# Conjugated Linoleic Acid in Canadian Dairy and Beef Products

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Conjugated linoleic acid (CLA) is a dietary fatty acid produced by ruminant animals and exhibits promising beneficial health effects. CLA has been identified as having anticancer, antiatherogenic, and body fat reducing effects. There are no published data on the CLA content of Canadian beef and dairy products. The purpose of this study was to assess the level and type of CLA isomers found in commercial beef and dairy products. Under the present experimental conditions only the Δ9*c*,11*t*-18:2 isomer was detected. Other minor isomers, which may be present, were not determined by the method used in this study. Levels of CLA ranged between 1.2 and 6.2 mg/g of fat or 0.001−4.3 mg/g or mg/mL of sample. On the basis of a usual serving size, levels of CLA ranged between 0.03 and 81.0 mg per serving. It is concluded that the Δ9*c*,11*t*-18:2 isomer is present in dairy and beef products and levels when expressed per gram of fat are not significantly different among products.

**Keywords:** *Conjugated linoleic acid; dairy and beef*

## INTRODUCTION

Conjugated linoleic acid (CLA) refers to a group of positional and geometrical isomers of linoleic acid. Interest in CLA has arisen due to reports of its many beneficial health effects. Animal and in-vitro models have shown that feeding a mixture of CLA is able to inhibit tumorigenesis in skin, colon, prostate, and breast tissues (Ip et al., 1991; Shultz et al., 1992a,b; Visonneau et al., 1997). Antiatherogenic properties have also been associated with dietary CLA in hamster and rabbit models (Lee et al., 1994; Nicolosi et al., 1997). Recently, CLA was reported to alter energy metabolism by decreasing body fat and increasing lean tissue mass (Chin et al., 1994; Park et al., 1997).

The major isomer detected in animal tissues is the Δ9*c*,11*t*-18:2 isomer, and this isomer has been reported in the phospholipid fraction (Chin et al., 1992; Fogerty et al., 1988; Ha et al., 1990; Ip et al., 1991). Therefore, detection of CLA in this fraction is suggestive of a functional role. CLA has been detected in various human tissues (Ackman et al., 1981; Cawood et al., 1983; Harrison et al., 1985; Iverson et al., 1984; Szebeni et al., 1986; Tay et al., 1987; Thompson Smith, 1985), and levels of CLA can be modulated by dietary intervention (Ackman et al., 1981; Britton et al., 1992; Fogerty et al., 1988; Huang et al., 1994). CLA intake is estimated to be several hundred milligrams per day (Fritsche and Steinhart, 1998). This level of intake is considerably less than the 3 g/day minimum value extrapolated from animal studies that would result in beneficial effects (Ip et al., 1994). However, exact intake levels in humans that confer these effects are still unknown. Nevertheless, increasing CLA consumption or assessment of its consumption requires documentation of CLA composition and content in the food supply.

CLA is produced naturally in ruminant animals such as cattle, sheep, and goats and is hence a component of most North American diets. The ruminant microorganism *Butyrivibrio fibrisolvens* is responsible for the synthesis of the Δ9*c*,11*t*-18:2 isomer as an intermediate in the biohydrogenation of linoleic (LA) to vaccenic acid (Kepler et al., 1966; Kepler and Tove, 1969; Kepler et al., 1971). Consequently, this single isomer of CLA is reported in dairy and beef products (Chin et al., 1992; Ha et al., 1989, 1987). Others have reported the presence of some of the positional and geometrical isomers of Δ9,11-18:2, and Δ10,12-18:2 (Chin et al., 1992; Ha et al., 1989; Lavillonniere et al., 1998).

Current compositional information of CLA in foods is limited and has only been conducted in a few countries, not including Canada. Therefore, it is of interest to assess the content of CLA isomers in commonly consumed Canadian foods.

## MATERIALS AND METHODS

**Materials.** A mixture of free fatty acids of CLA was obtained commercially (Nu-Chek-Prep, Inc., Elysian, MN, and Matreya, ON, Canada). A mixture of CLA isomers was also synthesized from safflower oil and is described elsewhere (Ma et al., 1999). A total of 20 different dairy and 5 beef products were obtained locally (Edmonton, Canada). All other chemical reagents were obtained from BDH (BDH Inc., Toronto, ON).

