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Pasteurization is a thermal treatment applied to the milk used in human milk banks so as to provoke the thermic inactivation of pathogenic micro-organisms, with the aim of avoiding contamination of milk that will be offered to new-born infants in clinical conditions very often demanding special care. The literature has very little data available relating to the effect of pasteurization on the concentration of oligo-elements in human milk. The objective of this study was to evaluate the effect of pasteurization on the concentrations of iron (Fe), copper (Cu) and zinc (Zn) in the colostrum of mothers of pre-term (PT) and term (T) infants. Samples were collected from the day of birth to the seventh day after birth. The oligo-elements were analyzed using the total reflection X-ray fluorescence technique with synchrotron radiation. The following results of Fe, Cu and Zn (means $\pm$ SD) were obtained for the PT and T colostrum samples, non-pasteurized and pasteurized, respectively: PT: 1.96 ± 0.73 mg/l Fe/ 1.71 ± 0.70 mg/l Fe, 0.67 ± 0.28 mg/l Cu/0.64 ± 0.28 mg/l Cu, 5.55 ± 2.71 mg/l Zn/5.39 ± 2.73 mg/l Zn; T: 1.71 ± 1.01 mg/l Fe/1.46 ± 0.99 mg/l Fe, 0.54 ± 0.29 mg/l Cu/0.49 ± 0.19 mg/l Cu, 6.97 ± 2.82 mg/l Zn/6.75 ± 2.62 mg/l Zn. There was a significant reduction in the levels of Fe, Cu and Zn in the samples of pasteurized colostrum. These results suggest that, despite the observance of a diminution in the levels of Fe, Cu and Zn in the samples of pasteurized colostrum, the values fell within the acceptable range for the specific nutritional needs of new-born infants during this period of lactation.

Introduction

Human Milk Banks (HMBs) are specialized centers, responsible for the collection and processing (including pasteurization and freezing) as well as quality control of milk (colostrum, transition milk and mature milk) (Ministério da Saúde, 1999). The main objective of HMBs is to stimulate the practice of maternal breast-feeding. However, not all

new-borns can be breast-fed by their mother, whether due to deficiency arising from the gestational age at birth causing difficulties in suckling, or due to pathologies that impede breast-feeding (Abdallah *et al.*, 1992). These new-borns need to receive the human milk supplied by HMBs. This milk is obtained from healthy nursing mothers who present a lacteal secretion above the amount required by their child and who consent to donate the excess milk through their own free will: this is later distributed, under medical prescription from doctors or nutritionists, for the alimentation of new-borns who require this feed (Ministério da Saúde, 1999).

The conservation treatment (pasteurization) of colostrum and mature milk — nowadays compulsorily employed in HMBs — aims to produce the thermic inactivation of pathogenic micro-organisms and of part of the saprophytic flora. A recent study demonstrated that the standard processing adopted by the majority of HMBs does not alter the net contents of macronutrients — fat, protein and lactose — in the human milk pool (Taveira & Taveira, 1972; Ministério da Saúde, 1993). However, the same author observed alteration in the distribution of zinc in the fractions of the human milk pool, leading to a decrease in this oligo-element in the fraction associated with the milk serum, which could result in a diminution in its availability to the new-born. No studies exist on the effects of pasteurization on the levels of oligo-elements in individual colostrum samples.

Allowing for the fact that oligo-elements fulfil important functions in enzyme production, growth regulation, development, activity and the functioning of the immune and reproductive systems (Flynn, 1992; Zlotkin *et al.*, 1995), it is important to know the concentration of oligo-elements in colostrum before and after pasteurization, in order to evaluate the quality of this lacteal secretion after thermic treatment (pasteurization), since it is the colostrum processed in HMBs which comprises the new-born's main aliment in the first days of life when for clinical reasons the child finds itself unable to obtain this aliment directly from the maternal breast. The objective of this study was to evaluate the effect of pasteurization on the concentration of iron (Fe), copper (Cu) and zinc (Zn) in colostrum derived from adult mothers of term (T) and pre-term (PT) new-born infants.

Methodology

Study design, population and sampling

The study was descriptive and transversal (Hennekens & Buring, 1987). The population studied was of adult mothers, interned in the maternity wards of the Fernandes Figueira Institute/Oswaldo Cruz Foundation — IFF/FIOCRUZ, Rio de Janeiro, Brazil, Public Health Unit of the municipality of Rio de Janeiro, part of the Ministry of Health.

The sample selection criteria were maternal age equal to or older than 21 years, with a sufficient inter-parturition period (a minimum of 2 years) and with no use of supplements.

