Journal of Trace Elements in Medicine and Biology 54 (2019) 199–205

Contents lists available at ScienceDirect

Journal of Trace Elements in Medicine and Biology

journal homepage: www.elsevier.com/locate/jtemb

Epidemiology

Trace mineral composition of human breast milk from Brazilian mothers

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ARTICLE INFO

Keywords:
Human breast milk
Mineral composition
ICP-MS
Pasteurization
PCA

ABSTRACT

Background: Human milk is a dynamic food and some important differences in composition can be found between the milk from preterm and terms infants. Additionally, in some situations, the mother's own milk is unavailable and the use of milk from human milk banks is considered as the most appropriate substitute. In this way, concentrations of trace elements (Ba, Cu, Fe, Mn, Mo, Se, Sr, and Zn) were determined in human milk, considering the differences about preterm and term human milk and its processing in a human milk bank.

Methods: A total of 156 samples were analyzed, which were divided in three groups: samples collected at the hospital at bedside (BS, 60 samples) from mothers of preterm infants and samples from mothers of term infants collected in a human milk bank without pasteurization (WP, 49 samples) and pasteurized by the Holder procedure (P, 47 samples). The analyzes were conducted by inductively coupled plasma mass spectrometry (ICP-MS) after the treatment of the samples with acid mineralization assisted by microwave radiation.

Results: Concentrations varied in a range of 0.6–88.2 μg/L for Ba, 78.6–954.5 μg/L for Cu, 24.2–5229.2 μg/L for Fe, 0.4–42.6 μg/L for Mn, 0.1–39.1 μg/L for Mo, 2.5–70.6 μg/L for Se, 8.9–187.5 μg/L for Sr and 76.3–17727.2 μg/L for Zn. Significant differences ($p < 0.05$) were found between preterm (BS) and term human milk (WP and P) for Ba, Cu, Mo, Se, and Zn, whereas the processing of the donated milk by Holder pasteurization did not influence the concentration of the studied trace elements. The milk of term infants does not attend the recommended daily intake (RDI) of Zn and for preterm infants the RDI of Fe and Mn is not achieved.

Conclusions: The higher concentrations of Cu, Mo, Se and Zn observed in milk from mothers of preterm infants indicate that the milk to be offered for these high-risk neonates in neonatal intensive care units should contain higher levels of these trace elements. Besides, considering the RDI, the milk of term infants should be fortified with Zn, whereas the milk of preterm infants should be fortified with Fe.

1. Introduction

Human breast milk is recommended to be the single source of nutrients and bioactive factors for newborns in their first six months of life, with continued breastfeeding up to two years or beyond along with the ingestion of complementary foods [1]. The ingestion of maternal milk is essential for achieving optimal health, growth, and development; it also contributes to reduce the incidence of infection diseases and the infant post-neonatal mortality [2]. In addition, children and adolescents who were breastfed usually present better performance in intelligence tests and, as adults, showed to be less prone to be overweight or to present obesity [3].

Sometimes, unfortunately, some mothers are not able to feed their own babies. In these cases, the milk provided by human milk banks and the use of artificial infant formulations are the most used alternatives. Donor human breast milk is the preferred substitute, regarding that artificial formulations may present some important differences in composition when compared to raw maternal milk, mainly related to the different chemical forms of the elements [4], which directly affects their oral bioavailability. Additionally, in some cases, the mother's own milk of premature newborns might need fortification with essential nutrients, such as Fe, considering the especially high nutritional demands of these infants. For this reason, human milk fortification is recommended for those infants who were born before 32 weeks of gestation [5].

The composition of human breast milk includes water, proteins,

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https://doi.org/10.1016/j.jtemb.2019.05.002
Received 29 March 2019; Received in revised form 28 April 2019; Accepted 2 May 2019
0946-672X/

fats, carbohydrates, vitamins, essential elements, and live cells [6]. Information about concentrations of major elements in human breast milk have already been investigated in several works [7–10]. In Brazil, the mineral composition of breast milk was studied by Morgano et al. [7] with the determination of Ca, P, Na, K, Zn, Fe, and Mn and by Codo et al. [10] with the determination of Ca, P, Mg, Na, and K, both studies applied inductively coupled plasma optical emission spectrometry (ICP OES) in the analyzes. The accurate knowledge about the mineral composition of human breast milk is particularly important for high-risk infants, such as preterm, underweight, and infants born with hematologic disorders, considering how challenging is their feeding [2,11]. Particularly for preterm infants, a recent report alarmed that small infants presented slow growth when fed with donor human milk [12], endorsing that nutrient fortification may be necessary in these cases. Moreover, regarding micronutrient information, some significant differences in composition were already pointed out between preterm and term milk for some elements such as Na, Zn, Ca, and Fe [5,13].

