The Impact of Fortification with Conjugated Linoleic Acid (CLA) on the Quality of Fluid Milk¹

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ABSTRACT

The effects of added conjugated linoleic acid (CLA) on the sensory, chemical, and physical characteristics of 2% total fat (wt/wt) fluid milk were studied. Milks with 2% (wt/wt) total fat (2% CLA, 1% CLA:1% milkfat, 2% milkfat) were made by the addition of cream or CLA triglyceride oil into skim milk followed by HTST pasteurization and homogenization. The effects of adding vitamin E (200 ppm) and rosemary extract (0.1% wt/wt based on fat content) were investigated to prevent lipid oxidation. HTST pasteurization resulted in a significant decrease of the *cis*-9/*trans*-11 isomer and other minor CLA isomers. The *cis*-9/*trans*-11 isomer concentration remained stable through 2 wk of refrigerated storage. A significant loss of both the *cis*-9/*trans*-11 and the *cis*-10/*trans*-12 isomers occurred after 3 wk of refrigerated storage. The loss was attributed to lipase activity from excessive microbial growth. No differences were found in hexanal or other common indicators of lipid oxidation between milks with or without added CLA ($P > 0.05$). Descriptive sensory analysis revealed that milks with 1 or 2% CLA exhibited low intensities of a “grassy/vegetable oil” flavor, not present in control milks. The antioxidant treatments were deemed to be ineffective, under the storage conditions of this study, and did not produce significant differences from the control samples ($P > 0.05$). CLA-Fortified milk had significantly lower L^* and b^* values compared with 2% milkfat milk. No significant differences existed in viscosity. Consumer acceptability scores ($n = 100$) were lower ($P < 0.05$) for CLA-fortified milks compared to control milks, but the addition of chocolate flavor increased acceptability ($P < 0.05$).

(**Key words:** conjugated linoleic acid, milk, lipid oxidation, sensory)

Abbreviation key: CLA = conjugated linoleic acid, S-GC = static headspace chromatography, RRF = relative response factor.

INTRODUCTION

Conjugated linoleic acid (CLA) is a group of fatty acids found predominantly in the milk and meat of ruminant animals, such as cows, goats, and sheep (Chin et al., 1992). Of the nine possible CLA isomers reported, the *cis*-9/*trans*-11 is the primary isomer found in nature and incorporated into the phospholipids (Chin et al., 1992). The level of CLA in milkfat commonly falls between 3 and 6 mg/g (Kelly et al., 1998). Factors such as seasonal fluctuations, feeding practices, and processing steps including heat, aeration, and the addition of whey proteins all affect the levels of CLA present in milkfat (O'Shea et al., 1998).

The *cis*-9/*trans*-11 isomer in CLA has been considered to be the most biologically important in terms of anticarcinogenic activity because it is the only isomer incorporated into the phospholipid fraction of rat tissues (Ip et al., 1991). However, most research examining the health benefits of CLA has been conducted with a 50:50 mixture of the *cis*-9/*trans*-11 isomer and the *cis*-10/*trans*-12 isomer. The *cis*-10/*trans*-12 isomer has been specifically linked with a decrease in body fat concurrent with an increase in lean body mass (Park et al., 1999). Other nutraceutical benefits of CLA include protection against arteriosclerosis (Lee et al., 1994), cachexia (Cook et al., 1993), and treatment of non-insulin-dependent diabetes (Houseknecht et al., 1998).

The level of CLA intake that would result in beneficial properties is 3 g/d, as extrapolated from animal studies (Ip et al., 1994). However, Fritsche and Steinhart (1998) reported total CLA intake for German men and women to be only 350 and 430 mg/d, respectively. In regards to marketing of foods with elevated CLA, consumers with a family history of cancer, female, and over the age of 25 were found to be more willing to pay extra

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Table 1. Composition of conjugated linoleic acid oil, as provided by Certificate of Analysis by Crokers Croklaan (Channahon, IL).

Fatty acid composition (as FAME)	% of FAME ¹
C16:0	5.2
C18:0	1.6
C18:1	12.5
C18:2	1.3
CLA, cis-9/trans-11	36.9
CLA, cis-10/trans-12	36.7
Other CLA isomers	8.3
CLA oxidized	0.2
C20:0	0.1

¹FAME = Fatty acid methyl esters.

for milk with increased concentrations of CLA. The growth of the functional beverage market has far outpaced the beverage category as a whole and has led all other functional food segments (Hollingsworth, 2000). This indicates a possible market for such products (Ramaswamy et al., 2001).

