Article

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Action of Successive Heat Treatments in Bovine Milk Fatty Acids

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O estudo mostra a ação sucessiva dos tratamentos térmicos de pasteurização (75 °C por 15 s) e esterilização comercial por troca indireta de calor (140 °C for 6 s) sobre o perfil lipídico de leite bovino. Amostras de leite cru foram submetidas à pasteurização e então, à esterilização comercial (ultra-alta temperatura, UHT). A gordura de amostras de leite cru, de leite pasteurizado e de leite esterilizados comercialmente foi extraída. Após transesterificação, os ésteres metílicos dos ácidos graxos (FAMEs) foram analisados por cromatografia gasosa com detecção por ionização de chama (GC-FID). A quantificação revelou que para a maioria dos ácidos graxos (FA) encontrados não houve diferença significativa ($p > 0,05$) entre as amostras de leite cru e leite pasteurizado. Entretanto, foram encontradas diferenças significativas para 21 dos 26 ácidos graxos analisados ($p > 0,05$) para as amostras de leite cru e de leite esterilizado, incluindo o isômero predominante no leite do ácido linoléico conjugado (CLA-c9t11). Este fato evidencia a ação sucessiva dos tratamentos térmicos no perfil lipídico do leite.

The action of successive pasteurization thermal treatments (75 °C for 15 s) and commercial sterilization by indirect heat exchange (140 °C for 6 s) was analyzed on the lipid profile of bovine milk. Raw milk samples were submitted to pasteurization and then were submitted to sterilization (ultra-high temperature, UHT). The fat of raw milk, pasteurized milk and commercially sterilized milk samples was extracted. After transesterification, the fatty acid methyl esters (FAMEs) were analyzed by gas chromatography with flame ionization detector (GC-FID). The quantification of fatty acids (FA) revealed that for most of the found fatty acids there was no significant difference ($p > 0.05$) between raw milk and pasteurized milk. However, it was found significant differences for 21 of the 26 analyzed fatty acids ($p > 0.05$) for the raw and sterilized milks, including the predominant isomer of the conjugated linoleic acid (CLAc9t11) of the milk. This fact evidences the successive action of heat treatments on milk lipid profile.

Keywords: milk, pasteurization, commercial sterilization, conjugated linoleic acid

Introduction

Currently, there is a growing the demand for high quality dairy products, leading to a trend of gradual adaptation by the dairy industry to the needs dictated by the consumers. In this context, there are challenges such as considering the preventive role that healthy eating habits have on certain pathologies.[1]

As a result, nutraceutical substances present in foods have been studied. However, most compounds that show some anticarcinogenic activity are of plant origin.[2] In the decade of 1980, conjugated linoleic acid isomers (CLA) were found in animal fats, with different physiological effects and confirmed biological activity.[3,4] Isomer t10c12 acted on the redistribution of fat of the muscle, being able to reduce body fat and to increase lean body mass.[5-7] Isomer c9t11 presented antitumor properties, acting in the reduction of breast cancer.[4,8] These factors are causing more and more producers to seek a supplementary diet of their animals in order to increase the amount of these beneficial compounds in the final product.

Due to the characteristics of raw milk, it is essential to keep it conditioned at low temperatures and submitted to heat treatment at processing phase for the destruction of microorganisms. Pasteurization is the most widely used heat treatment by the industries. Although this process completely eliminates pathogenic bacteria from milk, it does not eliminate spores of psychrotrophic bacteria.[9]

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Another widely used treatment is the commercial sterilization (ultra-high temperature, UHT), which is more advantageous than pasteurization due to its practicality of conservation and use.[10]

Works concern the influence of slow pasteurization on the lipid profile of human milk have been described in the literature and show that it is not changed by pasteurization.[11] Similar results were also obtained in studies on the effect of the conventional heat treatment on bovine milk.[12] However, no study was found about the successive action of heat treatments on the same sample. Thus, the objective of this study was to verify the effect of successive action of pasteurization and commercial sterilization (UHT) heat treatments on the fatty acids (FA) of a same sample of bovine milk.

Experimental

Sampling

Eleven milk samples from different lots were obtained from September to November 2010 in a dairy industry in Itapetinga City (Bahia, Brazil). The raw milk samples were submitted to pasteurization (75 ºC for 15 s) and then to sterilization (ultra-high temperature, UHT). Samples were collected and immediately frozen for later duplicate analysis (n = 22).

