Stability of fatty acid composition after thermal, high pressure, and microwave processing of cow milk as affected by polyunsaturated fatty acid concentration

L. M. Rodríguez-Alcalá,* L. Alonso,† and J. Fontecha*¹

*Department of Bioactivity and Food Analysis, Group of Lipids, Instituto de Investigación en Ciencias de la Alimentación (CIAL CSIC-UAM), Nicolás Cabrera, 9, Universidad Autónoma de Madrid, 28049, Madrid, Spain

†Instituto de Productos Lácteos de Asturias (IPLA CSIC), Carretera de Infesto s/n, 33300 Villaviciosa, Principado de Asturias, Spain

ABSTRACT

Interest has been increasing to enhance the contents of healthy polyunsaturated fatty acid (PUFA) in milk. However, *trans* fatty acids and conjugated linoleic acid (CLA) can be altered after thermal processing and high pressures disrupt the milk fat globule membrane, exposing the lipid core and helping its oxidation. The objective of the present research was to study whether processing can alter the fatty acid composition of milk and if these changes are affected by PUFA concentration as previous studies suggest. Two cow milk batches (500 L each), one naturally enriched in PUFA, were processed to obtain pasteurized; high temperature, short time; UHT; high pressure; and microwave pasteurized samples. The detailed fatty acid composition was analyzed with special attention to *trans* fatty acids and CLA isomers. Results showed that after high temperature, short time processing, total CLA content increased in both milk batches, whereas sterilization resulted in a sigmatropic rearrangement of C18:2 *cis*-9,*trans*-11 to C18:2 *trans*-9,*trans*-11. The extent of these effects was greater in milks naturally enriched in PUFA.

Key words: milk processing, conjugated linoleic acid isomers, *trans* fatty acids, unsaturated fatty acids

INTRODUCTION

Because of the relatively high amount of SFA and *trans* fatty acids (TFA) in dairy fat and the role of those compounds in the risk of chronic and other metabolic diseases, food industries are committed to decrease SFA, cholesterol, and TFA contents as much as is possible (Nishida et al., 2004; Haug et al., 2007). The high susceptibility of milk fat profile modulation to diet composition has led to the carrying out of an extensive number of research works supplementing

forage/ensilage diets with high content PUFA oils or oilseeds and resulting in dairy products naturally enriched in polyunsaturated bioactive lipids (from 3.6 to 4.8 g of PUFA/100 g of fat with control diets to 9 g of PUFA/100 g of fat with supplemented feeding) and lower SFA concentrations (from 65 to 68 g of PUFA/100 g of fat with control diets to 55–47 g of PUFA/100 g of fat with supplemented feeding; Jones et al., 2005; Lynch et al., 2005; Jenkins and McGuire, 2006).

Furthermore, PUFA have been demonstrated to exert important beneficial activities in human health: linoleic acid (C18:2 *cis* (c)-9c12) is a precursor of arachidonic acid, involved in the synthesis of molecules associated to inflammatory processes (Calder, 2006), and linolenic acid (C18:3 c9c12c15) is a precursor of eicosapentaenoic, docosapentaenoic, and docosahexaenoic acids (Williams, 2000). The rate of these 2 FA (n-6/n-3) in human erythrocytes is related to cardiovascular risk (Harris, 2008). Even more, CLA has been extensively associated with anticancer (Hagen et al., 2013) and body weight properties (Rodríguez-Alcalá et al., 2013b).

In milk, oxidation of lipids produces strong off-flavors and deterioration of the nutritional and health quality, affecting both the shelf-life and acceptability of the product by consumers (Rafalowski et al., 2014). These reactions are affected by the degree of unsaturation of the FA, and PUFA are highly prone to oxidation, mainly when radical starters are present. Because bioactivity of these compounds is associated with a dose [e.g., 3 g of CLA/d according to Ip et al. (1996)], any possible degradation would result in a daily intake below the amount needed to obtain the beneficial effect. Thus, in dual-homogenized (20,000 kPa) skim milk fortified with CLA oil, total *trans trans* CLA isomers concentration increased, whereas total double-bond *cis cis* decreased after industrial atomization processing (Rodríguez-Alcalá and Fontecha, 2007). Studies focused on the effects of pasteurization (homogenization at 13,789 kPa; 77.2°C, 12 s) reported that rumenic acid but not C18:2 *trans* (t)-10,*cis*-12 CLA decreased within 24 h after treatment and refrigerated storage at 5°C (Campbell et

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¹Corresponding author: j.fontecha@csic.es

al., 2003). Herzallah et al. (2005b) found that low pasteurization (63°C, 10 min) and microwaving (96.8°C, 5 min) processing of milk caused losses of TFA whereas HTST after 3 d at 5°C (85°C, 16 s), UHT after 5 d (140°C, 4 s), and microwave processing decreased the total concentration of CLA.

As a solution to this, new emerging technologies such as high pressure processing and homogenization (50–1,000 MPa) are being studied to evaluate their potential as alternative or complementary process to thermal pasteurization as 400 MPa, 15 min are conditions able to kill most bacteria in milk (Urala and Lahteenmaki, 2007; Chawla et al., 2011). However, it has not been found modifications of the lipid fraction when human (0–600 MPa) or cow, goat, and sheep milk (0–350 MPa) were processed by high pressure or high pressure homogenization (Rodríguez-Alcalá et al., 2009; Moltó-Puigmartí et al., 2011). It is known that those treatments can result in partial whey protein denaturation, casein micelle dissociation, and disruption of the milk fat globule membrane (Pereda et al., 2007; Zamora et al., 2007). There is also evidence that 200 MPa, 30 min and inlet temperature of 60°C increased the native lipase activity when compared with raw milk (Datta et al., 2005).

The aim of the present work is to study the possible effects of conventional heating (pasteurization, UHT, and sterilization), high pressure, and microwave processing on the fatty acid composition of naturally PUFA-enriched milk with special attention to CLA isomers and TFA.

MATERIALS AND METHODS

Chemicals

Hexane, methanol, and chloroform were purchased from LabScan (Dublin, Ireland); potassium hydroxide, sodium sulfate-1 hydrate, and 20% aqueous solution of AgNO₃ from Panreac (Barcelona, Spain); CLA standards from Nu-Chek Prep (Elysian, MN); stearic FAME (C18:0), elaidic FAME (C18:1 t9), rumenic acid (C18:2 c9,t11; **RA**), 1,2,3-tritridecanoylglycerol, and sodium azide from Sigma (St. Louis, MO); and high CLA concentration oil (Tonalin) was obtained from Cognis (Illertissen, Germany). All reagents were GC or HPLC grade. Reference milk fat butter BCR-164 (EU Commissions; Brussels, Belgium) was purchased from Fedelco Inc. (Madrid, Spain).

Samples

Two raw milk batches of 500 L labeled as B1 (batch 1) and B2 (batch 2), collected from different cows herds

(Holstein breed) from the Castilla-La Mancha region (Spain) were kindly donated by a Spanish dairy company (Leches Pascual, Aranda de Duero, Spain). Animals producing the B2 samples were fed with linseed. Milk was processed to obtain pasteurized (72°C, 30 s), HTST (85°C, 30 s), and UHT (**UHT1**, 135°C, 30 s, and **UHT2**, 150°C, 5 min) samples using an aseptic sterilizer (Rossi & Catelli, Parma, Italy) as follows: 30 L of raw milk per assay were preheated to 65°C and then homogenized at 18 MPa. The temperature was raised to pasteurization conditions and then cooled to an output value of 20°C. In UHT processing, the preheating step was carried out after homogenization (85°C). Sterilization (**STR**, 121°C, 15 min) was carried out using 1 L of raw milk from each batch in a laboratory autoclave (Selecta Autotester E DRY-PV, Barcelona, Spain). As control samples 4 L of raw milk were collected from the 2 batches. For high pressure processing (400 MPa, 25°C, 15 min), a laboratory-scale high pressure machine (ACB, GEC, Alsthom, Nantes, France) was used with 200 mL of raw milk fitted in pressure-resistant packages. Microwaving (650 W, 1.30 min) was performed using 100 mL of raw milk in a domestic apparatus (Moulinex AET1, Ecully Cedex, France). All samples were placed into amber-glass bottles and sodium azide (0.06 g/mL) added to avoid microbial growth. All samples were placed in refrigeration after processing. Assays were performed in duplicate.

Basic Parameter Analysis

Fat content, protein, lactose, and dried extract were measured using a Milkoscan (Foss, Hillerød, Denmark).

Lipid Extraction and FA Derivatization

Milk fat extraction was carried out according to standard methods (ISO-IDF, 2001). The fat residue was collected into amber vials and stored at –20°C until analysis. Fatty acid methyl esters were prepared by base-catalyzed methanolysis (2 *N* KOH in methanol) according to ISO-IDF (2002). As internal standard, 1,2,3-tritridecanoylglycerol, was added to samples (200 µL, 1.24 mg/mL). All analyses of samples were carried out in duplicate.

