

# Effect of Pasteurization, Freeze-drying and Spray Drying on the Fat Globule and Lipid Profile of Human Milk

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**Abstract** Human milk is the ideal food to nourish newborn babies; it contains important nutrients: proteins, carbohydrates, fat, vitamins and minerals, which are needed to provide a good health to the infants. Milk fat provides about 50% of energy to infants and its fatty acids are essential for brain and retina development. Therefore, analysis of the lipid fraction of human milk is an important task, especially when this milk is processed. The objective of this research was to study the effect of pasteurization, freeze-drying and spray drying on some characteristics of human milk fat. The fatty acid profile was analyzed by HPLC and gas chromatography. Fat content, globule size and distribution were measured. The HPLC method for the analysis of fatty acids showed accuracy, precision and linearity in the concentration range studied. Non-significant differences in fat content between the different processes were found; however, there was a decrease of 23% in the fat content of spray dried milk. The fat mean globule size decreased considerably in all treatments, varying from 2138 to 529 nm. The size distribution of fat globules increased during pasteurization and drying from 0.24 in raw milk to 0.78 in pasteurized milk. With respect to the fatty acid profile, we found that human milk samples had an elevated content of palmitic (27%), and oleic (30%) acids and significant variations were observed in the pasteurized samples for oleic and linoleic acid. Preservation processes applied to human milk caused a decrease on the fat globule diameter; the change in size increased the surface area and could improve the bioavailability of the fat components. This is the first report of human milk drying as a preservation method.

**Keywords:** human milk fat, fatty acids, pasteurization, lyophilization, spray drying

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## 1. Introduction

Human milk is recognized as a nutritional and immunologically important food, appropriate for newborn babies. It reduces the risk of disease and death, and contains nutritional components such as lactose, lipids, proteins, vitamins, immunoglobulins, hormones, growth factors, enzymes and protective agents, which are in suitable concentrations to contribute to the correct growth and development of the newborn ([1,2]).

Water is the major constituent of human milk (90%), that contains ca. 10% of solids; of which, the main components are lactose (6.7 to 7.8 g/dL), fat (3.2 to 3.6 g/dL) and protein (0.9 to 1.2 g/dL). This composition may be modified by factors such as, individual characteristics of the mother, lactation cycle, diet, number and length of gestation, seasonal and geographic factors ([3,4]). The most variable component is fat, which provides nearly 50%

of the energy, and facilitates the transport and absorption of fat-soluble vitamins to the infant ([4,5]).

Fat in human milk comes from fat depots of the mother as well as from endogenous synthesis by the mammary gland [6]. Milk fat is found in form of globules as part of a colloidal system, which is formed in the mammary gland by a biological mechanism of assembly of intracellular lipid droplets through the plasma membrane of the epithelial cells ([7,8]).

The fat globule in human milk has a size distribution ranging from 1 to 4  $\mu\text{m}$ . It is formed by a triacylglycerol core covered by a trilayer membrane composed of phospholipids, proteins, sphingolipids, cholesterol, glycoproteins and enzymes ([8,9]). The triacylglycerols present in the fat globule contain fatty acids involved in brain development and visual acuity. It is also important in the process of synthesis of prostaglandins and eicosanoids and in the calcium absorption process, which is necessary for infant development [10]. Palmitic and oleic acids are found in greater proportion in human milk. Saturated fatty acids

represent nearly 41%, while polyunsaturated fatty acids constitute 27% and monounsaturated fatty acids comprise 31% of the total fatty acids [5]. The differences in size, distribution and composition of fatty acids is attributed to dietary habits and the genetics of the mother, as well as the lactation stage, among other factors ([7,11,12]). Regarding the lipid content and given its biological importance to the health of infants, several studies have focused on the analysis of the fatty acids profile by gas chromatography (GC); which is currently the most widely used method. However, in this methodology, milk samples need to be methylated, which could be a laborious process, and also can lead to loss of highly volatile short-chain fatty acid methyl esters in the esterification reaction. Additionally, carrier gases, namely He or N<sub>2</sub> are expensive ([13,14,15,16,17]). Analysis by HPLC has some advantages over CG, since the sample does not require preparation before injecting and the mobile phase may be less expensive than gases used for CG.