Analysis of fatty acids

The analyses were carried out by Center of Chromatographic Analysis of Universidade Estadual do Sudoeste da Bahia (Itapetinga City). Lipid extraction followed the methodology proposed by Folch *et al*.[13] and the transesterification was carried out according to Bannon *et al.*,[14] with modifications according to Simionato *et al*.[15]

Fatty acid methyl esters (FAMEs) were analyzed by gas chromatography in Thermo model Trace-GC-Ultra, equipped with a flame ionization detector (GC-FID) and a fused silica capillary column BPX-70 (120 m × 0.25 mm i.d.). The established operating parameters after checks of best resolution condition were: injector and detector temperatures, 250 and 280 °C, respectively. Column temperature was set at 140 °C for 10 min, followed by a first ramp at 15 °C min^{-1} until 200 °C for 1 min. The second ramp was at 10 °C min^{-1} until 230 °C for 1 min, the third ramp at 0.4 °C min^{-1} until 233 °C for 3 min and the fourth ramp at 0.5 °C min^{-1} until 238 °C for 2 min. Total analysis time was 41.50 min. Gases flow rates (White Martins) were 30 mL min^{-1} for hydrogen, 30 mL min^{-1} for nitrogen and 250 mL min^{-1} for synthetic air.

Injections of 1.2 μL were performed in duplicate. The peak areas of FAMEs were determined by ChromQuest 4.1 software.

Identification and quantification of fatty acids

The identification of fatty acids was performed after verification of the equivalent length of the chain of peaks and comparison of retention times of samples with the standards of methyl esters of fatty acids (189-19, O-5632 and O-5626, Sigma, EUA), according to Simionato *et al*.[15]

Quantification of FA (in mg g^{-1} of total lipids) was made in relation to the internal standard, methyl tricosanoate (23:0) (Sigma). The sample FA concentrations were calculated according to Joseph and Ackman[16], using equation 1:

$$C\,(mg\ g^{-1}) = \frac{A_X\ M_{23:0}\ T_{RF}}{A_{23:0}\ M_A\ C_F} \quad (1)$$

where A_X = FAME area, $A_{23:0}$ = internal standard area, $M_{23:0}$ = internal standard mass added to the sample (mg), M_A = sample mass (g), T_{RF} = theoretical response factor of FAMEs and C_F = conversion factor to express the results in mg fatty acids *per* g total lipids (TL).

To assess the response of the FID, the theoretical response factors were calculated and the agreement verification between theoretical and experimental response factors was performed, as described by Simionato *et al.*[15]

Statistical analysis

The results were submitted to variance analysis (ANOVA) at 5% probability and means were compared by Tukey test with the software Statistical version 7.0.[17]

Results and Discussion

The quantification of fatty acids using internal standards has been widely applied to provide reliable results that can be easily interpreted. However, when using FID, the differential response must be considered and the correction factors must be increased.[18] Thus, it is necessary to validate the used equipment to verify the agreement between the theoretical response factors (T_{RF}) from those experimentally obtained (E_{RF}). The ideal situation is to get results in which the error factor is close to unity, so that the results have high accuracy. After verifying the agreement between E_{RF} and T_{RF}, the theoretical factors were used for the quantitative determinations of fatty acids, as proposed by Bannon *et al.*[19]

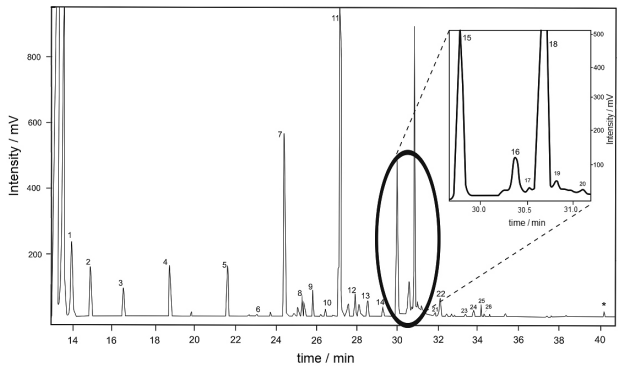

Figure 1. Gas chromatogram obtained for milk samples, the lipid profile: (1) 4:0, (2) 6:0, (3) 8:0; (4) 10:0, (5) 12:0, (6) 13:0, (7) 14:0, (8) 14:1, (9) 15:0, (10) 15:1, (11) 16:0, (12) 16:1, (13) 17:0, (14) 17:1, (15) 18:0, (16) 18:1n-9t, (17) 18:1n-7t, (18) 18:1n-9, (19) 18:1n-7, (20) 18:2t9t12, (21) 18:2t9c12, (22) 18:2c9c12, (23) 20:0, (24) 18:3n-3, (25) 18:2c9t11, (26) 18:2t10c12 and (*) 23:0 (standard).

