Hot Topic: Fatty Acid and Conjugated Linoleic Acid (CLA) Isomer Composition of Commercial CLA-Fortified Dairy Products: Evaluation After Processing and Storage

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ABSTRACT

Conjugated linoleic acid (CLA) exerts a strong positive influence on human health but intake of these fatty acids is typically too low, and increased consumption of CLA is recommended. A good way to raise the CLA content in the diet without a radical change in eating habits seems to be the enrichment of commonly consumed food products with CLA supplements. This study analyzed the total fatty acid content and the CLA isomer composition of 6 commercially available CLA-fortified dairy products during processing and 10 wk of refrigerated storage. Research was carried out by combining gas chromatography and silver-ion HPLC. The tested samples were a CLA oil supplement, and several skim milk dairy products fortified with the supplement (milk, milk powder, fermented milk, yogurt, fresh cheese, and milk-juice blend). The CLA oil supplement was added such that the consumer received 2.4 g/d of CLA by consuming 2 servings. The predominant isomers present, C18:2 *cis*-9, *trans*-11 CLA and C18:2 *cis*-10, *trans*-12 CLA, were in at a similar ratio, which ranged from 0.97 to 1.05. These major isomers were not significantly affected by processing but a decrease in total CLA in fresh cheese samples was detected after 10 wk of refrigerated storage. Refrigerated storage and thermal treatment resulted in significant decreases or disappearance of some of the minor CLA isomers and a significant increase of *trans*, *trans* isomers from both *cis*, *trans*, *trans*, *cis*, and *cis*, *cis* isomers especially in CLA-fortified milk powder but also in fermented milk, yogurt, and milk-juice blend.

Key words: conjugated linoleic acid, milk product, fatty acid composition

INTRODUCTION

The generic name “conjugated linoleic acid” (CLA) is a collective term embracing all octadecadienoic acids

(C18:2) with a conjugated double bond system in the 7–9, 8–10, 9–11, 10–12, 11–13, and 12–14 positions and a *cis*, *cis*, *cis*, *trans*, *trans*, *cis*, and *trans*, *trans* geometrical configuration. Recently, evidence has suggested that individual CLA isomers might act differently in biological systems and contribute in different ways in their beneficial or potential side effects (Belury, 2002a; Khanal, 2004; Pariza, 2004; Parodi, 2004; Terpsstra, 2004). Data from animal models have been used to suggest that the CLA isomer C18:2 *cis*-9, *trans*-11, also known as rumenic acid (**RA**), is responsible for the anticarcinogenic properties of CLA, as well as growth-promoting and antiatherogenic effects (Ip et al., 1994, 1996, 2002; Belury, 2002b; Masso-Welch, et al., 2002, 2004), whereas the C18:2 *cis*-10, *trans*-12 isomer is responsible for the observed weight loss and muscle-mass enhancement effects (Gaulhier et al., 2004; Malpuech-Brugere et al., 2004).

The advantageous nutritional properties and benefits associated with CLA have important implications for food industries whose challenge is the production of functional foods with high health-promoting capacities.

Most full-fat dairy products contain CLA in quantities varying from 6 to 16 mg/g of total fat content, with lesser amounts in meat (Parodi, 1977), 85 to 95% of which is present as the C18:2 *cis*-9, *trans*-11 isomer. Therefore, estimates of CLA daily intake from food sources range from 150 to 212 mg/d (McGuire et al., 1997) or from 300 mg to 1.5 g (Fritsche et al., 1999) although actual intake appears to be dependent on gender and intake of food from animal or vegetable origins. Ip et al. (1994) estimated that a 70-kg human should consume 3.0 g of CLA/d to achieve maximum health benefits. Similarly, CLA supplementation in overweight subjects after weight loss seems to aid the regain of fat-free mass at experimental doses of 1.8 and 3.6 g/d (Kamphuis et al., 2003). Nevertheless, the extrapolation of CLA effects observed in animals to the human situation should be made with caution.

There are different approaches to increasing the human dietary intake of CLA isomers from food. One is to modify the feeding diets of ruminants with supplements

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rich in polyunsaturated fatty acids (**PUFA**) that provide lipid substrates for the production of *cis*-9, *trans*-11 C18:2 or *trans*-11 C18:1 (*trans*-vaccenic acid; Stanton et al., 2003; Khanal and Olson, 2004; Luna et al., 2005b). This strategy has proved to be effective but the concentration of CLA in the milk that is richest in CLA is low compared with other commercial sources of CLA, such as CLA capsules or CLA-fortified dairy products, that provide an additional oral source of CLA to supplement the human diet and complement the CLA amount contained in foods.

