Nutrición Hospitalaria

Nutr Hosp. 2015;31(3):1386-1393
ISSN 0212-1611 • CODEN NUHOEQ
S.V.R. 318

Original/*Otros*

Are fat acids of human milk impacted by pasteurization and freezing?

Luiz Antônio Borgo[1], Wilma Maria Coelho Araújo[2], Maria Hosana Conceição[3], Inês Sabioni Resck[3] y Márcio Antonio Mendonça[1]

[1]Faculty of Agronomy and Veterinary Medicine, Laboratory of Food Analysis University of Brasilia. [2]Department of Nutrition. University of Brasilia. [3]Institute of Chemistry, University of Brasilia. Brazil.

Abstract

The Human Milk Bank undergo human milk to pasteurization, followed by storage in a freezer at -18° C for up to six months to thus keep available the stocks of this product in maternal and infant hospitals. The objective of this study was to evaluate the effects of processing on the lipid fraction of human milk. A sample of human milk was obtained from a donor and was subdivided into ten sub-samples that was subjected to the following treatments: LC = raw milk; T0 = milk after pasteurization; T30 = milk after pasteurization and freezing for 30 days; T60 = milk after pasteurization and freeze for 60 days, and so on every 30 days until T240 = milk after pasteurization and freezing for 240 days, with 3 repetitions for each treatment. Lipids were extracted, methylated and fatty acid profiles determined by gas chromatography. The fatty acids were characterized by nuclear magnetic resonance and functional groups were identified by infrared spectroscopy. There were variations in the concentration of fatty acids. For unsaturated fatty acids there was increasing trend in their concentrations. The IR and NMR analyze characterized and identified functional groups presents in fatty acids.

(*Nutr Hosp*. 2015;31:1386-1393)

DOI:10.3305/nh.2015.31.3.8120

Key words: *Human milk. Lipids. Pasteurization. Fatty acid. Freezing.*

¿SON LOS ÁCIDOS GRASOS DE LA LECHE HUMANA AFECTADOS POR LA PASTEURIZACIÓN Y LA CONGELACIÓN?

Resumen

Los Bancos de Leche Humana someten la leche la pasteurización, seguido del almacenamiento en un congelador a -18 grados por até seis meses, para así disponerlo a los Hospitales Materno Infantiles. El objetivo de este estudio fue evaluar los efectos del procesamiento de la fracción lipídica de la leche humana. Para esto, una muestra obtenida de una donante y sometida a diez diferentes tratamientos: LC = leche cruda; TO = leche después de la pasteurización; T30 = leche después de la pasteurización y congelación por 30 días; T60 = leche después de la pasteurización y congelación por 60 días y así sucesivamente a cada 30 días hasta T240 = leche después de la pasteurización y congelación por 240 días, con tres repeticiones a cada tratamiento. Los lípidos fueron extraídos y los ácidos grasos metilados fueron determinados por cromatografía gaseosa. Los ácidos grasos fueron caracterizados por resonancia magnética nuclear y los grupos funcionales identificados por espectroscopia infrarroja. Hubo variaciones en las concentraciones de ácidos grasos. Para los ácidos grasos insaturados hubo un aumento en sus concentraciones. Sin embargo, los grupos funcionales se caracterizaron por espectroscopia infrarroja y resonancia magnética nuclear, RMN.

(*Nutr Hosp*. 2015;31:1386-1393)

DOI:10.3305/nh.2015.31.3.8120

Palabras clave: *Leche humana. Lípidos. Pasteurización. Ácidos grasos. Congelador.*

Introduction

The Brazilian Network of Human Milk Bank is considered the largest and most complex in the world by the World Health Organization. Human Milk Bank (HMB) is a specialized center, obligatorily linked to a maternal and / or children's hospital, responsible for promoting breastfeeding and execution of collection, processing and quality control of colostrum, transition milk and mature human milk, for distribution under prescription from the doctor or dietician (American

Correspondence: Luiz Antônio Borgo.
Faculdade de Agronomia e Medicina Veterinária.
Laboratório de Análise de Alimentos, Universidade de Brasília.
Campus Universitário Darcy Ribeiro.
Asa Norte – CEP 70910-900. Brasília, DF - Brazil.
E-mail: luizborgo@gmail.com.br; borgo@unb.br

Recibido: 23-IX-2014.
Aceptado: 3-XI-2014.

