

# Nutrient Composition of Banked Human Milk in Brazil and Influence of Processing on Zinc Distribution in Milk Fractions

Heloísa C. A. Góes, MSc, Alexandre G. Torres, MSc, Carmen M. Donangelo, PhD, and Nadia M. F. Trugo, PhD

*From the Laboratorio de Bioquímica Nutricional e de Alimentos, Departamento de Bioquímica, Instituto de Química, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil*

**OBJECTIVE AND METHODS:** We measured the contents of fat, protein, lactose, calcium, phosphorus, zinc, iron, copper, and vitamin A in processed mature milk samples (individual,  $n = 60$ , and pooled,  $n = 10$ ) from a reference human milk bank in Brazil and assessed the effect of pasteurization followed by freezing on the nutrient composition and the pattern of zinc distribution in fractions (fat, whey, and casein) of milk samples ( $n = 15$ ).

**RESULTS:** Mean nutrient concentrations were within expected ranges in mature milk from healthy women, except fat, which was lower. Interindividual variability of nutrient concentrations was high (coefficient of variation, 21–62%) but reduced overall in pooled samples. Processing of milk samples did not affect the nutrient contents but did cause a significant shift ( $P < 0.04$ ) in the relative distribution of zinc, with a decrease in the whey fraction and an increase in the fat fraction.

**CONCLUSIONS:** Redistribution and possible alterations in the zinc-binding pattern during processing in human milk banks may reduce zinc bioavailability to the infant. *Nutrition* 2002;18:590–594. ©Elsevier Science Inc. 2002

**KEY WORDS:** human milk, processing, micronutrients, zinc, vitamin A

## INTRODUCTION

Human milk banks collect, process, and store milk from healthy lactating women. Infection control procedures for human milk banking support the use of banked milk as a safe and immunologically beneficial feeding alternative for infants.<sup>1,2</sup> In developing countries, human milk banks perform an additional important social role by promoting breast feeding, encouraging mothers to express milk for their babies when direct breast feeding is not possible, and instructing mothers on hygienic practices for infant feeding and care. This role is especially important for populations living in regions with inadequate sanitation, where morbidity and mortality of neonates caused by infections are more prevalent.<sup>3</sup> In Brazil, the human milk bank at the Instituto Fernandes Figueira (IFF-HMB; Fundação Oswaldo Cruz/Ministry of Health) in Rio de Janeiro is a national reference and offers professional training and consultation for all other Brazilian human milk banks.<sup>3</sup> However, despite its importance for milk banking in Brazil, the nutrient composition of the milk provided by the IFF-HMB has not been evaluated. In addition, data on milk composition provided by human milk banks in developing countries are scarcely available.

Banked human milk is an important alternative for the care and treatment of premature and low-birth-weight neonates, and sick newborns and infants with severe infectious disease, immunodeficiency, serious intestinal illness, intractable diarrhea, and heter-

olog protein intolerance.<sup>3</sup> There are nutritional and immunologic advantages for feeding human milk in such situations.<sup>2,4</sup> However, with regard to premature and low-birth-weight infants, feeding with banked human milk has raised concern, especially when mature milk is used, due to the infants' greater nutrient requirements in comparison with term infants.<sup>5</sup> Calcium, phosphorus, iron, copper, zinc, and vitamin A are among the nutrients that may be deficient in preterm infants.<sup>5</sup> Therefore, measurement of the concentrations of these micronutrients and macronutrients in banked human milk is useful to ascertain its adequacy for infant feeding and to establish levels for possible milk fortification or supplementation.

Milk processing at the IFF-HMB follows the worldwide guidelines for human milk banks<sup>1,6</sup> and includes pasteurization (62.5°C for 30 min, followed by rapid cooling) and freezing. This processing, however, reduces the content of some vitamins and immunologic factors and affects binding proteins of vitamins and minerals,<sup>7</sup> possibly reducing nutrient bioavailability. Distribution and binding patterns of minerals in milk fractions may play important roles in their intestinal absorption.<sup>8,9</sup> Reduced bioavailability due to milk processing may be critical for minerals whose requirements are high in infants, especially premature infants.

