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Fate of *Listeria monocytogenes* in Sweetened Condensed and Evaporated Milk During Storage at 7 or 21°C

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ABSTRACT

Sweetened condensed and evaporated milks were inoculated to contain ca. 10^3 to 10^7 cells of *Listeria monocytogenes* (strains Scott A, California, or V7)/ml. Both inoculated products were cooled from 25°C to 21°C in ca. 2 h or to 7°C in ca. 4 h. When inoculated sweetened condensed milk was held at 7°C for 42 d, there was no appreciable decrease in numbers of *L. monocytogenes* strains Scott A and V7, whereas the population of *L. monocytogenes* strain California decreased by ca. 1.2 orders of magnitude. Inoculum level had no effect on the magnitude of the decrease. At 21°C, 42 d of storage resulted in a more pronounced decrease in numbers of *L. monocytogenes* than it did during storage at 7°C, with numbers of the pathogen decreasing by 1.7, 1.6, and 3.4 orders of magnitude for strains Scott A, V7, and California, respectively. All strains of *L. monocytogenes* not only survived but grew in evaporated milk stored at 7 or 21°C for 56 or 28 d, respectively.

Listeriosis is the disease caused by *Listeria monocytogenes*. An outbreak of listeriosis which occurred in 1983 was linked to consuming a specific brand of pasteurized milk (*9*) and prompted concern about survival and growth characteristics of *L. monocytogenes* in dairy products.

Most healthy people can overcome infection by *L. monocytogenes* with only minor symptoms (stomach upset of "flu") or no symptoms at all (*5*). The incidence of listeriosis is greatest in newborns, pregnant women, and in elderly and immunosuppressed individuals (*3*). *L. monocytogenes* is ubiquitous in nature, raw foods, the home, and other environments (*7*). Refrigerators and dishcloths have been found to contain listeriae (*2,4,7*). The pathogen can grow between 3 and 45°C (*18*).

Listeriae are not fastidious; they can survive and often grow in feces, milk, soil, water, silage, and on plants. It is generally accepted that listeriosis can be transmitted to humans by contaminated foods, including dairy products (*11*). Several years ago the FDA found *Listeria* during inspections of dairy factories. Sixteen of 47 factories which yielded positive findings, also had finished products contaminated with *L. monocytogenes* (*1*).

Sweetened condensed milk is a concentrated dairy product prepared by addition of sucrose or glucose (about 18 to 20%, w/w) to whole milk. Federal standards require the product to contain 8.5% milk fat and 28% total milk solids (*8*). This non sterile product is widely used by bakers, confectioners, ice cream manufacturers, and in the prepared food industries. Thus, contamination of the product from environmental sources during handling is possible.

According to Federal standards (*8*), evaporated milk must contain at least 7.9% milk fat and 25.9% total solids. It is placed in small cans which are hermetically sealed and heat processed to make the product "commercially sterile" (some viable thermophilic spores may be present) and so it need not be refrigerated during storage and distribution (*10*). When in cans, sweetened condensed and evaporated milks need to be refrigerated after containers are opened. Evaporated milk is used primarily in the home for cookery, reconstitution and drinking, infant formulas, and in coffee and tea.

Condensed milks, with the exception of sweetened condensed milk and sour condensed milk (5-6% lactic acid), are favorable media for growth of many microorganisms found in the environment of milk processing factories (*10*). Since both sweetened condensed and evaporated milk, during handling after processing, can become contaminated from the environment which sometimes contains *L. monocytogenes*, it is important to know how the pathogen behaves in these foods. Thus, the purpose of this study was to evaluate behavior of *L. monocytogenes* when added at different levels to both milk products which were then refrigerated or held at room temperature.

MATERIALS AND METHODS

Milk products

Cans of commercial sweetened condensed milk (net wt. 396 g) and evaporated milk with vitamin D added [12 fl oz (354 ml)] were used for this work. Both milk products were obtained from a local supermarket in Madison, Wisconsin.