Fractioning by AgNO₃-Thin Layer Chromatography of FAME

Fatty acid methyl esters were fractionated according to the number and geometry of double bonds by thin layer chromatography (**TLC**) according to Alonso et al. (1999). The TLC glass plates (20 × 20 cm) with silica gel (0.25 mm; Merck, Darmstadt, Germany) were

incubated with 20% aqueous solution of AgNO_3 for 16 h, were partially air-dried, and activated at 120°C for 30 min. A 100- μL solution of FAME (100 mg/mL) was applied to the activated TLC glass plate in a narrow band. The plate was developed twice in a saturated chamber in hexane and diethyl ether (9:1, vol/vol) with 15 cm migration. At the end of chromatographic runs, the plates were air-dried and sprayed with a 0.20% ethanol solution of 2',7'-dichlorofluorescein, and the bands were visualized under UV light. The bands corresponding to saturated and *trans* monoenoic FAME, which were previously identified by a mixture of stearic FAME (C18) and elaidic FAME (C18:1 t9) running in AgNO_3 -TLC, were scraped into a flask. The FAME were extracted with 80 mL of diethyl ether in 4 extractions, and the solvent was evaporated in a rotary evaporator and stream of nitrogen. The residue was dissolved in 200 μL of hexane and used for GC analysis.

To calculate the total content of *trans* C18:1 isomers, the ratio of C18 to total *trans* C18:1 was determined in the saturated plus *trans* monoenoic AgNO_3 -TLC fraction and was related to the C18:0 content of total FAME. To calculate the total content of *trans* C16:1 isomers, the ratio of total C16:1 t to C18:1 t10 plus C18:1 t11 was determined in the *trans* monoenoic AgNO_3 -TLC fraction and related to the ratio of C18 to C18:1 t10 plus C18:1 t11 in the saturated plus *trans* monoenoic AgNO_3 -TLC fraction and to the C18 content of total FAME. All analyses were performed at least in triplicate.

GC-Flame-Ionization Detection Analyses of Total FA and TFA

Fatty acid methyl esters were analyzed on a Clarus 500 gas chromatograph (Perkin Elmer, Beaconsfield, UK) with a VF23ms, fused-silica capillary column (30 m $\times$ 0.25 mm i.d. $\times$ 0.25 μm film thickness, Varian, Middelburg, the Netherlands). Quick chromatography conditions were as previously described by Rodríguez-Alcalá et al. (2013a).

Trans FA, minor *cis* octadecenoic (C18:1 c), and octadecadienoic FA isomers were analyzed in an Autosystem chromatograph (Perkin-Elmer) with a flame-ionization detector. Fatty acids methyl esters were separated using a CP-Sil 88 fused-silica capillary column (100 m $\times$ 0.25 mm i.d. $\times$ 0.2 μm film thickness, Chrompack, Middelburg, the Netherlands). The column was held at 100°C for 1 min after injection, increased at $7^\circ\text{C}/\text{min}$ to 170°C , held there for 55 min, increased at $10^\circ\text{C}/\text{min}$ to 230°C and held there for 33 min. Helium was the carrier gas, with a column inlet pressure set at 214 kPa and a split ratio of 1: 20. The injection volume was 0.5 μL .

Response factors were calculated as obtained from a certified reference butter fat (BCR-164, EU Commissions, Brussels, Belgium, purchased from Fedelco Inc.) and used for sample peak area correction. All analyses were performed at least in triplicate.

Quantification of Total CLA Content by UV Spectroscopy

For total CLA quantification, samples were weighed to obtain a concentration of 0.2 mg/mL in hexane, placed into 2-mL quartz cuvettes, and absorbance was recorded with a scan program (190–350 nm) and data expressed as absorbance at 233 nm, in a Lambda650 spectrophotometer (Perkin-Elmer) as described by Rodríguez-Alcalá et al. (2011). Concentration was calculated by means of pure CLA FAME standard calibration curve.

Silver-Ion HPLC (Ag^+ -HPLC)

Separation of CLA as FAME was performed using HPLC (Shimadzu Vp Series, Duisburg, F.R. Germany) equipped with UV detector at 233 nm and a Chrom-Spher 5 Lipid analytical column (4.6 mm i.d. $\times$ 250 mm stainless steel, 5 μm particle size; Varian). The mobile phase was 0.1% acetonitrile in hexane, operated isocratically at a flow rate of 1.0 mL/min. The injection volume was 10 μL . For identification, a standard mixture of pure CLA FAME isomers was used.

Statistical Analysis

In the first instance, an exploratory analysis of data was performed to test normal distribution and homogeneity of variance. Then, a full factorial data analysis (one-way ANOVA and GLM) was conducted with the aid of the SPSS Statistics software v21.0 for Windows (IBM, Armonk, NY). Level of significance was fixed at $P < 0.05$ and $0.05 < P < 0.1$ was considered a trend.

RESULTS AND DISCUSSION

FA Composition of the Assayed Raw Cow Milk

In the assayed samples, values for basic composition parameters were as follows: fat content (3.53 g/100 mL $\pm$ 0.21 in B1 and 2.74 g/100 mL $\pm$ 0.07 in samples B2), protein (3.15 g/100 mL $\pm$ 0.01; 3.13 g/100 mL $\pm$ 0.05), lactose (4.71 g/100 mL $\pm$ 0.07; 5.09 g/100 mL $\pm$ 0.08) and dried extract (11.39 g/100 mL $\pm$ 0.23; 10.96 g/100 mL $\pm$ 0.29).

The FA composition of the assayed samples is shown in Tables 1 and 2. When compared, the total amount

of SFA in B2 raw milk (58.64%) was lower ($P < 0.05$) than in B1 (66.95%) as result of the differences in the concentration of lauric (C12), myristic (14), palmitic (C16), and stearic (C18) acids ($P < 0.05$). Previous studies reported that addition of linseed (rich in linolenic acid, C18:3) to the diets of cows led to lower contents of medium-chain fatty acids (C10–14) and increased the concentrations of stearic, linoleic (C18:2 c9c12), and oleic acids in milk (Lawless et al., 1998; Rego et al., 2005). These effects are related to changes in the rumen pH resulting in decreased acetate production (McNamee et al., 2002). This compound is directly involved in de novo synthesis of fatty acids (C4–C14) in the mammary gland.

Monounsaturated fatty acids concentration was 34.85% in the B2 raw milk and 28.63% for B1. Thus, in this fraction, oleic acid (21.97% in B2; 20.55% in B1),

C18:1 c (1.53% in B1 vs. 2.46% in B2), C18:1 t (3.57% in B1 vs. 7.23% in B2), and C16:1 c9 (1.84% in B1; 1.65% in B2) were the main FA. According to the results of the present research, the *cis* and *trans* MUFA moieties (Tables 3 and 4) were characterized by the concentrations of C18:1 t10 (0.43% in B1; 1.71% in B2), C18:1 t11 (0.87% in B1 vs. 1.82% in B2; *trans* vaccenic acid), C18:1 c11 (0.71% in B1 vs. 0.81% in B2), and C18:1 c12 (0.37% in B1 vs. 0.92% B2; $P < 0.05$). It has been described elsewhere that the alteration of the fermentative environment by the supplementation of the cows' diets with PUFA sources affects the metabolic pathways of the rumen biohydrogenation, increasing the concentration of the *trans* MUFA, mainly C18:1 t10 (Bauman et al., 2006; Elgersma et al., 2006; Li et al., 2007).

As with SFA and MUFA, similar significant differences among batches were observed for PUFA (4.42%

Table 1. Fatty acid composition (g/100 g of FA; means $\pm$ SD) in B1 milk samples¹

