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Impact of Conservation Processes on the Lipid Profile and Immunological Factors IL-10 and TGF-β1 in Whey Separated from Discarded Human Milk

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Reuse made by the separation of whey can reduce the waste of human milk. However, the authors are not aware of the literature on treatments in human whey, made available by pasteurization holder, freeze-drying, spray drying, and high hydrostatic pressure. In this context, the present study applied treatments to human whey and evaluated their lipid and immunological quality. Among the results, a different formation in the triacylglycerol profile was evidenced after the application of spray drying and high hydrostatic pressure, while pasteurization and freeze-drying presented ion intensity close to the control human whey. In addition, pasteurization proved to be adequate for lipid nutritional quality and transformation factor-β1 (TGF-β1) concentration, while an increase in interleukin-10 (IL-10) levels was promoted, between 73 and 80%, after freeze-drying, spray drying, and high hydrostatic pressure. Through the principal component analysis, it is noteworthy that the processes presented divergences in terms of the effects caused, with similarity only between pasteurization and freeze-drying in the composition of fatty acids. However, it was observed that all processes were able to maintain the nutrients. Nevertheless, it is relevant to consider individual characteristics presented and the interest in the desired quality, which can be promising as a complementary product to infant feeding.

Keywords: freeze-drying, spray drying, high hydrostatic pressure, fatty acid, triacylglycerol profile

Introduction

Human milk is essential for the organism development of newborns.[1] It is recommended that premature infants be fed with human milk from human milk banks in situations where breastfeeding is unavailable,[2] which perform actions such as promotion, protection, and support for breastfeeding, from the collection, selection, classification, quality control, processing, and distribution of human milk.[3] Among the activities of the human milk bank, quality control is worthy of mention, in which about 30% of the human milk received is discarded in the sewage system due to irregularities after its evaluation.[1]

The reuse of human milk discarded due to dirt involving exogenous causes (for example, hair, eyelashes) is promising since it can be redirected as a by-product for the development of new dairy products, as indicated by Castro *et al.*[4] The authors propose a new purpose for this essential food for babies, reusing discarded human milk to compose a prebiotic ice cream, meeting some parameters necessary for its production.[4] In addition, in the study by Alves *et al.*,[5] who used human milk discarded from a human milk bank due to the dirtiness attribute, and isolated and characterized the lipid content of human whey, it was obtained a product that consequently can be included in the introduction of food for children. On the other hand, in infant formulas and functional foods intended for children, the use of whey in their compositions is observed, which guarantees a wide variety of components with nutritional functions and important biological effects.[6,7]

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It is noteworthy that the compositions of casein and whey proteins differ between mammalian species, in this sense, obtaining human whey can be derived from the use of high centrifugal force, which consequently promotes the partial removal of fat, mammary gland cells, cellular debris, part of the casein, and yet the whey proteins that rests in the supernatant content.[5,8]

Some processes can offer greater conservation to human milk, identified by pasteurization holder and freeze-drying.[9] Pasteurization holder is considered a standard procedure in human milk banks, it is carried out by applying a temperature of 62.5 °C for 30 min.[3,10] On the other hand, the freeze-drying technique favors the removal of water from the matrix via sublimation, under conditions of vacuum pressure at low temperatures, followed by desorption, generating benefits such as a longer conservation time and smaller storage space.[11] Nevertheless, currently, new methods are being investigated in human milk, such as the spray drying technique[12] and high hydrostatic pressure (HHP) processing.[13] Spray drying processing can be an alternative since it is used industrially for powdered bovine milk production.[14] Furthermore, in this process, the milk is sprayed in drops inside a drying tower, where the product comes into contact with heated air.[15,16] While HHP is reputed to be efficient non-thermal processing in inactivating pathogens in food.[17]

Although there are studies on the impact of these processes on human milk, there are no reports of these in human whey, or the evaluation of its nutrients. Studies[12,17,18] report that the drying processes (freeze-drying and spray drying) and HHP performed on human milk were able to maintain the quality of an adequate nutritional composition, lipid profile, macronutrients, and immunological components. Given the above, this study aimed to evaluate the lipid quality through the determination of the triacylglycerol profile, fatty acids composition, nutritional indexes, as well as the maintenance of immunological compounds by the concentrations of the cytokines interleukin (IL-10) and transformation factor-β1 (TGF-β1) in human whey under different processes (pasteurization Holder, freeze-drying, spray drying, high hydrostatic pressure), comparing them with control whey human (untreated). The best treatment in terms of nutritional quality could be an alternative for inclusion in infant feeding.

Experimental

Reagents

The reagents used in the lipid extraction were chloroform (≥ 99.8%), *n*-heptane (≥ 99%), methanol (≥ 99.8%), and sodium chloride (≥ 99%), purchased from Synth (São Paulo, Brazil). For esterification, potassium hydroxide (≥ 85%) purchased from Dinâmica (São Paulo, Brazil), *n*-heptane (≥ 99%) from Synth (São Paulo, Brazil), methyl tricosanoate reference standards (23:0; ≥ 99%), and the fatty acid methyl ester (FAME) Mix C4-C24 unsaturated (≥ 97%), both purchased from Sigma-Aldrich (São Paulo, Brazil). For analysis of the triacylglycerol profile, high-performance liquid chromatography (HPLC) grade reagents were used, with methanol (≥ 99.8%) purchased from J.T. Baker® (Philipsburg, USA) and chloroform (≥ 99.8%) from Riedel de Haën (Seelze, Germany), respectively. In addition, the use of ammonium formate (≥ 97%) was purchased from Sigma-Aldrich (Darmstadt, Germany).

Sampling

The present study was approved by the local Research Ethics Committee, No. 3.430.478, of the Universidade Estadual de Maringá (UEM, Maringá, Brazil). The exclusion criteria for the study involve the requirements established by the Agência Nacional de Vigilância Sanitária (Anvisa).[3] In addition, the age group for samples collection was 18 to 33 years old, being non-smokers. This way, samples of mature raw human milk were collected at the Hospital Universitário Regional de Maringá, after evaluating the presence of dirt following a specific protocol for quality control of human milk from human milk banks,[3] under a temperature of cooling 4 °C. Subsequently, approximately 5.000 mL of mature human milk purchased from 10 different donors were mixed and stored in a single glass flask with a screw cap, which was subsequently stored at –18 °C until the following steps.

Whey protein isolation from human milk

Isolation of human whey was performed according to the methodology described by Alves *et al.*,[5] where human milk was initially centrifuged using a refrigerated centrifuge model Harrier 18/80 (Sanyo MSE, Kent, UK) at 1500 g for 10 min at 10 °C for partial separation of the fat layer manually. The skimmed human milk was then centrifuged again on the same equipment at 6000 g for 30 min at 30 °C for partial sedimentation of casein and separating of the human whey.

The volume obtained of the isolated human whey was mixed and separated into five portions of approximately 800 mL for comparison purposes between the following treatments proposed in the present study named as: control human whey (CW), pasteurized human whey (PW), freeze-drying human whey (FDW), human whey applied spray drying (SDW), and human whey applied high hydrostatic

pressure (HHPW). Thus, obtaining five samples in total, in which the control sample (untreated) named CW was stored in a glass flask with a lid threadable, and submitted to freezing at –18 °C until the time of analyzes. The other samples were followed through different processes, as described below.

Pasteurization Holder

Pasteurization was performed according to Anvisa;[3] 800 mL of human whey already stored in a glass flask were submitted to a digital refrigerated bath (Solab Cientifica, model SL-152/10L, Piracicaba, Brazil), for this, the volume was divided into four flasks containing 200 mL each, thus the treatment was carried out four times until reaching the total volume of 800 mL. For the treatment, heating was applied until reaching a temperature of 62.5 °C in the center of the flask, and kept for 30 min, with manual agitation every 5 min during processing. Subsequently, the sample titled PW was cooled by immersion in a bath containing water and ice until reaching 4 °C. Finally, the sample was stored at –18 °C until the time of analyzes.