Besides the differences found between the milk of preterm and term infants, the composition of human breast milk can also vary depending on the mode of storage and pasteurization processes [14–16]. In human milk banks, for instance, the standard treatment for the donated human milk is its freezing followed by Holder pasteurization (raise of the milk temperature to 62.5 °C and hold for 30 min), which is applied mainly for reasons of microbiologic safety [17]. Information about the effect of Holder pasteurization on the contents of organic compounds are available in the literature for carbohydrates, proteins, and lipids [18–21], but studies concerning trace metal composition were conducted in a very few studies [22,23] and there is no consensus if the processing could alter the concentration of trace elements.

Besides, in a general way, most studies considering the differences about preterm and term human breast milk composition and processing effects are focused in major elements. The quantification of other essential trace elements, such as Mn, Mo, and Se often requires the use of analytical techniques with better detectability and information about these elements are still scarce in the literature. Thus, this work has the aim to provide information about trace elemental composition of human breast milk and evaluate possible differences between the milk from mothers of preterm (premature) and term infants and between samples from a human milk bank before and after Holder pasteurization to investigate the possible effects of the application of this type of treatment in the concentration of trace elements and its nutritional consequences.

2. Experimental

2.1. Instrumentation

The analytical measurements for elemental determination were accomplished in an inductively coupled plasma mass spectrometer (ICP-MS), model 7700x (Agilent Technologies, Hachioji, Japan), equipped with a MicroMist nebulizer coupled to a Scott (double pass) spray chamber cooled at 2 °C, nickel sampler (1 mm) and skimmer (0.4 mm) cones and a third generation octapole collision/reaction system (ORS^3) using helium as collision gas in three different modes: no gas (Ba); He (Fe, Mo, and Sr) and HEHe (Cu, Mn, Se, and Zn). The ions lens setting was daily optimized using a solution of 10 μg/L of Li, Co, Y, and Tl. The instrumental conditions used in the equipment are displayed in Table 1.

A water bath (Dubnoff, Quimis, Diadema, Brazil) was used to defrost the samples and a microwave digestion system (ETHOS 1, Milestone, Sorisole, Italy), equipped with polytetrafluoroethylene vessels and temperature and pressure sensors for the reference vessel, was used for sample treatment.

Table 1
Instrumental conditions and data acquisition parameters used in the ICP-MS for the analysis of human breast milk samples for the determination of Ba, Cu, Fe, Mn, Mo, Se, Sr, and Zn.

Parameter	Value
Radio frequency power (W)	1550
Argon flow rate (L/min)	15
Auxiliary argon flow rate (L/min)	1.09
Sample depth (mm)	10.0
Nebulizer pump (rps)	0.10
Resolution (u)	0.5
Helium flow rate (mL/min)	No gas: 0.0, He: 4.3, HEHe: 10.0
Monitored isotopes	^{45}Sc,^{54}Fe, ^{55}Mn, ^{63}Cu, ^{66}Zn, ^{72}Ge, ^{80}Se, ^{88}Sr, ^{95}Mo, ^{115}In, ^{137}Ba, ^{205}Tl,

2.2. Reagents and materials

Calibration standards were prepared by using certified monoelemental solutions (1000 mg/L) of Ba, Cu, Fe, Mn, Mo, Se, Sr, and Zn after appropriate dilution, all purchased from Sigma Aldrich (TraceCERT®, Fluka Analytica, St. Louis, USA). Solutions of 100 mg/L of Sc, Ge, In, and Tl (Specsol®, São Paulo, Brazil) were used to prepare an internal standard solution. Nitric acid (65% w/w) and hydrogen peroxide (30% w/w) were acquired from Merck (Darmstadt, Germany) and ultrapure water (18.2 MΩ cm), obtained from Milli-Q Water Purification System (Millipore, Bedford, MA, USA), was used throughout.

The certified reference materials (CRM) whole milk powder (8534) and corn bran (8433), both from National Institute of Standards and Technology (NIST, Gaithersburg, USA), were used for accuracy evaluation.