Fatty acid ²	Raw	Past.	HTST	UHT1	UHT2	STR	HP	Micro.
C4	3.36 $\pm$ 0.36	3.15 $\pm$ 0.28	3.09 $\pm$ 0.55	3.03 $\pm$ 0.69	2.85 $\pm$ 0.69	3.20 $\pm$ 0.32	3.13 $\pm$ 0.14	3.32 $\pm$ 0.47
C6	2.31 $\pm$ 0.46	1.99 $\pm$ 0.17	2.19 $\pm$ 0.41	2.10 $\pm$ 0.28	2.12 $\pm$ 0.36	2.11 $\pm$ 0.26	2.11 $\pm$ 0.37	2.41 $\pm$ 0.83
C8	1.26 $\pm$ 0.22	1.39 $\pm$ 0.50	1.24 $\pm$ 0.10	1.25 $\pm$ 0.10	1.31 $\pm$ 0.13	1.31 $\pm$ 0.23	1.20 $\pm$ 0.22	1.23 $\pm$ 0.12
C10	2.80 $\pm$ 0.37	2.88 $\pm$ 0.81	2.76 $\pm$ 0.14	2.80 $\pm$ 0.17	2.99 $\pm$ 0.42	2.86 $\pm$ 0.43	2.65 $\pm$ 0.26	2.74 $\pm$ 0.15
C10:1	0.31 $\pm$ 0.07	0.33 $\pm$ 0.10	0.30 $\pm$ 0.05	0.31 $\pm$ 0.05	0.33 $\pm$ 0.08	0.31 $\pm$ 0.07	0.28 $\pm$ 0.05	0.30 $\pm$ 0.05
C12	3.05 $\pm$ 0.10	3.27 $\pm$ 0.40	3.11 $\pm$ 0.16	3.11 $\pm$ 0.13	3.28 $\pm$ 0.43	3.12 $\pm$ 0.32	3.04 $\pm$ 0.18	3.09 $\pm$ 0.15
C14	9.89 $\pm$ 0.36	10.30 $\pm$ 0.28	10.28 $\pm$ 0.26	10.31 $\pm$ 0.20	10.43 $\pm$ 0.54	10.04 $\pm$ 0.45	10.05 $\pm$ 0.28	10.19 $\pm$ 0.29
C15 <i>i</i>	0.20 $\pm$ 0.01	0.21 $\pm$ 0.01	0.21 $\pm$ 0.01	0.21 $\pm$ 0.01	0.21 $\pm$ 0.01	0.20 $\pm$ 0.02	0.20 $\pm$ 0.01	0.21 $\pm$ 0.01
C14:1	0.45 $\pm$ 0.03	0.47 $\pm$ 0.02	0.46 $\pm$ 0.02	0.47 $\pm$ 0.03	0.47 $\pm$ 0.02	0.40 $\pm$ 0.16	0.45 $\pm$ 0.03	0.46 $\pm$ 0.02
C15 <i>ai</i>	0.84 $\pm$ 0.03	0.89 $\pm$ 0.03	0.87 $\pm$ 0.02	0.87 $\pm$ 0.02	0.88 $\pm$ 0.04	0.85 $\pm$ 0.05	0.85 $\pm$ 0.03	0.86 $\pm$ 0.02
C15	1.07 $\pm$ 0.08	1.11 $\pm$ 0.03	1.11 $\pm$ 0.01	1.10 $\pm$ 0.02	1.12 $\pm$ 0.02	1.08 $\pm$ 0.05	1.10 $\pm$ 0.04	1.10 $\pm$ 0.02
C16 <i>i</i>	0.20 $\pm$ 0.01	0.21 $\pm$ 0.01	0.21 $\pm$ 0.01	0.21 $\pm$ 0.01	0.19 $\pm$ 0.07	0.20 $\pm$ 0.01	0.21 $\pm$ 0.01	0.21 $\pm$ 0.01
C16	31.97 $\pm$ 1.33	31.83 $\pm$ 2.08	32.1 $\pm$ 2.07	32.45 $\pm$ 1.64	33.00 $\pm$ 0.36	31.9 $\pm$ 1.92	32.06 $\pm$ 1.83	32.02 $\pm$ 1.63
C17 <i>i</i>	0.20 $\pm$ 0.12	0.27 $\pm$ 0.06	0.27 $\pm$ 0.07	0.29 $\pm$ 0.07	0.28 $\pm$ 0.07	0.26 $\pm$ 0.05	0.25 $\pm$ 0.05	0.24 $\pm$ 0.10
C16:1 c9	1.84 $\pm$ 0.04	1.86 $\pm$ 0.10	1.87 $\pm$ 0.12	1.90 $\pm$ 0.10	1.93 $\pm$ 0.03	1.81 $\pm$ 0.15	1.82 $\pm$ 0.15	1.86 $\pm$ 0.10
C17	0.53 $\pm$ 0.02	0.52 $\pm$ 0.04	0.53 $\pm$ 0.04	0.53 $\pm$ 0.03	0.55 $\pm$ 0.01	0.53 $\pm$ 0.03	0.53 $\pm$ 0.04	0.53 $\pm$ 0.03
C17:1	0.24 $\pm$ 0.05	0.25 $\pm$ 0.04	0.25 $\pm$ 0.04	0.25 $\pm$ 0.04	0.25 $\pm$ 0.04	0.25 $\pm$ 0.05	0.26 $\pm$ 0.05	0.24 $\pm$ 0.05
C18	9.10 $\pm$ 0.41	8.99 $\pm$ 0.82	9.15 $\pm$ 0.71	9.32 $\pm$ 0.52	9.56 $\pm$ 0.10	9.13 $\pm$ 0.77	9.18 $\pm$ 0.73	9.17 $\pm$ 0.60
C18:1 t ³	3.57 $\pm$ 0.11	3.43 $\pm$ 0.35	3.38 $\pm$ 0.33	3.40 $\pm$ 0.24	3.16 $\pm$ 0.08	3.48 $\pm$ 0.20	3.60 $\pm$ 0.15	3.41 $\pm$ 0.26
C18:1 c9	20.55 $\pm$ 1.02	20.68 $\pm$ 1.45	20.49 $\pm$ 1.39	20.21 $\pm$ 1.25	19.52 $\pm$ 0.45	20.71 $\pm$ 1.24	20.76 $\pm$ 1.54	20.49 $\pm$ 1.69
C18:1 c ⁴	1.53 $\pm$ 0.09	1.43 $\pm$ 0.19	1.44 $\pm$ 0.11	1.40 $\pm$ 0.11	1.24 $\pm$ 0.06	1.55 $\pm$ 0.10	1.59 $\pm$ 0.13	1.40 $\pm$ 0.14
C18:2 c9c12	2.63 $\pm$ 0.10	2.46 $\pm$ 0.24	2.49 $\pm$ 0.16	2.43 $\pm$ 0.17	2.36 $\pm$ 0.28	2.54 $\pm$ 0.18	2.53 $\pm$ 0.16	2.47 $\pm$ 0.18
C18:2 other ⁵	0.65 $\pm$ 0.03	0.54 $\pm$ 0.07	0.59 $\pm$ 0.05	0.57 $\pm$ 0.05	0.51 $\pm$ 0.06	0.61 $\pm$ 0.06	0.59 $\pm$ 0.06	0.56 $\pm$ 0.05
C20	0.13 $\pm$ 0.03	0.12 $\pm$ 0.02	0.12 $\pm$ 0.03	0.11 $\pm$ 0.03	0.11 $\pm$ 0.02	0.13 $\pm$ 0.03	0.12 $\pm$ 0.04	0.12 $\pm$ 0.02
C20:1	0.14 $\pm$ 0.02	0.16 $\pm$ 0.02	0.16 $\pm$ 0.01	0.16 $\pm$ 0.02	0.15 $\pm$ 0.01	0.17 $\pm$ 0.02	0.16 $\pm$ 0.02	0.10 $\pm$ 0.06
C18:3	0.31 $\pm$ 0.01	0.33 $\pm$ 0.04	0.33 $\pm$ 0.03	0.33 $\pm$ 0.03	0.30 $\pm$ 0.02	0.32 $\pm$ 0.02	0.33 $\pm$ 0.03	0.33 $\pm$ 0.03
C18:2 c9t11	0.39 $\pm$ 0.02	0.43 $\pm$ 0.04	0.43 $\pm$ 0.03	0.43 $\pm$ 0.02	0.39 $\pm$ 0.04	0.41 $\pm$ 0.03	0.41 $\pm$ 0.04	0.43 $\pm$ 0.03
CLA other	0.14 $\pm$ 0.02	0.13 $\pm$ 0.04	0.14 $\pm$ 0.03	0.13 $\pm$ 0.04	0.13 $\pm$ 0.04	0.13 $\pm$ 0.04	0.14 $\pm$ 0.03	0.14 $\pm$ 0.04
C22	0.04 $\pm$ 0.02	0.05 $\pm$ 0.01	0.05 $\pm$ 0.01	0.05 $\pm$ 0.01	0.05 $\pm$ 0.01	0.06 $\pm$ 0.03	0.05 $\pm$ 0.01	0.05 $\pm$ 0.02
C20:3	0.12 $\pm$ 0.01	0.14 $\pm$ 0.02	0.14 $\pm$ 0.02	0.14 $\pm$ 0.01	0.13 $\pm$ 0.01	0.12 $\pm$ 0.04	0.13 $\pm$ 0.01	0.14 $\pm$ 0.01
C20:4 ARA ⁶	0.18 $\pm$ 0.01	0.20 $\pm$ 0.03	0.21 $\pm$ 0.03	0.21 $\pm$ 0.03	0.19 $\pm$ 0.02	0.19 $\pm$ 0.02	0.20 $\pm$ 0.03	0.20 $\pm$ 0.03
Total SFA	66.95 $\pm$ 1.18	67.17 $\pm$ 1.77	67.31 $\pm$ 1.70	67.66 $\pm$ 1.27	68.94 $\pm$ 0.75	67.01 $\pm$ 1.49	66.74 $\pm$ 1.74	67.48 $\pm$ 1.76
Total MUFA	28.63 $\pm$ 1.12	28.6 $\pm$ 1.63	28.36 $\pm$ 1.57	28.11 $\pm$ 1.14	27.05 $\pm$ 0.42	28.68 $\pm$ 1.45	28.93 $\pm$ 1.70	28.25 $\pm$ 1.61
Total PUFA	4.42 $\pm$ 0.07	4.23 $\pm$ 0.39	4.33 $\pm$ 0.22	4.23 $\pm$ 0.21	4.01 $\pm$ 0.39	4.32 $\pm$ 0.18	4.33 $\pm$ 0.17	4.27 $\pm$ 0.24

¹Past. = low temperature pasteurization (72°C, 30 s); HTST = HTST pasteurization (85°C, 30 s); UHT1 = UHT processing (135°C, 30 s); UHT2 = non-commercial UHT processing (150°C, 5 min); STR = sterilization (121°C, 15 min); HP = high pressure processing (400 MPa, 25°C, 15 min); Micro. = microwave pasteurization (650 W, 1.30 min).