Infants born between 32 and 36 weeks, 6 days of conception, were considered PT, while those born between 37 and 41 weeks, 6 days of conception, were considered T infants (World Health Organization, 1995). Evaluation of the gestational age at birth was based on the date of last menstruation and/or ultra-sonographic evaluation (Ministério da Saúde, 2000).

Data collection

After the study's approval by the institution's Ethics Commission, collection of data and colostrum samples was initiated, following the consent of the mothers who met the sample selection criteria. Data collection was made by means of personal interviews with the mothers and consultation of medical records.

Eight-eight colostrum samples from mothers were collected, between the 1st and 7th day after birth, corresponding to 50 samples from mothers of T new-borns and 38 samples from mothers of PT new-borns.

Collection and pasteurization of the colostrum samples

First, the breasts were cleaned with de-ionized water and the skin of the areolæ

and nipples lubricated with the colostrum itself. The colostrum samples were collected by manual expression from both breasts between the 1st and 7th day of lactation, at a time between 10 a.m. and 2 p.m. (Neville *et al.*, 1984). Approximately 1.5 ml colostrum was collected directly into sterile polypropylene vials, previously treated (washed with distilled water, de-ionized water, a 5% solution of nitric acid and then washed again with de-ionized water) so as to avoid possible contamination. After the collection of colostrum from T and PT mothers, the vials were labeled and frozen ($-20°C$) for later analysis of their Fe, Cu and Zn contents.

Preparation of samples for quantitative analyses of oligo-elements

The pasteurized and unpasteurized colostrum samples were heated to $36°C$ for homogenization. Then 0.5 ml aliquots of colostrum, in duplicate, were transferred to polyethylene vials, free of oligo-elements, and treated with 0.5 ml HNO_3 and 0.5 ml H_2O_2 at $60°C$ for 5 days with the vials sealed. The solution was left to dry and the volume recuperated to 0.5 ml with a solution of 0.1 M HNO_3 as the diluting agent. A 50 μl solution of gallium (Ga) was added to the sample, which was used as an internal reference standard for the total reflection X-ray fluorescence measurements.

A 5 μl aliquot of the solution was placed, using a pipette, onto a Perspex support (used as a sample reflector) and then submitted to drying in a vacuum drier for 3 h, obtaining spots approximately 5 mm in diameter.

Quantitative analysis by total reflection X-ray fluorescence

The total reflection X-ray fluorescence analysis was conducted at the Fluorescence Line of the National Laboratory of Synchrotron Light, Campinas, São Paulo State. A white beam of irradiation was utilized with a maximum energy of 20 keV filtered by 0.5 mm of aluminum, with an angle of incidence of 1.0 mrad to excite the sample. The characteristic X-rays were detected by a silicon–lithium detector with a resolution of 165 eV for 5.9 keV. The distance between the detector and the sample was fixed at 6.0 mm, and a tantalum collimator with an aperture of 1.0 mm was used to limit the dead time of the measurements to a maximum value of 15%.

The spectrometer's sensitivity was determined using five multi-elemental standards with different concentrations containing aluminum, silicon, calcium, titanium, chromium, Fe, nickel, Zn, Ga and selenium. The standards were prepared from high-purity mono-elemental solutions (Induced Coupled Plasma standard; Sigma). Using the sensitivity curve, accuracy of the measurements was calculated by determining the concentration of the elements in a standard solution (ICP-Multi-element standard solution; MERCK), obtaining values that vary from 3.7 to 6.1% for the elements considered.

The measurement time was 300 sec for the samples and 150 sec for the standards. The spectrums were analyzed by an quantitative analysis program (Quantitative X-ray Analysis System) distributed by the International Atomic Energy Agency, from where the fluorescent count intensities for each element and the associated uncertainty were obtained. The concentrations of Fe, Cu and Zn were expressed in milligrams per liter.

Statistical analysis

Results are reported as means and standard deviation. The paired Student t test was employed to compare the oligo-element contents in the colostrum before and after pasteurization within each group (PT and T).

To compare the oligo-element contents between the T and PT groups, the Student t test was used for the colostrum before pasteurization and the pasteurized colostrum. The levels of significance were set at 1% and 5%.

