Behavior of Listeria monocytogenes in Avocado Pulp and Processed Guacamole

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MS 02-50: Received 5 March 2002/Accepted 7 July 2002

ABSTRACT

The potential ability of Listeria monocytogenes to grow or survive in avocado pulp (AP) and processed guacamole (PG) stored at 22, 4 to 7, and −18°C was studied. Both products were obtained from a factory in Michoacan, Mexico. PG consisted of AP mixed with dehydrated vegetables, antioxidants, and preservatives. Populations of L. monocytogenes in AP stored at 22°C increased from 2 to 6 and 9 log CFU/g after 24 and 48 h, respectively. At 4 to 7°C, the growth rate of L. monocytogenes in AP was greatly decreased; generation time was 8.2 h, in contrast with 1.35 h observed at 22°C. L. monocytogenes populations did not increase in PG either at 22°C for 48 h or at 4 to 7°C for 15 days. The bacteriostatic effect in PG may have resulted from the presence of added substances, especially citric acid and disodium dihydrogen pyrophosphate. Aerobic plate counts and coliforms increased in AP and PG stored at ambient temperature and under refrigeration. However, these increments did not affect the growth of the pathogen. L. monocytogenes (50,000 most probable number [MPN]/g) survived at least 58 weeks in both products stored frozen at −18°C; the final population was 335 MPN/g in AP and 23 MPN/g in PG. Although the composition of avocado fruit differs significantly (high content of lipids and scarcity of simple carbohydrates) from that typical of most fruits, these results underline AP as a potential vehicle of human listeriosis and indicate that freezing should not be used as the sole mechanism to control this pathogen.

MATERIALS AND METHODS

Strains and preparation of inoculum. A four-strain cocktail of L. monocytogenes consisting of (i) Scott A, from a patient in a milk-associated outbreak; (ii) V7, from raw milk; (iii) Q1, from cheese (all obtained from the culture collection of the University

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of Georgia Center for Food Safety, Griffin, Ga.); and (iv) N1, isolated in our laboratory from AP, was prepared. Working stock cultures of each strain were maintained at 4 to 7°C on tryptic soy agar (Bioxon, Becton Dickinson, Lomas de Chapultepec, Mexico) slants and transferred monthly. After two successive transfers in tryptic soy broth (Bioxon) containing 0.6% yeast extract at 35°C for 20 ± 2 h, cells were collected by centrifugation (3,000 × g, 15 min, 22°C), washed twice in isotonic saline solution (0.85% NaCl), and resuspended in isotonic saline solution. Equal volumes of suspensions of each strain were combined and diluted in isotonic saline solution to obtain the desired cell populations.

Source of AP and PG. AP and PG were obtained from a guacamole processing plant in Michoacan, Mexico. This is a modern factory with excellent sanitary facilities, which exports frozen guacamole. PG is prepared by mixing Hass AP with dehydrated vegetables (onion, tomato, jalapeno chili, and garlic), sugar, salt, sodium alginate, xanthan gum, and some preservatives (disodium dihydrogen pyrophosphate, ascorbic acid, and citric acid); it is then packed in plastic containers or polyethylene bags and finally subjected to a freezing process in air belt freezer (quick freezing, ~90 min). AP (10 kg) was collected from the production line before it was mixed with other ingredients. Five bags (2-kg size) of frozen PG were taken from the production line immediately after freezing. Samples were stored at 4 to 7°C and used within 3 days. Both AP and PG were negative for the presence of L. monocytogenes.

One day before analysis, samples were thawed under refrigeration at 4 to 7°C. pH values were measured with a calibrated potentiometer (Model 410-A, Orion, Boston, Mass.) in 1:2 (wt/vol) homogenized samples prepared with freshly boiled deionized water. Water activity was measured with a calibrated Aquamatic equipment model CX2 (Decagon Device Inc., Pullman, Wash.).

