

Effect of Freezing Raw Milk on Standard Plate Count

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Abstract

Samples of raw milk were frozen at -20 , -78 , and -196 C, stored for 3, 7, 14, or 28 days at -20 C, and examined for changes in Standard Plate Count. Freezing rates through the range of 0 to -20 C were 1, 21, and 93 C per minute for temperatures of -20 , -78 , and -196 C, respectively. Samples were thawed at 40 C and transferred to a 4.4-C bath when all visible ice had disappeared. There was a statistically significant reduction in the mean Standard Plate Count of the samples after each storage interval with all methods of freezing. The percentage change in plate counts varied widely from sample to sample, and this change exceeded 20% in 41, 46, and 49% of the milk samples frozen at -20 , -78 , and -196 C, respectively.

Introduction

Under some circumstances in which milk samples are taken for bacteriological examination for regulatory purposes, freezing the samples and maintaining them frozen would be easier than keeping them at refrigeration temperatures as recommended by Standard Methods for the Examination of Dairy Products (1). Another advantage of freezing samples is that they could be tested at a time convenient for the laboratory. Storage at freezing temperatures is believed by some to have no significant effect on Standard Plate Count, provided the storage time does not exceed two or three months.

Very few data are available on the effect of freezing on the Standard Plate Count. The only such published information on raw milk we are aware of is in a study of raw and finished products (5), in which samples of raw milk were frozen and stored at -17.8 or -10 C. With the lower freezing and storage temperature, the average Standard Plate Count was unchanged after 24 hr of storage but after 48 hr it was reduced by 25%. The samples frozen and held at -10 C had Standard Plate

Counts that were reduced by 14 and 31% after 24 and 48 hr of storage, respectively. Reports on the effect of freezing on the plate count of pasteurized milk vary from "practically unaltered" (9) and "no significant change" (6) to an average reduction of about 10% (5) and a "tendency to lower the Standard Plate Count" (2).

Several studies have been published on the effect of cooling rates on mammalian and bacterial cells. Rate of cooling has been shown to affect survival to the extent that, generally, a cooling rate of about 1 C per minute through the temperature range of 0 to -20 C resulted in the greatest survival of cells (7,8). None of these studies was done with milk as the suspending fluid. The data of Johns and Berzius (5) on the survival of bacteria in raw milk suggest that, of the two cooling rates studied, the faster gave the better survival.

The thawing technique is also important in cell survival. Evaluations reported to date indicate that fast thawing rates are least damaging to cells (8).

The purpose of our study was to determine the effect of freezing raw milk at -20 , -78 , and -196 C followed by storage at -20 C for 3, 7, 14, and 28 days on the Standard Plate Count.

Experimental Procedures

Four-liter samples of 48-hr bulk-tank raw milk were obtained, mixed thoroughly by repeated inversion, and divided into subsamples by dispensing 90-ml quantities to polypropylene bottles 46 mm in diameter and 96 mm high. Samples other than zero-hour controls were frozen at -20 , -78 , and -196 C. Those frozen at -20 C were placed upright on the expansion plate of a household-type freezer, and freezing was complete within 16 hr. Dry ice and acetone were used to freeze samples at -78 C, and liquid nitrogen was used for freezing at -196 C. The freezing times at -78 and -196 C did not exceed 25 and 12 min, respectively. After freezing, all samples were stored at -20 C for 3, 7, 14, or 28 days before assay.

Immediately before plating, the samples were

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thawed, 36 at a time, in an agitated water bath. Continuous addition of 40 C tap water and a suitable overflow device kept the water bath at constant temperature and water level. Upon the disappearance of visible ice, all samples were transferred from the 40 C water bath to one set at 4.4 C.

All Standard Plate Counts were performed on duplicate samples according to the Standard Methods for the Examination of Dairy Products (1). The hypothesis that the Standard Plate Count is not altered by freezing methods and storage time was tested. The number of samples of milk to be tested was determined by setting values for α (the probability that the hypothesis would be rejected when true), $1-\beta$ (the probability that the hypothesis would be rejected when false), and the critical difference that one wishes to detect with a probability of $1-\beta$ when the hypothesis is false. A 10% critical difference was selected with $\alpha = 0.01$ and $1-\beta \geq 0.99$. Data were analyzed using \log_{10} counts to make the usual assumptions for analysis of variance.