The interest in CLA as a nutritional supplement is high and different products are now offered commercially (Sæbø, 2003). Various methods are available to produce synthetic CLA but alkaline isomerization of linoleic acid is the most common (Villeneuve et al., 2005). These commercial supplements contain 50 to 80% CLA and correspond to a complex mixture of isomers, with the C18:2 *cis*-9, *trans*-11 and C18:2 *cis*-10, *trans*-12 isomers being the most abundant, accounting for approximately 90% at a 1:1 level, with the remaining isomers consisting of all *cis*- and all *trans*-isomers of 9,11-, 10,12-, and 11,13-C18:2 (Ma et al., 1999).

Studies of CLA-fortified products, including their behavior during production and storage, would support the development of consumer-acceptable strategies and processing systems to produce CLA-enriched products and enhanced dairy foods of proven quality.

Although Campbell et al. (2003) prepared and compared 3 samples of fluid milk containing 2% total fat (2% milk fat; 1% CLA oil:1% milk fat; and 2% CLA oil) on the sensory, chemical, and physical characteristics, the total fatty acid compositions, including CLA and CLA isomer distribution, have not been examined in commercially available CLA-fortified dairy products.

In the present study, a CLA oil supplement and commercial CLA-fortified dairy products were analyzed for CLA isomers and total fatty acid composition using a gas chromatographic method combined with a silver ion (Ag^+)-HPLC method. To our knowledge, this study demonstrated for the first time significant differences among commercially available CLA-fortified dairy products and their evaluation during processing and storage.

The results of the effect of processing conditions, storage, and aging on the CLA content of various types of dairy products are unclear. With regard to cheeses, reports and reviews present results for individual varieties, often in the belief that CLA levels may vary due to different processing conditions. Herzallah et al. (2005) reported CLA decreases of 21 and 53% in cheeses heated in a microwave oven for 5 and 10 min, respectively. Nevertheless, these effects are likely to be small, and variations in CLA levels are similar to the levels in the

starting milk (Shantha et al., 1995; Dhiman et al., 1999; Gnädig and Sébédio, 2002; Luna et al., 2005c). However, other studies detected new CLA isomers in ripened cheeses (Werner et al., 1992; Lavillonière et al., 1998; Sehat et al., 1998) and it was hypothesized that biohydrogenation of linolenic acid in cheese could lead to the formation of CLA isomers as intermediates.

In this article, industrial-scale production of milk products enriched with a commercial CLA oil supplement was conducted. Fatty acid composition and CLA isomer profiles were determined to investigate their development during processing and 10 wk of storage. Our aim was to determine whether there were any compositional differences in these products containing high concentrations of healthy fatty acids.

MATERIALS AND METHODS

Samples and Standards

Five commercially available CLA-fortified dairy products in Spain (milk, fermented milk, yogurt, fresh cheese, milk-juice blend) and a CLA-fortified milk powder (noncommercial) were shipped from the manufacturer (Capsa Inc., Oviedo, Spain) to the laboratory in isothermal containers at 4°C. Samples were maintained at this temperature until fat extraction was carried out. Each product consisted of a different concentration of a CLA oil added as a supplement such that the consumer received 2.4 g/d of CLA when consuming the recommended 2 servings (Table 1). Skim milk with CLA oil added was dual-homogenized ($20,000 \pm 1,000$ kPa) and treated by an indirect UHT process at 142°C for 6 s. Milk powder was obtained after atomization of the CLA-enriched milk sample. Fermented milk and yogurt with CLA oil added were dual-homogenized and HTST-pasteurized at 95°C, and traditional yogurt cultures (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*) were added. Fresh cheese was made from skim milk with CLA oil, salt, and rennet; no starter was added. Milk-juice blend (20% fruit from concentrate) was made from skim milk with CLA oil added, pasteurized at 100°C for 30 s, and single-homogenized. A sample of the CLA oil (Tonalin-80, Cognis, Düsseldorf, Germany) used to fortify the milk products was also donated by the manufacturer and analyzed. A total of 9 samples from each product (3 samples at 3 times: 1, 5 and 10 wk of storage at 4°C) were analyzed except for the CLA oil supplement and milk powder, for which storage studies were not carried out.

