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Distribution and Content of Geometric Isomers of Conjugated Linoleic Acid in Dairy Foods from the Quebec Province of Canada

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Abstract The distributions and content of geometrical isomers of conjugated linoleic acids (CLA) in dairy foods such as milk, yogurt, and cheese, produced or being sold in the Quebec province of Canada, were investigated by gas chromatographic analysis. The mean contents of total CLA (mg/g fat) were 5.06±0.74 in 4 low-fat milk samples, 14.14 ± 4.95 in 6 yogurt samples, and 18.22±7.89 in 5 natural ripened cheeses. Among the yogurt samples, YY contained the highest content of total CLA (20.68±5.17 mg/g fat). Among the cheese samples, Gruyere contained the highest amount of total CLA (29.86±0.62) as well as *c*-9,*t*-11 (22.03±0.36 mg/g fat), followed by Jarlsberg (22.76±0.14), Provolone (16.42±0.52), Cheddar (13.83±0.81), and Swiss (8.23±1.11). Based on the distribution ratios of CLA isomers in these dairy foods, the *c*-9,*t*-11 isomer appeared to be the major CLA isomer in both the low-fat milk (89.87±2.39%) and yogurt (90.98±4.42%). In the cheeses, however, the ratio of *c*-9,*t*-11 (54.86±13.06%) was slightly higher than that of *c*-10,*c*-12 (40.81±13.40%).

Keywords: conjugated linoleic acid (CLA), distribution ratio, *c*-9,*t*-11 isomer, *c*-10,*c*-12 isomer, Gruyere

Introduction

Conjugated linoleic acids (CLA) have been shown to (a) reduce body fat (1-5), (b) strengthen muscle fiber (6-8), (c) act as anti-carcinogens (9,10), (d) reduce low density lipoprotein (LDL) cholesterol (11,12), and (e) stimulate immune response (13-15). CLA is currently being produced from linoleic acid (LA) by chemical methods that create safety issues. Its production by microbial or enzymatic methods, rather than by chemical synthesis, should have an advantage from a consumer point of view; but no such method has been successful in producing high enough yields, due to culture inhibition, and the instability of the enzymes involved. CLA is one of the natural fatty acids contained in milk and milk products, as well as in meat products (16). Principally, the isomerization mechanism of CLA in milk is reported to be synthesized by a kind of lipase, which is produced by the rumen microflora of cows. The *c*-9,*t*-11 isomer of CLA is mainly produced as an intermediate product by the hydrogenation of linoleic acid through ester linkage within the molecular structure by the lipase. CLA isomers, transformed from *c*-12 into *t*-11, are directly adsorbed to the rumen or transformed into vaccenic acid via the rumen microflora, which is again transformed into the *c*-9,*t*-11 isomer by stearyl-Co A desaturase and excreted into the milk (17). The CLA production pathway by Δ^9-desaturase, which isomerizes $C_{18:1}$ *t*-11 vaccenic acid transformed by linoleic acid isomerase that is produced by lactic acid bacteria, has also been well-established by Jiang *et al.* (18) and Griinari *et al.* (19). In addition, Ogawa *et al.* (20) reported for the first time on the metabolic pathway of CLA production from linoleic acid. In regard to the CLA substrates in the rumen, such as linoleic acid (21) and linolenic acid (22,23), feed and feeding systems (24) have been studied. The formation of CLA in the rumen is influenced by the pH of the rumen fluid, but neither oil sources containing high levels of linoleic acid (25,26), nor the ratio of concentrated feed to grass (27,28), nor the levels and sources of carbohydrates (23) appear to be responsible for its formation. On the basis of 1 g of fat, 5.5 mg of total CLA is present in milk, 4.6 mg in butter and yogurt, 3.6 mg in Cheddar cheese, 6.0 mg in Colby cheese, and 5.0 mg in processed cheese (9). The contents of *c*-9,*t*-11 isomer of total CLA in milk and milk products are shown to range from 3.38-6.39 mg/g of lipid in milk, 3.82-4.66 mg/g of lipid in yogurt, and 3.59-7.96 mg/g of lipid in natural cheese (29). When one considers a daily intake of 0.4 g of CLA as estimated by multiplying approximate content, with 0.5% CLA in 80 g of fat for Americans (30), only 13% CLA is supplied by dietary intake, which is not enough to satisfy the daily allowance (3.0 g) of CLA (31). Therefore, it has been recommended to increase CLA intake by consuming new CLA enriched fermented milk products made by CLA producing microorganisms. Yun (32) has reported on the CLA concentration of yogurt that was enriched from 30 to 120 mg/mL with *Bifidobacterium breve*. Since *Propionibacterium shermanii* (18), *Lactobacillus acidophilus* (20), and *Lactobacillus reuteri* (33) are already used in manufacturing fermented dairy products, it is important to determine the precise quantities of CLA in milk and dairy products by improved analytical methods. For this reason, an improved extraction method for CLA

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was recently developed by Jung *et al.* (34), and purification and characterization of the linoleic acid isomerases are under investigation in this laboratory.

