed head lettuce is dominated by gram-negative, often pectinolytic psychrotrophic bacteria—mainly species of the genus *Pseudomonas (22).* The rate and extent of microbial proliferation in packaged lettuce depend mainly on initial microbial load and storage temperature *(22).* Attempts are therefore made to remove or inactivate contaminating microorganisms before packaging. Cold chlorinated water washes are widely practiced despite the limited effectiveness of such treatments. Attachment to the leaf surface is believed to limit contact with the sanitizing solution *(2, 5).* Most investigators report reductions of approximately 1 log CFU/g in water containing 100 to 150 μg/ml chlorine. Wash temperatures greater than 45°C improve the antimicrobial effect of chlorinated water. Reductions up to 3 log CFU/g have been reported in iceberg lettuce treated with 100 μg/ml chlorine at 47°C *(11).* Development of microbial populations determined from total aerobic plate counts was delayed for several days in chilled, packaged lettuce and the composition of the spoilage microflora was unaffected by wash temperature.

In addition to saprophytic species normally associated with plants, raw produce, including lettuce, may occasionally harbor pathogenic bacteria *(8, 23).* Several studies have shown that species such as *Listeria monocytogenes (15, 16, 28)* and *Escherichia coli* O157:H7 *(1, 12)* survive processing and may grow during storage. Efforts to eliminate such pathogens from produce by washing have met with mixed results. None of the treatments or sanitizers investigated to date can ensure consistent eradication, and behavior during subsequent storage remains unpredictable *(4, 14, 26, 27, 30).* We report here on the fate of *L. monocytogenes* and *E. coli* O157:H7 in ready-to-eat cut lettuce washed in cold and warm chlorinated water.

MATERIAL AND METHODS

Microorganisms, inoculum preparation, and inoculation procedures. *L. monocytogenes* (LCDC 81-861) and *E. coli* O157:H7 (ATCC 43895) were cultured for 24 h at 30°C in 100 ml tryptic soy broth (BBL, Cockeysville, Md.) amended with 5 g/

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liter yeast extract (TSBYE). Inocula for experiments were prepared by centrifugation of the cultures in a Sorvall RC5B (Du Pont, Newtown, Conn.) centrifuge at 3,000 rpm for 15 min at 4°C. The pellets were resuspended in sterile distilled water tempered to 4°C, and the optical density of the suspension was adjusted with distilled water in a spectrophotometer (600 nm) to obtain a cell density of 10^5 CFU/ml. Where required, inoculation with the test microorganisms was performed by adding 1 ml of the cell suspension per 100 g lettuce in a plastic bag to achieve initial populations of approximately 10^3 CFU/g lettuce. The contents were gently tossed and rolled for 5 min to distribute the inoculum evenly over leaf surfaces.

Lettuce processing. Experiments were conducted over a 3-month period using locally grown, boxed iceberg lettuce (cv. Salinas). The boxes were transported to the laboratory in a refrigerated transport and were stored at 4°C. All trials were carried out less than 48 h after harvest. The heads were trimmed of wrapper leaves and cores were removed with a stainless steel tube sharpened at the cutting end. Large rib tissues were removed and the remaining leaves were cut into square pieces (approximately 5 by 5 cm) with a knife. The cut lettuce was washed in 600-g batches in 20-liter stainless steel pails. Eight liters of distilled water at either 4 or 47°C were adjusted to 100 μg/ml total chlorine with a 10.8% sodium hypochlorite solution (Javex 12, Colgate-Palmolive Inc., Toronto, Canada) immediately before washing. Total chlorine concentrations were determined with a test kit from Hach (Model CN-66; Loveland, Colo.). The lettuce was added to the pails and stirred continuously with a stainless steel spoon for 3 min. Temperatures were monitored during treatment and were found to vary by 1°C above or below initial values for the 4 and 47°C washes, respectively (data not shown). The treated lettuce was immediately removed from the pail, spun in a household spinner to remove excess water, and transferred to a biological safety cabinet for 15 min prior to packaging. Samples (~100 g) were packed in bags fashioned from PD961EZ film (Cryovac, Duncan, S.C.; oxygen transmission rate: 6,000 to 8,000 cc/m^2/24 h) and sealed with a heating bar.

