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Thermal Resistance of *Listeria* spp. in Milk

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ABSTRACT

The thermal resistance of one strain each of *Listeria ivanovii*, *L. seeligeri*, and *L. welshimeri* and three *L. monocytogenes* strains was determined in raw and sterile milk. *Listeria* spp. suspended in milk at concentrations of 1×10^5 cells/ml were heated at temperatures ranging from 52.2 to 71.1°C for various contact times. The heat resistance of *L. monocytogenes* appeared somewhat greater than that of the other *Listeria* spp. in both milks, but the difference was not statistically significant ($\alpha = 0.05$). High-temperature, short-time processing is adequate for pasteurization of raw milk.

Three major U.S. epidemics of listeriosis, each implicating a dairy product, were documented during the past half decade (*20,27,35*). These outbreaks called into question the adequacy of current pasteurization procedures to inactivate *Listeria monocytogenes* in milk. Subsequently, numerous studies were undertaken to determine the ability of the organism to survive the minimum high-temperature, short-time (HTST) pasteurization process (71.7°C for 15 s) recommended by the Food and Drug Administration (FDA) (*21*). *L. monocytogenes* was found in naturally and artificially contaminated raw milk subjected to HTST processing (*13,17,18*); however, most investigators indicated that the organism could not survive HTST conditions in milk (*3-5,8,9,11,12,14,23,29,31,36*). Thermal resistance did not differ significantly among approximately a dozen strains of *L. monocytogenes* of several serotypes examined (*4,11*). Proposals that intracellular inclusion within bovine polymorphonuclear leukocytes conferred significant thermal protection upon *L. monocytogenes* (*13,20*) could not be supported (*8-10,32*). Suggestions that sublethal heat shock stimulates a marked, stable increase in the organism's heat resistance are open to question (*16,24*; R. G. Crawford, J. T. Tierney, J. T. Peeler, and V. K. Bunning, Abstr. Annu. Meet. Am. Soc. Microbiol. 1989, Q112, p. 348).

These observations reaffirmed the adequacy of pasteurization to eliminate the hazard posed by low numbers of *L. monocytogenes* in raw milk (*38*) and supported the assumption that *L. monocytogenes* cells in finished pasteurized milk are uninjured environmental contaminants (*10,38*). The risk of postpasteurization contamination was assessed under FDA's Dairy Safety Initiatives program (*25*). Results showed that all *Listeria* contamination involving processed dairy products resulted from an in-plant environmental source rather than from pasteurization process deficiencies (*9*).

Not surprisingly, other *Listeria* spp. that were recovered along with *L. monocytogenes* in up to 26% of farm bulk-tank, raw-milk test samples (*30*) were also found in the dairy plant environment (*22*). The recovery of *Listeria* spp. other than *L. monocytogenes* from finished dairy products (*2,15,34,37*) poses a problem for both public health and industry microbiologists. If the thermal resistance of such isolates is equivalent to that of *L. monocytogenes*, their presence in pasteurized products suggests either processing deficiencies or postpasteurization contamination; however, if their heat resistance is significantly greater than that of *L. monocytogenes*, they might survive pasteurization. In the latter case, the concern for the presence of these isolates in finished products would depend upon virulence considerations alone. Thus far, although *L. monocytogenes* is regarded as pathogenic, other *Listeria* spp., such as *L. ivanovii*, are thought to be only occasionally responsible for disease (*38*).

Data on the thermal inactivation of *Listeria* spp. available in the literature are limited to the relatively uniform heat resistance of more than a dozen strains of *L. monocytogenes* in a few foods, principally milk. The objective of this study was to provide information on the thermal resistance of *Listeria* spp. other than *L. monocytogenes*.

MATERIALS AND METHODS

Cultures and culture conditions

Five of the six *Listeria* cultures used in this study were isolated from soft cheese and were from FDA stock. They included *L. ivanovii* strain LA-30, *L. seeligeri* strain LA-34, *L. welshimeri* strain LA-36, and two strains of *L. monocytogenes* (BS-9 and SE-31). *L. monocytogenes* strain Scott A, a clinical isolate from the 1983 Massachusetts listeriosis outbreak, was obtained from D. W. Fleming, Centers for Disease Control, Atlanta, GA. Stock cultures were grown in trypticase soy broth-0.6% yeast extract (TSBYE) (BBL Microbiology Systems, Cockeysville, MD) at 37°C for 24 h and maintained in 40% glycerol at -20°C. Test cultures were grown in TSBYE at 37°C for 24 h in three serial transfers. The cell density of the final culture was adjusted at 625 nm to an absorbance of 0.20, yielding approximately 10^9 colony-forming units (CFU)/ml.