Medical Association, 2001). Regularly, the HMB undergo human milk, after physicochemical and microbiological testing, to pasteurization, followed by storage in a freezer at -18° C for up to six months to thus keep available the stocks of this product in maternal and infant hospitals.

Lipids and specially the essential fatty acids are among the nutrients that develop physiologically important functions such as the nervous system development and maturity of visual acuity, and provide energy and transport fat-soluble vitamins. The lipids are also susceptible to various types of chemical reactions, enzymatic or non-enzymatic. Oxidation is one of the main reactions; can occur in the presence or absence of oxygen and is catalyzed by light, heat, heavy metals, free radicals or irradiation, leading to the formation of toxic compounds (peroxides) (Heiman and Schandler, 2006; Hamosh, 1998; Bretillon *et al*., 1998).

The thermal process, in a greater or lesser extent, changes in food constituents according to the intensity of the binomial time / temperature and sensitivity of the food to heat. Specifically, the lipids present in milk are, among the macronutrients, those with the largest changes during processing. Some studies show that pasteurization and sterilization promoted slow variations, significant or not, in the lipid content and fatty acid fraction of human milk (Walstra *et al*., 2001; Fennema, 1993). But, none of these studies simulated the actual conditions of processing of milk followed by freezing and storage under freezing for eight months, thawing and heating before distribution, despite the Brazilian Network of Human Milk Bank recommending a time of 6 months.

Therefore, the objective of this study was to evaluate the effects of pasteurization and of freezing on the lipid fraction of human milk, considering the shelf life established by HMB.

Methods

This project was approved by the Ethics Committee of the Faculty of Medicine, University of Brasília (CEP-FM 072/2005 of 20/12/2005).

Analytic Sample

The sample of human milk (HM) was obtained from a single donor, without any kind of identification, and subdivided into ten sub-samples, followed these treatments: LC = raw milk; T0 = milk after pasteurization; T30 = milk after pasteurization and freezing for 30 days; T60 = milk after pasteurization and freeze for 60 days, and so on every 30 days until T240 = milk after pasteurization and freezing for 240 days, with 3 repetitions for each reference, totaling 30 samples. Pasteurization was taken to 63º C/30minutes by the Human Milk Bank (HMB), University Hospital, University of Brasilia, and the freezing temperature -18ºC.

Total Lipid Extraction and Methylation of Fatty Acids

The extraction of total lipids was taken by butyrometric method Gerber - Van Gulik and fat content read on butyrometer rod directly in percent (AOAC, 1998; Christie, 1990).

For methylation were taken aliquots of 20mg of lipid were transferred to a test tube with screw cap, 1.5 mL of 0.5 N potassium hydroxide in methanol was added, followed by stirring vortexing for 1 minute and heated in a water bath at 70º C for 5 minutes and cooling immediately in running water. After cooling, 2 mL of 12% BF_3 in methanol were added and new vortexing for 1 minute was made. Then, the tube was heated in a water bath at 70º C for 5 minutes and immediately cooled in water (Fidler *et al*., 1998).

Subsequently, 2.5 mL of saturated NaCl and 1 mL of hexane were added to the mixture, followed by centrifugation for 10 minutes at 1200 rpm. The supernatant was collected and transferred to a glass tube with 2 mL volumetric capacity, with screw cap, aluminium septum and saturated nitrogen atmosphere. The samples were stored in a freezer at -18º C until the time of analysis by chromatography, infrared spectroscopy and nuclear magnetic resonance (Fidler *et al*., 1998).

Chromatographic Analysis

The analysis of methylated fatty acids was taken into the gas chromatograph model Varian Star 3400cx with detector FID and split / splitless injector, with a fused silica capillary column SPTM-2380 (30m x 0.25 mm x 0.2 mM), Supelco brand (Fidler *et al*., 1998).

The chromatographic conditions were: temperatures of injector and detector equal to 260º C; initial temperature of column equal to 140° C, increase of 5° C/min up to 240° C after 30 min of chromatographic run. Carrier gas: nitrogen; 20cm/minutes flow; volume of sample injected equal 0.4 μL. Flue gases: hydrogen and medical oxygen. The total analysis time was 30 minutes and the results expressed as a percentage with respect to the area of total fatty acids. The fatty acids were identified by comparison with the retention time of the standard Supelco 37 fatty acid component FAME mix (Supelco, USA).

Analysis by Infrared Spectroscopy (IR)

The analysis by infrared spectroscopy equipment was made in the Hartmann & Braun, model Bomem (MB series). The samples were the same as used in gas chromatography after evaporation of solvent and placed as a film on sodium chloride tablet.