Treatments and experimental design. Three experiments were carried out with lettuce obtained on different dates over a 3-month period. Lettuce was inoculated separately with either pathogen before or after treatment for 3 min in chlorinated water at 4 or 47°C. Uninoculated controls were included to verify raw materials for the absence of the pathogens, as were inoculated controls to observe the behavior of the inocula in untreated lettuce; neither received further treatment before packaging. The bags containing controls and processed lettuce were divided in two lots for incubation at 1 or 10°C. Two bags from each control and treatment were removed for analysis after 0, 7, and 14 days in storage and mean populations at each sampling time were calculated from counts recorded for the three separate experiments.

Microbiological analyses. All samples were prepared for analysis by blending 25 g of lettuce with 225 ml of diluent for 2 min in a Lab stomacher (Colworth, UK). Total aerobic populations were estimated by surface-spreading duplicate aliquots (0.1 ml) of appropriate dilutions prepared in 0.1% peptone water onto plate count agar (Difco, Detroit, Mich.), followed by 2 days of incubation at 30°C. Where required, total populations of microorganisms other than *E. coli* O157:H7 or *L. monocytogenes* were estimated by subtracting counts derived from selective media for either test microorganism. *L. monocytogenes* populations were estimated by blending samples in Listeria enrichment broth (LEB; Oxoid, Nepean, Ontario, Canada). Dilutions prepared in LEB were spread onto PALCAM (polymyxin acriflavine LiCl ceftazidime esculin mannitol) agar (Difco) in duplicate and the plates were incubated at 30°C for 48 h. The blended samples were also placed in an incubator at 30°C for enrichment. Where colonies failed to develop on PALCAM after 24 h incubation, 0.1 ml of the enriched sample was transferred to 9.9 ml of modified Fraser broth (MFB; Difco) for further enrichment at 35°C for 48 h. Positive samples in MFB were streaked onto PALCAM and incubated as before. Presumptive *L. monocytogenes* colonies were transferred to tryptic soy agar (TSA; BBL) for confirmation by latex test (*L. monocytogenes* latex test, Oxoid).

Samples destined for estimation of *E. coli* O157:H7 populations were blended and diluted in modified TSB with Novobiocin (mTSB-n; Difco). Appropriate dilutions were spread onto modified sorbitol MacConkey (SMAC; Difco) plates, which were incubated at 42°C for 24 h. Where necessary, the initial samples were enriched by incubation at 37°C for 24 h and verified for the presence of *E. coli* O157:H7 by streaking onto SMAC. Presumptive colonies were transferred to TSA and confirmed with the *E. coli* latex test (Oxoid).

RESULTS

The lettuce used in this work was free of *L. monocytogenes* and *E. coli* O157:H7. Populations of each pathogen in inoculated lettuce samples withdrawn after 0, 7, and 14 days in storage are presented graphically in Figures 1 to 4. The effect of washing treatments on the fate of *E. coli* O157:H7 in lettuce stored at 1 and 10°C are shown in Figures 1 and 2. A storage temperature of 1°C was deleterious to the survival of this species, and the viability of inocula declined in unwashed lettuce over the 14-day storage period. Washing in cold chlorinated water after inoculation immediately reduced *E. coli* O157:H7 populations by 1 log CFU/g. The antimicrobial effect of chlorinated water was enhanced at 47°C, and reductions were in excess of 2 log CFU/g. *E. coli* O157:H7 populations continued to decrease in the packaged samples regardless of wash water temperature. In contrast, survival and growth in lettuce stored at 10°C were strongly influenced by wash treatments. Populations in inoculated controls and in lettuce washed at 4°C before inoculation did not change over time. A gradual decline in viability resulted from inoculation after washing at this temperature. In contrast, *E. coli* O157:H7 populations increased by 2 log CFU/g over 14 days in lettuce washed at 47°C.

Also given in Figures 1 and 2 are plots showing the change in populations of indigenous microorganisms in stored cut lettuce over time. The values represent estimates determined by subtracting counts on selective media from total plate counts. Raw lettuce harbored 10^6 microorganisms per gram, and overall growth was slightly slower in samples packaged without further processing. Washing in chlorinated water at 4°C reduced initial populations by 1 log CFU/g and 2 log CFU/g at 47°C. Variable differences between controls and treatments were evident after 7 days in storage at 1°C, but these were minimal after 14 days. Similar trends were observed in lettuce stored at 10°C, although final populations were slightly greater in samples washed at 47°C.