This study aimed to compare the level and distribution of geometric isomers of CLA in milk, yogurt, and naturally ripened cheese products marketed in Quebec province, Canada.

Materials and Methods

Materials Analytical grades of linoleic acid (linoleic acid methyl ester, L 1876, 1 g) and conjugated linoleic acid (octadecadienoic acid, conjugated, methyl ester, O 5632, 1 g) were purchased from Sigma Co. (St. Louis, MO, USA). Heptadecanoic acid (H 3500, 5 g, Sigma Co.) was chosen as the internal standard. The reagents were kept in a cold storage cabinet under −40°C, and were diluted with *n*-hexane (analytical grade, Sigma Co.) for the gas chromatographic analysis.

Extraction of fats from dairy products The fats from milk, yogurt, and cheese, purchased from the local markets in Quebec, Canada were extracted with a chloroform-methanol mixture (v/v 1 : 2), as described by Chin *et al.* (9). The dairy product samples were mixed with 50 mL of extraction solvent and were shaken vigorously for 5 min. After the mixtures were centrifuged at 10°C (15 min; 750×g), 30 mL of the bottom portion (chloroform layer) was taken from 3 layers (a methanol/water layer at the top and a protein layer in the middle) and flushed by N_2 gas until the layers were completely dry.

Saponification The extracted fat was hydrolyzed to free fatty acid by alkaline methanol containing a 1.0 N NaOH solution. One mL of methanol containing 1.0 N NaOH was transferred to each sample and then heated for 20 min to make sodium salts of the fatty acids.

Methyl esterification After cooling to room temperature, 6 mL of acidic methanol containing a 4% volume of H_2SO_4 was transferred into the saponified samples. The bottle was heated again to 80°C for 15 min to react the carboxyl groups of fatty acids with the alcohol to form methyl-esters. The methyl-esters of the fatty acids were extracted with 1 mL of *n*-hexane and transferred into a 1.5 mL vial for storage at −40°C in a refrigerator.

Gas chromatographic analysis A gas chromatography (Auto System GC; Perkin Elmer, Waltham, MA, USA), equipped with a flame ionization detector (FID) was used for the separation and quantification of the free fatty acid methyl esters. The samples were injected onto the capillary column using a split injector. Separation was performed on a Supelcowax-10 capillary column (25 m×0.32 mm, 0.25 m film thickness, Supelco Inc., Bellefonte, PA, USA), with helium as a carrier gas. The injector and detector temperatures of the gas chromatography were 250 and 260°C, respectively. The initial oven temperature was 170°C with a 5 min holding time, which was then increased to 190°C with a 2°C increase per min and maintained for 10 min at 190°C. The identification method for the CLA isomers was similar to that of Jung and Ha (35).

The CLA contents of the samples were calculated using the following formula:

$$CLA\ (mg/g)=(Ax)(WIS)(CFx)/(AIS)(Ws)(1.04)$$

where, Ax=the peak area of CLA, AIS=the peak area of the internal standard, CFx=the theoretical correction factor for CLA calculated based on an internal standard, WIS =the weight of the internal standard added to the sample (mg), and Ws=the sample weight (g). The conversion factor 1.04 was adopted from previous work (36) to express the results as mg of fatty acid per g of fat, rather than as methyl esters. Since the different CLA isomers have the same theoretical detector response, the same correction factor for the different isomers was used. The theoretical correction factor (CFx) used in this research was 0.9789, which was adapted from a previous paper (35).

Results and Discussion

Geometric isomers of CLA in milk The total contents of fat, LA, and total CLA, and their isomeric distributions in milk purchased from markets in Quebec, Canada are summarized in Table 1.

The mean LA content of the 4 milk samples was 52.61 mg/100 mL, which was about 2.47% of the total fat. The milk samples were low-fat (2%) and produced by different companies, but the total CLA contents were almost similar to those of Chin *et al.* (9). The total CLA in the 4 milk samples ranged from 8.92-12.51 mg/100 mL of milk, which corresponds to 4.31-6.07 mg of total CLA per g of milk fat. The milk sample NL appeared to contain the highest level of total CLA (6.07 mg/g fat), as shown in Table 1.

The extent of CLA excretion in milk may be influenced by different feed compositions, especially by the feeding of grasses suitable for synthesizing CLA in the rumen of cows. When Collomb *et al.* (37) studied the grass of the highlands of Scotland, they found that the *c*-9,*t*-11 geometric isomer of CLA was the most abundant (87.2-93.27%) among all the milk samples tested. The second most abundant isomer in milk, *c*-10,*c*-12, was detected in all milk samples at levels of 0.36 to 0.52 mg/g fat. In our studies, the *t*-10,*c*-12 isomer was detected at low levels (0.11 and 0.26 mg/g fat) in 2 milk samples (QN, NL), and was not detected at all in the other 2 samples (PT, LY). The GC-chromatogram of 1 milk sample (QN) is shown in Fig. 1B.