All the laboratory glassware was soaked and kept overnight in a 10% v/v HNO_3 bath and thoroughly rinsed with ultrapure water previously to their utilization to avoid metal contamination. Human breast milk samples were collected in sterile plastic tubes.

2.3. Samples

This study counted with the voluntary participation of mothers and a total of 156 samples were collected and divided into three groups: BS (bedside), WP (without pasteurization), and P (pasteurized). The BS group included raw milk samples from mothers of preterm infants hospitalized in the neonatal unit of the *Irmandade da Santa Casa de Misericórdia de Limeira* (Limeira, Brazil). In this case, the samples were collected at bedside in the presence of a health professional. The WP and P groups were composed of human breast milk samples from mothers of term infants who donated milk to the human milk bank of this hospital. Samples were collected before pasteurization (WP) and after the application of Holder pasteurization (P) at the human milk bank. Mothers who donated milk to the human milk bank collected the samples in their own residences after receiving a guidance and passing through pre-established tests to enable the donation, according to the rules preconized by the Brazilian Network of Human Milk Banks. The hospital staff was responsible for passing in mother's residences to receive the donated milk and maintenance of milk temperature (frozen). In the human milk bank, the samples were thawed, smelled and carefully inspected and only samples considered as adequate were selected for this study. Some characteristics of the donor mothers are shown in Table 2.

All participating mothers signed the term of free informed consent for donating the samples. The study was approved by the Research Ethics Committee (CEP) from the State University of Campinas (Campinas, Brazil) and it is registered in the *Plataforma Brasil* with the CAAE number 35545314.4.0000.5404.

Table 2
Characteristics of the mothers who donated milk for this study. Results market with an asterisk (*) indicate significant differences ($p < 0.05$) according to the Mann-Whitney U test. BS = bedside, WP = without pasteurization, P = pasteurized samples and sd = standard deviation.

Sample group (n)	Age/years (mean ± sd)	p-value
BS (60)	24.9 ± 6.0	BS × WP = 0.5459
WP (49)	24.3 ± 5.1	WP × P = 0.7262
P (47)	24.0 ± 5.1	BS × P = 0.3931
	Gestational age/months (mean ± sd)	p-value
BS (60)	33.0 ± 3.3	BS × WP < 0.0001*
WP (49)	38.9 ± 0.8	WP × P = 0.7648
P (47)	39.0 ± 0.7	BS × P < 0.0001*
	Vaginal delivery/%	p-value
BS (60)	28.3	BS × WP = 0.7627
WP (49)	22.5	WP × P = 0.9244
P (47)	21.3	BS × P = 0.5338
	Infant's birth weight/g (mean ± sd)	p-value
BS (60)	1871 ± 562	BS × WP < 0.0001*
WP (49)	3318 ± 505	WP × P = 0.7510
P (47)	3364 ± 463	BS × P < 0.0001*
	First pregnancy/%	p-value
BS (60)	48.3	BS × WP = 0.3846
WP (49)	55.1	WP × P = 0.8041
P (47)	57.5	BS × P = 0.2566

2.4. Sample treatment

The human breast milk samples were kept frozen (-20 °C) until analysis, when the samples were thawed and homogenized in a water bath at 37 °C and under agitation. Aliquots of 0.5 g of the samples were transferred to polytetrafluoroethylene vessels and 1.5 m L of nitric acid (65% w/w), 1.5 mL of hydrogen peroxide (30% w/w) and 4.5 m L of ultrapure water were added. Afterward, they were submitted to the following microwave heating program performed over four steps: heat from room temperature to 120 °C in 8 min; hold at 120 °C for 10 min; heat from 120 °C to 190 °C in 18 min and hold at 190 °C in 15 min. After cooling to room temperature, samples were transferred to polyethylene flasks and the volume was made up to 14.0 mL with ultrapure water.