The fate of *L. monocytogenes* in packaged lettuce

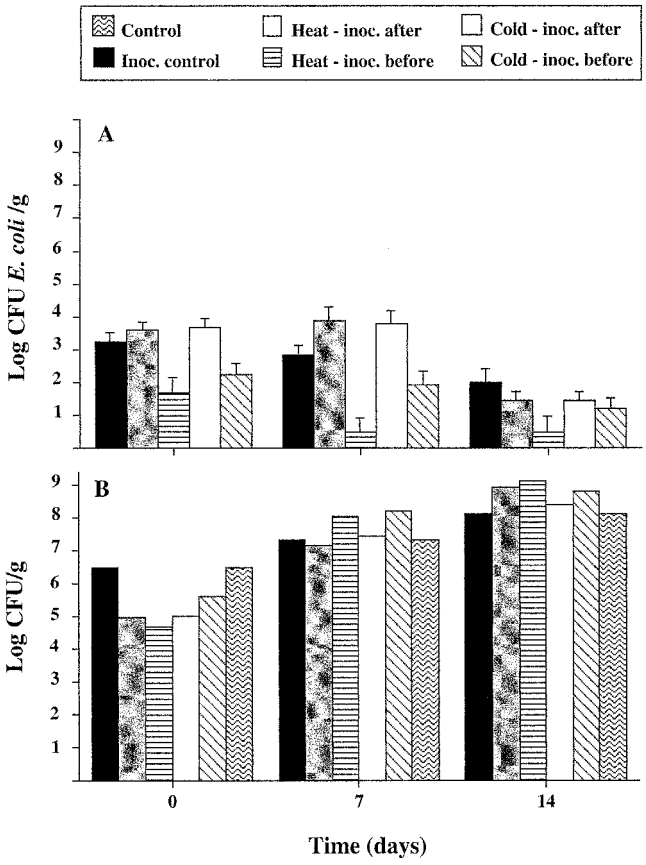

FIGURE 1. *Fate of* E. coli *O157:H7 in cut lettuce stored at 1°C. (A) Mean* E. coli *O157:H7 populations in unwashed lettuce (control) and samples inoculated before and after washing for 3 min in chlorinated water at 4 and 47°C. Each column shows the mean of three trials and the standard error of the mean. (B) Mean calculated populations of microorganisms other than* E. coli *O157:H7.*

