Determination of Conjugated Linoleic Acid Content and Isomer Distribution in Three Cheddar-Type Cheeses: Effects of Cheese Cultures, Processing, and Aging

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Conjugated linoleic acid (CLA) is a mixture of positional and geometric isomers of linoleic acid with conjugated double bonds. Several investigators have suggested that CLA has anticarcinogenic properties. This study describes the quantification of CLA isomers in three Cheddar-type cheeses relative to the effects of different cheese cultures, processing conditions, and aging periods. An improved method was developed for quantifying CLA isomers in cheese such that isomerization did not occur. Three 13-month-aged cheeses and one unaged cheese were analyzed for CLA concentration and isomer distribution. The CLA concentration in the fat of the aged and unaged cheeses ranged from 5.05 to 5.39 mg of CLA/g of fat. Isomers *c*-9,*t*-11-/*t*-9,*c*-11- and *t*-9,*t*-11-/*t*-10,*t*-12-octadecadienoic acids accounted for 82–88 and 8–11% of the total CLA, respectively. These results suggest that different starter cultures, processing conditions, and aging periods had negligible effects on the total CLA concentration but did influence the CLA isomer distribution in the cheeses studied.

INTRODUCTION

The term conjugated linoleic acid (CLA) describes a mixture of octadecadienoic acids with conjugated double bonds (i.e., 9,11-, 10,12-, and 11,13-octadecadienoic acids). Several investigators have reported that CLA has anticarcinogenic properties involving the inhibition of mouse skin carcinogenesis (Pariza and Hargraves, 1985; Ha et al., 1987) and mouse forestomach and rat mammary tumorigenesis (Ha et al., 1990; Ip et al., 1991). These properties have been attributed, in part, to the antioxidative characteristics of CLA (Ha et al., 1990; Ip et al., 1991). Shultz et al. (1992) recently found that physiological concentrations of CLA were cytostatic and cytotoxic to human malignant melanoma, colorectal, and breast cancer cells in vitro. These results suggested that tumor growth inhibition may be due to an ability of CLA to inhibit protein and nucleotide biosynthesis (Shultz et al., 1992). In terms of biological activity, *cis*-9,*trans*-11(*c*-9,*t*-11)-octadecadienoic acid has been implicated as the most important CLA isomer, since this isomeric derivative was the predominant isomer incorporated into the phospholipids of mouse forestomachs (Ha et al., 1990), rat livers, and mammary tumors (Ip et al., 1991).

Dietary sources of CLA include milk fat, natural and processed cheeses, meat products, and plant oils (Parodi, 1977; Fogerty et al., 1988; Ha et al., 1989; Chin et al., 1991; Shantha et al., 1991). Animal sources are richer in CLA than plant sources, and in general, foods from ruminants contain more CLA than foods from nonruminants (Chin et al., 1991). Dairy products are good sources of CLA, and a considerable amount of research has been conducted on the CLA content and isomer distribution in cow's milk.

Researchers have demonstrated that the concentration of CLA in milk fat is dependent on the diet and lactation number (age) of cows. The effect of diet on CLA formation has been known for over 50 years. Initially, Booth et al. (1935) reported that the ultraviolet (UV) absorbance of fatty acids (at 230 nm) in milk fat more than doubled during the summer months when cows had access to pasture. Further research indicated that the UV absorbance could be increased by feeding cows polyunsaturated oils which exhibit little or no UV absorbance at 230 nm (Dann et al., 1935; Houston et al., 1939). Shorland et al. (1955) established that CLA which absorbs strongly at 230–234 nm was produced from polyunsaturated oils by microorganisms of the rumen, and Bartlett and Chapman (1961) reported that CLA was an intermediate in the microbial biohydrogenation of linoleic acid to oleic acid. In a subsequent study, Kepler et al. (1966) discovered that a rumen bacterium, *Butyrivibrio fibrisolvens*, converted linoleic acid to oleic acid via CLA. More recently, Chin et al. (1991) studied a partially purified isomerase from *B. fibrisolvens* and found that most of the linoleic acid in hydrolyzed safflower oil was converted to CLA by this enzyme. The effect of lactation number on CLA concentration in milk fat was investigated by Lal and Narayanan (1984), who found that cows with seven or more lactations produced significantly more CLA in their milk fat than cows with one to three or four to six lactations.