Pure and mixed CLA isomer methyl esters (C18:2 *cis*-9, *trans*-11 CLA and C18:2 *cis*-10, *trans*-12 CLA) were purchased from Nu-Chek Prep (Elysian, MN). For quantitative determinations of total fatty acid methyl esters (**FAME**), an anhydrous milk fat (reference mate-

Table 1. Product information of the different fortified dairy products studied

Component	Product					
	Milk	Milk powder	Fermented milk	Yogurt	Cheese	Milk-juice blend
pH	6.7	6.7	4.0	4.6	6.5	3.7
Fat, %	1.0	10	2	1.6	1.5	0.8
Protein, %	3.2	32	2.6	5.0	13.4	0.3
Tonalin, ¹ %	0.6	6	1.5	1.2	1.2	0.6
Starter culture	No	No	Yes	Yes	No	No
Serving size, g	250	25	100	120	125	250
CLA ² per serving, g	1.2	1.2	1.2	1.2	1.2	1.2

¹Tonalin-80, Capsa Inc., Oviedo, Spain; a CLA-supplemented oil.²Conjugated linoleic acid.

rial BCR-164; EU Commission; Brussels, Belgium, purchased from Fedelco Inc., Madrid, Spain) was used. An internal standard (12.4 mg/mL of C13:0 as triacylglyceride; Sigma, St. Louis, MO) was also used.

Lipid Extraction and Fatty Acid Derivatization

Milk fat extraction was carried out according to standard methods (ISO-IDF, 2001). The fat residue extracted was stored frozen at -20°C until analysis. Fatty acid methyl esters were prepared by base-catalyzed methanolysis of the glycerides (2 N KOH in methanol) according to standard methods (ISO-IDF, 2002).

Gas Chromatography–Flame-Ionization Detection Analyses

Fatty acid methyl esters were analyzed on a Perkin-Elmer chromatograph (model Autosystem, Beaconsfield, UK) with a flame-ionization detector (FID). Fatty acid methyl esters were separated using a fused-silica capillary column (100 m $\times$ 0.25 mm i.d. $\times$ 0.2 μm film thickness, CP-Sil 88, Chrompack, Middelburg, the Netherlands). The column was held at 100°C for 1 min after injection, then the temperature was increased at $7^{\circ}\text{C}/\text{min}$ to 170°C , held for 55 min, then 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 (30 Psig) and a split ratio of 1:20. The injection volume was 0.5 μL . The CLA isomers were determined and identified by GC-FID by comparing the supplement CLA oil and CLA standards, in accordance with our laboratory's previous studies (Luna et al., 2005a).

Silver Ion-HPLC

Silver ion (Ag^+)-HPLC separation of CLA methyl esters was carried out using an HPLC (Shimadzu Vp Series, Duisburg, Germany) equipped with a UV detector operated at 233 nm. Fatty acid methyl esters were

separated using an analytical column (4.6 mm i.d. $\times$ 250 mm stainless steel; 5 μm particle size; ChromSpher 5 Lipid column, Varian-Chrompack Int., Middelburg, the Netherlands). The mobile phase was 0.1% acetonitrile in hexane, operated isocratically at a flow rate of 1.0 mL/min. The flow was initiated 0.5 h before the sample injection and the injection volume was 10 μL . Pure and mixed CLA FAME isomers from Nu-Chek Prep were used as standards.

Statistical Analysis

Data were analyzed using the ANOVA procedure of the SPSS package (SPSS 11.0 for Windows, SPSS Inc., Chicago, IL). Multiple range tests were applied to determine significance between different treatments.

RESULTS AND DISCUSSION

FAME Composition of CLA-Enriched Dairy Products by GC-FID

Table 2 shows FAME percentages in the CLA oil sample (Tonalin) and in 6 CLA-enriched products after 1, 5, and 10 wk of refrigerated storage. As expected, commercial CLA oil supplements typically comprised 2 major isomers: C18:2 *cis*-9, *trans*-11 CLA and C18:2 *cis*-10, *trans*-12 CLA. Minor CLA isomers detected by GC-FID (Figure 1) included *cis*-11, *trans*-13; *cis*-9, *cis*-11; and *cis*-10, *cis*-12. The *trans*-8, *cis*-10 isomer is included in the *cis*-9, *trans*-11 peak. Other minor CLA isomers in the chromatographic area studied included an overlapping of at least 3 *trans/trans* isomers that eluted as a single peak (*trans*-8, *trans*-10; *trans*-9, *trans*-11; and *trans*-10, *trans*-12).

