Effect of Heating and Processing Methods of Milk and Dairy Products on Conjugated Linoleic Acid and *Trans* Fatty Acid Isomer Content

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ABSTRACT

The conventional heating methods of milk did not cause any significant increase in the *trans* isomer content, with the exception of milk heated at $63 \pm 1.0^\circ\text{C}$ for 30 min and milk microwaved for 5 min, which were significantly increased by 19 and 31%, respectively. The chemical changes of lipids were generally accelerated with the severity of the heat treatment and duration of storage. The conjugated linoleic acid content of cheese heated in a microwave oven for 5 min decreased by 21%, and microwave heating for 10 min caused a decrease of 53% compared with that of freshly boiled cheese.

(**Key words:** Nabulsi cheese, microwave, conventional heating, conjugated linoleic acid)

Abbreviation key: CLA = conjugated linoleic acid.

INTRODUCTION

Milk lipids are considered one of the outstanding milk constituents with respect to presence of lipid classes, and variety and number of identified fatty acids, which was found to be more than 400 (Jensen et al., 1990, 1991). Milk lipids include anticarcinogenic compounds such as conjugated linoleic acid (CLA), sphingomyelin, and butyric acid (Ames et al., 1995; Parodi, 1999). Milk lipids may undergo chemical and physical changes during processing and storage such as auto-oxidation and formation of *trans* fatty acids (Semma, 2002).

Trans fatty acids are naturally found in low concentrations in dairy products because of the biohydrogenation process in the rumen but may also be formed during processing of dairy products at high temperatures such as in baking or frying. *Trans* fatty acids have been associated with biological and toxicological effects such as coronary heart disease, and disturbances of the metabolism of the essential fatty acids in the fetus, which could affect intrauterine human growth (Addis, 1990;

Addis and Warner, 1991; Kumar and Singhal, 1991; Willett and Ascherio, 1994; Boué et al., 2000).

Milk and milk products usually undergo different changes during their preparation (boiling and microwaving) or processing, which may include moderate or severe heat treatments that can lead to undesirable changes in lipids or proteins. Microwave ovens are widely used for cooking and reheating of foods in millions of kitchens throughout the world. Food heating by microwave results from the conversion of microwave energy into heat by friction of vibrating water molecules due to rapid fluctuations in the electromagnetic field (Decareau, 1992; Potter and Hotchkiss, 1996).

The trend of using microwave ovens in food preparation is attributed to the speed of heating and energy saving. Although microwave ovens are widely used as a means of food preparation, insufficient information is available on the consequences for the composition and nutritional quality of food. Some studies revealed that microwave heating affected fat oxidation and fatty acid isomer formations (Albi et al., 1997a,b).

Variations in heating treatments may have different effects on CLA and *trans* isomers. Therefore, the objective of this study was to evaluate the effect of heating treatments (microwave and conventional heating), processing steps of milk and milk products [yogurt, labaneh, brined white cheese (Nabulsi), and UHT], and storage conditions on CLA and *trans* fatty acid isomer contents.

MATERIALS AND METHODS

Raw cows' milk used in the study was obtained from the bulk tank of 3 dairy plants: Danish Jordan Dairy Company, Jordan University Dairy Plant, and Al-Sanabel Dairy Co. The milks were produced by the Cows' Breeder Society, the Jordan University farm, and the Haj Mustafa farm, respectively. Brined white cheese (Nabulsi) was produced from ewes' milk provided by Al-Sanabel Dairy Co.; yogurt and labaneh were from Danish Jordan Dairy Company and Jordan University Dairy Plant, respectively. The UHT (at $140 \pm 1.0^\circ\text{C}$) and pasteurized (at $85.0 \pm 1.0^\circ\text{C}$) milk were from Danish Jordan Dairy Company.

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Table 1. Cows' milk and milk products produced by different heat treatments.