Ha et al. (1989) determined the CLA content of cheese and milk fat. They found that the fat in cheese usually had greater concentrations of CLA than milk fat. Conjugated linoleic acid formation was attributed to two parameters: (1) isomerization of linoleic and linolenic acids in the rumen and (2) free-radical-type oxidation of linoleic acid caused by aging, heat treatment, and protein quality. However, few data are available on CLA in foods in which different processing practices are controlled. This study describes the identification and quantification of CLA isomers in three Cheddar-type cheeses relative to the effects of different cheese cultures, processing conditions, and aging periods. We also describe an improved method for quantifying CLA isomers in cheese.

MATERIALS AND METHODS

Materials. Acetonitrile, hexane, methanol, chloroform, and acetic acid (all of HPLC grade) were purchased from J. T. Baker, Inc. (Phillipsburg, NJ). Sulfuric acid (reagent grade) and methyl tertiary butyl ether (MTBE; HPLC grade) were obtained from Fisher Scientific (Fair Lawn, NJ). *c*-9,*c*-12-Octadecadienoic acid methyl ester, sodium *c*-9,*c*-12-octadecadienoate (methyl linoleate and sodium linoleate; 99% pure), and 14% boron trifluoride in methanol were purchased from Sigma Chemical Co. (St. Louis,

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MO). [9,10-^{3}H(N)]Triolein (26.8 Ci/mmol) was purchased from Du Pont (NEN Research Products, Boston, MA). [^{3}H]Triolein was purified with a silica Sep-Pak column (Waters Chromatography Division, Milford, MA) and the hexane–MTBE solvent system described by Hamilton and Comai (1988). Conjugated linoleic acid was obtained from Dr. Michael W. Pariza (Food Research Institute, University of Wisconsin, Madison, WI). Conjugated linoleic acid was analyzed in our laboratory for purity according to a capillary gas chromatography (GC) method (Ha et al., 1989) and was determined to be 98.5% pure. The *c*-9,*t*-11- and/or *t*-9,*c*-11- and the *t*-10,*c*-12-octadecadienoic acids comprised 46 and 50% of the total CLA, respectively. Minor components included *c*-9,*c*-11-, *c*-10,*c*-12-, and *t*-9,*t*-11- and/or *t*-10,*t*-12-octadecadienoic acids. The designation *c*-9,*t*-11-/*t*-9,*c*-11- and other similar designations used in this paper indicate that these are pairs of isomers that are not separated using the GC method described herein.

Cheese Cultures. Cougar Gold, Cheddar, and Viking cheeses were made at the Washington State University Creamery. These commercial cheeses rely on bacteria to provide essential enzymes and flavor compounds. *Lactococcus lactis* ssp. *lactis* and/or *L. lactis* ssp. *cremoris* were included in the starter cultures of all three cheeses. *Lactobacillus delbrueckii* ssp. *bulgaricus* was also added to the starter cultures of both the Cougar Gold and Viking cheeses, and *Streptococcus salivarius* ssp. *thermophilus* was included in the starter culture for the Viking cheese.

Cheese Preparation. Following pasteurization, starter cultures were added to milk (3.8% milk fat) at 31.1 °C. Rennet was used to coagulate the milk. The coagulated milk was cut into $^3/_8$-in. cubes which were then stirred and cooked. Cougar Gold and Cheddar cheeses were cooked from 31.1 to 36.7 °C over a period of 30 min and held at 36.7 °C for an additional 45 min. Viking cheese was cooked from 31.1 to 37.8 °C over a period of 50 min with no hold time. Following cooking, the curd was separated from the whey (dipped), and loaves were formed from the curd. The time between dipping and milling (cutting the loaves into $^5/_8 \times {^5/_8} \times 2$ in. pieces) was approximately 2.5 h for Cougar Gold and Cheddar cheeses and 30–45 min for Viking cheese. The milling acidity, expressed as percent lactic acid, of the Cougar Gold and Cheddar cheeses was 0.5–0.55%, while that of the Viking cheese was 0.21%. Following milling, the cheeses were salted, Cougar Gold and Cheddar with 0.27% NaCl and Viking with 0.32% NaCl (w/w). The milled and salted pieces of cheese were then put into hoops and pressed. Cougar Gold and Cheddar cheeses were pressed at 45 lb/in.^{2} (psi) for 14 h, while Viking cheese was pressed for 30 min at each of five different pressures (0, 5, 10, 15, and 20 psi). After pressing, the cheeses were cut into can-sized pieces and vacuum sealed in 603 × 204 enamel-coated steel cans. The aged cheese samples were stored for 13 months at 7.2 °C.

