APPLIED AND ENVIRONMENTAL MICROBIOLOGY, May 1991, p. 1367–1371
0099-2240/91/051367-05$02.00/0

Vol. 57, No. 5

Behavior of *Listeria monocytogenes* Inoculated into Raw Tomatoes and Processed Tomato Products

LARRY R. BEUCHAT* AND ROBERT E. BRACKETT

Department of Food Science and Technology, Agricultural Experiment Station, University of Georgia, Griffin, Georgia 30223-1797

Received 24 September 1990/Accepted 26 November 1990

Rates of death and growth of *Listeria monocytogenes* inoculated onto raw whole and into chopped tomatoes stored at 10 and 21°C were not influenced by prior treatment of tomatoes with chlorine or packaging under an atmosphere of 3% O_2 and 97% N_2. Growth of the pathogen occurred in whole tomatoes held at 21°C but not at 10°C, while death occurred in chopped tomatoes stored at these temperatures. Likewise, growth patterns of mesophilic aerobic microorganisms, psychrotrophic microorganisms, and yeasts and molds on whole and chopped tomatoes were essentially unaffected by chlorine and modified atmosphere packaging treatments. Populations of *L. monocytogenes* inoculated into commercially processed tomato juice and sauce and held at 5°C remained constant for 14 days. A gradual decrease in the number of viable *L. monocytogenes* cells was observed in juice and sauce held at 21°C. In contrast, the organism died rapidly when suspended in commercial tomato ketchup at 5 and 21°C. Unlike low-acid raw salad vegetables such as lettuce, broccoli, asparagus, and cauliflower on which we have observed *L. monocytogenes* grow at refrigeration temperatures, tomatoes are not a good growth substrate for the organism. Nevertheless, *L. monocytogenes* can remain viable on raw whole and chopped tomatoes and in commercial tomato juice and sauce for periods extending beyond their normal shelf-life expectancy.

Several outbreaks of human listeriosis in North America and Europe have been documented in recent years. Most cases have been caused by the consumption of foods from animal origin, particularly soft cheeses, but at least one outbreak has been attributed to the consumption of cabbage contaminated with *Listeria monocytogenes* (15). In another outbreak involving hospital patients, epidemiologic evidence suggested that raw celery, tomatoes, and lettuce may have been vehicles of *L. monocytogenes* infection (12).

L. monocytogenes is known to occur in the environment on a wide range of vegetation (19). Its presence on poor quality silage, sometimes in numbers in excess of 10^4/g (8), has been associated with listeriosis in cattle. A survey of 1,000 samples of 10 types of fresh produce at the retail level revealed the presence of *L. monocytogenes* on cabbage, cucumbers, potatoes, and radishes (11). Sizmur and Walker (16) detected *L. monocytogenes* in 4 of 60 prepacked, ready-to-eat salads. Vegetables included in two types of contaminated salads were cabbage, celery, carrots, lettuce, cucumber, onion, leeks, watercress, and fennel. In contrast, Farber et al. (7) did not detect *L. monocytogenes* in 110 raw vegetable samples. Likewise, Petran et al. (14) failed to detect the organism in market samples of fresh and frozen vegetables.

Nevertheless, documented evidence of the presence of *L. monocytogenes* on raw salad vegetables, coupled with its ability to grow on cabbage (3) and lettuce (2, 18) at 5°C, has given rise to concern relative to the risk of these vegetables to public health. The ability of *L. monocytogenes* to grow at pH 4.5 and below has been documented (9, 13, 17). However, survival and growth of the organism on tomatoes and in tomato products has not been reported. The objectives of the study reported here were to determine the behavior of *L. monocytogenes* on whole and chopped raw tomatoes and in commercially processed tomato products stored at refrigerated (5 and 10°C) and ambient (21°C) temperatures.

MATERIALS AND METHODS

Source and preparation of raw tomatoes and processed tomato products. Tomatoes (cherry type) were purchased from the Georgia State Farmers' Market, Forest Park. Whole and chopped (quartered) tomatoes were subjected to treatments outlined in Fig. 1. Two brands each of commercially processed tomato juice, tomato sauce, and tomato ketchup were purchased from a local grocer. Products were handled aseptically to avoid introduction of contaminants in the laboratory. The pH of raw tomatoes (internal portion) and commercial tomato products was determined with a pH meter (model 1805; Fisher Scientific Co., Pittsburgh, Pa.). The soluble solids content was measured with a Bausch & Lomb refractometer (Bausch & Lomb Analytical System Division, Westbury, N.Y.).