Milk producer/source	Heating treatment			Product
	Type	Temperature (°C)	Time	
Cow's Breeder Society/ Danish Jordan Dairy Co.	Tube pasteurization	85	16 s	Pasteurized milk
		95	5 min	Yogurt
		82	16 s	Labaneh
		140	4 s	UHT milk
	Lab-scale pasteurization	63 ± 1.0	30 min	Pasteurized milk
	Lab-scale boiling	97.5 ± 1.0	5 min	Boiled milk
	Microwave boiling	96.8 ± 1.0	5 min	Microwave-boiled milk
Mahmoud dairy farm/ Al-Sanabel Dairy Co.	Plate pasteurization	95	15 min	Yogurt
		95	15 min	Labaneh
	Lab-scale pasteurization	63 ± 1.0	30 min	Pasteurized milk
	Lab-scale boiling	97.5 ± 1.0	5 min	Boiled milk
	Microwave boiling	96.8 ± 1.0	5 min	Microwave-boiled milk
Jordan University dairy farm/ Jordan University dairy plant	Batch pasteurization	85 to 90	2 min	Yogurt
		85 to 90	2 min	Labaneh
	Lab-scale pasteurization	63 ± 1.0	30 min	Pasteurized milk
	Lab-scale boiling	97.5 ± 1.0	5 min	Boiled milk
	Microwave boiling	96.8 ± 1.0	5 min	Microwave-boiled milk

Heat Treatments of Milk and Milk Products

The raw cows' milk obtained from the 3 sources was subjected to different heat treatments as shown in Table 1.

Production of Set Yogurt

Set yogurt was produced from milk pasteurized at 95 ± 1.0°C for 5 min (tube pasteurization), 95 ± 1.0°C for 15 min (plate pasteurization), or 85 to 90°C for 2 min (batch pasteurization). The milk was cooled to 45 ± 1.0°C and inoculated with 2 to 3% freeze-dried mixed starter culture (Danisco, Denmark). It was then poured into plastic containers of different sizes, and incubated at 42 ± 1.0°C for up to 2.5 h. When the desired acidity of 0.7% (pH 4.5 to 4.6) was reached, the yogurt was cooled to 4.0 ± 1.0°C.

Labaneh Production

Traditional method (cloth sack). After cooling, the set yogurt was stirred and then poured into a cloth sack to drain off the whey for 12 to 24 h (overnight). The drained yogurt labaneh was salted with 1% NaCl, blended, poured into suitable plastic containers, and refrigerated at 4°C. The produced labaneh was 23 to 25% total soluble solids and had a pH of 5 to 5.5.

Separator method (centrifugal separator). Cream was separated, milk was pasteurized at 83 to 85°C for 16 s, salt (1% NaCl) was added, the pasteurized skim milk was inoculated with ~0.002% (wt/vol) powdered, freeze-dried, mixed starter culture (Danisco) and kept for 15 to 17 h at 42 to 44°C. When the pH of the

yogurt reached 4.5 to 4.6, the product was stirred and the whey separated via separator at 40°C. The concentrated skim yogurt and the cream (40% butter) were mixed to have total solids of not less than 23% and 10% fat. The produced labaneh was poured into suitable plastic containers and stored at 4°C.

Cheese Production and Heat Treatments

White brined cheese (Nabulsi) was produced according to the traditional method described by Humeid and Tukan (1986) and Humeid et al. (1990) from ewes' milk. Two desalted, grated cheese samples of approximately 200 g each were heated in a microwave oven (800 W, WD800B, Galanz, Korea) at 80% power. The first sample was heated at 96.3 ± 1.0°C until browning (~10 min), and the second was placed in a polyethylene bag and then in a Pyrex saucepan filled with water (distilled water), and boiled while floating in the microwave oven at 96.3 ± 1.0°C for 5 min. Another 2 cheese samples (~100 g each) were desalted, grated, placed in polyethylene bags in a Pyrex saucepan, covered with distilled water, and boiled on a gas stove for 5 min at 95.5 ± 1.0°C.

UHT Reconstituted Milk

Ultra-high temperature milk reconstituted from powdered milk (Kuwaiti Danish Dairy Co., Kuwait) was purchased from the local market for comparison (production date 09/09/02 and expired on 09/03/03).