Zinc concentration in breast milk may limit the weight and linear growth of term and preterm infants up to age 6 mo.<sup>10–12</sup> Preterm infants are more susceptible than term infants to zinc deficiency.<sup>13</sup> In human milk, zinc is present mainly in the whey fraction,<sup>8,14,15</sup> where its association with the low-molecular-weight components and proteins may contribute to its high bioavailability.<sup>9,13,16</sup> Pasteurization of human milk decreases zinc bioavailability in preterm infants.<sup>16</sup> Redistribution of zinc in milk fractions may occur during processing in human milk banks, thereby affecting its bioavailability for the recipient infant. To our knowledge, there are no data in the literature concerning zinc

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Correspondence to: Nadia M. F. Trugo, PhD, Departamento de Bioquímica, Instituto de Química, CT bloco A, UFRJ, Cidade Universitária, 21949-900 Rio de Janeiro, Brazil. E-mail: trugo@iq.ufrj.br

redistribution in human milk fractions due to combined pasteurization and freezing.

The purpose of the present work was to measure the content of selected nutrients, including fat, protein, lactose, calcium, phosphorus, zinc, iron, copper, and vitamin A, in banked mature milk samples provided by IFF-HMB and to assess the effect of processing on these components and on the pattern of zinc distribution in milk fractions (fat, casein, and whey). In addition, we investigated possible associations between nutrients in the milk samples.

## MATERIALS AND METHODS

### *Routine Procedures of Milk Collection and Processing at IFF-HMB*

Healthy nursing women who are producing milk in excess of their infants' demands are eligible as milk donors at the IFF-HMB. Age, parity, and socioeconomic level are not included in the selection criteria. In general, donors are adults, deliver term infants, and provide mature milk. The standard protocol adopted by IFF-HMB involves collection of the surplus milk by the donor at home. This protocol was approved by the Ethics Committee of the Fundação Oswaldo Cruz/Ministry of Health (Rio de Janeiro, Brazil) with which IFF-HMB is affiliated. Breast milk is obtained by hand expression or the use of a breast pump (manual or electric) and occasionally by collection of drip milk. Milk is collected into a 250-mL glass flask previously sterilized and provided by the milk bank. The flask is stored in the home freezer at  $-20^{\circ}\text{C}$ . When successive volumes of surplus milk are obtained, they are added on top of the previous sample and also frozen. The frozen milk is then transported in an ice box to the IFF-HMB by a staff member and remains frozen at  $-20^{\circ}\text{C}$  for up to 15 d before being processed.

The IFF-HMB protocol of milk processing consists of the following steps: 1) thawing in a microwave oven without warming up the samples; 2) pasteurization at  $62.5^{\circ}\text{C}$  for 30 min, followed immediately by submersion in ice-cold water for 10 min; and 3) freezing at  $-20^{\circ}\text{C}$  and storage for up to 6 mo. A sample aliquot is taken after pasteurization for microbiological control.

### *Source and Preparation of Milk Samples for the Present Study*

**CHARACTERIZATION OF NUTRIENT COMPOSITION IN MILK SAMPLES PROVIDED BY IFF-HMB.** Sixty individual samples of processed mature milk (1 to 6 mo postpartum) from IFF-HMB were selected at random for determination of the nutrient composition. These samples were obtained from 60 different donors. Fifty-nine percent of the donors were primiparas, 66% were of medium to high socioeconomic level, and 78% were at least 21 y of age.

In addition, 10 pools of processed milk samples from IFF-HMB, consisting of five different samples each, randomly chosen, were analyzed to determine the variability of nutrient composition of pooled samples.

Portions of individual ( $n = 60$ ) and pooled ( $n = 10$ ) milk samples obtained after pasteurization were separated in aliquots of polypropylene tubes for the analyses of fat, protein, lactose, calcium, phosphorus, zinc, iron, copper, and vitamin A and kept frozen at  $-20^{\circ}\text{C}$ . Immediately before analysis, the samples were thawed and equilibrated in an oven at  $37^{\circ}\text{C}$  to  $38^{\circ}\text{C}$ .