Pasteurization is a process commonly used to preserve human milk in the human milk banks (HMB). Different reports have shown that pasteurization can produce loss of nutrients in breast milk, as the loss of bioactive proteins, lipids and vitamins. Accordingly, new technologies are being developed to obtain safe human milk and maintaining its nutritional qualities [18]. Consequently, the aim of this study was to analyze the composition of fatty acids in human milk and the behavior of the lipid fraction after pasteurization, freeze-drying and spray drying, as an approach to evaluate these methods for the preservation and handling of human milk.

## 2. Materials and Methods

### 2.1. Reagents

Milli-Q water was employed for globule size determination. All reagents used were analytical grade or better, the HPLC grade solvents were obtained from Baker (Mexico city). Lauric (C12:0), myristic (C14:0), palmitic (C16:0), stearic (C18:0), oleic (C18:1) and linoleic (C18:2) acids as standards, together with all other reagents were purchased from Sigma-Aldrich (Mexico City).

### 2.2. Human Milk Sample Collection

The donors were healthy mothers selected from the Civil Hospital of Guadalajara, with ages ranging from 16 to 30 years; each signed a consent agreement. Sixty samples of mature human milk were collected under aseptic conditions using a sterile manual breast pump; the samples were then transported in an ice chest to the laboratory and immediately processed.

### 2.3. Milk Pasteurization

Pasteurization was conducted in sterile flasks containing the milk by immersion in a water bath that was maintained at 62, 72 or 85°C for 30, 15 and 5 minutes, respectively. After pasteurization, the samples were immediately transferred to an ice bath and analysed.

### 2.4. Freeze- and Spray Drying of Human Milk

Human milk was freeze-dried in a Liotop freeze-drier (São Carlos, SP, Brazil) at 10 pascals of vacuum and -

55 °C. For spray drying a LabPlant SD-Basic Spray Dryer (North Yorkshire, UK) was employed using a flow rate of 2 mL/min, a pressure 2.5 bar, inlet air temperature of 150 °C and outlet air temperature of 50 °C. Dehydrated samples were vacuum packed and further processed for lipid analysis.

### 2.5. Other Analysis of Human Milk

Lactose was quantified using the DNS method [19]; pH values were determined using a Thomas Scientific TS675 pH meter; proteins were measured by Lowry's method [20], and fat was extracted using Folch's method [21].

### 2.6. Fat globule Size Characterization

The fat globule size analysis was measured in human milk, under three conditions: fresh, pasteurized, freeze-dried and spray dried, according to [12]. Powdered milk was resuspended in Milli Q water to 10% total solids (w/v) and the fat globule size was expressed by the z-diameter, using laser light scattering through of a Zetasizer Nano-ZS90 (Malvern instruments, Malvern U.K.). Globule size distribution is reported as the polydispersity index (PDI), a dimensionless number ranging from 0 to 1.0. The surface charge of the globules (zeta potential) was measured using the Zetasizer. Samples were prepared diluting 25 µL of the nanoemulsion with 2000 µL of Milli-Q water and measurements were carried out at 25 °C. The fat globule size and zeta potential determinations were made in triplicate.

### 2.7. Analysis of Fatty Acids

For analysis of major fatty acids in mature human milk, a HPLC method was developed and validated, and the results were compared with those obtained by gas chromatography.

#### 2.7.1. Lipid Extraction

Human milk lipids were extracted by according to Folch [21], using chloroform:methanol (2:1, v/v). The solvent phase was evaporated and the extracted lipids were stored frozen until analysed.

#### 2.7.2. HPLC System and Conditions

Human milk lipids were hydrolyzed to obtain free fatty acids. Lipid hydrolysis was conducted by the method described by García and co-workers [22]. Free fatty acids obtained after hydrolysis of the human milk fat and mother solutions of lipid standards were solubilized in acetonitrile and filtered through a 0.22-µm nylon membrane filter before HPLC injection. The HPLC system was a Varian Prostar 210 equipped with a Perkin-Elmer LC-95 UV/Visible detector. An Agilent C18 Microsorb-MV column (250 x 4.6 mm, i.d. of 5 µm) was used for separation of fatty acids; the column was kept at 35 °C.