²*i* = *iso*; *ai* = *anteiso*; *c* = *cis*; *t* = *trans*.

³C18:1 t (t4, t5, t6–t8, t9, t10, t11, t12, t13–t14, t15, t16).

⁴C18:1 c (c11, c12, c13, c14, c15).

⁵C18:2 other (C18:2 t,t NMID, t9,t12, t9,t13, t9,t12), where NMID = non-methylene-interrupted dienoic.

⁶ARA = arachidonic acid.

Table 2. Fatty acid composition (g/100 g of FA; means $\pm$ SD) in B2 milk samples¹

Fatty acid ²	Raw ³	Past.	HTST	UHT1	UHT2	STR	HP	Micro.
C4	3.30 $\pm$ 0.06	2.92 $\pm$ 0.47	2.87 $\pm$ 0.43	2.94 $\pm$ 0.37	3.05 $\pm$ 0.34	3.00 $\pm$ 0.56	2.86 $\pm$ 0.52	3.05 $\pm$ 0.31
C6	2.12 $\pm$ 0.34	1.83 $\pm$ 0.22	1.80 $\pm$ 0.14	1.84 $\pm$ 0.40	1.88 $\pm$ 0.19	1.92 $\pm$ 0.17	1.81 $\pm$ 0.41	1.83 $\pm$ 0.16
C8	1.12 $\pm$ 0.09	0.97 $\pm$ 0.09	1.08 $\pm$ 0.10	0.99 $\pm$ 0.15	1.05 $\pm$ 0.10	1.12 $\pm$ 0.13	1.04 $\pm$ 0.17	1.09 $\pm$ 0.10
C10	2.38 $\pm$ 0.12	2.16 $\pm$ 0.17	2.40 $\pm$ 0.16	2.20 $\pm$ 0.26	2.31 $\pm$ 0.20	2.42 $\pm$ 0.21	2.26 $\pm$ 0.24	2.37 $\pm$ 0.17
C10:1	0.24 $\pm$ 0.04	0.21 $\pm$ 0.03	0.24 $\pm$ 0.04	0.22 $\pm$ 0.04	0.23 $\pm$ 0.04	0.24 $\pm$ 0.04	0.22 $\pm$ 0.04	0.21 $\pm$ 0.09
C12	2.65 $\pm$ 0.03	2.56 $\pm$ 0.13	2.71 $\pm$ 0.09	2.56 $\pm$ 0.14	2.64 $\pm$ 0.13	2.68 $\pm$ 0.09	2.60 $\pm$ 0.14	2.67 $\pm$ 0.07
C14	9.24 $\pm$ 0.16	9.28 $\pm$ 0.28	9.65 $\pm$ 0.22	9.22 $\pm$ 0.27	9.39 $\pm$ 0.25	9.42 $\pm$ 0.18	9.31 $\pm$ 0.25	9.54 $\pm$ 0.19
C15 <i>i</i>	0.13 $\pm$ 0.08	0.16 $\pm$ 0.02	0.17 $\pm$ 0.01	0.16 $\pm$ 0.02	0.16 $\pm$ 0.01	0.17 $\pm$ 0.01	0.15 $\pm$ 0.05	0.17 $\pm$ 0.01
C14:1	0.83 $\pm$ 0.02	0.84 $\pm$ 0.02	0.87 $\pm$ 0.02	0.83 $\pm$ 0.02	0.84 $\pm$ 0.02	0.90 $\pm$ 0.14	0.86 $\pm$ 0.06	0.86 $\pm$ 0.01
C15 <i>ai</i>	0.42 $\pm$ 0.03	0.43 $\pm$ 0.02	0.44 $\pm$ 0.01	0.38 $\pm$ 0.14	0.43 $\pm$ 0.02	0.39 $\pm$ 0.15	0.43 $\pm$ 0.02	0.44 $\pm$ 0.01
C15	1.03 $\pm$ 0.05	1.03 $\pm$ 0.04	1.07 $\pm$ 0.01	1.03 $\pm$ 0.04	1.04 $\pm$ 0.03	1.04 $\pm$ 0.02	1.03 $\pm$ 0.04	1.06 $\pm$ 0.01
C16 <i>i</i>	0.19 $\pm$ 0.01	0.17 $\pm$ 0.07	0.21 $\pm$ 0.01	0.19 $\pm$ 0.01	0.20 $\pm$ 0.01	0.18 $\pm$ 0.07	0.19 $\pm$ 0.01	0.20 $\pm$ 0.01
C16	24.70 $\pm$ 0.79	25.10 $\pm$ 0.52	25.80 $\pm$ 0.43	25.06 $\pm$ 0.48	25.26 $\pm$ 0.40	25.43 $\pm$ 0.43	25.07 $\pm$ 0.77	25.68 $\pm$ 0.18
C17 <i>i</i>	0.32 $\pm$ 0.10	0.28 $\pm$ 0.11	0.32 $\pm$ 0.11	0.33 $\pm$ 0.09	0.32 $\pm$ 0.09	0.23 $\pm$ 0.13	0.32 $\pm$ 0.08	0.32 $\pm$ 0.10
C16:1 c9	1.65 $\pm$ 0.06	1.68 $\pm$ 0.03	1.73 $\pm$ 0.05	1.68 $\pm$ 0.03	1.68 $\pm$ 0.04	1.68 $\pm$ 0.03	1.68 $\pm$ 0.04	1.72 $\pm$ 0.05
C17	0.55 $\pm$ 0.02	0.56 $\pm$ 0.01	0.58 $\pm$ 0.01	0.56 $\pm$ 0.01	0.57 $\pm$ 0.01	0.57 $\pm$ 0.01	0.57 $\pm$ 0.02	0.58 $\pm$ 0.01
C17:1	0.27 $\pm$ 0.01	0.28 $\pm$ 0.01	0.26 $\pm$ 0.04	0.26 $\pm$ 0.03	0.26 $\pm$ 0.03	0.25 $\pm$ 0.04	0.26 $\pm$ 0.04	0.26 $\pm$ 0.04
C18	10.23 $\pm$ 0.42	10.41 $\pm$ 0.26	10.63 $\pm$ 0.20	10.45 $\pm$ 0.24	10.51 $\pm$ 0.19	10.56 $\pm$ 0.13	10.43 $\pm$ 0.44	10.65 $\pm$ 0.16
C18:1 t ⁴	7.23 $\pm$ 0.78	8.02 $\pm$ 0.81	7.06 $\pm$ 0.38	7.72 $\pm$ 0.82	7.79 $\pm$ 0.68	7.53 $\pm$ 0.86	8.04 $\pm$ 1.14	7.38 $\pm$ 0.47
C18:1 c9	21.97 $\pm$ 1.17	21.89 $\pm$ 1.01	21.49 $\pm$ 0.42	22.17 $\pm$ 1.51	21.64 $\pm$ 1.03	21.61 $\pm$ 1.05	21.98 $\pm$ 1.22	21.38 $\pm$ 0.54
C18:1 c ⁵	2.46 $\pm$ 0.12	2.53 $\pm$ 0.12	2.13 $\pm$ 0.08	2.50 $\pm$ 0.20	2.17 $\pm$ 0.32	2.22 $\pm$ 0.13	2.31 $\pm$ 0.43	1.92 $\pm$ 0.26
C18:2 c9c12	3.82 $\pm$ 0.17	3.71 $\pm$ 0.22	3.57 $\pm$ 0.23	3.63 $\pm$ 0.30	3.60 $\pm$ 0.27	3.50 $\pm$ 0.26	3.67 $\pm$ 0.27	3.57 $\pm$ 0.21
C18:2 other ⁶	1.09 $\pm$ 0.03	1.01 $\pm$ 0.12	0.93 $\pm$ 0.05	1.03 $\pm$ 0.11	0.96 $\pm$ 0.09	0.89 $\pm$ 0.07	0.98 $\pm$ 0.10	0.96 $\pm$ 0.05
C20	0.13 $\pm$ 0.04	0.12 $\pm$ 0.04	0.11 $\pm$ 0.03	0.12 $\pm$ 0.03	0.11 $\pm$ 0.03	0.11 $\pm$ 0.02	0.12 $\pm$ 0.04	0.11 $\pm$ 0.03
C20:1	0.20 $\pm$ 0.06	0.18 $\pm$ 0.06	0.18 $\pm$ 0.06	0.19 $\pm$ 0.05	0.17 $\pm$ 0.03	0.18 $\pm$ 0.03	0.18 $\pm$ 0.06	0.18 $\pm$ 0.03
C18:3	0.34 $\pm$ 0.01	0.35 $\pm$ 0.01	0.36 $\pm$ 0.01	0.34 $\pm$ 0.01	0.35 $\pm$ 0.01	0.35 $\pm$ 0.01	0.34 $\pm$ 0.01	0.36 $\pm$ 0.01
C18:2 c9t11	0.84 $\pm$ 0.05	0.85 $\pm$ 0.03	0.88 $\pm$ 0.01	0.84 $\pm$ 0.02	0.85 $\pm$ 0.03	0.85 $\pm$ 0.03	0.84 $\pm$ 0.04	0.88 $\pm$ 0.02
CLA other	0.18 $\pm$ 0.01	0.18 $\pm$ 0.03	0.19 $\pm$ 0.01	0.18 $\pm$ 0.02	0.18 $\pm$ 0.02	0.18 $\pm$ 0.02	0.18 $\pm$ 0.02	0.19 $\pm$ 0.01
C22	0.05 $\pm$ 0.01	0.06 $\pm$ 0.01	0.05 $\pm$ 0.01	0.06 $\pm$ 0.01	0.06 $\pm$ 0.01	0.06 $\pm$ 0.01	0.06 $\pm$ 0.01	0.06 $\pm$ 0.01
C20:3	0.13 $\pm$ 0.01	0.13 $\pm$ 0.01	0.14 $\pm$ 0.01	0.13 $\pm$ 0.01	0.13 $\pm$ 0.01	0.13 $\pm$ 0.01	0.13 $\pm$ 0.01	0.13 $\pm$ 0.03
C20:4 ARA ⁷	0.18 $\pm$ 0.01	0.18 $\pm$ 0.02	0.19 $\pm$ 0.02	0.18 $\pm$ 0.01	0.18 $\pm$ 0.02	0.18 $\pm$ 0.02	0.18 $\pm$ 0.02	0.19 $\pm$ 0.02
Total SFA	58.64 $\pm$ 0.77	58.04 $\pm$ 1.31	59.89 $\pm$ 0.41	58.09 $\pm$ 1.91	58.97 $\pm$ 1.24	59.3 $\pm$ 0.57	58.24 $\pm$ 1.61	59.82 $\pm$ 0.36
Total MUFA	34.85 $\pm$ 0.83	35.55 $\pm$ 1.12	33.86 $\pm$ 0.23	35.57 $\pm$ 1.74	34.78 $\pm$ 1.11	34.61 $\pm$ 0.64	35.44 $\pm$ 1.47	33.91 $\pm$ 0.27
Total PUFA	6.50 $\pm$ 0.17	6.41 $\pm$ 0.28	6.25 $\pm$ 0.23	6.34 $\pm$ 0.31	6.25 $\pm$ 0.29	6.09 $\pm$ 0.27	6.32 $\pm$ 0.30	6.27 $\pm$ 0.23

