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Article

Effects of Pasteurization and Freeze Drying on Proximal Composition, Fatty Acids Composition and Lipid Quality in Colostrum Human Milk

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Colostrum human milk samples obtained from human milk banks received different treatments and were evaluated for their proximal composition, fatty acids composition and lipid quality. The total lipids content was maintained in the treatments. There was no difference in moisture, ash, total proteins, carbohydrates and energetic value for raw colostrum human milk and pasteurized colostrum human milk, and there was difference in ash, total proteins and energetic value for freeze-dried colostrum human milk and pasteurized + freeze-dried colostrum human milk. The fatty acids composition demonstrated no difference in the processes for 14 of 31 fatty acids identified. Furthermore, lipid quality indices were obtained, which were unpublished for colostrum human milk submitted to pasteurization and freeze drying. Principal component analysis showed similar behaviors for freeze-dried colostrum human milk and pasteurized and freeze-dried colostrum human milk. So, the processes used may be promising for application in colostrum human milk, due to the maintenance of its proximal characteristics and fatty acid composition.

Keywords: fatty acids, lipid quality indices, macronutrients, colostrum human milk

Introduction

Human milk (HM) is widely known as the ideal newborn nutrition, for presenting benefits until the adulthood.[1] Besides offering the necessary contents for child demand, it is a source of components deficient in commercial products, which makes its use excellent for preterm babies or under intensive care.[2]

In general, HM is mostly composed of water (about 90% of the total), followed by carbohydrates, lipids and proteins.[3,4] In addition to nutrition benefits, HM contributes to the increase of the intestine's beneficial microbiota and to specific functions in protein and cellular metabolism and immune response.[4]

Among these compounds, lipids present important functions for newborns, as energy source, and transport and absorption of fat-soluble vitamins. And there are evidence of their action on brain, neurological, and immunological development, due to the long-chain polyunsaturated fatty acids, such as the docosahexanoic acid (DHA, 22:6n-3), from the omega-3 family (n-3), and arachidonic acid (ARA, 24n-6) from the omega-6 family (n-6).[3-5] In addition, there is the possibility of future disease management through the lipid quality evaluation.[6] Lipids are present in HM in a fat globule form, and triacylglycerol is formed in the endoplasmic reticulum from circulating fatty acids (FAs) or synthesized in epithelial cells from glucose.[7,8]

According to the maternal lactation phase, the HM presents variations in its physical-chemical characteristics, proximal composition, and immunological functions. The classification in the different lactation phases is colostrum (from the 1st to the 7th day), transitional (8th to 14th day) and mature (from the 15th day). This variation follows the child's development, giving adequate nutritional aspects to each growing step.[9,10]

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Originally, colostrum human milk (CHM) presents a great role in immunological functions, due to the newborn first contact with the external environment.[11] Its complex composition, besides acting in the nutritional function of newborns, presents therapeutic characteristics, preventing diseases such as necrotizing enterocolitis, retinopathy, pulmonary broncho dysplasia, and sepsis. For all these benefits, the exclusive breastfeeding is recommended from 60 min of life until two years old.[9]

However, different factors can compromise HM access, such as neonatal hospitalization, pre-existing diseases, nutritional disorders, maternal death, and low production, among others.[12] In these cases, HM from human milk banks are a possibility for consumption, making them a strategic element of breastfeeding public policy.[13] Nevertheless, the availability of HM in human milk banks may be compromised by the donation incidence, stability, storage, and transportation. For these reasons, ways have been studied to ensure the safety and prolong the shelf life of raw HM, such as pasteurization and recently freeze drying.[3,5] Pasteurization is the most common process used in human milk banks. But the potential use of freeze-drying to provide HM has caused the growth of its study, because this technology, which consists of sublimation dehydration, allows the obtaining of powdered HM, which facilitates handling, storage, and transport.[9]

To achieve better HM compounds preservation, it is important studying its macronutrients according to the treatments employed, because the evaluation of particularities of HM composition in each lactation phase can aim a feeding supplemented according to the needs of the preterm life stage under hospitalization.[14] Thus, the information about the proximal composition, FA composition and nutritional indices of lipid quality of CHM are important, because characterizing the lipid quality of the diet that can serve as a basis for administration in the first stage of life.

In this context, the purpose of this study was to evaluate the proximal composition, FAs composition and lipid quality of CHM under different treatments (pasteurization and/or freeze drying), comparing them to raw CHM, to observe their effects on the aspects mentioned.

Experimental

Reagents

Reagents for lipid extraction: high performance liquid chromatography (HPLC) grade methanol and chloroform purchased from J.T. Baker® (Philipsburg, USA) and Riedel-de Haën (Seelze, Germany) were used. For esterification, chloroform, *n*-heptane, methanol, potassium hydroxide and sodium chloride were used, all with a high degree of purity > 99%, purchased from Synth (São Paulo, Brazil). For the realization of gas chromatography analysis, reference standard fatty acid methyl esters (FAME) FAME Mix, unsaturated C4-C24 (≥ 97%) was purchased from Sigma-Aldrich (Saint Louis, USA).

Ethical approval and sampling

The present study has a partnership with the human milk bank of the Hospital Universitário Regional de Maringá, and was approved by the Ethics Committee of the Universidade Estadual de Maringá under number 3.430.478/2019.

CHM was collected from the donation of 15 different mothers provided by the human milk bank of the Hospital Universitário Regional de Maringá. Each aliquot containing 100 mL was collected in glass bottles and identified as mother 1, mother 2, up to mother 15. Subsequently, they were homogenized in a pool with a total of 1500 mL. From this pool, four aliquots with 375 mL were separated and stored at −18 °C, for subsequent use of treatment.

