

Fatty Acid Content of Bovine Milkfat From Raw Milk to Yoghurt

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Abstract: Problem statement: The present study aimed to study the evolution of fatty acid content, focusing on rumenic acid content, from raw milk to yoghurt processed from this milk. **Approach:** Milk samples were collected in a dairy plant in the northwest of Paraná State weekly in January 2011 (Brazilian summer). It processed one truck load of 26,000 L of refrigerated type-C (whole standardized milk with a minimum of 3% fat) milk per day, mostly from the city of Lobato, Paraná, produced mainly by Gir (Bos indicus) cattle raised on stargrass (*Cynodon nlenfuensis* var. *nlenfuensis*) pasture. **Results:** Saturated Fatty Acid (SFA) were the most abundant, particularly palmitic (16:0), stearic (18:0) and myristic (14:0). Among the Monounsaturated Fatty Acid (MUFA), Polyunsaturated Fatty Acid (PUFA) and trans fatty acid, oleic acid (18:1n-9), linoleic acid (18:2n-6), elaidic acid (t9-18:1) and c9, t11-18:2 (rumenic acid) predominated. It was detected significant differences ($p < 0.05$) in the quantification of isomer c9, t11-18:2 of Conjugated Linoleic Acid (CLA). Raw milk had the largest content of rumenic acid ($14.91 \pm 0.17 \text{ mg g}^{-1}$ of lipids), decreasing to 6.22 ± 0.20 after pasteurization and to $5.41 \pm 0.18 \text{ mg g}^{-1}$ in yoghurt. **Conclusion/Recommendations:** It is demonstrated that pasteurization and yoghurt making affect the CLA contents.

Key words: Saturated Fatty Acid (SFA), Conjugated Linoleic Acid (CLA), Monounsaturated Fatty Acid (MUFA), High Temperature Short Time (HTST)

INTRODUCTION

The term Conjugated Linoleic Acid (CLA) refers to a mixture of linoleic acid isomers (c9, c12-18:2; t10, c12-18:2; c9, t11-18:2 (rumenic acid), t10, t12-18:2; t9, t11-18:2; t8, t10-18:2; t7, t9-18:2; t8, c10-18:2). This group of compounds is object of a large number of studies and represents a new and wide field of study on fatty acid and their relationship with human health (Autores, 2009). Among the potential benefits that have been reported are effects on the body composition, cardiovascular diseases and the immunologic system (Ip *et al.*, 2002; Ledoux *et al.*, 2007; Cook *et al.*, 1993).

Two CLA isomers have gained importance in food research worldwide. Isomer t10, c12-18:2 a powerful inhibitor of the synthesis of fat in milk and responsible for the redistribution of fat in muscle, contributing to reduce the fat mass and increase the lean mass (Mourão *et al.*, 2005; Dugan *et al.*, 1997; Chardigny *et al.*, 2003; Gaullier *et al.*, 2005; Degrace *et al.*, 2003). Isomer c9, t11-18:2 has demonstrated antitumoral properties, reducing the incidence of breast cancer (Mourão *et al.*, 2005; Dugan *et al.*, 1997; Chardigny

et al., 2003; Gaullier *et al.*, 2005; Degrace *et al.*, 2003; Ou *et al.*, 2007; Lavillonnière *et al.*, 2003; Belury *et al.*, 1996; Hubbard *et al.*, 2000; Molkentin, 1999; Chardigny *et al.*, 2008).

The pasture has major effects by decreasing saturated FA and increasing FA considered as favorable for human health (c9-18:1, 18:3n-3 and c9, t11-CLA), compared to winter diets, especially those based on maize silage and concentrates (Chilliard *et al.*, 2007).

As CLA is a product of incomplete biohydrogenation (Kepler *et al.*, 1966; Kramer *et al.*, 1998), foods derived from ruminants are the main sources of CLA in the human diet. Thus, after years of banning dairy products, the existence of such potentially beneficial product may be an opportunity for a new perception of animal-origin products by the consumers and the medical community.