Cheese Sampling. Conjugated linoleic acid in the three aged cheeses (Cougar Gold, Cheddar, and Viking) was assessed to determine the effects of different cheese cultures and processing conditions on CLA concentration and isomer distribution. Two cheese samples were analyzed from three different batches of each aged cheese; thus, 18 cans of cheese (2 cans × 3 batches × 3 cheeses) were sampled. Additionally, CLA in unaged Cougar Gold was evaluated to test for effects of aging on CLA concentration and isomer distribution. Two unaged cans of Cougar Gold from the same batch were analyzed for CLA and compared to the six cans of 13-month-aged Cougar Gold cheese.

Each block of cheese was cut in half, and approximately 25 g of cheese was grated from the cross section exposed by the cut. The grated cheese was mixed thoroughly, and a weighed 1-g sample was taken for analytical procedures.

Extraction. The fat from cheese was extracted and measured using the Folch extraction procedure described by Ha et al. (1989). The fat was then heated and mixed at 45 °C for 1 min to melt and distribute the fat uniformly in the rotoevaporating flask. A 15-mg portion of fat was then transferred to a screw-capped test tube for further analysis.

Hydrolysis. The fat was hydrolyzed to free fatty acids by adding 1 mL of 1 N NaOH in methanol. The test tube was then purged with nitrogen, sealed with a Teflon-lined cap, heated in a block heater for 15 min at 100 °C, and cooled at room temperature for 5 min. One milliliter of 2 N sulfuric acid and 2 mL of chloroform were added to the methanolic NaOH mixture and vortexed for 2 min. Following phase separation, the organic (lower) phase, containing free fatty acids, was collected and the chloroform evaporated at room temperature under a stream of nitrogen. The effect of hydrolysis on CLA formation was negligible and indicates that CLA was not formed as a result of saponification. This finding is consistent with a previous study by Ha et al. (1989).

[^{3}H]Triolein (7500 dpm) was added to fat extracts to determine the hydrolysis recovery. Following hydrolysis and extraction, neutral lipids and free fatty acids were separated (Hamilton and Comai, 1988), and [^{3}H]oleic acid was counted in an International Chemical Nuclear (ICN) Micromedic Model 36000 liquid scintillation counter (ICN Biomedical Inc., Huntsville, AL). Mean (±SD) hydrolysis recovery averaged 98 ± 1% (n = 3). Counting efficiency for ^{3}H was approximately 44%.

HPLC Fractionation. The free fatty acids were solubilized in 1 mL of acetonitrile/water/acetic acid (90:10:0.1 v/v/v), and 250 μL of this solution was fractionated at room temperature on a semipreparatory reversed-phase high-performance liquid chromatography (HPLC) column (Adsorbosphere ODS, 5-μm particle size, 250 × 10 mm; Alltech Associates, Inc., Deerfield, IL) fitted with a preparatory guard column containing similar C18 packing material (9.5 × 30 mm bed volume; Alltech Associates). An isocratic mobile phase of the aforementioned solvent was pumped through the column at 2.5 mL/min. An Isco Model 2350 HPLC pump with an Isco Model 2360 gradient programmer, a Hewlett-Packard 3394A integrator, and an Isco V^4 absorbance detector were used to conduct and monitor the CLA separation at 234 nm. Conjugated linoleic acid eluted between 23 and 29 min. The CLA fraction was collected and transferred to a 125-mL separatory funnel containing 15 mL of hexane and 9 mL of 0.2 N sulfuric acid. After vigorous shaking, the two phases were allowed to separate. The organic phase containing the CLA was transferred to a rotoevaporating flask, concentrated to a volume of 2 mL, dried with sodium sulfate, and collected in a test tube. The hexane was then evaporated at room temperature under a stream of nitrogen.

