

# Effects of Seasonality on the Proximate Composition and Fatty Acid Profile in Cow Milk

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**Abstract** The present study investigated the influence of seasonality (summer and winter) on the proximate composition and on the amount of polyunsaturated fatty acids in milk fat. The amounts of lipid and protein were greater in the winter. The quantity of conjugated linoleic acid (CLA, rumenic acid, 18:2c9t11) in type B pasteurized milk ( $10.38 \pm 0.69$  in summer and  $7.44 \pm 0.82$  in winter) was higher than in type C milk ( $9.90 \pm 0.64$  in summer and  $7.25 \pm 0.85$  in winter) in both seasons. The amount of trans linoleic acid (18:2n-6t), linoleic acid (18:2n-6) and linolenic acid (18:3n-3) had no variation between seasons and types of milk. Furthermore, the n-6/n-3 ratio of the milks analyzed after pasteurization has reduced significantly from ( $p < 0.05$ )  $7.50 \pm 0.85$  and  $7.46 \pm 1.71$  in winter to  $6.36 \pm 0.45$  and  $6.72 \pm 0.61$  in summer for milk Type C and B, respectively.

**Keywords** Linoleic Conjugated Acid, Rumenic Acid, Cow Milk, Seasonality

## 1. Introduction

Pasteurization is a thermal treatment applied to foods, including milk, to make them microbiologically safe for consumption. The most usual pasteurization process consists of raising temperature to 72°C for 15-20 s [1]. Many researchers have studied how thermal processes affect the nutritional and organoleptic properties of foods. The pasteurization affects mainly protein structures [1-4]. However, few reports have demonstrated the effect of pasteurization on the composition and amounts of fatty acids [5].

Fat is one of the most abundant components of milk, and the most variable one. Its concentration and composition are more influenced by nutrition and environmental conditions than its other fractions [6, 7]. Conjugated linoleic acid (CLA) is a fatty acid present in ruminant milk fat. CLA isomers have different physiological effects. The biological activity of two of them, 18:2 t10c12 and 18:2 c9t11, have already been demonstrated. The first is a potent inhibitor of the fat synthesis in milk and responsible for the redistribution of fat in the muscle, being able to reduce fat mass and increase lean mass. The other has antitumoral properties, reducing the incidence of breast cancer [7-12]. CLAs may be

produced synthetically and by the organism of ruminants in two ways: a) by incomplete biohydrogenation of dietary fatty acids and b) endogenously through the desaturation of trans-vaccenic fatty acid (18:1n-7t) by the enzyme stearoyl-CoA desaturase or  $\Delta$ -9-desaturase present in mammary glands and in adipose tissue. As 18:1n-7t is produced mainly by ruminal biohydrogenation, most CLA sources are ruminant products [13, 14]. Although the biological activities of c9t11 and t10c12 had already been confirmed, other CLA isomers and their interactions need further studies to determine their possible effects on human health. Furthermore, the use of CLA isomers in a variety of functional foods is a possibility that must not be ignored. However, it is also necessary to investigate the quantities of the isomers present in different food matrices. The separation and quantification of these isomers in dairy products by gas chromatography is challenging. Determining their mean amounts will enable setting a safe intake limit that is simultaneously suitable for the ingestion of CLA and saturated fatty acids.

In this work, analyses on proximate composition and fatty acid quantification were performed focusing on conjugated linoleic acid (rumenic acid, 18:2c9t11), and omega-3 and omega-6 essential fatty acids in milks produced during two seasons (winter and summer) in Southern Brazil.

## 2. Materials and Methods

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## 2.1. Sampling

Raw milk from 430 dairy cows aged from 3 to 7 years in lactation period (150 days) was analyzed. The animals were from 9 farms in Middle East of Paraná State and had excellent health. The animals were given feed complemented with high-quality forage ad libitum. Forage consisted of predominantly mombaça (*Panicum maximum* cv. Mombaça), tanzania (*Panicum maximum* cv. tanzania), and brachiaria (*Brachiaria brizantha* cv. Marandu) grasses. The milk containers were immediately cooled to 4°C after sampling and the milk was pooled, pasteurized (72°C for 15-20 s) for milk type B (MB) and C (MC) and frozen.