There were some studies on the effects of process, especially the effect of starter cultures on CLA contents in yoghurt and cheeses and the effect of ripening on CLA contents of cheeses and on the process of milk to cream and then to butter (Shantha *et al.*, 1995; Jiang *et al.*, 1998; Sieber *et al.*, 2004; Gnädig *et al.*, 2004;

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Ledoux and Laloux, 2006; Pereda *et al.*, 2008). However, there is a lack of data on the effect of the thermal processes used in the preservation of milk and its derivatives regarding their effects on the nutritional and organoleptic properties of dairy products (Pereda *et al.*, 2008; Raynal-Ljutovac *et al.*, 2007; Rynne *et al.*, 2004).

To our knowledge, there is no report on the effects of pasteurization on the fatty acid profile of milk and derivatives. The aim of the study was to determine the fatty acid, including CLA, contents of raw and pasteurized milk and yoghurt, focusing of the evolution of the rumenic acid content from raw milk to yoghurt during processing.

MATERIALS AND METHODS

Sampling: Milk samples were collected in a dairy plant in the northwest of Paraná State weekly in January 2011 (Brazilian summer). It processed one truck load of 26,000 L of refrigerated type-C (whole standardized milk with minimum of 3% fat) milk per day, mostly from the city of Lobato, Paraná, produced mainly by Gir (*Bos indicus*) cattle raised on stargrass (*Cynodon nlenfuensis* var. *nlenfuensis*) pasture. The tank outlets were then connected to stainless steel pipes and positively pumped through a closed system to a vertical isothermal storage tank with capacity of 30,000 L provided with a stirrer. A total of five lots of 250 mL raw milk were carried out weekly in January 2011 (Brazilian summer). Each lot was sampled three times in order to perform analysis in triplicates. Raw milk samples were transferred in sterilized tubes. The tubes were immediately cooled down to 20°C after sampling, then frozen (-18°C) and transported in thermal recipients.

From the vertical isothermal tank where the raw milk was initially stored, it is pumped through a stainless steel closed system to a High Temperature, Short Time (HTST) type pasteurizer provided with a digital temperature controller. The milk is heated to 75°C/15 s and immediately cooled to 5°C for pasteurization. Then, it follows to another vertical isothermal storage balloon with capacity of 30,000 L provided with a stirrer.

For each of the five week lots, three samples of 250 mL pasteurized milk were collected from the tank and transferred in sterilized tubes. The tubes were immediately cooled after sampling, frozen (-18°C) and transported in thermal recipients.

From the vertical storage balloon the pasteurized milk runs through a stainless steel closed system to a preparation tank, where it is added with sugar and stabilizers under constant stirring according to the plant

formulation. Next, the mixture is re-pasteurized (85°C/10 min) and goes to fermentation tanks, where the temperature reaches 42°C. The lyophilized starting mix Yo Mix from Danisco is added, the tank is sealed and the milk rests until pH 4.7 is reached, then it is cooled to 20°C under light agitation. To this mixture is added the wished fruit flavor and the yoghurt is cup-packed in a Brascop-type machine.

For each of the five week lots, three samples of 500 mL yoghurt were collected after packing and transferred in sterilized tubes. The tubes were immediately cooled after sampling, frozen (-18°C) and transported in thermal recipients. The three samples of each lot for all matrixes (raw milk, pasteurized milk and yoghurt) were analyzed individually and within the same time.

Lipid extraction and analysis of fatty acid: Total lipids were determined by the Folch *et al.* (1957) method using chloroform, methanol and water (2:1:1).

The lipids were converted into fatty acid methyl esters following Bannon *et al.* (1982) to a screw cap tube with approximately 150 mg of lipids was added 0.25 mol L⁻¹ 5.0 mL of sodium methoxide solution in methanol/ethylic ether (1:1). The tube was vigorously agitated for approximately 3 min. Next, 3.0 mL of isooctane and 15 mL of saturated sodium chloride were added. The tube was vigorously agitated again and rested for phase separation. The supernatant was collected in labeled Eppendorf tubes for chromatographic analysis.

The method originally involves fast heating under reflux after the addition of the transesterifying agent; however, this was not done to avoid isomerization of conjugated dienes of linoleic acid, as proposed by Simionato *et al.*, 2010).