**EFFECT OF THE PROCESSING PROCEDURE AT IFF-HMB ON NUTRIENT COMPOSITION AND ZINC DISTRIBUTION IN MILK SAMPLES.** A different set of 15 milk samples was used to test the effect of processing on the nutrient composition. The frozen samples provided by the donors were thawed and

equilibrated in an oven at  $37^{\circ}\text{C}$  to  $38^{\circ}\text{C}$  and divided into three portions. The first portion was analyzed immediately for vitamin A, fat, and zinc (total and in milk fractions). The second portion was separated in aliquots and frozen again at  $-20^{\circ}\text{C}$  for subsequent analysis of protein, lactose, calcium, phosphorus, iron, and copper. The first and second portions were called "unprocessed samples." The third portion was processed according to the IFF-HMB protocol and called "processed samples." After pasteurization and cooling, aliquots of the third portion were separated into polypropylene tubes and frozen at  $-20^{\circ}\text{C}$  for up to 6 mo, until analyzed. At the moment of analysis, all frozen samples were thawed and equilibrated in an oven at  $37^{\circ}\text{C}$  to  $38^{\circ}\text{C}$ .

### *Sample Analyses*

Fat in milk samples was determined by the crematocrit method<sup>17</sup> after equilibration in a water bath at  $50^{\circ}\text{C}$  followed by homogenization. Fat content (g/L) was calculated with the following equation:  $(\text{crematocrit}\% - 0.59)/0.146$ . Protein was determined according to the method of Lowry et al.<sup>18</sup> after treatment with sodium deoxycholate and trichloroacetic acid,<sup>14</sup> and results were expressed as grams per liter. Lactose was determined by the picric acid method of Perry and Doan<sup>19</sup> and the concentration was expressed in grams per liter.

For determination of minerals except zinc, milk samples were dry ashed at  $550^{\circ}\text{C}$  for 12 h and diluted with 1.2 mol/L of HCl. Calcium was determined by the methylthymol blue method with the use of a commercial kit (Biolab-Merieux, Rio de Janeiro, Brazil). Phosphorus was measured according to the method of Bartlett.<sup>20</sup> Iron was determined by the bathophenanthroline method as described by Vargas-Zapata et al.<sup>21</sup> Copper was determined by atomic absorption spectrometry (atomic absorption spectrometer, Model ICP5500, Perkin-Elmer, Norwalk, CT, USA). Concentrations were expressed in milligrams per liter. Recovery of added known amounts of standards to samples provided 93% to 96% of expected values for all minerals.

Zinc was determined in all whole-milk samples and milk fractions from milk samples that were compared before and after processing. Milk fractions were obtained according to the method of Fransson and Lonnerdal.<sup>8</sup> Briefly, whole milk was centrifuged at  $4000g$  for 30 min at  $4^{\circ}\text{C}$  to remove the milk fat. A portion of the skim milk was then centrifuged at  $106\,000g$  for 90 min at  $4^{\circ}\text{C}$  for separation of the casein fraction (sedimentable fraction) from the whey fraction (aqueous, non-sedimentable fraction). Portions (500  $\mu\text{L}$ ) of whole milk, skim milk, or milk whey were then transferred to Pyrex hydrolysis tubes with Teflon screw caps, followed by the addition of 500  $\mu\text{L}$  of 65% nitric acid (Ultrapure, Merck, Germany). The capped tubes were maintained in an oven at  $105^{\circ}\text{C}$  for 20 h. The hydrolysates were then diluted 1:1 (v/v) with deionized water, and zinc was measured by atomic absorption spectrometry. Zinc in the fat fraction was calculated by the difference between the zinc concentration in whole milk and that of skim milk corrected by the crematocrit equation:  $Zn_{fat} = Zn_{wm} - Zn_{sm} (1 - \text{crematocrit}\%/100)$ , where *wm* is whole milk and *sm* is skim milk. Zinc in the casein fraction was calculated by difference between zinc concentrations in skim milk and milk whey, corrected by the crematocrit equation:  $Zn_{casein} = (Zn_{sm} - Zn_{whey}) \cdot (1 - \text{crematocrit}\%/100)$ . Results of total, fat-bound, casein-bound, and whey-bound zinc concentrations were expressed in milligrams per liter of whole-milk samples. Zinc concentrations in milk fractions also were expressed as a percentage of total zinc content in whole milk. Recovery tests for zinc on average produced 93% of the expected values.