Freeze-drying

Another measure of approximately 800 mL of human whey was subjected to a pasteurization holder following the previously mentioned protocol, aiming at the integrity of pathogenic microorganisms of the sample, since freeze-drying not able to inhibit them, immediately after the volume was stored in appropriate containers for use in a tray freeze dryer, and frozen in a preparatory manner, at –18 ºC for 48 h. After that, according to Manin *et al*.,[19] the freeze-drying was performed in a freeze dryer (Alpha 1-2 LD Plus, model 101522, Osterode, Germany) at about –54 °C and 0.021 mbar. The freeze-drying process continued until a constant weight was obtained, for approximately 48 h. The dry sample was macerated until it acquired a fine powder, named FDW, then transferred to flexible polyethylene packaging, sealed under vacuum, and stored at –18 ºC until the analyzes were carried out. The sample was rehydrated for analysis with distilled water in the same mass of water removed during the drying process, using the initial weight of the sample as a reference.

Spray drying

The spray drying processing was performed according to Castro-Albarrán *et al*.,[20] about 800 mL of human whey divided into three aliquots of the same volume, and were subjected to the mini spray dryer process (model B-191, Buchi, Switzerland), with an inlet temperature of 175 °C and outlet temperature of 103 °C, with pressurized airflow of 15 mL min^{-1} using 100% compressed air. To perform the spray drying processing, it was necessary to calculate the yield after finishing the processing, so, the content of soluble solids was measured using a digital refractometer (Hanna Instruments, model HI96801, Brazil). From this, the theoretical value can be obtained, and after drying the actual value is obtained. Then, the yield would be calculated using equation 1.

$$\text{Yield}(\%) = \frac{(\text{Actual value})}{\text{Theoretical value}} \times 100 \quad (1)$$

The dried sample (SDW) was collected in flexible polyethylene packaging and kept at –18 ºC until the analysis was performed. For the rehydration of the obtained powder it was added distilled water (g) according to the processing yield, being 1 g of SDW for 11 g of distilled water.

High hydrostatic pressure (HHP) processing

The processing of the HHPW sample was performed according to the specifications by Manin *et al*.,[17] using high hydrostatic pressure equipment (QFP 2L-700 Avure Technologies, Middletown, USA). Sample containing 800 mL divided into four fractions stored in flexible polyethylene packaging, were submitted to processing. In the reservoir was used cold water as an indirect pressurizer, and the temperature was controlled for 5 min when a pressure of 600 MPa was reached, the initial temperature measured was 4 °C and the final temperature was 17.35 °C, respectively. The pressurization rate was 3 ºC min^{-1} and the decompression was practically instantaneous. The samples were mixed in a single glass vial with a screw cap and kept stored at –18 ºC until the analysis was performed.

Triacylglycerol (TAG) profile

The triacylglycerol (TAG) profile was obtained by direct infusion into a mass spectrometer (MS) using an electrospray ionization (ESI) source. The lipids of the samples were extracted according to Folch *et al*.,[21] for this, lipids from 10 mL of the samples were extracted with chloroform:methanol (2:1, v/v), using magnetic stirring (Fisatom, model 761-5, São Paulo, Brazil), thus transforming it into a final biphasic system, which was decanted and the upper organic phase was discarded. The lower organic phase was then recovered, the solvent was evaporated in a vacuum rotary evaporator and finally the extracted lipid was properly stored.

From extraction, the lipid samples of untreated human whey and in different processes applied were prepared according to Silveira *et al*.,[22] approximately 50.0 μL of the lipid were added to 950.0 μL of chloroform. Then, 5.0 μL of this solution was transferred to a vial and 1.0 mL of 9:1 methanol/chloroform solution (v v^{-1}) was added. To obtain the ammonium adults, $[M + NH_4]^+$, 20.0 μL of 0.10 mol L^{-1} ammonium formate prepared in methanol was added to the final solution. The prepared solutions were infused at a flow rate of 10.0 μL min^{-1} directly into a Xevo TQ-D™ triple quadrupole MS (Waters, Massachusetts, USA) equipped with an ESI Z spray™, operating in positive ion mode (ESI(+)) according to the following conditions: desolvation gas flow (500 L h^{-1}), source temperature (150 °C), desolvation temperature (200 °C), capillary and cone tension (3.00 kV and 20.00 V, respectively), evaluated in the mass-to-charge ratio (*m/z*), performing a scan on range of 500-1100. The results obtained were determined using MassLynx™ software.

Fatty acids composition

The lipids of the samples were extracted according to Folch *et al*.[21] Posteriorly, the FAMEs can be prepared by methylation of total lipids according to International Organization for Standardization (ISO) 12966:2017.[23]

Subsequently, all samples were analyzed on a gas chromatographer (GC) (Thermo Scientific, Trace GC Ultra, 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) (Agilent, Saint Clara, USA) and split/splitless injector. The detector and injector temperatures were at 250 and 230 °C, 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, as shown in Figure 1.

The gas flow rates used 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). Split injection mode was used with a ratio of 1:100 and the volume of sample injections was 2.0 μL. Consequently, in this study, internal standards methyl tricosanoate (23:0; ≥ 99%) and unsaturated FAME Mix C4-C24 (≥ 97%) were injected along with the samples. The FAMEs were identified by comparing the retention times of the sample's constituents with the analytical standards (FAME, C4-C24), and lipid mass was calculated related to 23:0. Peak areas were determined using ChromQuest 5.0 software and the fatty acids composition was expressed in mass (mg g^{-1} of lipid). All samples were analyzed in triplicate.

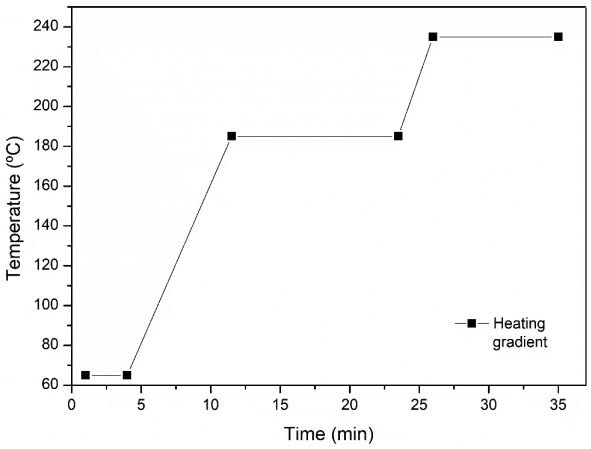

Figure 1. Heating gradient of the chromatographic run of the fatty acids composition.

Lipid nutritional quality of human whey

Considering the composition of fatty acids, lipid nutritional quality was evaluated using 8 indices: sum of polyunsaturated fatty acids as a ratio of the sum of saturated acids (equation 2), in addition to a sum of omega-6 fatty acids group as a ratio of the sum of a group of omega-3 fatty acids (equation 3),[24] a ratio of linoleic fatty acid to α-linolenic acid (equation 4),[25] a sum of eicosapentaenoic and docosahexaenoic fatty acids (equation 5), also the proportion of hypocholesterolemic/hypercholesterolemic (H/H) fatty acids (equation 6),[26] as well as the atherogenicity indices (AI) (equation 7), thrombogenic index (TI) (equation 8),[27] and finally, the health promotion index (HPI) (equation 9).[28] The values were submitted to the following equations:

$$\text{Proportion of polyunsaturated and saturated fatty acids} = \frac{\Sigma[\text{PUFAs}]}{\Sigma[\text{SFAs}]} \quad (2)$$

$$\text{Omega family proportion} = \frac{\Sigma[n-6]}{\Sigma[n-3]} \quad (3)$$

$$\text{LA/ALA} = \frac{18{:}2n-6}{18{:}3n-3} \quad (4)$$

$$\text{Sum of essential fatty acids} = \text{EPA} + \text{DHA} \quad (5)$$

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

$$\text{AI} = \frac{[12{:}0 + (4 \times 14{:}0) + 16{:}0]}{\text{MUFA} + n-6 + n-3} \quad (7)$$

$$TI = \frac{(14:0 + 16:0 + 18:0)}{\left[(0.5 \times MUFA) + (0.5 \times n-6) + (3 \times n-3) + \left(\frac{n-3}{n-6}\right)\right]} \quad (8)$$

$$HPI = \frac{MUFA + n-6 + n-3}{[12:0 + (4 \times 14:0) + 16:0]} \quad (9)$$

where PUFA: polyunsaturated fatty acid; SFA: saturated fatty acid; n-6: fatty acid from the omega-6 group; n-3: fatty acid from the omega-3 group; LA: linoleic acid; ALA: α-linolenic acid; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid; MUFA: monounsaturated fatty acid.

