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Thermal Destruction of *Listeria monocytogenes* in Ice Cream Mix

ABSTRACT

Thermal destruction of *Listeria monocytogenes* strain Scott A was studied in refrigerated ice cream mixes to evaluate the relationship of mix composition to heat resistance with differing heat treatments. A central composite response surface design with two independent variables (high fructose corn syrup solids content and milkfat content) and one dependent variable (viscosity of the mix) was developed. High fructose corn syrup solids (HFCSS) content ranged from 1 to 7%, milkfat (MF) content from 4 to 18%, and total solids content from 28 to 44%. Sucrose content (11%) and milk-solids-not-fat content (10%) were kept constant. $D_{140^{\circ}\text{F}}$ values were established in the mixes with a simulated batch pasteurization procedure using closed vials. Survivor data indicated sigmoidal responses with initial shoulders and tailing, but shoulder values were not significantly affected by either HFCSS or MF. D and F ($F = 7D + \text{shoulder}$) values were significantly ($p = 0.01$, $R^2 = .88$; $p = 0.01$, $R^2 = .89$, respectively) correlated to HFCSS content with increasing heat resistance conferred at higher concentrations. MF content had little or no effect on thermal death time but had a greater effect on viscosity of the mix than did content of HFCSS. Increased thermal resistance of LM was associated with the common ice cream stabilizer used; stabilizer contained guar gum and carrageenan. Pasteurization guidelines for ice cream mix are adequate to ensure inactivation of LM. Since results suggest that major ingredients in ice cream, ice milk, and shake mixes increase thermal resistance of LM, it is important that every precaution be taken to inactivate the organism.

The outbreaks of illness and fatalities associated with the consumption of milk and dairy products contaminated with *Listeria monocytogenes* (LM) has resulted in increased surveillance of all milk products by the U. S. Food and Drug Administration. This pathogenic microorganism is important to the dairy industry because of its ability to grow at refrigeration temperatures; its behavior in dairy products has been reviewed (8,10). Several recalls of milk products for LM contamination have involved ice cream or other frozen desserts, including frozen novelty products (11).

Ice cream varies in fat from 8 to 20%, sugar or sugar and corn syrup solids from 13 to 20%, milk-solids-not-fat from 8 to 15%, and stabilizer from 0 to 0.7%. To obtain legal weight, the total solids for mixes may range from 36 to 43% (3). Soft serve ice milk and milk shake mixes may vary from 23 to 31% total solids. Some information is available on the growth and survival of LM in various ingredients used in ice cream formulations. LM survived in nonfat dry milk stored at room temperature for at least 12 weeks but died off during storage with a greater than 4-log cycle reduction occurring by 16 weeks of storage (6). Survival studies in milk-chocolate flavored and cocoa-based ice cream coatings showed that viable cells were still present in both mixes after 28 d of storage at 25°C, but storage at temperatures close to 49°C seemed to eliminate LM as a problem at the inoculum levels used (14). Rosenow and Marth (12) studied the effects of addition of cane sugar, cocoa powder, and carrageenan to milk on growth of LM. They found that each of the major ingredients in chocolate milk appeared to enhance growth of LM when added to milk. More recently, Pearson and Marth (9) evaluated the behavior of LM in the presence of cocoa and casein, reporting that generation times under static incubation conditions were longer for 10% Dutch-processed cocoa in tryptose medium than in its absence; agitation and 2% or more cocoa content caused LM inactivation, but addition of casein reversed the antibacterial effect of the cocoa. Amelang and Doores (1) evaluated the effects of some ingredients in ice cream mix formulations on growth of LM, reporting that mixes held at 21 and 35°C showed significant differences due to mix formulation when fat and milk-solids-not-fat (MNF) ingredients and ratios were altered. In a second paper (2), they reported that the percentage of fat in the ice cream mix did not significantly affect growth of LM.

No information is available on the effect of the composition of the sweetener content on the survival of LM in ice cream mixes. The objective of our study, therefore, was to determine the effects of different levels of milk fat (MF) and high fructose corn syrup solids (HFCSS) on the thermal destruction of LM in ice cream mixes under various

heat treatments. The effects of MF and HFCSS on viscosity were also investigated.