Based on the equivalent chain length and the comparison with standard FA, 26 fatty acids in fat of milk were tentatively identified (Figure 1) and quantified to assess the interference of successive heat treatments.

Table 1 lists the saturated fatty acids (SFA) that were found in the samples. There was no significant difference ($p > 0.05$) between raw and pasteurized milk samples for all SFAs. These results agree with Herzallah *et al.*,[12] who evaluated the influence of the low pasteurization process on the lipid profile of milk. Souza *et al.*[20] also evaluated the composition and profile of raw and pasteurized milk fatty acids in mini dairies and found no significant difference ($p > 0.05$) for the influence of pasteurization heat treatment on raw milk.

Evaluating the commercial sterilization process by indirect heat exchange (UHT), only the obtained values for tridecanoid acid (13:0) and palmitic (16:0) SFA on different thermal treatments were not different ($p > 0.05$), evidencing that successive heat treatments have no influence in these substances. In contrast, all other analyzed SFA showed values statistically different ($p > 0.05$) between raw and sterilized milk samples. This indicates that successive heat treatments influenced these substances.

From the obtained data for unsaturated fatty acids (UFA) of the analyzed samples, it was observed that, there was no statistical difference ($p > 0.05$) between raw and pasteurized milk samples, as for SFA (Table 2). Exceptions were observed for heptadec-10-enoic acid (17:1) and elaidic acid (18:1n-9t) fatty acids, with decreasing of *ca.* 27% (from 5.600 to 4.081 mg g^{-1}) and 24% (from 25.51 to 19.46 mg g^{1}), respectively, between raw and pasteurized milk samples. However, as noted for SFA, most analyzed UFA presented statistically different values ($p > 0.05$) between raw and sterilized milk samples, indicating the action of successive heat treatments on these FA. Exceptions were observed for vaccenic (18:1n-7t) and trans-9-cis-12-octadienoic (18:2t9c12) acids and t10c12 isomer of the conjugated linoleic acid (18:2t10c12), in which heat treatment action was not statistically verified.

There was a significant reduction (*ca.* 21.80%) between raw and UHT milk samples for CLAc9t11 (from 10.18 to 7.96 mg g^{-1}). Herzallah *et al.*[12] found CLA levels in pasteurized milk (according to high temperature, short time (HTST) and low temperature, long time (LTLT) processes) lower than those found in raw milk. According to the same author, the trend in reduction of CLA could be attributed to an oxidation process, resulting in hydroperoxides that could cause the conversion or degradation of CLA.[12] This study evidences and confirms the action of successive heat treatments on this fatty acid.

Table 1. Saturated fatty acids (mg g^{-1}) of lipids for raw, pasteurized and sterilized milk samples

Fatty acid[a]		Raw milk / (mg g^{-1})	Pasteurized milk / (mg g^{-1})	Sterilized milk / (mg g^{-1})	VC[b]
Butyric	4:0	34.25^{A}	31.38^{AB}	23.36^{B}	38.59
Caproic	6:0	21.29^{A}	19.93^{A}	14.70^{B}	37.53
Caprylic	8:0	11.77^{A}	10.81^{AB}	8.39^{B}	37.99
Capric	10:0	22.46^{A}	20.71^{AB}	17.09^{B}	32.68
Lauric	12:0	25.52^{A}	23.03^{AB}	19.35^{B}	30.98
Tridecanoic acid	13:0	0.84^{A}	0.98^{A}	0.64^{B}	61.53
Myristic	14:0	93.78^{A}	82.31^{AB}	72.60^{B}	29.30
Pentadecenoic	15:0	13.80^{A}	12.03^{AB}	10.90^{B}	27.40
Palmitic	16:0	251.70^{A}	222.40^{A}	198.60^{B}	24.82
Margaric	17:0	8.07^{A}	7.08^{AB}	6.71^{B}	25.00
Stearic	18:0	103.90^{A}	89.38^{AB}	83.41^{B}	22.16
Arachidic	20:0	1.66^{A}	1.41^{AB}	1.310^{B}	26.73

[a]Usual nomenclature; [b]variation coefficient; means in the same row followed by different letters differ by Tukey test ($p > 0.05$).