Storage of Milk and Milk Products

The milk and milk products used in the study (pasteurized milk, UHT milk, yogurt, and labaneh) were

stored at $5.0 \pm 1.0^\circ\text{C}$ and analyzed after a storage period of 3, 5, 7, and 15 d for pasteurized milk, UHT milk, yogurt, and labaneh, respectively, as indicated on the package label (i.e., the commercial shelf life). The produced white brined cheese (Nabulsi) was evaluated after 1 mo of storage in tins at $18 \pm 1.0^\circ\text{C}$.

Milk Fat Extraction and Analysis

Lipids were extracted from the milk and milk products using chloroform and methanol as described by Bligh and Dyer (1959) with some modifications regarding sample weight, solvent volume, and centrifugation speed and time. Approximately 70 g of cheese, yogurt, or labaneh, or 100 mL of fluid milk product was homogenized with 100 mL of methanol and 100 mL of chloroform using a Hamilton Beach Scovel homogenizer (NSF, Racine, WI) for 2 min at medium speed. Then, 100 mL of chloroform was added to the mixture, and homogenized for an additional 2 min. The homogenate was centrifuged at 4000 rpm for 20 min using an Heraeus centrifuge (Heraeus Christ, GmbH, Osterode/Harz, Germany). The upper layer (methanol and water) was removed through aspiration. The middle and the lower layers (chloroform and precipitated protein, respectively) were filtered through filter paper to separate precipitate particles. The chloroform-lipid extracts were again filtered through anhydrous sodium sulfate (Na_2SO_4) and the Na_2SO_4 was rinsed 3 times with 30 mL of chloroform (10 mL each rinse). The lipid extracts were dried under nitrogen using a roto-evaporator (Laborota, 4001 WB, Heidolph, Germany) at 150 rpm and 50°C , and stored in 5-mL brown glass vials under nitrogen at -18°C . The lipid samples were then used for the analysis of CLA and fatty acids *trans* isomers.

Chemical and Instrumental Analyses

Fourier transform infrared spectroscopy was used to determine *trans* isomer contents in lipid extracts. The American Oil Chemists Society official method Cd 14-61 was used for the determination of the *trans* content of fats (Walker, 1980; Gunstone, 1986). The transmittance of the isolated *trans* double bond was measured in the infrared region at a wavenumber of 967 cm^{-1} , which is equivalent to a wavelength of $10.34\text{ }\mu\text{m}$. The transmittant band of deformed C-H bond about the *trans* double bonds is typical of the isolated *trans* group. On the contrary, the *cis* and saturated fatty acids do not have such a spectrum. Therefore, measurement of such band intensity forms the basis for the *trans* isomers determination (Conacher, 1976; Svensson et al., 1982; Yamaoka et al., 1987; Mossoba et al., 1990; Ulberth and Haider, 1992; Mossoba et al., 1993; Toschi et al., 1993; Guillen and Cabo, 1997).

Esterification of Lipid Samples and Elaidic Acid

Two milliliters of elaidic acid (2 mg/mL) and 5 selected lipid extracts were accurately weighed (50 to 100 mg) and dissolved in toluene (1 mL). Two milliliters of 2% sulfuric acid (Fisher Scientific Co., Fairlawn, NJ) in methanol (Lab-Scan, Dublin, Ireland) was added. The mixture was incubated overnight in stoppered tubes at 50°C , then 5 mL of water containing 5% NaCl (GCC Laboratory, Clwyd, UK) was added and the esters were extracted 3 times with 3 mL of hexane (Lab-Scan) using Pasteur pipettes to remove each layer. The hexane layer was washed with 4 mL of 2% potassium carbonate (BDH Laboratory, Poole, UK). The solution was filtered through a filter paper (Ederol, 5.5 mm, Hatzfeld, Eder, Germany), and was then dried over 99.0% anhydrous sodium sulfate (SDS Fine Chemicals, Ltd., Mumbai, India). The solvent was removed under a stream of nitrogen gas (Christie, 1992). The dried extract was dissolved in carbon disulfide (GCC Laboratory) and *trans* isomers spectra were collected by a Fourier transform infrared spectrometer 670 (Thermo Nicolet Nexus, Thermo Electron Corp., at the Chemistry Department, Jordan University. A demountable liquid cell of 150 μL capacity, 0.015 mm thickness, and 25 mm diameter NaCl windows (Buck Scientific, Inc., Germany) was used for measurement of the *trans* isomers of the standard and the lipid extract.