Consumer acceptability studies were conducted on milk with naturally increased levels of CLA, through modified feeding regimes of cows (Ramaswamy et al., 2001). Milk with 2.30 g of total CLA/100 g of fat and 2.58% (wt/wt) milkfat, or ~100 mg of CLA per serving was compared to milk from a control-feeding regime that resulted in milk with a CLA content of 0.56 g of total CLA/100 g of fat and 3.51% (wt/wt) milkfat. No significant differences in consumer acceptability between milks with or without elevated levels of CLA were noted. However, to receive the recommended 3 g/d (Ip et al., 1994), approximately 30 servings per day (8 oz) of milk with naturally increased levels of CLA would be necessary. Direct fortification of milk with CLA oil may be a more feasible and realistic solution to obtain maximum health benefits. The effects of CLA fortification on the quality of milk have not been analyzed to our knowledge and were the objectives of this study. The conjugated bonds in CLA decrease the oxidative stability of the CLA oil (Nawar, 1996), which may result in decreased nutritional quality and flavor characteristics (Karpinska et al., 2001). Two natural antioxidants, vitamin E (tocopherols) and rosemary extract were evaluated for their effectiveness in preventing lipid oxidation in milks containing added CLA. Natural antioxidants were chosen since consumers generally prefer them to synthetic antioxidants (Karpinska et al., 2001).

MATERIALS AND METHODS

Materials

The CLA oil (Clairinol G-80) was generously supplied by Loders Croklaan (Channahon, IL) (Table 1). The

CLA oil was produced from sunflower oil, and CLA content was increased enzymatically using a pilot-scale process. Reagent grade chemicals used in the study were hexane, hydrochloric acid, chloroform, methanol, and anhydrous sodium sulfate (Fisher Scientific, Pittsburgh, PA). StabiEnhance Water Soluble Rosemary (NaturEnhance, Product # 2411, Long Beach, CA) and vitamin E MTS 70 (minimum of 70% tocopherols with a maximum of 20% are d-alpha tocopherol, ADM, Decatur, IL) were used as the antioxidant treatments. For the chocolate milk formulation, cocoa powder mix (Super 8 Cocoa Mix (cocoa processed with alkaline, corn starch, salt, carageenan, and vanillin), Benjamin P. Forbes Company, Cleveland, OH) and cane sugar (local store) were utilized.

Milk Preparation

Raw skim milk and cream were collected separately from the NCSU Dairy and Process Applications Laboratory (Raleigh, NC) in sanitized stainless steel containers and held at 3°C until use (<8 h). Fat content of the cream and skim milk were determined using the Babcock test. Approximately, 3.7 L of milk was HTST pasteurized for each treatment. Skim milk with cream and/or CLA (for 2% total fat) were pasteurized for 16 s at 77.2°C (171°F) followed by dual stage homogenization at 13,789/3,447 KPa. If necessary, vitamin E was added at levels of 200 ppm (wt/wt of total milk) or rosemary extract at 0.1% (wt/wt based on fat content) per usage levels recommended by the manufacturers. Milks were bottled in Nalgene bottles (Rochester, NY) and cooled on ice to 3°C. For gas chromatography, samples were frozen at -80°C until analysis.

Chocolate milk (consumer testing only) was prepared by collecting raw skim milk and cream separately from the NCSU Dairy and Process Applications Laboratory (Raleigh, NC) in sanitized stainless steel containers and holding at 3°C until use (<8 h). Fat content of the cream and skim milk was determined using the Babcock test. As before, 2% total fat milk was made by adding cream and/or CLA to skim milk. Prior to HTST pasteurization, 1.2 g of cocoa powder mix/100 g of milk and 7.6 g of sugar/100 g of milk was mixed into the milk. Approximately, 10 L of milk was HTST pasteurized for each treatment for 25 s at 85°C (185°F) followed by dual-stage homogenization at 13,789/3447 KPa. Milks were cooled on ice and held at 5°C until sensory analysis.