The mobile phase consisted of two phases; phase A consisted of water:acetonitrile (75:25 v/v) and phase B was made of 100% acetonitrile. Phase B was acidified with 0.2% of acetic acid, using a gradient system that started with a flow that consisted of 100% of phase A over 10 min, then a change from 100 to 85% of phase A in 5 min, followed by a linear gradient to reach 50% of phase

A in 15 minutes, then a linear gradient from 50% to 30% phase A in 15 min, and kept for another 10 minutes. Then the composition was returned linearly to the starting condition in 3 min. The flow rate was 1 mL/min and the UV detector was set at 205 nm. Chromatographic peaks were identified by comparing their corresponding retention times with those of true standards. The linearity of calibration curves for each fatty acid was obtained by triplicate, at a concentration range of 0.48 - 2.88 mg/mL for lauric acid, 0.2 - 1.33 mg/mL for myristic acid, 1.1 - 6.9 mg/mL for palmitic acid, 0.01 - 0.25 mg/mL for stearic acid, 0.1 - 1.8 mg/mL for oleic acid and 0.05 - 0.4 mg/mL for linoleic acid. The calibration curve for each fatty acid was verified by the correlation coefficient ( $R^2$ ). Repeatability was calculated by intra-day precision, analyzing three replicates of each fatty acid standards on the same day. Reproducibility was determined by inter-day precision, analyzing the same standards over three different days. The relative standard deviation (RSD) of the peak area was determined for repeatability and reproducibility, considering as the acceptance criteria that the RSD value must be  $\leq 5\%$ . The limit of detection (LD) and limit of quantification (LQ) were measured according to IUPAC,

$$LD = \frac{3SB}{m} \quad (1)$$

$$LQ = \frac{10SB}{m} \quad (2)$$

where SB is defined as the standard deviation of the blank and m is the slope of the calibration curve.

### 2.7.3. GC System and Conditions

For GC analysis, extracted lipid samples were derivatized using sodium methoxide 0.5N (Supelco, Bellefonte, PA). Methylation was carried out by adding 1 mL of sodium methoxide to 50  $\mu$ L of fat samples and allowed to rest at room temperature for 5 minutes. The reaction was stopped by adding 100  $\mu$ L of water and the methyl esters formed were extracted with 2 mL of hexane; then, 1  $\mu$ L of the sample was injected into the GC. Separation of fatty acids was done on a HP 6890 GC fitted with a HP-INNOWax capillary column (60 m x 0.25 mm x 0.25 mm film thickness) and a flame ionization detector (FID); the program temperature for the fatty acids separation was carried out according to [23].

## 2.8. Statistical Analysis.

Comparison of means by Tukey's test ( $p < 0.05$ ) was carried out using the Minitab statistical software v.16 (Minitab Inc., State College, PA).

## 3. Results and Discussion

### 3.1. Chemical Composition of Human Milk

The proximate composition of mature human milk and its pH value are shown in Table 1. The pH value measured was comparable with those reported by [24] and [25], who found a range of pH values between 6.70 and 7.2. No differences were found in fresh milk for lactose, protein

and fat content; the macronutrient content was similar to values reported by [26,27,28] in human milk samples from Brazil, Spain and Korea, respectively. The fat content in human milk from Mexican mothers (2.70 g/dL), was similar to that reported by [28] in milk samples from South Korea (3.1 g/dL) and [26] in samples from Brazil (2.9 g/dL); however our values were lower compared to other reports by [29] and [30], in samples from Australia (ca. 4 g/dL) and Germany (4.1 g/dL). It has been reported that fat is the component with the highest variability in human milk, attributed to factors such as: race, culture, individuality, diet and climate, among others ([11,31]). It has been reported that preservation processes applied to human milk, could affect its chemical composition and cause loss of nutritional components, especially on heat-labile fat and protein components. The loss of fat during pasteurization, freezing and thawing, is normally attributed to its adherence on the surface of containers, pipelines and feeding systems ([4,32,33]).

**Table 1. Proximate analysis and pH value of mature human milk**

Component	Content (g/100 mL)
Protein	1.53 $\pm$ 0.447
Lactose	6.25 $\pm$ 0.82
Fat	2.70 $\pm$ 0.43
pH	6.88 $\pm$ 0.22

Table 2 depicts the fat content for each milk treatment evaluated; the fat content of the treatments to different processes compared to raw milk showed a reduction, although there was no significant difference. The reduction of fat content in spray-dried samples was about 23% ([4,26]); this reduction could be attributed to the adherence of fat to the walls or tubing of the equipment; or to a faulty milk homogenization process, causing loss of vitamins, essential fatty acids and decreased overall energy content of the milk. This type of loss has been reported in human milk to range between 18 and 34% ([4,32,33]).

