

Resistance of *Listeria monocytogenes* to freezing in foods

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The ability of L. monocytogenes to survive freezing and frozen storage at -18°C was studied in ground beef, ground turkey, frankfurters, canned corn, ice-cream mix, and tomato soup. Injury of L. monocytogenes as a result of freezing and frozen storage, as well as the ability of various Listeria-selective media to quantitatively recover the organism after freezing was also investigated. The responses of the organism, i.e. survival, injury, and quantitative on selective media, were related to the pH (acidity) of the food. Five of the examined foods had pH values of 5.8 or above, while tomato soup had a pH of 4.74. L. monocytogenes survived freezing and frozen storage well in five of the examined foods, was not injured, and was quantitatively recovered on Listeria-selective media. In contrast, the organism showed a decline in viable count after extended frozen storage in tomato soup, was injured, and could not be quantitatively recovered on Listeria-selective media. These results indicate that for most foods, freezing prior to analysis for L. monocytogenes should not hamper quantitative determination of the organism.

Introduction

Listeria monocytogenes is widely distributed in the environment, particularly in soil, water and vegetation, and thus can be present in/on various raw foods. It is also recognized that *L. monocytogenes* can be transmitted to humans via foods (Shelef 1989) either from raw, inadequately processed food, or from adequately processed foods which are then recontaminated by contact with a contaminated plant environment. The ability of different processing treatments, especially heat, to kill *L. monocytogenes* has received intense scrutiny, and several have been extensively investigated (Shelef 1989, Zaika et al. 1990). Recent work from this laboratory has indicated that *L. monocytogenes* can

remain viable for very long periods of time when held at temperature and relative humidity conditions which simulate various food plant environments (Palumbo and Williams 1990) and thus can contaminate processed food which comes into contact with these surfaces.

L. monocytogenes possesses characteristics which make it a unique foodborne pathogen. Perhaps the most important characteristic is its ability to grow in foods held at 5°C, a temperature previously thought adequate to retard the growth of foodborne pathogens (Palumbo 1986). This ability to grow at 5°C means that any food sample taken for qualitative or quantitative determination of *L. monocytogenes* should be analyzed immediately or frozen to avoid skewing the results. Based on the isolation of *L. monocytogenes* from ice cream (Buchanan et al. 1988) and *Listeria* spp. from frozen

seafood (Weagant et al. 1988), it is generally recognized that the organism can withstand freezing, but quantitative data are lacking. The purpose of this study was (a) to obtain quantitative data on the resistance or sensitivity of *L. monocytogenes* to freezing; (b) to determine the influence of different foods on the survival of the organism during frozen storage; (c) to determine if *L. monocytogenes* is injured during freezing and frozen storage; and (d) to investigate the ability of different *Listeria*-selective media to recover *L. monocytogenes* which had been frozen.

Materials and Methods

Organism

The following strains of *L. monocytogenes* were used in this study: Scott A, RM I, RM II, Murray B, ATCC 7644, V7, and V9. Each of the seven strains were grown individually in 50 ml of tryptose phosphate broth (Difco, Detroit, MI.) overnight at 37°C with shaking. After growth, equal portions of the individual cultures were mixed together and added to the specific food to yield a *L. monocytogenes* count of approximately 1×10^7 g⁻¹ at the time of freezing.

Foods

The following foods were used in this study: (a) ground sirloin; (b) ground turkey (55% white meat, 45% dark meat, and 7% fat)—both the ground sirloin and turkey were irradiated at 300 krad at 0°C to reduce the background microflora; (c) commercial all-meat frankfurters (ground through a sterile grinder with a one-eighth-inch plate); (d) simulated ice-cream mix (45 g sucrose, 34 ml distilled water, and 0.6 g commercial stabilizer, sterilized by autoclaving in a large beaker; after cooling, 140 g canned evaporated milk and 80 g ultrapasteurized cream were added. This mixture contained 11.73% fat, 11% milk solids, 15% sucrose, and 37.93% total solids); (e) canned whole kernel corn; and (f) canned tomato soup (diluted 1:1 with sterile distilled water). The cocktail of the seven strains was added to the individual foods and mixed thoroughly with the food; the inoculated foods were then frozen.

Freezing

After addition of the culture, 25-g portions of the individual foods were placed in Stomacher bags, frozen at -18°C, and held at that temperature until sampling.

Bacteriology

At intervals, duplicate samples of each food were removed from the freezer and 225 ml of 0.1% peptone water added. The sample was processed immediately (without thawing) in a Stomacher 400 laboratory mixer, and appropriate dilutions (made with 0.1% peptone water) surface plated in duplicate on various media. The zero time counts were done on the food before freezing. Colonies were counted after 48 h at 37°C. Since the foods used were sterile or had very low background microflora counts, all colonies were considered to be *L. monocytogenes*; in general, the colonies appeared typical of *L. monocytogenes* on the respective media.

