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Conjugated Linoleic Acid in Processed Cheeses during the Manufacturing Stages

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Conjugated linoleic acid (CLA) is a naturally occurring micronutrient in milk fat and dairy products consisting of a group of geometric and positional isomers. The purpose of this study was to assess the level and type of CLA isomers found in two commercial processed cheeses (portions and slices) as well as to monitor their evolution during the different manufacturing stages. Total CLA concentrations ranged from 7.5 to 7.9 mg/g of fat, and rumenic acid (*cis*-9,*trans*-11 C18:2), the isomer responsible for the biological functions, represented >80% of total CLA. *trans*-11,*cis*-13 and *trans*-11,*trans*-13 were, with ∼4% each, the second main CLA isomers. *trans*−*trans* isomers accounted for <10% of total CLA. The processing parameters used in this research had negligible effects on the CLA content of processed cheese and did not modify the isomer profile in these dairy products, thereby confirming the stability of rumenic acid during manufacturing.

KEYWORDS: Processed cheese; CLA; processing; isomer; GC-MS; Ag^+-HPLC

INTRODUCTION

CLA, the acronym for conjugated linoleic acid, is a collective term used to describe one or more positional and geometric isomers of linoleic acid that have been recognized as anticarcinogens in animal models. Other potential benefits include the action of CLA as an antioxidant, an antiatherosclerosis agent, and an immune system modulator. Of these isomers, *cis*-9,*trans*-11-octadecadienoic acid (also referred to as rumenic acid, RA) is the most common natural form with biological activity. Other physiological effects have also been more recently attributed to *trans*-10,*cis*-12 (*1*) and *cis*-9,*cis*-11 (*2*) isomers. Dairy products and other foods derived from ruminant animals are recognized as major dietary sources of CLA, thereby contributing to the positive nutritional value of these foods. This has led to a series of studies to determine the production of CLA contents (*3*−*6*). The effects of diet, animal, and postharvest factors on CLA concentrations in foods have also been reviewed (*7*−*10*). These studies have reported a significant variability in CLA concentration among and in dairy products.

The effect on CLA content of postharvest-related factors, such as processing conditions, storage, cooking, aging, etc., or conversion one product into another, such as milk into cheese is still, however, controversial. Processed cheese is produced by blending shredded natural cheeses of different types with emulsifying agents and then heating the blend under partial vacuum with constant agitation until a homogeneous mass is obtained. Ha et al. (*11*) reported increased levels of CLA in processed cheeses as compared to natural cheeses. Shanta et al. (*12, 13*) showed that an increase in processing temperature and the addition of whey protein concentrate could increase CLA concentration during the preparation of processed cheese. Yet their papers, along with those by others (*14, 15*), did not determine the CLA concentration in the raw material from which the final products were manufactured in order to directly validate how processing may have influenced CLA concentrations.

Few data are available on CLA in processed cheeses made under controlled processing practices. Most of the works mentioned above did not monitor the evolution of the CLA content during the different processing stages. Furthermore, information on the isomer profile in processed cheese is usually very scant (*11, 16*). In fact, very few researchers have reported CLA isomers other than RA, because the techniques used were unable to detect and identify them with reliability. Moreover, acid methylation procedures, the most commonly used methods in the 1990s, can substantially alter the CLA isomer profile by decreasing the RA contents (*17*−*19*). Some of the previous studies on processed cheeses showed a high content of isomers other than RA, and further research using proper techniques would therefore be needed.

This study deals with the effects of processing during the preparation of processed cheese (portions and slices) at two different temperatures and conditions. The objective of this research was to determine CLA levels and evaluate the isomer profile in processed cheese at various points in the processing line. The newly developed methods reported here combining Ag^+-HPLC columns in series with GC-MS may be used to determine CLA and its isomer forms with reliability at the different stages of processed cheese production.

MATERIALS AND METHODS

Samples. Two types of processed cheeses (portions and slices) were manufactured under normal operation conditions at a Kraft Foods factory (Mahón, Spain). These conditions are described in **Figure 1**.