MATERIALS

Ice cream mixes

Ice cream mix formulations (Table 1) were made up in either 2.7- or 4.6-kg lots to accommodate the capacity of the homogenizer. Mixes were formulated with low heat nonfat dry milk (Maryland-Virginia Milk Producers, Capitol Heights, MD). Sucrose was U. S. Sugar brand (Clewiston, FL) and was purchased locally. HFCSS was purchased from American Sweeteners, Inc., Frazer, PA; it was distinctive in that it contained 55% fructose, 41% dextrose, and only 4% higher saccharides. Citation brand stabilizer (containing guar gum and carrageenan) was from Germantown Manufacturing Corp., Broomall, PA. Cream, containing 55% milkfat, was purchased at a local dairy. Dry ingredients were dry blended before dispersing in 48.9°C (120°F) water, cream, and corn syrup mixture; total solids of the mixes varied with the sum of milkfat plus HFCSS used. Mixes were pasteurized at 71.1 - 73.9°C (160 - 165°F) for 30 min, homogenized in two stages at 1000 and 500 psi (6.89 and 3.45 MPa), cooled, and refrigerated overnight below 4.4°C. To reduce the number of interfering microorganisms, the mix was repasteurized at 79.4°C (175°F) for 10 min, cooled to below 4.4°C (40°F), divided into 100-ml aliquots in sterile containers, and frozen until used.

Mix viscosity measurements were performed on unfrozen mix aliquots at 10°C (50°F) and 60°C (140°F) with a Model DV-II Brookfield digital viscometer (Brookfield Engineering, Stoughton, MA) equipped with a UL adapter. Seventeen-g samples were weighed into the cup and equilibrated for 2 min in a water bath at the desired temperature before reading.

Mix pH was measured at 20°C (68°F) with an Orion Digital pH meter 611 fitted with a Cell 91-63 combination electrode (Orion Research Inc., Boston, MA). Water activity (a_w) of the mixes was measured at 25°C (77°F) and 10°C (50°F) with a Rotronic Hygroskop DT Relative Humidity Instrument (Rotronic Instrument Corp., Huntington, NY) equipped with a DMS 100H Sensor. The instrument was calibrated with salt solutions in the range of mix a_w , according to Stoloff (15).

Bacteriology and heating procedure

A 0.3-ml volume of a refrigerated (5°C) stock culture of LM strain Scott A was inoculated into 100 ml of brain heart infusion + glucose broth (Difco Laboratories, Detroit, MI) and incubated at 37°C (98.6°F) for 18 h with shaking. The cells were centrifuged and dispersed into the thawed 100-ml aliquots of ice cream mix so that the starting inoculum was 10^9 cells/ml. Three-ml aliquots of inoculated mix were placed in sterile 9-ml screw-cap vials with hole caps and Teflon-coated rubber septa and the sealed vials placed in a rack in an ice bath. Another vial, with 3 ml of mix,

was equipped with a pierced septum and a thermocouple hooked to an Esterline-Angus MRL Model 250 data logger (Esterline-Angus, Indianapolis, IN), for measuring the heating profile and recording temperature variations during the holding period. The vials were weighted down and completely submerged in a circulating water bath at 60.3°C (140.5°F). The rack was manually shaken until the internal temperature reached 60 ± 0.1 °C (140 ± 0.2 °F). The come-up time to 60°C (140°F) was recorded for each mix, before holding time commenced. Come-up time averaged 3 min, ranging from 2.67 to 3.42 min. Vials were removed at selected holding time intervals, ranging from 0 to 60 min, and immediately cooled in a ice bath before analysis. Plating was done at appropriate dilutions on tryptose agar (Difco) with a Model D spiral plater (Spiral Systems, Beltsville, MD). Incubation was at 37°C for 48 h; plates were read on a Spiral Systems Laser Colony Counter or manually counted.

The temperature of 60°C (140°F) was selected because it was the highest temperature where sufficient numbers of organisms survived over time to provide meaningful counts.

Survivor plots (\log_{10} CFU/ml versus time) were generated and D values calculated as the negative reciprocals of the slopes on the estimated straight line portions of the curves by linear regression. The General Linear Models Procedure from the SAS Software system (13) was used for the response surface analysis. All survivor curves (\log_{10} number vs time) showed both an initial shoulder and tailing (data not shown). Lag periods were measured by extrapolation to zero time as part of the program.

Because of the sigmoidal nature of the survivor curves obtained, an additional analysis was conducted using the Gompertz equation as described by Bhaduri et al. (4) and Buchanan et al. (5). D values were calculated as the negative reciprocals of the inactivation rates (equal to $\frac{BC}{e}$ where B and C are Gompertz parameters and e = the Euler constant).