The main differences in the FAME profiles between the analyzed products were related to their CLA content. The CLA oil supplement contained around 80% of total CLA whereas the supplemented samples contained in the range of 50 to 75% of total CLA, due to

Table 2. Fatty acid composition of conjugated linoleic acid (CLA) oil supplement and CLA-fortified products, after 1, 5, and 10 wk of refrigerated storage

Sample ¹ (wk of storage)	Fatty acids, ² % of total fatty acids									
	C16:0	C18:0	C18:1 c9	C18:2 c9, c12	C18:2 c9, t11	C18:2 t10, c12	SFA	MUFA	PUFA	CLA
CLA oil supplement	0.20 ± 0.02	2.54 ± 0.12	13.37 ± 0.57	0.43 ± 0.06	39.65 ± 0.76	38.12 ± 0.76	2.82 ± 0.13	14.12 ± 0.79	82.37 ± 1.07	81.27 ± 1.04
Milk powder	7.37 ± 0.26	4.14 ± 0.15	13.21 ± 0.45	0.47 ± 0.07	31.11 ± 0.79	32.03 ± 0.69	18.17 ± 0.54	15.33 ± 0.69	66.12 ± 0.94	64.71 ± 0.84
Milk										
1	11.68 ± 0.61	5.46 ± 0.21	17.52 ± 0.67	1.31 ± 0.17	24.61 ± 1.83	23.55 ± 1.14	26.61 ± 1.85	20.37 ± 1.31	52.41 ± 3.32	49.91 ± 0.77
5	12.14 ± 0.76	5.24 ± 0.35	15.80 ± 0.94	1.32 ± 0.25	24.45 ± 0.73	24.05 ± 0.67	26.51 ± 1.15	18.58 ± 1.47	53.70 ± 1.53	50.70 ± 0.52
10	11.87 ± 0.93	5.15 ± 0.14	16.80 ± 0.49	1.39 ± 0.18	24.01 ± 0.56	23.50 ± 0.47	26.09 ± 0.73	19.88 ± 1.37	52.71 ± 1.41	49.69 ± 0.85
Fermented milk										
1	1.62 ± 0.17	2.54 ± 0.20	14.55 ± 0.48	0.35 ± 0.02	38.03 ± 0.74	37.75 ± 0.39	4.98 ± 0.17	15.51 ± 0.59	79.01 ± 0.66	77.34 ± 0.99
5	1.63 ± 0.12	2.33 ± 0.22	14.62 ± 0.31	0.36 ± 0.04	37.90 ± 0.67	37.42 ± 0.57	4.76 ± 0.14	15.65 ± 0.44	78.94 ± 0.58	77.19 ± 0.89
10	1.75 ± 0.19	2.44 ± 0.18	14.74 ± 0.53	0.37 ± 0.03	37.21 ± 0.86	37.00 ± 0.35	5.08 ± 0.21	15.86 ± 0.58	78.19 ± 0.82	76.52 ± 0.76
Yogurt										
1	5.96 ± 0.83	3.79 ± 0.34	16.18 ± 0.52	0.76 ± 0.15	32.10 ± 1.00	31.11 ± 1.61	14.24 ± 0.93	17.80 ± 0.51	67.34 ± 2.07	65.22 ± 2.07
5	5.27 ± 0.56	3.45 ± 0.50	15.68 ± 1.69	0.68 ± 0.13	32.68 ± 2.10	32.28 ± 0.71	13.60 ± 0.68	17.46 ± 1.73	68.60 ± 1.17	66.58 ± 1.30
10	6.08 ± 0.50	3.77 ± 0.66	15.36 ± 1.86	0.77 ± 0.21	32.12 ± 3.20	31.28 ± 0.81	14.66 ± 0.44	17.17 ± 1.15	67.42 ± 1.27	65.28 ± 1.54
Cheese										
1	1.82 ± 0.12 ^a	2.82 ± 0.16 ^a	16.49 ± 1.01	0.63 ± 0.16	36.81 ± 0.95	34.79 ± 1.43	5.48 ± 0.38 ^a	17.58 ± 1.16	76.57 ± 0.82 ^a	75.34 ± 0.92 ^a
5	3.78 ± 0.19 ^b	3.78 ± 0.29 ^b	15.06 ± 1.31	0.62 ± 0.12	36.73 ± 1.30	34.14 ± 1.22	7.48 ± 0.47 ^b	16.29 ± 1.19	74.82 ± 0.89 ^a	74.41 ± 1.53 ^a
10	3.30 ± 0.12 ^b	3.42 ± 0.13 ^b	16.47 ± 0.73	0.82 ± 0.21	34.59 ± 1.47	32.86 ± 1.15	8.42 ± 0.54 ^b	18.01 ± 1.14	72.32 ± 1.33 ^b	70.85 ± 1.26 ^b
Milk-juice blend										
1	2.37 ± 0.22	5.02 ± 0.45	13.71 ± 0.81	0.37 ± 0.11	37.50 ± 0.94	36.83 ± 0.96	8.08 ± 0.54	14.46 ± 1.44	77.46 ± 1.21	76.22 ± 1.24
5	2.69 ± 0.52	3.98 ± 0.50	12.25 ± 1.04	0.30 ± 0.15	39.14 ± 1.08	37.69 ± 1.15	7.85 ± 0.26	12.94 ± 1.10	78.83 ± 1.10	78.03 ± 1.37
10	2.42 ± 0.15	4.89 ± 0.53	14.03 ± 0.84	0.40 ± 0.07	36.72 ± 1.29	36.71 ± 0.51	8.36 ± 0.36	14.92 ± 0.63	76.69 ± 1.32	75.61 ± 1.31