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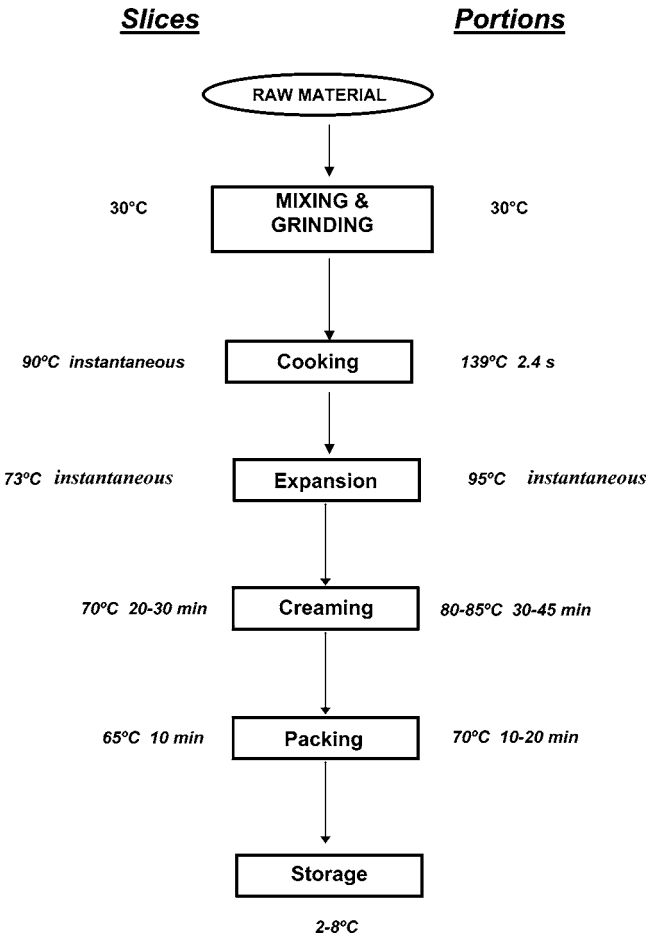

Figure 1. Scheme of the processing line during the production of processed cheeses (portions and slices).

To evaluate the effect of processing on CLA content and profile, aliquots of cheese were collected at different points in the processing line: raw material and after cooking, creaming, and packing. All samples were stored at refrigeration temperature until the fat extraction was performed. The manufacturing process (portions and slices) was done in triplicate from the same raw material (Menorca island cheese made from cow's milk).

Standards and CLA Quantification. A mixture (*cis*-9, *trans*-11, *trans*-8, *cis*-10, *cis*-11, *trans*-13, *trans*-10, *cis*-12 C18:2 and small amounts of a variety of *cis*-*cis* and *trans*-*trans* C18:2 isomers) and pure CLA methyl ester isomers (*cis*-9,*trans*-11 and *trans*-10,*cis*-12 C18:2) were purchased from Nu-Chek Prep. Inc. (Elysian, MN). The areas of the CLA peaks were calculated as milligrams per gram of fat using nonadecanoic acid (C19) as the internal standard. To obtain response factors, an anhydrous milk fat (reference material CRM-164) consisting of known amounts of fatty acids obtained from the European Commission (Brussels, Belgium) was also used.

Lipid Extraction and Fatty Acid Derivatization. Milk fat extraction was carried out according to the ISO method (*20*). The fat residue extracted was stored frozen at −20 °C until analysis. Fatty acid methyl esters (FAME) were prepared by base-catalyzed methanolysis of the glycerides (KOH in methanol) according to the ISO method (*21*). The preparation of dimethyloxazoline (DMOX) derivatives from FAME was based on that of Fay and Richli (*22*).

GC Analysis. FAME were separated using a CP-Sil 88 fused-silica capillary column (100 m × 0.25 mm i.d. × 0.2 μm film thickness, Chrompack, Middelburg, The Netherlands) on a Perkin-Elmer chromatograph (model 8420, Beaconsfield, U.K.) equipped with a flame ionization detector. The column was held at 100 °C for 1 min after injection, temperature-programmed at 7 °C/min to 170 °C, held there for 55 min, then temperature programmed at 10 °C/min to 230 °C, and held there for 23 min. Helium was the carrier gas with a column inlet pressure set at 214 KPa and a splitless injection system. The volume of injection was 1.0 μL.