Table 2. Mono and polyunsaturated fatty acids (mg g^{-1}) of lipids for raw, pasteurized and sterilized milk samples

Fatty acid[a]		Raw milk / (mg g^{-1})	Pasteurized milk / (mg g^{-1})	Sterilized milk / (mg g^{-1})	VC[b]
Myristoleic	14:1	8.991^{A}	7.953^{AB}	7.01^{B}	26.25
Palmitoleic	16:1	12.13^{A}	11.01^{AB}	9.52^{B}	30.15
Heptadec-10-enoic acid	17:1	5.60^{A}	4.081^{B}	3.78^{b}	38.96
Elaidic	18:1n-9t	25.51^{A}	19.46^{B}	18.48^{B}	29.13
Oleic	18:1n-9c	216.70^{A}	187.50^{AB}	170.60^{B}	23.62
Vaccenic acid	18:1n-7t	9.47^{A}	8.47^{A}	7.84^{B}	55.75
Cis vaccenic acid	18:1n-7c	5.28^{A}	3.58^{AB}	2.67^{B}	76.99
Linolelaidic	18:2n-6t	5.56^{A}	4.47^{AB}	3.66^{B}	52.86
Trans-9, cis-12 acid octadienoic	18:2t9c12	2.54^{A}	1.99^{A}	2.26^{A}	37.04
Gamma-linoleic	18:2n-6	8.01^{A}	6.96^{AB}	6.41^{B}	21.30
Alpha-linolenic	18:3n-3	3.82^{A}	3.381^{AB}	3.10^{B}	24.78
Dihomo-gamma-linolenic acid	20:3n-6	11.99^{A}	10.33^{AB}	9.26^{B}	25.03
Conjugated linoleic acid	CLAc9t11	10.18^{A}	8.82^{AB}	7.96^{B}	24.02
Conjugated linoleic acid	CLAt10c12	0.78^{A}	0.81^{A}	0.54^{A}	57.16

[a]Usual nomenclature; [b]variation coefficient; means in the same row followed by different letters differ by Tukey test ($p > 0.05$).

Figure 2 shows octadienoic acid profiles to raw, pasteurized and sterilized milk samples. It is evidenced the significant difference between raw and UHT milks for CLAc9t12 and 18:2c9c12 and no difference between raw and UHT milks for CLAt10c12.

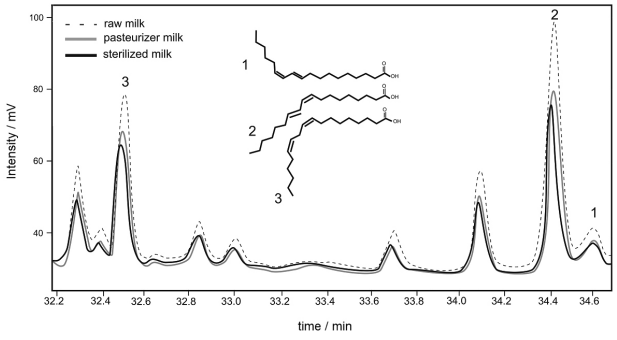

Figure 2. Gas chromatograms obtained for (---) raw, (—) pasteurized and (**—**) sterilized milk samples, the octadienoic acid profiles: (1) CLA(t10c12), (2) CLA(c9t11) and (3)18:2c9c12.

Fanti *et al.*[21] report that CLA content in fat milk is usually between 0.3 and 1.0%. Data from this study are higher than these, but close to those found by Kelsey *et al.*[22] The found difference in conjugated linoleic acid contents may be caused by season, feed, breed, food supplied to the animal, lactation phase and thermal processing.[23]

Table 3 shows the sums of saturated (4:0, 6:0, 8:0, 10:0, 12:0, 13:0, 14:0, 15:0, 16:0, 17:0, 18:0, 20:0), monounsaturated (14:1, 16:1, 17:1, 18:1n9t, 18:1n9c, 18:1n7t, 18:1n7c) and polyunsaturated (18:2n6t, 18:2t9c12, 18:2n-6, 18:3n-3, CLAc9t11, CLAt10c12, 20:3n-6) fatty acids, the relation between polyunsaturated and saturated fatty acids (AGPI/AGS), the sums of omega-3 (18:3n-3, 20:3n-3) and omega-6 (18:2n-6, 20:3n-6) fatty acid series and the relation between the fatty acids of these series (n-6/n-3).