Evaluation of IL-10 and TGF-β1 concentration in human whey samples in different processes

The samples from each process were separated into 10 mL aliquots and stored in an ultra-freezer at −80 ºC until the performance of immunological analyses. IL-10 and TGF-β1 were measured in all samples, control human whey (untreated) and the different processes applied. IL-10 levels were assessed using the IL-10 Human Enzyme Linked Immuno Sorbent Assay (ELISA) kit (Invitrogen™, Thermo Fisher Scientific Inc., Burlington, Canada). The dosage of TGF-β1 was performed by enzyme-linked immunosorbent assay. For this, the Human TGF beta Platinum Enzyme linked immuno sorbent assay kit (Invitrogen™, Thermo Fisher Scientific, Inc., Burlington, Canada) was used. Both follow the manufacturer's recommendations. The absorbance of the tests was interpreted in an ASYS™ microplate reader, model EXPERT PLUS (ASYS Expert Plus, Cambridge, UK). All samples were analyzed in triplicate and your concentrations were estimated in pg mL^{-1}.

Statistical analysis

Statistical analyzes were performed using the Rstudio software.[29] The data of the fatty acid composition, lipid nutritional quality indices, and immunological analyzes were submitted to analysis of variance (ANOVA) and Tukey's test ($p < 0.05$) for comparison of means. Additionally, the multivariate exploration technique of the principal component analysis (PCA) was applied to these data, performed using the Factorextra and FactoMineR commands.

Results and Discussion

Triacylglycerol profile

Information on the TAG lipid profile enables an orientation of the functional properties of lipids, and fatty acid content present in the diet,[18] in addition to the behavior after processing, thus characterizing its range of lipid class. However, in the present study, the TAGs were identified by their mass spectra considering the mass-to-charge values *m/z* $[M + H]^+$, which makes it possible to base on the methodologies already consolidated in the literature.[5,17,19,22,30-37] By mass spectrometric conditions is not possible to identify TAGs isomeric, that is, those that differ in the positions of the acyl radicals. This way, the abbreviations of the acyl radicals of the TAGs evaluated in this study are not necessarily informed regarding the positions of the molecules of each TAG.

The TAG lipid profile of the samples is shown in Figure 2 and Table 1. In Figure 2, the intensities determined by direct infusion in ESI-MS $[M + NH_4]^+$ are shown in specific regions of *m/z* in which the TAGs ions are presented in each sample.

The sample titled CW (Figure 2a) presents the most intense ion (100%) in the region *m/z* 903, while in the PW (Figure 2b) it is displayed in the *m/z* 877, for FDW (Figure 2c) in the region *m/z* 874, for SDW (Figure 2d) in the region *m/z* 684, and HHPW (Figure 2e) in the region *m/z* 656. However, the human milk fat globule has about 98% (wt.%) of neutral lipids (triacylglycerols, diacylglycerols, and monoacylglycerols), which are presented as the predominant lipid molecules. However, it is also involved in its structural membrane with small amounts of phospholipids and cholesterol, and other components.[38]

Therefore, as demonstrated by Silveira *et al.*,[22] the technique by direct infusion in ESI-MS proves to be effective to characterize the TAG composition, as it has the ability to separate by mass and charge (*m/z*), which makes it possible to analyze complex chemical mixtures, characterizing a molecular composition. In the present study, we investigated the TAG profile of the samples between the region *m/z* 530-1110, following the study of Tu *et al.*[39] which characterized the identification of TAGs in mature human milk between the *m/z* 572-984. Another scan identifying profile of TAGs were evaluated between the range *m/z* 500-1000.[36]

The FDW sample was similar to the study by Manin *et al.*,[19] presenting 98.63% of intensity in the region *m/z* 874, during your assessment of the lipid stability of freeze-dried HM and exhibited this TAG profile on day 0 after the freeze-drying. As for the SDW sample, this is the first study that evaluates the lipid profile of TAG by direct infusion in ESI-MS of human milk derivative after the application of the spray drying process. It is noticeable that the lipid profile of TAG in the spectra of the SDW and HHPW samples took another format, presenting the most

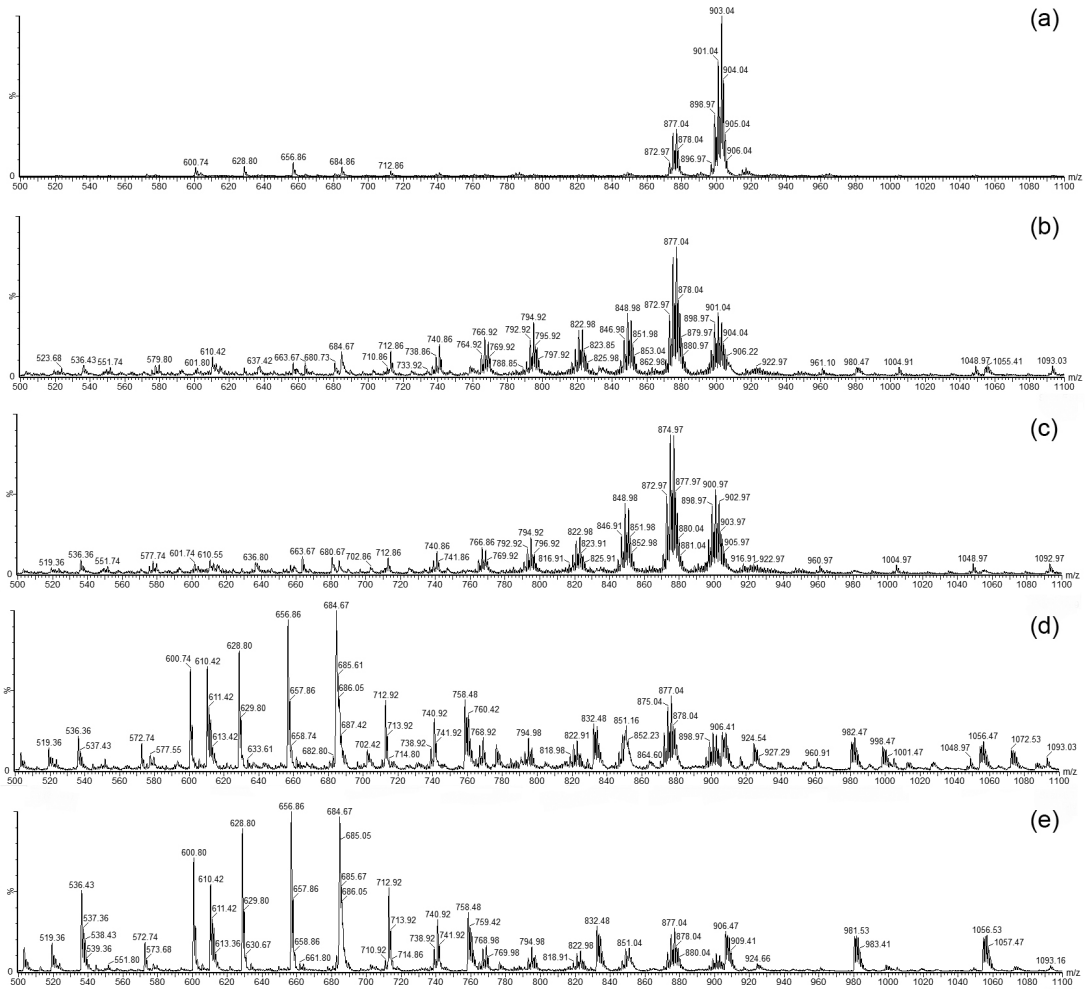

Figure 2. TAG ESI (+) mass spectra ranging from *m/z* 500-1100 evaluated in untreated human whey (CW) and other human whey applied different processes. (a) CW; (b) PW; (c) FDW; (d) SDW; (e) HHPW.

intense spectral peak between the region *m/z* 684-656, respectively, this is because they present a different fatty acid profile when compared to other samples of human whey evaluated in this study.