DMOX derivatives were analyzed on an Agilent chromatograph (model 6890N, Palo Alto, CA) equipped with a mass spectrometry detector. The filament trap current was 400 μA at 70 eV. Injections were under data system control with an autoinjector and a glass split injector insert packed with silanized glass wool. One microliter of solution of DMOX derivatives was separated in the same column in the following conditions: oven temperature was 75 °C for 2 min after injection, then temperature-programmed at 5 °C/min to 180 °C, held there for 30 min, then temperature-programmed at 5 °C/min to 220 °C, and held there for 30 min. The column inlet pressure was set at 197 kPa, and a splitless injection system.

Silver Ion HPLC (Ag^+-HPLC). Ag^+-HPLC separation of CLA methyl esters was carried out using an HPLC (Agilent Technologies, series 1100, Palo Alto, CA) equipped with a photodiode array detector operated at 234 nm. Three ChromSpher 5 Lipids analytical silver-impregnated columns (250 mm × 4.6 mm i.d. stainless steel; 5 μm particle size; Varian) were used in series. The mobile phase was 0.1% acetonitrile in hexane and with an isocratic flow rate of 1.0 mL/min. The flow was initiated 0.5 h prior to sample injection, and the injection volume was 10 μL. All fatty acid analyses by GC and Ag^+-HPLC were carried out in duplicate.

Statistical Analysis. The significance of the differences was evaluated using one-way ANOVA. The Tukey HSD test was used to identify significant differences. Statistical processing was carried out using the SPSS computer program (SPSS Inc., Chicago, IL).

RESULTS AND DISCUSSION

CLA Total Content and Evolution during Processing. Table 1 shows the CLA concentration based on lipid content determined by GC during the different manufacturing stages of processed cheese portions and slices. The total CLA levels ranged from 7.4 to 7.9 mg/g of fat in the different samples analyzed and were similar in both types of processed cheeses. In general, cheeses from cow's milk contain ∼3−6 mg/g CLA in their fat (*23*) and, therefore, the levels found in this research can be considered to be high.

As it can be seen (**Table 1**), no significant differences were observed in total CLA content during the manufacturing of processed cheese slices or portions. These results are apparently in contradiction with earlier research studies in this field. Information on changes in CLA concentration during the processing of these dairy products was published at the beginning of the 1990s by other authors. Ha et al. (*11*) proposed that CLA formation in cheese resulted from the oxidation of linoleic acid by free radicals during processing. Then, the radicals rearrange to form conjugated dienyl structures, which react with protons from hydrogen donors, such as proteins, to form CLA. The heating temperature and protein content were considered to be primarily responsible for accelerating oxidative reactions and were used to explain CLA formation in processed cheeses.

First, it was suggested that CLA is formed on heating during the manufacture of processed cheese. In a comparison of commercially available natural and processed cheeses, Ha et al. (*11*) attributed the higher CLA content in processed cheeses to the additional heat treatment. Their observations were confirmed in a study by Shanta et al. (*12*), where total CLA content increased significantly for processed cheeses heated at 80 and 90 °C, but not at 70 °C. García-López et al. (*16*) reported that heating was the only stage in processing that increased CLA content in processed cheeses. Examining other dairy materials Aneja and Murthi (*24*) also detected a increase of CLA content in ghee samples when butter was clarified at a temperature higher (120 °C) than 110 °C, the temperature traditionally used for making ghee.

Table 1. Mean Content and Standard Deviation (Milligrams per Gram of Fat) of Conjugated Linoleic Acid (CLA) in Processed Cheeses (Portions and Slices) Determined by GC during the Different Processing Stages[c]