Analysis by Nuclear Magnetic Resonance (^1H)

The nuclear magnetic resonance of hydrogen (^1H NMR) was performed on Varian Mercury plus spectrometer (300 MHz, 7.05T). The samples were the same as used in gas chromatography, but replacing the solvent (hexane) 99.8% deuterated chloroform ($CDCl_3$), Sigma-Aldrich.

Statistical Analyses

Statistical analyses were performed using SAS ® V. 9.1.3 (SAS, Cary Indiana, 2003). The statistical treatment of the data considered the method of analysis and the time at which the sample was taken from the milk bank. There were three replications in a completely randomized design. The analysis of variance for the amount of each fatty acid was made using repeated measures and, when significant effects ($P<0.05$) on the time factor, linear regression analysis was performed.

Results

Saturated Fatty Acids

The data obtained for the concentration of the saturated fatty acids in the samples indicate that there were variations, higher or lower, for the concentrations of all fat acids esters in the treatments studied (Table I).

The concentration of capric acid was almost constant between LC and T0 treatments; however, it decreased when compared to treatment T90. There was a low coefficient of determination ($r^2 = 0.16$) and correlation coefficient $r = 0.4$ (Fig. 1a); therefore, 40% of the variation in the concentration of this acid are related with the time. There was also a trend towards reduction in acid concentration with time storage of milk (Fig. 1a). The coefficient of determination indicates that 16% of the variation in the concentration of this fatty acid may be explained by the statistical model applied; 84% vary depending on other factors not accounted for by this model.

For lauric acid, there was an increase of concentration after pasteurization and during the other treatments. The quadratic regression shows that the concentration tends to remain constant with time (Fig. 1b). For myristic acid, a trend of increasing concentration with time was observed, this relationship being 47%. For stearic acid there was a slight tendency to decrease in the concentration with respect to time (14%) (Fig. 1b). The same occurred for the arachidic acid, with trend to reductions and 51% ratio with time (Fig. 1a).

The esters of capric and lauric fatty acids presented quadratic regression; myristic and arachidic, linear regression (Fig. 1a and 1b), both significant. The stearic and palmitic acids showed no significant regressions (Fig. 1b and 1c).

Unsaturated fatty acids

Regarding the fraction of unsaturated fatty acids, it appears, as for the saturated fatty acids, there were also changes in the unsaturated fatty acid profiles, but with an upward trend in their concentrations, except for linolenic acid, which decreased (Table I).

Statistical analysis showed that only the ZZ-linoleic acid showed a significant quadratic regression (Fig. 1d). For the ZE-linoleic acid linear regression was significant (Fig. 1e). The palmitoleic, oleic and linolenic acids showed no significant regression (Fig. 1d and 1e).

Infrared Spectroscopy

The stretching vibrational frequencies ($2950cm^{-1}$ and $2850cm^{-1}$) and deformation ($1360cm^{-1}$ and $1461cm^{-1}$) were observed for aliphatic CH (CH_3 and CH_2) esters of saturated and unsaturated derivatives. Only in oleic, ZE-linoleic, linolenic fat acids, which have double bonds, were identified in the band $3004cm^{-1}$; on ZZ-linolenic the absence of vibration was due to its low concentration to obtain the spectrum. The vibrations relating to acylals groups (C = O) esters were recorded between $1733cm^{-1}$ and $1743cm^{-1}$ and corresponding frequencies to vibrations C-O between $1169cm^{-1}$ and $1201cm^{-1}$. The low intensity of stretch relative to C = C (cis or *trans*) fatty acids of the studied can be related to the long carbon chain (16 to 18 carbons) that disadvantages the vibration of the double bond at $1650cm^{-1}$ and $600cm^{-1}$ - $700cm^{-1}$, in which the vibrational frequency of the C = CH double bond with cis stereochemistry should occur. However, for linoleic ZE the dual *trans* was shown in the $969cm^{-1}$ band. For all fatty acids, except lauric and linolenic, the presence of vibration between $720cm^{-1}$ and $732cm^{-1}$, due to rock deformation (> $4CH_2$), was constant in the spectra analyzed (Tables IIIa and IVa).

Resonance Spectroscopy

By spectroscopic data, it is observed that the chemical shifts (in ppm δ) are very close and the spectra are very similar, differing in relation to the numbers of hydrogen atoms coming from the integrations, obtained by means of the areas of each peak noted (Tables IIIb and IVb).