Measurement of *Trans* Isomers

A calibration curve was made with elaidic acid ($\text{C}_{17}\text{H}_{33}\text{COOH}$ standard; BDH Laboratory Supplies) in the form of methylelaidate. The standards were dissolved in carbon disulfide at concentrations of 0.04, 0.08, 0.2, 1.0, 2.0, and 5.0 mg/mL. The demountable cell of the spectrometer was filled carefully to avoid air bubble entrapment. The cell was washed 3 times with pure carbon disulfide and 3 times with the analyzed fatty acid methyl ester before each analysis. Thirty analytical scans were used; the range spectrum was obtained at wavenumbers between 800 and 1200 cm^{-1} . Each sample was scanned 3 times in succession and scanned again after 10 min. The recovery test for methyl elaidate was conducted by spiking a starch sample (10 g) with 3 mL of methyl elaidate (20 mg/mL), followed by 4 replicate complete analyses. Then, the methyl ester was extracted, dried under nitrogen gas, dissolved in approximately 2 mL of CS_2 , transferred into a 25-mL volumetric flask, and the volume completed with CS_2 (GCC Laboratory). The minimum detection limit of *trans* isomers as methyl elaidate was found to be 20 ppm. The calibration curve is shown in Figure

Figure 1. Linearity of the results for methyl elaidate as measured by Fourier transform infrared spectroscopy.

1 and the averaged value of recoveries was 97.8% for methyl elaidate, as shown in Table 2.

CLA

Conjugated linoleic acid was determined by HPLC (Pump 600, 486 UV detector, manual injection Reodyne and 2010-Mellenium software, Waters Co., Milford, MA) using the chromatograph at the Water and Environmental Study and Research Center at Jordan University.

Derivatization of CLA from Lipid Extracts

A sample of 40 to 100 mg of the extracted lipids was weighed and derivatized in screw-capped tubes with 6 mL of 4% HCl (Frutarom, Kettering, UK) in HPLC-grade methanol (Lab-Scan) for 40 min at 60°C. The tubes were cooled to $18 \pm 1^\circ\text{C}$ and diluted with 2 mL of double distilled water. The CLA methyl ester was then extracted 3 times with 2 mL of HPLC-grade hexane (Lab-Scan). The hexane extract was washed twice with double distilled water and dried over anhydrous sodium sulfate (SDS Fine Chemicals). A 1-mL portion

Table 2. Recoveries and confidence range of methyl elaidate (addition of 60 μg).

Determination	Recovery (%)	Confidence range ¹
1	101.900	61.1 ± 0.54
2	96.000	57.6 ± 1.18
3	108.000	64.8 ± 3.94
4	85.300	51.2 ± 1.95
Mean recovery	97.800	
Standard deviation	8.372	
Coefficient of variability	8.560	

¹CL = Confidence limit calculated at the 95% level.

Table 3. High performance liquid chromatography conditions.

Time (min)	Flow (mL)	%A (methanol)	%B (acetonitrile)
—	0.5	100	0.0
2.0	0.5	100	0.0
3.0	0.5	80	20
8.0	0.5	100	0.0

of the hexane extract was dried under nitrogen and redissolved in 1 mL of methanol for HPLC analysis of total CLA. The remaining portion of hexane was stored at -18°C (Chin et al., 1992).