Sampling were conducted in three consecutive weeks in January and July 2009 (summer and winter, respectively). The weekly samples (n=81) were analyzed in triplicate.

## 2.2. Chemical Analysis

Moisture was determined according to the AOAC method[15] by oven heating (105°C) and the ash content, by oven incineration (600°C). Nitrogen was determined by the Kjeldahl method.

Total lipids were determined by the Folch[16] method with chloroform, methanol, and water in the ratio of 2:1:1.

## 2.3. Preparation of Fatty Acid Methyl Esters

Fatty acid methyl esters were prepared as described by Bannon et al.[17] with modifications.

To a screw cap tube with approximately 150 mg lipids was added 5.0 mL of 0.25 mol L<sup>-1</sup> sodium methoxide in methanol diethyl ether (1:1). The tube was stirred vigorously for about 3 min. The mixture was added with 3.0 mL isooctane and approximately 15 mL of a saturated sodium chloride solution. The tube was vigorously stirred again and left at rest for phase separation. The supernatant was collected in labeled Eppendorf tubes for later chromatographic analysis. The original method used short heating under reflux after the addition of the transesterification reagent; however, this was not done to prevent the isomerization of the conjugated linoleic acid dienes.

## 2.4. Chromatographic Analysis of Fatty Acid Methyl Esters

Fatty acid esters were analyzed in gas chromatographer CP-3380 (Varian, EUA) equipped with a flame ionization detector and fused silica capillary column CP-7420 (100 m, 0.25 mm and 0.39 µm i.d., 100% bound cyanopropyl, Varian, EUA).

The flows of gas (White Martins) were 1.4 mL min<sup>-1</sup> carrier gas (H<sub>2</sub>), 30 mL min<sup>-1</sup> auxiliary gas (N<sub>2</sub>), and 30 and 300 mL min<sup>-1</sup> for H<sub>2</sub> and flame synthetic air, respectively. The sample split rate was 1/80.

The injector and detector temperatures were both 240°C. The column temperature was set at 65°C for 4 min, followed by a first 16°C/min ramp up to 185°C for 12 min. The second ramp was set at 20°C/min up to 235°C,

remaining at this temperature for 14 min. The total analysis time was 40 min. The peak areas were determined with the software Star (Varian).

The injections were made in triplicate with injection volume of 2 µL. The fatty acids were identified based on the comparison of the retention times of the methyl esters with those of standards with geometric isomers c9t11 and t10c12 of linoleic acid (189-19 and O-5626, Sigma, EUA).

The limits of detection and quantification were estimated at 0.148 and 0.476 mg/g lipids, respectively, according to the ACS[18] considering the signal/noise ratio equal to 3 and 10, respectively, from successive dilutions of a standard solution of methyl arachidate.

## 2.5. Quantification

Total lipid fatty acids were measured in mg g<sup>-1</sup> in relation to the internal standard, methyl tricosanoate (23:0), Sigma. Before transesterification, 1.00 mL of internal standard solution (1 mg mL<sup>-1</sup>) was added to each sample and the solvent was evaporated under N<sub>2</sub> flow.

Fatty acid quantification was carried out after verifying the agreement between the theoretical and the experimental response factors.

The sample fatty acid concentrations were calculated according to Joseph and Ackman[19] following the equation 1:

$$C \text{ (mg g}^{-1}\text{)} = A \times M_{23:0} \times FRT / A_{23:0} \times MA \times FCT \text{ (Equation 1)}$$

where:

AX = Area of the fatty acid methyl esters

A<sub>23:0</sub> = Internal standard area

M<sub>23:0</sub> = Mass of Internal standard added to the sample (mg)

MA = sample mass (g)

FRT = theoretical response factor of fatty acid methyl esters,

FCT = conversion factor to express results in mg fatty acids g<sup>-1</sup> total lipids

Certified reference material (power milk) was used to confirm the accuracy of the method. The reference material (RM-8435) was obtained from the Canadian National Institute of Standards and Technology (NIST).