The signals related to the terminal methyl groups (CH_3) of saturated and unsaturated carbon chains are between 0.88 ppm and 0.89 ppm, with a single exception to linoleate derivative which has the methyl group at 0.98 ppm. It is common the overlapping of the methylenic hydrogen atoms $(-CH_2)_n$, which is shown with different shapes of the peaks, depending on the studied fatty acid.

The signs of the methylenic hydrogen atoms positions β ($-CH_2CH_2COOCH_3$) and α ($-CH_2CH-$

Table I

Concentration of saturated fatty acids in human milk samples for LC, T0, T90, T120, T150, T180, T210, T240 (mg% ± SD%) treatments and of unsaturated fatty acids in human milk samples, for LC, T0, T30, T90, T120, T150, T180, T210, T240 (mg% ± SD%) treatments. 2010

Fatty acids	*Treatments*							
	LC	*T0*	*T90*	*T120*	*T150*	*T180*	*T210*	*T240*
Saturated								
Capric acid	0,1740±0,0653	0,1605±0,0275	0,0313±0,0183	0,1818±0,0811	0,1354±0,0247	0,1149±0,0280	0,0873±0,0325	0,0873±0,0107
Lauric acid	0,2413±0,0381	0,3373±0,0247	0,3069±0,0521	0,4990±0,0428	0,4859±0,0543	0,4340±0,0679	0,4788±0,0603	0,4113±0,0271
Myristic acid	0,5007±0,0800	0,7416±0,0475	0,5805±0,0809	0,8496±0,0631	0,8777±0,0805	0,7171±0,1055	0,7928±0,0655	0,7809±0,0358
Palmitic acid	2,6926±0,3440	4,5001±0,2754	2,3471±0,4180	3,5613±0,2304	3,6718±0,3178	2,9540±0,4210	3,2575±0,2121	3,5979±0,2392
Stearic acid	0,7799±0,0948	1,2898±0,0818	0,5679±0,1601	0,3901±0,0255	0,9401±0,0906	0,7741±0,1113	0,8479±0,0567	0,9795±0,0610
Arachidic acid	0,0466±0,0028	0,1008±0,0052	0,0334±0,0063	0,0475±0,0020	0,0517±0,0029	0,0369±0,0039	0,0424±0,0012	0,0469±0,0028
Unsaturated								
Palmitoleic acid	0,2757±0,0338	0,3763±0,0219	0,2758±0,0444	0,3901±0,0255	0,4469±0,0341	0,3244±0,0456	0,4033±0,0271	0,3485±0,0158
Oleic acid	3,5132±0,4176	5,9298±0,3727	2,9796±0,5987	4,3029±0,2592	5,1995±0,3874	3,6860±0,5214	4,4580±0,3058	4,3102±0,3365
Linoleic (*ZZ*) acid	1,1375±0,1384	4,2389±0,2516	0,8206±0,2022	0,8368±0,0411	1,8560±0,1349	1,2423±0,1756	1,5919±0,1082	1,1793±0,1494
Linoleic (*ZE*) acid	0,0471±0,0063	0,0810±0,0079	0,0513±0,0206	0,0793±0,0029	0,1089±0,0097	0,0880±0,0106	0,0911±0,0087	0,1132±0,0401
Linolenic acid	0,0593±0,0071	0,0429±0,0019	Nd	0,0252±0,0002	Nd	0,0492±0,0083	0,0619±0,0018	0,0271±0,0021

The values for the T30 and T60 treatments were discarded because they were not consistent; probably the esterification reaction was incomplete. *(P<0,05). SD = standard deviation. Nd = not detected