CLA isomer	raw material	cooking	creaming	final product
		Portions		
c9,t11 + t7,c9	6.927 ± 0.485[a]	6.566 ± 0.413[a]	6.530 ± 0.108[a]	7.060 ± 0.399[a]
t9,c11	0.039 ± 0.009[a]	0.038 ± 0.007[a]	0.036 ± 0.003[a]	0.037 ± 0.005[a]
t10,c12	0.023 ± 0.004[a]	0.019 ± 0.006[a]	0.019 ± 0.004[a]	0.018 ± 0.003[a]
t11,c13 + c9,c11	0.422 ± 0.023[a]	0.373 ± 0.028[b]	0.352 ± 0.018[b]	0.372 ± 0.008[b]
t12,t14	0.095 ± 0.006[a]	0.087 ± 0.009[a]	0.086 ± 0.011[a]	0.085 ± 0.006[a]
t11,t13	0.229 ± 0.011[a]	0.230 ± 0.022[a]	0.232 ± 0.007[a]	0.233 ± 0.014[a]
t10,t12 + t9,t11 + t8,t10	0.101 ± 0.030[a]	0.103 ± 0.027[a]	0.118 ± 0.012[a]	0.111 ± 0.006[a]
total CLA	7.836 ± 0.373[a]	7.416 ± 0.599[a]	7.373 ± 0.133[a]	7.916 ±0.134[a]
		Slices		
c9,t11 + t7,c9	6.910 ± 0.436[a]	7.004 ± 0.338[a]	6.878 ± 0.356[a]	6.862 ± 0.351[a]
t9,c11	0.037 ± 0.005[a]	0.040 ± 0.010[a]	0.047 ± 0.007[a]	0.043 ± 0.008[a]
t10,c12	0.017 ± 0.003[a]	0.018 ± 0.002[a]	0.024 ± 0.009[a]	0.026 ± 0.012[a]
t11,c13 + c9,c11	0.366 ± 0.034[a]	0.380 ± 0.023[a]	0.389 ± 0.047[a]	0.381 ± 0.027[a]
t12,t14	0.086 ± 0.012[a]	0.087 ± 0.010[a]	0.089 ± 0.011[a]	0.089 ± 0.010[a]
t11,t13	0.274 ± 0.048[a]	0.276 ± 0.038[a]	0.264 ± 0.039[a]	0.280 ± 0.044[a]
t10,t12 + t9,t11 + t8,t10	0.067 ± 0.019[a]	0.069 ± 0.016[a]	0.079 ± 0.020[a]	0.069 ± 0.018[a]
total CLA	7.757 ± 0.495[a]	7.874 ± 0.368[a]	7.770 ± 0.512[a]	7.750 ± 0.072[a]

[a,b] Values in the same row without a common superscript letter are significantly different: $p \leq 0.05$. [c] c, cis; t, trans.

Table 2. Contents (Gram per 100 g) in Protein, Carbohydrates, Lipids, and Calcium in Processed Cheese (Portions and Slices)

	processed cheese	
	portions	slices
proteins	9.2	12.4
carbohydrates	4.5	4.2
lipids	18.5	21.4
calcium	0.289	0.432

However, Chin et al. (*14*), who noted no differences in the CLA content of natural and processed cheeses, suggested that processing parameters other than heat would contribute to CLA formation in processed cheeses. More recent studies (*25*, *26*) support the idea that heating at a high temperature does not raise CLA levels in milk fat. What is more, high-temperature−short-time pasteurization resulted in a significant decline of RA and other minor CLA isomers (*27*), whereas drastic heat treatment, at a higher temperature (200 °C), induced more significant losses of CLA in milk fat (*28*). These detrimental effects caused by heating were not found in this research. Although processing temperatures were more severe in the manufacturing of processed cheese portions (**Figure 1**) than in slices, such differences did not lead to significant changes in CLA levels throughout the production process. Creaming and packing stages at temperatures >70 °C for prolonged periods of time, for instance, hardly modified the amounts of those fatty acids (**Table 1**) during the cheese portion processing chain, thereby confirming how good these treatments are for preserving CLA in processed cheese.

Second, a positive relationship between CLA and protein content was also identified in earlier studies (*6*, *11*, *12*). Proteins, functioning as hydrogen donors, were thought to enhance CLA formation in dairy products. In this study, however, the highest proportion of protein during the manufacturing of processed cheese slices (**Table 2**) did not improve CLA levels. These results could be justified by taking into account the following considerations. Oxidative reactions could also cause destruction of the conjugated double-bond systems, thus potentially causing destruction of CLA. The addition of the whey protein concentrate (WPC) at concentrations normally found in processed cheeses resulted in an increase in CLA concentrations, but only when the fraction of low molecular weight was incorporated as an ingredient. The high molecular weight fraction of WPC did not increase the CLA concentration (*12*). Furthermore, in other works (*13*) air was also incorporated into ice cream during processing, and no increases in CLA were observed.

Linoleic acid content found in raw material did not alter during the manufacturing of processed cheese (data not shown). No changes during the different processing stages would be evidence that the assayed conditions do not favor CLA formation from linoleic acid. If oxidation reactions took place, decreases in linoleic acid should be observed during processing. Then, under the conditions (time and temperature) used in this study, the mechanism of CLA formation during the manufacturing of processed cheese based on linoleic acid oxidation would not be confirmed. It should also be remembered that some of the changes reported in previous studies in CLA levels that were claimed to be due to processing variables were often less than the expected measurement error. Even if such changes were real, they appear to be insignificant against the large variations due to feeding regimens and individual physiological factors regulating CLA synthesis (*7*, *9*).