Strains and preparation of inocula. *L. monocytogenes* Scott A and LCDC 81-861 were cultured in tryptic phosphate broth (pH 7.3) (10) at 30°C. Cultures which had been loop transferred at 24-h intervals for a minimum of three successive days were diluted in 0.1 M potassium phosphate buffer (pH 7.0) to yield suspensions ranging from 2.3×10^5 to 9.9×10^5 CFU/ml, in which whole tomatoes were dipped, or 2.7×10^6 to 9.7×10^6 CFU/ml, which was added directly to chopped tomatoes (10 ml/1,000 g).

Preparation of chlorine dip. Water containing 210 to 280 μg of free chlorine per ml was prepared and analyzed by the procedure described by Brackett (4).

Procedure for inoculation. Whole raw tomatoes (4 kg) were subjected to the washing and chlorine treatments outlined in Fig. 1. The volume of dipping solutions was 12 to 14 liters. Two kilograms of whole tomatoes were dipped in 10 liters of

* Corresponding author.

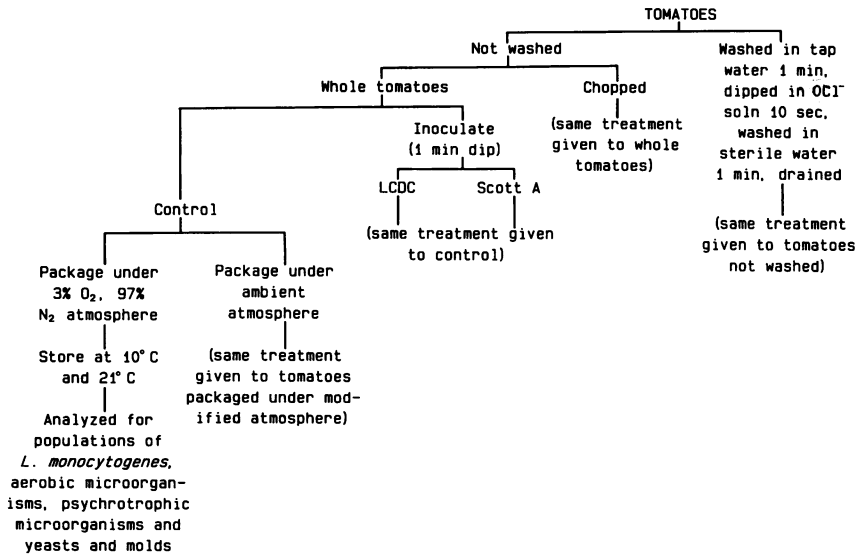

FIG. 1. Scheme for investigating the effect of chlorine wash treatment, chopping, and modified atmosphere packaging on survival and growth of *L. monocytogenes* on tomatoes stored at 10 and 21°C.

L. monocytogenes suspension and then drained, whereas 10 ml of diluted suspension was thoroughly mixed with 1,000 g of chopped tomatoes. Preparation of controls consisted of dipping whole tomatoes in 0.1 M phosphate buffer (pH 7.0) instead of *L. monocytogenes* suspension and adding buffer in place of suspension to chopped tomatoes.

Tomato juice, sauce, and ketchup (490 ml) were inoculated with 10 ml of a 10^{-1} dilution of 24-h cultures of test strains of *L. monocytogenes*. The inoculum was thoroughly distributed in tomato products by vigorously mixing with a sterile stainless-steel spoon.

Packaging procedure. Uninoculated (control) and inoculated raw tomatoes (120 to 140 g) were placed in D-955 Film bags (oxygen transmission rate is 8,750 ml/m^2/24 h) (Cryovac Inc., Duncan, S.C.). One-half of the samples were flushed three times with a modified gas mixture containing 3% O_2 and 97% N_2 before the bags were sealed. The other half of the samples, sealed under air atmosphere, served as controls. Samples were incubated at 10°C for 0, 5, 12, and 20 days (whole tomatoes) and 0, 3, 6, and 10 days (chopped tomatoes) and at 21°C for 0, 2, 4, and 8 days (whole and chopped tomatoes) before being subjected to microbiological analyses.

Inoculated tomato juice, sauce, and ketchup (25 g) were deposited in stomacher bags, sealed, and incubated at 5 and 21°C for 0, 2, 4, 8, 10, and 14 days before analyzing for viable *L. monocytogenes* cells.