The same trend previously reported was observed in the present article, i.e., there are no significant differences ($p > 0.05$) for the sums of saturated, monounsaturated and polyunsaturated fatty acids and omegas 6 and 3 between raw and pasteurized milks. However, significant difference was found ($p > 0.05$) between raw and sterilized milk samples. Successive action of pasteurization and sterilization treatments generally decreased the fatty acids in milk, but not to the point of interfering in the ratios between omegas 3 and 6 and between saturated and polyunsaturated fatty acids, as evidenced by the statistical similarity of the results.

The ratios between the omega-6 and omega-3 fatty acids in the samples that were subjected to different heat treatments were equal to 2.10, 1.97 and 2.07 for raw, pasteurized and commercially sterilized milks, respectively. Simopoulos[24] suggests that this ratio cannot be over than 4. The United Kingdom Department of Health[25] suggests that the intake ratio of omega-6 and omega-3 is between 5 and 10. The values from this study show that milk despite of presenting high levels of saturated fatty acids is still a great source of essential fatty acids. Results indicate that there was no significant difference between raw, pasteurized and UHT milks in the ratio of n-6/n-3. Moreover, there were no significant differences for most analyzed fatty acids, as

Table 3. Totals of saturated, monounsaturated and unsaturated fatty acids; ratio unsaturated/saturated fatty acids, omega-3, omega-6 and ration between omega-6 and omega-3 (mg g^{-1}) of lipids for raw, pasteurized and sterilized milk

Fatty Acid[a]	Raw milk / (mg g^{-1})	Pasteurized milk / (mg g^{-1})	Sterilized milk / (mg g^{-1})	VC[b]
Saturated	589.10^{A}	521.40^{AB}	457.10^{B}	26.06
Monounsaturated	283.60^{A}	242.10^{AB}	219.90^{B}	23.53
Polyunsaturated	33.77^{A}	28.85^{AB}	26.16^{B}	24.52
PUFA/SFA[c]	0.06^{A}	0.05^{A}	0.06^{A}	13.28
n-3	4.60^{A}	4.19^{AB}	3.65^{B}	24.86
n-6	9.07^{A}	7.86^{AB}	7.32^{B}	20.03
n-6/n-3	2.10^{A}	1.97^{A}	2.07^{A}	10.72

[a]Usual nomenclature; [b]variation coefficient; [c]ratio polyunsaturated/saturated fatty acids; means in the same row followed by different letters differ by Tukey test ($p > 0.05$).

confirmed by Ford and Thompson.[26] According to these authors, there are no alterations of nutritional importance on lipid content after UHT processing, although there might be some unsaturated fatty acid loss in milk lipids.[26] The results from that study indicate that in milk obtained from animals fed with special diets (aimed to increase such substances), the thermal processing will not negatively affect the quality of the final product as to the presence of essential fatty acids.

However, results also show that, when a final product rich in CLA is desirable (either by animal supplementation or direct addition), one must be careful with the processing since it was found significant differences after successive heat treatments, the evidence of the reduction of the isomer c9t11. The isomer c9t11 according to Kelsey *et al.*[22] is the most abundant, corresponding to 75 to 90% of total CLA in milk fat.

Conclusions

Pasteurization does not significantly alter the quality and quantity of fatty acids in milk. However, significant differences can be found when raw milk passes through successive heat treatments of pasteurization and commercial sterilization. These differences were found in 21 of the 26 analyzed fatty acids, including the predominant isomer of conjugated linoleic acid (CLA) in milk. This evidenced the action of successive heat treatments on the lipid profile of milk.

Supplementary Information

Experimental (E_{RF}) and theoretical (T_{RF}) correction factors and error factor (E_F) of the TRACE GC Ultra Gas Chromatograph Thermo Scientific are available free of charge as PDF file at http://jbcs.sbq.org.br.

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Supplementary Information

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Table S1. Experimental (E_{RF}) and theoretical (T_{RF}) correction factors and error factor (E_F) for the gas chromatograph thermo trace-GC-ultra

Fatty acids	E_{RF}	T_{RF}	EF
12:0	1.224	1.114	1.099
14:0	1.032	1.080	0.956
16:0	0.961	1.055	0.912
18:0	0.925	1.035	0.894
18:1	0.914	1.028	0.889
18:2	1.012	1.021	0.991
18:3	1.136	1.014	1.121
20:0	0.900	1.019	0.883
20:3	1.094	0.999	1.094

E_{RF} = experimental correction factor; T_{RF} = theoretical correction factor; E_F = error factor.

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