Results

The results described in Tables 1 and 2 show a diminution in the Fe, Cu and Zn contents in the colostrum of PT mothers after the pasteurization process and the same alterations observed in the colostrum of T mothers. The levels of Fe were the most affected, with

Table 1. Comparison of the concentration of oligo-elements before and after pasteurization in the colostrum of pre-term mothers*

Component	Amplitude	Means $\pm$ SD	N	Observed reduction (%)
Iron (mg/l)				
Before	0.54–3.29	1.96 ± 0.73	22	12.75%**
After	0.18–3.00	1.71 ± 0.70	22	
Copper (mg/l)				
Before	0.20–1.51	0.67 ± 0.28	38	4.48%***
After	0.20–1.46	0.64 ± 0.28	38	
Zinc (mg/l)				
Before	1.54–13.25	5.55 ± 2.71	38	2.88%***
After	1.43–12.79	5.39 ± 2.73	38	

*n = Number of samples taken. Significant effect as shown by paired Student t test. **$P < 0.01$, ***$P < 0.05$.

a reduction of 12.75% registered against 4.48% in the concentration of Cu, and just 2.88% in the concentration of Zn in the colostrum samples from PT mothers; and a reduction in the concentrations of Fe, Cu and Zn of 14.62, 9.26 and 3.16%, respectively, in the colostrum samples of T mothers.

Table 3 presents the average concentration of the Fe, Cu and Zn oligo-elements in the colostrum of T mothers, before and after pasteurization. Despite our observation of higher average values of Fe in the colostrum of T mothers, no significant statistical differences were found in relation to the colostrum of PT mothers. Comparing the Cu and Zn concentrations between the two groups (the colostrum of PT mothers and the colostrum of T mothers) before and after pasteurization, significant differences were found before and after pasteurization for both elements. The mean concentration of Cu was significantly higher in the unpasteurized and pasteurized colostrum of PT mothers in

relation to the T group, while the concentration of zinc was significantly lower in the colostrum of PT mothers.

Discussion

It is well known that thermic treatment (pasteurization) of milk leads to the destruction of some vitamins, in addition to deactivating lipase, altering the link between some vitamins and proteins, and causing a reduction of approximately 67% in the lactoferric content (Jensen, 1995; Lepri *et al.*, 1997). Taveira & Taveira (1972) relate that the processing of cow's milk causes a diminution in the calcium salt content. However, there exist few studies on the effect of pasteurization on the concentration of minerals and oligo-elements in human milk. It is known that there is a reduction in the concentration of selenium (Alaejos & Romero, 1995) and an alteration in the distribution of Zn in the fractions of human milk,

Table 2. Comparison of the concentration of oligo-elements before and after pasteurization in the colostrum of term mothers*

Component	Amplitude	Means $\pm$ SD	N	Observed reduction (%)
Iron (mg/l)				
Before	0.24–3.76	1.71 ± 1.01	37	14.62%**
After	0.01–3.36	1.46 ± 0.99	37	
Copper (mg/l)				
Before	0.20–1.63	0.54 ± 0.29	50	9.26%***
After	0.16–0.97	0.49 ± 0.19	50	
Zinc (mg/l)				
Before	2.41–14.49	6.97 ± 2.82	48	3.16%***
After	1.95–13.20	6.75 ± 2.62	48	

*n = Number of samples taken. Significant effect as shown by paired Student t test. **$P < 0.01$, ***$P < 0.05$.

Table 3. Comparison between the average concentrations of iron, copper and zinc in the colostrum of pre-term (PT) mothers and the colostrum of term (T) mothers before and after pasteurization*

Oligo-element	Before pasteurization			After pasteurization		
	PT colostrum	T colostrum	t Test	PT colostrum	T colostrum	t Test
Iron (mg/l)	1.96 ± 0.73	1.71 ± 1.01	0.9546	1.71 ± 0.70	1.46 ± 0.99	1.0116
(n)	(22)	(37)	$P > 0.05$	(22)	(37)	$P > 0.05$
Copper (mg/l)						
(n)	0.67 ± 0.28	0.54 ± 0.29	2.0340	0.64 ± 0.28	0.49 ± 0.19	3.000
	(38)	(50)	$(0.01 < P < 0.02)$*	(38)	(50)	$P < 0.005$*
Zinc (mg/l)	5.55 ± 2.71	6.97 ± 2.82	2.2671	5.39 ± 2.73	6.75 ± 2.62	2.2527
(n)	(38)	(48)	$(0.01 < P < 0.02)$*	(38)	(48)	$(0.01 < P < 0.02)$*

*n = Number of samples taken. Significant effect as shown by Student t test. Data presented as means ± SD.

leading to a decrease in the fraction of this oligo-element associated with the serum (Góes, 1999).