Enumeration of microorganisms. Duplicate samples from two replicate trials were examined on each day of analysis.

Raw whole and chopped tomatoes (50 g) were combined with 200 ml of sterile 0.1 M potassium phosphate buffer (pH 7.0) and pummeled with a stomacher for 2 min. The stomacher bag was rinsed with 250 ml of buffer which was then combined with the tomato-buffer mixture. Commercial tomato products (25 g) were combined with 225 ml of buffer and pummeled for 2 min.

To determine populations of *L. monocytogenes*, appropriately diluted sample-buffer mixtures were surface spread (0.1 ml) in duplicate on modified Vogel-Johnson agar plates (5) and incubated for 44 to 48 h at 30°C before presumptive *L. monocytogenes* colonies were counted. Confirmation of *L. monocytogenes* was done as described by Golden et al. (10). Populations of *L. monocytogenes* in suspensions immediately before and after dipping whole tomatoes and before inoculating chopped tomatoes and commercial tomato products were also determined. Samples were analyzed by surface plating 0.1 ml of diluted suspensions on modified Vogel-Johnson agar.

Pummeled raw tomato samples, appropriately diluted in buffer, were analyzed for total mesophilic aerobic microorganism populations by surface spreading duplicate 0.1-ml samples on plate count agar. Colonies were counted after 48 h incubation at 30°C.

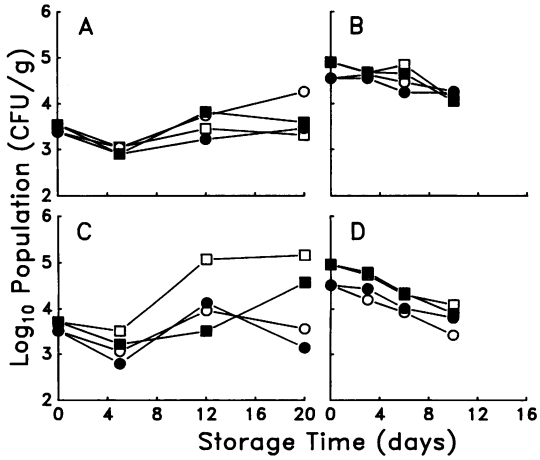

FIG. 2. Effects of chlorine treatment, chopping, and modified atmosphere storage of tomatoes at 10°C on changes in *L. monocytogenes* populations. (A and C) Whole tomatoes; (B and D) chopped tomatoes; (A and B) not treated with chlorine; (C and D) chlorine treated; circles, inoculated with *L. monocytogenes* LCDC 81-861; squares, inoculated with *L. monocytogenes* Scott A; open symbols, packaged under ambient air atmosphere; closed symbols, packaged under 3% O_2 and 97% N_2.

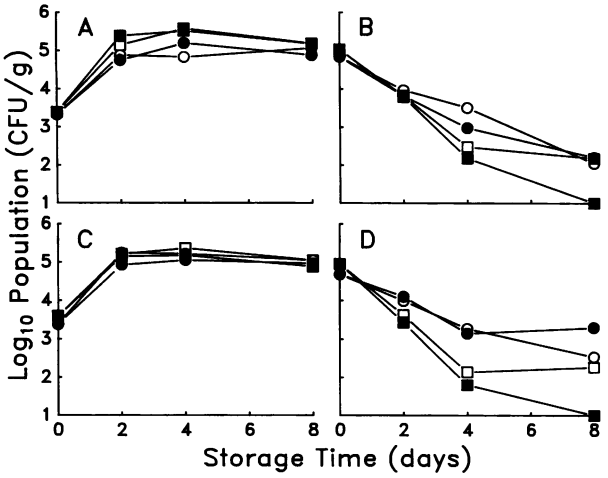

FIG. 3. Effects of chlorine treatment, chopping, and modified atmosphere storage of tomatoes at 21°C on changes in *L. monocytogenes* populations. See the legend to Fig. 2 for key.