Preparation of samples

To insure uniformity of contents, immediately before transfer to sample containers the viscous sweetened condensed milk was warmed in a water bath to a temperature not above 45°C,

contents were mixed thoroughly, and 50 ml of product was transferred promptly from the can to a sterile sample container. Evaporated milk is not as viscous as sweetened condensed milk, and thus it was not necessary to warm the product before its transfer to sample containers. Sweetened condensed and evaporated milks (50-ml amounts at 25°C) each were inoculated to contain ca. 10^3 to 10^7 CFU of *L. monocytogenes*/ml. Both products were cooled to 21°C in ca. 2 h or to 7°C in ca. 4 h. These samples were then held at 21 or 7°C, respectively, and numbers of listeriae were determined periodically using a selective medium (McBride Listeria Agar) for sweetened condensed milk, and a non selective medium (Tryptose Agar, TA) for evaporated milk since this product is free of other microorganisms. Before inoculating with *L. monocytogenes*, samples were checked for yeasts and molds using acidified potato dextrose agar.

Preparation of cultures

L. monocytogenes strains used in these experiments include Scott A (clinical isolate, serotype 4b) and V7 (milk isolate, serotype 1) both provided by R. M. Twedt, Food and Drug Administration, Cincinnati, OH, and California (serotype 4b isolate from Mexican-style cheese) obtained from Silliker Laboratories, Inc., Carson, CA. Stock cultures were maintained through bimonthly transfer on TA (Difco Laboratories, Detroit, MI) and storage at 4°C. To prepare for an experiment, inocula from stock cultures were transferred to Tryptose Broth (TB) (Difco) and incubated in air for 24 h at 35°C. Subsequent transfers of these cultures to new TB were then made followed by incubation as just described. The population of cells in the culture was adjusted by dilution and sufficient volume of culture was dispersed in 50 ml of sweetened condensed and evaporated milks to yield three initial levels of *L. monocytogenes*, ranging from ca. 10^3 to 10^7 CFU/ml.

RESULTS AND DISCUSSION

Tests on uninoculated samples of sweetened condensed milk failed to recover *L. monocytogenes*, yeasts, or molds. Results (Fig. 1 A, B, C) indicate that all strains of *L. monocytogenes* survived in sweetened condensed milk and persisted for at least 42 d at 21°C. Inoculum levels had no apparent effect on the magnitude of the decrease in numbers during the storage period. Populations of *L. monocytogenes* under these storage conditions decreased by 1.7, 1.6, and 3.4 orders of magnitude for strains Scott A, V7, and California, respectively.

Storage of inoculated sweetened condensed milk at 7°C for 42 d did not cause a noticeable decrease in the numbers of *L. monocytogenes* strain Scott A and V7 (Fig. 2 A, C). Under the same conditions, however, numbers of *L. monocytogenes* strain California decreased by 1.2 orders of magnitude (Fig. 2, B). thus, the amount of sugar added to milk, e.g., low water activity, likely was more detrimental to survival of one than the other two strains of *L. monocytogenes*. Pearson and Marth (*15*) indicated that the maximum population of the pathogen increased significantly with an increasing sugar concentration in milk, but the highest level of sugar they used was 12%. Sweetened condensed milk is prepared by addition of 18-20% sucrose to whole milk; thus, it contains more sugar than