Quantification of CLA in Lipid Extracts

Quantification of the total CLA methyl esters in fat was performed with a Waters HPLC system. This HPLC is equipped with solvent delivery system capable of pumping 4 different solvents that make a gradient solvent delivery system. The CLA methyl ester in methanol (20 μL) was injected into the HPLC column through the injection port. Separation was performed on a reversed phase analytical column (Sherisorb ODS2, 5 μm , 150 mm $\times$ 4.6 mm i.d., Waters). The gradient mobile phase was delivered at a flow rate of 0.5 mL/min as shown in Table 3. Eluents were monitored at 245 nm using a Waters 486 UV tunable detector, controller system 600, pump 600E, and Millennium software 2010 Chromatography Manager. Quantification of total CLA in a sample was based on the external standard calibration curve.

Preparation of CLA Methyl Ester Calibration Curve

The CLA-methyl ester standard (250 mg; Sigma Chemical Co., St. Louis, MO) was dissolved in 25 mL of HPLC-grade hexane. Concentrations of 1.0, 2.0, 4.0, 5.0, 10.0, 20.0, 40.0, 50.0, 60.0, 80.0, and 100.0 $\mu\text{g/mL}$ (ppm) were prepared and measured by HPLC using the same conditions as described for the lipid extracts. The calibration curve was built and used in quantifications of CLA in the lipid extracts. Recovery of CLA was checked by spiking a starch sample (~10 g) with 3 mL of the CLA standard (10 mg/mL), and performing a re-extraction by the Bligh and Dyer (1959) method. The extracted CLA that was dried under nitrogen gas, dissolved in methanol, and measured by HPLC under the same conditions was used for the standard and the lipid extracts analysis. The linearity was checked and found to be up to 60 ppm; the detection limit was 1 ppm. The calibration curve is shown in Figure 2 and the linearity is represented by R^2 .

Recoveries of CLA were determined by adding 10 μL of 100-ppm standard CLA to a starch sample, followed

Figure 2. Conjugated linoleic acid calibration curve as measured at 245 nm by HPLC.

by 3 replicate complete analyses. Each sample was injected in triplicate into the HPLC on 3 separate days. Recoveries and confidence limits of CLA are shown in Table 4. The mean recovery values were 98.4, 98.2, and 94.0% for d 1, 2, and 3, respectively.

Statistical Analyses

Data were analyzed using the ANOVA procedure of SAS (version 7, 1998; SAS Institute, Inc., Cary, NC). Duncan's multiple range test was applied to determine significance between different treatments.

RESULTS AND DISCUSSION

Milk and Milk Products

Influence of heating of milk on CLA and trans isomer contents. *Trans* isomer and CLA contents of milk and milk products are shown in Table 5. Infrared spectra of the lipid methyl ester of the fat samples are illustrated in Figure 3. The results indicate that milk pasteurization ($85 \pm 1.0^\circ\text{C}$ for 16 s or $95 \pm 1.0^\circ\text{C}$ for 5 min) and UHT ($140 \pm 1.0^\circ\text{C}$ for 4 s) had no significant

($P > 0.05$) influence on *trans* isomer formation. On the other hand, pasteurization at $63 \pm 1.0^\circ\text{C}$ for 30 min or heating in a microwave at $95.8 \pm 1.0^\circ\text{C}$ for 5 min caused a significant ($P < 0.05$) increase in the *trans* isomers. For example, the *trans* isomer values for raw, pasteurized ($95.8 \pm 1.0^\circ\text{C}$), and microwaved milk were 1.69, 1.76, and 2.22% (as methyl elaidate), respectively.

The significant increase in *trans* isomer formation due to microwave heating could be attributed to the unique mechanism of microwave heating. Moreover, heating for a prolonged period (30 min) at relatively low temperature ($63 \pm 1.0^\circ\text{C}$) under aerobic conditions seems to contribute to lipid oxidation more than heating at higher temperature for 5 min. This unexpected result may be explained as follows: heating at 63°C is not effective in expelling the dissolved oxygen in the liquid milk, whereas heating above 80°C caused a rapid escape of the dissolved oxygen.