**Lipid Extraction.** Samples were analyzed from 2 or 5 g samples with the exception of butter, skim milk powder, and milk, for which 0.5 g, 25 g, and 5 mL quantities were used, respectively. Beef products were analyzed as both raw and cooked preparations from 10 g samples. The consistency of the cooked beef samples was monitored by measuring internal temperatures at the outer edge and inner portion of the cooking sample. Lipid material was extracted in duplicate according to the method of Folch et al. (1957). All lipid extracts

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were reconstituted in chloroform and stored at −70 °C. Fat content was determined from dry lipid weights.

**Saponification and Methylation of Lipid Extracts for GC Analysis.** In a screw-cap tube, 5 mg of sample and 25 μg of C19:0 of free fatty acid standard were saponified in NaOH/MeOH (2 mL, 0.5 M). Samples were heated for 1 h at 110 °C in a sand bath and then cooled. Hexane (2 mL) and 14% $BF_3$/MeOH (2 mL) were then added to each sample and methylated at room temperature as described in the methods of Werner et al. (1992) for 30 min with shaking. Double-distilled $H_2O$ (1 mL) was added immediately, and samples were vortexed briefly and then centrifuged at 2000 rpm for 10 min. The upper hexane phase was collected and the lower phase re-extracted with hexane (2 mL).

**Chromatographic Analyses.** Food products were analyzed for CLA content using an SP-2560 fused silica capillary column (100 m × 0.25 mm i.d. × 0.2 μm film thickness; Supelco Inc., Bellefonte, PA). Column conditions were set as follows: samples were dissolved in hexane; the injector port was set at 250 °C; detector temperature was 270 °C; the gas carrier was He with 2 mL/min flow rate; column pressure was 50 psi; and a 100:1 split mode was used. Samples were eluted off the column using a temperature program set from 130 to 225 °C over 110 min. Samples were injected in duplicate from each lipid extraction. CLA was identified by comparison of retention time to commercial and prepared CLA references.

**Statistical Analysis.** CLA content was expressed relatively, per gram of fat or per gram of sample. Content was also expressed per serving using values from food labels or the Canadian Food Guide sizes. Orthogonal comparisons were used to analyze the assorted dairy and beef samples used in this study. Samples were compared in the following groupings: dairy versus beef; milk versus nonmilk; cheeses versus all other dairy foods; and cooked versus raw beef. The relationship between the level of fat and CLA was analyzed by ranked correlation analysis. A possible producer difference was analyzed by two-way ANOVA. Differences between cooked and raw meats were analyzed using a paired *t* test. All analyses were done using SAS version 6.11.

## RESULTS

**CLA Content in Commercial Dairy and Beef Foods.** Orthogonal comparisons showed no difference in levels of CLA when expressed on a per gram of fat basis, which varied between 1.2 and 6.2 mg/g of fat. Levels of CLA differed in several orthogonal comparisons when expressed relative to sample size, which varied between 0.001 and 4.3 mg/g or mg/mL of sample. Differences were observed in the milk versus nonmilk ($p = 0.001$) and cheese versus all other dairy foods ($p = 0.005$). Differences were also observed when levels of CLA were expressed per usual serving size, which varied between 0.03 and 81.0 mg/serving. Levels were significantly different for dairy versus beef ($p = 0.0001$), milk versus nonmilk ($p = 0.0001$), and cheese versus all other dairy foods ($p = 0.0003$).

Orthogonal comparisons showed significant differences in the percent fat content (wt %); however, these differences did not correlate with levels of CLA when expressed per gram of fat (mg/g of fat) by Spearman ranked correlation (0.008). Levels of CLA were correlated to the percent fat content (wt %) when expressed on a per unit of sample (mg/g or mg/mL) (0.90) or per usual serving size (0.58).