The mean ratios of 3 geometric isomers for the 4 milk samples were 89.87% of *c*-9,*t*-11; 8.49% of *c*-10,*c*-12; and 1.64% of *t*-10,*c*-12. The total CLA contents and their isomeric distributions in the milk samples are in a good agreement with the results of Chin *et al.* (9), in which the total CLA content of milk was 5.55 mg/g of fat, with a ratio of 92% for the *c*-9,*t*-11 isomer.

Geometric isomers of CLA in yogurt Among 5 low-fat yogurts and 1 non-fat yogurt, the mean LA content of the samples was 48.33 mg/100 g, including the non-fat yogurt (DS) that contained only 2.78 mg/100 g. The mean CLA content of the 6 yogurts was 14.14±4.95 mg/g of fat, ranging from 5.95 to 20.68 mg/g of fat (Table 2).

Table 1. Distribution of geometric isomers of CLA and their contents in commercial milks from Quebec province

Milk	Fat	Linoleic acid			Total CLA			Geometric isomers of conjugated linoleic acid											
								c-9,t-11			t-10,c-12			t-9,t-11			c-10,c-12		
	g/ 100 mL	mg/ 100 mL	mg/ g fat	%	mg/ 100 mL[1)]	mg/ g fat[2)]	%[3)]	mg/ 100 mL	mg/ g fat	%	mg/ 100 mL	mg/ g fat	%	mg /100 mL	mg /g fat	%	mg/ 100 mL	mg/ g fat	%
QN	2.33	51.36	22.03	2.20	11.28	4.83	100.00	10.19	4.37	90.37	0.25	0.11	2.19	ND[4)]	-	-	0.84	0.36	7.44
PT	2.07	55.57	26.86	2.69	8.92	4.31	100.00	7.95	3.84	89.16	ND	-	-	ND	-	-	0.97	0.47	10.84
LY	2.08	52.75	25.34	2.53	10.42	5.01	100.00	9.68	4.65	92.84	ND	-	-	ND	-	-	0.75	0.36	7.16
NL	2.06	51.04	24.76	2.48	12.51	6.07	100.00	10.90	5.29	87.12	0.54	0.26	4.35	ND	-	-	1.07	0.52	8.53
Mean	2.14	52.61	24.72	2.47	10.78	5.06	100.00	9.68	4.54	89.87	0.40	0.09	1.64	-	-	-	0.90	0.43	8.49
±SD	±0.13	±2.07	±2.01		±1.51	±0.74		±1.26	±0.60		±0.21	±0.12		-	-	-	±0.14	±0.08	

[1)]mg/100 mL of fatty acid was obtained from gas chromatographic analysis.
[2)]mg/g of fat was calculated by each fatty acid divided by 1 g of milk fat.
[3)]% means the ratio of geometric isomers of total CLA.
[4)]Not detected.

Table 2. Distribution of geometric isomers of CLA and their contents in commercial yogurts from Quebec province

Yogurt		Fat	Linoleic acid			Total CLA			Geometric isomers of conjugated linoleic acid											
									c-9,t-11			t-10,c-12			t-9,t-11			c-10,c-12		
		g/ 100 g	mg/ 100 g	mg/ g fat	%	mg/ 100 g[2)]	mg/ g fat[3)]	%[4)]	mg/ 100 g	mg/ g fat	%	mg/ 100 g	mg/ g fat	%	mg/ 100 g	mg/ g fat	%	mg/ 100 g	mg/ g fat	%
DD	Mean	1.41	50.05	35.47	3.55	22.06	15.64	100.00	18.55	13.14	84.05	0.38	0.18	1.15	1.15	0.81	5.18	2.11	1.50	9.58
	±SD[1)]		±2.09	±1.48		±4/15	±2.94		±5.93	±4.20		±0.11	±0.17		±1.29	±0.92		±1.52	±1.08	
DS	Mean	0.33	2.78	13.38	0.83	0.99	5.95	100.00	0.77	5.45	91.54	0.13	0.13	2.19	0.02	0.02	0.25	0.08	0.36	6.02
	±SD		±3.01	±8.08		±0.30	±4.42		±0.55	±4.48			±0.23			±0.03		±0.10	±0.26	
YP	Mean	2.11	64.01	31.26	3.04	27.96	13.45	100.00	26.72	12.81	95.24	0.35	0.16	1.22	0.39	0.14	1.07	0.69	0.33	2.47
	±SD		±22.38	±12.84		±7.78	±4.51		±7.26	±4.21		±0.07	±0.03		±0.31	±0.18		±0.47	±0.27	
YM	Mean	2.51	64.46	25.68	2.57	31.08	12.38	100.00	27.10	10.80	87.21	2.10	0.84	6.75	0.56	0.22	1.80	1.32	0.52	4.24
	±SD		±14.15	±5.76		±3.33	±1.33		±4.69	±1.87		±2.69	±1.07		±0.08	±0.03		±0.57	±0.23	
LB	Mean	2.01	52.60	34.24	2.61	25.72	16.75	100.00	24.19	15.75	94.03	0.39	0.17	1.01	0.56	0.24	1.44	1.36	0.59	3.51
	±SD		±12.36	±8.05		±3,17	±2.06		±2.52	±1.64		±0.03	±0.15		±0.34	0±.26		±1.08	±0.71	
YY	Mean	1.54	56.12	36.53	3.65	31.76	20.68	100.00	29.79	19.40	93.79	0.88	0.38	1.84	0.47	0.21	1.00	1.07	0.70	3.37
	±SD		±5.66	±3.69		±7.95	±5.17		±7.57	±4.93		±0.25	±0.35		±0.11	±0.19		±0.60	±0.39	
Mean		1.65	48.33	29.50	2.97	23.26	14.14	100.00	21.19	12.89	90.98	0.70	0.31	2.36	0.52	0.27	1.80	1.10	0.67	4.87
±SD		±0.76	±23.08	±8.61		±11.48	±4.95		±10.70	±4.70		±0.73	±0.27		±0.37	±0.29		±0.69	±0.43	