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Thermal resistance studies

The heating medium was farm bulk-tank milk used raw or sterilized in an autoclave at 121°C for 20 min. An adjusted culture was serially diluted in phosphate-buffered dilution water (*1*) and inoculated into the heating medium in a dilution ratio of 1:100 to yield about 1 x 10^5 *Listeria* cells/ml. Portions of 1.5 ml were dispensed into 13 x 100-mm borosilicate glass tubes that were then sealed and heated in a water bath at temperatures between 52.2 and 71.7°C, according to methods described previously (*6,7*). During each heating interval, sterile milk studies were made with duplicate tubes and raw milk studies were made with quadruplicate tubes. Tests at each temperature were repeated twice.

Microbiological procedures

Surviving *Listeria* spp. in pooled tube contents of inoculated sterile milk at each heating interval were enumerated in duplicate plate counts on trypticase soy agar-0.6% yeast extract (TSAYE) after 7 d at 25°C. To recover *Listeria* that survived heating in raw milk, 1 ml was transferred from each heated tube to 9 ml of enrichment broth (EB) (*30*) and incubated at 30°C for 4 d. Each EB culture was streaked to modified McBride agar (MMA) (*30*). Plates were incubated at 35°C for 24 h and examined in 45° incident-transmitted, intense white light with a dissecting microscope (*28*). Five presumptive colonies having the bluish, granular appearance typical of *Listeria* spp. on MMA were transferred from each plate and streaked for purity and subsequent verification tests (*5*) on TSAYE.

Well-isolated colonies were tested for catalase production and subcultured into TSBYE. Motility was demonstrated by the tumbling movement seen in wet mounts of TSBYE cultures after 24 h at 30°C or by umbrelliform growth around SIM (BBL) agar stabs.

Statistical methods

Rates of thermal inactivation were determined for each *Listeria* sp. tested at each temperature. D-value calculations from enrichment detection of the organism in raw milk were estimated using the 50% end point statistical procedure (*19,26*). A linear regression of $\log_{10}$ CFU/ml versus heating times was computed (*33*) for each species tested in sterile milk at each temperature. The least-square estimate of slope is the rate of thermal inactivation at constant temperature. An estimate of D-value was obtained by taking the absolute value of the inverse of the slope. The D-values were corrected for the lethality during heating and cooling, from data gathered with both milks at each heating temperature using sealed tubes, thermocouples and a temperature recorder, as previously described (*7*). A linear regression was computed from the $\log_{10}$ D-value versus temperature, and the z_D-value was computed as the absolute value of the inverse of the slope.

RESULTS AND DISCUSSION

In a preliminary investigation, the thermal resistance of four strains of *L. ivanovii*, two of *L. seeligeri*, two of *L. welshimeri*, and 29 of *L. monocytogenes* isolated from food was compared in duplicate trials at 57.8°C in sterile milk. The rates of thermal destruction of these several *Listeria* spp. in milk were linear, confirming our previous observation with *L. monocytogenes* (*4*).

In a recent study, Knabel et al. (*24*) plotted a linear thermal destruction curve for *L. monocytogenes* strain F5069, tested under conditions used in the present study. Their cells were grown at 37°C for 18 h, suspended in broth or sterile milk, heated in sealed tubes at 62.8°C, and recovered aerobically. However, when they subjected cells to heat shock by limited prior exposure to or growth at 43°C, thermal inactivation rates became nonlinear, unless recovery was made under strict anaerobiosis. Their findings suggest that the metabolic alterations associated with the heat shock phenomenon in this species determine, in part, conditions optimal for recovering heat-injured cells but do not alter, a priori, the linearity of thermal destruction. Two strains of *L. monocytogenes* (BS-9 and SE-31), *L. ivanovii* strain LA-30, *L. seeligeri* strain LA-34, and *L. welshimeri* strain LA-36 were chosen for further study on the basis of demonstrated heat resistance in the preliminary trial. *L. monocytogenes* strain Scott A was also studied as a reference to previous work (*4,5,8*).

The thermal resistance characteristics of the six *Listeria* strains suspended in raw and sterile farm bulk-tank milk and heated at temperatures between 52.2 and 71.7°C are shown as D-values in Tables 1 and 2. The z_D-values ranged from 5.3 to 7.0°C. As reported previously (*5,10*), the D-values estimated in sterile milk (Table 2) were somewhat higher than those in raw milk (Table 1). Heat-injured *Listeria* cells in raw milk are inhibited by competition from thermoduric milk flora (*1*). Heating times permitting *Listeria* recovery tend to be longer in sterile milk.

The heat resistance of the three *Listeria* spp. other than *L. monocytogenes* was somewhat less than that of the *L. monocytogenes* strains in both menstrua, but the differences were not statistically significant ($\alpha = 0.05$). The $\log_{10}$ of D-values for *L. welshimeri* strain LA-36, a representative member of the first group, and of *L. monocytogenes* strain BS-9 heated in raw and in sterile milk were plotted against heating temperatures (Fig. 1 and 2).