Milk samples, 1% milk, 2% milk, and half/half cream (12% fat), were assessed for possible brand differences. No brand effect was observed. Differences in the level of CLA occurred when expressed per volume (mL) of sample ($p = 0.0001$) and per usual serving (250 mL) ($p = 0.0001$).

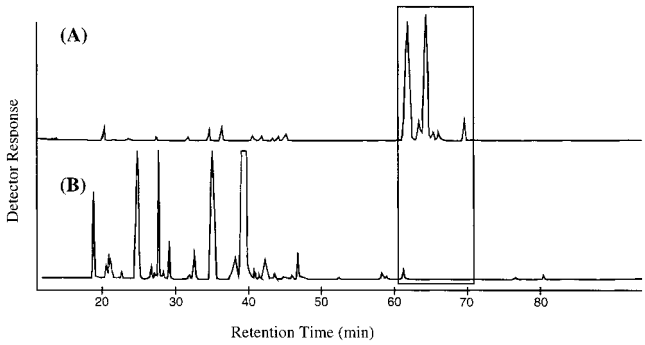


**Figure 1.** Typical SP-2560 GLC chromatogram of (A) a CLA standard synthesized from safflower oil and (B) a sample of beef. CLA peaks are enclosed within the boxed region. The beef sample contains a single peak corresponding to the Δ9*c*,11*t*-18:2 isomer.

Split plot analysis of the raw and cooked meats show significant differences among the types of meats ($p = 0.006$) per gram of fat. Therefore, pairwise *t*-test comparisons were conducted between raw and cooked samples for each type of meat. The level of fat was significantly different ($p < 0.05$) between the raw and cooked states for all but the extra lean ground beef. Only the sirloin tip roast had significantly higher content of CLA after cooking ($p = 0.02$) when expressed on a per gram of fat basis. Both the rib ($p = 0.005$) and sirloin ($p = 0.05$) roast had significantly higher levels of CLA after cooking when expressed per gram of sample.

## DISCUSSION

Only the Δ9*c*,11*t*-18:2 isomer of CLA was detected in dairy and beef foods (Tables 1 and 2) (Figure 1). *B. fibrisolvens* is the main producer of CLA and produces mainly the Δ9*c*,11*t*-18:2 isomer via an enzymatic mechanism. Minor isomers may also be produced by other ruminant microorganisms (Pariza, 1997). Typically, the Δ9*c*,11*t*-18:2 peak appeared as a small but distinguishable peak among all of the remaining other fatty acid peaks (Figure 1). In the present study, no prepurification was undertaken, such as HPLC, to concentrate the level of CLA, and due to the high concentration of saturated and monounsaturated fatty acids, the minor CLA isomers (<10% of total CLA) may have escaped detection by GLC. New methods using HPLC in the silver ion mode in conjunction with GLC offer greater sensitivity in the detection of these very minor isomers (Kramer et al., 1998; Sehat et al., 1998). However, little is known about the biological and physiological importance of these isomers found in only trace amounts.

Methylation of lipids by $BF_3$ at reduced temperature (Werner et al., 1992) or by sodium methoxide (Shantha et al., 1993) minimizes intraisomerization and artifact formation. Our initial assessment of the method described by Werner et al. (1992) also indicated that minimal artifact formation (<1%) was produced (data not shown). Therefore, minor isomers not detected were not due to loss from methylation using $BF_3$.

Cooking meat, such as ground beef, has been suggested to increase the amount of total CLA (Ha et al., 1989). However, Shantha et al. (1994) have shown that various cooking methods at different temperatures do not affect CLA content. Of the four cuts of meats, only the sirloin tip roast cut was observed to contain more CLA on a fat basis after cooking (increased from 1.2 to 2.8 mg/g of fat) (Table 2). Although CLA content appears