The accuracy of the method was confirmed by the analysis of the certified reference material (powder milk) with a quantitative recovery (>80%) of the analyzed fatty acids. This method may be applied to the quantification of fatty acids in commercial samples[20].

## 2.6. Statistical Analysis

The results obtained were submitted to an analysis of variance (ANOVA) at 5% probability and the means were compared by Tukey's test, using the software Statistica 7.0 [21].

# 3. Results and Discussion

Table 1 lists the results of the analysis of variance for the chemical composition of milk after pasteurization in the summer and winter.

**Table 1.** Proximate composition (%) of type B (MB) and C (MC) pasteurized milk during different seasons

	Moisture	Ash	Protein	Total Lipids
Winter				
MC	88.20a±0.04	0.62a±0.05	3.43ab±0.23	3.39a±0.23
MB	88.93a±1.38	0.44b±0.03	2.43cd±0.13	3.08ab±0.35
Summer				
MC	89.12ab±0.17	0.48ab±0.03	2.88bc±0.06	3.04bc±0.10
MB	90.23a±0.49	0.63a±0.06	2.53cd±0.25	3.14ab±0.17

Results in percentage as mean ± standard deviation of 9 different milk producer (n=81). Means followed by different letters in the same row are significantly different by Tukey's Test at 5% probability between seasons

The content of lipid and protein were greater for the type C milk produced in the winter; for the type B milk there was no variation due to the standardization process for the fat content. The quantification of fatty acids for type C and type B milk produced during the winter and summer is presented in the Table 2. In total, 29 fatty acids were

identified, primarily palmitic acid (14:0), oleic acid (18:1n-9), stearic acid (18:0) and myristic acid (14:0). The amounts of fatty acids were always high for milk produced in the summer, both the type C and B.

As for the CLA (ruminic acid c9t11), the seasons had a significant influence on its content. Bugaud *et al.*, (2001) suggested that the biohydrogenation in ruminants is lower in winter than in summer. The higher temperature may increase the activity of the isomerase, the enzyme that converts linoleic acid into CLA in the rumen, which is transported to tissues and mammary glands, or biohydrogenated to vaccenic acid by *B. Fibriosolvens*. Thus, apparently, the transport of this substance to tissues and mammary glands is greater during warm season than its later biohydrogenation.

Herzallah *et al.*[5] reported a decrease in ruminic acid after pasteurization. Noteworthy, these authors used derivatization in acid medium with HCl. It is known that the methylation by acid catalysis using BF<sub>3</sub>, HCl, and H<sub>2</sub>SO<sub>4</sub> favors the extensive isomerization of conjugated dienes and contributes to the formation of artefacts [11].

**Table 2.** Mean amounts (g 100 g<sup>-1</sup> milk fat) of polyunsaturated fatty acids in type B and type C pasteurized milk in different seasons