^{a,b}Means within a product and column with different superscripts differ ($P < 0.05$).¹The CLA oil supplement and milk powder were not evaluated over the storage period.²c = *cis*; t = *trans*; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids.

Figure 1. Fatty acid methyl ester profile by gas chromatography with flame-ionization detection of conjugated linoleic acid (CLA) oil used as a supplement to fortify the studied dairy products and profile of the standard CLA methyl ester mixture (Nu-Chek Prep Inc., Elysian, MN). c = *cis*, t = *trans*.

the presence of individual non-CLA fatty acids. Thus, the lower occurrence of CLA in some of the samples, such as milk and yogurt (50 and 65%, respectively) was correlated with the higher presence of milk fat fatty acids. The percentage of saturated fatty acids (SFA) in milk and yogurt (26 and 14%, respectively), and of short- and medium-chain fatty acids (2.5 and 6.9% in milk; 1.2 and 3.2% in yogurt, respectively) was high, which is related to the presence of milk fat in the products. The occurrence of milk fat fatty acids in some of the studied products (especially in milk) was higher than would be expected in a skim milk.

The high ratio of SFA to PUFA in dairy fats is undesirable from a nutritional perspective due to the link between saturated fats and increased levels of serum cholesterol and heart disease. Nevertheless, in all of the products studied, C18:2 *cis*-9, *trans*-11 and C18:2 *cis*-10, *trans*-12 CLA were the predominant fatty acids present. All samples had a similar ratio of C18:2 *cis*-9, *trans*-11 to *trans*-10, *cis*-12 CLA, which ranged from 0.97 to 1.05. Moreover, in these studied samples, the ratio of CLA to PUFA was greater than 0.95 in all cases. In addition to CLA, oleic acid (C18:1 *cis*-9) was the most abundant fatty acid (around 15%) and linoleic acid (C18:2 *cis*-9, *cis*-12) was also present, giving all of the studied dairy products a balanced SFA to PUFA ratio.

As mentioned earlier, other minor CLA isomers did not occur as pure chromatographic peaks but were overlapped as indicated by a single peak (*trans*-8, *trans*-10; *trans*-9, *trans*-11; and *trans*-10, *trans*-12; Figure 1).

These compounds were detected in all products at levels ranging from 1.6 to 3.5%.

Effects of Processing and Refrigerated Storage of Fatty Acid Composition by GC

Due to the existence of variable amounts of milk fat present in the CLA-fortified samples, comparisons of the fatty acid content between CLA oil and CLA-enriched samples immediately after preparation and treatment could not be determined by GC analysis. Instead, it was studied by Ag⁺-HPLC of the total CLA fraction and individual isomers as discussed below.