Chromatographic analysis was performed in a gas chromatograph Varian, model CP 3380, equipped with a flame ionization detector, split/splitless-type injector, fused silica capillary column CP Select CB-Fame (100% bonded cyanopropyl, 100 m, 0.25 mm i.d., 0.39 µm stationary phase). The best resolution operation parameters were: injector and detector temperature 235°C; column temperature 65°C for 4 min, followed by a 16°C/min ramp up to 185°C, for 12 min. A second ramp of 20°C/min up to 235°C for 14 min was programmed. The total analysis time was 40 min.

The following gas flows were used: Carrier gas (H₂) 1.4 mL.min⁻¹, auxiliary (N₂) 30 mL.min⁻¹ and H₂ and flame synthetic air, 30 and 300 mL.min⁻¹, respectively. The sample split rate used was 1/80. Injections were performed in duplicate in volumes of 2 µL. The peak

areas of fatty acid methyl esters were determined with the software workstation version 5.0 (Varian).

Identification of fatty acid: The fatty acid methyl esters were identified by comparison of retention times of the sample constituents with a mixture of 37 fatty acid methyl ester standards (189-19 Sigma, USA) and comparison with retention times of methyl ester standards containing the geometric isomers *c9*, *t11-18:2* and *t10,c12-18:2* of linoleic acid (O-5626, Sigma, USA).

Fatty acids were quantified as mg g⁻¹ of total lipids compared to the internal standard, methyl tricosanoate (23:0) from Sigma. Before weighing the sample, 1.00 mL of internal standard solution (1 mg mL⁻¹) was added to the esterification recipient, after which the solvent was evaporated under N₂ flow.

The fatty acid methyl esters were quantified after verification of agreement between the theoretical and experimental response factor. The fatty acid concentration was calculated according to Joseph and Ackman (1982) using the equation:

$$C(\text{mg g}^{-1}) = \frac{A_X \cdot M_{23:0} \cdot T_{RF}}{A_{23:0} \cdot M_A \cdot F_{CT}}$$

Where:

- A_X = Area of fatty acid methyl esters
- A_{23:0} = Area of the internal standard
- M_{23:0} = Mass of internal standard added to the sample (mg)
- M_A = Sample mass (g)
- TRF = theoretical response factor of fatty acid methyl esters
- FCT = conversion factor for results as mg fatty acid /g of Total Lipids (TL)

Statistical analysis: The results were submitted to Variance Analysis (ANOVA) at 5% significance level using the software StatSoft (2005). The mean values were compared with Tukey's test.

RESULTS

The total lipids of the samples were determined and are given in Table 1. They varied from 2.41% in yoghurt to 3.66% in raw milk.

Figure 1 shows the 26 Fatty Acid (FA) identified in the samples and the internal standard used. The analyzed samples presented 26 fatty acid, which were identified and quantified (Table 2).

Figure 2 shows that women would need to take 2.5 glasses of raw milk or 6 glasses of pasteurized milk or 9

cups of yoghurt a day and men, 3 glasses of raw milk or 8 glasses of pasteurized milk or 11 cups of yoghurt to ingest the required amount of CLA recommended by Sieber *et al.* (2004).

Table 1: Total lipid content of raw milk, pasteurized 1 milk and yoghurt

Sample	Total lipids (%)
Raw milk	3.66 ^a ±0.08
Pasteurized milk	3.04 ^b ±0.03
Yoghurt	2.41 ^c ±0.03

* Results in percentage as mean ± standard deviation of triplicate analysis results of five different lots (n =15). Means followed by different letters in the same column are significantly different by Tukey's test at 5% probability level

Table 2: Fatty acid content (mg/g milkfat)* in raw milk, pasteurized 1 milk and yoghurt