Treatments employed

The four aliquots of CHM were identified according to the treatment received.

(*i*) Raw colostrum human milk (RCHM): sample not subjected to treatment, sent to storage at −18 °C;

(*ii*) Pasteurized colostrum human milk (PastCHM): colostrum human milk sample submitted to pasteurization;

(*iii*) Freeze-dried colostrum human milk raw (FdCHM): raw colostrum human milk sample submitted directly to freeze-drying;

(*iv*) Pasteurized + freeze-dried colostrum human milk (PastFdCHM): sample of pasteurized and subsequently freeze-dried colostrum human milk.

Figure 1 shows the sample segmentation.

Application of treatments

Control

Approximately 375 mL of RCHM was kept as a control sample (untreated), without application of treatment and evaluated in the following steps in a liquid state, called RCHM. The sample was stored in a glass bottle with a suitable screw cap.

Holder pasteurization

The application of Holder pasteurization occurred according to Agência Nacional de Vigilância Sanitária

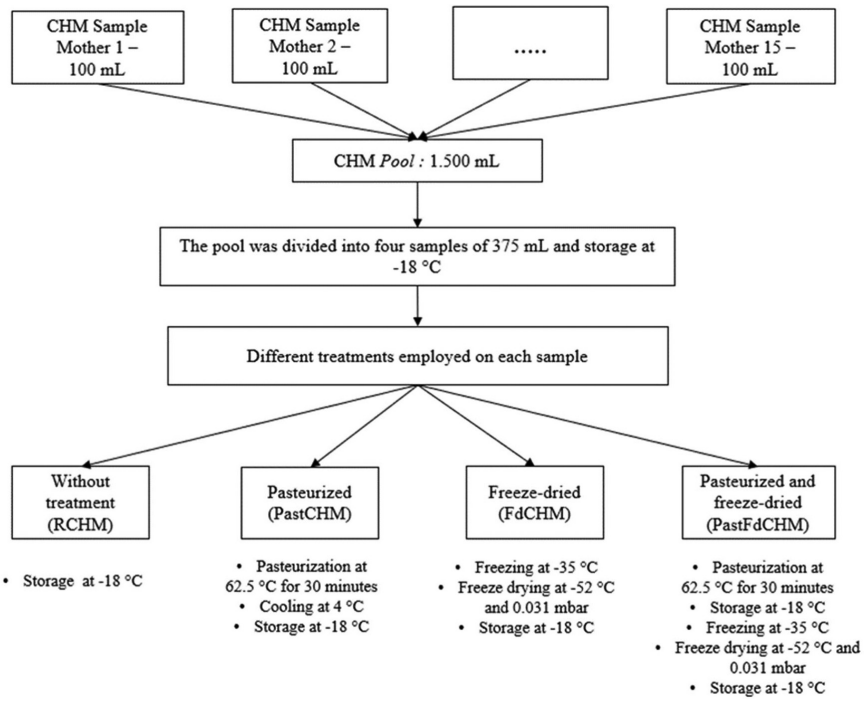

Figure 1. Sample segmentation and identification according to treatment received.

(ANVISA) protocol: [15] 375 mL of CHM contained in a glass bottle was submitted to a water bath (SL-152 Ultra-thermostated Digital Refrigerated Bath, Solab Cientifica, Brazil). The thermal treatment lasted for 30 min after reaching a temperature of 62.5 ºC in the center of the bottle, manual agitation was also provoked every 5 min. The water bath temperature was maintained at 68.4 ºC. Next, the bottle was immersed in a water bath containing ice until it reached 4 ºC, and then frozen at −18 ºC until other steps.

Freeze-drying

First, the appropriate container containing 375 mL of CHM was stored for 48 h at −18 ºC. Then, freeze-drying was performed according to Manin *et al*.,[3] where the freeze-drying processing application was submitted in a freeze-dryer (model 101522 - Christ Alpha 1-2 LD Plus, Martin Christ, Osterode, Germany) at about −52 °C and 0.031 mbar. The freeze-drying time was maintained for approximately 48 h, until constant weight for the samples was obtained on an analytical balance.

Holder pasteurization followed by freeze-drying

Preliminarily, 375 mL of stored RCHM were conducted to heat treatment by Holder pasteurization as mentioned above.[15] Afterwards, the pasteurized sample was frozen at −18 ºC for 48 h in preparation for the freeze-drying process. Then, it was submitted to drying with the same previously mentioned parameters.[3] It is worth mentioning that the freeze-drying time was maintained for approximately 48 h, when a constant weight was obtained for the samples on an analytical balance.