**Table 2. Effect of processes on milk fat content**

Treatment	Fat content (g/100 mL)
Raw	2.70 $\pm$ 0.43 <sup>a</sup>
Pasteurization	2.46 $\pm$ 0.39 <sup>a</sup>
Lyophilization	2.31 $\pm$ 0.54 <sup>a</sup>
Spray dried	2.08 $\pm$ 0.25 <sup>a</sup>

\*Means  $\pm$  SD with different letters in the same column are not significantly different at the 0.05 probability level.

### 3.2. Fat Globule Size Analysis

In this study the fat globule size in fresh milk ranged from 0.94  $\mu$ m to 3.2  $\mu$ m, confirming that globule size is attributed to individual differences. The fat globule size of human milk varies with race and the individual factors of the donor mother. The mean globule size in this study (2.2  $\mu$ m) is smaller than 4.7  $\mu$ m, reported by [12] in mature milk. This might be caused by the difference in height and body composition of Danish and Mexican donors, as well as the genetics. However, there have been reported globule sizes ranging from from 0.3 to 15  $\mu$ m ([34] and [35]). Globule size measured in samples from the different treatments of this study are shown in Table 3. Comparing the globule size of the fresh milk globules with sizes

obtained after processing, a significant decrease caused by processing was observed. A reduction in fat globule size was observed with increased pasteurization temperatures (75 and 85°C). Globule size significantly decreased, because the temperature very likely caused rupture of the fat globule membranes. In addition, adhesion and separation of fat in the processing vessel and fittings was observed, caused by the instability of the fat globule. Regarding the results of freeze-drying and spray drying processes (Table 3), we found that the fat globules had a measurable size reduction, attributed to a partial mechanical homogenization during drying. Additionally, passing through the atomizing nozzle probably produced the fat phase to break into smaller globules. Globule size was reduced in all treatments and thus the overall surface area increased; hence, it would be expected to have greater availability and absorption of fatty acids and other lipophilic compounds (i.e. vitamins) as has been reported in other studies ([36,37]).

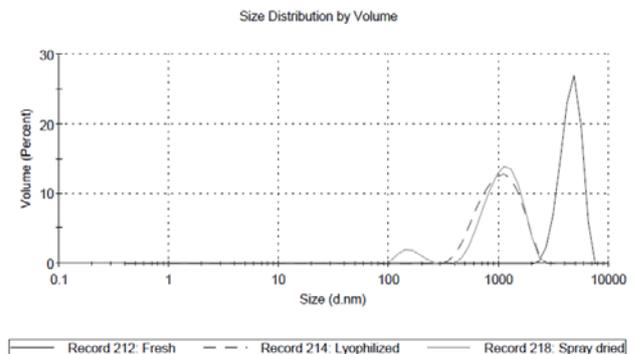
**Table 3. Globule size and distribution for processed milk**

Treatment	Globule size (nm)	Polydispersity index*
Fresh milk	2137.56 ± 626.77 <sup>a</sup>	0.24 ± 0.08 <sup>a</sup>
Pasteurized milk (62 °C, 30 min)	830.95 ± 79.43 <sup>b</sup>	0.78 ± 0.13 <sup>b</sup>
Pasteurized milk (75 °C, 15 min)	773.95 ± 149.16 <sup>bc</sup>	0.57 ± 0.12 <sup>bc</sup>
Pasteurized milk (85 °C, 5 min)	628.88 ± 84.03 <sup>cd</sup>	0.59 ± 0.16 <sup>cd</sup>
Spray dried	575.33 ± 30.76 <sup>d</sup>	0.45 ± 0.07 <sup>d</sup>
Lyophilized	528.55 ± 52.34 <sup>d</sup>	0.41 ± 0.11 <sup>e</sup>

\*The polydispersity index (PDI) is an estimate of the amplitude of the globule size distribution, is a dimensionless number ranging 0 to 1.0