Fatty acids	Raw milk	Pasteurized milk	Yoghurt
4:0	31.39a±0.55	31.18a±0.65	15.05b±0.06
6:0	9.67a±0.32	9.60a±0.24	8.81a±0.12
8:0	7.46a±0.25	7.53a±0.19	4.34b±0.14
10:0	19.10a±0.23	17.76a±0.55	10.89b±0.09
11:0	2.51 ^a .b±0.20	3.14a±0.06	1.83b±0.04
12:0	23.04a±0.22	20.66a±0.43	13.54b±0.07
14:0	93.56a±1.24	97.85b±0.45	57.87c±0.56
14:1n-11	2.54a±0.33	2.38a±0.17	2.33a±0.04
14:1n-9	10.06a±0.45	7.98b±0.32	6.78b±0.03
14:1n-7	4.70a±0.37	4.73a±0.08	3.67b±0.03
15:0	5.90a±0.28	5.45a±0.16	6.15a±0.07
15:1n-7	2.59a±0.19	2.47a±0.14	1.26b±0.06
16:0	291.99a±2.27	288.58a±2.02	157.95b±0.83
16:1n-11	1.74a±0.04	1.77a±0.08	1.50a±0.02
16:1n-9	1.71a±0.07	1.68a±0.03	1.27a±0.03
16:1n-7	12.33a±1.18	14.40a±0.12	2.67b±0.03
17:0	5.78a±0.35	6.10a±0.04	4.94a±0.12
17:1n-7	2.30a±0.03	2.34a±0.15	1.65b±0.04
18:0	101.97a±0.94	104.33a±0.97	71.93b±0.38
18:1n-11t	32.54a±0.49	27.55a±0.51	18.01b±0.38
18:1n-9	208.40a±1.09	217.45a±1.96	125.97b±0.81
18:1n-7	2.82a±0.15	2.65a±0.18	2.72b±0.03
18:2n-6t	2.28a±0.15	2.21a±0.04	2.22a±0.05
18:2n-6	15.03a±0.22	13.88a±0.39	10.70b±0.25
18:3n-3	4.71a±0.13	4.05a±0.06	2.16b±0.03
18:2 c9t11	14.91a±0.17	6.22b±0.20	5.41b±0.18
SFA	571.45a±14.87	596.87b±1.01	356.37c±0.41
MUFA	276.46a±5.23	285.38a±2.24	167.82b±0.68
PUFA	36.92a±0.20	26.36a±0.42	20.50b±0.27
n-6	32.21a±0.26	22.31b±0.39	18.32b±0.28
n-3	4.71a±0.13	4.05a±0.06	2.16b±0.03
PUFA/SFA	0.06a±0.02	0.04a±0.03	0.06a±0.02
n-6/n-3	6.85a±0.23	5.52a±0.09	8.49b±0.15

* mean ± standard deviation of the results of triplicate analysis of five different lots (n = 15). SFA = saturated fatty acids, MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids, n-6= omega-6 fatty acids, n-3 = omega-3 fatty acids and CLA (conjugated linoleic acid). Means followed by different letters in the same line are significantly different by Tukey's test at 5% probability level

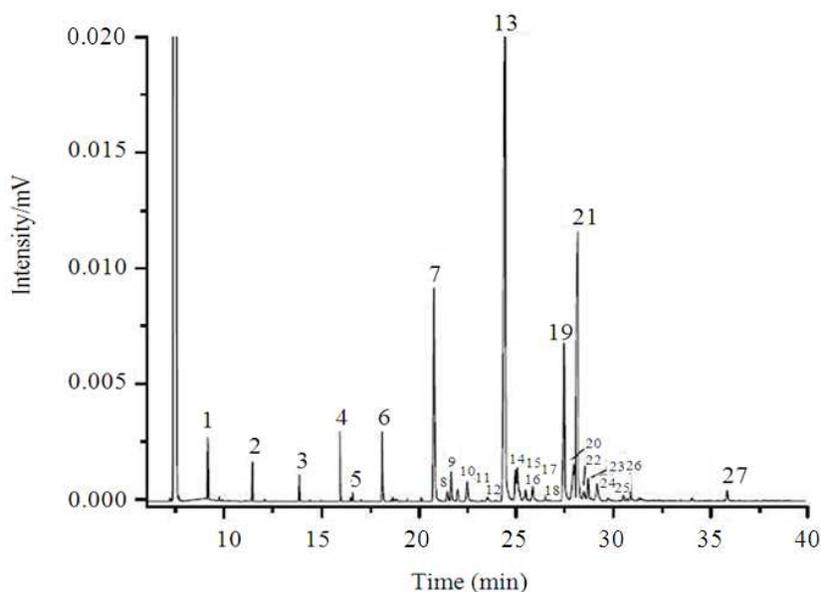


Fig. 1: Representative chromatogram of the analyzed milk and yoghurt samples (1) 4:0. (2) 6:0.(3) 8:0. (4) 10:0. (5) 11:0. (6) 12:0. (7) 14:0. (8) 14:1n-11. (9) 14:1n-9. (10) 14:1n-7. (11) 15:0. (12) 15:1n-7. (13) 16:0. (14) 16:1n-1. (15) 16:1n-9. (16) 16:1n-7. (17) 17:0. (18) 17:1n-7. (19) 18:0. (20) 18:1t. (21) 18:1n-9. (22) 18:1n-7. (23) 18:2n-6t. (24) 18:2n-6. (25) 18:3n-3. (26) 18:2c9t11. (27) 23:0 (internal standard)