CLA Isomer Profile. A partial GC-MS chromatogram of the CLA region from processed cheese fat FAME is shown in **Figure 2**. Some isomers were identified on the basis of FAME retention times and were compared with a commercial CLA methyl ester standard mixture separated in the same conditions. The elution order of the CLA isomers using the 100 m CP Sil88 column is well established and could also be another very useful tool for identifying CLA isomers: *cis*/*trans* and *trans*/*cis* elute simultaneously followed by *cis*/*cis* and, finally, *trans*/*trans*. Although the standard mixture containing comparable amounts of eight CLA isomers is partially resolved by GC, such separation is not apparent when one isomer such as RA predominates. The biggest peak in the CLA area in the cheese fat chromatogram (**Figure 2**), for instance, would correspond to *cis*-9,*trans*-11 C18:2, but other minor isomers such as 7−9 and 8−10 C18:2 could be masked. RA and *trans*-8,*cis*-10 C18:2 were unresolved, even when both were in similar proportions (standard mixture in **Figure 2**). The use of other chromatographic techniques (GC-MS of CLA DMOX and Ag^+-HPLC of FAME) made it possible to determine 7−9 positional isomers but did not confirm the existence of the 8−10 isomer. Other

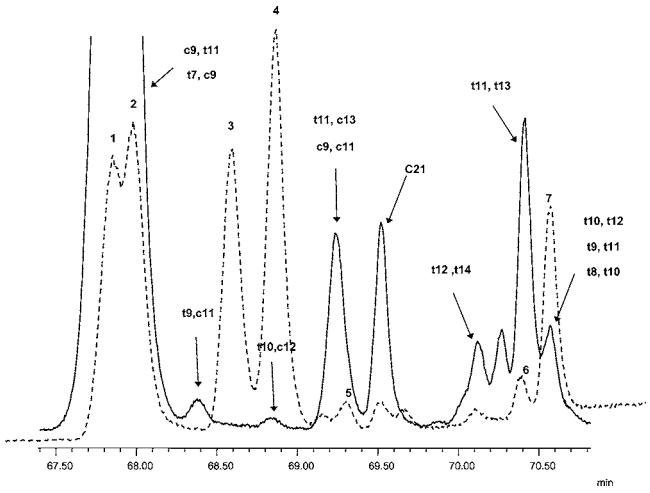

Figure 2. GC-MS (total ion) partial chromatograms showing the profiles of CLA methyl esters from processed cheese fat (solid line) and a standard mixture (Nu-Chek Prep., Inc.) (dotted line): 1, *cis*-9,*trans*-11; 2, *trans*-8,*cis*-10; 3, *cis*-11,*trans*-13; 4, *trans*-10,*cis*-12; 5, *cis*-9,*cis*-11; 6, *trans*-11,*trans*-13; 7, *trans*-8,*trans*-10 + *trans*-9,*trans*-11 + *trans*-10,*trans*-12 (c, *cis*; t, *trans*).

CLA molecules do not occur as pure peaks and overlap, as do *trans*/*trans* isomers (last peak in **Figure 2**). In the *cis*/*cis* area (**Figure 2**), *cis*-9,*cis*-11 C18:2 could not be identified because this isomer was masked by *trans*-11,*cis*-13 C18:2. However, analysis by GC-MS of CLA DMOX supplied the evidence to confirm the existence of traces of this isomer in processed cheeses. Finally, it is remarkable that concentrations of the isomers corresponding to the peaks in **Figure 2** remained unchanged during the manufacturing of cheese slices or portions (**Table 1**), and CLA isomer levels were stable at the different stages monitored.

For most of the peaks described in **Table 1** statistical analysis did not reveal significant differences at the different stages of manufacturing. This would support the idea that the processing parameters used in this research had negligible effects on the CLA content of processed cheese and would not modify the isomer profile in these dairy products.