[1)]Means and SD from triplicates, except YP, which is data from 4 analyses.
[2)]mg/100 g of fatty acid was obtained from gas chromatographic analysis.
[3)]mg/g of fat was calculated by each fatty acid divided by 1 g of milk fat.
[4)]% means the ratio of geometric isomers of total CLA.

The total CLA contents of the 5 low-fat yogurt samples were higher than those of low-fat (4.4±0.21 mg/g fat) and non-fat yogurts (1.7±0.10 mg/g fat) reported by Chin *et al.* (9). Also, the total CLA contents of the yogurts were much higher than those of the milk samples in this experiment, except for the non-fat yogurt product DS (0.33 g fat/100 g), which contained 5.95±4.42 mg CLA/g of fat. It is interesting to note that a yogurt brand, YY, contained an exceptionally high amount of total CLA (20.68±5.17 mg/g fat) as compared to the other 4 low-fat yogurt brands. In Fig. 1C, GC-chromatogram of a yogurt (DD) compares the CLA isomers with that of a standard (A).

The amount of total CLA in the yogurts (23.26 mg/100 g) was almost 2 fold higher than that in the milk (10.78 mg/100 mL), suggesting that the higher CLA in the yogurts might be the result of CLA isomerization by linoleic acid isomerase, from the starter bacteria during lactic acid fermentation. There is an evidence indicating increases in total CLA content during yogurt fermentation. Yun (32) reported that the CLA concentration of yogurt increased from 30 to 120 mg/100 mL by using *B. breve* LMC 017 with a commercial strain of *Streptococcus thermophilus*, after 9 hr of fermentation and 12 hr of post-acidification.

In the examination of the CLA geometric isomers in the 6 yoghurt samples, the distribution ratio of the *c*-9,*t*-11 isomer was 84.05-95.24%, as presented in Table 2. This distribution ratio for the *c*-9,*t*-11 isomer, among the CLA geometric isomers obtained from this experiment, was much higher than the results of Chin *et al.* (9), where the ratio of the *c*-9,*t*-11 isomer was in the range of 83-85%. Although the mean distribution ratio of the *c*-10,*c*-12 isomer by fermentation was decreased from 8.49% in the milk to 4.87% in the yogurt, the actual content was slightly increased from 0.43±0.08 mg/g of fat in the milk to 0.67± 0.43 mg/g of fat in the yogurt. Meanwhile, the *c*-9,*t*-11 isomer content of the yogurts (21.19±10.70 mg/100 g) was over 2 times higher than that of the tested milk samples (9.68±1.26 mg/100 mL). Thus, the increase of total CLA content during milk fermentation may mostly be contributed by the production of the *c*-9,*t*-11 isomer. The variation of total CLA content in the yogurts might be explained by their fermentation processes, which are dependent on the combination of *St. thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*.

Geometric isomers of CLA in cheese The fat contents, LA and CLA contents, and isomeric distributions of CLA in the cheese samples are shown in Table 3. The fat contents of the cheese samples were 29.1-41.28 g/100 g of cheese. The fat content (41.28%) of the Gruyere cheese, which is made from cow's milk without standardization, was more than 37% on the label. The LA contents were 1.28-1.91 g/100 g of cheese, which corresponds to 3.64-6.31% of the total fat in the cheeses.

The mean content of total CLA/g of fat in the cheeses was 18.22±7.89 mg. Gruyere contained the highest amount of total CLA (29.86±0.62 mg/g fat), followed by Jarlsberg (22.76±0.14 mg/g fat), Provolone (16.42±0.52 mg/g fat), Cheddar (13.83±0.81 mg/g fat), and Swiss (8.23±1.11 mg/g fat). Zlatanos *et al.* (38) studied the total CLA content in the fat of 10 Greek Feta and other Feta-type cheeses, 3 whey cheeses, and 17 short- and long-aged hard cheeses. They found that CLA contents were highest in the long-aged hard cheeses (9.4 mg/g fat) and in the Greek Feta