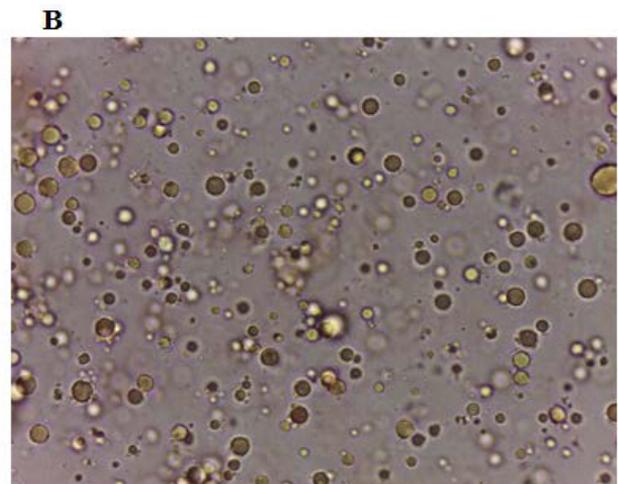
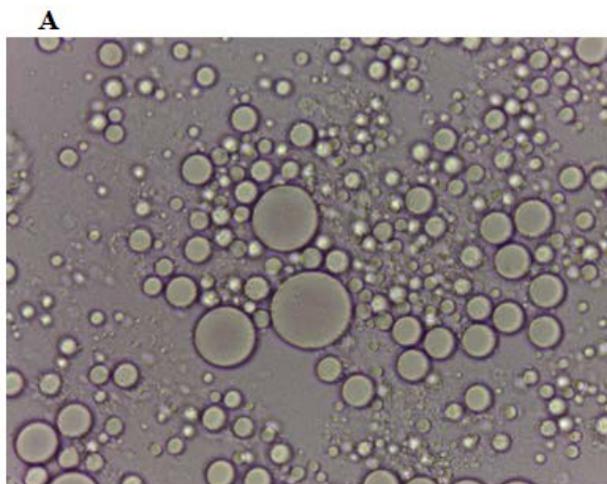
The polydispersity index (PDI) is used as a measure of the distribution broadness of globule size, samples having values close to zero indicate uniform and unimodal globule size. Raw milk had the lowest PDI value (0.24, Table 3); this value defines a homogeneous and unimodal globule size. Freeze-dried and spray dried milk samples had wider globule size distributions (PDI values of 0.41 and 0.45, respectively); however, they were significantly

different from raw milk. Changes in PDI were caused by the decrease of globule size by drying, so the globule size is not uniform and the amplitude of the size distribution increased. Figure 1 shows the changes in the globule size for fresh, spray dried and freeze-dried milk. Spray dried milk had a broader globule size distribution showing a bimodal behavior which is related to high PDI. It can be observed in Figure 2 that globule size in spray dried milk compared to fresh milk is smaller.



**Figure 1.** Globule Size distribution of human milk under different treatments

Pasteurized samples showed a wide globule size distribution (ranging from 0.59 to 0.78); in these treatments, globule size changed by the effect of temperature and there was no homogenization effect as during spray drying, in which samples were stirred while drying. Reduction in globule size during pasteurization was not uniform and samples were produced having a wider globule size distribution. There were no significant differences between pasteurization samples, showing higher PDI values; hence, pasteurization temperatures exerted an effect on fat globule stability. The PDI reflects the influence of treatments applied on the globule size of the samples. A larger value of PDI showed greater variation in globule size that may favor some mechanism of destabilization (Ostwald ripening, creaming) causing fat separation.



**Figure 2.** Microstructure of human milk fat globule observed by optical microscopy 100 X (A: Fresh milk, B: Spray dried milk)

The zeta potential was  $-17.1 \pm 1.17$ ,  $-17.4 \pm 3.32$  and  $-14.7 \pm 2.46$  mV for fresh, freeze-dried and spray dried milks, respectively. These values are similar to those reported by [38] for buffalo milk ( $-18$  mV) and [8] for

cow's milk ( $-13$  mV); however, they differ from [12] for mature human milk ( $-7$  mV). This was possibly caused by the adsorption of caseins which has been reported to increase the zeta potential [8].

### 3.3. Fatty Acid Composition by HPLC and GC

Fatty acids were adequately separated by the HPLC procedure. For evaluation of detector linearity, the calibration curves for each fatty acid were obtained, calculating the  $R^2$  (Table 4). Results proved a satisfactory correlation ( $R^2 > 0.97$ ); therefore, the method showed linearity in all calibration curves in the concentration ranges employed. The limit of detection (LD) defines the

capacity of the method to detect a minimum concentration of the analyte, the quantities of fatty acids to be detected ranged from 1 to 13  $\mu\text{g/mL}$ . The limit of quantification (LQ) determines the concentration that can be analyzed with precision and accuracy. The values found ranged from 3 to 45  $\mu\text{g/mL}$ . Overall, the method showed high sensitivity for all fatty acids evaluated.