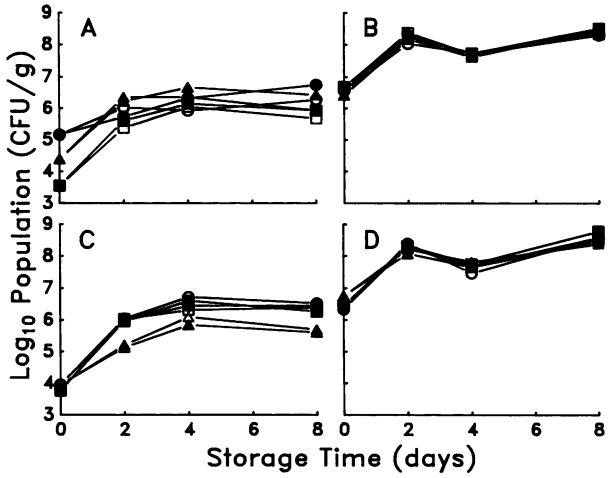

FIG. 5. Effects of chlorine treatment, chopping, and modified atmosphere storage of tomatoes at 21°C on changes in populations of mesophilic aerobic microorganisms. See the legends to Fig. 2 and 4 for key.

Raw tomato samples were analyzed for psychrotrophic microorganism populations by surface spreading duplicate 0.1-ml samples of pummeled tomato-buffer mixture on plate count agar; plates were incubated at 7°C for 10 days before colonies were counted.

Total yeast and mold populations were determined by surface spreading duplicate diluted raw tomato samples (0.1 ml) on plate count agar supplemented with 100 μg of chloramphenicol per ml. Plates were incubated at 25°C for 4 days before colonies were counted.

RESULTS AND DISCUSSION

***L. monocytogenes* in raw tomatoes.** Populations of *L. monocytogenes* detected on whole and chopped tomatoes stored at 10 and 21°C are shown in Fig. 2 and 3, respectively. Chopped tomatoes stored at 10°C were subjectively judged to be inedible after 10 days due to deterioration of sensory qualities, and thus analysis for *L. monocytogenes* was not done thereafter. Changes in populations were not affected by chlorine treatment or modified atmosphere packaging of tomatoes, regardless of storage temperature.

Initial populations of *L. monocytogenes* on whole tomatoes were lower than on chopped tomatoes, and this can be attributed to differences in inocula. Although increases appeared to occur on whole tomatoes stored at 10°C for 20 days (Fig. 2A and C), these increases were not significant ($P \leq 0.05$). Significant increases in populations on whole tomatoes did, however, occur when storage was at 21°C (Fig. 3A and C). Significant decreases in *L. monocytogenes* populations occurred in chopped tomatoes stored at 10 and 21°C. The rate of death was slower at 10°C compared with that at 21°C. We have also observed that death of *L. monocytogenes* at pH ≤ 4.8 in cabbage juice was more rapid at refrigeration (5°C) temperature compared with 30°C (6).

Contact of *L. monocytogenes* cells with tomato juice (pH

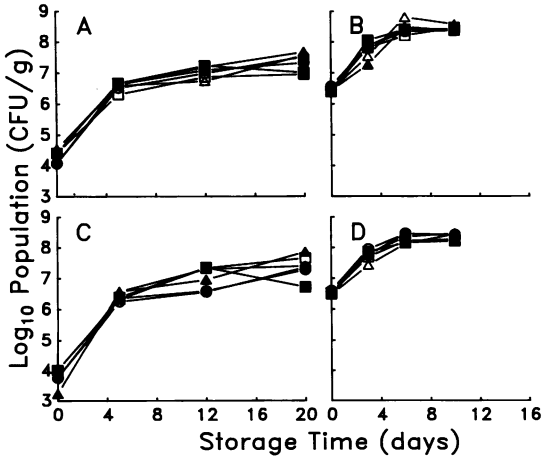

FIG. 4. Effects of chlorine treatment, chopping, and modified atmosphere storage of tomatoes at 10°C on changes in populations of mesophilic aerobic microorganisms. Triangles, tomatoes not inoculated with *L. monocytogenes*. See the legend to Fig. 2 for remainder of key.

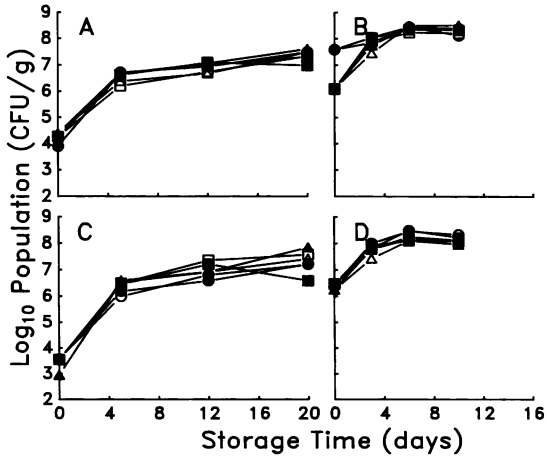

FIG. 6. Effects of chlorine treatment, chopping, and modified atmosphere storage of tomatoes at 10°C on changes in populations of psychrotrophic microorganisms. See the legends to Fig. 2 and 4 for key.