TABLE 1. *Mean D-value estimates for* Listeria *spp. in raw milk.*

	D-value (s)					
Temperature	*L. ivanovii*	*L. seeligeri*	*L. welshimeri*		*L. monocytogenes*	
(°C)	LA-30	LA-34	LA-36	BS-9	Scott A	SE-31
52.2	1334.5(2)[a]	1054.9(2)	1629.8(2)	-[b]	-	-
57.8	128.7(2)	150.9(2)	151.3(2)	435.6(2)	330.0(2)	528.6(2)
63.3	18.8(2)	21.0(2)	32.0(2)	38.7(2)	31.0(2)	46.1(2)
68.9	2.9(2)	2.8(2)	2.1(2)	3.3(2)	4.0(2)	2.8(2)
71.7	-	-	-	2.2(2)	2.0(2)	1.5(2)
Z value, °C	6.3	6.4	6.9	5.8	6.2	5.3

[a]Number of determinations in parentheses.

[b]Not determined.

TABLE 2. *Mean D-value estimates for* Listeria *spp. in sterile milk.*

Temperature (°C)	D-value (s) *L. ivanovii* LA-30	*L. seeligeri* LA-34	*L. welshimeri* LA-36	*L. monocytogenes* BS-9	Scott A	SE-31
52.2	1523.3(2)[a]	1247.9(2)	1779.1(2)	2848.3(2)	1704.8(2)	-
57.8	179.1(2)	190.9(2)	228.7(2)	409.0(5)	290.2(2)	440.5(2)
63.3	20.1(2)	28.5(2)	25.5(2)	68.0(2)	50.6(2)	49.6(2)
68.9	4.8(2)	5.2(2)	3.4(2)	9.1(2)	7.3(2)	6.2(2)
71.7	-	-	-	-	-	4.4(3)
Z value, °C	6.6	6.9	6.1	6.7	7.0	6.8

[a]Number of determinations in parentheses.
[b]Not determined.

Our results suggest that, at least for the strains tested in this study, *Listeria* spp. other than *L. monocytogenes* possess a thermal resistance that is equivalent to, or possibly less than, that of pathogenic *L. monocytogenes*. Thus, we conclude that our previous finding, which advocated the continued use of HTST processing to eliminate *L. monocytogenes* from dairy products (*4,5,8-10*), should apply equally to all other *Listeria* spp. The presence of *Listeria* spp., regardless of pathogenic potential, in finished dairy products denotes either a deficiency in the pasteurization process or a postpasteurization contamination. In either case, the finding of *Listeria* spp. in pasteurized dairy products should signal immediate attention to the offending dairy plant's environment, process, and sanitary practices. Deficiencies can best be approached and controlled by applying the hazard analysis critical control point (HACCP) concept at all levels of dairy processing.

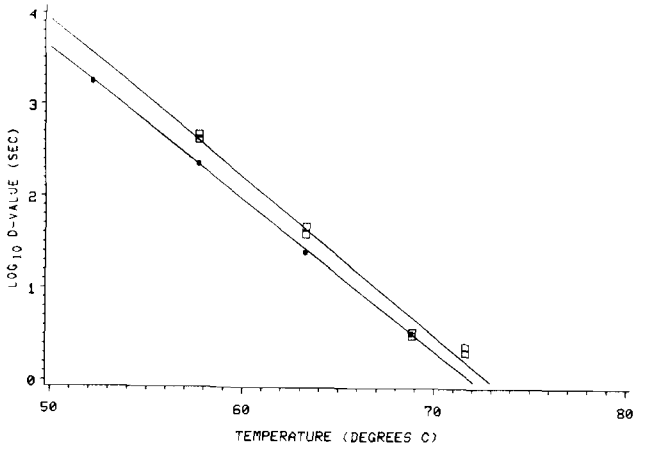

Figure 1. *Thermal death-time curves for* Listeria *spp. in raw milk.* ■ = L. monocytogenes *strain BS-9;* ● = L. welshimeri *strain LA-36.*

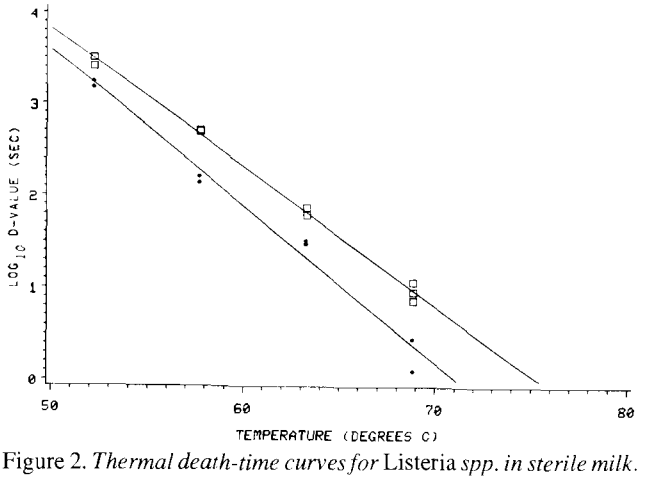

Figure 2. *Thermal death-time curves for* Listeria *spp. in sterile milk.* ■ = L. monocytogenes *strain BS-9;* ● = L. welshimeri *strain LA-36.*

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