Fatty Acid	Summer		Winter	
	Pasteurized type B	Pasteurized type C	Pasteurized type B	Pasteurized type C
4:0	36.32c±5.67*	37.32c±2.67	29.17a±4.66	23.65b±3.32
6:0	17.19a±0.86	14.51b±1.69	18.68a±2.57	15.25b±1.95
8:0	9.79a±0.48	8.37b±0.92	10.45a±1.24	9.03b±1.08
10:0	20.78a±1.02	17.77b±1.80	22.00a±2.31	19.91b±2.47
11:0	2.45a±0.20	2.84b±0.39	3.53a±0.35	3.20b±0.37
12:0	24.65a±1.44	21.32b±2.01	25.69±2.22	24.27±2.91
14:0	94.99a±5.88	84.55b±6.67	93.62a±7.57	87.19b±5.72
14:1n-11	4.41a±0.21	4.15b±0.41	4.37a±0.44	3.79b±0.23
14:1n-9	12.73a±0.98	11.31b±0.80	12.10a±1.18	11.10b±0.58
14:1n-7	6.78a±0.45	6.17b±0.50	5.54a±0.38	5.05b±0.38
15:0	11.83a±0.75	10.79b±0.97	10.19±0.91	10.41±0.58
15:1n-7	2.90a±0.20	2.66b±0.25	2.82±0.24	2.59±0.28
16:0	275.85a±17.13	248.47b±20.45	271.77a±21.01	261.85a±17.74
16:1n-11	14.51b±0.88	13.73b±1.29	11.78a±1.17	9.76b±0.72
16:1n-9	11.80a±1.09	10.75b±0.88	14.25c±0.81	14.63c±0.83
16:1n-7	4.34a±0.41	4.10a±0.35	3.99a±0.31	3.70b±0.35
17:0	5.48a±0.34	4.92b±0.40	4.88b±0.30	4.67b±0.30
17:1n-7	8.59a±0.56	8.24a±0.86	6.48b±0.58	6.33b±0.96
18:0	3.19a±0.49	2.56b±0.26	2.93a±0.29	2.98a±0.36
18:1	113.45c±6.01	113.67c±10.50	103.65a±9.02	91.44b±8.38
18:1 t	30.97a±1.48	30.75a±3.25	21.66b±2.66	21.51b±1.67
18:1n-9	215.11a±13.26	213.01a±19.76	223.14a±17.69	203.27b±19.06
18:1n-7	4.05a±0.69	4.34a±0.60	2.44b±0.34	2.19b±0.27
18:2n-6t	9.65±0.68	9.84±1.01	9.70±1.69	9.77±1.56
18:2n-6	13.59±1.12	13.81±1.01	14.96±1.66	15.59±2.30
18:3n-3	3.87±0.32	3.66±0.44	3.32±0.43	3.15±0.40
18:2c9t11	10.38a±0.69	9.90a±0.64	7.44b±0.82	7.25b±0.85
SFA	616.50a±19.49	563.57a±24.35	600.11a±24.93	553.91b±21.21
MUFA	310.40a±13.49	302.04a±20.13	304.38a±18.01	277.08b±19.19
PUFA	37.54±1.52	35.33±1.63	35.15±2.54	30.93±2.94
n-6	23.30±1.31	23.12±1.43	24.67±2.37	25.95±2.78
n-3	3.84±0.32	3.66±0.44	3.32±0.43	3.17±0.40
PUFA/SFA	0.06±0.00	0.06±0.00	0.06±0.00	0.06±0.00
n-6/n-3	6.36a±0.45	6.72a±0.61	7.50b±0.85	7.42b±0.94

Results in percentage as mean ± standard deviation of 9 different milk producer (n=81). Means followed by different letters in the same row are significantly different by Tukey's between seasons

In relation to the trans-octadecadienoic fatty acids, the amount of conjugated linoleic acids and linoleic acid has increased, and the quantity of cis-linoleic fatty acids decreased during the study seasons, from summer to winter. Herzallah et al. [5] observed no significant increase in the amount of trans isomers in milk submitted to conventional heating, except when heated to 63°C for 30 min and milk microwaved for 5 minutes, which increased in a range of 19-31%. Santos Jr et al. [22] registered higher amounts of rumenic acid (conjugated linoleic acid – CLA) in the raw milk, and lower amount in pasteurized milk, compared with the present study, evidencing that the pasteurization reduces the amount of CLA. Test at 5% probability.  $\Sigma$ SFA=summation of saturated fatty acids;  $\Sigma$ MUFA=summation of monounsaturated fatty acids;  $\Sigma$ PUFA=summation of polyunsaturated fatty acids.

Table 2 shows that the lowest n-6/n-3 ratios were found in pasteurized milk produced in the summer. Clinical studies in the last decade pointed out the need of reducing the n-6/n-3 ratio. The English health department [23] recommends that the n-6/n-3 ratio should be below 4. Simopoulos [24] suggested values between 5 and 10. In France and Switzerland, the recommended value is 5.1. At last, it is observed a convergence to values between 4 and 5.1 [25].

## 4. Conclusions

In summary, the amount of CLA (rumenic acid) was higher in the summer, and the amount of trans linoleic acid (18:2n-6t), linoleic acid (18:2n-6) and linolenic acid (18:3n-3) had no variation between seasons neither between types of milk.

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