It is worth noting that low-temperature, long-time pasteurization was not more effective in *trans* isomer formation than UHT (which uses a very short time) but was more effective than heating at $95 \pm 1.0^\circ\text{C}$ for 5 min, because it is known that isomerization of oils generally occurs as a result of severe heating (Semma, 2002). It is assumed that this isomerization was due to the action of microbes or specific enzymes found in raw milk, especially because heating treatment in this experiment was very slow (heating in glass beakers at $63 \pm 1.0^\circ\text{C}$ for 30 min in a water bath of 70 to 80°C). The increase of *trans* isomers of about 30% after microwaving for 5 min demonstrates the detrimental effect of this energetic electronic heating.

Conjugated linoleic acid contents of raw and heated milk (Table 5) indicated that there was no significant ($P > 0.05$) difference among the different heating treatments with the exception of microwave heating and UHT, which caused significant decrease of the CLA content. The average values of CLA for raw and microwave-heated milk were 5.67 and 4.48 mg/g of fat, respectively.

On the other hand, refrigerated storage at $5.0 \pm 1.0^\circ\text{C}$ had no significant effect on CLA content of milk, except

Table 4. Recoveries of conjugated linoleic acid (CLA; addition of 1000 μg) on d 1, 2, and 3.

Determination	Recovery (%)					
	d 1	CL ¹	d 2	CL	d 3	CL
1	97.1	970 $\pm$ 3.8	97.0	970 $\pm$ 13.1	95.8	958 $\pm$ 15.1
2	102.0	120 $\pm$ 18.7	95.6	965 $\pm$ 18.7	97.3	973 $\pm$ 19.9
3	96.0	960 $\pm$ 18.7	102.0	120 $\pm$ 25.9	89.0	890 $\pm$ 28.6
Mean recovery	98.4		98.2		94.0	
Standard deviation	2.6		2.7		3.6	
Coefficient of variation	2.7		2.7		3.8	

¹CL = Confidence limit calculated at the 95% level.

Table 5. Effect of different heat treatments and commercial shelf life storage of milk and milk products on conjugated linoleic acid (CLA) and *trans* isomers (as % elaidic acid or methyl elaidate) contents.¹

Treatment and storage ²	<i>Trans</i> isomers AS methyl elaidate, %	CLA, mg/g of lipid
Raw cows' milk	1.69 ± 0.02 ^c	5.67 ± 0.14 ^{ab}
Pasteurized milk (85 ± 1.0°C, 16 s)	1.68 ± 0.12 ^c	5.53 ± 0.11 ^{ab}
Pasteurized milk (85 ± 1.0°C, 16 s) stored for 3 d	1.76 ± 0.11 ^c	4.71 ± 0.03 ^c
Pasteurized milk (63 ± 1.0°C, 30 min)	2.02 ± 0.18 ^b	5.34 ± 0.12 ^b
Pasteurized milk (95 ± 1.0°C, 5 min)	1.76 ± 0.12 ^c	5.37 ± 0.13 ^b
Pasteurized milk (85 to 90°C, 2 min)	1.73 ± 0.11 ^c	5.33 ± 0.14 ^b
Boiled cows' milk (96.3 ± 1.0°C, 5min)	1.76 ± 0.11 ^c	5.34 ± 0.22 ^b
Microwaved milk (95.8 ± 1.0°C, 5min)	2.22 ± 0.07 ^a	4.48 ± 0.13 ^d
UHT Milk (140 ± 1°C, 4s)	1.76 ± 0.02 ^c	4.82 ± 0.22 ^c
UHT Milk (140 ± 1°C, 4s) stored for 5 d	1.84 ± 0.12 ^{bc}	4.67 ± 0.14 ^c
Yogurt (from milk pasteurized at 95 ± 1.0°C, 5 min)	1.73 ± 0.01 ^c	4.93 ± 0.17 ^{bc}
Yogurt stored for 7 d	1.78 ± 0.04 ^c	4.71 ± 0.14 ^c
Yogurt (from milk pasteurized at 85 to 90°C, 2 min)	1.76 ± 0.02 ^c	5.03 ± 0.13 ^b
Yogurt stored for 7 d	1.82 ± 0.02 ^{bc}	4.68 ± 0.13 ^d
Labaneh (strained in cloth)	1.75 ± 0.02 ^c	5.99 ± 0.17 ^a
Labaneh (strained in cloth) stored for 15 d	1.87 ± 0.02 ^b	5.44 ± 0.16 ^b
Labaneh (strained by separator)	1.77 ± 0.02 ^c	5.72 ± 0.14 ^{ab}
Labaneh (strained by separator) stored for 15 d	1.74 ± 0.02 ^c	5.39 ± 0.03 ^b

^{a,b,c,d}Mean values in the same column with different superscript letters are significantly different ($P \leq 0.05$) according to (ANOVA) Duncan's multiple range test.