Fate of L. monocytogenes in AP and PG at 22 and 4 to 7°C: inoculation procedure. Portions (10 g) of AP or PG were aseptically weighed, placed individually in polyethylene bags, and tempered at 22 and 4 to 7°C. Each bag was inoculated with 0.5 ml of the L. monocytogenes suspension at a final concentration of approximately 100 CFU/g. The inoculum was thoroughly distributed by external hand massage for 1 min. The bags were closed with several folds and incubated at 22 and 4 to 7°C, and three samples were periodically taken for microbiological analyses. For PG, the procedure described for AP was followed.

Fate of L. monocytogenes in AP and PG at 22 and 4 to 7°C: microbiological analyses. Each bag containing 10 g of AP or PG was combined with 90 ml of 0.1% peptone water and homogenized with a stomacher (Stomacher 400; Seward Medical, London, UK) for 2 min at normal speed. The resulting suspensions were serially diluted and surface-plated in duplicate onto modified Oxford medium (Oxoid, Basingstoke, UK) supplemented with 0.01 g of colistin methane sulfonate (Sigma Chemical Co., St. Louis, Mo.). After incubation at 35°C for 48 h, presumptive colonies of L. monocytogenes were counted, and two to five typical colonies were confirmed by Gram stain, catalase, tumbling motility at 25°C, carbohydrate fermentation (rhamnose but not xylose), and agglutination testing against commercial antiserum (Difco Laboratories, Sparks, Md.) (14).

Statistical analysis. Slopes were calculated from growth or inactivation curves (assuming linearity), and data were subjected to the Statistica 4.3 program for analysis of variance and to Tukey’s test to determine significant differences (α = 0.05) between means.

RESULTS AND DISCUSSION

As with other fruits that are consumed raw, avocado may be a vehicle of pathogenic bacteria. The sources of these bacteria in the field may be soil, water, wild and domestic animals, drift and runoff from adjacent farms, and manure (12). In the processing plant, guacamole may be contaminated if sanitation programs are deficient or if workers do not take care to avoid cross-contamination at the time the pulp is removed from avocados. Apart from the problem of contamination, to prevent listeriosis, it is important to know the potential of L. monocytogenes to survive and multiply in AP and PG.

pH and water activity values of AP and PG were within the limits recognized as appropriate for L. monocytogenes growth (9). The pH values showed an average of 6.7 (AP) and 5.3 (GP), whereas the water activity values of both products ranged between 0.980 and 0.990. Differences in pH can be associated with the citric acid used in the formulation of PG.

Although the composition of AP differs significantly from that typical of most fruits and would not seem to be
a good substrate for \textit{L. monocytogenes} growth, the pathogen multiplied actively in AP stored at 22°C (Fig. 1A). After a short lag period (approximately 3 h), its population increased from 2 log to 6 and 9 log CFU/g after 24 and 48 h, respectively. During the storage of AP at 4 to 7°C, the growth rate of \textit{L. monocytogenes} was greatly decreased (Fig. 1B); generation time was 8.2 h, in contrast with 1.35 h observed at 22°C. It has been reported that the minimal infective dose of \textit{L. monocytogenes} for compromised people is unknown and may be as few as 1 to 10 cells, depending on the strain and person (20). Thus, under refrigeration, even a few cells of \textit{L. monocytogenes} per gram of AP may, in a day, reach 100 cells, which is about the regulatory limit in some countries. The danger is higher if AP is stored at 22°C. In this case, the time required to reach the minimal level of \textit{L. monocytogenes} would be close to 4.5 h when the initial concentration in AP is only 1 cell per gram. In a similar study, Arvizu-Medrano et al. (2) reported that \textit{Escherichia coli} O157:H7, \textit{Salmonella}, and \textit{Staphylococcus aureus} multiplied actively in AP stored at 22°C; no growth but survival was observed at 4 to 7°C. Any temperature abuse after thawing by the consumers may result in a significant growth of \textit{L. monocytogenes}.