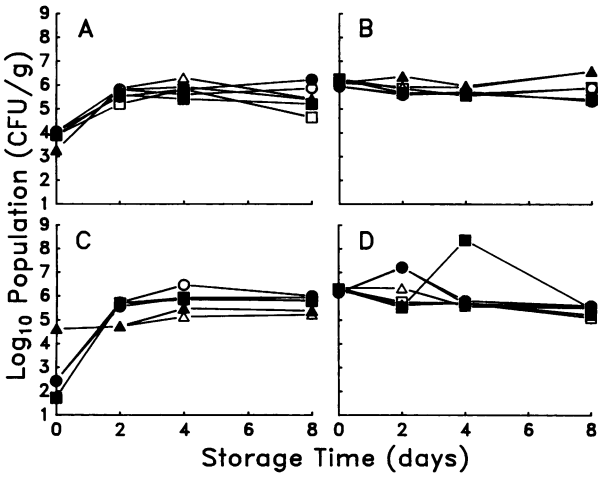

FIG. 7. Effects of chlorine treatment, chopping, and modified atmosphere storage of tomatoes at 21°C on changes in populations of psychrotrophic microorganisms. See the legends to Fig. 2 and 4 for key.

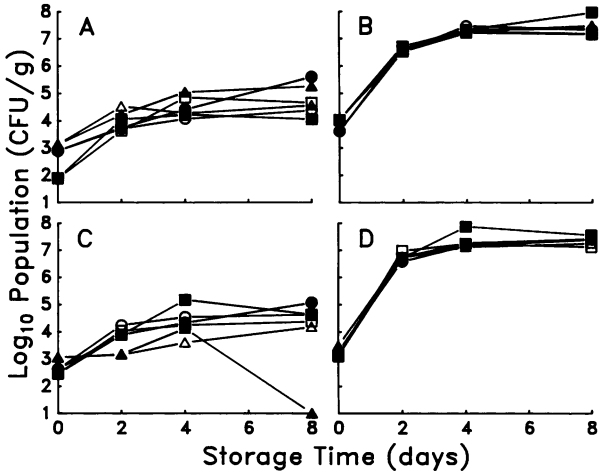

FIG. 9. Effects of chlorine treatment, chopping, and modified atmosphere storage of tomatoes at 21°C on changes in total populations of yeasts and molds. See the legends to Fig. 2 and 4 for key.

4.1) in samples of chopped tomatoes resulted in death. Others have reported that the type of acidulant, molarity, and temperature, as well as pH, influence the ability of *L. monocytogenes* to grow (17). At the same pH, acetic acid was more effective than lactic, citric, malic, and hydrochloric acids. Acetic acid is the predominant acid in tomatoes and undoubtedly had a lethal effect on the two test strains of *L. monocytogenes* examined in this study.

Mesophilic aerobic microorganisms. Changes in populations of total mesophilic aerobic microorganisms in whole and chopped tomatoes stored at 10 and 21°C are shown in Fig. 4 and 5, respectively. Significant ($P \leq 0.05$) increases occurred in both types of product, and although growth was more rapid at 21°C than at 10°C, increases in mesophiles as storage time progressed were not influenced by chlorine or modified atmosphere packaging treatments.

Psychrotrophic microorganisms. Populations of psychrotrophic bacteria on whole and chopped tomatoes stored at 10 and 21°C are shown in Fig. 6 and 7, respectively. The initial number of psychrotrophs decreased as a result of chlorine treatment. Populations of psychrotrophs on tomatoes stored at 10°C (Fig. 6) were similar to populations of mesophiles (Fig. 4). At 21°C, psychrotrophs failed to grow as rapidly on chopped tomatoes (Fig. 7B and D) as did mesophiles (Fig. 5B and D). Growth of psychrotrophs was not affected by chlorine or modified atmosphere packaging treatments.