¹Values represent means ± SD (n = 4).

²All stored products underwent standard commercial refrigeration at $5.0 \pm 1.0^\circ\text{C}$.

Figure 3. Fourier-transform infrared spectra of methyl elaidate standard of 0.2 mg/mL, microwave-heated milk, and UHT milk.

Table 6. Effect of different heat treatments and commercial shelf life storage of white brined cheese (Nabulsi) on its conjugated linoleic acid (CLA) and *trans* isomers (as % methyl elaidate) contents.¹

Treatment	% <i>Trans</i> isomers as methyl elaidate	CLA, mg/g of lipid
Raw sheep's milk	1.66 ± 0.11 ^c	6.16 ± 0.12 ^{ab}
Fresh curd	1.68 ± 0.13 ^c	5.85 ± 0.12 ^{ab}
Boiled cheese ²	1.79 ± 0.12 ^c	6.32 ± 0.12 ^a
Boiled cheese, reheated at 94.3 ± 1.0°C for 5 min on gas stove ³	1.76 ± 0.13 ^c	6.26 ± 0.16 ^{9a}
Boiled cheese stored for 1 mo ⁴	1.76 ± 0.10 ^c	5.87 ± 0.15 ^{ab}
Boiled cheese, reheated at 94.3 ± 1.0°C for 5 min on gas stove and stored for 1 mo	1.78 ± 0.03 ^c	5.40 ± 0.19 ^{8bc}
Boiled cheese, reheated in microwave oven at 94.3 ± 1.0°C for 5 min ⁵	2.18 ± 0.17 ^b	4.96 ± 0.16 ^c
Boiled cheese, reheated in microwave oven at 94.3 ± 1.0°C for 5 min and stored for 1 mo	2.13 ± 0.20 ^b	4.91 ± 0.11 ^c
Boiled cheese reheated in microwave oven at 94.3 ± 1.0°C for 10 min ⁶	3.17 ± 0.13 ^a	2.93 ± 0.19 ^d
Boiled cheese, reheated in microwave oven at 94.3 ± 1.0°C for 10 min and stored for 1 mo	3.21 ± 0.12 ^a	2.96 ± 0.17 ^d

^{a,b,c,d}Mean values in the same column with different superscript letters are significantly different ($P \leq 0.05$) according to (ANOVA) Duncan's multiple range test.

¹Values represent means ± SD ($n = 4$).

²Brined white cheese pieces boiled in brine (17% NaCl) for 15 min at 94.3 ± 1.0°C (temperature measured at the center of the cheese pieces).

³Desalted, grated, brined white boiled cheese reheated in distilled water on gas stove.

⁴Brined white boiled cheese pieces stored in tins at room temperature (18 ± 1.0°C) for 1 mo.

⁵Desalted, grated, brined white boiled cheese reheated in distilled water in a microwave oven at 80% power.

⁶Brined white desalted, grated, cheese heated until cheese particles browned.

for pasteurized (at 85 ± 1.0 for 16 s) milk stored for 3 d, which resulted in a decrease in CLA content. The CLA levels generally were in agreement with the results reported by others (Warner et al., 1992; Lin et al., 1998; DeMan, 1999; Ma et al., 1999).

The tendency toward a decrease in CLA content upon heating could be attributed to fat oxidation resulting in formation of hydroperoxides, which might cause CLA conversion or degradation.

The significant decrease ($P < 0.05$) in CLA content of microwave-heated milk samples compared with raw milk could be due to the scavenging act of CLA toward free radicals that are formed as a result of lipid oxidation (Okada et al., 1996; Leung and Liu, 2000).