2.5. Quality control and statistical treatment

The quantification of the analytes was carried out with external calibration using aqueous elemental standards into ranges of 0.1–25.0 μg/L for Ba, Mo, Sr, and Mo and 0.1–1000 μg/L for Cu, Fe, and Zn. Internal standards (Sc for Cu, Fe, Mn, and Zn; Ge for Se; In for Sr, and Mo; Tl for Ba) were added *online* in the equipment to compensate possible instrumental drifts and matrix effects. The CRM whole milk powder (8534) was used to evaluate the accuracy of the method for the determination of Cu, Fe, Mn, Mo, Se, Sr, and Zn. Alternatively, the CRM corn bran (CRM 84 33) was used to evaluate the accuracy of Ba determination, as there is no CRM of milk for the determination of this element. Precision was expressed as standard deviations and relative standard deviations (RSD). Analytical blanks were prepared by using the same procedure used for sample preparation. Limits of detection (LOD) and quantification (LOQ) were calculated as 3 and 10 times, respectively, the standard deviations of the mean obtained for analytical blanks determinations. All the measurements were carried out at least in triplicate and the results are expressed as median, interval and interquartile range 25th–75th (Box plots).

The independent groups (BS, WP, and P) were compared in relation to their trace elemental content by using the Mann-Whitney U test (Statistica 7) with a statistical significance of $p < 0.05$. Additionally, a multivariate data exploration was done by principal component analysis (PCA) using the software The Unscrambler × 10.4 and the algorithm NIPALS. Previously to the PCA model, the data were organized in a matrix form and auto-scaled, *i.e.*, each elemental concentration were centered by subtracting the mean and dividing by its standard deviation. In this way, all quantified elements present the same weight in the model independent of their concentration ranges.

Table 3
Analytical features of the analytical method, including certified and determined concentrations for whole milk powder certified reference material (CRM 8534) (n = 3), RSD and limits of detection (LOD) and quantification (LOQ).

Element	Determined (mg/kg)	Certified (mg/kg)	RSD (%)	LOD (μg/L)	LOQ (μg/L)
Ba	2.4 ± 0.3[a]	2.40 ± 0.52[a]	12.0	0.17	0.56
Cu	0.44 ± 0.02	0.46 ± 0.08	4.5	1.20	4.00
Fe	2.5 ± 0.1	1.8 ± 1.1	4.0	5.39	19.95
Mn	0.198 ± 0.008	0.17 ± 0.05	4.0	0.16	0.52
Mo	0.24 ± 0.04	0.29 ± 0.13	16.7	0.05	0.18
Se	0.147 ± 0.005	0.131 ± 0.014	3.4	0.09	0.30
Sr	3.99 ± 0.04	4.35 ± 0.5	1.0	0.44	1.47
Zn	26.1 ± 0.8	28.0 ± 3.1	3.1	6.41	21.38

[a] Values for Corn Bran (CRM 8433) certified reference material.

3. Results and discussion

3.1. Analytical characteristics of the method

The evaluation of the accuracy of the method together with RSD, LOD and LOQ values obtained for each element are displayed in Table 3. The results obtained for the CRM whole milk powder for the determination of Cu, Fe, Mn, Mo, Se, Sr, and Zn are in agreement with the certified values. For Ba determination, there is no milk certified reference material commercially available; thus, alternatively, corn bran certified material was analyzed. In this case, the corn bran sample was treated by the same procedure used for the treatment of milk samples and the results obtained are also in agreement with the certified value. The LOD and LOQ obtained using ICP-MS were suitable for the quantification of the studied elements in all samples analyzed. The analytical curves correlation coefficients were at least of 0.999 and RSD values of less than 5% were obtained in general, except for Ba and Mo.

3.2. Trace elemental content of human breast milk

The trace elements Ba, Cu, Fe, Mn, Mo, Se, Sr, and Zn were determined in the samples of human breast milk, being Cu, Fe, Mn, Mo, Se, and Zn essential elements for humans, whereas for Ba and Sr there is no current evidence of their essentiality.

Copper is an essential component for the structural and catalytic properties of several enzymes, such as ceruloplasmin, cytochrome-c oxidase, catechol oxidase, etc, which present several functions such as electron transport, oxidation, and synthesis of melanin, for example [24]. Iron plays a key role in several biological phenomena of the human body, being an important part of an array of enzymes, systems of energy transduction, and oxygen carriers [25]; its deficiency in the first stages of human life is particularly important and can impair brain development and cause anemia [26]. Manganese is important for development, metabolism and antioxidant system, acting mainly as an activator and components of enzymes [25,27]. In mammalian, four molybdenum-dependent enzymes are currently known, mainly related to oxygen transfer reactions. Selenium is an essential element in trace levels, being part of the structure of twenty-five selenoproteins, which are mostly involved in redox systems and signaling pathways. Zinc is part of more than three thousand proteins found in the human body, playing also regulatory functions in mammalian cells [25].