The complementary use of Ag^+-HPLC is currently the most effective way of separating and quantifying CLA isomers. CLA are selectively detected by their characteristic UV absorbance at 234 nm, and nonconjugated FAME respond poorly at this wavelength. Inspection of **Figure 3** indicates that up to 17 different peaks could be attributed to CLA in processed cheese fat, and 12 of them were identified. Due to retention time irreproducibility, Ag^+-HPLC identification of CLA isomers was based on co-injection with reference material (**Figure 3**) as well as on the elution order previously reported in studies in similar chromatographic conditions.

Ag^+-HPLC made it possible to separate the different *trans*/*trans* compounds (from 12−14 to 7−9 C18:2) followed by a chromatographic zone where *cis*/*trans* and *trans*/*cis* isomers eluted. Although some geometrical isomers were not resolved (*cis*/*trans* from *trans*/*cis*), species differing in positional double bonds eluted separately. The use of three columns in series was sufficient to resolve 7−9, 8−10, 9−11, 10−12, 11−13, and 12−14 C18:2. The most prominent peak corresponds to 9−11 positional isomers. It was preceded by three small peaks assigned to 12−14, 11−13, and 10−12 positional isomers. A tiny shoulder eluting after the 9−11 isomer was attributed to *trans*-9,*cis*-11 C18:2. This was followed by a greater peak corresponding to 7−9 C18:2. The difference in retention time

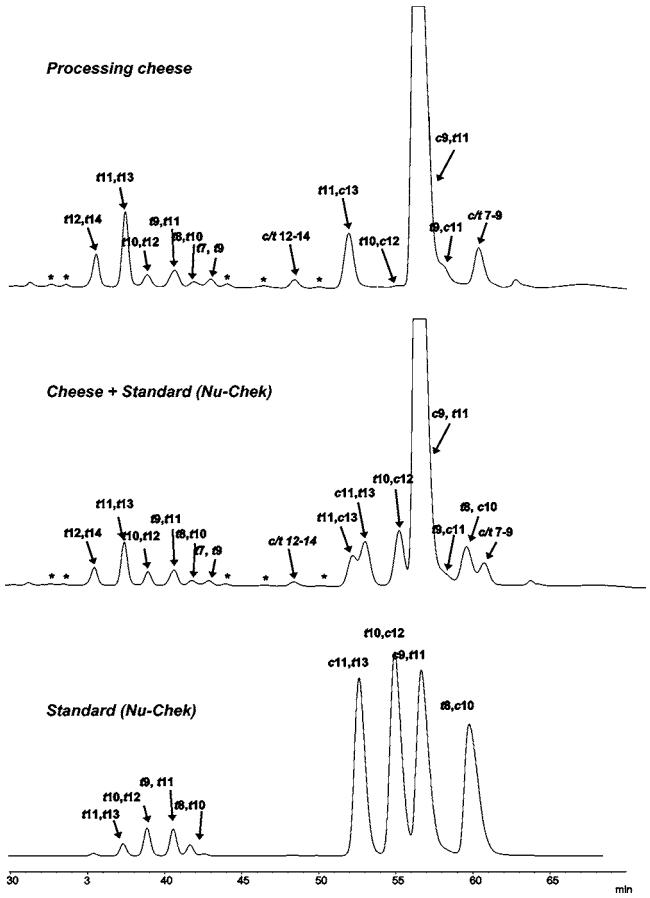

Figure 3. Ag^+-HPLC profiles at 234 nm of methyl esters of CLA isomers from processed cheese fat, a standard mixture (Nu-Chek Prep., Inc.), and a co-injected mixture of processed cheese fat and standard. The asterisks correspond to unidentified peaks (c, *cis*; t, *trans*).

between the 8−10 and 7−9 C18:2 was demonstrated chromatographically by co-injecting a CLA standard mixture added to a processed cheese fat sample (**Figure 3**). With the addition of the standard, 8−10 C18:2 emerged before the 7−9 C18:2 peak as more abundant in the co-injected mixture. Likewise, the co-injection of the CLA standard made it possible to assign the 11−13 C18:2 peak in the processed cheese chromatogram to the *trans*/*cis* geometric isomer.