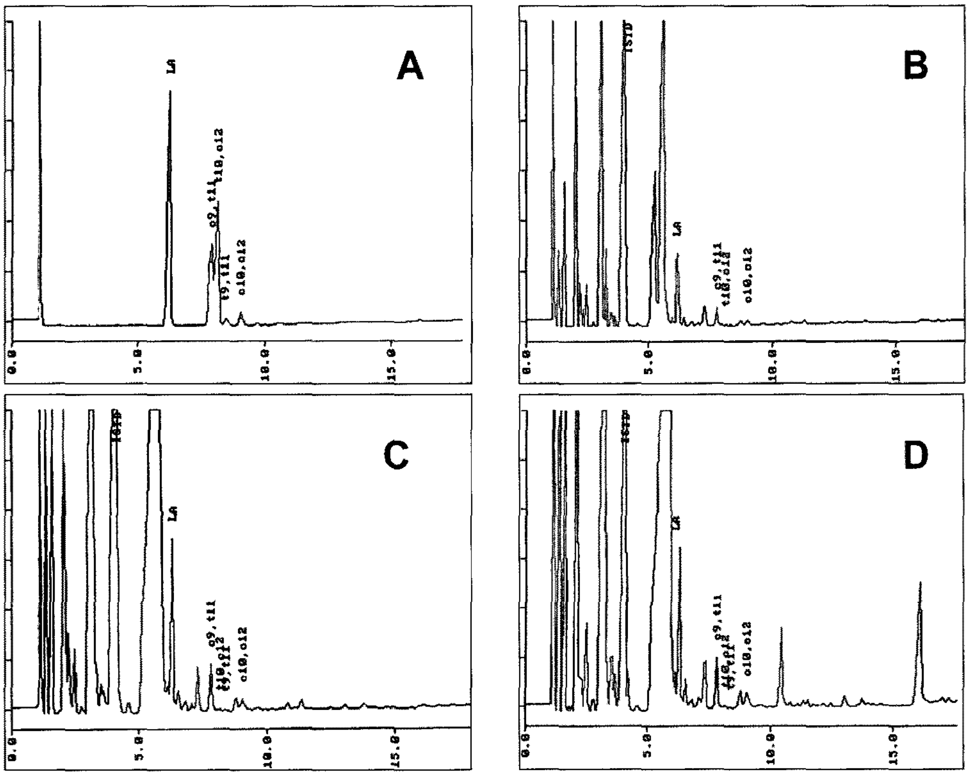

Fig. 1. GC-Chromatgrams of geometric isomers of CLA for a sample each of milk, yogurt, and cheese. A: Standard CLA (octadecadienoic acid, conjugated, methyl ester) composed of approximately 42% *c*-9,*t*-11, 44% *t*-10,*t*-12, 5% *t*-10,*t*-12, and 10% *c*-10,*c*-12; B: a milk sample QL; C: a yogurt sample DD; and D: Jarlsberg cheese.

Table 3. Distribution of geometric isomers of CLA and their contents in commercial cheeses from Quebec province

Cheese		Fat	Linoleic acid			Total CLA			Geometric isomers of conjugated linoleic acid											
									c-9,t-11			t-10,c-12			t-9,t-11			c-10,c-12		
		g/100 g	mg/ 100 g	mg/ g fat	%	mg/ 100 g[2]	mg/ g fat[3]	%[4]	mg/ 100 g	mg/ g fat	%	mg/ 100 g	mg/ g fat	%	mg/ 100g	mg/ g fat	%	mg/ 100g	mg/ g fat	%
Gruyere	Mean	41.28	1500.81	36.36	3.64	1232.60	29.86	100.00	909.23	22.03	73.77	43.18	1.05	3.50	5.44	0.13	0.44	274.73	6.66	22.29
	±SD[1]		±2.78	±0.07	±0.01	±25.63	±0.62		±15.02	±0.36	±0.31	±1.32	±0.03	±0.03	±0.25	±0.00	±0.01	±9.02	±0.22	±0.27
Jarlsberg	Mean	29.10	1279.31	43.96	4.40	662.24	22.76	100.00	304.03	10.45	45.91	10.09	0.35	1.52	5.04	0.17	0.76	343.11	11.79	51.80
	±SD		±33.19	±1.14	±0.11	+4.14	±0.14		±11.63	±0.40	±2.05	±0.30	±0.01	±0.4	±0.23	±0.01	±0.03	+15.27	±0.52	±1.98
Cheddar	Mean	32.62	1757.47	53.88	5.39	454.90	13.83	100.00	207.44	6.35	46.02	11.41	0.35	2.55	1.16	0.00	0.34	230.52	7.07	51.09
	±SD		±11.97	±0.37	±0.04	±20.92	±0.81		±9.26	±0.28	±0.65	±2.70	±0.08	±0.75	±0.07	±0.00	±0.49	+17.64	±0.54	±0.91
Provolone	Mean	29.24	1844.67	63.09	6.31	464.65	16.42	100.00	213.45	7.30	44.35	36.06	1.23	7.53	ND[5]	-	-	230.51	7.88	48.13
	±SD		±64.11	±2.19	±0.22	±39.07	±0.59		±39.15	±0.13	±6.55	±2.18	±0.07	±0.73	-	-	-	±19.63	±0.67	±5.83
Swiss	Mean	35.24	1905.11	54.05	5.40	290.02	8.23	100.00	184.92	5.25	64.27	13.80	0.39	5.00	ND	-	-	91.30	2.59	30.73
	±SD		±89.25	±2.53	±0.25	±38.79	±1.11		±2.78	±0.08	±7.64	±8.68	±2.46	±3.66	-	-	-	±44.69	±1.27	±11.30
Mean		33.50	1657.47	50.27	5.03	620.88	18.22	100.00	363.81	10.28	54.86	22.91	0.67	4.02	2.42	0.07	0.31	234.03	7.20	40.81
±SD		±4.76	±249.70	±9.79		±346.22	±7.89		±291.02	±6.48		±14.97	±0.42		±2.55	±0.08		±88.76	±3.14	