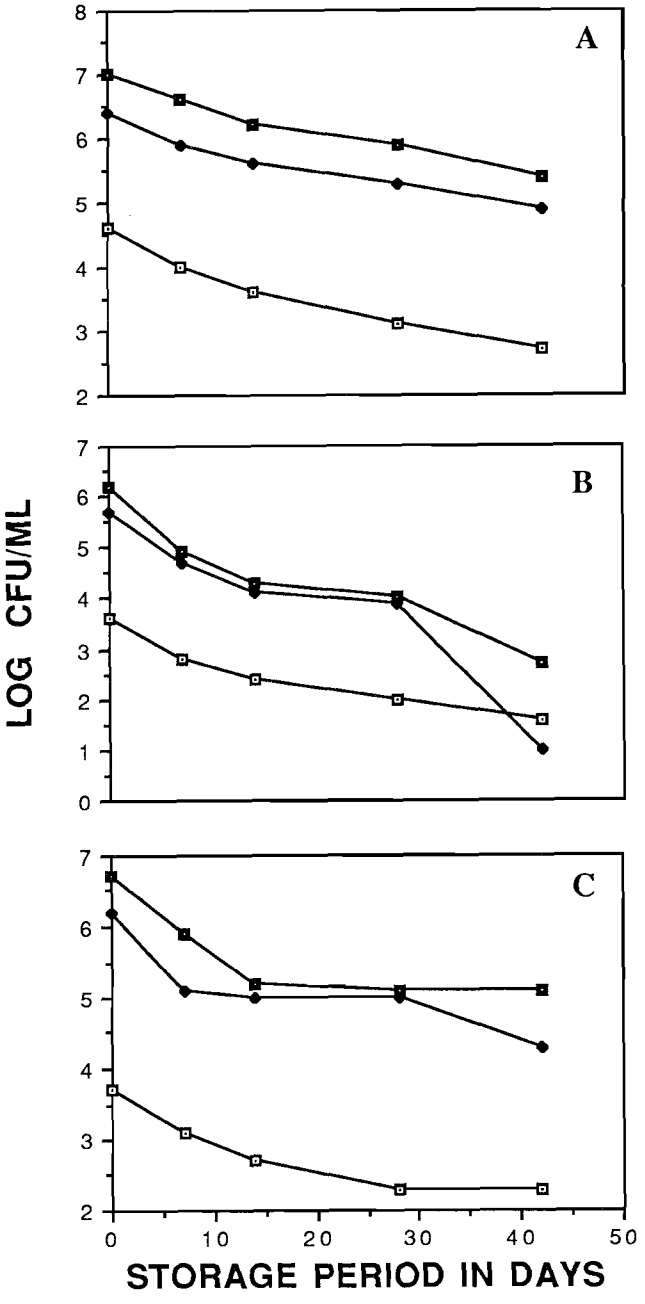

Figure 1. *Survival of* L. monocytogenes *strains Scott A (A), California (B), and V7 (C) in sweetened condensed milk held at 21°C for 42 d.*

the maximum used by Pearson and Marth (*15*) in their work.

In sweetened condensed milk, added sugar has reduced the level of available moisture to a point where bacteria and most yeasts will not grow because of the high osmotic pressure and low water activity (a_w). The a_w of sweetened condensed milk is about 0.83 and that of fluid milk is ca. 0.993 (*17*). Maximum growth rates of many bacteria occur at a_w values between 0.990 and 0.995. As the a_w is decreased, the growth rate diminishes until the minimum a_w for growth is reached (*16*). Conner et al. (*6*) reported that no growth of *L. monocytogenes* was observed at a_w values below 0.932 (glycol) or 0.942 (sucrose or