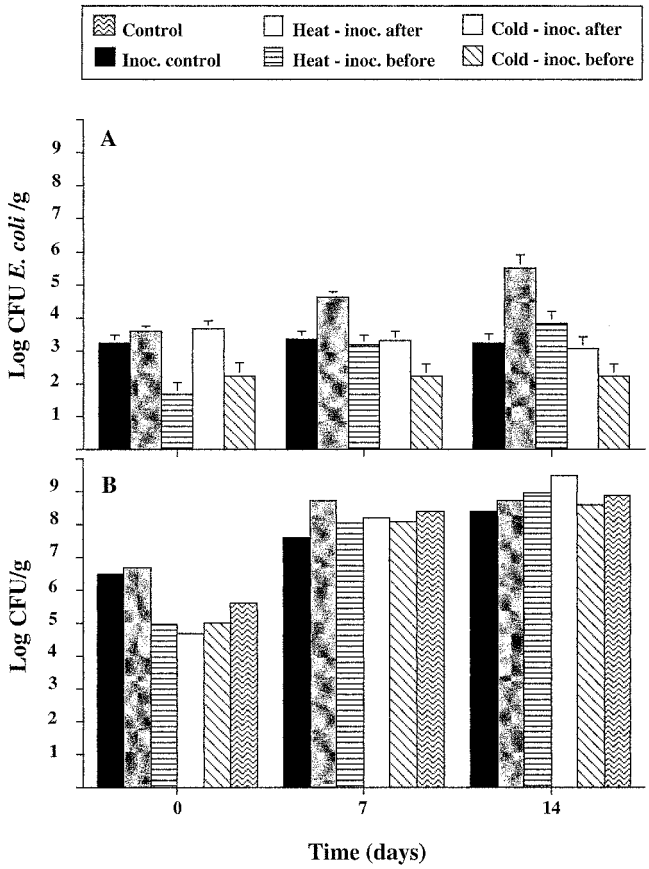

FIGURE 2. *Fate of* E. coli *O157:H7 in cut lettuce stored at 10°C. (A) Mean* E. coli *O157:H7 populations in unwashed lettuce (control) and samples inoculated before and after washing for three minutes in chlorinated water at 4 and 47°C. Each column shows the mean of three trials and the standard error of the mean. (B) Mean calculated populations of microorganisms other than* E. coli *O157:H7.*

stored at 1 and 10°C is shown in Figures 3 and 4, respectively. Populations in inoculated lettuce remained stable at 1°C for 14 days. Washing at either 4 or 47°C reduced inocula by 1 log CFU/g, and the fate of survivors was influenced both by chlorinated water and storage temperatures. *L. monocytogenes* populations in lettuce inoculated before or after washing at 4°C declined during storage. In contrast, inoculation by both procedures led to an increase of approximately 1 log CFU/g in lettuce washed at 47°C after 14 days. Growth of *L. monocytogenes* was enhanced at 10°C, and populations increased by 1 log CFU/g in inoculated, unwashed lettuce. Washing at 47°C resulted in more extensive growth of the pathogen, and populations >6 log CFU/g were obtained after 14 days, regardless of inoculation procedure. In contrast, washing in cold chlorinated water suppressed the growth of *L. monocytogenes* inoculated before treatment throughout the storage period. Populations in lettuce inoculated after washing increased by 2 log CFU/g during the first 7 days but were reduced by a similar measure after 14 days.

The lettuce used for experiments with *L. monocytogenes* carried slightly smaller initial microbial populations (10^5 CFU/g) than that used in experiments with *E. coli* O157:H7. Growth was again slower in samples packaged without further processing, and total populations increased at a faster rate in the inoculated, unwashed lettuce. Mean populations were similar across treatments in lettuce stored at 10°C for 7 days but were slightly higher in lettuce washed at 47°C after 14 days.

DISCUSSION

Cut iceberg lettuce was inoculated with *E. coli* O157:H7 and *L. monocytogenes* at a density of 3 log CFU/g before and after washing in cold (4°C) and warm (47°C) chlorinated water to simulate pre- and postprocess contamination. Control over physiological degradation and ensuing quality defects is best achieved by storage at 0°C, a temperature considered optimal for cut iceberg lettuce *(19).* In practice, temperature regimes vary widely and abusive conditions are frequently encountered in distribution and retail. The samples were therefore incubated at 1°C and 10°C to examine the fate of both pathogens during storage at near ideal and abusive temperatures.

E. coli O157:H7 populations declined in cut lettuce stored at 1°C irrespective of inoculation timing or treatment applied before packaging. Similar findings have been described for raw or chlorine-washed iceberg lettuce stored at ≤5°C, temperatures that are well below the minimum re-