¹Past. = low temperature pasteurization (72°C, 30 s); HTST = HTST pasteurization (85°C, 30 s); UHT1 = UHT processing (135°C, 30 s); UHT2 = non-commercial UHT processing (150°C, 5 min); STR = sterilization (121°C, 15 min); HP = high pressure processing (400 MPa, 25°C, 15 min); Micro. = microwave pasteurization (650 W, 1.30 min).

²*i* = *iso*; *ai* = *anteiso*; *c* = *cis*; *t* = *trans*.

³Bold numbers in raw milk sample column indicate significant differences among batches ($P < 0.05$).

⁴C18:1 t (t4, t5, t6-t8, t9, t10, t11, t12, t13-t14, t15, t16).

⁵C18:1 c (c11, c12, c13, c14, c15).

⁶C18:2 other (C18:2 t,t NMID, t9,t12, t9,t13, t9,t12), where NMID = non-methylene-interrupted dienoic.

⁷ARA = arachidonic acid.

in B1 raw milk; 6.50% in B2). Linoleic acid (2.63% vs. 3.82%) was higher ($P < 0.05$) in the B2 samples as was total CLA (4.62 mg/g of fat vs. 7.19 mg/g of fat; Tables 5 and 6). From this total amount, 81.40% (Table 5) corresponded to C18:2 c9t11 (RA) in B1 raw milk, whereas 84.31% corresponded to RA (Table 6) in B2 ($P > 0.05$). The second major CLA isomer was C18:2 t7c9, with 9.76% in B1 samples and 11.22% in B2 samples ($P < 0.05$).

Data in the present study are supported by other previous research works reporting that supplementation with different lipid sources is a useful way to increase the CLA content in milk (Loor et al., 2005; Nudda et al., 2006). The concentrations of RA and other CLA isomers are result of the biohydrogenation in the rumen

of dietary PUFA and the action of the Δ^9 -desaturase on *trans* vaccenic acid in the mammary gland.

Effect of Processing on the Fatty Acid Composition

Both milk batches (B1 and B2) were processed to obtain pasteurized, UHT, STR, microwaved, and HP milk samples. The experiment was designed to test if milk fat could be affected by processing and if those changes may be associated with the fatty acid composition because preliminary results showed that B2 raw milk had higher concentration unsaturated FA than B1 raw milk.

Previous results from other authors are not conclusive. Herzallah et al. (2005a) conducted a study focused

Table 3. *trans* monoenoic and C18:2 minor isomers composition (g/100 g of FA; means $\pm$ SD) in B1 milk samples¹

Fatty acid ²	Raw	Past.	HTST	UHT1	UHT2	STR	HP	Micro.
C16:1 t	0.14 $\pm$ 0.03	0.15 $\pm$ 0.03	0.14 $\pm$ 0.02	0.15 $\pm$ 0.02	0.16 $\pm$ 0.02	0.14 $\pm$ 0.02	0.14 $\pm$ 0.02	0.15 $\pm$ 0.02
C18:1 t4	0.03 $\pm$ 0.02	0.03 $\pm$ 0.01	0.03 $\pm$ 0.01	0.03 $\pm$ 0.01	0.03 $\pm$ 0.01	0.03 $\pm$ 0.01	0.03 $\pm$ 0.01	0.03 $\pm$ 0.01
C18:1 t5	0.03 $\pm$ 0.02	0.03 $\pm$ 0.02	0.03 $\pm$ 0.01	0.03 $\pm$ 0.02	0.03 $\pm$ 0.02	0.03 $\pm$ 0.02	0.03 $\pm$ 0.01	0.03 $\pm$ 0.01
C18:1 t6-t8	0.23 $\pm$ 0.08	0.23 $\pm$ 0.06	0.26 $\pm$ 0.04	0.23 $\pm$ 0.06	0.26 $\pm$ 0.01	0.28 $\pm$ 0.04	0.29 $\pm$ 0.02	0.27 $\pm$ 0.02
C18:1 t9	0.28 $\pm$ 0.04	0.28 $\pm$ 0.07	0.29 $\pm$ 0.08	0.24 $\pm$ 0.03	0.26 $\pm$ 0.06	0.34 $\pm$ 0.07	0.33 $\pm$ 0.10	0.32 $\pm$ 0.06
C18:1 t10	0.43 $\pm$ 0.03	0.45 $\pm$ 0.06	0.48 $\pm$ 0.09	0.43 $\pm$ 0.04	0.41 $\pm$ 0.02	0.49 $\pm$ 0.07	0.49 $\pm$ 0.07	0.45 $\pm$ 0.08
C18:1 t11	0.87 $\pm$ 0.08	0.85 $\pm$ 0.04	0.82 $\pm$ 0.08	0.78 $\pm$ 0.06	0.70 $\pm$ 0.03	0.84 $\pm$ 0.06	0.83 $\pm$ 0.05	0.80 $\pm$ 0.09
C18:1 t12	0.39 $\pm$ 0.02	0.36 $\pm$ 0.04	0.40 $\pm$ 0.07	0.35 $\pm$ 0.04	0.33 $\pm$ 0.03	0.39 $\pm$ 0.09	0.39 $\pm$ 0.02	0.36 $\pm$ 0.03
C18:1 t13-t14	0.71 $\pm$ 0.07	0.58 $\pm$ 0.08	0.51 $\pm$ 0.05	0.70 $\pm$ 0.09	0.47 $\pm$ 0.03	0.51 $\pm$ 0.04	0.57 $\pm$ 0.04	0.55 $\pm$ 0.06
C18:1 t15	0.22 $\pm$ 0.02	0.21 $\pm$ 0.03	0.21 $\pm$ 0.02	0.25 $\pm$ 0.02	0.25 $\pm$ 0.01	0.21 $\pm$ 0.02	0.24 $\pm$ 0.02	0.22 $\pm$ 0.03
C18:1 t16	0.22 $\pm$ 0.02	0.24 $\pm$ 0.03	0.21 $\pm$ 0.02	0.23 $\pm$ 0.02	0.27 $\pm$ 0.01	0.22 $\pm$ 0.02	0.26 $\pm$ 0.02	0.23 $\pm$ 0.03
C18:1 c11	0.71 $\pm$ 0.04	0.67 $\pm$ 0.08	0.67 $\pm$ 0.05	0.66 $\pm$ 0.04	0.60 $\pm$ 0.03	0.71 $\pm$ 0.06	0.75 $\pm$ 0.07	0.66 $\pm$ 0.06
C18:1 c12	0.37 $\pm$ 0.02	0.33 $\pm$ 0.05	0.33 $\pm$ 0.03	0.32 $\pm$ 0.03	0.29 $\pm$ 0.01	0.35 $\pm$ 0.03	0.36 $\pm$ 0.03	0.31 $\pm$ 0.04
C18:1 c13	0.08 $\pm$ 0.01	0.07 $\pm$ 0.01	0.06 $\pm$ 0.01	0.07 $\pm$ 0.01	0.06 $\pm$ 0.01	0.08 $\pm$ 0.01	0.08 $\pm$ 0.01	0.07 $\pm$ 0.01
C18:1 c14	0.29 $\pm$ 0.02	0.28 $\pm$ 0.04	0.29 $\pm$ 0.03	0.27 $\pm$ 0.03	0.23 $\pm$ 0.02	0.31 $\pm$ 0.03	0.32 $\pm$ 0.03	0.28 $\pm$ 0.03
C18:1 c15	0.08 $\pm$ 0.01	0.08 $\pm$ 0.01	0.08 $\pm$ 0.01	0.08 $\pm$ 0.01	0.06 $\pm$ 0.01	0.09 $\pm$ 0.01	0.09 $\pm$ 0.01	0.08 $\pm$ 0.01
C18:2 NMID ³	0.25 $\pm$ 0.02	0.21 $\pm$ 0.03	0.22 $\pm$ 0.02	0.21 $\pm$ 0.02	0.19 $\pm$ 0.01	0.24 $\pm$ 0.02	0.23 $\pm$ 0.02	0.22 $\pm$ 0.02
C18:2 t9t12	0.12 $\pm$ 0.01	0.09 $\pm$ 0.01	0.10 $\pm$ 0.01	0.10 $\pm$ 0.01	0.08 $\pm$ 0.01	0.11 $\pm$ 0.01	0.10 $\pm$ 0.01	0.10 $\pm$ 0.01
C18:2 c9t13	0.11 $\pm$ 0.01	0.08 $\pm$ 0.01	0.09 $\pm$ 0.01	0.08 $\pm$ 0.01	0.07 $\pm$ 0.01	0.10 $\pm$ 0.01	0.09 $\pm$ 0.01	0.09 $\pm$ 0.01
C18:2 c9t12	0.09 $\pm$ 0.01	0.08 $\pm$ 0.01	0.08 $\pm$ 0.01	0.08 $\pm$ 0.01	0.07 $\pm$ 0.01	0.09 $\pm$ 0.01	0.10 $\pm$ 0.01	0.08 $\pm$ 0.01
C18:2 t11c15	0.03 $\pm$ 0.01	0.02 $\pm$ 0.01	0.03 $\pm$ 0.01	0.03 $\pm$ 0.01	0.02 $\pm$ 0.01	0.03 $\pm$ 0.01	0.03 $\pm$ 0.01	0.02 $\pm$ 0.01
C18:2 t9c12	0.03 $\pm$ 0.01	0.05 $\pm$ 0.02	0.06 $\pm$ 0.02	0.05 $\pm$ 0.02	0.05 $\pm$ 0.03	0.03 $\pm$ 0.01	0.02 $\pm$ 0.01	0.03 $\pm$ 0.02
C18:2 c9c15	0.03 $\pm$ 0.01	0.02 $\pm$ 0.01	0.02 $\pm$ 0.01	0.02 $\pm$ 0.01	0.02 $\pm$ 0.01	0.02 $\pm$ 0.01	0.02 $\pm$ 0.01	0.02 $\pm$ 0.01