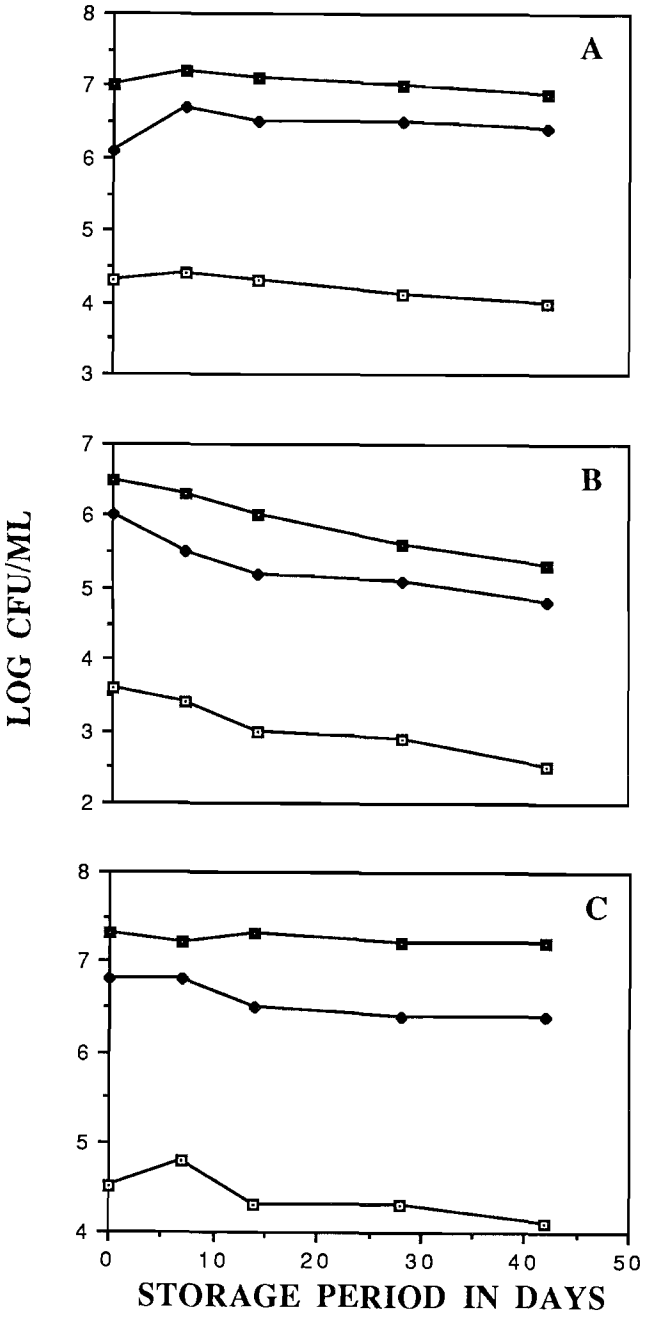

Figure 2. *Survival of* L. monocytogenes *strain Scott A (A), California (B), and V7 (C) in sweetened condensed milk held at 7°C for 42 d.*

NaCl). Cells of the pathogen can remain viable at salt concentrations of 5%, w/v, although no proliferation takes place (*6*).

When the a_w of the external medium is reduced, cells are subjected to osmotic shock and rapidly loose water, a process called plasmolysis. Koujima et al. (*12*) showed that *Staphylococcus aureus* loses about 50% of its intracellular water when switched from medium of a_w 0.995 to one of a_w 0.950. During plasmolysis a cell will not grow, it will either die or remain dormant. Our results on behavior of *L. monocytogenes* in sweetened condensed milk at 7 or 21°C are in accord with the observations just described.

Ockerman (*14*) found that 1-10% sugar added to comminuted meat or meat products accelerated growth of most microorganisms, whereas a higher percentage of sugar was needed to retard bacterial growth. Furthermore, Kuo and Ockerman (*13*) found that addition of high rather than low levels of sugar was more effective in retarding growth of microorganisms when they studied the effect of sugar and salt on the microbiology of dried pork.

The added carbohydrate in sweetened condensed milk "binds" water, making it unavailable for metabolic functions, and this "binding" may be thought of as a type of drying process. Moreover, increasing the concentration of milk solids in the condensing process also is effective in raising the osmotic pressure, binding water and reducing the a_w value (*10*).

Generally, microbial spoilage of evaporated milk can conveniently be divided into two categories. Spoilage of one type results from action of heat-resistant bacteria; they may survive a marginal or slightly deficient heat treatment. The other type of microbial spoilage results from organisms entering the product because of an imperfect seal or because of subsequent damage to the container; these organisms may not be heat resistant. Contamination also can occur after a container of product is opened and stored awaiting use.

All strains of *L. monocytogenes* not only survived but grew in evaporated milk stored either at 21 or 7°C for 28 or 56 d, respectively (Fig. 3,4). Evaporated milk has an a_w value of ca. 0.986 (*17*) and thus permitted growth of *L. monocytogenes*, whereas sweetened condensed milk with an a_w value of ca. 0.83 did not.

Population increases ranged from 1.3 to 3.9, 1.1 to 4.3, and 1.2 to 3.8 orders of magnitude for strains Scott A, California, and V7, respectively, at the end of 28 d at 21°C (Fig. 3). At 7°C, increases in population were 1 to 4.3, 1.5 to 4.0, or 0.6 to 4.0 orders of magnitude (Fig. 4) after 56 d.