Strontium is considered a non-essential element. However, its presence in humans showed to be correlated with dental cavities prevalence and bone compression strength. Regarding its toxicity, toxic symptoms have not been reported in humans [28]. Barium has also no recognized essential function in the human body and the ingestion of

Table 4
Determined concentrations of Ba, Cu, Fe, Mn, Mo, Se, Sr and Zn (μg/L) in samples of human breast milk and statistical treatment (Mann-Whitney). Results market with an asterisk (*) indicate significant differences ($p < 0.05$). BS = bedside, WP = without pasteurization and P = pasteurized samples.

Sample group (n)	Median	Interval	p-value
Ba			
BS (60)	11.6	0.6–88.2	BS × WP = 0.0012*
WP (49)	8.1	3.7–20.3	WP × P = 0.7168
P (47)	7.2	2.7–22.7	BS × P = 0.0066*
Cu			
BS (60)	618.3	95.4–954.5	BS × WP < 0.0001*
WP (49)	288.8	81.6–829.0	WP × P = 0.5357
P (47)	275.4	78.6–813.1	BS × P < 0.0001*
Fe			
BS (60)	380.0	110.5–3594.0	BS × WP = 0.1488
WP (49)	313.0	24.2–2157.1	WP × P = 0.8864
P (47)	320.2	98.3–5229.2	BS × P = 0.0502
Mn			
BS (60)	4.5	1.6–22.2	BS × WP = 0.8934
WP (49)	5.5	0.4–19.1	WP × P = 0.8174
P (47)	4.9 0.4–42.6		BS × P = 0.7064
Mo			
BS (60)	9.3 0.1-39.1		BS × WP < 0.0001*
WP (49)	1.8	0.1–24.3	WP × P = 0.8460
P (47)	1.7	0.1–15.1	BS × P < 0.0001*
Se			
BS (60)	12.6	3.0–70.6	BS × WP < 0.0001*
WP (49)	8.4	2.5–38.1	WP × P = 0. 7058
P (47)	7.4	2.7–15.2	BS × P = < 0.0001*
Sr			
BS (60)	43.5	14.9–71.1	BS × WP = 0.3149
WP (49)	45.7	11.3–187.5	WP × P = 0.2365
P (47)	40.9	8.9–80.0	BS × P = 0.6879
Zn			
BS (60)	2614.4	422.8–17727.2	BS × WP = 0.007*
WP (49)	1434.3	76.3–8632.0	WP × P = 0.8921
P (47)	1415.2	125.3–6601–6	BS × P = 0.0054*

food is one of the main routes of exposure to this element. The ingestion of high levels of soluble barium compounds can cause various symptoms such as gastroenteritis, hypertension, cardiac arrhythmias, among others [29].

The concentrations of Ba, Cu, Fe, Mn, Mo, Se, Sr, and Zn determined in the samples of human breast milk are presented in Table 4 and Fig. 1 (box plots) in relation to the type of sample (BS, WP, and P). As can be seen, variations in the concentrations of all studied elements were observed even in the same group, which was already expected considering the dynamic nature of breast milk. Regarding the trace elements determined, the most prominent variations were observed for Cu and Mo (Fig. 1).

In this case, it is also important to point out the limitations of this study and how difficult is to estimate accurate mean values of trace elements in a specific group of mothers due to their different characteristics. Factors such as diet, race, smoking status, stage of lactation (colostrum, transition and mature milk) and the collection period of the day may influence the chemical composition of the milk [30]. In this way, future studies still should be conducted correlating other characteristics of the donor mothers and their influence on the contents of trace elements, considering that in this study we only considered the gestational age (mothers of preterm and term infants) and the processing of the milk in a human milk bank.

Comparing the different groups, the statistical analysis indicated significant differences ($p < 0.05$) between milk samples of mothers of preterm (BS) and term infants (WP and P) in relation to their contents of Ba, Cu, Mo, Se, and Zn. For these five elements, higher concentrations were found in the milk collected from mothers of premature infants (BS). Differences between the contents of essential trace elements in preterm and term human breast milk were already pointed out in other papers [5,13], in which higher levels of elements such as Na, Zn, Ca, and Fe were found in preterm milk. However, for other trace elements, information about differences in preterm and term milk are still scarce.