Methylation. Methyl esters were prepared by modifying the procedure described by Morrison and Smith (1964). Before samples were analyzed, an appropriate methylating procedure was developed, since intraisomerization of CLA isomers or interisomerization of linoleic acid and CLA would have generated inaccurate CLA isomer data. The CLA standard (400 μg) and 1 mL of 14% boron trifluoride in methanol were added to a screw-capped test tube. The mixture was purged with nitrogen, sealed with a Teflon-lined cap, vortexed, and methylated for various lengths of time (0.5–120 min) at a variety of temperatures (20–100 °C; see Table I). Two milliliters of hexane and 1 mL of water were then added, and the mixture was vortexed thoroughly. Following phase separation, the aqueous (bottom) layer was discarded. The organic (top) layer was dried with sodium sulfate and quantified by capillary GC (Ha et al., 1989).

The CLA fraction from cheese samples was methylated for 30 min at room temperature as described above. After drying with sodium sulfate, the organic layer was transferred to another test tube. The methyl esters were then concentrated at room temperature under a stream of nitrogen to a total volume of 250 μL and transferred to a 1-mL tapered screw-capped vial, where the remaining hexane was evaporated. Finally, the CLA methyl esters were redissolved in 75 μL of hexane and analyzed as previously described (Ha et al., 1989). Duplicate injections were made for each sample.

GC Analysis. Samples containing 150–400 ng of CLA in 1 μL of hexane were injected through the on-column injection port onto a Supelcowax 10 fused silica capillary column (60 m × 0.32 mm, 0.25-μm film thickness; Supelco, Inc., Bellefonte, PA). An HP 5880A GC system (Hewlett-Packard, Avondale, PA) fitted with a flame ionization detector was used for the GC analysis. The GC conditions were set according to the method of Ha et al. (1989). The helium carrier gas flowed at 2 mL/min, and the oven temperature was programmed as follows: 50–200 °C at 20 °C/min and held for 40 min; then from 200 to 240 °C at 20 °C/min and held for 30 min. The detector temperature was 250 °C. Conjugated linoleic acid methyl esters eluted between 33 and 39 min.

Table I. Effects of Methylation Times and Temperatures on Conjugated Linoleic Acid (CLA) Isomerization[a]

		% distribution of CLA isomers								
reaction time, min	temp, °C	*c*-9,*t*-11-/ *t*-9,*c*-11-	*c*-10,*t*-12-	*t*-10,*c*-12-	*c*-11,*c*-13-	*c*-9,*c*-11-	*c*-10,*c*-12-	*t*-9,*t*-11-/ *t*-10,*t*-12-	unidentified peaks, %	total peak area
30.0	20	46.7		50.0		1.1	1.2	1.0		3220
120.0	20	46.7		50.1		1.1	1.2	0.9		3301
15.0	50	46.7		49.9		1.1	1.2	0.8	0.3	3163
0.5	100	45.8		49.1		1.1	1.4	2.3	0.3	3225
1.5	100	42.4		45.6		1.2	1.3	4.0	5.5	3087
2.0	100	40.5		42.9		1.2	1.3	5.8	8.3	3218
3.0	100	37.5		39.6	0.1	1.2	1.2	10.5	9.9	3108
5.0	100	32.7		33.6	0.3	1.0	1.4	19.4	11.6	3188
10.0	100	19.2	0.3	21.5	0.9	1.1	1.5	44.1	11.4	3059
15.0	100	15.9	4.5	14.1	1.8	1.3	1.2	50.8	10.4	3334
20.0	100	14.0	5.4	11.8	2.2	1.4	1.5	53.5	10.2	3105

[a] Conjugated linoleic acid standard (400 μg) was methylated in 1 mL of 14% boron trifluoride in methanol, extracted with 1 mL of water and 2 mL of hexane, and dried with sodium sulfate. Methylated CLA (200 ng in 1 μL of hexane) was analyzed by capillary gas chromatography (Ha et al., 1989). The area of each peak was divided by the sum of all the peaks to calculate percentages.

CLA Quantification. Ha et al. (1989) initially identified the isomers in a CLA standard. We obtained an aliquot of this standard, analyzed the standard according to their GC method (Ha et al., 1989), and identified the CLA peaks by comparing retention times and peak areas. Methylated CLA standard was cochromatographed with the CLA fraction. Those peaks in the cheese CLA fraction that cochromatographed with the standard CLA methyl esters were identified as such.