Table 1 expresses the results as a relative intensity, in which it was possible to observe 41 ion intensities from the profile TAGs of control whey (untreated) and after process, being found in the region between *m/z* 536 and 906.

In Table 1, the CW sample presents its largest spectral ions between the region *m/z* 900-904, while the PW sample in the *m/z* 874-878, besides the FDW sample presents in the region *m/z* 874-877, and 60.68% in the region *m/z* 900. As for the SDW and HHPW samples, both showed an altered lipid profile when compared to the other samples, where SDW presented the highest intensity in the region *m/z* 684, while HHPW in the region *m/z* 656.

According to Mitsutake *et al.*,[40] the precursor ion *m/z* 903 visualizes the TAG OOO and characterizes the ion present in the CW sample. In addition, accompanied the theory of Manin *et al.*,[19] who verified the ion *m/z* 874 with characteristics of the TAG molecules composed of oleic acid (O, 18:1n-9), palmitoleic acid (Po, 16:1n-9), and palmitic acid (P, 16:0), defining the OPoO and LPO molecules, these were found mainly in the FDW sample. It is important to define the TAG profile of human whey since the constitution of the TAG molecule of human milk is known for about 65% of the 16:0 that is located in the central position (Sn-2), while the Sn-1 and Sn-3 positions are usually occupied by oleic acid (O, 18:1n-9) and linoleic acid (L, 18:2n-6), which favors the hydrolysis of fatty acids 18:1n-9 and 18:2n-6 by pancreatic lipase, when positioned in the extremities of the TAG molecule, while 16:0 can be absorbed together with bile salts.[41]

Furthermore, rapid hydrolysis can be provided by the presence of SFA and MUFA in the TAG profile, thus the particularity present in human whey becomes important for a product that is easily absorbed and helps in the baby's digestion process, a fact that has been proven to be present

Table 1. Relative intensity of ions of triacylglycerol profile determined by ESI(+)-MS of untreated human whey (CW) and other human whey applying different processes

m/z	Relative intensity of ions / %				
	CW	PW	FDW	SDW	HHPW
536	0.22	8.76	10.47	21.47	50.70
600	5.31	5.01	3.52	63.43	71.14
601	3.30	6.59	7.49	31.14	37.41
610	0.54	15.21	10.09	64.94	54.32
611	0.36	9.18	5.86	39.34	33.00
628	5.95	6.45	4.00	74.71	89.56
629	3.12	3.80	2.40	45.89	56.21
656	8.35	10.54	6.42	94.31	100.00
657	5.32	6.55	3.94	68.35	74.58
658	2.54	6.27	6.06	31.44	33.76
684	5.72	19.04	9.79	100.00	96.95
685	4.15	13.90	6.94	76.46	81.84
686	2.02	8.13	4.05	45.23	43.34
712	3.23	19.51	11.71	43.72	52.61
713	2.63	16.29	9.71	38.04	45.09
740	2.19	24.59	16.25	32.67	32.52
758	0.61	8.27	3.31	44.40	37.19
794	1.17	41.62	26.15	20.42	15.41
848	2.60	48.57	51.06	22.14	13.63
849	2.48	46.75	47.38	22.24	14.12
850	1.76	43.28	47.18	24.64	14.44
851	1.74	42.97	45.39	28.16	14.79
872	8.67	47.37	56.13	23.19	12.13
873	8.26	44.14	51.92	23.73	11.85
874	26.91	91.79	100.00	38.86	22.19
875	26.27	90.86	96.56	40.04	23.13
876	28.30	98.56	99.82	44.40	26.47
877	29.13	100.00	99.23	46.74	27.14
878	16.33	58.93	56.84	27.47	15.60
879	6.14	48.49	43.99	26.36	14.71
896	7.25	20.16	24.95	8.49	3.82
897	6.65	19.12	23.19	8.45	3.49
898	38.40	40.50	49.18	18.09	7.90
899	37.86	37.39	46.58	17.98	8.24
900	70.89	48.24	60.68	22.96	10.77
901	71.74	49.27	59.85	23.51	11.19
902	96.78	41.02	51.41	22.19	10.32
903	100.00			22.54	10.34
904	59.68	25.86	29.76	13.73	6.15
905	25.87	20.99	25.27	10.83	5.41
906	9.44	15.05	15.01	23.90	25.03

Results are expressed as the mean of three spectral replicates. CW: control human whey; PW: pasteurized human whey; FDW: freeze-drying human whey; SDW: human whey applied spray drying; HHPW: human whey applied high hydrostatic pressure.

in all evaluated samples.[6] Therefore, the study of the TAG lipid profile becomes relevant, as the stereospecific numbering (Sn) defines the position in which fatty acids in the TAG molecule, which consequently determines their lipid nutritional quality, influence human metabolism, such as digestion, absorption, and distribution in the organism.[18]

Fatty acids composition by GC-FID

Defining the composition of fatty acids allows the detailing of the nutritional and physical-chemical value of foods. According to Table 2, 29 fatty acids were identified and quantified (mg g^{-1} of lipid) in human whey samples applied to different processes.

The highest concentrations found specify in the PW and FDW samples were the oleic acid (O, 18:1n-9), while in the SDW and HHPW samples the fatty acid palmitic (P, 16:0) is found with a higher concentration, and for CW was lauric acid (La, 12:0). Thus, the highest concentration of SFA presents in the samples was palmitic acid (P, 16:0), except in the CW sample that had lauric acid (La, 12:0) who it was the majority ($p < 0.05$). The fatty acid 12:0 is related to antimicrobial activity, these are incorporated into the lipid layer, where they cause instability of the lipid membrane, which results in the rupture of the lipid coating and death of the microorganism.[42] Still, generally in human milk, the fatty acid 16:0 were found in the central position (i.e., Sn-2) of the TAG molecule, in this way, it is easily absorbed, resulting in several benefits for the newborn, such as improvement in intestinal discomfort and reduction of colic, in addition, it also influences the levels of neurotransmitter anandamide which has an analgesic effect.[43]

In the literature, it is found that the fatty acids 15:0, 17:0, and 21:0 can be related to maternal food intake, which generally allows these fatty acids as markers of ruminant fat, extra virgin olive oil or eggs.[44-46] Other studies have also found odd-carbon FAs in human milk.[5,18,47,48] In addition, it is noteworthy that in the present studies the process showed potential for the maintenance of these fatty acids.

The MUFA found in greater quantity in all samples was oleic acid (O, 18:1n-9), thus agreeing with the studies by Manin *et al.*[19] who evaluated the lipid quality of freeze-dried human milk for six months. It also corroborates the results of Rydlewski *et al.*,[49] who evaluated the lipid profile and fatty acid composition in human milk, and both studies determined oleic acid as the highest concentration of MUFA. Manin *et al.*[17] mention the importance of fatty acid 18:1n-9 in the diet of infants as an influential source of energy, and brain structural component, in addition to aiding in the absorption of fat by the intestine.