In the present study, we observe that pasteurization adopted in the Human Milk Bank of the Fernandes Figueira Institute — namely, the so-called Holder pasteurization that comprises heating to 62.5°C for 30 min — affected the mean composition of the analyzed elements, causing a reduction in the concentrations of Fe, Cu and Zn in the colostrum of T mothers of approximately 15, 9 and 3%, respectively. In the colostrum of PT mothers, this reduction was found to be approximately 13, 4 and 3%, respectively. These results, as well as those in the literature, demonstrate that pasteurization can cause alterations in the nutrient contents of human milk. This is especially important when the target population comprises premature or low-weight new-borns, who need an adequate input of oligo-elements for their growth and development. However, although we found a reduction in the concentration of these elements in the colostrum in the present study, the average concentrations of these elements after pasteurization fell within the range of values observed in the literature: Fe, 0.45 mg/l; Cu, 0.46 mg/l; and Zn, 5.4 mg/l (Worthington-Roberts, 1997), adequate for the specific nutritional needs of new-born infants during this period of lactation.

There are reports in the literature that the bio-availability of oligo-elements in human milk is related mainly to the nature of the complexes in which these are found to be linked. In fact, the high bio-availability of Fe in human milk is probably influenced by its distribution in fractions of the latter (Casey *et al.*, 1995). Thus, investigations of the characteristics of the different composites that link oligo-elements in human milk can assist in elucidating the mechanisms of absorbing these elements. Such knowledge is essential to the optimization of the bio-availability of oligo-elements in the colostrum. Consequently, future studies are needed on the distribution of Fe, Cu and Zn in fractions of fat, casein and serum in the colostrum before and after pasteurization, in the studied population, with the aim of verifying whether these elements may also suffer alteration with pasteurization in the different fractions of the colostrum. In addition to this, it is important to investigate whether the reduction of oligo-elements in the total sample of pasteurized colostrum shown in the present study also occurs in the fractions of colostrum, or whether it simply occasions a possible re-distribution of these oligo-elements in the fractions.

Another aspect that deserves deeper investigation is the effect of freezing/defrosting during the processing of colostrum in the milk bank routine, which could lead to important physio-chemical modifications that affect the distribution of these elements in human milk.

The type of pasteurization employed in the HMBs is Holder pasteurization. In the case of cow's milk, the type of pasteurization more commonly employed is high-temperature short-time (HTST), which involves heating to 70°C for 15 sec. This treatment results in minimal losses of immunological factors (Jensen, 1995) and vitamins (Goldblum *et*

al., 1984) from the milk, although it is not used in HMBs due to the absence of equipment for pasteurization of small sample quantities in HTST (Jensen, 1995).

Loaharanu (1997) indicates the use of irradiation as another method of eliminating pathogenic micro-organisms in aliments of animal origin, although other studies would be necessary to verify whether this method alters the milk's chemical composition.

Cases of Zn deficiency were reported in neonates born at T and PT (Aggett, 1994), mainly in PT. It is known that the accumulation of Zn in the colostrum of mothers of PT new-borns were lower when compared with mothers whose infants were born were at T, taking into consideration the same lactation period: this could suggest a risk of Zn deficiency for PT. Similar findings were observed in the study by Trugo *et al.* (1988) in analyzing the concentration of oligo-elements in the colostrum of Brazilian mothers of PT and T new-borns, which described lower Zn values in the colostrum of mothers of PT new-borns, although without statistical significance. In relation to Cu and Fe, the present study observed higher levels of these elements in the colostrum of PT mothers, in both the situations before and after pasteurization, in comparison with the colostrum of T mothers — a significant increase in the case of Cu. Atinmo & Omololu (1982) found significantly higher levels of Cu and Fe in the milk of mothers of PT infants when compared with that of mothers of T infants, during the same period of lactation. Lemons *et al.* (1982), studying the composition of human milk from mothers who gave birth to PT and T during the first weeks of lactation, also observed that the concentration of Fe in the milk of PT mothers presented significantly higher values than that of T mothers (1.51 mg/l versus 0.84 mg/l, respectively). Gupta *et al.* (1984) found no significant differences in the levels of Zn and Cu in the milk of Indian mothers who had PT or T infants. On the contrary, Lawrence (1985) indicates that the milk of mothers of PT infants presents a different composition to that of mothers of T infants, containing higher concentrations of Fe, Mg, Na and Cl. These findings from the literature, as well as those reported in the present study, therefore demonstrate that some physiological adaptation may occur during this post-natal period, in such a way that an adequate supply of Cu and Fe is guaranteed for PT new-borns.

The present study's results allow us to conclude that there was a difference in the mean concentrations of these analyzed elements between the pasteurized samples in comparison with the unpasteurized samples, indicating that Holder pasteurization caused a reduction in the levels of these elements in the analyzed samples of colostrum. The physiological and biochemical importance of these findings to T and PT deserves future investigation, including the analysis of these elements in the different fractions of colostrum.

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