Individual CLA peak areas were quantified using a methyl linoleate standard curve. The mean (±SD) recovery of [^{3}H]-triolein added to fat samples and subjected to hydrolysis, extraction, and methylation was 80 ± 1% ($n = 3$). Methyl [^{3}H]-oleate was separated from [^{3}H]oleic acid and glycerides of [^{3}H]-oleic acid using silica Sep-Pak cartridges (Hamilton and Comai, 1988). Cheese CLA concentrations were corrected for recovery of the tritiated standard.

Statistical Analysis. Cheese CLA concentrations were compared using the protected least significant difference (protected LSD) test of the program Statistix (version 3.1; Analytical Software, St. Paul, MN). Mean differences were considered to be significant at the $p < 0.05$ level (two-tailed).

RESULTS AND DISCUSSION

The CLA standard was methylated for various lengths of time at a variety of temperatures with 14% boron trifluoride in methanol (Table I). Methylation of the CLA standard for 15 min at 100 °C and subsequent capillary GC analysis revealed seven CLA peaks and other unidentified peaks (Figure 1A). These peaks were also detected by Ha et al. (1989) using a high-temperature methylating procedure. However, room temperature methylation of the CLA standard for 30 min followed by GC analysis revealed only five CLA peaks and no unidentified peaks (Figure 1B). Methylation with heat and boron trifluoride in methanol caused a shift in the CLA isomers from the *c*-9,*t*-11-/*t*-9,*c*-11- and *t*-10,*c*-12-octadecadienoic acids to the *c*-10,*t*-12-, *c*-11,*c*-13-, *t*-9,*t*-11-/*t*-10,*t*-12-octadecadienoic acids and other unidentified peaks (Table I and Figure 1). Importantly, total peak areas (mean ± SD; 3182 ± 88) of the CLA standard methyl esters were similar for all tested times and temperatures, thereby allowing accurate within and between treatment comparisons (Table I). We found that the 30-min room temperature methylating procedure prevented intraisomerization of CLA isomers and was more time efficient than the 2-h procedure. Consequently, CLA fractions of cheese were routinely methylated in 14% boron trifluoride in methanol for 30 min at room temperature.

The effects of the analytical procedure on the intraisomerization of CLA (Table I) and interisomerization of linoleic acid and CLA (see Materials and Methods, Hydrolysis) were negligible. As previously mentioned, Ha et al. (1989) used a high-temperature methanolic boron trifluoride methylating procedure to analyze CLA in milk, cheese, and ground beef. Although this procedure did not result in interisomerization (Ha et al., 1989), their method was not adequately tested for its effect on CLA intraisomerization. Unfortunately, intraisomerization of CLA isomers during the methylation procedure may have affected the CLA isomer profiles of those food samples. Consequently, the reported isomer distributions should be interpreted with caution.

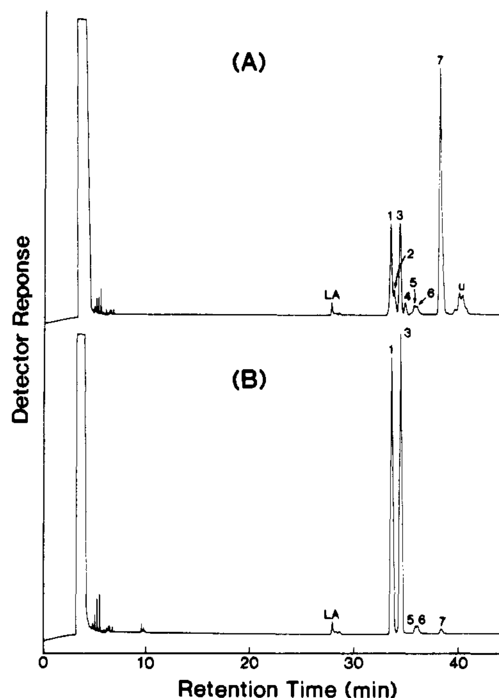

Figure 1. Capillary (Supelcowax 10 column) gas chromatography elution profiles of conjugated linoleic acid (CLA) standard after methylation with 14% boron trifluoride in methanol for (A) 15 min at 100 °C and (B) 30 min at room temperature. A *c*-9,*c*-12-linoleic acid peak (LA), unknown peaks (U) and seven CLA peaks (1–7) were identified using the GC method reported by Ha et al. (1989). The seven CLA peaks represent the following octadecadienoic acid methyl esters (Ha et al., 1989): (1) *c*-9,*t*-11-/*t*-9,*c*-11-; (2) *c*-10,*t*-12-; (3) *t*-10,*c*-12-; (4) *c*-11,*c*-13-; (5) *c*-9,*c*-11-; (6) *c*-10,*c*-12-; (7) *t*-9,*t*-11-/*t*-10,*t*-12-.