Table 2. Composition of fatty acids of untreated human whey (CW) and other human whey applying different processes

Fatty acids composition	Fatty acid / (mg g^{-1} of the lipid)				
	CW	PW	FDW	SDW	HHPW
8:0	0.68 ± 0.05^{a}	0.07 ± 0.01^{b}	0.04 ± 0.01^{b}	0.09 ± 0.02^{b}	0.04 ± 0.00^{b}
10:0	14.35 ± 1.30^{a}	2.14 ± 0.05^{c}	3.82 ± 0.46^{c}	6.37 ± 0.76^{b}	2.11 ± 0.26^{c}
12:0	174.87 ± 6.07^{a}	34.48 ± 1.68^{c}	55.08 ± 10.78^{b}	68.30 ± 7.97^{b}	22.71 ± 0.18^{c}
14:0	87.58 ± 2.19^{a}	29.28 ± 1.43^{cd}	37.55 ± 4.83^{bc}	43.50 ± 4.63^{b}	22.01 ± 0.03^{d}
14:1n-9	0.09 ± 0.01^{b}	0.17 ± 0.02^{a}	0.17 ± 0.01^{a}	0.09 ± 0.01^{b}	0.12 ± 0.00^{b}
15:0	0.43 ± 0.02^{d}	0.79 ± 0.05^{b}	0.93 ± 0.05^{a}	0.62 ± 0.08^{c}	0.10 ± 0.02^{e}
16:0	90.54 ± 1.33^{a}	78.51 ± 2.52^{bc}	86.51 ± 6.85^{ab}	71.24 ± 3.14^{cd}	65.38 ± 2.49^{d}
16:1n-7	0.33 ± 0.02^{b}	0.71 ± 0.04^{a}	0.66 ± 0.05^{a}	0.37 ± 0.07^{b}	0.45 ± 0.03^{b}
16:1n-9	1.77 ± 0.02^{c}	3.31 ± 0.05^{a}	3.18 ± 0.06^{a}	1.88 ± 0.09^{c}	2.30 ± 0.09^{b}
17:0	0.61 ± 0.06^{c}	1.12 ± 0.06^{a}	0.88 ± 0.09^{b}	0.91 ± 0.04^{b}	0.88 ± 0.05^{b}
17:1n-9	0.21 ± 0.02^{c}	0.44 ± 0.02^{a}	0.44 ± 0.00^{a}	0.34 ± 0.02^{b}	0.35 ± 0.07^{ab}
18:0	32.43 ± 0.41^{a}	26.80 ± 0.57^{b}	27.60 ± 0.79^{b}	24.91 ± 0.69^{c}	24.55 ± 0.73^{c}
18:1n-9	99.48 ± 1.34^{b}	112.83 ± 1.74^{a}	94.59 ± 3.78^{b}	64.95 ± 2.91^{c}	63.99 ± 2.02^{c}
18:2n-6	53.28 ± 0.74^{b}	79.42 ± 0.10^{a}	72.00 ± 5.95^{a}	36.49 ± 2.72^{c}	37.03 ± 1.44^{c}
18:2n-6 *cis*9,*trans*11	0.10 ± 0.00^{b}	0.21 ± 0.02^{a}	0.19 ± 0.01^{a}	0.13 ± 0.00^{b}	0.12 ± 0.01^{b}
18:2n-6 *trans*10,*cis*12	0.20 ± 0.02^{a}	0.14 ± 0.01^{b}	0.15 ± 0.00^{b}	0.09 ± 0.02^{c}	0.17 ± 0.02^{ab}
18:3n-3	1.25 ± 0.05^{c}	2.34 ± 0.15^{b}	3.67 ± 0.37^{a}	1.46 ± 0.20^{c}	1.65 ± 0.12^{c}
18:3n-6	0.08 ± 0.01^{d}	0.30 ± 0.02^{a}	0.31 ± 0.03^{a}	0.15 ± 0.00^{c}	0.25 ± 0.00^{b}
20:0	0.85 ± 0.00^{a}	0.56 ± 0.06^{b}	0.56 ± 0.11^{b}	0.50 ± 0.03^{b}	0.49 ± 0.06^{b}
20:1n-9	0.67 ± 0.00^{b}	0.69 ± 0.02^{ab}	0.72 ± 0.04^{a}	0.38 ± 0.01^{d}	0.58 ± 0.01^{c}
21:0	0.49 ± 0.00^{b}	0.67 ± 0.09^{a}	0.63 ± 0.00^{a}	0.56 ± 0.02^{ab}	0.62 ± 0.05^{a}
20:3n-6	0.38 ± 0.02^{c}	0.85 ± 0.01^{ab}	0.94 ± 0.00^{a}	0.72 ± 0.06^{b}	0.78 ± 0.02^{b}
20:3n-3	0.40 ± 0.03^{e}	1.01 ± 0.02^{a}	0.87 ± 0.04^{b}	0.55 ± 0.00^{d}	0.74 ± 0.05^{c}
20:4n- 6	0.06 ± 0.00^{c}	0.16 ± 0.02^{b}	0.14 ± 0.01^{b}	0.21 ± 0.00^{a}	0.13 ± 0.02^{b}
22:0	0.54 ± 0.04^{a}	0.48 ± 0.07^{a}	0.53 ± 0.07^{a}	0.25 ± 0.01^{b}	0.50 ± 0.04^{a}
20:5n-3	0.10 ± 0.01^{c}	0.21 ± 0.03^{ab}	0.19 ± 0.01^{b}	0.18 ± 0.02^{b}	0.24 ± 0.02^{a}
24:0	0.36 ± 0.02^{ab}	0.38 ± 0.01^{ab}	0.43 ± 0.03^{a}	0.34 ± 0.05^{bc}	0.27 ± 0.04^{c}
24:1n-9	0.09 ± 0.00^{d}	0.22 ± 0.02^{a}	0.21 ± 0.00^{a}	0.13 ± 0.01^{c}	0.17 ± 0.00^{b}
22:6n-3	0.16 ± 0.00^{c}	0.34 ± 0.02^{a}	0.34 ± 0.00^{a}	0.26 ± 0.00^{b}	0.27 ± 0.01^{b}
Σ(n-3)	1.91 ± 0.01^{d}	3.90 ± 0.08^{b}	5.07 ± 0.40^{a}	2.45 ± 0.22^{cd}	2.90 ± 0.11^{c}
Σ(n-6)	54.09 ± 0.72^{b}	81.08 ± 0.06^{a}	73.72 ± 6.09^{a}	37.79 ± 2.68^{c}	38.48 ± 1.43^{c}
ΣSFA	403.72 ± 11.45^{a}	175.28 ± 6.15^{cd}	214.58 ± 23.46^{bc}	217.60 ± 17.17^{b}	139.66 ± 3.04^{d}
ΣMUFA	102.64 ± 1.28^{b}	118.37 ± 1.76^{a}	99.98 ± 3.76^{b}	68.14 ± 2.96^{c}	67.95 ± 2.14^{c}
ΣPUFA	56.00 ± 0.70^{b}	84.98 ± 0.02^{a}	78.79 ± 6.49^{a}	40.24 ± 2.90^{c}	41.38 ± 1.32^{c}
ΣTotals FA	562.37 ± 13.44^{a}	378.62 ± 7.93^{bc}	393.36 ± 33.70^{b}	325.98 ± 23.03^{c}	248.99 ± 6.50^{d}

Results are 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; CW: control human whey; PW: pasteurized human whey; FDW: freeze-drying human whey; SDW: human whey applied spray drying; HHPW: human whey applied high hydrostatic pressure; PUFA: polyunsaturated fatty acid; SFA: saturated fatty acid; MUFA: monounsaturated fatty acid.

The PUFA found in greater quantity was linoleic acid (L, 18:2n-6), this is considered a strictly essential FA and a precursor to arachidonic acid (AA, 20:4n-6), and moreover, both were present in the samples. However, other long-chain polyunsaturated fatty acids (LC-PUFAs) were also found, such as alpha-linolenic acid (Ln, 18:3n-3), a precursor of eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3), considered sources important in visual and brain development during perinatal progress. Therefore, it can be affirmed that all samples of human whey present both strictly essential fatty acids and their precursors, essential fatty acids.

Long-chain polyunsaturated fatty acids, considered essential, have several important functions for the baby

as they are responsible for cognitive, visual, and immune development, besides protecting against allergies, asthma, and childhood obesity. For babies, it is necessary to directly consume these fatty acids from breastfeeding because, despite these fatty acids being metabolized by the human body, the baby does not have the total capacity to convert them, due to physiological immaturity.[43]

When evaluating conjugated linoleic acid *cis*9,*trans*11, the results of the human whey samples when subjected to different process did not show a statistical difference between the PW and FDW samples, thus representing the highest concentrations, while the other CW, SDW, and HHPW samples also showed no differences between them, but with lower results. For conjugated linoleic acid *trans*10,*cis*12, the CW and HHPW samples showed higher values, while the others were similar between them, resisting the applied process.

