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Effect of Environmental Conditions on Unpasteurized Donor Human Milk

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ABSTRACT

As a result of concerns over the transmission of infectious diseases by donor milk, as well as the possible loss of nutritional value of donor milk through exposure to a variety of environmental conditions, the practice in the United States has been to discard unpasteurized donor milk that has thawed or sat for several hours at room temperature or in the refrigerator rather than (re)freezing it. We undertook an experiment to measure the effects of ambient temperature conditions and refreezing on the bioburden and nutritional content of human milk. We conclude that unpasteurized human milk is robust and can be used after storage under certain conditions.

INTRODUCTION

HUMAN BREAST MILK is the ideal food for human infants.[1] However, it is an unfortunate fact that not every mother can or will breastfeed her baby for any one of a variety of reasons. Data published over the past few decades indicate that human milk has particular benefits for infants being treated in the neonatal intensive care unit (NICU), and these benefits are seen with both fresh and banked human milk.[2,3] One question related to the use of banked milk that previously had not been rigorously studied is the effect of thawing and refreezing on microbial growth and milk constituents. We undertook this study to determine whether repeated freeze–thaw cycles and particularly thawing conditions adversely affect human milk.

MATERIALS AND METHODS

Human milk that was expressed by donors who were breastfeeding their own children was collected without any special sanitary precautions beyond what the mother normally would take when feeding her child. The milk was stored at −20°C for 2 months and then at −80°C until used for the experiment.

At the start of the experiment, the milk was thawed from −80°C overnight to 4°C and apportioned in 25-mL aliquots, which were then refrozen at −80°C. One day before the experiment began, the samples were placed in a −20°C freezer. The milk was thawed and brought to room temperature (23ºC). The samples were stored under the conditions described in Table 1.

The samples were tested for total bacterial burden, vitamin A and C levels, and free fatty acids (FFA). Bacterial burden testing was performed by diluting all samples up to 1:4 in peptone physiologic salt solution (PPS) and then plating 1 mL of the resulting solution on plate count agar. The plates were incubated for 3 days at 20 ± 1°C, after which the colonies were counted. Vitamin A and C analyses were performed by high-performance liquid chromatography. FFAs were ex-

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Table 1. Storage Conditions for Milk Samples

Sample	*Storage condition*
A	8°C for 4 hours
B	8°C for 24 hours
C	23°C for 4 hours
D	23°C for 8 hours
E	Repeat freeze-thaw 8°C for 4 hours, 8°C for 24 or 4 hours, refrozen to −20°C for 20 hours, rethawed and stored at 8°C for 4 hours, refrozen at −20°C for 20 hours, rethawed and stored at 8°C for 24 hours.
F	Control (−20°C throughout)

tracted by the combined steam distillation and extraction method of Likens and Nickerson.[4] The FFAs were methylated using diazomethane and were analyzed by gas chromatograph mass spectroscopy (GCMS) after derivitization. Special attention was directed toward the presence of aldehydes and FFAs.

RESULTS

The results of the microbial analysis show that breast milk that had been frozen at −20°C and then thawed did not develop a microbial load approaching 10^4 CFU/mL, which has been the accepted limit for bacterial growth for raw (unpasteurized) milk for most milk banks in North America. These results were the same for milk stored at 23°C for 8 hours or that had undergone repeated freeze–thaw cycles. The microbiologic results are shown in Table 2.

Vitamin analysis showed that there was no appreciable change in the concentration of vitamin A regardless of the test conditions under which the samples were stored. Vitamin C did show a decrease in concentration, losing about one-half of the initial concentration when kept at room temperature for 8 hours. When kept refrigerated for 24 hours, about one-fourth of the initial concentration was lost. These results are shown in Table 3.