Yeasts and molds. Growth of yeasts and molds on whole and chopped tomatoes at 10 and 21°C is illustrated in Fig. 8 and 9, respectively. Significant increases in populations were

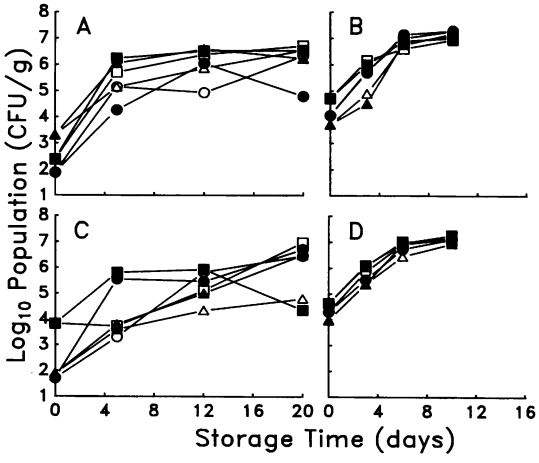

FIG. 8. Effects of chlorine treatment, chopping, and modified atmosphere storage of tomatoes at 10°C on changes in total populations of yeasts and molds. See the legends to Fig. 2 and 4 for key.

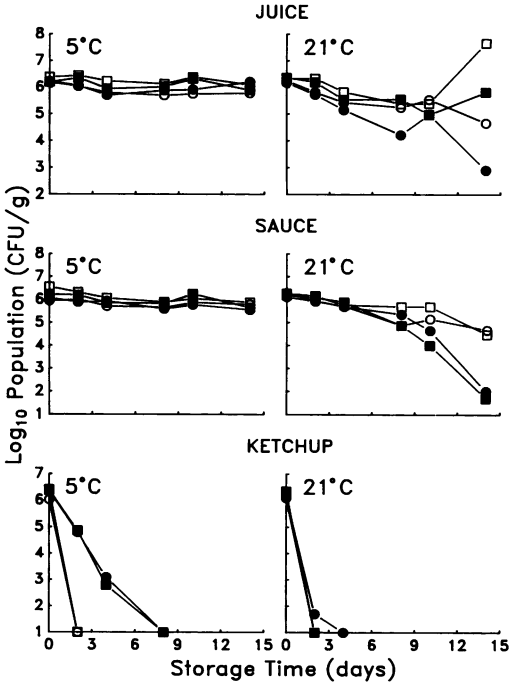

FIG. 10. Survival of *L. monocytogenes* LCDC 81-861 (circles) and Scott A (squares) in tomato juice, sauce, and ketchup stored at 5 and 21°C. Two brands of each tomato product are differentiated by open and closed symbols.

TABLE 1. pH and soluble solids of tomato products inoculated with *L. monocytogenes*

Tomato product	Brand	pH	Soluble solids (%)[a]
Juice	A	4.21	6.2
	B	4.21	6.2
Sauce	C	4.18	8.2
	D	4.07	8.8
Ketchup	E	3.60	23.2
	F	3.69	21.2

[a] At 21°C.

noted during storage at both temperatures. Changes appeared to be unaffected by chlorine or modified atmosphere packaging treatments, but the rate of growth was enhanced in chopped tomatoes (Fig. 9B and D) compared with whole tomatoes (Fig. 9A and C) stored at 21°C.

***L. monocytogenes* in commercial tomato products.** Behavior of *L. monocytogenes* when inoculated into commercial tomato products was somewhat surprising. Populations remained constant in juice and sauce held at 5°C for 14 days (Fig. 10). Gradual decreases were observed when juice and sauce were held at 21°C. In contrast, both test strains of *L. monocytogenes* died at a comparatively rapid rate when suspended in tomato ketchup. Death was more rapid at 21°C than at 5°C. The lethal effect of ketchup can be attributed in part to a lower pH (Table 1). In addition, the acetic acid content in ketchup was probably higher than that in juice and sauce due to the removal of more water (i.e., concentration of other tomato constituents, including acetic acid) and the addition of vinegar during processing.

In conclusion, unlike low-acid raw salad vegetables such as lettuce, broccoli, asparagus, and cauliflower which support the growth of *L. monocytogenes* at temperatures ranging from 5 to 15°C (1, 2, 16, 18), tomatoes are not a good growth substrate for the organism. Nevertheless, *L. monocytogenes* can remain viable on raw whole and chopped tomatoes and in tomato juice and sauce for periods extending beyond their normal shelf-life expectancy.