Recently, Ip et al. (1991) used a sodium methylate/HCl/methanol methylating procedure to produce CLA methyl esters from phospholipids of tissue samples. This procedure can also be used with triglycerides and other biological lipids (Carreau and Dubacq, 1978). If the aforementioned procedure does not cause interfering side

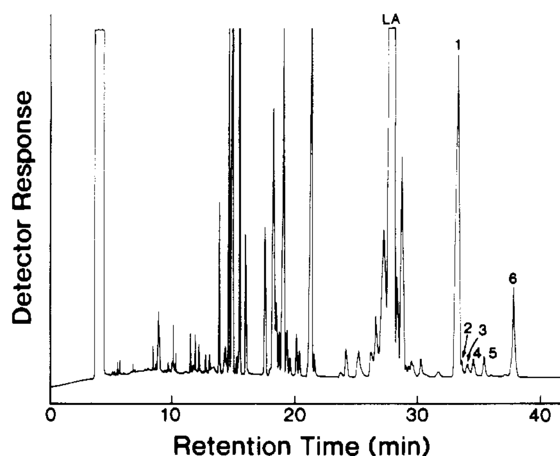

Figure 2. Capillary (Supelcowax 10 column) gas chromatography elution profile of the conjugated linoleic acid (CLA) fraction of Cheddar cheese. A *c*-9,*c*-12-linoleic acid methyl ester peak (LA) and six CLA peaks (1–6) were identified using the GC method reported by Ha et al. (1989). The six CLA peaks represent the following octadecadienoic acid methyl esters (Ha et al., 1989): (1) *c*-9,*t*-11-/*t*-9,*c*-11-; (2) *c*-10,*t*-12-; (3) *t*-10,*c*-12-; (4) *c*-11,*c*-13-; (5) *c*-9,*c*-11-; (6) *t*-9,*t*-11-/*t*-10,*t*-12-.

reactions (i.e., inter- or intraisomerization), this method (Carreau and Dubacq, 1978) may be advantageous to the boron trifluoride in methanol procedure reported herein. The procedure described by Carreau and Dubacq (1978) and used by Ip et al. (1991) apparently requires less time (1–2 min at 55 °C) and may involve one less analytical step than our method.

A typical capillary GC chromatogram (Figure 2) presents the profile and identities of CLA isomers obtained from Cheddar cheese. The CLA concentration in the fat of the aged and unaged cheeses ranged from 5.05 to 5.39 mg of CLA/g of fat (Table II). Of the isomers, *c*-9,*t*-11-/*t*-9,*c*-11- and *t*-9,*t*-11-/*t*-10,*t*-12-octadecadienoic acids accounted for 82–88 and 8–11% of the total CLA, respectively. The *c*-10,*t*-12-, *t*-10,*c*-12-, *c*-11,*c*-13-, and *c*-9,*c*-11-octadecadienoic acids each made up less than 2% of the total CLA. Cheese did not contain *c*-10,*c*-12-octadecadienoic acid. These results are in agreement with those of Chin et al. (1991), who reported that the CLA concentration in various cheeses ranged from 2.9 to 6.1 mg of CLA/g of fat and that greater than 75% of this CLA was *c*-9,*t*-11-octadecadienoic acid. Ha et al. (1989) reported previously that the CLA concentration in four aged cheeses (i.e., blue, Romano, Cheddar, and Parmesan) varied from 0.55 to 1.93 mg of CLA/g of fat, while processed cheeses, in general, contained more CLA than aged cheeses. Additionally, of the major isomers, these investigators (Ha et al., 1989) reported that *c*-9,*t*-11-/*t*-9,*c*-11- and *t*-9,*t*-11-/*t*-10,*t*-12-octadecadienoic acids contributed 18 and 61% of the total CLA in Cheddar cheese, respectively. Blue, Romano, and Parmesan cheeses showed similar isomer profiles.