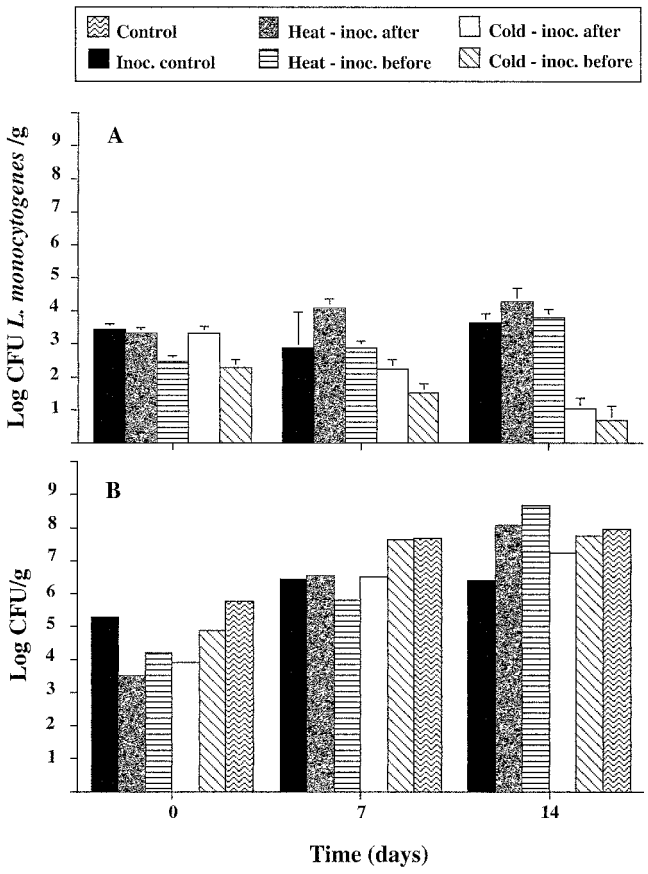

FIGURE 3. *Fate of* L. monocytogenes *in cut lettuce stored at 1°C. (A) Mean* L. monocytogenes *populations in unwashed lettuce (control) and samples inoculated before and after washing for 3 min in chlorinated water at 4 and 47°C. Each column shows the mean of three trials and the standard error of the mean. (B) Mean calculated populations of microorganisms other than* L. monocytogenes.

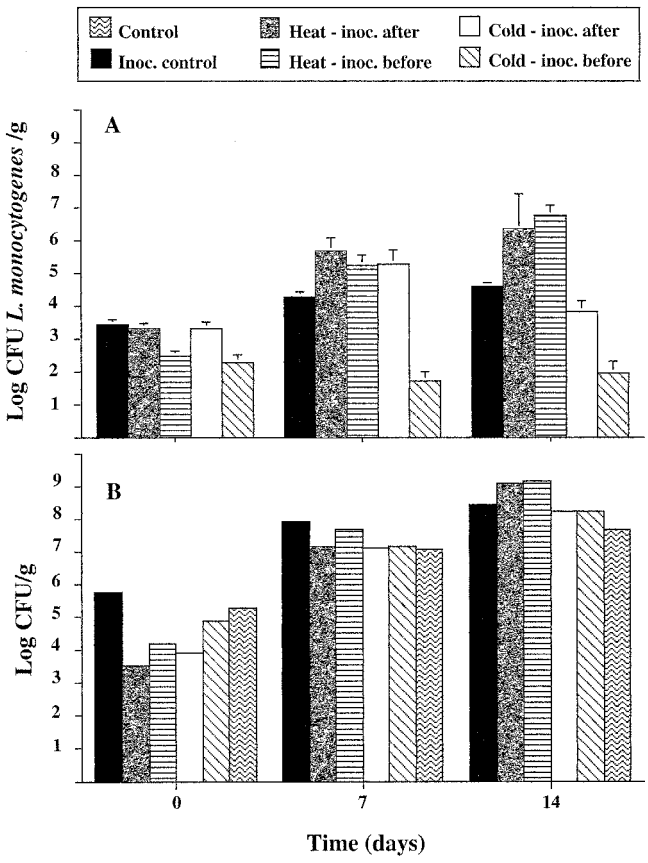

FIGURE 4. *Fate of* L. monocytogenes *in cut lettuce stored at 10°C. (A) Mean* L. monocytogenes *populations in unwashed lettuce (control) and samples inoculated before and after washing for 3 min in chlorinated water at 4 and 47°C. Each column shows the mean of three trials and the standard error of the mean. (B) Mean calculated populations of microorganisms other than* L. monocytogenes.

quired for growth of this species *(1, 6).* Growth in cut lettuce has been demonstrated at 12 and 13°C *(1, 12).* In the present investigation, inocula applied to lettuce washed in cold chlorinated water or in untreated controls did not expand at 10°C. Storage trials carried out under processing conditions that approximated commercial practice therefore indicated that this temperature is below the minimum required for growth of *E. coli* O157:H7. The increase in population observed in lettuce washed at 47°C negated this hypothesis, however, and showed clearly that temperature was not the only factor governing the fate of this species. It has been suggested that competition with spoilage microorganisms may affect the survival of *E. coli* O157:H7 in lettuce *(12, 18).* The lack of growth in unwashed controls stored at 10°C could be viewed as evidence for inhibition by the spoilage microflora. However, no clear relationship between total microbial populations and the fate of *E. coli* O157:H7 emerged from the data. While washing in chlorinated water at both temperatures removed part of the initial microflora, differences between treatments and controls had essentially disappeared after 7 days in storage, particularly at 10°C. It is therefore doubtful that the behavior of *E. coli* O157:H7 in stored lettuce was influenced by the effect of chlorinated water on indigenous microorganisms.