Results obtained throughout the refrigerated storage of CLA-fortified dairy products for 1, 5, and 10 wk showed that significant differences were found only in the fresh cheese sample. Both C18:2 *cis*-9, *trans*-11 CLA and C18:2 *cis*-10, *trans*-12 CLA isomers decreased but not significantly after 5 wk of storage; subsequently, a significant loss of the total CLA and total PUFA fraction occurred within the same period of storage (Table 2). Campbell et al. (2003) found a significant loss of C18:2 *cis*-9, *trans*-11 CLA after HTST pasteurization of 2% CLA-fortified skim milk. The same authors reported a significant decrease of C18:2 *cis*-9, *trans*-11 after 3 wk of refrigerated storage compared with the levels of this isomer at 1 and 2 wk of storage. These reductions of C18:2 *cis*-9, *trans*-11 were attributed to the heat processing and to excessive microbial growth during the storage of the milk samples.

In the fermented milk studied in this work (a CLA-enriched sample with yogurt culture), declines of C18:2 *cis*-9, *trans*-11 and C18:2 *trans*-10, *cis*-12 CLA were also detected after wk 5 of storage, but were not statistically significant. Nevertheless, no similar decreases were found in yogurt. No change was observed in milk or milk-juice blend samples during storage. Xu et al. (2005) demonstrated that the combination of most probiotic bacteria with the yogurt cultures produced slightly higher contents of C18:2 *cis*-9, *trans*-11 and C18:2 *trans*-10, *cis*-12 CLA, but it did not occur in yogurt culture alone (i.e., without probiotic bacteria) after 14 d of storage.

CLA Isomer Composition of CLA-Enriched Products by Ag⁺-HPLC

The use of Ag⁺-HPLC is currently the most effective way of separating and quantifying CLA isomers. Conjugated linoleic acid FAME are selectively detected by their characteristic UV absorbance at 233 nm; nonconjugated FAME respond poorly at this wavelength. There are about 20 different CLA isomers in natural milk fat based on Ag⁺-HPLC separation (Sehat et al., 1998).

Table 3. Conjugated linoleic acid (CLA) isomer composition of CLA oil supplement and CLA-enriched products after 1, 5, and 10 wk of storage

Sample (wk of storage)	CLA isomer composition, ¹ % of total CLA							
	t11, t13	t10, t12	c11, t13	t10, c12	c9, t11	t8, c10	c9, c11	c10, c12
CLA oil supplement	0.56 ± 0.06 ^x	0.49 ± 0.05 ^x	1.65 ± 0.23 ^x	45.74 ± 1.84	48.57 ± 0.61	1.76 ± 0.32 ^x	0.54 ± 0.16	0.55 ± 0.08
Milk powder	0.99 ± 0.09 ^y	0.89 ± 0.07 ^y	0.24 ± 0.04 ^y	47.81 ± 1.14	48.48 ± 0.45	0.31 ± 0.18 ^y	0.59 ± 0.08	0.57 ± 0.07
Milk								
1	0.65 ± 0.12	0.63 ± 0.12	1.49 ± 0.28	44.96 ± 1.85	49.46 ± 1.88	1.65 ± 0.28	0.51 ± 0.15	0.52 ± 0.10
5	1.10 ± 0.45	1.04 ± 0.49	1.64 ± 0.15	46.12 ± 1.57	47.28 ± 1.31	1.49 ± 0.52	0.43 ± 0.09	0.58 ± 0.05
10	1.08 ± 0.45	1.03 ± 0.46	1.37 ± 0.11	47.58 ± 1.70	45.91 ± 1.67	1.82 ± 0.13	0.23 ± 0.08	0.53 ± 0.04
Fermented milk								
1	0.43 ± 0.06	0.38 ± 0.05	0.11 ± 0.12 ^y	49.45 ± 1.82	48.35 ± 1.90	0.25 ± 0.03 ^y	0.50 ± 0.06	0.51 ± 0.05
5	0.43 ± 0.06	0.37 ± 0.04	0.29 ± 0.10	47.82 ± 1.42	49.82 ± 1.82	0.24 ± 0.04	0.50 ± 0.05	0.51 ± 0.04
10	0.45 ± 0.04	0.41 ± 0.03	0.34 ± 0.20	47.78 ± 1.02	49.11 ± 1.75	0.27 ± 0.05	0.45 ± 0.08	0.45 ± 0.07
Yogurt								
1	0.58 ± 0.20	0.54 ± 0.11	ND	46.18 ± 1.07	49.30 ± 0.64	1.11 ± 0.15	0.48 ± 0.25	0.61 ± 0.13
5	0.44 ± 0.17	0.39 ± 0.17	ND	48.51 ± 1.47	48.72 ± 0.46	1.23 ± 0.11	0.47 ± 0.18	0.51 ± 0.07
10	0.55 ± 0.14	0.48 ± 0.13	ND	46.77 ± 1.87	49.31 ± 0.69	1.05 ± 0.15	0.39 ± 0.10	0.49 ± 0.14
Cheese								
1	0.53 ± 0.06	0.46 ± 0.10	2.38 ± 0.27 ^a	44.01 ± 0.70	49.09 ± 0.49	2.33 ± 0.30	0.56 ± 0.15	0.61 ± 0.09
5	0.69 ± 0.05	0.67 ± 0.05	1.38 ± 0.21 ^b	47.03 ± 0.51	48.49 ± 0.66	1.75 ± 0.26	ND	ND
10	0.70 ± 0.08	0.62 ± 0.14	1.42 ± 0.14 ^b	46.88 ± 0.89	48.48 ± 0.53	1.85 ± 0.15	ND	ND
Milk-juice blend								
1	0.52 ± 0.04	0.47 ± 0.04	ND	47.40 ± 1.27	49.80 ± 2.04	0.55 ± 0.15 ^y	0.61 ± 0.18	0.58 ± 0.14
5	0.49 ± 0.05	0.44 ± 0.04	ND	46.67 ± 1.52	52.20 ± 1.16	0.37 ± 0.06	ND	ND
10	0.50 ± 0.06	0.46 ± 0.04	ND	46.93 ± 1.03	52.08 ± 1.92	ND	ND	ND