The processing of human breast milk in a human milk bank with the application of Holder pasteurization did not affect the total contents of Ba, Cu, Fe, Mn, Mo, Se, Sr, and Zn, considering that no significant differences were found in the concentrations determined in the groups WP and P. The application of Holder pasteurization is nowadays a practice commonly adopted in most human banks around the world [17,31–35] aiming to avoid disease transmission through consumption of donor human breast milk. However, pasteurization is a thermal treatment that can affect some nutritional properties of the milk, considering that physiochemical changes may occur in some milk compounds as a result of the elevation of the temperature. This is more critical in the case of human milk, which should ideally be the single source of nutrients for infants. Despite that, few studies concerning trace elements can be found [22,23].

The effects of the application of Holder pasteurization on Fe, Cu and Zn concentrations were investigated by Costa et al. [22] in colostrum milk samples, where a reduction on the levels of these three elements were noticed after the application of the thermal treatment, which is not in agreement with the results obtained in this work. On the contrary, Mohd-Taufek et al. [23] did not observe significant changes in the contents of Zn, Cu, Fe, Se, Mn, I, Mo, and Br in human breast milk after Holder pasteurization, as observed in this work.

In this case, it is important to point out that although total concentration of trace elements did not change after the application of Holder pasteurization, this does not mean that the nutritional properties of donor human milk breast remained unchanged regarding its elemental composition, considering that the oral bioavailability of metallic elements are deeply related to their chemical species. In the case of human breast milk, particularly, some essential elements are mainly found as associated to the protein fraction of milk [4,36] and proteins are prone to denaturation with the rise of the temperature. Thus, future studies still need to be done considering the effect of Holder pasteurization on the chemical species of essential elements.

3.3. Data exploration by principal component analysis (PCA)

PCA is a chemometric method widely used to simplify complex data which allows their visualization in a bi or tridimensional plot, facilitating exploratory analysis and patterns recognition [37,38]. This method reduces the information correlated with different elements by concentrating them into a same PC (principal component). The first PC contains the greatest variance and the variance explained decreases with the increase of the number of PCs. The original sample information and variables are then projected into the space of the PCs.

Therefore, a PCA model was built with the auto-scaled concentrations of all studied elements. The samples distribution on PC-1 and PC-2 (scores) are shown in Fig. 2**a**), whereas in Fig. 2**b**) is presented the projection of each trace element concentration about PC-1 and PC-2 (loadings). These two PCs explains about 67% of the total variance. According to the scores graphic, there is a tendency to form two groups between milk samples from mothers of preterm infants (BS) and term infants (WP and P), which were separated along to PC-1. There was no separation between the samples of the groups WP and P, considering that the application of Holder pasteurization did not affect the concentration of the studied elements.

The separation into two groups is mainly associated with the elements that have the greatest contribution to PC-1. According to the Fig. 2**b**), Cu and Mo present a larger contribution to PC-1, which explains 49% of the variance of the dataset. PC-2 presents a lower influence on the separation of the samples. Although the information contained in this last PC is responsible for 18% of the observed variance, there is a no significant discriminant character on it.