Fatty Acid Analysis

Fat was extracted from 1 g of milk using the Folch procedure as modified by Chin et al. (1992). Internal

standard (10 mg/ml of C17:0) was added to the milk before extraction. Twenty milliliters of chloroform and methanol (2:1) was added and homogenized with a tissue homogenizer for 1 min. Five milliliters of KCl (4%) in water was added. The mixture was shaken gently for 10 s and centrifuged at $650 \times g$ for 10 min. The top layer was removed, and the bottom layer filtered. The filtered solution was washed 2 times with double deionized water, and anhydrous sodium sulfate 1 g was added to remove any remaining water. The solution was filtered into a preweighed test tube and dried under nitrogen before weighing to calculate the amount of fat extracted. After the fat was extracted, 5 ml of 4% HCl in methanol was added (Chin et al., 1992). The tube was heated in a water bath at 50°C for 30 min. The tube was cooled to room temperature and 1 ml of double-deionized water was added and the tube was vortexed for 10 s. Hexane (5 ml) was added, and the mixture was shaken for 2 min. The bottom layer was removed and discarded. The remaining layer was washed twice with 1 ml of double deionized water, the bottom water layer was removed, and anhydrous sodium sulfate (1 g) was added to remove any remaining water.

Gas Chromatography

The fatty acid composition of fat in the milks was analyzed by gas chromatography using a Perkin Elmer Gas Chromatograph XL Autosampler (Shelton, CT) equipped with a flame-ionization detector. Turbochrom software Version 5.1 (Perkin Elmer, Shelton, CT) was used for data collection and peak integration. The column used was a BPX-70-1, 30 m $\times$ 0.25 mm i.d., 0.25- μ m film (J & W Scientific, Folsom, CA). Helium was used as a carrier gas with a head pressure of 20 Psi with a split flow rate of 40:1. The initial temperature was set at 60°C with a 2-min hold. A rate of 10°C/min was used until the column temperature was 180°C. The rate was decreased to 4°C/min to a final temperature of 265°C. Peak identification was confirmed by verifying retention times with pure standards.

Static Headspace Gas Chromatography

Static headspace gas chromatography (**S-GC**) was used to determine lipid oxidation headspace volatiles of milks. Milks were defrosted in a covered water bath (25°C) for approximately 2.5 h, or until completely thawed. Milk (2 g) and 1 g of sodium sulfate were added to a 25-ml headspace vial (Perkin Elmer, Shelton, CT). Ten microliters of 3-heptanone (200 ppm) (Sigma-Aldrich, St. Louis, MO) in n-hexadecane was added as an internal standard (I. S.). A Perkin Elmer (Shelton, CT) Gas Chromatograph Autosampler XL equipped with a

flame-ionization detector was used with Turbochrom (Perkin Elmer, Shelton, CT) software used for peak integration. The samples were heated for 30 min at 100°C. The temperature of the transfer line was 105°C and the needle temperature was 110°C. The vial was pressurized for 4 min and injected for 1 min. The detector temperature was set at 280°C and the injector temperature at 140°C. Helium was used as the carrier gas with a flow rate of 10 Psi and a split flow rate of 5:1. The column used was a Zebron (Torrance, CA) ZB-5 30 ml $\times$ 0.25 mm ID $\times$ 1.0 μ m film thickness with 5% phenyl polysiloxane. The initial column temperature was 35°C and held for 1 min. The column was then heated to 300°C at a rate of 15°C. Identification was performed using a Hewlett Packard MSD 5972 mass spectrometer set at 70 eV ionization potential with a scan range of 35 to 350 atomic mass units along with matching of retention times of known compounds. All the reference compounds were obtained from Sigma-Aldrich (St. Louis, MO). Volatiles that were identified and quantified included hexanal, pentanal, 2-4 decadienal, nonanal, and total volatiles. Quantification was determined as the ratio of the internal standard to the compound of interest multiplied by the relative response factor (**RRF**).

Concentration of volatile =

$$\frac{\text{Concentration of IS}}{\text{Peak area of IS}} \times \text{Peak area of volatile} \times \text{RRF}$$

$$\text{RRF} = \frac{\text{Concentration of known volatile standard}}{\text{Peak area of known volatile standard}} \times \frac{\text{Peak area of IS}}{\text{Concentration of IS}}$$

Physical Measurements

Total fat of processed milks was confirmed by the Babcock test. Color was measured with a hand-held colorimeter (Minolta Corporation, Ramsey, NJ) using the Hunter color scale (L^* , a^* , b^*). The viscosity of the milk was measured using a StressTech Controlled Stress Rheometer (ATS Rheosystems, NJ/ReoLogica Instruments AB, Lund, Sweden) using a concentric cylinder geometry (CC25). Samples were presheared for 30 s at 15 1/s to ensure sample uniformity and kept at a constant temperature of 20°C. Apparent viscosity and shear rates were recorded as the shear stress was ramped from 0.05 to 25 Pa. Aerobic Plate Count Petrifilm (3M, St. Paul, MN) was used to enumerate bacteria in milk. Milks were diluted in 0.1% (wt/wt) peptone water.