**Table 1. Levels of CLA in Dairy Products**[a]

| product | % fat | mg/g fat | mg/g sample | mg/serving | serving size (g) |
|---|---|---|---|---|---|
| | | Milk and Cream | | | |
| skim milk powder | 0.1 ± 0.01 | 1.8 ± 0.2 | 0.001 ± 0.0003 | 0.03 ± 0.01 | 25 |
| whole milk | 3.2 ± 0.2 | 3.4 ± 0.2 | 0.1 ± 0.002[b] | 25.6 ± 0.5 | 250 mL |
| 1% milk | 1.0 ± 0.04 | 4.3 ± 0.4 | 0.04 ± 0.003[b] | 10.5 ± 0.8 | 250 mL |
| 2% milk | 2.1 ± 0.1 | 5.0 ± 0.3 | 0.1 ± 0.004[b] | 25.8 ± 1.2 | 250 mL |
| half/half cream | 12.1 ± 0.2 | 5.5 ± 0.4 | 0.7 ± 0.05[b] | 10.0 ± 0.7 | 15 mL |
| | | Cheese | | | |
| goat cheese | 28.5 ± 1.8 | 2.7 ± 0.2 | 0.7 ± 0.03 | 34.3 ± 1.5 | 50 |
| Brie cheese | 27.9 ± 1.7 | 3.8 ± 0.5 | 1.0 ± 0.1 | 52.1 ± 6.9 | 50 |
| Italian Parmesan cheese[c] | 28.3 ± 2.5 | 4.2 ± 0.5 | 1.2 ± 0.3 | 12.0 ± 2.7 | 10 |
| mozzarella cheese | 24.9 ± 2.5 | 4.6 ± 0.2 | 1.1 ± 0.2 | 57.1 ± 8.3 | 50 |
| Cheddar cheese | 34.6 ± 2.4 | 4.2 ± 0.6 | 1.4 ± 0.1 | 71.7 ± 7.4 | 50 |
| Imperial Cheddar cheese | 33.0 ± 0.9 | 4.7 ± 0.2 | 1.5 ± 0.05 | 76.4 ± 2.3 | 50 |
| farmer cheese | 28.9 ± 3.1 | 4.7 ± 0.7 | 1.3 ± 0.2 | 64.7 ± 10.0 | 50 |
| | | Processed Products | | | |
| cream cheese | 33.8 ± 0.9 | 2.7 ± 0.2 | 0.9 ± 0.06 | 13.8 ± 0.9 | 15 |
| yogurt | 5.4 ± 0.6 | 4.4 ± 1.1 | 0.2 ± 0.1 | 42.7 ± 12.5 | 175 |
| butter | 91.1 ± 3.1 | 4.7 ± 1.9 | 4.3 ± 1.8 | 64.1 ± 26.5 | 15 |
| Cheese Whiz | 19.1 ± 2.7 | 4.9 ± 0.3 | 0.9 ± 0.1 | 13.7 ± 1.8 | 15 |
| sour cream | 12.6 ± 0.3 | 5.0 ± 1.4 | 0.6 ± 0.2 | 9.2 ± 2.4 | 15 |
| processed Parmesan cheese | 28.5 ± 1.2 | 5.3 ± 0.6 | 1.5 ± 0.2 | 15.0 ± 1.8 | 10 |
| cottage cheese[d] | 3.1 ± 0.1 | 5.9 ± 1.4 | 0.2 ± 0.03 | 26.7 ± 5.1 | 150 |
| processed cheese | 24.3 ± 1.8 | 6.2 ± 1.2 | 1.4 ± 0.2 | 70.4 ± 11.2 | 50 |

[a] All products were obtained locally during the Canadian spring and summer months. Each product was assessed from $n = 4$ brands of the same product or 4 of the same product from different locations. Values are expressed as mean ± SEM. [b] mg of CLA/mL of sample. [c] $n = 2$. [d] $n = 3$.