¹Past. = low temperature pasteurization (72°C, 30 s); HTST = HTST pasteurization (85°C, 30 s); UHT1 = UHT processing (135°C, 30 s); UHT2 = non-commercial UHT processing (150°C, 5 min); STR = sterilization (121°C, 15 min); HP = high pressure processing (400 MPa, 25°C, 15 min); Micro. = microwave pasteurization (650 W, 1.30 min).

²t = *trans* double bond; c = *cis* double bond.

³NMID = non-methylene-interrupted dienoic.

Table 4. *trans* monoenoic and C18:2 minor isomers composition (g/100 g of FA; means $\pm$ SD) in B2 milk samples¹

Fatty acid ²	Raw ³	Past.	HTST	UHT1	UHT2	STR	HP	Micro.
C16:1 t	0.15 $\pm$ 0.02	0.15 $\pm$ 0.02	0.16 $\pm$ 0.01	0.15 $\pm$ 0.01	0.16 $\pm$ 0.01	0.15 $\pm$ 0.01	0.16 $\pm$ 0.02	0.16 $\pm$ 0.02
C18:1 t4	0.05 $\pm$ 0.01	0.05 $\pm$ 0.01	0.04 $\pm$ 0.01	0.04 $\pm$ 0.01	0.04 $\pm$ 0.01	0.04 $\pm$ 0.01	0.04 $\pm$ 0.01	0.04 $\pm$ 0.01
C18:1 t5	0.05 $\pm$ 0.02	0.05 $\pm$ 0.02	0.04 $\pm$ 0.02	0.04 $\pm$ 0.01	0.04 $\pm$ 0.02	0.04 $\pm$ 0.02	0.04 $\pm$ 0.02	0.04 $\pm$ 0.02
C18:1 t6-t8	0.55 $\pm$ 0.07	0.55 $\pm$ 0.10	0.50 $\pm$ 0.07	0.53 $\pm$ 0.11	0.54 $\pm$ 0.08	0.51 $\pm$ 0.10	0.53 $\pm$ 0.12	0.48 $\pm$ 0.11
C18:1 t9	0.60 $\pm$ 0.14	0.54 $\pm$ 0.06	0.47 $\pm$ 0.06	0.54 $\pm$ 0.06	0.50 $\pm$ 0.05	0.48 $\pm$ 0.04	0.49 $\pm$ 0.03	0.45 $\pm$ 0.06
C18:1 t10	1.71 $\pm$ 0.70	1.96 $\pm$ 0.54	1.83 $\pm$ 0.43	1.93 $\pm$ 0.55	1.88 $\pm$ 0.53	1.86 $\pm$ 0.53	1.97 $\pm$ 0.57	1.87 $\pm$ 0.44
C18:1 t11	1.82 $\pm$ 0.03	1.91 $\pm$ 0.10	1.74 $\pm$ 0.09	1.93 $\pm$ 0.14	1.91 $\pm$ 0.15	1.73 $\pm$ 0.13	1.82 $\pm$ 0.17	1.67 $\pm$ 0.08
C18:1 t12	0.70 $\pm$ 0.17	0.80 $\pm$ 0.10	0.72 $\pm$ 0.10	0.78 $\pm$ 0.14	0.78 $\pm$ 0.12	0.80 $\pm$ 0.07	0.81 $\pm$ 0.13	0.74 $\pm$ 0.08
C18:1 t13-t14	0.81 $\pm$ 0.02	0.96 $\pm$ 0.05	0.92 $\pm$ 0.05	1.06 $\pm$ 0.08	1.04 $\pm$ 0.08	0.95 $\pm$ 0.08	1.10 $\pm$ 0.10	1.09 $\pm$ 0.05
C18:1 t15	0.38 $\pm$ 0.01	0.49 $\pm$ 0.03	0.31 $\pm$ 0.02	0.37 $\pm$ 0.03	0.43 $\pm$ 0.04	0.49 $\pm$ 0.04	0.54 $\pm$ 0.05	0.40 $\pm$ 0.02
C18:1 t16	0.42 $\pm$ 0.01	0.55 $\pm$ 0.03	0.33 $\pm$ 0.02	0.35 $\pm$ 0.03	0.47 $\pm$ 0.04	0.48 $\pm$ 0.04	0.53 $\pm$ 0.05	0.43 $\pm$ 0.02
C18:1 c11	0.81 $\pm$ 0.04	0.85 $\pm$ 0.05	0.78 $\pm$ 0.02	0.85 $\pm$ 0.07	0.80 $\pm$ 0.06	0.75 $\pm$ 0.02	0.84 $\pm$ 0.08	0.78 $\pm$ 0.04
C18:1 c12	0.92 $\pm$ 0.04	0.98 $\pm$ 0.04	0.85 $\pm$ 0.05	0.92 $\pm$ 0.08	0.70 $\pm$ 0.26	0.82 $\pm$ 0.06	0.76 $\pm$ 0.30	0.53 $\pm$ 0.28
C18:1 c13	0.11 $\pm$ 0.01	0.09 $\pm$ 0.03	0.06 $\pm$ 0.02	0.11 $\pm$ 0.01	0.09 $\pm$ 0.02	0.10 $\pm$ 0.01	0.10 $\pm$ 0.04	0.07 $\pm$ 0.03
C18:1 c14	0.47 $\pm$ 0.02	0.46 $\pm$ 0.02	0.32 $\pm$ 0.11	0.46 $\pm$ 0.04	0.44 $\pm$ 0.03	0.42 $\pm$ 0.04	0.45 $\pm$ 0.05	0.40 $\pm$ 0.02
C18:1 c15	0.14 $\pm$ 0.01	0.14 $\pm$ 0.01	0.12 $\pm$ 0.02	0.15 $\pm$ 0.01	0.15 $\pm$ 0.01	0.14 $\pm$ 0.01	0.15 $\pm$ 0.02	0.14 $\pm$ 0.01
C18:2 NMID ⁴	0.48 $\pm$ 0.02	0.44 $\pm$ 0.05	0.39 $\pm$ 0.02	0.44 $\pm$ 0.04	0.41 $\pm$ 0.04	0.37 $\pm$ 0.02	0.42 $\pm$ 0.04	0.39 $\pm$ 0.02
C18:2 t9t12	0.17 $\pm$ 0.01	0.15 $\pm$ 0.02	0.13 $\pm$ 0.01	0.16 $\pm$ 0.02	0.14 $\pm$ 0.01	0.13 $\pm$ 0.01	0.15 $\pm$ 0.02	0.13 $\pm$ 0.01
C18:2 c9t13	0.17 $\pm$ 0.01	0.16 $\pm$ 0.01	0.14 $\pm$ 0.01	0.16 $\pm$ 0.02	0.14 $\pm$ 0.01	0.13 $\pm$ 0.01	0.15 $\pm$ 0.02	0.14 $\pm$ 0.02
C18:2 c9t12	0.14 $\pm$ 0.01	0.14 $\pm$ 0.01	0.12 $\pm$ 0.01	0.13 $\pm$ 0.02	0.13 $\pm$ 0.02	0.11 $\pm$ 0.01	0.13 $\pm$ 0.02	0.12 $\pm$ 0.01
C18:2 t11c15	0.05 $\pm$ 0.01	0.05 $\pm$ 0.01	0.04 $\pm$ 0.01	0.05 $\pm$ 0.01	0.04 $\pm$ 0.01	0.04 $\pm$ 0.01	0.04 $\pm$ 0.01	0.04 $\pm$ 0.01
C18:2 t9c12	0.05 $\pm$ 0.01	0.04 $\pm$ 0.01	0.08 $\pm$ 0.03	0.07 $\pm$ 0.02	0.07 $\pm$ 0.03	0.08 $\pm$ 0.04	0.06 $\pm$ 0.03	0.11 $\pm$ 0.01
C18:2 c9c15	0.03 $\pm$ 0.01	0.03 $\pm$ 0.02	0.02 $\pm$ 0.01	0.03 $\pm$ 0.01	0.02 $\pm$ 0.01	0.02 $\pm$ 0.01	0.03 $\pm$ 0.01	0.03 $\pm$ 0.01