Table 2. Definitions and references of sensory attributes.

Attribute	Reference	Description
Grassy/vegetable oil	CLA ¹ oil	Grassy/vegetable oil aromatic associated with CLA oil
Milk fat	Heavy cream	Aromatic associated with milk fat
Astringency	Tea	Mouthfeel (drying out sensation) after consuming tea or coffee
Sweet taste	5% sucrose solution	Basic taste associated with sugars

¹CLA = Conjugated linoleic acid.

Sensory Analyses

All preparation of milks for sensory analyses were conducted with overhead lights turned off to minimize light exposure and the possibility of light induced flavors. Descriptive sensory analysis of selected flavor attributes was conducted on milks using nine panelists (seven females, two males). All panelists had previous experience (>50 h) with descriptive sensory analysis of dairy products. Two 1-h training sessions were conducted where panelists evaluated and discussed flavor and tastes of milks with or without added CLA. The following attributes were selected: milkfat, grassy/vegetable oil, sweet taste, and astringency (Table 2). Milks were dispensed into 2-oz soufflé cups with lids and coded with random three-digit codes. Each treatment was evaluated in duplicate by each panelist weekly. Milks were tasted at time 0 (within 24 h of pasteurization), 1 wk, and 2 wk. Samples were not tasted on wk 3 due to microbial spoilage (which was determined by aroma, appearance, and plate counts). Panelists evaluated each descriptor for each milk sample using a 10-cm line scale anchored on the left with “none” and on the right with “extreme.” Milks were evaluated at 5°C.

Consumer acceptability testing was conducted on two different days. On the first day, white milks were evaluated: three treatments (2% CLA, 1% CLA:1% milkfat, and 2% milkfat). Consumers (n = 101) evaluated overall acceptability, overall flavor, and freshness of milks using a 9-point hedonic scale. Milks were served in randomized balanced order of presentation in Styrofoam cups with three-digit codes and lids with straws. Before tasting milks, consumers also filled out an informed consent form consistent with NCSU Human Subjects Approval and filled out a brief questionnaire on demographic information, milk consumption habits, and interest in nutraceutical/functional foods. Subsequently, consumer acceptability testing (n = 100) was conducted with chocolate milks (2% CLA, 1% CLA:1% milkfat, and 2% milkfat) analogously.

Experimental Design

A three × three factorial arrangement of treatments (type of fat × antioxidant) with repeated measures was

implemented. The fat level was kept constant 2% (wt/wt): 2% CLA, 1% CLA:1% milk fat, and 2% milk fat. The following antioxidants were incorporated: control (no antioxidant), 200 ppm (wt/wt of total milk) vitamin E, and 0.1% (wt/wt of total milkfat) rosemary extract. The entire experiment was replicated twice with different lots of milk. For FAME analysis, sensory analysis, and viscosity, measurements were taken in duplicate. For color, volatiles, and APC, measurements were taken in triplicate. Data was analyzed using analysis of variance (PROC GLM) with means separation to determine differences among treatments (SAS version 8.0, Cary, NC). Significance was established at $P < 0.05$.

RESULTS

Fatty Acid Analysis

According to the Certificate of Analysis (Table 1) for the CLA triglyceride oil, the *cis-9/trans-11* isomer accounted for 36.9% of the FAME and the *cis-10/trans-12* isomer accounted for 36.7% (wt/wt). Our analysis indicated the oil was comprised of 33.04% of the *cis-9/trans-11* isomer and 33.68% of the *cis-10/trans-12* isomer before HTST pasteurization (Table 3). Park et al. (2001) investigated the effects of various methylation procedures on CLA composition. The use of HCl/MeOH (4%) at 50°C for 20 min did not result in the formation of artifacts or impurities; however, the methylation was found to be incomplete. No single method will methylate CLA without problems, and caution must be used when derivatizing CLA. The method of analysis may account for the differences in reported isomer concentrations or actual differences may exist.

A significant loss of the *cis-9/trans-11* isomer was seen immediately after HTST pasteurization, within less than 24 h of refrigerated storage (Table 3). A slight, insignificant increase in the *cis-9/trans-11* isomer was seen at wk 1 and 2 of refrigerated storage. The *cis-10/trans-12* isomer was not significantly reduced due to pasteurization and storage time of up to 3 wk did not result in a significant decline in concentration. All other minor CLA isomers declined after pasteurization ($P < 0.05$), but remained stable during 3 wk of refrigerated storage. The loss of the *cis-9/trans-11* isomer at wk 3

Table 3. The effects of HTST pasteurization and 3 wk of refrigerated storage on the composition of conjugated linoleic acid (CLA) isomers in fortified milk (2% CLA).