Proximal composition

The analysis of protein (method 990.03), moisture (method 934.01), ash (method 942.05), and carbohydrates (calculated by difference), were made according to official methods of analysis of the Association of Official Analytical Chemists (AOAC).[16] There was also the determination of the lipid content.[17] The energy value of the food was expressed by the sum of the macronutrients that compose it, using nutrient conversion factors that potentially provide energy for the human body, such as total lipids, carbohydrates and proteins, in which 1 g of carbohydrate and 1 g of protein correspond to 4 kcal each, and 1 g of fat to 9 kcal.[18]

Total lipids from all samples were extracted according to Folch *et al*.[17] FAMEs were prepared by the methylation indicated in International Organization for Standardization (ISO) No. 15885: 2000.[19] The upper phase obtained was collected, stored in an appropriate vial, and then analyzed on a gas chromatograph (GC) (Trace Ultra 3300, Waltham, USA) with a flame ionization detector (FID), CP-7420 capillary column (100.0 m in size, 0.25 mm inside diameter and 0.25 µm thin film of cyanopropyl as stationary phase) and split/split less injector. The flows used for separation were 1.4 mL min^{-1} for hydrogen (H_2) carrier gas, 30 mL min^{-1} for nitrogen (N_2) make-up gas, and 30 and 300 mL min^{-1} for the flame gases (H_2 and synthetic air,

respectively). The GC-FID oven was programmed to 65 °C and held for 4 min, then heated to 185 °C to 16 °C min^{-1} and held for 12 min, then heated to 235 °C to 20 °C min^{-1} and held for 9 min. The detector and injector temperatures were at 250 and 230 °C, respectively. Split injection mode was used with a ratio of 1:100 and the volume of sample injections was 2.0 µL. The FAMEs were identified by comparing the retention times of the sample's constituents with the analytical standards (FAME standard mixture, C4-C24, Sigma-Aldrich, Saint Louis, USA). Peak areas were determined using LabSolutions software and composition in FA were expressed in percentage of relative area. All samples were analyzed in triplicate.

Lipid nutritional quality of CHM

The lipid nutritional quality was calculated by the average composition of FAs determined by gas chromatography (n = 3) according to the following equations, using the six indices: atherogenicity index (AI) (equation 1), thrombogenicity index (TI) (equation 2),[20] and the hypocholesterolemic/hypercholesterolemic (H/H) FAs ratio (equation 3),[21] in addition to the sum of n-6 in relation n-3 (equation 4),[22] the sum of eicosapentaenoic (EPA) and docosahexaenoic (DHA) FAs (equation 5); and finally, the sum of the polyunsaturated fatty acids (PUFAs) due to the sum of the saturated fatty acids (SFAs) (equation 6).[23] The equations are shown below:

$$AI = \frac{\left[12{:}0 + \left(4 \times 14{:}0\right) + 16{:}0\right]}{MUFA + n6 + n3} \quad (1)$$

$$TI = \frac{\left(14{:}0 + 16{:}0 + 18{:}0\right)}{\left(0.5 \times MUFA\right) + \left(0.5 \times n6\right) + \left(3 \times n3\right) + \left(\frac{n3}{n6}\right)} \quad (2)$$

$$\frac{H}{H} = \frac{\left[\left(18{:}1n\text{-}9 + 18{:}2n\text{-}6 + 18{:}3n\text{-}3 + 20{:}3n\text{-}6 + 20{:}4n\text{-}6 + 20{:}5n\text{-}3 + 22{:}6n\text{-}3\right)\right]}{\left(12{:}0 + 14{:}0 + 16{:}0\right)} \quad (3)$$

$$OFP = \frac{\Sigma\left[n6\right]}{\Sigma\left[n3\right]} \quad (4)$$

$$SEFA = EPA + DHA \quad (5)$$

$$\text{Proportion of polyunsaturated and saturated fatty acids} = \frac{\Sigma\left[PUFAs\right]}{\Sigma\left[SFAs\right]} \quad (6)$$

where OFP is omega family proportion; SEFA is the sum of the essential fatty acids EPA and DHA; and MUFA is monounsaturated fatty acids.

Statistical analysis

The averages of triplicates of all analyzes were submitted to analysis of variance (ANOVA) and Tukey's test for their comparisons ($p < 0.05$), using the Statistica software version 7.0.[24] The data obtained regarding lipid quality were subsequently submitted to multivariate principal component analysis (PCA) exploration using the software previously mentioned.

Results and Discussion

Proximal composition

The proximal composition (moisture, ash, total protein, total lipids and carbohydrates), in g *per* 100 g, and the energy value, in kcal *per* 100 g of sample, of the RCHM and PastCHM (liquid samples) are exhibited in Table 1. There was no significant difference in moisture, ash, total proteins, total lipids, carbohydrates, and energetic value between RCHM and PastCHM. These results indicate that pasteurization did not impact the PastCHM proximal composition when compared to RCHM, and are similar to those obtained by Santos *et al.*,[25] that found difference only for lipids content when compared CHM pasteurized

Table 1. Proximal composition and energy value of RCHM and PastCHM

	Sample	
	RCHM	PastCHM
Moisture / (g *per* 100 g)	88.720 ± 0.203[a]	88.417 ± 0.115[a]
Ash / (g *per* 100 g)	0.377 ± 0.012[a]	0.363 ± 0.018[a]
Total proteins / (g *per* 100 g)	2.190 ± 0.087[a]	2.080 ± 0.112[a]
Total lipids / (g *per* 100 g)	2.357 ± 0.055[a]	3.141 ± 0.694[a]
Carbohydrates / (g *per* 100 g)	6.357 ± 0.243[a]	6.000 ± 0.512[a]
Energetic value / (kcal *per* 100 g of sample)	55.397 ± 0.550[a]	60.588 ± 3.788[a]

Results expressed as mean ± standard deviation of triplicate. Values with different letters on the same line are significantly different ($p < 0.05$) by Tukey's test. RCHM: raw colostrum human milk; PastCHM: pasteurized colostrum human milk.

and raw, and observed maintenance for moisture, ash, total proteins, carbohydrates and energetic value.[25]

Table 2 presents the composition (g *per* 100 g) and the energy value (kcal *per* 100 g of sample) of the FdCHM and PastFdCHM (solid samples).