Results and Discussion

Since cooling rates through the range of 0

to -20 C have been shown to affect survival of bacteria in suspending fluids other than milk (8), initial studies were concerned with determining the usefulness of three milk-freezing techniques that gave a wide range of cooling rates. No attempt was made to develop a system for programming cooling rates, because the apparatus involved would make the method cumbersome, and its application for freezing milk samples in the field would be limited. Cooling rates were determined by placing thermocouples in 46- by 96-mm polypropylene bottles containing 90 ml of raw milk and immersing the bottles in a bath at -196 or -78 C or placing them directly on the expansion plate of a household-type freezer operating at -20 C. Maximum cooling rates through the range of 0 to -20 C were 93.0, 21.0, and 1.0 C per minute for respective exposure temperatures of -196 , -78 , and -20 C.

The rate of warming during thawing also was determined with thermocouples. Warming was slow until the milk had thawed, and this was followed by a rapid increase in temperature until all ice in the sample had disappeared. The maximum temperature reached

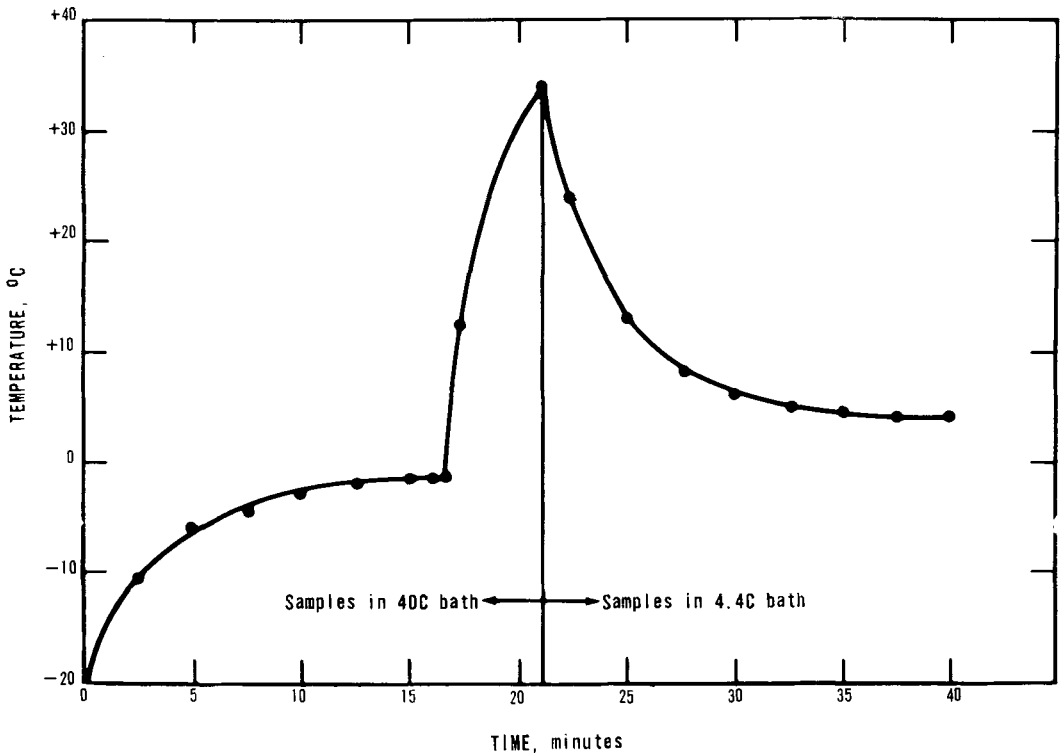


FIG. 1. Temperatures and times observed during the thawing of milk.

during the thawing was 34 C; however, the total time that the sample was above 10 C did not exceed 10 min (Fig. 1).

To evaluate the effect of cooling rate on the Standard Plate Count of raw milk, subsamples from 11 samples of 48-hr bulk-tank milk were prepared and frozen at three different temperatures. Enough subsamples were made so that all plate count determinations could be done on duplicate samples after 3, 7, 14, and 28 days of storage at -20 C without refreezing any of the subsamples. All three freezing techniques reduced the mean plate count significantly. The greatest changes occurred with the faster cooling rates and the longer periods of storage.

Since the slowest cooling rate (a maximum of 1.0 C per minute) caused the smallest reduction in counts, a larger series of determinations was done at this cooling rate to more adequately determine the effect of freezing raw milk samples at -20 C on the Standard Plate Count. These were made in August, whereas the initial determinations had been made in January. Twenty-one 48-hr bulk-tank milk samples were obtained, subsamples were prepared in the usual manner, and plate counts were done before freezing and after storage at -20 C. As before, the mean Standard Plate Count of the frozen samples of milk was significantly different from the counts obtained before freezing. The reductions in plate count for the milk samples examined in this series were greater than those encountered in the initial series. This might reflect a change in susceptibility to freezing of the normal flora of milk in the summer as opposed to winter. The plate counts in this series of determinations and those obtained in the initial series are shown in Table 1.