Vitamin A was determined by high-performance liquid chromatography according to the method of Giuliano et al.<sup>22</sup> Briefly, samples were saponified and extracted twice with hexane. Remaining water-soluble compounds were removed with deionized water

TABLE I.

NUTRIENT CONTENT OF SAMPLES OF MATURE (1–6 MO) HUMAN MILK FROM THE IFF-HMB			
Nutrient	Milk samples ( <i>N</i> = 60)		
	Median	Mean ± SD	CV (%)
Fat (g/L)	17.4	17.5 ± 10.7	61
Protein (g/L)	11.4	11.6 ± 3.2	27
Lactose (g/L)	72.1	73.2 ± 15.5	21
Calcium (mg/L)	236	237 ± 53	22
Phosphorus (mg/L)	131	132 ± 31	24
Calcium:phosphorus	1.83	1.82 ± 0.38	21
Iron (mg/L)	0.54	0.66 ± 0.31	47
Copper (mg/L)	0.48	0.52 ± 0.12	23
Zinc (mg/L)	1.49	1.65 ± 1.00	61
Vitamin A (μmol/L)	1.40	1.51 ± 0.93	62

CV, coefficient of variation of milk samples; IFF-HMB, Instituto Fernandes Figueira; SD, standard deviation

and ethanol and re-extracted with hexane. The combined hexane layers were then evaporated under a gentle nitrogen stream and the residue dissolved in dichloromethane:methanol (1:1, v/v). Separation was achieved using a liquid chromatograph (Shimadzu LC-10 AD, Kyoto, Japan) equipped with a Shimadzu SPD-10 AV UV-Vis detector and a Hewlett-Packard 3396A electronic integrator (Corvallis, OR, USA). A reverse-phase column (Spherisorb ODS-2, 1.5 μm, 250 × 4.6 mm, Supelco, St. Louis, MO, USA) was used, and the mobile phase was a dilution of methanol:water (95:5, v/v) at 1.0 mL/min. Injection was carried out with a Rheodyne injection valve (Supelco, St. Louis, MO, USA) with a 20-μL fixed loop. Retinol was detected at 325 nm and the concentration was determined with the aid of external calibration (synthetic all *trans*-retinol, Sigma, St. Louis, MO, USA), based on peak areas, and expressed in micromoles per liter. All steps were carried out under subdued light. All solvents used were of chromatographic grade (Merck, Darmstadt, Germany). Milk samples (six replicates) were spiked with retinyl palmitate to verify the extent of saponification of the retinyl esters and extraction of retinol. Results showed complete saponification and extraction.

All analytical procedures were carried out at least in duplicate for each sample. Within-sample coefficient of variations (CVs) for each analytical procedure were determined in six replicates of one pooled milk sample. CVs were less than 5% for fat, protein, lactose, and total minerals, 6.5% for zinc fractions, and 9.2% for vitamin A.

### Statistical Analyses

Pearson's correlation analyses were used for determining relations between nutrients. Paired *t* test was used for comparison of results before and after milk processing. For comparison between two different groups of samples, unpaired *t* test was used. *P* < 0.05 was considered statistically significant.

## RESULTS

Nutrient concentrations of individual samples (*n* = 60) of processed mature milk from IFF-HMB are shown in Table I. A high interindividual variability in fat, iron, zinc, and vitamin A concentrations in the milk samples was observed, with CVs higher than 40%. However, in pooled milk samples (*n* = 10), the variability in composition was generally lower than in the individual samples,

TABLE II.

NUTRIENT CONTENTS OF SAMPLES OF MATURE (1–6 MO) HUMAN MILK BEFORE AND AFTER PROCESSING				
Nutrient	Unprocessed milk		Processed milk*	
	Mean ± SD (N)	CV (%)	Mean ± SD (N)	CV (%)
Fat (g/L)	18.4 ± 13.2 (15)	72	18.6 ± 13.1 (15)	70
Protein (g/L)	14.3 ± 3.5 (15)	24	12.9 ± 2.3 (15)	18
Lactose (g/L)	80.1 ± 8.8 (15)	11	76.4 ± 17.5 (15)	23
Zinc (mg/L)	1.50 ± 1.02 (15)	68	1.49 ± 0.96 (15)	64
Fat fraction (mg/L)	0.19 ± 0.10 (10)	53	0.28 ± 0.12 (10)	43
Casein fraction (mg/L)	0.63 ± 0.40 (10)	63	0.77 ± 0.61 (10)	79
Whey fraction (mg/L)	0.73 ± 0.70 (10)	96	0.48 ± 0.31 (10)	65
Vitamin A (μmol/L)	1.21 ± 0.63 (15)	52	1.10 ± 0.69 (15)	63

\* Mean nutrient concentrations were not significantly different from those in unprocessed milk (*t* test).