Still, about Table 2, in the sum of total fatty acids (Σtotals FA), the sample that presented the highest value was CW (562.37 ± 13.44), and the lowest was HHPW (248.99 ± 6.50). The evaluated samples present their highest quantification in the sum of saturated fatty acids (ΣSFA), followed by the sum of monounsaturated fatty acids (ΣMUFA) and the sum of polyunsaturated fatty acids (ΣPUFA) when compared to each other, respectively. Regarding the ΣTotals FA, the ΣSFA of the samples represents about 72% (CW) to 46% (PW), while the ΣMUFA ranged from 31% (PW) to 18% (CW), and ΣPUFA from 22% (PW) and close to 10% for CW. Representing in general, that there was a change in the composition of fatty acids when applying the process.

Therefore, the fatty acids composition found indicates that the less favorable process was the HHPW, as it presented lower values for the sum of fatty acids ΣSFA, ΣMUFA, ΣPUFA and consequently ΣTotals FA, followed by SDW, which also has a low content when verified between the process. In comparison, PW and FDW were similar in terms of ΣTotal FA composition, so these results found in the study corroborate with those of Manin *et al*.,[19] who showed that the fatty acid composition of human milk does not show significant differences between pasteurized and freeze-dried human milk.

Lipid nutritional quality

The fatty acids composition has individual characteristics, influenced by factors such as storage, freezing, and thawing conditions, including process temperature, therefore, to determine the lipid nutritional value, the relationships between individual fatty acids or their groups are indicated. Table 3 shows the results of the lipid nutritional quality indices of control human whey (untreated) and in different processes.

PUFA/SFA is the most commonly used index to assess the nutritional value of foods, as it hypothesizes that PUFAs can restrict low-density lipoprotein (LDL) cholesterol and lower serum cholesterol levels, while the SFAs shift to high levels of serum cholesterol.[50] Determination techniques stimulate body composition by comparing diets based on the PUFA/SFA ratio and are associated with resting energy expenditure and/or fat oxidation after exposure to a PUFA-rich diet.[51,52] There are stipulated values for foods such as seaweed, meat, fish, shellfish, and diet products, with results ranging from 0.42-2.12, 0.11-2.042, 0.50-1.62, 0.20-2.10, 0.02-0.175, respectively, where both sources show different effects on cardiovascular health. In the

Table 3. Lipid nutritional quality indices of untreated human whey (CW) and other human whey applying different processes

Index	Sample				
	CW	PW	FDW	SDW	HHPW
ΣPUFA/SFA	0.14 ± 0.00^{e}	0.47 ± 0.02^{a}	0.36 ± 0.01^{b}	0.17 ± 0.00^{d}	0.28 ± 0.01^{c}
Σ(n-6)/(n-3)	28.29 ± 0.59^{a}	20.78 ± 0.46^{b}	14.54 ± 0.06^{cd}	15.46 ± 0.32^{c}	13.29 ± 1.00^{d}
LA/ALA	42.55 ± 2.36^{a}	34.01 ± 2.26^{b}	19.63 ± 0.36^{d}	25.14 ± 1.59^{c}	22.59 ± 2.54^{cd}
Σ(EPA) + (DHA)	0.26 ± 0.01^{c}	0.55 ± 0.05^{a}	0.53 ± 0.02^{a}	0.44 ± 0.02^{b}	0.51 ± 0.03^{ab}
H/H	0.44 ± 0.01^{d}	1.37 ± 0.04^{a}	0.95 ± 0.06^{b}	0.56 ± 0.02^{c}	0.93 ± 0.01^{b}
AI	3.88 ± 0.05^{a}	1.13 ± 0.04^{d}	1.63 ± 0.11^{c}	2.89 ± 0.12^{b}	1.61 ± 0.03^{c}
TI	2.50 ± 0.02^{a}	1.21 ± 0.03^{e}	1.48 ± 0.03^{d}	2.31 ± 0.01^{b}	1.81 ± 0.01^{c}
HPI	0.26 ± 0.00^{d}	0.88 ± 0.03^{a}	0.62 ± 0.04^{b}	0.35 ± 0.01^{c}	0.62 ± 0.01^{b}

Results are 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. CW: control human whey; PW: pasteurized human whey; FDW: freeze-drying human whey; SDW: human whey applied spray drying; HHPW: human whey applied high hydrostatic pressure; PUFA: polyunsaturated fatty acid; SFA: saturated fatty acid; n-6: fatty acid from the omega-6 group; n-3: fatty acid from the omega-3 group; LA: linoleic acid; ALA: α-linolenic acid; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid; MUFA: monounsaturated fatty acid; H/H: hypocholesterolemic/hypercholesterolemic fatty acids; AI: atherogenicity indices; TI: thrombogenic index; HPI: health promotion index.

present study, all samples showed significant differences for the PUFA/SFA index, ranging from 0.14 ± 0.00 (CW) to 0.47 ± 0.02 (PW), which allows, therefore, new equations to be used to assess the effect of fatty acid composition on cholesterol.[50]

The Σ(n-6)/(n-3) ratio showed no statistical difference between FDW and HHPW, and between FDW and SDW, although they presented statistical differences between the other samples. The highest value found was for CW (28.29 ± 0.59), while the HHPW had the lowest value (13.29 ± 1.00). The estimation of the n-6/n-3 ratio is important since it is understood that the series of fatty acids (n-3, n-6, n-7, and n-9) compete with each other for the metabolic pathways of elongation and desaturation for the proper functioning of the organism. Furthermore, the n-6/n-3 ratio is important to ensure the balance of anti (n-3) and pro-inflammatory (n-6) activities.[50] It refers to the high value displayed coming from the fatty acid of the n-6 series (L, 18:2n-6) found in the composition of fatty acids, this is an indispensable structural component of certain dermal ceramides with importance for the maintenance of the barrier of epidermal water.[50,53]

The linoleic/α-linolenic (LA/ALA) ratio is a guide associated with the diet, acting as a coadjuvant to maintain neurodevelopment and brain function under normal conditions.[54] Among the samples, there was a variation from 19.63 ± 0.36 (FDW) to 42.55 ± 2.36 (CW). It is understood that smaller proportions are beneficial to human health. Toro-Ramos *et al*.[55] identified an LA/ALA ratio of 23:1 and elucidated that excess LA can compete for the same enzymes in desaturase and elongase during the metabolism of EPA and DHA precursors, due to competition in the synthesis of ALA. Van Der Westhuyzen *et al*.[56] identified an LA/ALA content of 40:1 in the milk of infants on a traditional corn diet,[57] thus corroborating the results of the findings obtained in the present study. However, the standards for infant formulas stipulated by CODEX mean an LA/ALA ratio between 5:1 and 15:1.[58] The LA/ALA ratio is also often elucidated as an influencer of the immune system, however, Jensen *et al*.[59] recommend in-depth studies of the effects of LA/ALA ratios on infant growth, before the adoption of any regimented indication. Chen *et al*.[50] state that the LA/ALA ratio does not have a significant impact on tissue development during older age, therefore, it is suggested to enable the use of processed human whey during food introduction, as a food supplement for childhood.

Regarding the Σ(EPA) + (DHA) results, the PW, FDW, and HHPW samples showed no significant differences ($p > 0.05$), with values of 0.55 ± 0.05, 0.53 ± 0.02 and 0.51 ± 0.03, respectively. The highest value found was in the sample PW (0.55 ± 0.05), while the lowest value was the CW (0.26 ± 0.01). Studies claim that EPA and DHA fatty acids play roles in the development of nerve and brain cells, from the prenatal period to after birth.[54] Although there is no specific recommendation for adequate intake of EPA for children up to six months of age, the Food and Agriculture Organization of the United Nations[60] recommends that the intake of EPA for children aged 2 to 4 years is 100 to 150 mg day^{-1}. For breastfed babies, an intake of at least 20 mg day^{-1} of DHA is indicated to reach the amount accumulated in metabolism.[54] Therefore, human whey presents these fatty acids in the sample, and consumption becomes responsible for a portion of the intake of EPA + DHA.