L. monocytogenes failed to grow in unwashed iceberg lettuce stored at 1°C, and a modest population increase of 1 log CFU/g was attained over 14 days at 10°C, results that are in general agreement with previous observations. Inocula applied before packaging are reported to lose viability at 3 *(16)* and 4°C *(21),* remain unchanged *(7)* or increase at 5°C *(28),* and grow exponentially at higher temperatures. The effect of other unit operations in lettuce processing on the fate of *L. monocytogenes* was also examined in these studies. Beuchat and Brackett *(7)* found that washing in chlorinated water (200–250 mg/liter free chlorine) did not affect subsequent growth. In contrast, Francis and O'Beirne *(16)* observed enhanced growth after washing in 100 mg/liter total chlorine. Anoxic storage atmospheres are frequently reported to confer selective advantage to *L. monocytogenes* in cut lettuce, particularly at higher storage temperatures. Francis and O'Beirne *(16)* found that growth was enhanced at 8°C in impermeable bags flushed with 100% N_2 compared to bags made from a microperforated film that maintained an aerobic atmosphere. Jacxsens et al. *(20)* recorded similar observations for shredded iceberg lettuce stored at 7°C under a 2 to 3% O_2, 2 to 3% CO_2, 94 to 96%

N_2 atmosphere. However, contradictory conclusions were drawn from a similar study by Beuchat and Brackett *(7),* where no difference was found between samples stored at 10°C in air or in a 3% O_2/97% N_2 gas mixture. The effect of chlorine washes and modified atmospheres on the fate of the pathogen in cut lettuce therefore remain uncertain.

In the present study, the fate of *L. monocytogenes* was mainly affected by chlorinated water temperature. Washing in cold chlorinated water limited growth during subsequent storage, whereas washing at 47°C had the opposite effect. In our experience, the packaging film used in this work maintains atmospheres containing a minimum of ~10% O_2 and a maximum of ~3% CO_2 for packaged iceberg lettuce over 15 days at 1°C, conditions which are considered aerobic from a microbiological perspective *(10).* In the latter study, atmosphere composition was not significantly affected by washing, and aerobic spoilage microflorae dominated by *Pseudomonas* spp. developed during storage of lettuce washed at either temperature. Enhanced growth and survival of *L. monocytogenes* in lettuce stored under modified atmospheres or in product disinfected with chlorine are often ascribed to reductions or alterations in the background microflora. Francis and O'Beirne *(17)* tested this hypothesis in vivo using *L. innocua* as a model microorganism. Background populations ranging from 10^3 to 10^7 CFU/g had no effect on the survival of inocula in iceberg lettuce. A similar conclusion must be drawn from the present study because there was no evidence that the size of background populations affected experimental outcomes for either species. These observations suggest that increased competitiveness due to partial removal of indigenous microorganisms does not fully account for the behavior of *L. monocytogenes* in iceberg lettuce washed in chlorinated water.

The conversion of iceberg lettuce into a ready-to-eat product elicits several physiological responses associated with stress and wound healing. Oxygen-dependant phenolic metabolism is particularly relevant in lettuce quality with respect to browning, notably a group of reactions known collectively as phenylpropanoid metabolism *(13).* Interestingly, key intermediates in phenylpropanoid metabolism exhibit antimicrobial activity. Barber et al. *(3)* recently showed that several compounds, including coumaric, caffeic, ferulic, and sinapic acids; coumaraldehyde; and coniferaldehyde, inhibit fungi, yeast, and bacteria, including *E. coli.* Although plant pathologists have long suspected that these and other compounds synthesized in response to stress or wounding are implicated in defense against microbial invasion, their influence on the microbial ecology of human bacterial pathogens in stored, packaged commodities such as lettuce are unknown.