In conclusion, storage of contaminated sweetened condensed milk for 6 weeks at 21°C was accompanied by a decrease in populations of added *L. monocytogenes*. The magnitude of the decrease was strain-dependent. At 7°C, the decrease was minimal or there was none. In contrast, all test strains of the pathogen grew well in evaporated milk. Results obtained with both products suggest it is prudent to protect them from contamination with *L. monocytogenes*, particularly during handling after processing.

ACKNOWLEDGMENTS

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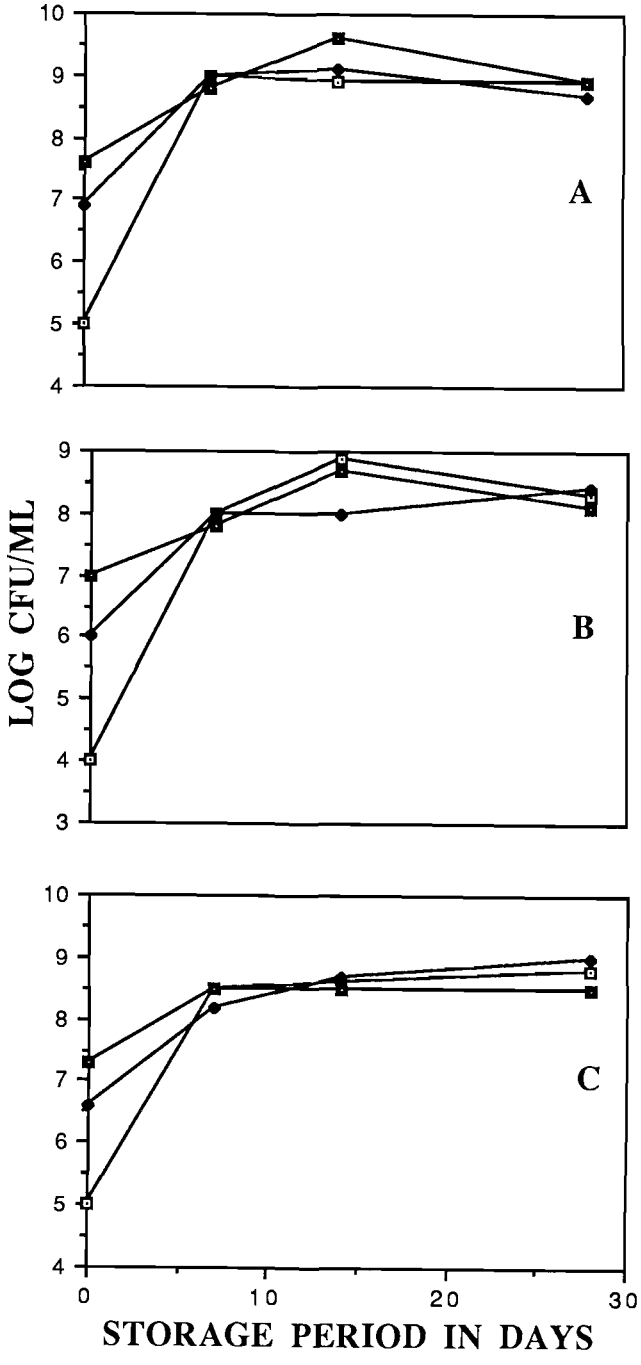

Figure 3. *Behavior of* L. monocytogenes *strain Scott A (A), California (B), and V7 (C) in evaporated milk held at 21°C for 28 d.*