The proportion of fatty acids hypocholesterolemic/hypercholesterolemic (H/H) showed significant differences between samples, except for the FDW and HHPW samples, which showed statistical similarity to each other ($p > 0.05$). Among them, the highest result was in the PW (1.37 ± 0.04), while the CW showed the lowest (0.44 ± 0.01). According to Chen and Liu,[50] it is required out that the H/H index will have ratios ranging from 0.32 to 1.29 for dairy products. Evidence points that the H/H ratio indicates the specific effects of fatty acids on cholesterol metabolism, therefore it is recommended that increasing the H/H ratio in the lipid portion of human milk benefits the organism human, since hypocholesterolemic fatty acids act in the reduction of low-density lipoprotein (LDL), preventing cardiovascular diseases.[26]

Other relevant findings restrict themselves to the atherogenicity (AI) and thrombogenicity (TI) indices, which become more appropriate for characterizing estimates of the atherogenic and thrombogenic potential of the diet than the PUFAs/SFAs ratio, suggesting that these indices are associated with the blockage of the arteries.[50] The main groups of SFAs are related to pro-atherogenic, which include 12:0, 14:0, and 16:0, because they are considered to favor the adhesion of lipids to the circulatory and immune systems. However, unsaturated fatty acids are pointed as antiatherogenic, as they inhibit plaque accumulation and reduce the levels of phospholipids and cholesterol.[51] For TI, the tendency to form clots in blood vessels is characterized, and thus the functions of anti-thrombogenic fatty acids (MUFA, groups n-3, and n-6) contribute between pro-thrombogenic fatty acids (12:0, 14:0, 16:0).[27]

Because of this, the AI reduced could be related to a healthy lipid index, capable of preventing the incidence of coronary heart disease, and is related to healthy lipid indexes.[51] AI showed no significant difference between FDW and HHPW, however, all other process showed a statistical difference. The highest value found was related

to CW (3.88 ± 0.05) and the lowest value to PW (1.13 ± 0.04), in which, when comparing the samples, the quantification of 12:0, 14:0, 16:0 was higher in the sample CW, while the ΣMUFA, Σn-3, Σn-6 were higher in PW, thus corroborating the results obtained. AI is an efficient marker for predicting the risk of cardiovascular disease, as it considers an available evidence of specific saturated fatty acids considered proatherogenic.[50] Modifications in the lipid profile of milk consequently affect the AI value.[61] Chen and Liu[50] prescribed AI variations in milk between 1.42 to 5.13, with differences between different mammalian species and lactation stages.

The thrombogenicity index (TI) showed a statistical difference concerning all process, with the highest value being in the CW sample (2.50 ± 0.02) and the lowest in the PW (1.21 ± 0.03). Balthazar *et al*.[62] obtained higher values, between 2.75 ± 0.01 to 5.28 ± 1.48, and the authors also consider their results healthy for human consumption. The reduction in the values of these indices (AI and TI) is important, as it occurs both due to the decrease in the concentrations of fatty acids 12:0, 14:0, and 16:0 (atherogenic and thrombogenic) and due to the increase in MUFA and PUFA, which are considered beneficial to health.

The authors did not find evidence in the literature on reference values for childhood consumption for AI and TI, however, according to Santos-Silva *et al*.,[26] these indices indicate the potential for platelet aggregation, therefore low levels are desirable, as they indicate a lipid dietary quality and its potential effects on the development of coronary diseases. Thus, the reduction of these indices demonstrates the potential of applying process for the most appropriate nutritional quality for health, since diets that include products whose fatty acid composition is lower than AI and TI can help reduce the risk of disease in coronary arteries due to the better nutritional quality of these products.[62]

The health promotion index (HPI) proposed by Chen *et al*.[28] enables an evaluation of the lipid nutritional value which focuses on the protective effect of fatty acid composition on cardiovascular disease, inversely of AI. In researches for dairy products, the indicated values range from 0.16 to 0.68.[50] It was possible to observe in the present study that the HPI is between 0.26 ± 0.00 (CW) to 0.88 ± 0.03 (PW), with significant differences ($p > 0.05$), therefore, the PW sample showed a value higher than expected. This occurrence can be justified by the statement by Chen *et al*.,[28] who points out that the polyunsaturated content in the profile of dairy products interferes with viscosity, and the application of temperature causes its release, that is, when consumed, it decreases plasma concentrations of cholesterol and will promote human health. In this sense, it is worth noting that the sample with the highest value found (PW) had an applied pasteurization temperature of 62.5 ºC, while the CW sample was not submitted to any treatment.

Another study relates fatty acids from buffalo milk powder produced by spray drying with technological aspects, lipid oxidation, and conversion into free fatty acids.[63] The HPI of the SDW sample (0.44 ± 0.02), applied spray drying, can favor the degradation of fatty acids, consequently clarifying the value obtained in the sample. While the FDW and HHPW samples did not show significant differences ($p < 0.05$), which elucidates the efficiency of these processes employed, as demonstrated by Neia *et al*.[18] and Manin *et al*.,[17] who indicate them as viable alternatives for the treatment of human milk, maintaining adequate lipid quality.

Barreto *et al*.[64] describe that the nutritional quality of the lipid fraction composed of amounts of 18:1n-9 and 18:2n-6 greater than 18:0 and 16:0 supports the recommendations for humans. Differences in the composition of fatty acids and the lipid nutritional quality between the samples can be explained by the individual application of the processes performed, consequently modifying their lipid profile, since the lipid fraction is altered.

Concentration of cytokines IL-10 and TGF-β1

The content of IL-10 and TGF-β1 cytokines obtained from control human whey (untreated), and after the processes of pasteurization, freeze-drying, spray drying, and high hydrostatic pressure are described in Table 4.

Evaluating the influence of process on the concentration of IL-10 and TGF-β1 becomes primordial, as they are

Table 4. Concentration of cytokines IL-10 and TGF-β1 in untreated human whey (CW) and other human whey applying different processes

Cytokine	Sample				
	CW	PW	FDW	SDW	HHPW
IL-10 / (pg mL^{-1})	1.65 ± 0.91^{c}	3.21 ± 0.92^{bc}	6.11 ± 0.40^{ab}	7.43 ± 1.67^{a}	8.09 ± 2.17^{a}
TGF-β1 / (pg mL^{-1})	413.70 ± 18.93^{ab}	461.42 ± 57.14^{a}	210.55 ± 12.34^{d}	311.44 ± 13.40^{c}	339.66 ± 4.45^{bc}

Results are expressed as mean ± standard deviation of duplicates. Values with different letters on the same line are significantly different ($p < 0.05$) by Tukey's test. CW: control human whey; PW: pasteurized human whey; FDW: freeze-drying human whey; SDW: human whey applied spray drying; HHPW: human whey applied high hydrostatic pressure; IL-10: interleukin-10; TGF-β1: transformation factor-β1.

indispensable anti-inflammatory cytokines detected in serum fractions.[65] When comparing IL-10 levels in CW samples and after the processes, the PW sample was the only one that remained similar in IL-10 levels. In contrast, the IL-10 concentrations increased for FDW (6.11 ± 0.40 pg mL^{-1}), for SDW (7.43 ± 1.67 pg mL^{1}), and HHPW (8.09 ± 2.17 pg mL^{-1}) process when compared with the control (1.65 ± 0.91 pg mL^{-1}). On the other hand, the concentration of TGF-β1 remained similar between the PW (461.42 ± 57.14 pg mL^{-1}) and CW (413.70 ± 18.93 pg mL^{-1}) samples ($p > 0.05$). However, the levels of the TGF-β1 decreased in the FDW (210.55 ± 12.34 pg mL^{-1}), SDW (311.44 ± 13.40 pg mL^{-1}), and HHPW (339.66 ± 4.45 pg mL^{-1}) samples, when compared to CW (413.70 ± 18.93 pg mL^{-1}).