According to Table 2, the sample submitted to pasteurization followed by freeze-drying (PastFdCHM) presented differences at the level of 5% significance in ash and total protein when compared to the sample submitted to freeze drying without previous pasteurization (FdCHM). These results can be related to the fact that the two processes use temperatures in distinct ranges may have caused mineral degradation and protein decrease.[26] More than 400 proteins are present on HM, and they are divided into whey proteins (60-80%), caseins (20-40%) and milk fat globule membrane proteins (1-4%).[4] Meng *et al*.[4] related in their review that some whey proteins, as α-lactalbumin and lactoferrin present heat resistance and decreasing levels at freezing at −20 °C, but it was not found reports about heating or freezing influence on caseins. In another hand, cooling causes β-casein dissociation and decrease, so they concluded that there is a gap on knowledge about properties of HM proteins. Another factor to note is that the PastFdCHM sample was submitted a higher number of handling cycles compared to the FdCHM sample, which may also have implied changes in the total protein content.

Once that energetic value is obtained by calculation using the amount of nutrients, its value also presented difference. It is important to observe that FdCHM sample presented a value less than PastFdCHM, related to the fact that PastFdCHM presented a higher value in lipids content, the nutrient that presents more contribution to energetic value (9 kcal *per* gram of lipid).[18] No difference was observed for moisture, lipids, and carbohydrates. It demonstrates the greater need for studies to evaluate the impacts of the use of the two treatments in a combined way.

Fatty acid composition

Thirty-one FAs were identified using GC-FID (Table 3). The omega system was used to describe FA from omega-3, omega-6 and omega-9 family, in which the letter n followed by a number refers to the position of the double bond, starting the counting of carbons from the methyl group. The results are expressed in percentage of relative area. The columns show the results according to the treatment applied on the samples. The FAs identified are divided into about 50% SFAs, 25% monounsaturated fatty acid (MUFAs), and 25% PUFAs, values similar to those mentioned by Schipper,[27] which indicates 50% SFAs and 20% PUFAs. The prevalent FA was oleic acid (OA, 18:1n-9), followed by palmitic acid (PA, 16:0) and linoleic acid (LA, 18:2n-6). Published data[28-30] show similarities in the total content of FAs found for the colostrum lactation phase.

According to Visentainer *et al*.,[31] FA composition determines in the HM its nutritional and physical-chemical properties. Therefore, defining the FA composition based on diet and maternal age, as well as factors such as phase (colostrum, transition and mature) and feeding time (previous, intermediate, and posterior) is extremely important for the newborn's health.[11,32]

It can be considered that the 18:1n-9 is synthesized by infants mainly as an energy source, besides promoting fat absorption by the small intestine.[8] PA (16:0) is mostly in the central position (Sn-2) of the triacylglycerol molecule, presenting great importance for calcium absorption and intestinal composition.[29,33] It favors the action of pancreatic lipase, enabling the conversion of 16:0 into Sn-2 monoacylglycerol, directly related to hormonal levels, such as anandamide, a brain neurotransmitter with calming power for neonates, and also presenting potential improvement in intestinal discomfort.[7,31]

In addition to the above, among the prominent constituents of HM are the LA (18:2n-6) and α-linoleic (LNA,18:3n-3), which are precursors of ARA (20:4n-6) and DHA (22:6n-3), main sources in visual and cerebral development during

Table 2. Proximal composition and energy value of FdCHM and PastFdCHM

	Sample	
	FdCHM	PastFdCHM
Moisture / (g *per* 100 g)	4.287 ± 0.117[a]	3.840 ± 0.356[a]
Ash / (g *per* 100 g)	3.103 ± 0.308[a]	2.487 ± 0.137[b]
Total proteins / (g *per* 100 g)	19.190 ± 1.082[a]	16.500 ± 1.154[b]
Total lipids / (g *per* 100 g)	18.330 ± 0.590[a]	20.790 ± 1.467[a]
Carbohydrates / (g *per* 100 g)	55.090 ± 1.102[a]	56.383 ± 1.555[a]
Energetic value / (kcal *per* 100 g of sample)	462.094 ± 4.061[b]	478.643 ± 9.225[a]

Results expressed as mean ± standard deviation of triplicate. Values with different letters on the same line are significantly different ($p < 0.05$) by Tukey's test. FdCHM: freeze dried colostrum human milk; PastFdCHM: pasteurized and freeze-dried colostrum human milk.

Table 3. Fatty acids composition (relative area percentage) of CHM for each treatment received