¹Past. = low temperature pasteurization (72°C, 30 s); HTST = HTST pasteurization (85°C, 30 s); UHT1 = UHT processing (135°C, 30 s); UHT2 = non-commercial UHT processing (150°C, 5 min); STR = sterilization (121°C, 15 min); HP = high pressure processing (400 MPa, 25°C, 15 min); Micro. = microwave pasteurization (650 W, 1.30 min).

²t = *trans* double bond; c = *cis* double bond.

³Bold numbers in the raw milk sample column indicate significant differences among batches ($P < 0.05$).

⁴NMID = non-methylene-interrupted dienoic.

Table 5. Total CLA (mg/g of fat) and isomers distribution (g of isomer/100 g of CLA; means $\pm$ SD) in B1 milk samples¹

Item ²	Raw	Past.	HTST	UHT1	UHT2	STR	HP	Micro.
Total CLA	4.61 $\pm$ 0.12 ^{ab}	4.51 $\pm$ 0.20 ^{ab}	4.84 $\pm$ 0.05 ^b	4.39 $\pm$ 0.09 ^a	4.37 $\pm$ 0.19 ^a	4.55 $\pm$ 0.06 ^a	4.53 $\pm$ 0.26 ^a	4.42 $\pm$ 0.07 ^{ab}
% CLA isomers								
t,t compounds	7.64 $\pm$ 1.18	7.60 $\pm$ 0.52	8.18 $\pm$ 0.44	7.76 $\pm$ 0.41	8.17 $\pm$ 0.60	8.66 $\pm$ 0.76	7.66 $\pm$ 0.29	7.59 $\pm$ 0.20
12,14	0.88 $\pm$ 0.22	0.83 $\pm$ 0.06	0.87 $\pm$ 0.14	0.75 $\pm$ 0.20	1.01 $\pm$ 0.13	0.75 $\pm$ 0.10	0.74 $\pm$ 0.09	0.81 $\pm$ 0.10
11,13	1.31 $\pm$ 0.01	1.17 $\pm$ 0.12	1.16 $\pm$ 0.06	1.15 $\pm$ 0.15	1.19 $\pm$ 0.04	1.18 $\pm$ 0.10	1.25 $\pm$ 0.15	1.13 $\pm$ 0.06
10,12	2.14 $\pm$ 0.09	2.08 $\pm$ 0.09	2.11 $\pm$ 0.12	1.99 $\pm$ 0.10	1.98 $\pm$ 0.10	2.12 $\pm$ 0.12	2.01 $\pm$ 0.07	1.96 $\pm$ 0.07
9,11	1.43 $\pm$ 0.34 ^a	1.55 $\pm$ 0.21 ^a	1.77 $\pm$ 0.10 ^{ab}	1.81 $\pm$ 0.06 ^{ab}	1.95 $\pm$ 0.29 ^{ab}	2.16 $\pm$ 0.29 ^b	1.49 $\pm$ 0.07 ^a	1.58 $\pm$ 0.12 ^a
8,10	1.27 $\pm$ 0.42	1.24 $\pm$ 0.19	1.51 $\pm$ 0.11	1.28 $\pm$ 0.02	1.29 $\pm$ 0.10	1.56 $\pm$ 0.29	1.33 $\pm$ 0.17	1.35 $\pm$ 0.10
7,9	0.61 $\pm$ 0.11	0.74 $\pm$ 0.23	0.75 $\pm$ 0.11	0.78 $\pm$ 0.15	0.75 $\pm$ 0.17	0.88 $\pm$ 0.07	0.85 $\pm$ 0.10	0.75 $\pm$ 0.14
ct/tc compounds	92.30 $\pm$ 1.18	92.40 $\pm$ 0.52	91.82 $\pm$ 0.44	92.24 $\pm$ 0.41	91.83 $\pm$ 0.60	91.34 $\pm$ 0.76	92.34 $\pm$ 0.29	92.41 $\pm$ 0.20
12,14	0.39 $\pm$ 0.02	0.47 $\pm$ 0.06	0.46 $\pm$ 0.07	0.69 $\pm$ 0.17	0.71 $\pm$ 0.20	0.60 $\pm$ 0.11	0.55 $\pm$ 0.10	0.58 $\pm$ 0.19
11,13	0.80 $\pm$ 0.24	0.62 $\pm$ 0.13	0.54 $\pm$ 0.04	0.74 $\pm$ 0.20	0.71 $\pm$ 0.19	0.72 $\pm$ 0.11	0.87 $\pm$ 0.30	0.76 $\pm$ 0.24
9,11	81.40 $\pm$ 0.94 ^a	81.49 $\pm$ 0.26 ^a	81.04 $\pm$ 0.36 ^{ab}	80.96 $\pm$ 0.25 ^{ab}	80.62 $\pm$ 0.51 ^{ab}	80.05 $\pm$ 0.60 ^b	81.01 $\pm$ 0.43 ^{ab}	81.20 $\pm$ 0.52 ^a
7,9	9.76 $\pm$ 0.02	9.82 $\pm$ 0.61	9.77 $\pm$ 0.32	9.85 $\pm$ 0.34	9.78 $\pm$ 0.15	9.99 $\pm$ 0.27	9.92 $\pm$ 0.31	9.88 $\pm$ 0.37

^{a,b}Values within a row with different superscripts differ significantly ($P < 0.05$).

¹Past. = low temperature pasteurization (72°C, 30 s); HTST = HTST pasteurization (85°C, 30 s); UHT1 = UHT processing (135°C, 30 s); UHT2 = non-commercial UHT processing (150°C, 5 min); STR = sterilization (121°C, 15 min); HP = high pressure processing (400 MPa, 25°C, 15 min); Micro. = microwave pasteurization (650 W, 1.30 min).

²t,t = *trans trans*; ct/tc = *cis trans/trans cis*.

on pasteurized, UHT, boiled, and microwaved milk, reporting no changes in the concentrations of total SFA, MUFA, and PUFA. Those authors concluded that the continuous aqueous phase of milk acts as an oxygen barrier that hinders the oxidation reactions, and therefore triglycerides are not altered during processing. In further studies from other authors, cow milk naturally enriched in RA was assayed in the elaboration of UHT milk (Jones et al., 2005). The provided FA composition also showed absence of variations after processing. In a similar assay, Lynch et al. (2005) did not find alterations in pasteurized milk naturally enriched in C18:1 t11 and PUFA.

According to the results in the present research, the total concentration of SFA, MUFA, and PUFA (Tables 1 and 2) remained stable when milk from both batches were processed to obtain pasteurized, HTST, UHT1, UHT2, STR, HP, and microwave pasteurized milks in agreement with the previously cited studies. Furthermore, when the detailed FA composition was examined, stability was found for most of the compounds in all samples.