Examination of the effect of freezing on the plate counts of samples of raw milk by each

of the three methods shows that individual samples of milk reacted quite differently. Counts after freezing ranged from 26 to 200% of those for the unfrozen controls. These data are summarized in Figure 2. There was a tendency for milks with low initial counts to have a greater reduction in bacterial count from freezing than had high-count milks. When the percentage of change by all freezing methods was compared with samples with initial Standard Plate Count ranges of 0 to 49,000, 50,000 to 99,000, 100,000 to 190,000, 200,000 to 290,000, and 300,000 and over per milliliter of milk, the Standard Plate Count after freezing by all freezing methods expressed as mean per cent recoveries after freezing was 70, 85, 89, 83, and 112, respectively. Since samples with Standard Plate Counts of >300,000 per milliliter would be expected to have high psychrophilic populations, these results might indicate that psychrophilic bacteria are more stable to freezing than mesophilic bacteria that would be expected to predominate in raw milks having plate counts below 50,000 per milliliter.

The survival of bacteria in milk frozen and thawed by our techniques compares very favorably with the results reported with other methods, including ultrarapid freezing and thawing (4), lyophilization, and the use of special suspending media (3, 7). However, significant changes in the means of Standard Plate Counts resulted with all the procedures studied. These changes exceeded 20% in 41, 46, and 49% of the raw milk samples frozen at -20, -78, and -196 C, respectively. The magnitude and frequency of these changes in count preclude the use of these freezing techniques in milk-testing programs in which attempts are being made to obtain accurate information on the Standard Plate Count of the raw milk supply.

TABLE 1. Effect of three different freezing rates and temperatures on the mean standard plate count of raw milk.

Freezing rate ^a	Freezing temp	Month, samples taken	Sample storage time (days)				
			0 ^b	3	7	14	28
(C/min)	(C)		————(SPC/ml of milk)————				
1.0	-20	January	68,000	63,000	64,000	62,000	55,000
1.0	-20	August	68,000	52,000	51,000	47,000	43,000
21	-78	January	68,000	61,000	56,000	57,000	52,000
93	-196	January	68,000	52,000	51,000	50,000	47,000

^a Freezing rate through 0 to -20 C temperature range.

^b Unfrozen control.

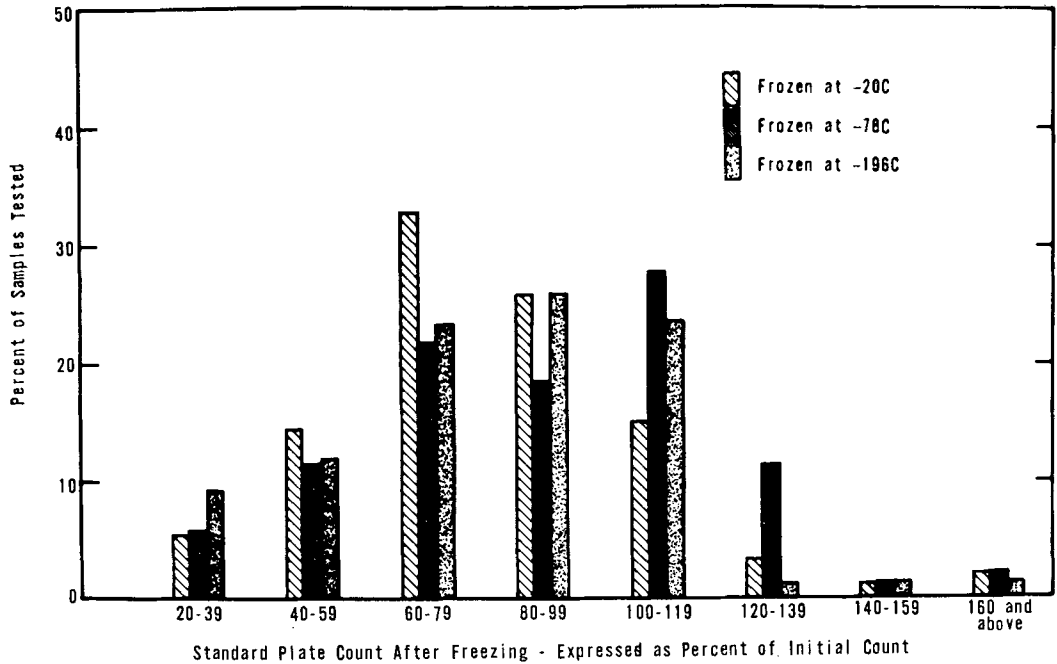


FIG. 2. Frequency of occurrence of eight ranges of change in standard plate count after freezing raw milk at three temperatures and storing at -20°C for 3, 5, 7, 14, and 28 days.

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