RESULTS AND DISCUSSION

Data from 18 runs representing nine different ice cream mixes were evaluated. The data range used in our experimental design does not represent the full range of permissive ingredients in ice cream. We believe that compositions of the nine ice cream mixes we used are representative of industry practice, however. The numerical values for D, the shoulder interval and F for the 18 runs (Table 2) were calculated by both linear regression and ANOVA (13) and by the Gompertz equation (nonlinear regression) approach (5). The F value was considered to represent a more complete description to predict the necessary heat treatment. Highest D values, and therefore highest F values, indicating greatest heat resistance, were found in mix 8,

TABLE 1. Ice cream mix formulations for comparing effects of milkfat and high fructose corn syrup solids (HFCSS) on survival of *L. monocytogenes* after heat treatment.

Ingredient	Mix 1	Mix 2	Mix 3	Mix 4	Mix 5	Mix 6	Mix 7	Mix 8	Mix 9
Milkfat	6.00	17.00	6.00	17.00	4.00	18.00	10.20	10.20	10.20
MSNF ¹	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Sucrose	11.00	11.00	11.00	11.00	11.00	11.00	11.00	11.00	11.00
HFCSS	1.00	1.00	6.00	6.00	3.00	3.00	0.00	7.00	3.00
Stabilizer	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18
Total solids	28.18	39.18	33.18	44.18	28.18	42.18	31.18	38.35	34.15

¹MSNF = Milk-solids-not-fat.

containing highest levels of HFCSS, 7%. Mixes 3 and 4 with the next highest D values contained 6% HFCSS but Mix 4 contained 17% MF compared to 6% MF in Mix 3. Length of the lag period was variable. D and F values obtained by the two methods of calculation were similar but lower in most cases when the Gompertz equation method was used for calculation. Bhaduri et al. (4) pointed out that calculation of D values by the Gompertz method takes advantage of all data points, so this approach is likely to provide a more accurate estimate of the thermal resistance when sigmoidal survivor curves are obtained. In this study, however, results indicate that a greater margin of safety may be obtained by using the linear regression analysis approach for prediction of D values.

TABLE 2. Influence of mix on lag period, D and F values at 60°C (140°F) for nine ice cream mixes. $F = 7D + \text{lag period}$.

Mix	Calculated by linear regression		
	D min	Lag period min	F min
1	2.40 ¹	2.04	18.84
2	2.21	4.49	19.96
3	2.97	4.60	25.44
4	3.73	4.60	30.71
5	2.41	5.24	22.11
6	2.74	4.35	23.53
7	2.13	4.70	19.61
8	4.79	3.78	37.28
9	2.53	4.84	22.55
Mix	Calculated by Gompertz equation (4,5)		
	D	Lag period	F
1	1.80	2.17	14.77
2	1.97	3.60	17.12
3	2.64	4.29	22.77
4	3.12	5.92	27.76
5	1.80	4.10	16.68
6	2.37	4.19	20.78
7	1.83	1.94	14.96
8	3.15	8.97	31.02
9	2.26	3.36	19.18

¹Average of duplicate determinations.

Multiple regression analysis (13) of the shoulder values showed that concentration of HFCSS or MF had no significant effect ($R^2 = 0.16$) on the initial lag period, even though D and F values were highly related to HFCSS and MF concentrations.

Mix pH varied from 6.3 to 6.5 with the more acid pH associated with higher milkfat content. Water activity at 25°C averaged 0.969 (s.d. = .003) for all mixes. At 10°C, a_w varied from 0.930 to 0.957 (s.d. = .009) and was slightly but not significantly ($p > .05$) correlated with higher HFCSS content.

Using a central composite design and ANOVA, a second order response surface in HFCSS and MF was fitted to the viscosity, D and F values. All three response surfaces were significant at $p = 0.01$. The resulting equations and coefficients of determination (R^2) are listed (Table 3). The response surfaces for the D and F values were largely dominated by the concentration of HFCSS; MF concentration had little or no effect on thermal death time. The converse is true for the viscosity data, where the MF concentration dominated, with the exception that with higher MF concentration, there is a linear increase with HFCSS concentration due to the interaction term (HFCSS-MF). Viscosities at 60°C (140°F) ranged from 12 (Mix 5) to 108 (Mix 6) centipoise.

TABLE 3. Coefficients and R^2 for second order response surfaces of D and F values and viscosity of ice cream mixes.