Table 2. Microbial Analysis of Frozen and Thawed Breast Milk

Conditions	*CFU/mL*
8°C for 4 hours	8.6×10^1
8°C for 24 hours	3.5×10^1
23°C for 4 hours	1.0×10^2
23°C for 8 hours	3.7×10^2
Repeated freeze-thaw	1.1×10^2
Control	1.1×10^2

Table 3. Vitamin Analysis of Frozen and Thawed Breast Milk

Conditions	*Vitamin A (IE/100 mL)*	*Vitamin C (mg/100 mL)*
8°C for 4 hours	100	2.2
8°C for 24 hours	100	1.7
23°C for 4 hours	105	1.6
23°C for 8 hours	100	1.0
Repeated freeze-thaw	100	1.5
Control	100	2.2

Analysis of fat failed to detect the presence of aldehydes in any sample at >0.1 ppm and were absent altogether in many of the samples. FFAs were measured by comparison of the heights of their chromatographic peaks. Then the samples were ranked for each FFA tested; the data are presented in Table 4.

DISCUSSION

From the data presented, unpasteurized human milk is a fairly robust product that can withstand a certain amount of environmental fluctuation without posing a health risk or losing its nutritional value.

The bacterial growth data failed to indicate any direct correlation between time and temperature on milk bioburden. The study did not go beyond 24 hours, and it is reasonable to assume that a longer observation period would have resulted in the type of growth kinetics

Table 4. Ranking of Samples

Fatty acid	*Highest peaks* ⟶ *Lowest peaks*					
C6	F	C	D	B	E	A
C8	F	C	D	B	E	A
C10	F	D	C	E	B	A
C12	C	D	F	B	E	A
C14	C	D	F	B	E	A
C16:1	C	F	B	D	A	E
C16	C	D	F	B	A	E
C18:2	C	B	F	D	A	E
C18:1	C	F	B	D	A	E

A = 8°C for 4 hours; B = 8°C for 24 hours; C = 23°C for 4 hours; D = 23°C for 8 hours; E = represented freeze-thaw; F = control.

typical for the organisms found in breast milk, particularly in those samples held at room temperature. It is particularly important that repeated freeze–thaw cycles did not result in a significant increase in bacterial growth, which has been a general concern in the milk banking community. It is also significant that milk held at room temperature for 8 hours still had a bacterial count about 1.67 orders of magnitude lower than the cutoff value generally used in North American milk banks[5] for acceptability of unpasteurized milk, and 2.67 orders of magnitude below the cutoff suggested by the United Kingdom Association of Milk Banks[6] for acceptability before pasteurization.

The analyses of vitamins A and C showed no effect on the former and some loss of vitamin C if left at room temperature for 8 hours or at refrigerated temperatures for 24 hours. In either case, unfortified human milk is not an adequate source of these vitamins for very-low-birth-weight infants even if handled optimally. This is based on the adequate intake recommendations for these infants as suggested by the National Academy of Medicine.[7] For older infants, the levels found after storage in the refrigerator for 24 hours or at room temperature for 8 hours still should be adequate to meet these recommendations, assuming the child is receiving sufficient milk.

The FFA analysis showed that unpasteurized milk held at 23°C for 4 hours as well as the control sample had the highest levels of FFA, whereas milk stored at 8°C for 4 hours and milk that was repeatedly frozen and thawed had the lowest levels. There was no clear correlation between time and temperature for unfrozen conditions with FFA levels. Moreover, although differences were seen among storage conditions, they appeared to be small and, presumably, clinically insignificant.

CONCLUSION

Human milk has been singled out as the best form of nutrition for human babies. Although direct feeding from the breast to a mother's own child is the optimal delivery method, this is not always practical or possible. Pumping of milk for storage for later use often is necessary for many mothers and always is necessary for donor milk provided to milk banks.

The data generated by the authors support the contention that milk is relatively robust. Milk that has been left unrefrigerated for less than 8 hours, or placed in the refrigerator for a day, is safe to use and retains a good portion of its nutritional value. Moreover, it appears that unpasteurized milk that has been accidentally thawed remains safe to use provided it has not been left too long in an unthawed condition. Based on these data, it appears that unpasteurized milk that has thawed in the refrigerator for up to 8 hours may be safely refrozen. Moreover, this data would seem to support the use of frozen milk to which fresh milk has been added and then refrozen. This should allow for more convenient storage and for the salvage of milk that mothers might otherwise have been told to discard.

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