Mild heat treatments provide an alternative to storage under anoxic atmospheres through the inhibition of enzymatic browning *(10).* Loaiza-Velarde et al. *(24)* have shown that the activity of phenylalanine ammonia lyase, the first committed enzyme in phenylpropanoid metabolism, is inhibited by the application of heat shocks to iceberg lettuce at temperatures above 45°C, an observation we have verified in our laboratory (data not shown). It is therefore plausible that reduced phenylpropanoid metabolism and a corresponding decrease in the ability of the plant tissues to resist microbial invasion following washing in warm chlorinated water led to enhanced survival and growth of both *E. coli* O157:H7 and *L. monocytogenes.* Presumably, these reactions were less affected by cold chlorinated water. Inhibition of browning reactions provides the rationale for reducing the availability of oxygen in storage, either by packaging in gas-impermeable films or by the introduction of modified atmospheres. Previous reports of enhanced growth under modified atmospheres provide further evidence for the potential role of phenolic metabolism in the microbial ecology of cut lettuce because atmospheres low in oxygen inhibit the synthesis of phenylalanine ammonia lyase. Clearly, the role of physiological status, phenolic metabolism, and other plant defence mechanisms on the growth of microorganisms in ready-to-eat lettuce needs further study.

Washing in cold and warm chlorinated water yielded partial elimination of *E. coli* O157:H7 and *L. monocytogenes* in cut lettuce stored at near-optimum temperature. However, the microbiological benefits derived from washing were lost in storage at an abusive temperature, which reinforces the need to strictly control this parameter during storage of ready-to-eat vegetables. Although washing at temperatures >45°C provides improvements in the overall quality of ready-to-eat lettuce, the survival and growth of pathogenic bacteria could be enhanced by inappropriate handling during distribution and retail display.

REFERENCES

1. Abdul-Raouf, U. M., L. R. Beuchat, and M. S. Ammar. 1993. Survival and growth of *Escherichia coli* O157:H7 on salad vegetables. Appl. Environ. Microbiol. 59:1999–2006.
2. Adams, M. R., A. D. Hartley, and L. J. Cox. 1989. Factors affecting the efficacy of washing procedures used in the production of prepared salads. Food Microbiol. 6:69–77.
3. Barber, M. S., V. S. McConnell, and B. S. DeCaux. 2000. Antimicrobial intermediates in the general phenylpropanoid and lignin specific pathways. Phytochemistry 54:53–56.
4. Bershing, J., S. Winkler, P. Franz, and R. Premier. 2000. Efficacy of chlorine for inactivation of *Escherichia coli* on vegetables. Postharvest Biol. Technol. 19:187–192.
5. Beuchat, L. R. 1992. Surface disinfection of raw produce. Dairy Food Environ. Sanit. 12:6–9.
6. Beuchat, L. R. 1999. Survival of enterohemorrhagic *Escherichia coli* O157:H7 in bovine feces applied to lettuce and the effectiveness of chlorinated water as a disinfectant. J. Food Prot. 62:845–849.
7. Beuchat, L. R., and R. E. Brackett. 1990. Survival and growth of *Listeria monocytogenes* on lettuce as influenced by shredding, chlorine treatment, modified atmosphere packaging and temperature. J. Food Sci. 55:755–758, 870.
8. Brackett, R. E. 1999. Incidence, contributing factors and control of bacterial pathogens in fresh produce. Postharvest Biol. Technol. 15: 305–311.
9. Burmeister, D. M.., S. Ball, S. Green, and A. B. Woolf. 1997. Interaction of hot water treatments and controlled atmosphere storage on quality of 'Fuyu' persimmons. Postharvest Biol. Technol. 12:71–81.
10. Delaquis, P. J., S. Stewart, M. Cliff, P. M. Toivonen, and A. L. Moyls. 2000. Sensory quality of ready-to-eat lettuce washed in warm, chlorinated water. J. Food Qual. 23:553–563.
11. Delaquis, P. J., S. Stewart, P. M. A. Toivonen, and L. A. Moyls.