	D*	F*	Viscosity*
Intercept	1.92	15.8	38.1
HFCSS	0.233	-0.614	-1.23
Milkfat	0.098	0.773	-4.72
HFCSS ²	0.059	0.304	-0.035
Milkfat ²	0.005	-0.034	0.439
HFCSS-Milkfat	0.012	0.059	0.342
R^2	0.88	0.89	0.98

*Significant at $p = 0.01$.

Stabilizer concentration was held constant at 0.18% of the ice cream mix total solids. Because Rosenow and Marth (12) had reported that the stabilizer (carrageenan) in chocolate milk appeared to enhance the growth response of LM, we examined the effect of stabilizer concentration on the heat resistance of LM more closely. D values were compared at three temperatures, 54.4°C (130°F), 57.2°C (135°F), and 60°C (140°F) for LM in 0.1 M potassium phosphate buffer (pH 7.13) to those for homogenized milk. Results are also shown for 0.5% stabilizer in buffer and for 0.18 and 5% stabilizer concentrations in a standard ice milk mix (mix without added stabilizer contained 4.0% MF, 9.0% sucrose, 5.0% HFCSS, 12.0% MSNF and 30.0% total solids). Results clearly show that at $D_{135^\circ\text{F}}$, increased stabilizer concentration (stabilizer contained guar gum and carrageenan) increased the D value in both ice milk mix and in buffer solution (Table 4). The addition of 0.5% buffer salts had no effect on the D value. D values calculated by the nonlinear regression method were again lower in all cases, and differences brought about by added stabilizer, although not as marked, were observed.

Rosenow and Marth also showed that both cocoa powder and cane sugar contributed to enhanced growth of LM in 2% milk, although not always significantly (12). They proposed that carrageenan also enhanced growth by keeping cocoa in suspension, making it more accessible to the organism rather than by adding nutrients to the mixture. They also suggested that casein in the milk may have prevented the cocoa anthocyanins from suppressing growth

TABLE 4. Effect of heating menstruum and temperature on D value of *L. monocytogenes*.

Sample	D _{130°F} (54.4°C)		D _{135°F} (57.2°C)		D _{140°F} (60°C)	
	min a ¹	a	b ¹	a	b	min
Homogenized milk	3.45	3.39	---	2.10	---	
0.1 M Potassium phosphate buffer	7.87	2.10	1.62	1.14	0.84	
Buffer + 0.5% buffer solids	---	2.13	1.59	1.14	0.74	
Buffer + 0.5% stabilizer	---	7.01	4.72	1.32	0.79	
Ice milk mix ² + 0.18% stabilizer	14.71	5.63	4.10	---	---	
Ice milk mix + 0.5% stabilizer	21.70	6.23	4.04	---	---	

¹ a = Calculation by linear regression; b = Calculation by nonlinear regression (Gompertz equation) (4,5).

² Composition: 4.0% milkfat; 12.0% MSNF; 9.0% Sucrose; 5.0% high fructose corn syrup solids; 30.0% total solids.

of LM; it was later demonstrated that this was true (9). They suggested further that the presence of sucrose in chocolate milk might cause an increase in the heat resistance of LM and indicated the need for further work so appropriate processing conditions might be used to assure the safety of chocolate milk. However, they did not consider the effects of the added ingredients on the viscosity of the milk system.

Carrageenan prevents or alters the agglomeration of casein micelles during processing. It cannot be used in ice cream mix alone since it greatly increases mix viscosity. It is usually used in combination with guar or other gums with good water binding and swelling properties that confer good heat shock resistance and smooth meltdown to the ice cream (7). Under our experimental design, where stabilizer and cane sugar concentrations were held constant, mix viscosity was viewed as a variable dependent on the mix composition in terms of HFCSS and MF; MF content was demonstrated to have a highly significant effect on mix viscosity but little or no effect on D or F values.

Our results clearly show that increased thermal resistance of LM is associated with the common ice cream stabilizer we used. Since this effect was also observed in phosphate buffer, it appears that the presence of stabilizers must also be taken into account when considering heat resistance of LM in dairy foods. However, it remains to be determined if the combination of carrageenan and guar gum is the only stabilizer that enhances heat resistance of LM, possibly through entrapment of the organism in the three-dimensional network formed with the casein micelle (7).

Although Scott A has been found to be among the most heat resistant strains of LM in dairy products (10), pasteurization guidelines for ice cream mix are adequate to ensure inactivation of LM. Since results of our experiments and those of others (9,11,12,14) suggest that major ingredients in ice cream, ice milk, and milk shake mixes increase the thermal resistance of LM, it is important that every precaution be taken to inactivate the organism.

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