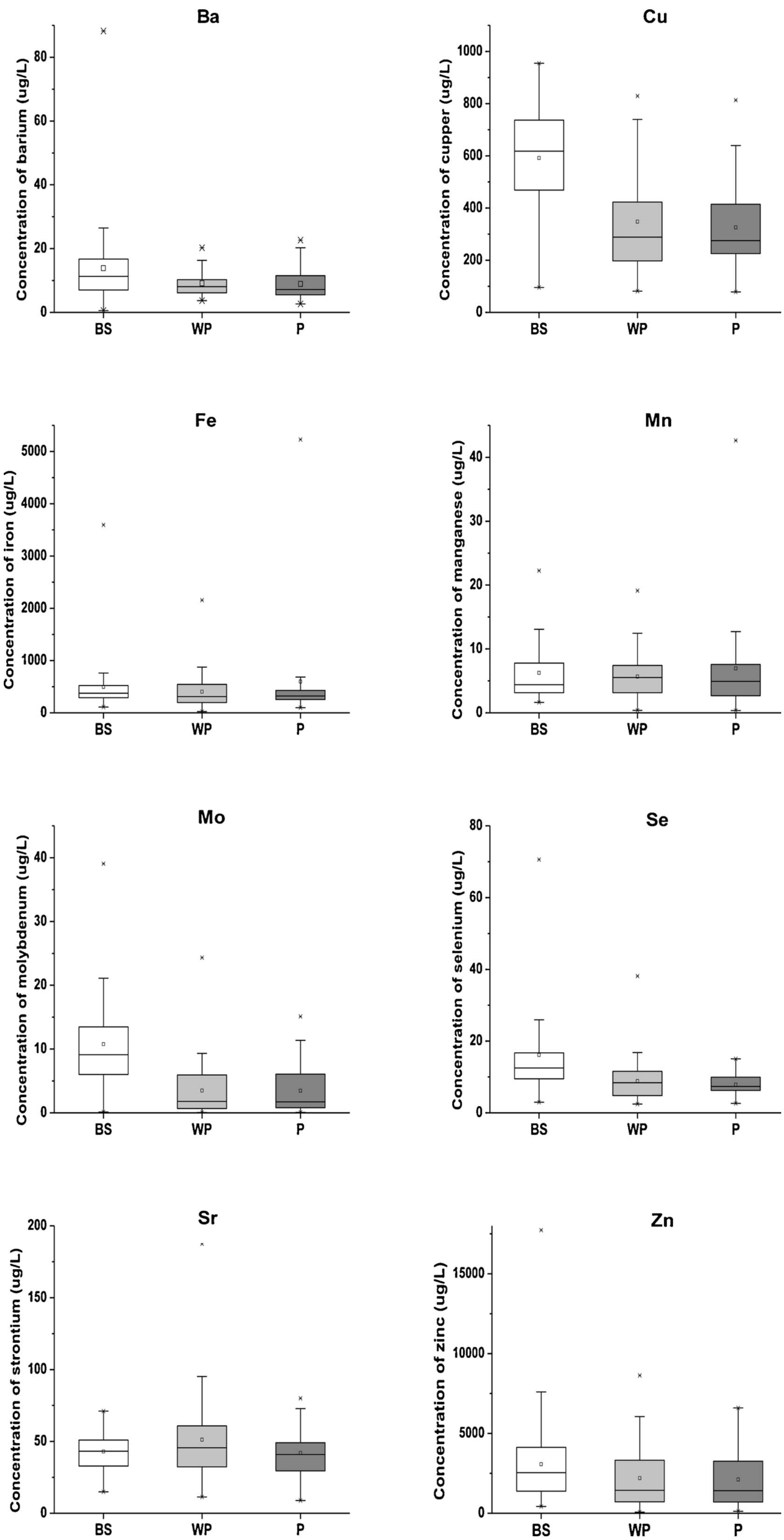

Fig. 1. Determined concentrations of Ba, Cu, Fe, Mn, Mo, Se, Sr and Zn. in samples of human breast milk in relation to the type of sample (BS = bedside; WP = without pasteurization and P = pasteurized).

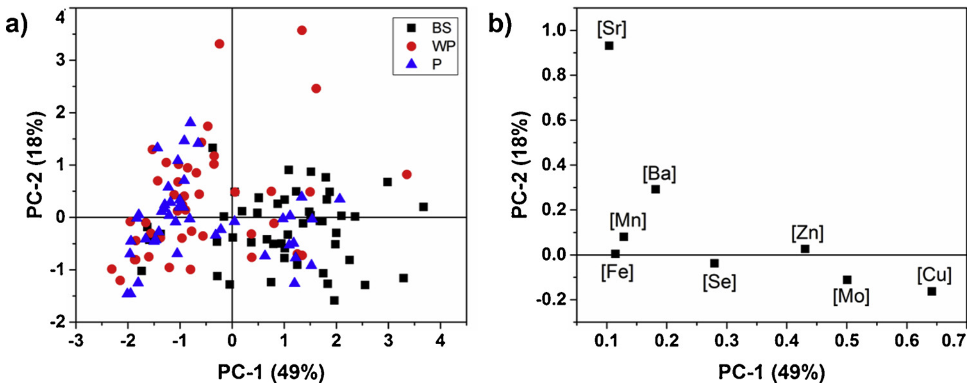

Fig. 2. a) Scores graphic and **b)** Loadings graphic for PCA analysis of human breast milk samples in relation to their contents of Ba, Cu, Fe, Mn, Mo, Se, Sr and Zn.

Table 5
Contribution of human breast milk consumption to the recommended daily intakes (RDIs) of essential elements for preterm and term infants, considering as daily ingestions 324 mL of milk/day and 720 of milk/day for preterm and term infants, respectively.