^{x,y}Means within CLA oil supplement and a wk 1 storage product with different superscripts differ ($P < 0.05$); differences by industrial treatment.

^{a,b}Means within a product and column with different superscripts differ ($P < 0.05$); differences by refrigeration storage.

¹c = *cis*; t = *trans*; ND = not determined.

The CLA isomer compositions (% of total CLA) of the 6 CLA-enriched products studied after 1, 5, and 10 wk of storage are shown in Table 3. To determine the possible variations in CLA isomer composition during the processing of the CLA-enriched products, CLA-supplemented oil was used as a control during the first week of storage of the different products. Silver ion-HPLC identification of more than 10 different peaks attributed to CLA isomers was based on coinjection with reference material because of retention time irreproducibility and in accordance with the elution order, as we reported in previous studies with the same chromatographic conditions (Luna et al., 2005a). The Ag⁺-HPLC profile of lipid sample was shown to separate the different C18:2 *trans*, *trans* compounds followed by a chromatographic zone where *cis*, *trans* and *trans*, *cis* isomers were located and finally the *cis*, *cis* CLA isomers eluted. The most prominent peaks in all products corresponded, as expected, to C18:2 *cis*-9, *trans*-11 and *trans*-10, *cis*-12 CLA isomers, which accounted for about 95% of total CLA. All enriched milk products showed a similar ratio of C18:2 *cis*-9, *trans*-11 to *trans*-10, *cis*-12 CLA, which ranged from 0.95 to 0.98.

The CLA isomer percentages of the enriched products studied differed substantially from proportions reported for total CLA fatty acids in cow's milk fat, in which most of the CLA corresponds to RA (about 80%)

and which would be a poor source of the C18:2 *trans*-10, *cis*-12 CLA isomer (Sehat et al., 1998). Similarly, the C18:2 *trans*-7, *cis*-9 CLA isomer is the second greatest in cow's milk fat (5 to 10% of total CLA), whereas its presence in the products of the current study seemed to be negligible.

Noticeable percentages of the isomers C18:2 *cis*-11, *trans*-13 and *trans*-8, *cis*-10 were found in the fortified products (1.8 and 1.7%, respectively, in the CLA-supplemented oil and up to 2.4% in CLA-enriched cheese). Amounts of 4.2% of C18:2 *cis*-11, *trans*-13 and lower than 1% of C18:2 *trans*-8, *cis*-10 of total CLA were reported by Luna et al. (2005a) in ewe's milk.

Another 4 peaks eluting in the C18:2 *trans*, *trans* CLA region were assigned to *trans*-9, *trans*-11; *trans*-10, *trans*-12; *trans*-11, *trans*-13; and *trans*-12, *trans*-14. Two of these isomers (*trans*-9, *trans*-11 and *trans*-12, *trans*-14) were in trace amounts in the CLA oil supplement and not detectable in the analyzed samples, whereas the 2 peaks identified as *trans*-10, *trans*-12 and *trans*-11, *trans*-13 accounted for 0.5 and 0.6% of the CLA-oil supplement, respectively.