	Sample			
Fatty acids / %	RCHM	PastCHM	FdCHM	PastFdCHM
4:0	0.156 ± 0.014^{ab}	0.132 ± 0.010^{b}	0.195 ± 0.037^{a}	0.200 ± 0.044^{ab}
6:0	0.547 ± 0.082^{a}	0.418 ± 0.027^{a}	0.611 ± 0.106^{a}	0.620 ± 0.079^{a}
8:0	0.081 ± 0.011^{a}	0.055 ± 0.020^{a}	0.072 ± 0.008^{a}	0.057 ± 0.006^{a}
10:0	0.137 ± 0.044^{a}	0.096 ± 0.036^{ab}	0.049 ± 0.010^{b}	0.055 ± 0.004^{ab}
12:0 (LAU)	2.322 ± 0.226^{a}	0.896 ± 0.038^{b}	1.050 ± 0.021^{b}	1.011 ± 0.042^{b}
14:0 (MYR)	5.487 ± 0.316^{a}	3.395 ± 0.127^{b}	3.771 ± 0.076^{b}	3.567 ± 0.089^{b}
14:1n-9	0.031 ± 0.005^{a}	0.042 ± 0.021^{a}	0.027 ± 0.001^{a}	0.027 ± 0.006^{a}
15:0	0.192 ± 0.006^{a}	0.159 ± 0.020^{b}	0.165 ± 0.003^{ab}	0.160 ± 0.006^{b}
15:1n-9	0.051 ± 0.026^{a}	0.038 ± 0.002^{a}	0.034 ± 0.003^{a}	0.031 ± 0.007^{a}
16:0 (PA)	30.822 ± 1.109^{a}	27.342 ± 0.586^{b}	27.662 ± 0.315^{b}	27.241 ± 0.177^{b}
16:1n-7	0.101 ± 0.020^{a}	0.141 ± 0.007^{a}	0.119 ± 0.024^{a}	0.109 ± 0.017^{a}
16:1n-9	1.078 ± 0.066^{b}	1.211 ± 0.065^{b}	1.188 ± 0.035^{b}	1.314 ± 0.203^{a}
17:0	0.252 ± 0.032^{a}	0.277 ± 0.061^{a}	0.301 ± 0.011^{a}	0.322 ± 0.013^{a}
17:1n-9	0.091 ± 0.011^{b}	0.119 ± 0.004^{a}	0.110 ± 0.011^{ab}	0.116 ± 0.015^{ab}
18:0	6.525 ± 0.256^{a}	7.147 ± 0.354^{a}	6.852 ± 0.137^{a}	6.928 ± 0.086^{a}
18:1n-9 (OA)	34.966 ± 4.018^{a}	38.622 ± 0.824^{a}	38.364 ± 0.054^{a}	38.490 ± 0.658^{a}
18:2n-6 (LA)	14.026 ± 2.005^{a}	15.769 ± 0.493^{a}	15.269 ± 0.657^{a}	15.644 ± 0.720^{a}
CLA; *cis*9,*trans*11	0.033 ± 0.005^{a}	0.043 ± 0.009^{a}	0.041 ± 0.010^{a}	0.019 ± 0.006^{a}
CLA; *trans*10,C12	0.051 ± 0.004^{b}	0.090 ± 0.005^{a}	0.086 ± 0.003^{a}	0.110 ± 0.021^{a}
18:3n-3 (LNA)	0.600 ± 0.008^{a}	0.564 ± 0.031^{a}	0.595 ± 0.001^{a}	0.597 ± 0.045^{a}
18:3n-6 (GLA)	0.078 ± 0.004^{a}	0.100 ± 0.009^{a}	0.089 ± 0.011^{a}	0.098 ± 0.011^{a}
20:0	0.090 ± 0.009^{b}	0.127 ± 0.023^{ab}	0.132 ± 0.015^{a}	0.109 ± 0.011^{ab}
20:1n-9	0.254 ± 0.019^{b}	0.395 ± 0.007^{a}	0.435 ± 0.010^{a}	0.371 ± 0.058^{a}
21:0 (HEN)	0.683 ± 0.018^{b}	0.897 ± 0.024^{a}	0.874 ± 0.008^{a}	0.861 ± 0.038^{a}
20:3n-6	0.367 ± 0.021^{b}	0.530 ± 0.007^{a}	0.497 ± 0.021^{b}	0.484 ± 0.025^{b}
20:3n-3 (DLA)	0.356 ± 0.019^{b}	0.486 ± 0.008^{a}	0.449 ± 0.013^{a}	0.464 ± 0.030^{a}
20:4n- 6 (ARA)	0.126 ± 0.029^{a}	0.170 ± 0.034^{a}	0.147 ± 0.024^{a}	0.169 ± 0.026^{a}
22:0	0.054 ± 0.007^{a}	0.072 ± 0.009^{a}	0.065 ± 0.011^{a}	0.072 ± 0.007^{a}
20:5n-3 (EPA)	0.067 ± 0.007^{b}	0.098 ± 0.021^{b}	0.225 ± 0.070^{a}	0.093 ± 0.019^{b}
24:0 (LIG)	0.191 ± 0.011^{c}	0.334 ± 0.011^{a}	0.297 ± 0.012^{b}	0.300 ± 0.013^{b}
24:1n-9	0.061 ± 0.006^{b}	0.102 ± 0.009^{a}	0.100 ± 0.005^{b}	0.093 ± 0.011^{b}
22:6n-3 (DHA)	0.135 ± 0.008^{a}	0.133 ± 0.013^{a}	0.130 ± 0.004^{a}	0.134 ± 0.006^{a}
Σ (n-3)	1.158 ± 0.015^{b}	1.281 ± 0.012^{ab}	1.398 ± 0.084^{a}	1.288 ± 0.071^{ab}
Σ (n-6)	14.681 ± 2.032^{a}	16.702 ± 0.501^{a}	16.129 ± 0.669^{a}	16.523 ± 0.717^{a}
Σ SFA	47.528 ± 1.884^{a}	41.346 ± 0.589^{b}	42.096 ± 0.631^{b}	41.485 ± 0.323^{b}
Σ MUFA	36.633 ± 3.926^{a}	40.670 ± 0.769^{a}	40.377 ± 0.016^{a}	40.704 ± 0.409^{a}
Σ PUFA	15.839 ± 2.045^{a}	17.983 ± 0.510^{a}	17.528 ± 0.617^{a}	17.811 ± 0.725^{a}