Comparison of the aged Cheddar, Viking, and Cougar Gold cheeses revealed that they did not differ significantly in total CLA concentration (Table II). However, the concentrations of individual CLA isomers (i.e., *c*-9,*t*-11-/*t*-9,*c*-11-, *c*-10,*t*-12-, *c*-9,*c*-11-, and *t*-9,*t*-11-/*t*-10,*t*-12-octadecadienoic acids) were significantly different between cheeses, possibly due to differences in cheese cultures and processing conditions.

Ha et al. (1989) suggested a positive relationship between the aging period and CLA content of four cheeses aged between 3 and 10 months. Our data imply, however, that the total CLA concentration of unaged Cougar Gold cheese did not differ significantly from that of 13-month-aged Cougar Gold (Table II). Nonetheless, aging may have affected the CLA isomer distribution, since the concentration of *c*-9,*c*-11-octadecadienoic acid was greater in the aged than unaged cheese (Table II).

In summary, we present an improved method for quantification of CLA isomers. This method prevents intra- and interisomerization of fatty acids and may be used to determine the concentration and isomeric forms of CLA in various dairy products. Furthermore, we found that different starter cultures, processing conditions, and aging periods had a negligible influence on the total CLA concentration but significantly affected the CLA isomer distribution of Cougar Gold, Cheddar, and Viking cheeses. The cheese CLA concentrations and isomer distributions reported in this study are similar to those of milk fat. The concentration of CLA in milk fat is variable and has been reported to range from 2.4 to 21.8 mg of CLA/g of fat (Riel, 1963), while the major CLA isomer in milk fat is *c*-9,*t*-11-octadecadienoic acid (Parodi, 1977). Further research is warranted to determine more precisely the role of the cheese-making process in the production and/or isomerization of CLA. Such information would be of interest given the cancer-inhibitory properties of CLA (Pariza and Hargraves, 1985; Ha et al., 1990; Ip et al., 1991; Shultz et al., 1992) and the fact that the presence of CLA in biological fluids (Cawood et al., 1983; Dormandy and Wickens, 1987; Fogerty et al., 1988) may represent a protective factor against cancer.

ABBREVIATIONS USED

CLA, conjugated linoleic acid; *c*, cis; *t*, trans; UV, ultraviolet; MTBE, methyl tertiary butyl ether; HPLC, high-performance liquid chromatography; GC, gas chromatography; psi, pounds per square inch; ICN, International Chemical Nuclear; HP, Hewlett-Packard; LSD, least significant difference.

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Table II. Conjugated Linoleic Acid (CLA) Isomers in Cheddar, Viking, and Cougar Gold Cheeses[a]

cheese	fat, %	CLA isomers, μg/g of fat: *c*-9,*t*-11-/*t*-9,*c*-11-	*c*-10,*t*-12-	*t*-10,*c*-12-	*c*-11,*c*-13-	*c*-9,*c*-11-	*t*-9,*t*-11-/*t*-10,*t*-12-	total CLA, mg/g of fat
aged Cheddar	31.31 ± 0.48[b]	4320 ± 260[b]	58.6 ± 10.7[b]	117.0 ± 93.0	92.6 ± 13.5	90.7 ± 12.3[c]	596 ± 59[c]	5.28 ± 0.32
Viking	29.65 ± 0.97[c]	4670 ± 230[c]	43.7 ± 4.7[c]	51.2 ± 22.7	81.7 ± 8.0	60.4 ± 15.0[b]	485 ± 81[b]	5.39 ± 0.21
Cougar Gold	31.50 ± 0.24[b]	4280 ± 180[b]	54.9 ± 5.1[b]	91.2 ± 46.1	85.8 ± 13.7	67.4 ± 5.0[b]	472 ± 28[b]	5.05 ± 0.20
unaged Cougar Gold	31.47 ± 0.42	4560 ± 0	48.5 ± 1.4	57.9 ± 8.1	61.6 ± 4.2	39.9 ± 4.5[d]	401 ± 42	5.17 ± 0.04

[a] Two cans from three batches of each aged cheese and one batch of the unaged Cougar Gold cheese were analyzed. Means and standard deviations are reported for aged ($n = 6$) and unaged ($n = 2$) cheeses. [b,c] Conjugated linoleic acid isomers in the same column sharing a common superscript do not differ significantly ($p < 0.05$). [d] The aged and unaged Cougar Gold cheeses contained significantly different concentrations of this isomer ($p < 0.05$).

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