ACKNOWLEDGMENTS

This research was supported in part by the International Life Sciences Institute–Nutrition Foundation, Committee on Food Microbiology, Washington, D.C.

We are grateful to Cryovac Inc. for supplying the D-955 bags and to Brenda Nail and Lynn Perkins for technical assistance.

REFERENCES

1. **Berrang, M. E., R. E. Brackett, and L. R. Beuchat.** 1989. Growth of *Listeria monocytogenes* on fresh vegetables stored under controlled atmosphere. J. Food Prot. **52**:702–705.
2. **Beuchat, L. R., and R. E. Brackett.** 1990. Growth of *Listeria monocytogenes* on lettuce as influenced by shredding, chlorine treatment, modified atmosphere packaging, temperature and time. J. Food Sci. **55**:755–758, 870.
3. **Beuchat, L. R., R. E. Brackett, D. Y. Y. Hao, and D. E. Conner.** 1986. Growth and thermal inactivation of *Listeria monocytogenes* in cabbage and cabbage juice. Can. J. Microbiol. **32**:791–795.
4. **Brackett, R. E.** 1987. Antimicrobial effect of chlorine on *Listeria monocytogenes*. J. Food Prot. **50**:999–1003.
5. **Buchanan, R. L., H. G. Stahl, and D. L. Archer.** 1987. Improved plating media for simplified, quantitative detection of *Listeria monocytogenes* in foods. Food Microbiol. **4**:269–275.
6. **Conner, D. E., R. E. Brackett, and L. R. Beuchat.** 1986. Effect of temperature, sodium chloride, and pH on growth of *Listeria monocytogenes* in cabbage juice. Appl. Environ. Microbiol. **52**:59–63.
7. **Farber, J. M., G. W. Sanders, and M. A. Johnston.** 1989. A survey of various foods for the presence of *Listeria* species. J. Food Prot. **52**:456–458.
8. **Fenlon, D. R.** 1986. Rapid quantitative assessment of the distribution of *Listeria* in silage implicated in a suspected outbreak of listeriosis in calves. Vet. Rec. **118**:240–242.
9. **George, S. M., B. M. Lund, and T. F. Brocklehurst.** 1988. The effect of pH and temperature on inhibition of growth of *Listeria monocytogenes*. Lett. Appl. Microbiol. **6**:153–156.
10. **Golden, D. A., L. R. Beuchat, and R. E. Brackett.** 1988. Evaluation of selective direct plating media for their suitability to recover uninjured, heat-injured, and freeze-injured *Listeria monocytogenes* from foods. Appl. Environ. Microbiol. **54**:1451–1456.
11. **Heisick, J. E., D. E. Wagner, M. L. Nierman, and J. T. Peeler.** 1989. *Listeria* spp. found on fresh market produce. Appl. Environ. Microbiol. **55**:1925–1927.
12. **Ho, J. L., K. N. Shands, G. Friedland, P. Ecklind, and D. W. Fraser.** 1986. An outbreak of type 4b *Listeria monocytogenes* infection involving patients from eight Boston hospitals. Arch. Intern. Med. **146**:520–524.
13. **Parish, M. E., and D. P. Higgins.** 1989. Survival of *Listeria monocytogenes* in low pH model broth systems. J. Food Prot. **52**:144–147.
14. **Petran, R. L., E. A. Zottola, and R. B. Gravini.** 1988. Incidence of *Listeria monocytogenes* in market samples of fresh and frozen vegetables. J. Food Sci. **53**:1238–1240.
15. **Schlech, W. F., P. M. Lavigne, R. A. Bortolussi, A. C. Allen, E. V. Haldane, A. J. Wort, A. W. Hightower, S. E. Johnson, S. H. King, E. S. Nicholls, and C. V. Broome.** 1983. Epidemic listeriosis—evidence for transmission by food. N. Engl. J. Med. **308**:203–206.
16. **Sizmur, K., and C. W. Walker.** 1988. *Listeria* in prepacked salads. Lancet **i**:1167.
17. **Sorrells, K. M., D. C. Enigl, and J. R. Hatfield.** 1989. Effect of pH, acidulant, time, and temperature on the growth and survival of *Listeria monocytogenes*. J. Food Prot. **52**:571–573.
18. **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.
19. **Welshimer, H. J.** 1968. Isolation of *Listeria monocytogenes* from vegetation. J. Bacteriol. **95**:300–320.