The precision of the method was determined by the relative standard deviation in the repeatability and reproducibility analysis; data are shown in Table 5.

**Table 4. Linearity and sensitivity of HPLC method**

Fatty acid	Concentration range (mg/mL)	Regression equation	$R^2$	LD ( $\mu\text{g/ml}$ )	LQ ( $\mu\text{g/ml}$ )
C12:0	0.48 - 2.88	$y = 1495.1x + 60.83$	0.9912	9.97	33.23
C14:0	0.27 - 1.33	$y = 4033.2x - 284.50$	0.9748	3.69	12.32
C16:0	1.15 - 6.90	$y = 1109.7x + 137.84$	0.9953	13.43	44.76
C18:0	0.05 - 0.25	$y = 7314x + 18.431$	0.9955	2.04	6.79
C18:1	0.10 - 1.80	$y = 3744.5x + 414.58$	0.9831	3.98	13.27
C18:2	0.10 - 0.40	$y = 14862x + 264.59$	0.9898	1.00	3.34

Determination coefficient ( $R^2$ ); limit of detection (LD); limit of quantification (LQ).  $y$  represents the fatty acid concentration and  $x$  means the peak area.

The relative standard deviation values for each fatty acid in precision analysis were less than 5%. According to the acceptance criteria used, these values are acceptable; so the precision of the method has to be considered also as acceptable.

**Table 5. Precision of the HPLC method by the relative standard deviation (%)**

Fatty acid	Repeatability	Reproducibility
C12:0	2.057	0.832
C14:0	1.958	3.129
C16:0	2.458	4.020
C18:0	1.622	1.892

It has been reported that more than 80% of the total fatty acids were composed of C12:0 (lauric acid), C14:0 (myristic acid), C16:0 (palmitic acid), C18:0 (stearic acid), C18:1 (oleic acid) and C18:2 (linoleic acid), which were analyzed in fresh milk by gas chromatography (GC), and the data were compared with those obtained by the HPLC method (Table 6). The fatty acid composition of human milk is the result of amount and type of fat consumed in the maternal diet, the stage of lactation, which may produce variations in the fatty acid profile. Lauric acid was detected in a concentration of 7% in both methods, and myristic acid in a concentration of 10.2% and 7.5% for GC and HPLC, respectively, without significant statistical difference. The amount of palmitic acid was 27% in both methods; while in other studies contents of 20 to 23% of the total fatty acids was reported [39]. The content of oleic acid was similar for GC and HPLC from 28.5 to 29.8%, respectively; which is comparable to those reported by other authors ([5-40]). As it can be seen, no significant differences were found on the profile of fatty acids, as analyzed by GC and HPLC.

Therefore, our data suggest that the HPLC method is adequate for fatty acids analysis as the GC method, so this method can be used when a GC equipment is not available. The fatty acid profile found in the fresh human milk samples appears similar to samples from other countries analyzed by GC as the USA and Canada [41]; Turkey [5]; Greece [42]; Italy [40] and Denmark [12]. In all these

studies including the present work, palmitic and oleic acids were reported as the major fatty acids with concentrations of 19.3 to 28.8 wt% and 27.8 to 37.3 wt%, respectively. The analysis of fatty acids in human milk fat is usually conducted by gas chromatography; however, the method requires derivatization in order to prepare volatile fatty acids derivatives and the detection system destroys the fatty acids, impeding the recovery and further utilization.

**Table 6. Fatty acids concentration (wt %) in human fresh milk**

Fatty acid	Fresh milk	
	GC	HPLC
C12:0	$7.42 \pm 2.87^a$	$6.96 \pm 1.98^a$
C14:0	$10.20 \pm 3.81^a$	$7.48 \pm 1.96^a$
C16:0	$27.66 \pm 3.07^a$	$27.33 \pm 3.97^a$
C18:0	$6.76 \pm 3.02^a$	$3.76 \pm 2.24^a$
C18:1	$29.83 \pm 2.05^a$	$28.49 \pm 3.72^a$
C18:2	$5.79 \pm 2.02^a$	$6.89 \pm 4.06^a$

\*Means  $\pm$  SD with different letters in the same line are not significantly different at the 0.05 probability level.

