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BRIEF REPORT

Effect of freezing on the ''creamatocrit'' measurement of the lipid content of human donor milk☆,☆☆

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KEYWORDS
Donor human milk;
Lipid content;
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Creamatocrit;
Freezing

Abstract
Objective: To determine, by the creamatocrit measurement, the effect on fat content of freezing raw and pasteurised donor milk at −20 °C for 3 months.
Methods: The evolution of the creamatocrit measurement (following Lucas technique) on frozen (−20 °C), raw and pasteurised human milk, was analysed during 3 months.
Results: The fat content of raw milk ($n = 44$) was 3.19 g/dl at the beginning and 2.86 g/dl after 3 months frozen ($p = 0.02$). In pasteurised milk ($n = 36$) fat content at the first determination was 2.59 g/dl and 2.20 g/dl after 1 month frozen ($p = 0.01$). Afterwards there were no significant changes up to 3 months frozen. Variability was observed in the intermediate values.
Conclusions: A reduction on the fat content measurement of raw and pasteurised donor human milk after freezing was observed. Freezing does not inactivate the milk lipase but does destroy the fat globule. Creamatocrit measurement may not be the best method to determine the fat content of processed human milk.

PALABRAS CLAVE
Leche materna donada;
Contenido graso;

Medida por crematocrito del contenido calórico de la leche materna donada congelada

Resumen
Objetivo: Determinar, mediante crematocrito, las modificaciones del contenido graso de la leche materna cruda y pasteurizada a lo largo de 3 meses de congelación.

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Contenido energético;
Crematocrito;
Congelación

Método: Se analizó la evolución del crematocrito (fórmula de Lucas) en leche cruda y pasteurizada a lo largo de 3 meses de congelación a −20 °C.
Resultados: La grasa en leche cruda (n = 44) fue 3,19 g/dl al inicio y 2,86 g/dl a los 3 meses de congelación (p = 0,02). En leche pasteurizada (n = 36), al inicio fue 2,59 g/dl y 2,20 g/dl al mes de congelación (p = 0,01), posteriormente, hasta los 3 meses, no hubo cambios significativos. Se observó variabilidad en los valores intermedios.
Conclusiones: Se observó una disminución en la medida de la grasa tras congelación en leche cruda y pasteurizada. La congelación no impide la acción de la lipasa y también afecta al glóbulo de grasa. Probablemente, el crematocrito no sea el método óptimo para cuantificar la grasa en leche ya procesada.

Introduction

Human milk is the ideal food for newborns, especially if they are premature or ill,[1–4] but its properties may alter during its manipulation and storage.

Freezing human milk at −20 °C guarantees its microbiological quality, but its effects on nutritional quality have not been researched thoroughly. Some studies have observed a decrease in the bactericidal and antioxidant activities of breast milk[5–7] and a reduction in the energy and fat content over the freezing time.[8]

At times, the nutritional content of breastmilk does not meet the requirements of preterm and low-birth-weight infants,[9] so it is important that we learn its nutritional value. Lipids constitute the main source of energy, but they are the component that shows the highest degree of variation.[10] The creamatocrit method is a simple and inexpensive means to calculate the fat and energy content of breast milk. It was described by Lucas et al.[11] in 1978. Several authors have observed a good correlation between the energy content of milk and the creamatocrit.[12–14]

The purpose of our study was to determine, by means of the creamatocrit method, the variations in the fat content of raw and pasteurised human milk throughout 3 months of freezing at −20 °C.

Materials and methods

We performed an experimental study. We obtained the samples from women who donate to the Milk Bank, and gave informed consent. The samples were expressed manually or with a breast pump, and stored in sterilised glass containers.

The samples of raw (unpasteurised) milk were refrigerated at 4–5 °C following extraction for a maximum of 24 h. In those first 24 h the samples were homogenised by rocking them in an arc-like fashion 10 times and then were divided in 7 aliquots. We analysed the first aliquot (time 0) and the 6 remaining aliquots were frozen at −20 °C.

The samples of pasteurised milk were homogenised immediately following Holder pasteurisation by rocking them in an arc-like fashion 10 times, after which they were divided into 7 aliquots. We analysed the first aliquot after pasteurisation, and the 6 remaining aliquots were frozen at −20 °C.

The raw and pasteurised milk aliquots were stored in polypropylene tubes and were labelled based on freezing time (7, 14, 21, 30, 60, or 90 days).

To analyse the aliquots, they were thawed in a 40 °C water bath until a frozen pellet remained in the middle, and moved to a refrigerator to complete the thawing at 4–5 °C.

Creamatocrit analysis

We used the Lucas method.[11] Each aliquot was heated in a 40 °C water bath for 10 min and homogenised with a Vortex® mixer. Three 75 μl capillary tubes were drawn from each aliquot for the creamatocrit. The capillary tubes were sealed at one end and centrifuged for 15 min at 12 000 rpm in a Hettich® haematocrit centrifuge.

The cream and aqueous fraction layers were measured with callipers. The creamatocrit was expressed as the percentage of cream relative to the length of the entire milk column. We calculated the mean value of the 3 creamatocrits, and applied the Lucas formula to determine the fat content (%cream − 0.59)/0.146 = g/l and energy content (%cream × 66.8 × 290 = kcal/l).

Statistical methods

Sample size: we conducted a pilot study beforehand. We found a difference in fat content of 0.7 g in raw milk and 0.5 g in pasteurised milk. The sample size required to detect this difference with a power of 80% and a confidence interval of 95% was of 39 raw milk samples and 34 pasteurised milk samples.

We have used the mean ± standard deviation to describe continuous variables. The proportion of samples analysed by means of 1, 2, or 3 creamatocrits was expressed in percentages. To evaluate the differences in fat and energy content at the initial time point and the different freezing times, we used Student's *t*-test for paired samples. We tested the normality of the data distribution by means of the Kolmogorov–Smirnov test.

Results

We collected 44 samples of raw donated breastmilk. Table 1 shows the number and percentage of raw milk aliquots on which 1, 2, and 3 creamatocrits were performed.