Results expressed as mean ± standard deviation (SD) of triplicate. Values with different letters on the same line are significantly different ($p < 0.05$) by Tukey's test. LAU: lauric acid; MYR: myristic acid; PA: palmitic acid; OA: oleic acid; LA: linoleic acid; CLA: conjugated linoleic acid; LNA: alpha-linolenic acid; GLA: gamma- linolenic acid; HEN: heneicosyl acid; DLA: di-homo-α-linolenic acid; ARA: arachidonic acid; EPA: eicosapentaenoic acid; LIG: lignoceric acid; DHA: docosahexaenoic acid; n-3: fatty acids from omega 3 family; n-6: fatty acids omega 6 family; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; RCHM: raw colostrum human milk; PastCHM: pasteurized colostrum human milk; FdCHM: freeze dried colostrum human milk; PastFdCHM: pasteurized and freeze-dried colostrum human milk.

perinatal progress and of great importance for neonates up to 7 days postpartum, or premature.[34] Although they can be synthesized from their respective precursors, conversion rates are insufficient. Thus, providing ARA and DHA at adequate concentrations in the diet is important for optimal development during pregnancy.[35]

It can be said that the structuring of physiological processes, as well as brain development and function, and immunological tissues required supply of certain rate of DHA *per* day, reaching a DHA content of at least ca. 0.3% of total FAs.[36,37] Among the findings in the present study regarding CHM even with applied treatments, the concentration is equal to 0.13% for DHA, indicating an important interaction of 18:3n-3 as its precursor, so that daily DHA levels are met and observing an adequate newborn development. While availability in HM is relatively low on EPA (0 to 0.12% by weight) and higher for ARA (0.08-1.1% by weight), similarly DHA (0.01-0.56% by weight).[38] In view of the results found, they presented within the standard, with significant differences ($p > 0.05$) in EPA (0.067 ± 0.007 to 0.225 ± 0.070), and similarly for ARA ranging from 0.126 ± 0.029 to 0.170 ± 0.034, both cases of the FAs were negatively affected in their concentrations.

Most official agencies around the world establish alternatives to maintain the biological safety of HM.[9,15,39-41] Consequently, studies evaluated Holder pasteurization with a temperature of 62.5 ºC for 30 min, freeze drying, and/or pasteurization followed by freeze drying.[42-46] Changes in lipid content are predicted after application of treatments such as pasteurization and freeze-drying due to the temperature variations to which the samples are submitted.[47] Therefore, it is necessary the observation the occurrence of significant differences between the processing performed and the untreated sample, to verify the processes interference in the conservation of the CHM lipid profile.

The results expressed in Table 3 demonstrate that the application of the treatments decreased the medium chain fatty acids concentration, such as lauric acid (LAU 12:0), myristic acid (MYR 14:0) and 16:0, in relation to RCHM levels. Evaluating the numbers, the PastFdCHM sample in relation to RCHM had a decrease of 61% for 12:0, 38% for 14:0 and 12% for 16:0. For conjugated linoleic acid (CLA *trans*10, *cis*12), heneicosyl (HEN 21:0), di-homo-α-linolenic (DLA 20:3n-3) and lignoceric (LIG 24:0) there was a significant increase achieved for the treated samples in relation to RCHM.

Most of the MUFAs found (about 50%) and PUFAs (about 60%), among which are included some of the n-6, namely LA 18:2n-6 and GLA 18:3n-6, did not present significant differences ($p < 0.05$) among the samples evaluated. Moltó-Puigmartí *et al.*[48] state that they did not find significant differences in the proportions of these FAs between pasteurized and untreated samples.

There were effects on the saturated fatty acids (SFA) sum, which presented total concentrations of 47.528 ± 1.884, 41.346 ± 0.589, 42.096 ± 0.631 and 41.485 ± 0.323 for the samples RCHM, PastCHM, FdCHM, and PastFdCHM, respectively, so that the RCHM sample differed significantly from the others. This fact demonstrates similarity to Neia *et al.*[30] results, which identified implications for SFA concentration when pasteurization and freeze-drying were applied in combination on CHM.

In general, 48% of the monounsaturated fatty acids found did not present significant difference between treatments, followed by 26% in which the RCHM differed from the others, 10% in which the PastCHM differed from the others and 16% which presented different behaviors. Thus, it was observed in the present study that the pasteurization and freeze drying partially influenced the concentration of FAs in the CHM. According to Meng *et al.*,[4] this may be related to the fact that temperature affects the stability of nutrients in HM, since the lipid structure can be modified. Another authors[25] points out that the pasteurization applied by human milk banks may imply the maintenance of lipid characteristics. A greater number of steps may also explain the possible differences obtained between the samples.[47]

Despite the observed changes, it is interesting to highlight that the sums related to the FA from the n-6 family, MUFA and PUFA showed no difference between the treatments, which points to an important result because these FA act in the synthesis of DHA, an essential component for brain development, constituting one of the reasons why breastfeeding premature babies with breast milk overlaps the use of commercial infant formulas.[27]

Lipid nutritional quality

The importance performed by CHM lipid components on newborns is poorly documented, thus needing to be better investigated, since a lipid nutritional quality can promote health maintenance at different ages of life.[6,49] Six lipid nutritional quality indices were calculated according to the FA quantification obtained preliminary. Table 4 shows CHM lipid nutritional quality indices in different processes.