Media

The following media were used during this study: (a) tryptose phosphate broth agar and 1% sodium pyruvate (TPB[Difco], 2% agar and 1% sodium pyruvate; TPBAP) (Smith and Archer 1988); (b) tryptose phosphate broth agar, 5% NaCl (TPB[Difco], 2% agar and 5% NaCl; TPBAS) (Smith and Archer 1988); (c) lithium chloride-phenylethanol-moxalactam medium (LPM) (Lee and McClain 1986); (d) cyclohexanedione-naladixic acid phenolethanol agar (CNP medium) (Loessner et al. 1988); (e) McBride *Listeria* agar [Difco] without the addition of blood (McB); (f) modified Vogel Johnson agar (MVJ) (Buchanan, Stahl and Archer 1987); and (g) ARS-modified McBride's agar (ARS-MMcB) (Buchanan, Stahl and Archer 1987).

pH

The pH of the individual foods was determined by inserting a combination electrode (Sensorex, semi-micro, A. H. Thomas, Philadelphia, PA) into the 1:10 dilution food slurry in the Stomacher bag. An Orion model 601A pH meter was used for this determination.

Results and Discussion

The basic responses of *L. monocytogenes* to freezing, survival, injury, and recov-

ery on *Listeria*-selective media were dependent on the pH of the food. The pH values of the foods used in this study are given in Table 1. As can be seen from this data, except for tomato soup, the foods had pH values of 5.8 and above (non-acidic foods).

Table 1. pH value of the foods used in this study

Food	pH
Ground beef	5.84
Ground turkey	6.44
Frankfurters	5.95
Canned corn	6.98
Ice-cream mix	6.71
Tomato soup	4.74

The survival of *L. monocytogenes* during extended frozen storage in ground beef and tomato soup is shown in Fig. 1(a) and (b). The viable count of *L. monocytogenes* (on TPBAP) remained essentially constant when the organism was suspended in ground beef [Fig. 1(a)]; similar responses were observed when the

organism was suspended in ground turkey, frankfurters, ice-cream mix, and corn (data not shown). In contrast, the response of *L. monocytogenes* was erratic when the organism was suspended in tomato soup [Fig. 1(b)], but the trend was a slight decline. Except for suspension in tomato soup, *L. monocytogenes* was highly resistant to freezing and frozen storage.

When the organism was suspended in tomato soup, the viable counts at two weeks of storage were the same as before freezing, indicating that *L. monocytogenes* is not damaged by the freezing process itself.

It is well known that bacterial cells can be injured by stresses such as heating, acidification, drying, and freezing among others (Ray, 1989). The TPBAP/TPBAS plating system described by Smith and Archer (1988) was employed to detect the possible occurrence of freeze-injured cells. With their system, the difference between TPBAP and TPBAS is a reflection of the number of injured cells.

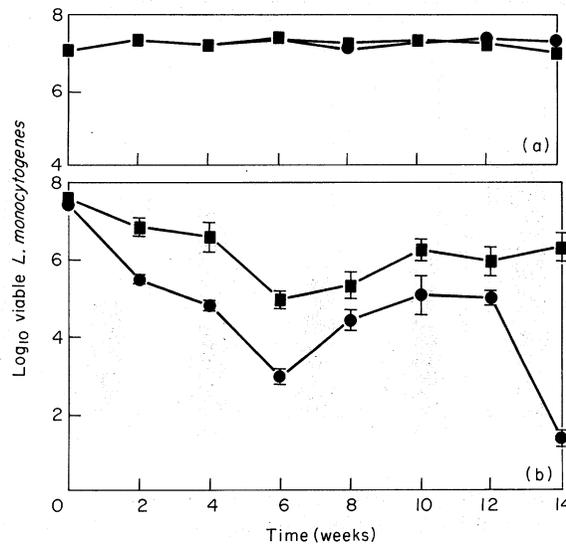


Fig. 1. Effect of food and plating media [TPBAP, ■—■ vs TPBAS (●—●)] on the number of viable *L. monocytogenes* detected after storage at -18°C: Each point represents duplicate plates of duplicate samples. Error bars represent 1 s.d. Error bars not presented for ground beef data; s.d. ranged from 0.3 to 0.21 for both TPBAP and TPBAS. (a) Ground beef, and (b) tomato soup.

L. monocytogenes is quite resistant to killing and/or injury when suspended in ground beef [Fig. 1(a)]. Similar effects were observed when the organism was suspended in ground turkey, frankfurters, corn, and ice-cream mix (data not shown). In contrast, *L. monocytogenes* showed substantial injury when frozen in tomato soup [Fig. 1(b)].