Time	% <i>cis</i> -9/ <i>trans</i> -11 Isomer (as FAME ¹)	% <i>cis</i> -10/ <i>trans</i> -12 Isomer (as FAME)	Other isomers (as FAME)
Oil before HTST pasteurization	33.04 ^a	33.68 ^a	4.7 ^a
Time 0	29.69 ^{bc}	30.12 ^a	2.07 ^b
wk 1	31.18 ^{ab}	31.81 ^a	0.93 ^b
wk 2	31.96 ^{ab}	33.30 ^a	1.35 ^b
wk 3	27.79 ^c	30.88 ^a	1.07 ^b

^{a,b,c}Means within the same column with different letters are significantly different ($P < 0.05$).

¹FAME = Fatty acid methyl esters.

was attributed to excessive microbial growth. The addition of antioxidants had no effect on the oxidative stability of the CLA isomers ($P > 0.05$, data not shown).

The milkfat in this study had a higher proportion of C18:1 and lower concentration of C16 than other published values (Jensen et al., 1991; Table 4). While most dairy cattle are confined and fed grain, the cattle used in this study are allowed to naturally graze and this may explain the higher concentrations of unsaturated fat. Diets of pasture resulted in threefold variation of CLA concentration in milk fat among individual cows fed the same diet (Kelly et al., 1998). Typical concentrations of CLA in milkfat range from 3 to 6 mg/g, with large variations existing by herd. Typical CLA concentrations of unfortified 2% milk fat milk in this study were 5 to 10 mg CLA/g of milk fat.

Volatile Analysis

No significant differences in selected volatiles (hexanal, nonanal, 2-4 decadienal, pentanal) or total volatiles were found between the treatments nor was there a storage effect for these volatiles ($P > 0.05$, data not shown). The average concentration of hexanal was 43.2 ppb (SD = 12.6 ppb) and the average concentration of

pentanal was 18.7 ppb (SD = 5.7 ppb). 2-4 Decadienal and nonanal were found to be below the limits of detection (10 ppb).

Color

Instrumental color differences were observed between milks with varying levels of CLA (Table 5) and color changes occurred with storage time (Table 6). There was not a treatment $\times$ time interaction nor did the presence of antioxidants impact color ($P > 0.05$, data not shown). Milk fortified with 2% CLA had a significantly lower L* value (95.01) than 2% milkfat (103.49), indicating milk fortified with CLA is less white compared with typical milkfat (Table 5). The b* value is an indicator of blue (–) and yellow (+). The 2% CLA had a b* value of 0.55 indicating less yellow color compared to 2% milkfat milk (6.82). The a* values indicates that milk fortified with CLA has less red color compared with typical milkfat. L*, a*, and b* values decreased within all treatments after wk 1 and 2 of refrigerated storage (Table 6). A significant increase in the L*, a*, and b* values occurred after 3 wk of storage and was attributed to excessive microbial growth. Lipases are produced during the late log and early stationary phases of growth after cell numbers reach 10^6 to 10^7 (Rowe et al., 1990) and initiate agglutination of the fat globules into cream flakes or flecks (Labots and Galesloot, 1959).

Table 4. Composition of milk fat used in this study to other published values.

FAME ¹	Control milk (% FAME)	Previously published values (% FAME)
< C12:0	2.01	9.2
C12:0	3.19	3.1
C14:0	11.42	14.2
C14:1	1.24	0.0
C15:0	1.33	1.3
C16:0	34.99	42.7
C16:1	1.57	3.7
C18:0	15.77	5.7
C18:1	24.93	16.7
C18:2	1.68	1.6 (includes conjugated)
C18:3	0.08	1.8
CLA	1.51	N/A

¹FAME = Fatty acid methyl esters.

Table 5. Color values (L*, a*, and b*)¹ of milks with and without conjugated linoleic acid (CLA).

Treatment	L*	a*	b*
2% CLA	95.01 ^c	–1.04 ^c	0.55 ^c
1% CLA:1% Milk fat	100.53 ^b	0.58 ^b	4.99 ^b
2% Milk fat	103.49 ^a	1.64 ^a	6.82 ^a

^{a,b,c}Means within the same column with different letters are statistically different ($P < 0.05$).