[1]Mean and SD from duplicates.
[2]mg/100 g of fatty acid was obtained from gas chromatographic analysis.
[3]mg/g of fat was calculated by each fatty acid divided by 1 g of milk fat.
[4]% means the ratio of geometric isomers of total CLA.
[5]Not detected.

cheese (9.2 mg/g fat). On the other hand, the whey cheese (7.0 mg/g fat) and the short-aged hard cheese (7.40 mg/g fat) produced less amounts of CLA. Others found that the CLA/g of fat was 4.1 mg in Canadian cheese (16), 4.6 mg in U.S. cheese (39), and 5.9 mg in Swedish cheese (40). Werner *et al.* (41) determined that the *c*-9,*t*-11 isomer of CLA in Cheddar cheese was 4.5 mg/g of fat, and the CLA content was influenced by the manufacturing conditions such as cheese cultures, processing, and ageing.

The mean distribution ratios of 4 geometric isomers of CLA for the 5 cheese samples were 54.86% of *c*-9,*t*-11; 40.81% of *c*-10,*c*-12; 4.02% of *t*-10,*c*-12; and 0.31% of *t*-9,*t*-11. The GC-chromatogram for Jarlsberg cheese is shown in Fig. 1D.

Among the 5 cheese samples, the distribution of the *c*-9,*t*-11 isomer was highest in Gruyere cheese, at 73.77% of the total CLA content. The content (22.03±0.36 mg/g fat) of the *c*-9,*t*-11 isomer in the Gruyere cheese was almost 3 or 4 times higher than those of the Cheddar (6.35±0.28 mg/g fat), Provolone (7.30±0.13 mg/g fat), and Swiss (5.25±0.08 mg/g fat) cheeses. The isomeric distributions of *c*-9,*t*-11 CLA in the other cheese samples, except for the Gruyere (73.77%) and Swiss (64.27%), were less than 50% of the total CLA. This distribution ratio of *c*-9,*t*-11 isomer was much lower than the results of Chin *et al.* (9). The *c*-10,*c*-12 isomer proportion (7.20 mg/g fat) in the cheeses was 40.81% of total CLA and much higher than those in the milk and yogurt samples. After studying the bacteria involved in the experiments, the high distribution ratio of the *c*-10,*c*-12 isomer could not be explained by either the cheese starter or by the adjunct starter bacteria. Another CLA isomer, *t*-10,*c*-12, which is known for reducing body fat (3), has also been detected in 0.67 mg/g of fat in cheese with 4.02% of total CLA.

Both Gruyere and Swiss cheese belong to the same cheese variety, which originates from a high-mountain area of Switzerland. Swiss cheese varieties are commonly fermented with thermophilic stater bacteria as well as ripened for 2 or 3 months at 15-18°C, to allow for the formation of the eyes in the cheese structure, which are contributed by *Propionibacterium freudenreichii* subsp. *freudenreichii* or subsp. *shermanii* (42). However, Gruyere cheese can be differentiated, in particular, by the appearance of *Brevibacterium linens* in the smear on the cheese surface, unlike other Swiss varieties (43). Cheddar cheese is made with mesophlic lactic acid bacteria, *Lactococcus lactis* subsp. *lactis* and *Lc. lactis* subsp. *cremoris*; while Provolone cheese is a variety of pasta-filata cheese inoculated with a mixture of thermoduric streptococci, and often, *Lb. delbreuckii* subsp. *bulgaricus*.

As stated by Werner *et al.* (41) on the CLA content of Cheddar cheese, CLA production is influenced by the manufacturing conditions. The highest content of total CLA found in the Gruyere cheese may thus be related to the activity of *B. linens* during ripening, but rarely has CLA isomerization by this microorganism been studied. Another possible consideration for the high total CLA content in the Gruyere cheese could be the origin of the cheese. The Gruyere and Jarlsberg used in this experiment were imported in the shredded form from Switzerland and Norway, respectively, and the Swiss, Provolone, and Cheddar were made in Canada. Zlatanos *et al.* (38) stated that the CLA contents of cheeses produced in different geographical areas might be influenced by seasonal variation, ageing time, and temperature conditions in cheese ripening.