Element	Preterm			Term		
	RDI (µg)	Mean for BS (µg/L)	% RDI	RDI (µg)	Mean for P (µg/L)	% RDI
Cu	100-132	592.0	192-145	200	325.6	117
Fe	2000-3000	497.1	8-5	270	597.8	159
Mn	≤ 27.5	6.3	7	3	7.0	167
Mo	0.3-5	10.8	1163-70	2	3.5	124
Se	5-10	16.1	105-52	6	7.9	94
Zn	1.100-2000	3074.5	91-50	2800	2116.1	54

BS = bedside and P = pasteurized samples.

Thus, the application of PCA showed that the grouping of the samples is mainly associated to the higher levels of Cu and Mo determined in the samples of the BS group in comparison to the WP and P groups.

3.4. Contribution of human breast milk consumption for the RDI of elements for infants

In Table 5 are shown the contribution of human breast milk consumption to the recommended daily intakes (RDI) of Cu, Fe, Mn, Mo, Se, and Zn for preterm and term infants. For preterm infants, specifically, there are no Brazilian recommendations regarding the ingestion of essential elements, so the recommended intakes suggested by the European Society of Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) were taken into account [26]. For term infants, it was used the daily intakes suggested by the National Health Surveillance Agency of Brazil (ANVISA) [39]. Another difference was the volume of milk ingested by preterm and term infants. Preterm newborns normally ingest a lower volume of milk per day, being a rate of 150–180 m L/kg day considered for routine purposes [26]. Thus, regarding a maximum weight of 1.8 kg for preterm newborns and a rate of 180 mL/kg·day, we considered a daily ingestion of 324 m L of milk/day for the calculations. Term infants, otherwise, normally consume a higher volume of milk and for these, we considered daily ingestion of 720 m L of milk/day. For preterm and term infants, the mean concentration obtained for the BS and P groups, respectively, were taken into account.

According to the results displayed in Table 5 for term infants the ingestion of pasteurized human breast milk (P group) meets the recommended daily intakes for the majority of the elements considered, except for Zn, for which the ingestion of human milk would contribute with half (54%) of the RDI.

For preterm infants, the RDI through human breast milk consumption (BS group) is achieved for the elements Cu, Mo, Se, and Zn. On the other hand, for Fe and Mn the contribution for the RDI of preterm infants is very low, representing less than 10%, indicating a critical situation in relation to the ingestion of these essential trace elements. Of course, in a more accurate analysis, each case has to be examined separately, regarding that in this work the calculations were made with mean values. However, considering the special high requirements of preterm infants, our results indicated the necessity of fortification of the human breast milk to be offered for preterm newborns with Fe, as already stated [5]. In the case of Mn, futures studies still should be conducted regarding Mn accumulation and toxicity in infants. In patients fed with parenteral nutrition, for example, Mn toxicity is a recognized problem. It is also important to consider that infants are more prone to accumulate Mn than adults due to the immaturity of their regulatory mechanisms [40].

4. Conclusion

The accurate knowledge about human breast milk composition is a question of public matter, regarding the great importance of this food matrix on infant's nutrition. Thus, in this work, a significant sampling (156 samples) was performed to study the trace elemental composition of human breast milk, including samples of mothers of preterm and term infants and the processing of milk in human milk banks, which are very common in Brazil and other parts of the world.

Regarding essential elements, higher levels of Cu, Mo, Se, and Zn were observed in the milk produced by mothers of preterm infants in comparison to the milk from mothers of term infants. These data are particularly important for neonatal intensive care units and fortification programs to assure adequate intakes of these trace essential elements for high-risk neonates, such as preterm infants.

The application of Holder pasteurization to donor human breast milk did not affect the total concentrations of Ba, Cu, Fe, Mn, Mo, Se, Se, and Zn. Nevertheless, new studies still should be done considering the effects of the application of this thermal treatment on the chemical species of essential elements.

The contribution of human breast milk consumption does not attend the recommended daily intakes of Zn for term infants and of Fe and Mn for preterm infants, being critical for these two last elements, marking the necessity of human milk fortification with Fe for preterm infants.

Conflict of interest

None.

Acknowledgments

This work was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and INCTAA (Instituto Nacional de Ciências e Tecnologias Analíticas Avançadas). This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001 (R.L.R. thanks CAPES for the fellowship, process 88887.195518/2018-00).

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