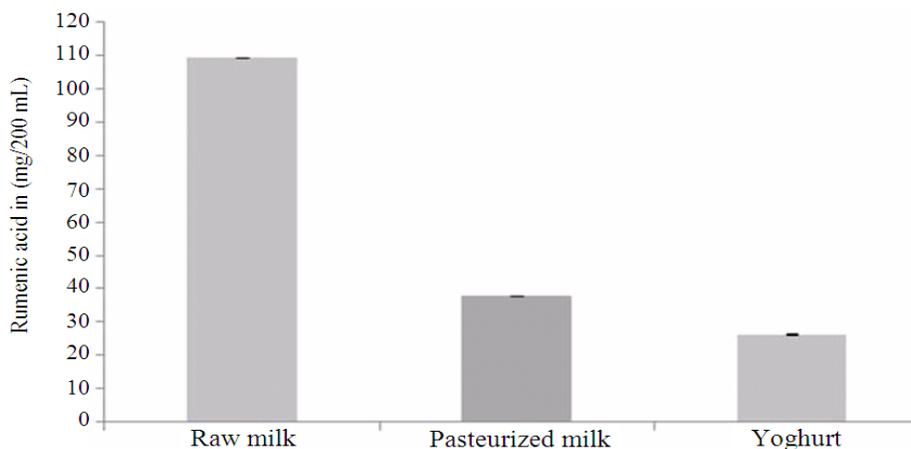


Fig. 2: CLA content in (mg/200 mL) of raw milk, pasteurized milk and yoghurt

DISCUSSION

As can be observed in Table 1, there were significant differences in total lipid content between raw milk, pasteurized milk and yoghurt. The results of both milk types were within the requirements of minimum fat content of 3% (BRASIL, 2002).

The fat contents are in agreement with the literature data for milk and yoghurt produced in ummer (Simionato *et al.*, 2010). However, these values may vary largely with breed, lactation stage

and animal age and diet (Chilliard *et al.*, 2007; Simionato *et al.*, 2010; Collomb *et al.*, 2002; 2004).

The difference in total lipids after pasteurization resulted from standardization by high rotation equipment in order to remove eventual dirtiness. Then milk stored in the vertical tank after agitation was stored at low temperature (5°C) and a thin fat layer with possible dirtiness was formed on the surface which was kept on the equipment. Thus its removal would reduce the lipid content.

The difference in lipid content of yoghurt is due to the addition of stabilizers to increase product viscosity, which may reduce the quantity of milk in the formulation and the total lipids in the final product when compared to raw and pasteurized milk. The lipid content of yoghurt may vary with the yoghurt type (skimmed, partially skimmed and whole).

The main fatty acid were myristic (14:0), palmitic (16:0), stearic (18:0) and oleic (18:1n-9). As shown in Table 2, 65% of the fatty acid was saturated, 32% were monounsaturated and 3% were polyunsaturated, those results are similar to those published by Ordonez (2005).

Many researchers have studied how the thermal processes used in the preservation of milk and its derivatives affect their nutritional and organoleptic properties (Pereda *et al.*, 2008; Raynal-Ljutovac *et al.*, 2007; Rynne *et al.*, 2004).