Cytokines are soluble multifunctional peptides, commonly present in picograms in human milk, and even so act as autocrine-paracrine factors by binding to specific cell receptors, with functions of the immune system.[66] Some factors become relevant to the maintenance of cytokine levels, namely, storage conditions, such as temperature, freezing and thawing cycles, therefore their exposure to handling and process conditions can influence the stability of the cytokine structure.[67] Thus, it was observed that the drying processes (freeze-drying and spray drying) were appropriate to maintain the biological structure and guarantee immune functions in the human milk matrix.

Still, looking at IL-10 levels (Table 4), Wesolowska *et al.*[13] associated the preservation of cytokine activity with high pressure, because the protective effect of the technology remains intimately dependent on intermolecular interactions, such as hydrogen bonding and the structure of the proteins that the matrix has, that is, it reinforces the levels of IL-10 defined in HHPW. Untalan *et al.*[68] culminate in their study that pasteurization reduces the concentration of IL-10 by 66.67% in human milk, while in the present study when compared the PW to the other methods applied FDW, SDW, and HHPW had a reduction of 47.46 to 60.32%, in addition, the study adds that the pasteurization also does not affect TGF-β1 levels, as found in the present study.

This is the first study that evaluated cytokines in HW processed by freeze-drying, spray drying, and high hydrostatic pressure. In general, cytokines are fundamental components, as they are multifunctional peptides that bind to cell receptors and act in the immune system.[69] Previously, it was consolidated by different authors that the cytokines of the IL group are perceptible in the aqueous phase of human milk for IL-2, IL-4, IL-5, and IL-8.[70,71] Therefore, it can be reported that the levels of cytokines found after process became highly available to IL-10. This finding can be justified because the process is indirectly related to the liberation of non-bound IL protein, making it capable of binding to the IL antibody spontaneously in the ELISA assay.[72,73]

It was expected that after the pasteurization the TGF-β1 level would obtain a superior result, as well as observed by Riskin,[74] TGF-β1 concentrations are influenced by the liberation of fatty or cellular compartments in the aqueous fraction, in this case, that involved granulocytes macrophages of the evaluated matrix. The decrease after the application of spray drying corroborates the statements by Silva *et al.*,[15] who identified a 19% reduction in the yield of growth factors in the serum fractions of cow's milk, in addition to defining the concentration of TGF-β to be intensely dependent on the integrity of the protein content.

IL-10 and TGF-β1 are anti-inflammatory cytokines that play a fundamental role in the regulation of the immune response of newborns. IL-10 is involved in the maturation of cells in the gastrointestinal tract, in the regulation of intestinal inflammation, and in the tolerance of newborn intestinal microbiome antigens. However, TGF-β1 is an abundant cytokine in human milk, that is, its functions in the newborn's organism are the control of the immune system and stimulation of cell growth, especially of the connective tissue and wound healing.[65] Thus, in the present study, the levels of IL-10 in all samples obtained adequate levels, however, the opposite was observed about TGF-β1, although the PW sample is the only sample that presented an advantage over the others, where maintained TGF-β1 concentrations similar to CW.

Principal component analysis

PCA was performed to measure the similarity between the samples, so it was possible to assess by this analysis that 86.8% of the data was justified, with PC1 representing 57.4% of the data, thus being the majority axis, and PC2 explaining approximately 29.4% of the data. The variables (FAs, lipid nutritional indices, and immunological analyses) were represented in the form of loadings, and the samples are represented in the form of scores, as can be seen in Figure 3.

The variables on the right of PC1 contribute positively, as we have EPA, DHA, and AA, and the variables on the left contribute negatively, where it is possible to observe AI and TI. The PCA analysis showed that scores (samples) 2 and 3 are similar to each other, corroborating with the analysis of the fatty acids composition. The loadings did not present an equal distance from the center of the graph, thus evidencing that the variables had different significance

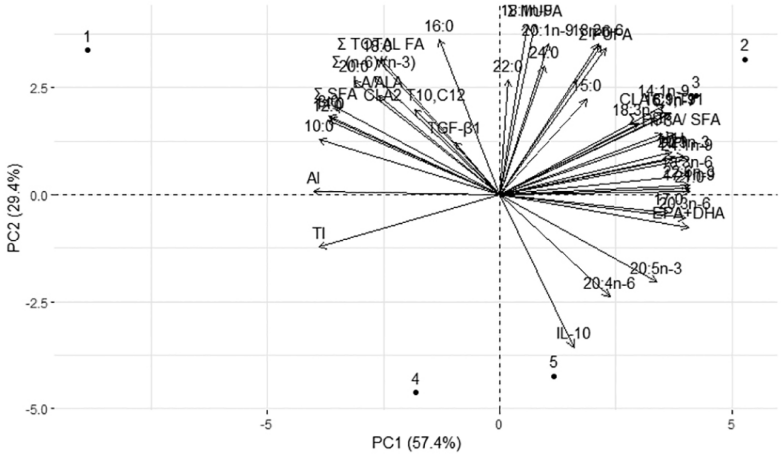

Figure 3. PCA biplot graph of the fatty acids composition, lipid nutritional indexes and immunological analysis of the samples untreated human whey (CW) and other human whey applied different processings. (1) CW; (2) PW; (3) FDW; (4) SDW; (5) HHPW.

in the context of expressing the PCA. In general, it is noted that the processes showed divergences in the caused effects, and, furthermore, the pasteurization and freeze-drying were the vast majority similar in terms of fatty acid composition, and the high hydrostatic pressure influenced IL-10, while no treatment proved to be positive for TGF-β1 maintenance due to being only distinguished in the PCA for CW.

Conclusions

Processes with the potential for conservation of dairy products were evaluated in the present study, evidenced by the lipid profile of triacylglycerols and its fatty acids composition, which were demonstrated by the influence on the lipid quality of the product after different processes. Among the treatments employed, it was evident that high hydrostatic pressure and spray drying caused significant changes in the configuration of the lipid profile. On the other hand, the rest techniques showed similar behavior, preserving the majority of its lipid constituents, considered beneficial to health.

Regarding cytokine levels, all samples were adequate for IL-10, with high concentrations after process; on TGF-β1, only pasteurization presented an advantage over the others, maintaining its concentration.

Consequently, on the PCA, it was observed that the effects of pasteurization and freeze-drying were similar to each other in terms of fatty acid composition. High hydrostatic pressure is characterized by high concentrations of the cytokine IL-10. However, the spray drying differed from the other processes in all aspects evaluated, remaining only singular to TI.

Given the alterations in the quality of the treated human serum investigated, the applications of pasteurization and freeze-drying showed many similarities, in addition to being generally adequate. In this sense, it was observed that after each process application, individual characteristics were presented in the conservation of nutrients, this way, when indicating process to future applications, the interest of the quality sought, and the individual organism of each consumer should be relevant.

Because of the above, processed human whey can be promising as a complementary product for introducing infant food, as it guarantees the conservation of its nutrients. Therefore, the findings in this study demonstrate the potential of human whey applied to different processes, for use as a complementary product for the introduction of infant food, due to the guarantee of the quality of essential nutrients for children's nutrition. In this context, research on the impact on the lipid profile and on the cytokines IL-10 and TGF-β1, both studies are scarce in the literature and can be considered as starting points for future studies that confirm the hypotheses found in our study.

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Author Contributions

Eloize S. Alves was responsible for conceptualization, methodology, data curation, formal analysis, investigation, writing original draft, review and editing; Matheus C. Castro for methodology, formal analysis, writing original draft; Bruno Henrique F. Saqueti for investigation, visualization, writing original draft; Luciana P. Manin for methodology; Josiane B. Alencar for formal analysis, data curation; Joana Maira V. Zacarias for formal analysis; Andressa Rafaella S. Bruni for writing review and editing; Grasiele S. Madrona for writing review and editing; Jeane E. L. Visentainer for methodology, resources; Marcelo Cristianini for visualization, resources; Oscar Oliveira Santos for supervision, resources; Jesui V. Visentainer for supervision, conceptualization, funding acquisition, resources.

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