**Table 2. Levels of CLA in Beef Products**[a]

| product | % fat | mg/g fat | mg/g sample | mg/100 g serving |
|---|---|---|---|---|
| | | Raw Meats | | |
| sirloin roast tip | 4.4 ± 0.6 | 1.2 ± 0.4 | 0.1 ± 0.01 | 5.0 ± 1.4 |
| extra lean ground beef | 9.1 ± 1.5 | 1.4 ± 0.2 | 0.1 ± 0.03 | 11.2 ± 2.9 |
| ground beef | 26.0 ± 1.7 | 1.6 ± 0.1 | 0.4 ± 0.02 | 41.8 ± 2.0 |
| rib roast | 13.4 ± 1.6 | 3.0 ± 0.7 | 0.4 ± 0.1 | 40.3 ± 11.6 |
| | | Cooked Meats | | |
| sirloin roast tip | 9.2 ± 2.0 | 2.8 ± 0.4 | 0.3 ± 0.1 | 28.7 ± 11.2 |
| extra lean ground beef | 10.0 ± 1.5 | 1.2 ± 0.1 | 0.1 ± 0.03 | 11.5 ± 2.5 |
| ground beef | 20.4 ± 1.2 | 1.8 ± 0.2 | 0.4 ± 0.03 | 36.2 ± 2.7 |
| rib roast | 27.8 ± 1.2 | 2.9 ± 0.5 | 0.8 ± 0.1 | 77.6 ± 11.5 |
| fast food burger | 25.6 ± 0.4 | 3.2 ± 0.4 | 0.8 ± 0.1 | 81.0 ± 7.7 |

[a] All products were obtained locally during the Canadian spring and summer months. Each product was assessed from $n = 4$ brands of the same product or 4 of the same product from different locations. Values are expressed as mean ± SEM.


**Figure 2.** Comparison of levels of CLA (mg/g of fat) reported by (A) others to (B) levels observed in this study. The ranges of values are reported for each category indicated. Values by others are compiled from Ha et al. (1989), Werner et al. (1992), Chin et al. (1992), Shantha et al. (1992, 1994, 1995, 1997), and Lin et al. (1994).

to increase in the sirloin tip roast, the increase may reflect other changes in the edible portion. Cooking can alter the level of CLA on a per gram of edible sample basis (mg/g of sample) (Shantha et al., 1994). Both the sirloin and rib roasts had significantly greater levels of CLA (mg/g of sample) after cooking, which coincided with an increase in fat content. The fat content most likely increased relative to moisture loss.

Compared to previously published values, the concentration of CLA in dairy products is similar. However, levels detected in the meat cuts are lower than those reported by others (Figure 2) (Chin et al., 1992; Ha et al., 1989; Lin et al., 1995; Shantha et al., 1992, 1994, 1995, 1997; Werner et al., 1992). Some variation in CLA content may be attributed to seasonal feeding practices and diets used by farmers to feed their animals. Diet restriction (Jiang et al., 1996) and diets containing grasses increase CLA content in milk (Jahreis et al., 1996, 1997; Jiang et al., 1996; Stanton et al., 1997) and meat (Shantha et al., 1997). Diets containing cereals and maize or grain decrease milk (Jahreis et al., 1997) and meat CLA (Shantha et al., 1997), respectively.

Differences in feeding practices may not be the only factor contributing to low levels of CLA in our beef samples. Foods were obtained during the Canadian spring and summer months; therefore, cows and cattle would likely be pasture fed, and thus a higher CLA content would be expected. No differences in CLA content were observed in this study among different milk producers and products. Investigators analyzing raw milk note that there is high variability of CLA content among experimental animals (Jahreis et al., 1997; Jiang et al., 1996; Stanton et al., 1997). Lactation number has been reported to influence the level of milk fat CLA (Stanton et al., 1997). Hence, animal genetics may have a role in altering levels of CLA in milk and meat.

Beef and dairy products were found to contain one isomer of CLA, Δ9*c*,11*t*-18:2. The lower concentration of CLA in beef products detected in this study is attributed to various factors such as seasonal variation, animal genetics, and production practices. Overall, levels of CLA in dairy and beef products do not significantly differ on a per gram of fat basis. Hence, current foods containing CLA vary in direct relation to the amount of fat provided per usual serving size.

## ABBREVIATIONS USED

CLA, conjugated linoleic acid; c, cis; t, trans; GLC, gas−liquid chromatography; HPLC, high-performance liquid chromatography,

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