¹L* = Black (0) to white (100+); a* = green (–) to red (+); b* = blue (–) to yellow (+).

Table 6. Color changes in milks during refrigerated storage.¹

Time	L*	a*	b*
Time 0	100.62 ^a	0.72 ^a	4.28 ^a
wk 1	97.84 ^b	-0.19 ^b	3.84 ^a
wk 2	97.50 ^b	-0.25 ^b	2.70 ^b
wk 3	103.89 ^c	1.63 ^c	6.42 ^c

^{a,b,c}Means with the same column with different letters are significantly different ($P < 0.05$).

¹L* = Black (0) to white (100+); a* = green (-) to red (+); b* = blue (-) to yellow (+).

Viscosity

Viscosity was not significantly different among milks nor was a change seen during storage (data not shown). The average mean viscosity of the milk samples was 170 $\mu\text{Pa}\cdot\text{s}$ (SD = 1.5 $\mu\text{Pa}\cdot\text{s}$).

Microbial Growth

Microbial counts were determined as an additional quality control measure. Since we were primarily looking at the effects of CLA on flavor, we did not want to confuse microbial spoilage flavors with other milk flavors. Microbial counts were not different among treatments and similar increases were seen during storage. Time zero counts were 1.2×10^2 cfu/ml (SD 4.4×10^1 cfu/ml). For wk 1 and 2, plate counts were 1.1×10^2 and 4.0×10^3 cfu/ml (SD 8.3×10^1 and 4.3×10^2 cfu/ml, respectively). Microbial counts of approximately 1×10^6 cfu/ml were seen after 3 wk of refrigerated storage, and the milk was considered spoiled (Hayes et al., 2002).

Descriptive Sensory Analysis

The presence of antioxidants had no effect on the sensory properties of milks ($P > 0.05$, data not shown). Differences in flavor were observed between treatments with different CLA concentrations (Table 7) and differences occurred during refrigerated storage (Table 8). There was not a treatment $\times$ time interaction, so only main effects are shown ($P > 0.05$; Tables 7 and 8). No significant differences were found in astringency or sweet taste between milks ($P > 0.05$). Milks containing CLA were all significantly higher in grassy flavor and

lower in milkfat flavor compared to the 2% milk fat control. Significant decreases in grassy/vegetable oil and milkfat flavor were seen at wk 1 and 2 wk of refrigerated storage (Table 8).

Consumer Acceptability

Consumers were fairly evenly split between the sexes (46% female, 54% male) and 66% were between the ages of 18 and 35 yr. Seventy-five percent consumed dairy products more than once per day; 73% consumed milk at least 3 to 4 times per week. Forty-one percent normally purchased skim milk, whereas 32% regularly consumed 2% fat milk. Consumers indicated taste and nutrition (78 and 70%) as primary reasons for consumption and purchase of milk. Ninety-six percent indicated some interest in nutraceutical/functional foods, and 89% indicated they definitely or might be willing to pay more for nutraceutical or functional foods. (A definition and examples of nutraceutical/functional foods were provided in the questionnaire.) Consumers also noted differences between milks with and without added CLA ($P < 0.05$).

The addition of CLA to milk decreased overall acceptability, overall flavor, and freshness perception of milk (Table 9). Ten panelists made comments that the milk with added CLA seemed watered down and less creamy, even though fat levels were kept constant.

In an effort to increase consumer acceptability, testing was conducted with chocolate-flavored milks with and without added CLA (Table 10). Population demographics were similar to the previous consumer test; 53% of consumers were male, 47% were female and 61% were between the ages of 18 and 35 yr old. Seventy-one percent consumed milk at least 3 to 4 times per week (97% at least once per week). All consumers indicated that they liked chocolate milks, although regular consumption or purchase of chocolate milks was not as frequent; 20% consumed chocolate milk at least once per week, 40% at least once per month. Chocolate milks with 2% milkfat were the most frequent fat content purchased, (51%) with skim/nonfat and whole also purchased (21 and 22%, respectively). The addition of chocolate flavor increased acceptability, flavor, and freshness scores of control (no CLA) and 1% CLA:1% milkfat

Table 7. Descriptive sensory results (10-cm line scale) of milk fortified with different levels of conjugated linoleic acid (CLA).

Sample	Milk fat	Grassy	Sweet taste	Astringency
2% CLA	1.35 ^b	2.95 ^a	1.98 ^a	2.55 ^a
1% CLA/1% milk fat	2.50 ^{ab}	2.93 ^a	1.48 ^a	2.38 ^a
2% milk fat	3.45 ^a	0.50 ^b	2.03 ^a	2.33 ^a

^{a,b}Means within the same column with different letters are significantly different ($P < 0.05$).