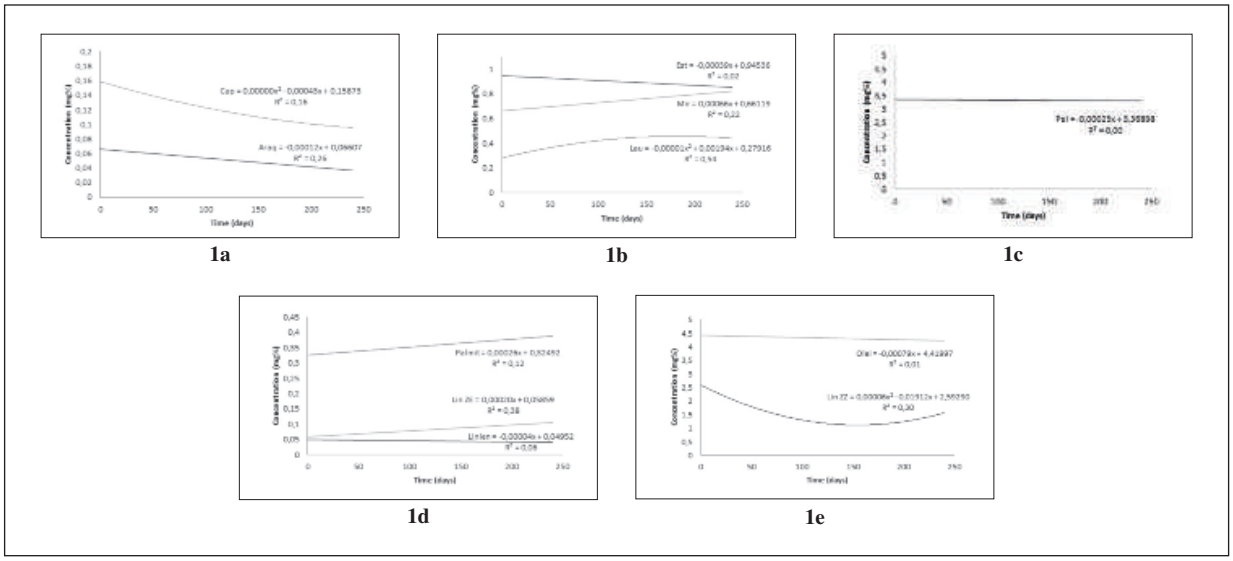

Fig. 1.—Regression of capric acid (Cap) and arachidic (Araq) (1a); of stearic acid (Est), myristic (Mir) and lauric (Lau) (1b); palmitic acid (PAL) (1c); of oleic acid (Olei) and ZZ-linolenic acid (Lin ZZ) (1d); of palmitoleic acid (Palmit), ZE-linoleic acid (Lin-ZE) and linolenic (Linlen) (1e).2010.

Table II
Concentration of unsaturated fatty acids in human milk samples, for LC, T0, T30, T90, T120, T150, T180, T210, T240 (mg% ± SD%) treatments. 2010

Treatments	*Palmitoleic acid*	*Oleic acid*	*Linoleic (ZZ) acid*	*Linoleic (ZE) acid*	*Linolenic acid*
LC	0,2757±0,0338	3,5132±0,4176	1,1375±0,1384	0,0471±0,0063	0,0593±0,0071
TO	0,3763±0,0219	5,9298±0,3727	4,2389±0,2516	0,0810±0,0079	0,0429±0,0019
T90	0,2758±0,0444	2,9796±0,5987	0,8206±0,2022	0,0513±0,0206	Nd
T120	0,3901±0,0255	4,3029±0,2592	0,8368±0,0411	0,0793±0,0029	0,0252±0,0002
T150	0,4469±0,0341	5,1995±0,3874	1,8560±0,1349	0,1089±0,0097	Nd
T180	0,3244±0,0456	3,6860±0,5214	1,2423±0,1756	0,0880±0,0106	0,0492±0,0083
T210	0,4033±0,0271	4,4580±0,3058	1,5919±0,1082	0,0911±0,0087	0,0619±0,0018
T240	0,3485±0,0158	4,3102±0,3365	1,1793±0,1494	0,1132±0,0401	0,0271±0,0021

SD = standard deviation (P <0.05). Nd = not detected

$_2COOCH_3$) to the acyl group were identified between 1.58 ppm and 1.64 ppm and between 2.30 ppm and 2.31 ppm, respectively.

The signals esters of unsaturated groups identified between 1.97 ppm and 2.17 ppm are related to the methylene hydrogens attached to the double bonds, known as allylic hydrogens. As for the ZE-linoleate and linolenate there are methylenes between 2.66 ppm and 2.81 ppm, called bisallylic position. The methoxy groups (CH_3O) absorb all of the fatty acids at 3.67 ppm and olefinic protons (-CH=CH-) are only shown for the unsaturated esters between 5.30 ppm and 5.46 ppm.

Discussion

The results obtained in this study are novel because no other study was conducted in conditions approaching those that occur in HMB and because they showed the behavior of fatty acids in the same sample of human milk subjected to pasteurization and freezing for eight months.