These changes affect mainly the structure of proteins. However, we have not found any similar report on how pasteurization affects the fatty acid composition and content. Table 2 shows the quantity of each fatty acid quantified in both raw and pasteurized milk samples and also in yoghurt samples. The total FAs (sum SFAs + MUFAs + PUFAs) are 544.69 mg g⁻¹, 884.83 mg g⁻¹ and 908.61 mg g⁻¹ for yoghurt, raw milk and pasteurized milk, respectively. Both the latest results seem correct, the remaining 100 mg being probably glycerol and unsaponifiable. However the value for yoghurt lipids looks questionable. One hypothesis is that there are a lot of Free Fatty Acid (FFAs) in yoghurt due to microbiological starter culture activity during process. Several studies have reported that the addition of lactic acid bacteria to dairy products may contribute to the production of FFAs by lipolysis of milk fat (Coskun and Ondul, 2004; Kurmann, 1988). FFAs are not methylated in the analytical conditions applied in this study, so they are not taking into account in the results. The total FFAs in terms of acid degree values significantly increased during fermentation and storage of yoghurts (Yadav *et al.*, 2007). SFA, MUFA and PUFA contents decrease in yoghurt, but, the percentage de SFA in relation of total lipids is equal 65% in all raw milk and pasteurized milk samples.

The Polyunsaturated Fatty Acid (PUFA) (Table 2) decreased, as expected, by 28.6% after pasteurization, from 36.92±0.20 to 26.36±0.42 mg g⁻¹ demonstrating that heating degrades its structure. Probably the acidic pH of milk and heating favor the attack of the double bonds by oxygen, forming volatile compounds such as alcohols and ketones, thus decreasing the amount of PUFA. The decrease in PUFA from pasteurized milk to yoghurt is smaller (22.23%) due to the addition of

starch and gelatin as stabilizers, which form a network when heated that retains the fat, moisture and protein and prevents syneresis on the shelf.

The PUFA that has the greatest variation was rumenic acid, ranging from 14.91±1 0.17 mg g⁻¹ in raw milk to 6.22±0.20 mg g⁻¹ in pasteurized milk, a reduction of 58.28%. Looor and Herbein (2003) found that concentration of c9, t11-18:2 was greater in response to soybean oil or soybean oil plus conjugated linoleic acids (7 mg g⁻¹) compared with canola oil or canola oil plus conjugated linoleic acids (5 mg g⁻¹) and accounted for the effect of oil on its yield in milk fat. The concentration of c9, t11-18:2 in milk fat tended (p = 0.08) to be lower when cows were fed soybean oil plus conjugated linoleic acids.

Processing yoghurt from pasteurized milk showed a 13.14% reduction in the CLA content which was smaller than the % CLA reduction from raw to pasteurized milk, due milk heating in the production of yoghurt (85°C/10 min) and the elimination of a large part of the dissolved oxygen Ordonez (2005). Thus, the formation of volatile compounds caused by the attack of double bonds by oxygen is smaller and so is the reduction of the fatty acid content in yoghurt.

The n-6/n-3 ratios of raw milk, pasteurized milk and yoghurt samples were 6.85±0.23, 5.52±0.09 and 8.49±0.15, respectively. All values were within the recommendations of United Nations agency for food and agriculture (FAO, 2007) which suggests a ratio between 5 and 10. The decrease of this ratio in pasteurized milk is due to the reduction in CLA, thus, of n-6. Its increase in yoghurt is due to the reduction in n-3.

Recent research shows that CLA and its precursor, vaccenic acid (a naturally occurring trans fatty acid), in milk fat may protect against development and progression of atherosclerosis (Kurmann, 1988). Studies performed in countries such as Germany indicate that to provide anticarcinogenic effects, women must ingest 360 mg of CLA per day and men, 440 mg, 2/3 being from milk and dairy products and 1/4 from meat and derivatives (Sieber *et al.*, 2004). Figure 2 gives the amounts of CLA in mg/200 mL of food for raw milk, pasteurized milk and yoghurt.

Safety concerns regarding the use of CLA in humans are not conclusive and need further investigation (Park and Pariza, 2007).

CONCLUSION

Pasteurization drastically reduces the CLA content in milk, showing that animal diet supplementation to increase CLA in milk is not advantageous, as pasteurization is a requirement for milk conservation.

As to the consumption of milk and its derivatives, the intake of yoghurt is recommended since it has a lower content of saturated fatty acid to compared pasteurized milk, is rich in calcium, has easily digestible proteins due to denaturation by heating, has less lactose than milk, which is mostly digested by microorganisms during fermentation, thus being acceptable by those with allergy to lactose and has a large CLA content.

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