The AI refers to the FA atherogenic potential. Among the samples analyzed, RCHM has the highest value found, and a significant difference was found between RCHM and samples that underwent a conservation treatment. Reference values for AI were not found in the literature, but it is known that the lower the AI value, the higher the lipid quality of food.[50] TI index presents the FA thrombogenic potential, the propensity to form clots in blood vessels. So, like AI, low values for TI are expected. For the analyzed samples, RCHM was the highest value found, and it presented a significant difference when compared to the treatments applied. Therefore, for both TI and AI, The RCHM presents lower lipid nutritional quality than samples

Table 4. Lipid nutritional quality indices of CHM for each treatment received

Indice	Sample			
	RCHM	PastCHM	FdCHM	PastFdCHM
AI	1.052 ± 0.085[a]	0.713 ± 0.021[b]	0.757 ± 0.019[b]	0.727 ± 0.008[b]
TI	1.468 ± 0.100[a]	1.162 ± 0.028[b]	1.177 ± 0.023[b]	1.159 ± 0.016[b]
H/H	1.305 ± 0.109[b]	1.767 ± 0.053[a]	1.700 ± 0.039[a]	1.753 ± 0.012[a]
Σ (n-6)/Σ (n-3)	12.668 ± 1.623[a]	13.039 ± 0.307[a]	11.577 ± 1.094[a]	12.856 ± 0.892[a]
(EPA) + (DHA)	0.202 ± 0.015[b]	0.231 ± 0.025[b]	0.354 ± 0.071[a]	0.227 ± 0.019[b]
Σ PUFA/Σ SFA	0.332 ± 0.031[b]	0.435 ± 0.014[a]	0.417 ± 0.021[a]	0.429 ± 0.021[a]

Results expressed as mean ± standard deviation (SD) of triplicate. Values with different letters on the same line are significantly different ($p < 0.05$) by Tukey's test. AI: atherogenicity index; TI: thrombogenicity index; H/H: ratio of hypocholesterolemic/hypercholesterolemic fatty acids, Σ (n-6)/Σ (n-3): sum of the omega-6 family in ratio to the omega-3 family; Σ PUFA/Σ SFA: sum of polyunsaturated fatty acids in ratio of saturated fatty acids; (EPA) + (DHA) sum of eicosapentaenoic and docosahexaenoic fatty acids; SFA: saturated fatty acids; PUFA: polyunsaturated fatty acids; n-6: fatty acids from omega-6 family; n-3: fatty acids from omega-3 family; RCHM: raw colostrum human milk; PastCHM: pasteurized colostrum human milk; FdCHM: freeze dried colostrum human milk; PastFdCHM: pasteurized and freeze-dried colostrum human milk.

submitted to some heat treatment.[50]

The H/H, according to Santos-Silva *et al.*,[21] indicates the specific effects of FAs on cholesterol metabolism in the human body, and higher values are desirable, as they generate greater health benefits. This is because hypocholesterolmic FAs reduce low density lipoprotein, preventing cardiovascular diseases, while hypercholesterolemic FAs increase blood cholesterol levels, consequently, increasing the coronary heart disease risk. The values found support the hypothesis that the samples of CHM that were submitted to some heat treatment present a higher nutritional quality lipidic than the RCHM, because the values for these samples were higher.

For the Σ (n-6)/Σ (n-3) ratio, high values indicate the pathogenesis of many diseases, while lower values exert a disease suppression effect. The ideal value of this ratio should be between 5 and 10. For the analyzed samples, no statistical difference was found, and all values are above 10,[51] which demonstrates that the treatments applied did not influence this aspect.

The sum of EPA + DHA can simulate the dietary fat intake interaction, demonstrating the effects of these long-chain polyunsaturated fatty acids on human health, which perform functions to the pathophysiological mechanisms underlying immune function and neurodevelopment in newborns.[52] Among EPA + DHA, significant differences were found ($p > 0.05$) between the FdCHM sample, being superior to the others, justified by the distinct EPA concentration.

The Σ PUFA/Σ SFA ratio allows point the generalized lipid nutritional quality, as it considers PUFA activity on SFA, and PUFAs can restrict low density lipoprotein (LDL) cholesterol and decrease plasma cholesterol levels.[50] However, the Σ PUFA/Σ SFA index may not be efficient to evaluate the fat nutritional value, since some SFA does not influence the increase in plasma cholesterol, besides ignoring the effects of MUFAs.[53] However, the data presented in Table 4 show that there was a significant difference ($p > 0.05$) when comparing the pasteurization and freeze-drying processes to RCHM. Thus, it is suggested that the temperature applied in the samples, together with the melting point between the FAs saturation, influences the concentrations identified,[49,54] in addition to other factors such as handling and storage conditions.

Principal component analysis (PCA)

A multivariate exploratory technique to the PCA was performed to expand the visualization of data involving the CHM lipid quality. Figure 2 illustrates the plot graphs representing the loadings (variables, that are FA composition and lipid nutritional quality) and scores (namely as score 1-RCHM, score 2-PastCHM, score 3-FdCHM and score 4-PastFdCHM) from the similarity of the comparison between the samples, being possible to justify the 88.81% total variance of the data by the analysis of PCA, where PC1 represents 71.90% and PC2 16.91%.

There was a cluster between the vast majority of FA data and lipid nutritional quality indices related to the negative quadrant of PC1 and PC2 (Figure 2a), thus indicating that the variables with significant differences are short chain fatty acids 4:0, 6:0, 8:0, 10:0, some monounsaturated like 14:1n-9 and 15:1n-9, in addition to 18:3n-3 and 22:6n-3. Thus, variations in lipid quality influenced by treatments such as pasteurization and freeze-dried are distinguished by PCA.