Table 8. Effect of refrigerated storage on the sensory attributes (10-cm line scale) of the milk samples.

Time	Cream	Grassy	Sweet taste	Astringency
Time 0	2.75 ^a	3.70 ^a	2.18 ^a	2.38 ^{ab}
wk 1	2.18 ^b	1.95 ^b	1.63 ^a	2.28 ^b
wk 2	2.00 ^b	1.80 ^b	1.55 ^a	2.73 ^a

^{a,b}Means within the same column with different letters are significantly different ($P < 0.05$).

milks ($P < 0.05$). In particular, acceptability of the 1% added CLA milk scores increased by nearly 1 point for overall acceptability, flavor, and freshness (5.6 to 6.41, 5.3 to 6.2, and 5.5 to 6.4, respectively). Scores for 2% CLA milks were not impacted much by the addition of chocolate. Scores remained less than 5.5 on a 9-point hedonic scale (Table 10).

DISCUSSION

The bland taste of milk makes it susceptible to flavor defects arising from the autooxidation of unsaturated fatty acids (O'Connor and O'Brien, 1994). Yang et al. (2000) compared the stability of CLA against linoleic acid when exposed to heat and air and found CLA more susceptible to oxidation, suggesting a conjugated bond is more susceptible to autooxidation than a nonconjugated double bond. Our study found no significant differences in volatile profiles between the different milk samples or during refrigerated storage. Volatile lipid oxidation products present in small concentrations may be undetectable using S-GC alone.

Lipid oxidation appears to result in both the production and decomposition of CLA. CLA is formed as an intermediate product during the biohydrogenation of linoleic acid to stearic acid in rumenic animals (Chin et al., 1992). In cheeses, for example, processing temperatures, incorporation of air, and aging all play a role in lipid autooxidation and final CLA concentrations (Chin et al., 1992; Shantha et al., 1995).

HTST pasteurization resulted in an initial significant decline of the *cis-9/trans-11* isomer and other minor CLA isomers. A slight increase in the concentration of the *cis-9/trans-11* isomer seen at wk 1 and 2 of refrigerated may be seen as evidence of free radical oxidation resulting in linoleic acid being converted to CLA. Although a similar decrease of the *cis-10/trans-12* isomer occurred after pasteurization followed by an increase during storage, the changes were not significant. Leung and Liu (2000) compared the oxidative stability of the *cis-9/trans-11* and *cis-10/trans-12* isomers using total oxyradical scavenging capacity assays. At concentrations ranging from 2 to 200 μmol , the *cis-10/trans-12* isomer exhibited antioxidant behavior. Conversely, the *cis-9/trans-11* isomer possessed only weak antioxidant activity at low concentrations (2 and 20 μmol) and pro-

oxidant activity at higher concentrations (200 μmol). The significant changes that occurred in the concentration of the *cis-9/trans-11* isomer after HTST pasteurization confirm the results of Leung and Liu (2000). One viable option to increase the oxidative stability of the CLA is to encapsulate the oil with cyclodextrins (Kim et al., 2000).

The significant decrease in the concentration of the *cis-9/trans-11* isomer seen after 3 wk of refrigerated storage was attributed to high microbial counts ($>\log 6$ cfu/ml). Lipolysis by bacterial enzymes contributes to an increased content of free fatty acids (Nawar, 1996). Free fatty acids are significantly more susceptible to lipid oxidation compared to the esterified form.

Antioxidants were incorporated into the experimental design as a means to prevent lipid oxidation. However, the use of antioxidants did not have a significant effect on descriptive sensory data, volatiles, color, or FAME profiles. Perhaps if the milk samples had been exposed to light and/or temperature abuse, the antioxidants may have shown a protective effect.

The research of Phillips et al. (1995) indicated that the appearance (color) of milk had a profound effect on the perceived mouthfeel of milk and consumer acceptability of skim milk. Our research indicated that milk fortified with CLA had significantly less white color and more blue color than typical milkfat at the same concentration. The L^* , a^* , and b^* values for 2% CLA-fortified milk were 95.01, -1.04 , and 0.55 and 103.49 , 1.64 , and 6.82 for 2% milkfat milk. The high concentration of saturated fat found naturally in milk results in a significantly whiter appearance than milk fortified with CLA oil, which is comprised primarily of unsaturated bonds.