In addition to survival and injury in various foods during freezing and frozen

storage, we also studied the ability of various media selective for *L. monocytogenes* to recover the organism from frozen foods by direct plating. Again, as with survival and injury, the recoveries were food-related, apparently attributable to the pH of the food, though other factors may also contribute [Fig. 2(a) and (b)]. When *L. monocytogenes* was frozen in ground beef, the selective media were able to recover the same

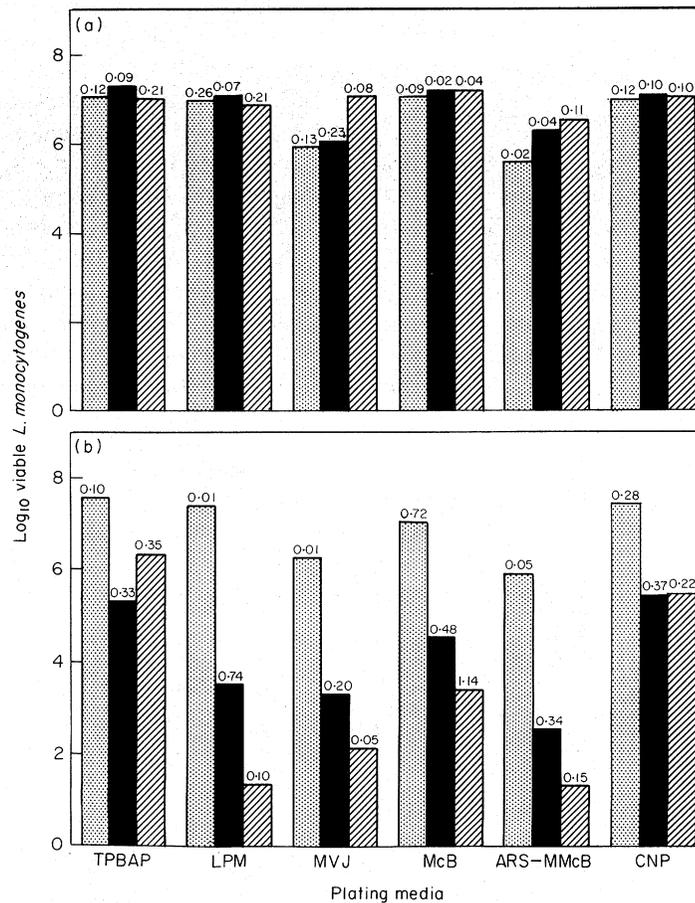


Fig. 2. Effect of food and *Listeria* plating media on the number of viable *L. monocytogenes* detected after 0 (□), 8 (■) and 14 (▨) weeks of storage at -18°C . Each count represents duplicate plates of duplicate samples. The number on top of each bar is the standard deviation. (a) Ground beef, and (b) tomato soup. TPBAP, tryptose phosphate broth and sodium pyruvate; LPM, lithium chloride-phenylethanol-moxalactam medium; MVJ, modified Vogel Johnson agar; McB, McBride *Listeria* agar without blood; ARS-MMcB, ARS-modified McBride's agar; and CNP, cyclohexanedione-naladixic acid phenoethanol agar.

number of viable cells after 14 weeks as at zero time (not frozen). Similar results were obtained for ground turkey, frankfurters, corn, and ice-cream mix (data not shown). In contrast to these observations, four of the selective media (LPM, MVJ, McB, and MMcB) showed substantially reduced recoveries of *L. monocytogenes* after 14 weeks frozen storage in tomato soup [Fig. 2(b)], while the fifth (CNP) showed about a 2 log decline in recovery. Thus, direct plating to recover *L. monocytogenes* from frozen acidic foods using selective media is not recommended; with these foods, a non-selective enrichment broth or a resuscitation step should be included in the procedure.

Golden, Beuchat and Brackett (1988) studied the survival of *L. monocytogenes* when frozen in tryptose phosphate broth. They observed that *L. monocytogenes* was fairly resistant to freezing and frozen storage at -18°C over a 14-day period. Our observations of the organisms' survival in frozen foods (Fig. 1) support and expand their initial observations. However, in contrast to their findings of injury to *L. monocytogenes* when frozen in broth, we found that *L. monocytogenes* was not injured when frozen in foods that had pH values of 5.8 or above (Fig. 1). It is possible that this difference, injury vs non-injury, can be explained by the

presence of food. Speck and Ray (1977) have indicated that viscous foods and food components such as proteins, carbohydrates, and triglycerides, increased the resistance of bacterial cells to freezing damage.

L. monocytogenes is known to be fairly acid tolerant, with growth observed at pH values of 4.5 and above (Parrish and Higgins 1989). The organism survives (with a gradual decline in number) in various acidic environments. Choi, Schaack and Marth (1988) observed survival of *L. monocytogenes* in buttermilk (pH 4.30) and plain yogurt (pH 4.06) held at 4°C for up to 23 days in both foods. Parrish and Higgins (1989) observed a decrease in viable *L. monocytogenes* held at 4°C in orange serum adjusted (with HCl) to pH values of 4.8 to 3.6; the rate of decline was proportional to the decrease in pH.

In summary, this study provides quantitative data which indicate that *L. monocytogenes* is resistant to freezing and freeze injury when present in most foods and that the organism can be quantitatively recovered on various *Listeria*-selective media after frozen storage. When frozen in tomato soup (pH 4.74), *L. monocytogenes* showed a decline in viable count, was freeze-injured, and exhibited decreased recoveries in *Listeria*-selective media.

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