In the C18:2 *cis*, *cis* CLA chromatographic area, 2 isomers with comparable amounts (0.6%) were detected in the CLA oil and assigned to *cis*-9, *cis*-11 and *cis*-10, *cis*-12. These isomers were not detected in different dairy foods when CLA profiles were researched using

Ag⁺-HPLC in similar conditions (Sehat et al., 1999; Luna et al., 2005a).

Effects of Processing and Storage on CLA Isomers by Ag⁺-HPLC

The important quality issues for CLA-supplemented products are total CLA content and isomeric distribution that could be altered by the effect of processing conditions, storage, and aging.

To determine significant variations in CLA isomer composition during processing of the CLA-enriched products, CLA-supplemented oil was used as a control in the first week of storage of the different products. The CLA isomer contents as a percentage of total CLA are shown in Table 3. No significant changes were found in the content of the major isomers (C18:2 *cis*-9, *trans*-11 and C18:2 *trans*-10, *cis*-12 CLA) as a consequence of treatment or refrigerated storage up to 10 wk in any of the CLA-enriched dairy products studied. Nevertheless, this study showed that the industrial process of the product or thermal treatment resulted in a significant decrease of some of the minor CLA isomers such as *cis*-11, *trans*-13 and *trans*-8, *cis*-10 in milk powder, fermented milk, yogurt, and milk-juice blend.

In the CLA-enriched fresh cheese studied, the formation of new isomers was not found, but a significant decrease of the minor isomer C18:2 *cis*-11, *trans*-13 during refrigerated storage, and even the disappearance of some other C18:2 *cis*, *cis* isomers as *cis*-9, *cis*-11 and *cis*-10, *cis*-12, was observed. Decreases in these minor isomers resulted in undetectable levels in other samples, such as the milk-juice blend.

Isomer contents in the C18:2 *trans*, *trans* region (mainly *trans*-9, *trans*-11 and *trans*-10, *trans*-12 isomers) were found to be low in all CLA-enriched products except for milk powder, in which levels increased significantly after the thermal process. The level of C18:2 *trans*, *trans* isomers was also positively influenced in milk after 5 wk of storage but not significantly. The increase in the content of C18:2 *trans*, *trans* isomers has been related to lipid transformation by heat treatment (Precht et al., 1999; Juanéda et al., 2003; Herzallah et al., 2005). It has been reported that the *trans* double bond is more stable compared with the *cis* bond in the same environmental conditions, and that the level of *trans*/*trans*-CLA may be elevated by reaction conditions that favor thermodynamic products such as a higher reaction temperature and longer reaction time (Bruce and Lightstone, 1999). Destailats et al. (2005) found that severe thermal processes result in sigmatropic isomerization of RA resulting in the formation of C18:2 *trans*-8, *cis*-10, which can be used as a marker of heat treatment of natural fats and oils containing CLA. Sig-

matropic rearrangement has been also described between the isomers C18:2 *trans*-10, *cis*-12 and *cis*-11, *trans*-13, and between C18:2 *cis*-9, *trans*-11 and *trans*-8, *cis*-10, with a significant increase of C18:2 *trans*, *trans* isomers from *cis*, *trans*; *trans*, *cis*; and *cis*, *cis* isomers.

In summary, this study indicates that the total CLA content of different commercially available CLA-enriched dairy products (supplemented using Tonalin-80, an oil supplement with 80% CLA) varied considerably from 50 to 75% depending on the presence of milk fat in the products. The CLA isomers C18:2 *cis*-9, *trans*-11 and C18:2 *trans*-10, *cis*-12 were the predominant fatty acids present in all products, at a ratio ranging from 0.97 to 1.05. These major isomers were not affected by the processing used and did not significantly decrease after 10 wk of storage. Only a significant loss of total CLA throughout the refrigerated storage of fresh cheese sample was found, possibly related to an increase in microbiota growth. Nevertheless, refrigerated storage and, particularly, thermal treatment resulted in significant decreases or disappearance of some of the minor CLA isomers and a significant increase of C18:2 *trans*, *trans* isomers from *cis*, *trans*; *trans*, *cis*; and *cis*, *cis* isomers especially in CLA-fortified milk powder but also in fermented milk, yogurt, and milk-juice blend.

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