1999. Effect of warm, chlorinated water on the microbial flora of shredded lettuce. Food Res. Int. 32:7–14

12. Diaz, C., and J. H. Hotchkiss. 1996. Comparative growth of *Escherichia coli* O157:H7: spoilage organisms and shelf-life of shredded lettuce stored under modified atmospheres. J. Sci. Food Agric. 70: 433–438.
13. Dixon, R. A. and N. L. Paiva. 1995. Stress-induced phenylpropanoid metabolism. Plant Cell 7:1085–1097.
14. Escudero, M. E., L. Velasquaez, M. S. Di Genaro, and A. M. de Guzman. 1999. Effectiveness of various disinfectants in the elimination of *Yersinia enterocolitica* on fresh lettuce. J. Food Prot. 62: 665–669.
15. Farber, J. M., S. L. Wang, Y. Cai, and S. Zhang. 1998. Changes in populations of *Listeria monocytogenes* inoculated on packaged fresh-cut vegetables. J. Food Prot. 61:192–195.
16. Francis, G. A., and D. O'Beirne. 1997. Effects of gas atmosphere, antimicrobial dip and temperature on the fate of *Listeria innocua* and *Listeria monocytogenes* on minimally processed lettuce. Int. J. Food Sci. Technol. 32:141–151.
17. Francis, G. A., and D. O'Beirne. 1998. Effects of the indigenous microflora of minimally processed lettuce on the survival and growth of *Listeria monocytogenes*. Int. J. Food Sci. Technol. 33:477–488.
18. Francis, G. A., C. Thomas, and D. O'Beirne. 1999. The microbiological safety of minimally processed vegetables. Int. J. Food Sci. Technol. 34:1–22.
19. Gorny, J. R. 1997. A summary of CA and MA recommendations for selected fresh-cut fruits and vegetables. Postharvest Horticulture Series No. 19, University of California, Davis, p. 30–66.
20. Jacxsens, L., F. Devlieghere, P. Falcato, and J. Debevre. 1999. Behavior of *Listeria monocytogenes* and *Aeromonas* spp. on fresh-cut produce packaged under equilibrium-modified atmosphere. J. Food Prot. 62:1128–1135.
21. Kakiomenou, K., C. Tassou, and G.-J. Nychas. 1998. Survival of *Salmonella enteriditis* and *Listeria monocytogenes* on salad vegetables. World. J. Microbiol. Biotechnol. 14:383–387.
22. King, A. D., J. A. Magnuson, T. Torok, and N. Goodman. 1991. Microbial flora and storage quality of partially processed lettuce. J. Food Sci. 56:459–461.
23. Lin, C.-M., S. Y. Fernando, and C. Wei. 1996. Occurrence of *Listeria monocytogenes, Salmonella* spp., *Escherichia coli* and *E. coli* O157: H7 in vegetable salads. Food Control 3:135–140.
24. Loaiza-Velarde, J. G., F. A. Tomás-Barberá, and M. F. Saltveit. 1997. Effect of intensity and duration of heat-shock treatments on wound-induced phenolic metabolism in iceberg lettuce. J. Am. Soc. Hortic. Sci. 122:873–877.
25. Lurie, S., and J. D. Klein. 1990. Heat treatment of ripening apples: differential effects on physiology and biochemistry. Physiol. Plant. 78:181–186.
26. Park, C. M., and L. R. Beuchat. 1999. Evaluation of sanitizers for killing *Escherichia coli* O157:H7, *Salmonella* and naturally occurring microorganisms on cantaloupes, honeydew melons and asparagus. Dairy Food Environ. Sanit. 19:842–847.
27. Simons, L. K., and P. Sanguansri. 1977. Advances in the washing of minimally processed vegetables. Food Aust. 49:75–80.
28. Steinbruegge, E. G., R. B. Maxcy, and M. B. Liewen. 1988. Fate of *Listeria monocytogenes* on ready to serve lettuce. J. Food Prot. 51: 596–599.
29. Tian, M. S., T. Islam, D. G. Stevenson, and D. E. Irving. 1997 Color, ethylene production, respiration, and compositional changes in broccoli dipped in hot water. J. Am. Soc. Hortic. Sci. 122:112–116.
30. Zhang, S., and J. M. Farber. 1996. The effect of various disinfectants against *Listeria monocytogenes* on fresh-cut vegetables. Food Microbiol. 13:311–321.