From the previous discussion, we may conclude that both the amount of total CLA and the distribution ratio of CLA isomers are derived from the fermentation conditions and ripening periods, as well as the microflora involved in cheese ripening. To warrant these findings from Gruyere cheese, further studies on linoleate isomerase and the identification of *Brevibacterium* by 16S RNA ribotyping are underway.

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References

1. Ha YL, Grimm NK, Pariza MW. Newly recognized anti-carcinogenic fatty acid: Identification and quantification in natural and processed cheese. J. Agr. Food Chem. 37: 75-81 (1989)
2. Delany JP, Blohm F, Truett AA, Scimeca JA, West DB. Conjugated linoleic acid rapidly reduce body fat content in mice without affecting energy intake. Am. J. Phys. 276: 1172-1179 (1999)
3. Baumgard LH, Corl BA, Dwyer DA, Saebo A, Bauman DE. Identification of the conjugated linoleic acid isomer that inhibits milk fat synthesis. Am. J. Phys. 278: 179-184 (2000)
4. Martin JC, Sebedio JL, Caselli C, Pimont C, Martine L, Beruard A. Lymphatic delivery and *in vitro* pancreatic lipase hydrolysis of glycerol esters of conjugated linoleic acid in rat. J. Nutr. 130: 1108-1114 (2000)
5. Miner JL, Cederberg CA, Nielson MK, Chen X, Baile CA. Conjugated linoleic acid (CLA), body fat, and apoptosis. Obes. Res. 9: 129-134 (2001)
6. Park YJ, Albright J, Storkson JM, Cook ME, Pariza MW. Effects of conjugated linoleic acid on body composition in mice. Lipids 32: 853-858 (1997)
7. Delany JP, West DB. Changes in body composition with conjugated linoleic acid. J. Am. Coll. Nutr. 19: 487-493 (2000)
8. Mueller HL, Kirchgessner M, Roth FX, Stangle GI. Effects of conjugated linoleic acid on energy metabolism in growing finishing pigs. J. Anim. Physiol. An. N. 83: 85-94 (2000)
9. Chin SF, Liu W, Storkson JM, Ha YL, Pariza MW. Dietary sources of conjugated dienoic isomers of linoleic acid, a newly recognized class anticarcinogens. J. Food Compos. Anal. 5: 185-197 (1992)
10. Riera JB, Hontecillas R, Beitz DC. Colonic anti inflammatory mechanism of conjugated linoleic acid. Clin. Nutr. 21: 451-459 (2002)
11. Nicolosi RT, Rogers EJ, Kirtchersyky D, Scimeca JA, Huth PJ. Dietary conjugated linoleic acid reduces plasma lipoproteins and early aortic atherosclerosis in hypercholesterolemic hamsters. Artery 22: 266-277 (1997)
12. Stangle GI. High dietary levels of a conjugated linoleic acid mixture alter hepatic glycerophospholipid class profile and cholesterol carrying serum lipoprotein of rats. J. Nutr. Biochem. 11: 184-191 (2000)
13. Miller CC, Park Y, Pariza MW, Cook ME. Feeding conjugated linoleic acid to animals partially overcomes catabolic responses due to endotoxin injection. Biochem. Bioph. Res. Co. 198: 1107-1112 (1994)
14. Liu KL, Belury MA. Conjugated linoleic acid residues arachidonic acid content and PGE_2 synthesis in murine keratinocytes. Cancer Lett. 127: 15-22 (1998)
15. Pariza MW, Park YJ, Cook ME. Conjugated linoleic acid and the