CV, coefficient of variation of milk samples; SD, standard deviation

especially for those nutrients with the highest CVs. The CVs for the pooled samples were 36% for fat, 34% for protein, 16% for lactose, 13% for calcium, 15% for phosphorus, 35% for iron, 38% for copper, 24% for zinc, and 46% for vitamin A.

Table II shows the analytic results of a different set of 15 milk samples before and after processing as carried out at IFF-HMB. Reported nutrients are fat, lactose, protein, zinc (total and in milk fractions), and vitamin A. There was no significant effect of processing on all nutrients analyzed.

In contrast, processing of the milk samples affected the relative zinc distribution in milk fractions (Fig. 1). In unprocessed milk, 16.5 ± 8.8% (mean ± standard deviation) of total zinc was present in the fat fraction, 42.5 ± 12.3% in the casein fraction, and 41.0 ± 14.2% in the whey fraction. In processed milk, the distributions were 22.5 ± 9.3% in fat, 48.8 ± 12.8% in casein, and 28.7 ± 12.8% in the whey fraction. There was a significant increase in the percentage of zinc in fat (*P* = 0.029) and a decrease in the percentage of whey (*P* = 0.032) in processed versus unprocessed samples (*t* test). There was no significant difference in the percentage of zinc in the casein fraction, although there was a tendency for its increase in the processed samples.

Pearson's correlation analysis between the nutrient concentrations was carried out in all individual samples of processed (*N* = 75) and unprocessed (*n* = 15) milk samples. The following significant correlations were found for processed samples: calcium

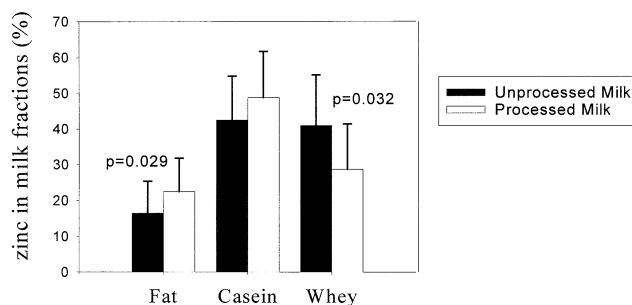


FIG. 1. Relative zinc distribution in milk fractions before and after processing of milk samples (*n* = 10, mean ± standard deviation). The percentage of zinc in milk fractions was calculated in relation to total zinc in whole milk.

and lactose,  $r = 0.51$ ,  $P < 0.001$ ; phosphorus and lactose,  $r = 0.34$ ,  $P = 0.004$ ; calcium and phosphorus,  $r = 0.39$ ,  $P < 0.001$ ; and zinc and protein,  $r = 0.64$ ,  $P < 0.001$ . In unprocessed milk samples, significant correlations also were observed for calcium and lactose ( $r = 0.58$ ,  $P < 0.05$ ) and phosphorus and lactose ( $r = 0.56$ ,  $P < 0.05$ ), but in the cases of phosphorus and calcium and of protein and zinc, correlation coefficients were similar to those of processed samples ( $r = 0.39$  and  $0.59$ , respectively) but were not significant, possibly due to the smaller number of samples.

## DISCUSSION

The nutrient composition of banked human milk depends on two main factors: the composition of the milk of the donors and the effect of processing. The individual nutrient contents of donated milk are subject to longitudinal, circadian, and interindividual variations. Variations in diet and sampling procedures also can affect milk nutrient composition. Pasteurization and cycles of freezing and thawing can affect the contents of several milk components.<sup>7</sup> In addition, modification of physical and chemical characteristics of human milk during processing may modify the binding patterns and redistribution of minerals in milk fractions,<sup>23</sup> which might affect their bioavailability. Such might be the case with zinc. To our knowledge, the effect of pasteurization on the distribution of zinc in human milk fractions has not been reported. Moreover, data on the nutritional quality of processed milk from human milk banks from developing countries, especially those from South America, are scarce.