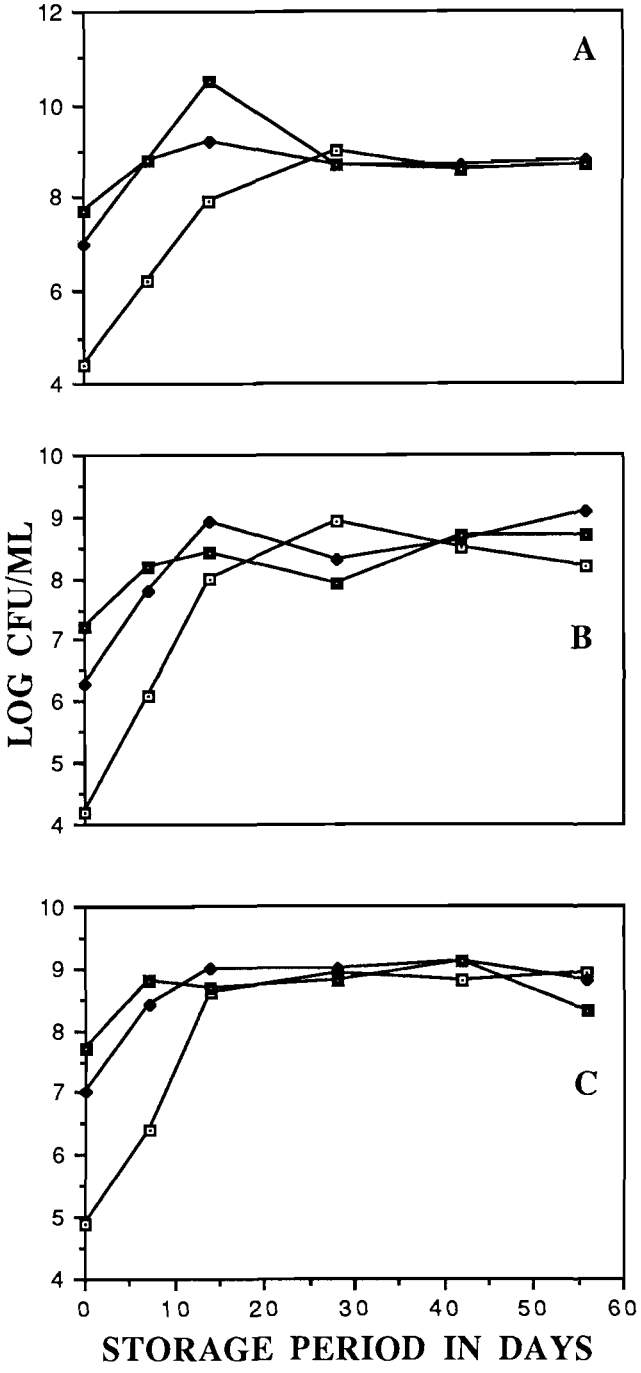

Figure 4. *Behavior of* L. monocytogenes *strain Scott A (A), California (B), and V7 (C) in evaporated milk held at 7°C for 56 d.*

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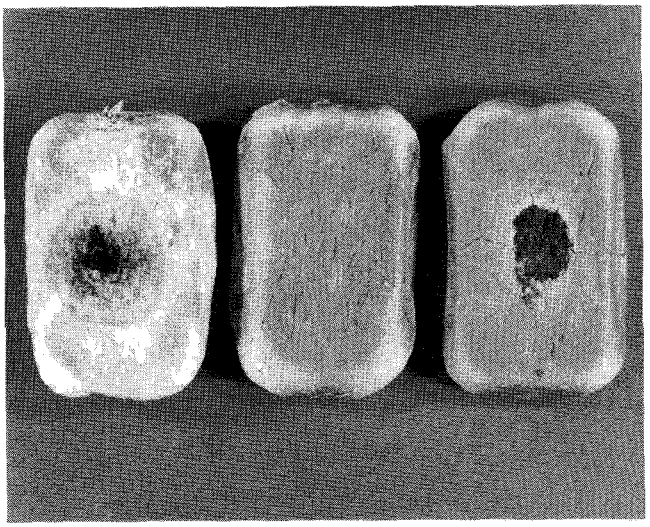

Figure 2. *Growth of a mixed mold inoculum in MRE bread after 13 months at 25°C. The center loaf was enclosed in a trilaminated pouch with one oxygen scavenging packet, and is clear of growth. The loaf on the left is the same loaf, showing mold growth, following exposure to air, in a Whirlpak bag, for 6 d. The loaf on the right shows the diameter of the mold colony, highlighted with methylene blue dye, that developed in the absence of an oxygen scavenging packet in the pouch. (Note color photo on following page).*

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