control of cancer and obesity. Toxicol. Sci. 52: 107-110 (1999)
16. Ma DWL, Wierzbicki AA, Field CJ, Clandinin MT. Preparation of conjugated linoleic acid from safflower oil. J. Am. Oil Chem. Soc. 76: 729-730 (1999)
17. Pariza MW, Park YJ, Cook ME. The biologically acitive isomers of conjugated linoleic acid. Prog. Lipid Res. 40: 283-287 (2001)
18. Jiang J, Bjorck L, Fonden R. Production of conjugated linoleic acid by dairy starter cultures. J. Appl. Microbiol. 85: 95-102 (1998)
19. Griinari JM, Cort BA, Lacy SH, Chouinard PY, Nurmela KVV, Bauman DE. Conjugated linoleic acid is synthesized endogenously in lactating dairy cows by Δ^9-desaturase. J. Nutr. 130: 2285-2291 (2000)
20. Ogawa J, Matsumurs K, Kishino S, Omura Y, Shimizu S. Conjugated linoleic acid accumulation via 10-hydroxy-12-octadecanoic acid during microaerobic transformation of linoleic acid by *Lactobacillus acidophilus*. Appl. Environ. Microb. 67: 1246-1252 (2001)
21. Kelly ML, Berrym JR, Ewyer DA, Griinari JM, Choinard PY, Vanamburgh ME, Bauman DE. Dietary fatty acid sources affect conjugated linoleic acid concentrations in milk from lactating dairy cow. J. Nutr. 128: 881-885 (1998)
22. Bessa RJ, Santos-Silva J, Ribeiro JM, Portugal AV. Reticulo-rumen bio-hydrogenation and the enrichment of ruminal edible products with linoleic acid conjugated isomers. Appl. Environ. Microb. 63: 201-211 (2000)
23. Choi SH, Song MK. Effects of the level of carbohydrates on bio-hydrogenation and CLA production by rumen bacteria when incubated with soybean oil and flaxseed oil *in vitro*. J. Anim. Sci. Technol. 48: 521-532 (2005)
24. Jiang J, Bjorck L, Fonden R, Emanuelson M. Occurence of conjugated 9,11-octadecadienoic acid (CLA) in bovine milk: Effects of feed and feeding regimen. J. Dairy Sci. 79: 438-445 (1996)
25. Wang JH, Song MK. pH affects the *in vitro* formation of *cis*-9,*trans*-11 octadecanoic acid by ruminal bacteria when incubated with oilseeds. Asian-Austral. J. Anim. 16: 1743-1748 (2003)
26. Choi SH, Song MK. Effects of C_{18}-polyunsaturated fatty acids on their direct incorporation into the rumen bacterial lipids and CLA production *in vitro*. Asian-Austral. J. Anim. 18: 512-515 (2006)
27. Dhiman TR, Helmin ED, Mcmahon DJ, Fire RL, Pariza MW. Conjugated linoleic acid content in milk and cheese from cows fed extruded oilseeds. J. Dairy Sci. 82: 412-419 (1999)
28. Choi NJ, Imm JY, Oh SJ, Kim BC, Hwang HJ, Kim YJ. Effect of pH and oxygen on conjugated linoleic acid (CLA) production by mixed rumen bacteria from cows fed high concentrate and high forage diets. Anim. Feed Sci. Tech. 123-124: 643-653 (2005)
29. Lin H, Boylston TD, Chang MJ, Luedecke LD, Shultz TD. Survey of the conjugated linoleic acid contents of dairy products. J. Dairy Sci. 78: 2358-2364 (1995)
30. Ha YL, Pariza MW. Naturally occuring novel anticarcinogens: Conjugated dienoic derivatives of linoleic acid (CLA). J. Korean Soc. Food Nutr. 24: 401-407 (1991)
31. Chin SF, Storkson JM, Lui M, Albright KJ, Pariza MW. Conjugated linoleic acid (9,11- and 10,12-octadecadienoic acid) is produced on conventional but not germ-free rats fed linoleic acid. J. Nutr. 124: 694-701 (1994)
32. Yun CS. Screening of dairy starter culture for CLA producing and development of functional fermented milk by the starter culture. Research Report No. 501026-2. Agriculture R&D Promotion Center, Seoul, Korea (2003)
33. Pariza MW. Method of producing conjugated fatty acids. U.S. patent 6,060,304 (2000)
34. Jung MY, Kim GB, Jang ES, Jung YK, Park SY, Lee BH. Improved extraction method with hexane for gas chromatographic analysis of conjugated linoleic acids. J. Dairy Sci. 89: 90-94 (2006)
35. Jung MY, Ha YL. Conjugated linoleic acid isomers in partially hydrogenated soybean oil obtained during nonselective and selective hydrogenation processes. J. Agr. Food Chem. 47; 704-708 (1999)
36. Shantha NC, Decker EA, Henning B. Comparison of methylation methods for the quantification of conjugated linoleic acid isomer. J. Assoc. Off. Ana. Chem. Int. 73: 644-649 (1993)
37. Collomb M, Butikofer U, Sieber R, Bosset JO, Jeangros B. Conjugated linoleic acid and trans fatty acid composition of cows' milk fat produced in lowland and highland. J. Dairy Res. 68: 519-523 (2001)
38. Zlatanos S, Laskaridis K, Feist C, Sagredos A. CLA content and fatty acid composition of Greek Feta cheese and hard cheese. Food Chem. 78: 471-477 (2002)
39. Lin H, Boylston TD, Luedecke LO, Shultz TD. Factors affecting the conjugated linoleic acid content of Cheddar cheese. J. Agr. Food Chem. 46: 801-807 (1998)
40. Jiang J, Björck L, Fondén R. Conjugated linoleic acid in swedish dairy products with special reference to the manufacture of hard cheeses. Int. Dairy J. 7: 863-867 (1997)
41. Werner SA, Luedecke LO, Schultz TD. Determination of conjugated linoleic acid content and isomer distribution in three Cheddar-type cheese: Effects of cheese cultures, processing, and aging. J. Agr. Food Chem. 41: 1817-1821 (1992)
42. Steffen C, Eberhard P, Bosset JO, Ruegg M. Swiss-type varieties. pp. 83-110. In: Cheese Chemistry. Fox PF (ed). Chapman & Hall, London, UK (1993)
43. Robinson RK. A Colour Guide to Cheese and Fermented Milks. Chapman & Hall, London, UK. pp. 103-104 (1995)