Refrigeration is used extensively to retard spoilage and extend the shelf life of fresh foods including salads. However, refrigeration still allows the growth of psychrotrophic pathogens like \textit{L. monocytogenes}. Our results illustrate that AP supports the growth of \textit{L. monocytogenes} at 22 and 4 to 7°C. The pathogen’s viability in PG was not altered at either temperature; little or no growth was observed at 4 to 7°C for 15 days or at 22°C for 48 h (Fig. 1). The inhibitory effect of PG may have been enhanced by some added substances, especially citric acid and disodium dihydrogen pyrophosphate; pH decreased from 6.7 in AP to 5.3 in PG.

Populations of naturally occurring microflora represented by aerobic plate counts and coliforms increased in uninoculated AP and PG stored at ambient temperature and under refrigeration (Fig. 2). Initial populations in PG were higher than in AP, suggesting that these microorganisms represent rather a passive contamination of AP and other ingredients or that microbial growth occurred during the preparation of PG.

The behaviors of \textit{L. monocytogenes} in AP and PG stored at −18°C are illustrated in Figure 3. In spite of the inaccuracies inherent to the MPN procedure, we used it to quantify \textit{L. monocytogenes} populations, including those cold-stressed in frozen AP and PG. After 58 weeks of storage, the viable population was reduced by 2 and 3 log in AP and GP, respectively. Inactivation occurred during the storage of both products at a similar rate ($P > 0.05$). The initial population of \textit{L. monocytogenes} did not change after the freezing process in either product (4.7 log CFU/g). Although it is recognized that microbial death may occur during the storage period, as well as during the freezing and thawing processes (6), these results show that \textit{L. monocytogenes} cells were not significantly affected by these treatments. During freezing, cells and tissues may be protected from damage by certain solutes, a phenomenon known as “cryoprotection.” In addition to affecting the quality of frozen foods, cryoprotectants are also likely to protect microorganisms from damage during freezing (7). Some natural foods contain cryoprotectants, and these are present in foods such as dairy desserts (19). For instance, Lammerding and Doyle (18) reported that \textit{L. monocytogenes} survived 5 months at −18°C in ice cream without any drastic change in the number of viable cells. It is possible that such cryoprotective effects in AP are related to its high content of lipid compounds.

Considering the low death rate observed in AP and PG (0.05 and 0.04 log MPN/wk, respectively), it can be speculated that if the initial level of \textit{L. monocytogenes} contamination were 10 cells/25 g, it would be necessary to store AP for at least 19 weeks, and PG for 27 weeks, to reduce this number to less than 0.04 MPN/g (i.e., to theoretically obtain a negative result by examining portions of 25 g). Therefore, reliance on freezing as the sole mechanism to eliminate the pathogen in frozen guacamole would not be congruent with the hygienic principles recognized to obtain foods free of pathogens (11). It is well known that bacteriocins such as nisin produced by lactic acid bacteria inhibit food spoilage and pathogenic bacteria, including \textit{L. monocytogenes} (1). The safety of PG could be improved by the addition of bacteriocins as one part of a multiple-barrier inhibitory system.

This work has shown that \textit{L. monocytogenes} is able to
FIGURE 2. Storage temperature effects on aerobic plate counts (filled circles) and coliforms (open circles) in avocado pulp (A and B) and processed guacamole (C and D). (A and C) 22°C and (B and D) 4 to 7°C.

FIGURE 3. Survival of L. monocytogenes in avocado pulp (filled symbols) and processed guacamole (open symbols) stored at -18°C.

grow in AP during storage at 22 and 4 to 7°C. Whereas the pathogen did not grow in PG, its viability was not affected at either temperature. The pathogen survived at least 58 weeks in frozen AP and PG. It is clear that strict control of raw materials and control of hygiene by good manufacturing practices are required during the production of GP to ensure its safety as well as its quality.

ACKNOWLEDGMENTS

We thank Dean O. Cliver, Professor of Food Safety at the University of California, Davis, for editorial assistance with this manuscript, and the Consejo Nacional de Ciencia y Tecnología, México, for providing the students with financial support. This work was conducted in collaboration with a manufacturer of frozen guacamole in Michoacan, Mexico.

REFERENCES