HPLC analysis was performed on fatty acids mixtures in vegetable oils, cow's milk fat and biological samples; in these studies good results have been found in terms of method validation and considered the use of HPLC as an alternative to the GC methods for accurate quantitative analysis that can be applied using a UV detector ([14,15,43,44,45,46]). The variation in fatty acids in this study, compared to studies from other countries is a consequence of the different maternal diets and eating habits of the countries of the milk donors. Reference [5] found a correlation between the maternal diet and the fatty acids profile during pregnancy and lactation; hence, variations found in this study may be attributed to this relationship.

The effects of freeze-drying, pasteurization and spray drying on the lipid profile were evaluated (Table 7). For lauric, myristic and stearic acids, there was no significant difference between the different treatments. However, for samples pasteurized at 85 °C the lowest values were obtained. This behavior may be explained by fat globule

destabilization caused by pasteurization, which in turn produced fat separation and adherence to the container. During fatty acid analysis samples were homogenized; however, the working temperature was refrigeration in order to avoid deterioration of lipids, the fat attached to the container was solid, possibly rich in saturated fatty acids, and thus the content of these fatty acids decreased in the fluid milk.

**Table 7. Fatty acids concentration (wt %) in human milk under different treatments**

Fatty acid	Fresh milk	Pasteurization	Freeze-dried	Spray dried
C12:0	6.96±2.0 <sup>a</sup>	6.55±1.1 <sup>a</sup>	8.04 ± 3.4 <sup>a</sup>	7.74 ± 2.5 <sup>a</sup>
C14:0	7.48±2.0 <sup>a</sup>	6.79±1.1 <sup>a</sup>	11.22 ± 5.2 <sup>a</sup>	11.10 ± 4.1 <sup>a</sup>
C16:0	27.33±4.0 <sup>a</sup>	20.78±1.1 <sup>b</sup>	27.69 ± 2.7 <sup>a</sup>	27.31 ± 3.5 <sup>a</sup>
C18:0	3.76±2.2 <sup>a</sup>	5.85±1.1 <sup>a</sup>	7.04 ± 3.1 <sup>a</sup>	6.96 ± 3.1 <sup>a</sup>
C18:1	28.49±3.7 <sup>a</sup>	34.5±3.2 <sup>a</sup>	31.25 ± 3.7 <sup>a</sup>	32.78 ± 3.1 <sup>a</sup>
C18:2	6.89±4.1 <sup>a</sup>	13.9 ± 1.9 <sup>b</sup>	7.75 ± 3.2 <sup>a</sup>	8.63 ± 2.9 <sup>a</sup>

\*Means ± SD with different letters in the same line are not significantly different at the 0.05 probability level. Pasteurization was conducted a 85°C/5 min.

Metha and co-workers [14] reported adherence of saturated medium chain fatty acids present in milk formulas to the container, so the decrease in the content of saturated fatty acids on pasteurized samples may be caused by this effect. There was a significant variation in palmitic acid concentration in samples pasteurized at 85 °C, in which the concentration decreased from 27% to 20% in fresh samples and pasteurized samples, respectively. This was possibly also caused by selective adhesion to the container. Oleic acid concentration increased from 28.5 % in fresh samples to 34.5% in treated milks. The decreased content of saturated fatty acids was most likely caused the increase in unsaturated fatty acids, as in the case of oleic and linoleic fatty acids present in significantly greater concentration in human milk samples under different treatments. Despite this, the fatty acids profile of pasteurized samples contained palmitic acid in concentrations similar to those reported by [5] and [39].

## 4. Conclusions

The macronutrient content in human milk from Mexican women was similar to those reported by other authors in samples from different countries. The processes used in this work did not affect the total fat content, nor significant changes were observed in the fatty acids profile. Pasteurization decreased fat globule size, the concentration of saturated fatty acids and conversely increased the concentration of oleic and linoleic acid. The fatty acid composition of human milk from Mexican donors from the state of Jalisco appeared similar to milks from other countries. To the best of our knowledge, this work is a first report of human milk drying intended for preservation; however, more impact studies on other nutrients as well as the biological activities should be assessed.

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## Statement of Competing Interests

The authors have no competing interests

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