The fortification of fluid milk with CLA resulted in the presence of a grassy/vegetable oil flavor coupled

Table 9. Consumer sensory results (9-point hedonic scale) of milk with different levels of conjugated linoleic acid (CLA).

Sample	Overall acceptability	Overall flavor	Freshness
2% CLA	4.8 ^c	4.6 ^c	5.0 ^b
1% CLA:1% Milk fat	5.6 ^b	5.3 ^b	5.5 ^b
2% Milk fat	6.4 ^a	6.4 ^a	6.3 ^a

^{a,b,c}Means within columns with different letters are statistically different ($P < 0.05$).

Table 10. Consumer sensory results (9-point hedonic scale) of chocolate flavored milk with different levels of conjugated linoleic acid (CLA).

Sample	Overall acceptability	Overall flavor	Freshness
2% CLA	5.01 ^c	4.65 ^c	5.20 ^c
1% CLA:1% Milk fat	6.41 ^b	6.22 ^b	6.43 ^b
2% Milk fat	7.04 ^a	7.07 ^a	7.07 ^a

^{a,b,c}Means within columns with different letters are statistically different ($P < 0.05$).

with a significant decrease in milkfat flavor. The CLA oil itself was used as a reference grassy/vegetable oil flavor by the descriptive sensory panel, indicating that this flavor at some intensity was present in the CLA oil before addition to milk. Native fats, such as butterfat, contain a large number of naturally occurring flavor compounds (Mela and Raats, 1994) that contribute to the flavor of milk. Because we were unable to confirm lipid oxidation by instrumental volatile analysis, and grassy/vegetable oil flavors did not increase with storage, it seems probable that flavors in CLA fortified milk were inherent in the CLA oil and not a product of storage induced oxidation of CLA oil.

Factors other than color and viscosity may play a role in the mouthfeel of milk. For consumer testing, the milk samples were poured into Styrofoam cups with lids, and panelists were given straws to prevent color from having an influence on flavor perception and acceptability (and to prevent light-induced oxidation). With white (unflavored) milks, 10 consumers made comments indicating perceived differences in mouthfeel; however, no significant differences in viscosity between samples were measured. Descriptive panelists also indicated differences in mouthfeel. Mela et al. (1994) found that a fat source with a relatively higher amount of saturated fat is perceived as having a higher fat content than foods containing higher amounts of unsaturated fat, which may provide insight into the results found in this study. Consumers and descriptive panelists may have been responding to the decrease in milkfat and milkfat-associated flavors.

Consumers rated milk fortified with CLA as having significantly lower acceptability, flavor liking, and perceived freshness compared to the control (2% milkfat milk). The combination of 1% CLA/1% milkfat resulted in increased consumer acceptability and flavor scores compared with the 2% CLA treatment; however, freshness scores were not significantly different between the two CLA milks. In contrast, the addition of chocolate flavor increased acceptability of control (no CLA) and 1% CLA/1% milkfat milks. Although scores for chocolate-flavored 1% CLA milks were still lower than control chocolate milks, hedonic scores did indicate the product

was acceptable (6.4, 6.2, and 6.4 for acceptability, flavor, and freshness, respectively). The optimization of a chocolate flavor specifically for 1% CLA/1% milkfat milk or addition of other flavors might further improve consumer acceptability.

The survey given to consumers before evaluation indicates the market potential for a CLA-fortified milk beverage. Consumers indicated taste and nutrition (78 and 70%) as primary reasons for consumption and purchase of milk. The current market for functional foods in the United States is \$18.25 billion, with beverages projected to remain the largest segment of the market, accounting for \$8.9 billion of the US market (NBJ, 2001). One-third of consumers agree that fortified foods are worth paying a premium for because fortified foods help them to take a more active role in their own health management (HealthFocus, 2001).

CONCLUSIONS

The addition of 2% CLA to skim milk, while a useful treatment for studying overall effects in this study, probably represents a level that would be unrealistic in the food industry due to cost constraints and limited production. It is estimated that the addition of CLA at the 2% (wt/wt) level in milk would increase the cost of milk an estimated \$0.20 to \$0.25 per 8-oz serving. A lower concentration of CLA (i.e., 0.5% CLA/1.5% milk fat (wt/wt)) may help to further increase consumer acceptability of CLA-fortified milk and would be more economically feasible for industry application. The addition of chocolate or strawberry flavors is another realistic solution to increase consumer acceptability. Regular consumption of milk and other dairy products coupled with consumers' interest in nutraceutical/functional foods indicates a market for a CLA-fortified milk beverage.

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