It can also be observed from Figure 2b that the RCHM sample was distinct from the others, positioned in the positive quadrant. It is possible to observe a predominant peculiarity referring to the SFA, 14:0, 15:0, and TI about the

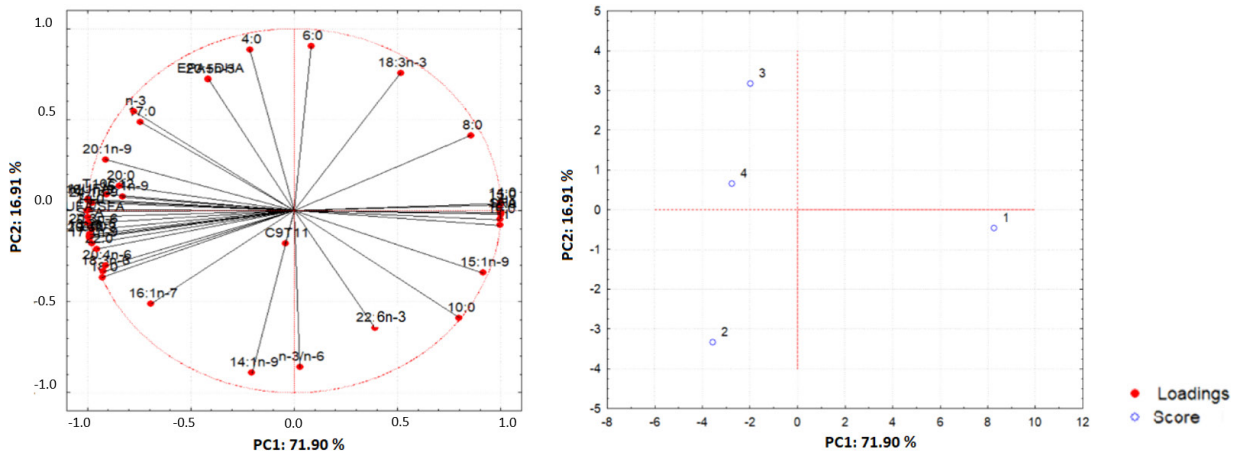

Figure 2. Representation by graphs of lipid quality variables among treatments in human colostrum milk. (a) Graph of the similarity assessment between the variables (loadings). (b) Graph of the similarity assessment between the samples (scores).

RCHM. It is justified that the degree of unsaturation of FAs in lipids influence fluidity, preventing FAs from grouping steadily, thus decreasing the melting temperature.[55] Temperature applications can degrade short-chain FAs, such as butyric (4:0), caproic (6:0), caprylic (8:0) and capric (10:0) acids present in the HM samples, which consequently will not be identified.[8]

In addition, the singularity to score 1 (RCHM) is restricted to the originality of the sample not treated by any treatment. On the other hand, the samples represented by scores 3 (FdCHM) and 4 (PastFdCHM) in Figure 2b show similarity with proximity to the positive PC2, which can be based on the effects of the application of freeze-drying, while score 2 (PastCHM) in negative PC2 particularizes the effects of pasteurization, with a proximity to score 4, a sample that was also applied pasteurization processing. Therefore, from the PCA it was possible to evaluate the similarity of lipid quality among the samples evaluated in this study.

Conclusions

This study demonstrated that there are variations in the proximal composition, in the FAs composition and consequently in the lipid quality of human colostrum milk submitted to different conservation treatments (pasteurization, freeze-drying or pasteurization combined with freeze-drying) when compared to raw colostrum milk.

Regarding the proximal composition, no significant difference was observed between the pasteurized sample and the untreated sample. However, the sample in which there was a combination of pasteurization and freeze-drying differed from the sample only freeze-dried in relation to the contents of proteins, ash and, consequently, energy value. Moisture, carbohydrates and lipid content showed no significant difference.

Regarding the FA composition, of the 31 identified FAs, 14 did not present a significant difference between the different treatments, and the sum of FA of the n-6 family, MUFA and PUFA remained preserved. Among the FA influenced by the treatments, the medium chain (12:0, 14:0, 15:0, 16:00) stands out.

The results obtained for lipid nutritional quality are complementary for the evaluation of CHM, and the sum of the FA ratio of the n-6 and n-3 family did not show a difference for the different treatments. For the other aspects, there was difference, being interesting its evaluation in other studies.

When applying PCA on the lipid quality of the samples, different behaviors were observed for RCHM and PastCHM samples and similar behavior for FdCHM and PastFdCHM.

The results suggest that the application of pasteurization and freeze-drying processes are adequate from the point of view of maintaining lipids in CHM, in addition to the prevalence of preserved FA compared to the total identified. However, new investigations must be conducted in view of the complexity of the HM matrix and the specificities of the processes employed, such as the evaluation of temperatures used in processes and storage.

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Author Contributions

Gislaine A. S. Ientz was responsible for investigation, visualization, formal analysis, writing original draft, conceptualization; Eloize S. Alves for data curation, writing- original draft, review and editing; Matheus C. Castro for writing original draft; Giovana Frigo for writing-review and editing; Christyna Beatriz G. Tavares for visualization, resources; Jeane E. L. Visentainer for writing review and editing, resources; Oscar Oliveira Santos for investigation, resources, supervision; Jesui V. Visentainer for visualization, resources, conceptualization, supervision, funding acquisition.

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