In the present study, the contents of fat, protein, lactose, vitamin A, zinc, calcium, phosphorus, iron, and copper of banked mature (1 to 6 mo) human milk from a Brazilian human milk bank (IFF-HMB) were determined. Also, the combined effects of pasteurization and freezing on the contents of these nutrients and on the distribution of zinc in milk fractions were studied.

The mean nutrient contents of the milk samples provided by the IFF-HMB, except for fat, were within the range of those reported for freshly expressed samples or samples frozen only once from well-nourished mothers during the same lactation period in developed countries<sup>24–28</sup> and Brazil.<sup>14,29</sup>

The low mean fat content observed in milk samples from IFF-HMB has also been reported in milk samples from other milk banks,<sup>1</sup> which may be of concern for the recipient infant, especially the premature or low-birth-weight infant who must gain weight quickly. Fat contributes up to 55% of the non-protein-energy content of human milk and contains the very long-chain  $\omega$ -3 fatty acids 20:5 $\omega$ -3 and 22:6 $\omega$ -3 essential for the neural development of newborn infants, which may not be synthesized in appropriate amounts from  $\alpha$ -linolenic acid (18:3 $\omega$ -3) in term and preterm infants.<sup>30</sup>

The low fat values reported in the present study may result from the milk collection procedure and from losses during milk processing (freezing and pasteurization followed by refreezing). Most samples in the present study consisted of foremilk (low fat content) or mixed (average fat content) milk, and a few consisted of dripped (low fat content) milk. Hand expression of breast milk also may have contributed to the low fat content because hand expression compared with suction usually produces less fat.<sup>31</sup> Further, the range of the lactation period of the donors was long (5 mo) and may have led to longitudinal influences on the fat content of the mature milk, which tends to decrease as lactation continues.<sup>28</sup> Processing of human milk can affect the physical structure of the milk fat globules and lead to a decrease in available fat content. Milk freezing and thawing cause disassembling and coalescence of the milk fat globules, resulting in loss of fat by its adherence to the storage flasks.<sup>23</sup> The pasteurization process per se could have led to adherence and losses of milk fat, but this does not seem to be the case because there was no difference of fat content between unprocessed and processed milk samples (Table II). For

human milk samples frozen only once, such as the unprocessed samples in our study, the crematocrit method was appropriate for determination of fat content<sup>32</sup> when compared with other methods. The similar fat contents in unprocessed and processed samples (Table II) indicated that the crematocrit method for measuring fat in processed samples (Tables I and II) does not underestimate fat content.

In the case of vitamin A, 34% of milk samples had concentrations lower than 1.1  $\mu\text{mol/L}$ , which is considered less than adequate in breast milk.<sup>33</sup> These low values may be due to poor maternal vitamin A status or may be a consequence of the loss of vitamin A due to possible light exposure of milk during collection and storage and possible loss of milk fat by adherence to the storage flasks. However, the mean vitamin A content in the samples from IFF-HMB (1.51  $\mu\text{mol/L}$ ; Table I) was above 1.1  $\mu\text{mol/L}$  and can be considered acceptable for the use of the banked milk by term infants from well-nourished mothers. However, for infants with low vitamin A reserves at birth, such as premature infants or infants from mothers deficient in vitamin A, milk vitamin A concentrations greater than 1.75  $\mu\text{mol/L}$  may be necessary to guarantee higher vitamin A reserves and protection from deficiency after weaning.<sup>33</sup> Therefore, human milk from IFF-HMB should be used with caution for these special infants.

High intersample variabilities were observed in the concentrations of all nutrients studied in milk samples from IFF-HMB, particularly for fat, iron, zinc, and vitamin A. This high variability can result from individual characteristics of the donors, stage of lactation, milk sampling (foremilk or hindmilk, pump-expressed, hand-expressed, or drip milk), and variable losses during milk storage and processing. Pooling of the milk samples reduced variability in milk nutrient concentrations, especially for zinc and fat, whose CVs were reduced to about one-third and one-half, respectively, compared with those of individual samples.