In general, the data of infrared spectroscopy (IR) shows organic functions present in methyl esters derived from fatty acids, while spectroscopic data show the signs of the methylenic hydrogen atoms positions ($-CH_2CH_2COOCH_3$) and ($-CH_2CH_2COOCH_3$) to the

Table III

Absorption bands observed in the IR to saturated fatty acids and their assignments (3a) and ^1H NMR chemical shifts of saturated fatty acids (3b) in human milk samples. 2010

III(a)						
Assignments to Functional Groups	*Capric* N^o *wave* cm^{-1}	*Lauric* N^o *wave* cm^{-1}	*Myristic* N^o *wave* cm^{-1}	*Palmitic* N^o *wave* cm^{-1}	*Stearic* N^o *wave* cm^{-1}	*Arachidic* N^o *wave* cm^{-1}
$CH_{3\ est}$	2950	2925	2924	2953	2925	2950
$CH_{2\ est}$	2855	2854	2854	2850	2849	2887
$C{=}O_{est}$	1743	1742	1744	1742	1742	1743
$CH_{3\ def.\ ass.}$	1465	1465	1466	1473	1474	-
$CH_{2\ def.}$	1436	1436	1436	1464	1463	1436
$CH_{3\ def.\ sim.}$	1363	-	1362	1383	1384	1384
$C\text{-}O_{est}$	1247	1169	1248	1199	1178	1227
$CH_{2\ def\ rock}$	723	-	722	732	720	720
III(b)						
Nature of the fatty acids characterized hydrogens	*Capric* δ *(ppm)*	*Lauric* δ *(ppm)*	*Myristic* δ *(ppm)*	*Palmitic* δ *(ppm)*	*Stearic* δ *(ppm)*	*Arachidic* δ *(ppm)*
CH_3	0,88	0,88	0,88	0,88	0,88	0,88
$-(CH_2)_n-$	1,20-1,40	1,20-1,40	1,20-1,40	1,20-1,40	1,20-1,40	1,20-1,40
$-CH_2CH_2OCOCH_2-$	1,62	1,62	1,62	1,62	1,62	1,62
$-CH_2CH_2COOCH_3$	2,31	2,31	2,30	2,30	2,30	2,30
$-CH_2COOCH_3$	3,67	3,67	3,67	3,66	3,67	3,67

acyl group and the signals of unsaturated esters groups, confirming that the observed changes were, in fact, the fatty acid molecules.

Studies evaluated the effects of pasteurization and sterilization on the content of lipids and fatty acids of human milk and concluded that pasteurization does not alter the lipid content available, not its composition. On the other hand, the sterilization reduces the concentration of about 13% and may cause small reductions in the concentrations of arachidonic and linoleic acids without, however, considering them significant. However, these two processes may also induce loss of unsaturated lipids by oxidation, both in the presence of oxygen or light (Henderson *et al*., 1998).

Another search concluded that both slow pasteurization (62.5° C/30min) and fast pasteurization (100° C/5min) did not alter the concentration of fatty acids, including the long chain unsaturated. These researchers also noted that storage of fresh milk at 4° C for 96 hours, at -20º C for 12 months and at -80º C for 12 months did not significantly alter the profile of saturated and unsaturated fatty acids (Wardell *et al.,* 1981).

The pasteurization does not affect the long-chain fatty acids due to the high antioxidant activity of human milk (Goldblum *at al.,* 1984). However, heating milk at 62.5° C caused partial hydrolysis of triglycerides and, after 30 minutes (holding time), a significant reduction in the concentrations of myristic fatty acids (15%), stearic (6%), palmitic (8%), and oleic (8%). The percentage of constituent fatty acids of triglycerides was not changed, except that linolenate reduced its concentration by 22% after heat treatment (Lepri *et al.,* 1997).

Research indicates that the time lapse between collection and pasteurization of milk favored the hydrolysis of lipids by up to 0.5%; pasteurization (62.5º C/30min) increased lipase activity with increased hydrolysis products up 0.9%; storage at -20° C for 90 days of pasteurized milk caused further hydrolysis of lipids and small reduction in the level of triglycerides, with a decrease of 6% fat; the total procedure (pasteurization and freezing / thawing) did not alter the percentage composition of fatty acids substantially (Weber *et al.,* 2001).