The evolution of the contents of all these isomers during the manufacturing of processed cheeses (portions and slices) is shown in **Table 3**. Overall, these data confirm that RA is quantitatively the most important CLA isomer in processed cheese. More than 80% of CLA could be attributed to the *cis*-9,*trans*-11 molecule. The second most abundant isomers, with ∼4% of total CLA, were *trans*-11,*cis*-13 and *trans*-11,*trans*-13 in processed cheese portions and slices, respectively. From a quantitative point of view, 7−9 (*cis*/*trans* + *trans*/*cis*) C18:2 were the third CLA molecules. Total *trans*/*trans* isomers represented <10% of total CLA, and *trans*-10,*cis*-12 content was negligible (<0.2%). Furthermore, no significant difference was observed during the different production stages, thereby confirming that processing does not affect the CLA isomer profile in these dairy products.

Most of the works published on the CLA isomer profile in processed cheeses report only percentages of RA in the total CLA content (*3*, *4*, *6*, *12*, *14*, *23*). In general terms, RA values in the total CLA fraction ranged from 40 to 80%, clearly lower than the percentages reported in this work. Differences in the CLA isomer profile with this research should be attributed to

Table 3. Evolution of the Content (Mean Value ± Standard Deviation) of Conjugated Linoleic Acid (CLA) Isomers in Processed Cheeses (Portions and Slices) during Processing Determined by Ag^+-HPLC[c]

isomer	raw material	cooking	creaming	final product
	Portions (Percent of Total CLA)			
t/t 12–14	1.86 ± 0.06[a]	1.84 ± 0.04[a]	1.85 ± 0.03[a]	1.83 ± 0.06[a]
t/t 11–13	3.91 ± 0.24[a]	4.02 ± 0.16[a]	4.04 ± 0.03[a]	3.98 ± 0.22[a]
t/t 10–12	0.69 ± 0.06[a]	0.67 ± 0.04[a]	0.67 ± 0.03[a]	0.68 ± 0.03[a]
t/t 9–11	1.11 ± 0.06[a]	1.16 ± 0.04[ab]	1.22 ± 0.01[ab]	1.23 ± 0.06[b]
t/t 8–10	0.20 ± 0.04[a]	0.17 ± 0.03[a]	0.19 ± 0.01[a]	0.21 ± 0.02[a]
t/t 7–9	0.37 ± 0.01[a]	0.38 ± 0.01[a]	0.37 ± 0.01[a]	0.39 ± 0.02[a]
c/t 12–14	0.60 ± 0.04[a]	0.60 ± 0.04[a]	0.62 ± 0.02[a]	0.60 ± 0.02[a]
c/t 11–13	4.71 ± 0.28[a]	4.62 ± 0.20[a]	4.78 ± 0.19[a]	4.57 ± 0.28[a]
c/t 10–12	0.15 ± 0.06[a]	0.16 ± 0.06[a]	0.12 ± 0.02[a]	0.12 ± 0.02[a]
c/t 9–11	82.93 ± 0.58[a]	82.96 ± 0.39[a]	82.56 ± 0.35[a]	82.79 ± 0.45[a]
c/t 7–9	3.04 ± 0.25[a]	2.87 ± 0.06[a]	2.98 ± 0.16[a]	2.98 ± 0.12[a]
∑ t,t	8.03 ± 0.51[a]	8.14 ± 0.09[a]	8.34 ± 0.06[a]	8.32 ± 0.25[a]
∑ c,t + t,c	91.43 ± 0.54[a]	91.20 ± 0.15[a]	91.05 ± 0.09[a]	91.07 ± 0.29[a]
∑ unidentified	0.54 ± 0.04[a]	0.66 ± 0.06[a]	0.59 ± 0.03[a]	0.59 ± 0.02[a]
	Slices (Percent of Total CLA)			
t/t 12–14	1.99 ± 0.15[a]	2.00 ± 0.13[a]	2.01 ± 0.12[a]	1.94 ± 0.21[a]
t/t 11–13	4.77 ± 0.65[a]	4.73 ± 0.55[a]	4.68 ± 0.51[a]	4.68 ± 0.46[a]
t/t 10–12	0.63 ± 0.07[a]	0.62 ± 0.05[a]	0.61 ± 0.08[a]	0.58 ± 0.09[a]
t/t 9–11	1.25 ± 0.08[a]	1.29 ± 0.07[a]	1.29 ± 0.05[a]	1.30 ± 0.03[a]
t/t 8–10	0.15 ± 0.02[a]	0.15 ± 0.02[a]	0.16 ± 0.01[a]	0.17 ± 0.02[a]
t/t 7–9	0.39 ± 0.03[a]	0.38 ± 0.01[a]	0.38 ± 0.01[a]	0.38 ± 0.01[a]
c/t 12–14	0.66 ± 0.06[a]	0.64 ± 0.06[a]	0.66 ± 0.08[a]	0.65 ± 0.05[a]
c/t 11–13	4.63 ± 0.13[a]	4.53 ± 0.13[a]	4.54 ± 0.12[a]	4.57 ± 0.11[a]
c/t 10–12	0.10 ± 0.03[a]	0.13 ± 0.04[a]	0.17 ± 0.03[a]	0.16 ± 0.04[a]
c/t 9–11	81.87 ± 1.14[a]	82.00 ± 1.07[a]	81.90 ± 1.01[a]	81.93 ± 0.96[a]
c/t 7–9	3.00 ± 0.12[a]	2.93 ± 0.14[a]	3.00 ± 0.13[a]	3.00 ± 0.27[a]
∑ t,t	9.17 ± 0.81[a]	9.18 ± 0.70[a]	9.13 ± 0.61[a]	9.12 ± 0.54[a]
∑c,t + t,c	90.25 ± 0.88[a]	90.22 ± 0.74[a]	90.27 ± 0.69[a]	90.30 ± 0.58[a]
∑ unidentified	0.58 ± 0.03[a]	0.60 ± 0.03[a]	0.60 ± 0.04[a]	0.58 ± 0.04[a]