However, CLA concentration in both B1 and B2 milks (Tables 5 and 6) increased after HTST processing ($P < 0.05$). These variations represented an increment of 5% for B1 and 11% for B2 compared with the raw

Table 6. Total CLA (mg/g of fat) and isomers distribution (g of isomer/100 g of CLA; means $\pm$ SD) in B2 milk samples¹

Item ²	Raw ³	Past.	HTST	UHT1	UHT2	STR	HP	Micro.
Total CLA	7.19 $\pm$ 0.14^a	6.81 $\pm$ 0.23 ^a	7.99 $\pm$ 0.08 ^b	7.32 $\pm$ 0.37 ^{ab}	6.78 $\pm$ 0.28 ^a	6.99 $\pm$ 0.13 ^a	7.58 $\pm$ 0.26 ^a	7.29 $\pm$ 0.28 ^a
% CLA isomers								
t,t compounds	3.68 $\pm$ 0.19	4.06 $\pm$ 0.39	4.11 $\pm$ 0.71	4.22 $\pm$ 0.80	4.38 $\pm$ 0.48	4.92 $\pm$ 0.65	4.41 $\pm$ 0.15	4.31 $\pm$ 0.54
12,14	0.56 $\pm$ 0.05	0.66 $\pm$ 0.07	0.62 $\pm$ 0.05	0.62 $\pm$ 0.15	0.67 $\pm$ 0.07	0.60 $\pm$ 0.07	0.70 $\pm$ 0.10	0.62 $\pm$ 0.03
11,13	0.74 $\pm$ 0.01	0.79 $\pm$ 0.08	0.78 $\pm$ 0.03	0.77 $\pm$ 0.03	0.77 $\pm$ 0.03	0.78 $\pm$ 0.08	0.90 $\pm$ 0.22	0.84 $\pm$ 0.14
10,12	1.06 $\pm$ 0.08	1.13 $\pm$ 0.08	1.08 $\pm$ 0.15	0.99 $\pm$ 0.13	0.99 $\pm$ 0.07	1.09 $\pm$ 0.10	1.09 $\pm$ 0.13	1.32 $\pm$ 0.24
9,11	0.69 $\pm$ 0.01^a	0.76 $\pm$ 0.08 ^a	0.82 $\pm$ 0.29 ^a	1.05 $\pm$ 0.21 ^{ab}	1.11 $\pm$ 0.11 ^{ab}	1.27 $\pm$ 0.19 ^b	0.79 $\pm$ 0.18 ^a	0.76 $\pm$ 0.13 ^a
8,10	0.31 $\pm$ 0.03	0.39 $\pm$ 0.14	0.43 $\pm$ 0.19	0.38 $\pm$ 0.20	0.37 $\pm$ 0.11	0.59 $\pm$ 0.20	0.49 $\pm$ 0.09	0.39 $\pm$ 0.09
7,9	0.31 $\pm$ 0.01^a	0.33 $\pm$ 0.06 ^a	0.39 $\pm$ 0.08 ^a	0.41 $\pm$ 0.09 ^{ab}	0.47 $\pm$ 0.10 ^{ab}	0.59 $\pm$ 0.09 ^b	0.44 $\pm$ 0.11 ^{ab}	0.38 $\pm$ 0.08 ^a
ct/tc compounds	96.32 $\pm$ 0.19	95.94 $\pm$ 0.39	95.89 $\pm$ 0.71	95.78 $\pm$ 0.8	95.62 $\pm$ 0.48	95.08 $\pm$ 0.65	95.59 $\pm$ 0.15	95.69 $\pm$ 0.54
12,14	0.39 $\pm$ 0.03	0.40 $\pm$ 0.14	0.37 $\pm$ 0.13	0.36 $\pm$ 0.04	0.36 $\pm$ 0.08	0.40 $\pm$ 0.08	0.41 $\pm$ 0.10	0.48 $\pm$ 0.13
11,13	0.41 $\pm$ 0.08	0.36 $\pm$ 0.03	0.40 $\pm$ 0.05	0.37 $\pm$ 0.02	0.51 $\pm$ 0.07	0.36 $\pm$ 0.10	0.33 $\pm$ 0.08	0.39 $\pm$ 0.13
9,11	84.31 $\pm$ 0.26	84.04 $\pm$ 0.60	84.23 $\pm$ 0.57	84.15 $\pm$ 0.68	83.87 $\pm$ 0.26	83.44 $\pm$ 0.55	83.95 $\pm$ 0.26	83.75 $\pm$ 0.29
7,9	11.22 $\pm$ 0.12	11.14 $\pm$ 0.23	10.89 $\pm$ 0.24	10.89 $\pm$ 0.16	10.87 $\pm$ 0.33	10.89 $\pm$ 0.18	10.89 $\pm$ 0.21	11.07 $\pm$ 0.27

^{a,b}Values within a row with different superscripts differ significantly ($P < 0.05$).

¹Past. = low temperature pasteurization (72°C, 30 s); HTST = HTST pasteurization (85°C, 30 s); UHT1 = UHT processing (135°C, 30 s); UHT2 = non-commercial UHT processing (150°C, 5 min); STR = sterilization (121°C, 15 min); HP = high pressure processing (400 MPa, 25°C, 15 min); Micro. = microwave pasteurization (650 W, 1.30 min).

²t,t = *trans trans*; ct/tc = *cis trans/trans cis*.

³Bold numbers in raw milk sample column indicate significant differences among batches ($P < 0.05$); superscript bold letters within a row for significant differences by processing ($P < 0.05$).

milk samples. The contents of C18:2 c9t11 (Tables 1 and 2) showed a trend to increase in the HTST samples from 0.39% in raw milk from B1 to 0.43% and 0.84% in raw milk from B2 to 0.88% ($0.05 < P < 0.1$). The concentration of C18:2 c9c12 also showed lower levels after elaboration (2.63% raw milk from B1 vs. 2.49% in HTST; 3.82% raw milk from B2 vs. 3.57%; $0.05 < P < 0.1$).

A significant alteration of the distribution of CLA isomers in the STR samples was found: the proportion of C18:2 t9t11 (Table 5) changed from 1.43 g of isomer/100 g of CLA in B1 raw milk to 2.16 g of isomer/100 g of CLA, representing a variation of 51%. This change was accompanied with a lower C18:2 c9t11 (RA) concentration (81.40 g of RA/100 g of CLA in B1 raw milk vs. 80.05 g of RA/100 g of CLA in STR samples). The level of this compound was also affected ($P < 0.05$) in B2 samples (0.69 g of C18:2 t9t11/100 g of CLA to 1.27 g of C18:2 t9t11/100 g of CLA; Table 6), whereas variation of RA was not significant (84.30 g of RA/100 g of CLA to 83.44 g of C18:2 RA/100 g of CLA; $P > 0.05$). The present results can be explained by taking into account that *trans* double bonds are more thermodynamically stable than *cis*. Thus, previous studies found that methyl linoleate can isomerize to C18:2 t9t11 and 18:2 t10t12 after heating at 200 and 220°C for 24 h (Destailats and Angers, 2005). Assays with trilinolein suggested that reactions occur in the triglyceride molecule without previous release of the FA (Christy, 2010). In food such as milk butter, heating at 225°C for 15 min leads to isomerizations of C18:1 c9 to C18:1 t9, whereas 200°C for 2 h resulted in a sigmatropic rearrangement of rumenic and C18:2 t10, c12 into *trans*, *trans* CLA compounds (Precht et al., 1999; Destailats et al., 2005). All these reactions were oxidative in nature. Because the observed changes were greater in the B2 samples than in B1, this seems to be related to the higher PUFA concentration in those samples. Thus, a high amount of substrate is available for the reaction.

Furthermore, in research works conducted with CLA-fortified milks, Campbell et al. (2003) found that pasteurization diminished the concentration of RA whereas Rodríguez-Alcalá and Fontecha (2007) described that thermal processing of CLA enriched dairy products resulted in higher contents of *trans*, *trans* isomers, pointing to sigmatropic rearrangement reactions of the CLA fraction.

However, other authors found results different from those in the present research, regarding the total CLA content (Herzallah et al., 2005b): elaboration of microwave and UHT processing resulted in lower contents of total CLA when compared with untreated samples.

Low temperature treatments such as pasteurization create oxidative environments caused by oxygen solubility, which is inversely proportional to temperature, whereas H^+ proton donors proteins are denatured less than at higher temperatures (Giroux et al., 2008). According to this, the obtained results may suggest that in HTST processing, oxidative reactions leads to isomerization of linoleic acid into CLA isomers, while at sterilization temperatures, denatured proteins acting as H^+ proton donors are responsible for the sigmatropic rearrangement of the CLA fraction. It is stated that oxidation of thiol groups from proteins to produce thiyl radicals can lead to the formation of *trans* double bonds in FA (Geißler et al., 2003).

CONCLUSIONS

The results of the present study clearly showed a differential effect of processing on the CLA fraction of dairy fat. Although total CLA concentration increased in the elaboration of HTST milk, sterilization processing led to alterations in the distribution of these fatty acids, leading to higher concentrations of C18:2 t9t11 resulting from a possible sigmatropic rearrangement of RA. The PUFA concentration of the milk affects the extent of those changes.

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