Studies show that *in natura* human milk hydrolysis reactions occur mediated by bile salt-dependent lipase and that this reaction is inactivated by pasteurization (Henderson *et al.,* 1998; Romeu-Nadal *et al.,* 2008). However, it should be considered possible hydrolysis of lipids by microbial enzymes, more resistant to thermal process, and the subsequent oxidation of fatty acids by microbial enzymes or by chemical process (catalysed by light). Part of the lipase is inactive by slow pasteurization, but still allows partial hydrolysis

Table IV

Absorption bands observed in the IR for unsaturated and their assignments (4a) and ^1H NMR chemical shifts of saturated fatty acids (4b) in samples of human milk fatty acids. 2010

IV(a)

Assignments to Functional Groups	*Palm-9 Nº wave cm^{-1}*	*Oleic Nº wave cm^{-1}*	*ZZ-Linol Nº wave cm^{-1}*	*ZE-Linol Nº wave cm^{-1}*	*Linolenic Nº wave cm^{-1}*
CH_{est} sp^2	3004	3004	-	3010	3004
$CH_{3\ est}$	2926	2925	2950	2926	2929
$CH_{2\ est}$	2855	2854	2858	2855	2857
$C{=}O_{est}$	1743	1744	1733	1743	1736
$CH_{3\ def.\ ass.}$	1461	1463	1461	1461	1461
$CH_{2\ def.}$	1436	1436	1438	1436	1439
$CH_{3\ def.\ sim.}$	1361	1360	1366	1360	1366
$C\text{-}O_{est}$	1197	1197	1201	1197	1198
C=CH sp^2E	*	*	*	969	*
$CH_{2\ def\ rock}$	725	723	728	725	-

* The vibrational frequency for the cis double bond (C = CH sp^2Z) were not noted in the spectra due to the low intensity of the respective bands.

IV(b)

Nature of the fatty acids characterized hydrogens	*Palmitoleic δ (ppm)*	*Oleic δ (ppm)*	*Linoleic (ZZ) δ (ppm)*	*Linoleic (ZE) δ (ppm)*	*Linolenic δ (ppm)*
CH_3	0,88	0,88	0,89	0,88	0,98
$-(CH_2)_n-$	1,20-1,40	1,20-1,40	1,20-1,40	1,20-1,40	1,20-1,40
$-CH_2CH_2COOCH_3$	1,62	1,60	1,61	1,58-1,64	1,62
$-CH_2CH{=}CHCH_2-$	1,98-2,14	1,98-2,04	1,92-2,10*	1,97-2,04	2,01-2,17
$-CH_2CH_2COOCH_3$	2,30	2,30	2,31	2,30	2,31
$=CHCH_2CH=$	-	-	2,66-2,80*	2,66-2,72	2,81
$-CH_2COOCH_3$	3,67	3,67	3,67	3,67	3,67
-CH=CH-	5,30-5,40	5,30-5,40	5,34-5,46*	5,37-5,42	5,30-5,45

* Data cited according to Silverstein, Webster, Kiemle (2005) due to the low concentration of linoleic acid in the sample.

of lipids and release of fatty acids (Weber *et al.,* 2001).

The obtained data are not unanimous with the literature because show wide variation in the concentration of fatty acids of the samples. In addition to the operating conditions of the processes of preservation, it is still possible to judge that the storage under slow freezing conditions allow the formation of ice crystals that can pierce the fat globules resulting in the separation of molecules lipids after thawing.

To interpret the observed changes in the lipid fraction of the samples, one should also consider aspects concerning the conservation of human milk in HMB. The heat exchangers in use are not equipped with stirrers and, occasionally, the containers are manually agitated to promote homogenization of the product. Such conditions allow the formation of a surface layer of fat (cream) after cooling. The hydrolysis of lipids originating in the release of fatty acids alters the density of these acids, as well as the action of microbial lipases, which are resistant to pasteurization, unlike endogenous lipases.

Moreover, it is also possible to consider some degree of adhesion of fat globules and free fat acids in the inner walls of the containers containing the products (glasses) acids, even after thawing under heating in a water bath at 40° C, or even adhesion caused by fat globules released during the steps of freeze / thaw by the ice crystals (large, pointy) formed during slow freezing. The lack of homogeneity of the milk before freezing can affect the fat content, as well as the different sampling techniques, since the milk is first collected and frozen at home by the donor (Weber *et al.,* 2001). Such considerations suggest that these factors may have contributed to the large variation of the samples observed month by month.