The process of pasteurization followed by refreezing and thawing as used in IFF-HMB for human milk samples did not affect the concentrations of the nutrients studied, not even that of vitamin A, which is a very labile compound (Table II). Human milk seems to be a very efficient matrix for the protection of vitamin A during pasteurization combined with freezing and thawing cycles, as tested in the present study. Interaction with hydrophobic amino acid residues in casein may contribute to vitamin A stabilization.<sup>34</sup> Also, this processing did not affect the variability in nutrient contents because the CVs were similar for unprocessed and processed samples.

Correlations between nutrient concentrations in processed and unprocessed milk samples from IFF-HMB were investigated. Because the nutrient contents did not differ between processed and unprocessed samples and associations between nutrients were found in both sets of samples, these associations may reflect events in the mammary gland. Positive correlations were found between concentrations of lactose and calcium, lactose and phosphorus, calcium and phosphorus, and zinc and protein in processed and unprocessed samples, although for the last two correlations significance was not attained in unprocessed samples, possibly due to the smaller number of samples. Correlations between calcium, phosphorus, and lactose might reflect their high concentrations in the Golgi apparatus of the mammary gland epithelial cells and simultaneous secretion into milk. Phosphate is a secondary product of the lactose synthesis, which in turn may influence calcium concentration in the Golgi apparatus, where calcium and phosphorus are incorporated into the structure of casein micelles.<sup>23,25</sup> The positive correlation between zinc and protein likely reflects the fact that proteins are major zinc ligands in milk.<sup>15</sup>

The binding pattern and distribution in milk fractions are factors that contribute to the high bioavailability of minerals in human milk.<sup>9</sup> In the case of zinc, 40% to 70%, 10% to 30%, and 10% to 45% is present in whey, fat and casein fractions, respectively.<sup>8,14–16</sup> Major zinc ligands are serum albumin and citrate in the whey fraction; alkaline phosphatase, bound to the fat globule

membrane, in the fat fraction; and casein phosphoserine residues in the casein fraction.<sup>27</sup> The superior bioavailability of zinc from human milk compared with that from cow's milk and infant formulas was demonstrated by several studies and might be related to the differences in zinc associations with ligands and distribution in milk fractions.<sup>13</sup> Therefore, redistribution of zinc among the milk fractions and alterations in the binding pattern that could occur during processing of milk in human milk banks may reduce its bioavailability to the infant.

The combined process of pasteurization and refreezing of milk samples in IFF-HMB caused a shift in the relative zinc distribution from the whey fraction to the fat fraction and possibly to the casein fraction. The percentage of zinc in the whey fraction decreased about one-third on average, whereas that in the fat fraction increased about one-third, accompanied by a tendency to increase by about one-sixth in the casein fraction in the processed milk compared with unprocessed milk. This redistribution could result from different physicochemical causes. Pasteurization (heating of milk at 62.5°C, followed by a thermal shock) might have provided an appropriate environment for exchange of zinc between ligands in the milk fractions and likely caused denaturation of proteins. Freezing before pasteurization might have disrupted the milk fat globule membranes, leading to the exposure of new binding sites for zinc in the fat fraction, such as the carboxyl groups of free fatty acids.<sup>23</sup> Denaturation of whey proteins that are major ligands for zinc in milk<sup>9</sup> may allow zinc transfer to the casein fraction.

Pasteurization of human milk sufficiently decreased zinc bioavailability to markedly affect zinc balance in preterm infants.<sup>16</sup> This decreased zinc bioavailability might be explained, at least in part, by zinc redistribution in milk fractions after combined pasteurization and refreezing, as observed in our study. However, it remains to be ascertained whether shifts of the magnitude reported in the present study have any effect on zinc bioavailability for infants.

## SUMMARY

Concentrations of fat, protein, lactose, calcium, phosphorus, zinc, iron, copper, and vitamin A in mature milk samples from a human milk bank in Brazil were measured. Processing of samples did not affect the nutrient contents but did cause a significant shift in the relative distribution of zinc in milk fractions, with a decrease in the whey fraction and an increase in the fat fraction.

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