[a,b] Values in the same row without a common superscript letter are significantly different: $p \leq 0.05$. [c] c, cis; t, trans.

the analytical methods, mainly the methylation procedure. Among the methods examined, CLA isomerization was greatest with the BF_3 catalyst (*18*). Increasing the temperature and/or incubation time with this catalyst for either method decreased the *cis*-9,*trans*-11, but *trans*-9,*trans*-11, *trans*-10,*trans*-12, and artifacts increased (*19*).

The acid-catalyzed methylation with the BF_3 procedure was used extensively to analyze CLA contents in cheeses in the 1990s and showed a high content of isomers other than RA. Ha et al. (*11*) measured very high levels of 11−13 and *trans*/*trans* CLA isomers in different dairy products, whereas García-López et al. (*16*) also detected noticeable amounts of *trans*/*trans* CLA in processed cheeses. Werner et al. (*15*) reported that CLA changed from *cis*/*trans* or *trans*/*cis* isomers into *trans*/*trans* isomers or unknown substances during the BF_3 methylation procedure. These authors (*15*) recognized the isomerization of the conjugated dienes during acid-catalyzed methanolysis and recommended that the reaction of BF_3/methanol be carried out at room temperature to reduce this isomerization. This modified methylation procedure was subsequently used to evaluate CLA content in a variety of dairy products (*3*, *4*, *29*, *30*). However, other studies (*17*) showed that methylation was not complete either with BF_3 as a catalyst under these mild conditions. GC analyses showed evidence of isomerization and artifact formation. Therefore, not even mild BF_3 methylation at room temperature would be appropriate for the analysis of conjugated dienes.

Careful analysis of the conjugated dienes in biological material, using only base-catalyzed methods that do not isomerize the conjugated diene system, would therefore be required. Although base-catalyzed methods do not methylate free fatty acids and ignore the sphingomyelin lipids, this is not a concern in processed cheese, where most fatty acids are sterified as triglycerides. Base-catalyzed methods used in this work would cause no isomerization and produce no methoxy artifacts, thereby enhancing the reliability of the results.

Given these results, processed cheeses can be considered as a good source of CLA. At the end of the manufacturing, CLA levels ranged between 7.4 and 7.9 mg/g of fat or 1.46−1.66 mg/g of sample. Therefore, in a usual serving size (a single slice or portion of processed cheese weighing ∼20 g), CLA content was ∼30 mg. An additional value of these dairy products comes from the CLA isomer detected in processed cheeses. RA, the biologically active form, accounted for >80% of total CLA, whereas *trans*/*trans* and other isomers were minor contributors.

These results suggest that, in the conditions studied, manufacturing practices had negligible effects on the total CLA concentration as well as on the CLA isomer distribution in commercial processed cheeses. No losses of RA were detected. CLA concentrations in processed cheeses are primarily dependent on the CLA content of the unprocessed raw material and the final fat content. The stability of RA during processing is very important, because this potential anticarcinogen isomer could be obtained from commercial dairy products such as processed cheeses.

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