In light of the obtained results, it is possible to make the following recommendations in order to minimize the effects of pasteurization and of freezing concerning

the lipidic fraction of the human milk: a) reducing the time lapse between collection and pasteurization not to please the hydrolysis of lipids; b) choosing rapid pasteurization in order to inhibit lipolytic activity, rising of concentration of free fatty acids and reducing the exposure time to heat the milk; c) for slow pasteurization, the use of equipment with stirring system to prevent the coalescence of fat and thus their partial loss; d) during and after thawing the milk in a double boiler, stirring it until it is ready to use to prevent the adhesion of molecules of fat in the walls of the container and its nonhomogeneous distribution of the newborn mode; e) considering that the sampling techniques are a factor that implies the variation of lipid content, guiding and overseeing of the donors continuously to ensure homogeneity of the milk before processing (pasteurization and freezing), since the fat content varies according to a number of factors and mainly increases between the beginning and end of milking.

One can consider the loss of two samples (T30 and T60) as a limitation of the search; in the same way as mentioned above, it is necessary to optimize operating conditions in human milk banks, especially for the pasteurization and thawing of milk.

Acknowledgments

The authors thank the Human Milk Bank, University Hospital, University of Brasilia for the donation of the milk sample.

References

1. AOAC, (1998) "Association of Official Analytical Chemists. Official Methods of Analysis", 16th ed. AOAC International, Maryland.
2. American Medical Association [homepage on the Internet], 2001. Chicago: The Association; c1995-2002 [updated 2001 Aug 23; cited 2002 Aug 12]. AMA Office of Group Practice Liaison; [about 2 screens]. Available at: http://www.ama-assn.org/ama/pub/category/1736.html .
3. Bretillon, L., Chardgny, J.M., Sébédio, J.L., Poullain, D., Nöel, J.P. and Vatèle, J.M. (1998), "Oxidative metabolism of [1-^{14}C] mono-*trans* isomers of linoleic and α-linolenic acids in the rat", *Biochimica et Biophysica Acta* Vol. 1390 No. 2, pp. 207-214.
4. Christie W.W., (1990) "Gas chromatography and lipids: a practical guide", The Oily Press, Ayr.
5. Fennema, O.R., (1993) "Química de los alimentos". 2nd Editorial Acribia, Zaragoza; 1993, pp. 1095.
6. Fidler, N., Sauerwald, T.U., Koletzko, B. and Demmelmair, H. (1998), "Effects of human milk pasteurization and sterilization on available fat content and fat composition", *Journal of Pediatric Gastroenterology and Nutrition* Vol. 27 No. 3, pp. 317-322
7. Goldblum, R.M., Dill, C.W., Albrecht, T.B, Alford E.S., Garza, C. and Goldman, A.S. (1984), "Rapid high-temperature treatment of human milk", *Journal of Pediatrics* Vol. 104 No. 3, pp. 380-385.
8. Hamosh, M. (1998), "Protective function of proteins and lipids in human milk", *Biology of the Neonate* Vol. 74 No. 2, pp. 163-176.
9. Heiman, H. and Schandler, R.J. (2006), "Benefits of maternal and donor human milk for premature infants", *Early Human Development* Vol. 82 No. 12, pp. 781-787.
10. Henderson, T.R., Fay, T.N. and Hamosh, M. (1998), "Effect of pasteurization on long chain polyunsaturated fatty acid levels and enzyme activities of human milk", *Journal of Pediatrics* Vol. 132 No. 5, pp. 876-878.
11. Lepri, L., Del Bubba, M., Maggini, R., Donzelli, G.P. and Galvan, P. (1997), "Effect of pasteurization and storage os some components of pooled human milk", *Journal of Chromatography B* Vol. 704, pp.1-10.
12. Romeu-Nadal, M., Castellote, A.I. and López-Sabater, M.C. (2008), "Effect of cold storage on vitamins C and E and fatty acids in human milk", *Food Chemistry* Vol. 106 No. 1, pp. 65-70.
13. Walstra, P., Geurts, T.J., Noomen, A.J. and van Boekel, M.A. JS. (2001), "Ciencia de la leche y tecnología de los productos lácteos". Editorial Acribia, Zaragoza, pp. 730.
14. Wardell, J.M., Hill, C.M. and D'Souza, S.W. (1981), "Effect of pasteurization and of freezing and thawing human milk on its triglyceride content", *Acta Paediatrica Scandinavica* Vol. 70, pp. 467-471.
15. Weber, A.L., Loui, A., Jochum, F, Bücher, C. and Obladen, M. (2001), "Breask milk from mothers of very low birthweight infants: variability in fat and protein content", *Acta Paediatrica* Vol. 90 No. 7, pp. 772-775.