Yogurt and Labaneh

The CLA and *trans* isomers contents of yogurt and labaneh are presented in Table 5. The results of the *trans* isomers levels calculated as percentage of methyl elaidate indicated that processing steps or refrigerated storage of yogurt and labaneh had no significant effects ($P > 0.05$) on *trans* isomer formation. This result is in agreement with the results of Jenkins et al. (2003) and Jenkins (1992), who reported that storage conditions of fermented dairy products did not affect the level of CLA.

The results in Table 5 show that conversion of milk to yogurt and yogurt to labaneh had no significant effect on either *trans* fatty acid or CLA content. Labaneh produced by conventional method (strained in cloth) showed a relative increase in its CLA level compared with raw milk, yogurt, and labaneh strained by separator. This increase could be attributed to the straining time of labaneh in cloth (which takes more than 18 h at room temperature) that may enhance the fermentation process, and hence, formation of CLA through microbial biohydrogenation. Because biohydrogenation is an enzymatic process in which microbial isomerase enzymes (that may be produced during fermentation) are needed to convert *cis*-9 and *cis*-12 of the fatty acid (C_{18:2}) to CLA, the *cis*-9 and *trans*-11 form (Jenkins, 1992).

On the other hand, the significant decrease in CLA content of yogurt stored with refrigeration for 7 d compared with that of raw milk may be explained by the scavenging effect of CLA against free radicals that are formed because of lipid oxidation (Okada et al., 1996; Leung and Liu, 2000). However, the CLA content of labaneh was not affected by refrigerated storage conditions as shown in Table 5. This result could be explained by the assumption that the rate of CLA formation due to prolonged fermentation was in balance with its conversion and degradation during storage periods.

Figure 4. High performance liquid chromatograms of conjugated linoleic acid (CLA) from (A) microwave heated brined white cheese (Nabulsi), (B) raw sheep milk, (C) CLA standard 0.4 ppm, and (D) standard fatty acid methyl ester. The conditions of the HPLC were as described in Materials and Methods.

Brined White Cheese (Nabulsi)

The effects of processing and heating of brined white boiled cheese (Nabulsi) on the CLA and *trans* isomer contents are presented in Table 6. Four chromatograms are presented in Figure 4: 2 chromatograms showing the levels of CLA in the fat extracted from microwave-heated and raw sheep milk, 1 chromatogram for the standard CLA methyl ester, and 1 chromatogram for the fatty acid methyl esters. The chromatograms illustrate the successful separation of CLA from fatty acid methyl esters.

The results in Table 6 show that no significant difference exists in *trans* isomer content of fat extracted from raw sheep milk and that extracted from cheese samples heated by a conventional method (boiling on gas stove). On the contrary, reheating of cheese in a microwave oven increased the *trans* isomer content significantly. For example, the values of *trans* isomers for raw sheep milk, boiled cheese, and microwave-heated cheese were 1.66, 1.79, and 2.18% (calculated as methyl elaidate), respectively. Furthermore, reheating time of cheese had a significant effect on increasing the *trans* isomers level. The values for cheese reheated in a microwave for 5 and 10 min were 2.18 and 3.17%, respectively.

It is worth noting that the increase in *trans* isomers of microwave-heated cheese compared with that of raw milk or cheese boiled on gas stove could be because isomerization needs high activation energy that is achieved at high temperature (Taylor et al., 1983; Semma, 2002). Microwave ovens have the power to provide the activation energy required to form the *trans* isomers. The results in Table 6 show that the processing steps of the Nabulsi cheese (renneting, pressing, and boiling) did not cause any significant changes in *trans* isomers level.

The results show that microwave reheating had a significant effect on lowering the CLA content of the reheated cheese. Furthermore, reheating time had a significant effect on CLA levels. For example, the values of CLA in the cheese microwaved for 5 and 10 min were 4.96 and 2.93 mg/g of fat, respectively. The decrease in CLA content of cheese reheated in a microwave oven compared with that of the raw milk, fresh curd, and boiled cheese may be partially attributed to acceleration of lipid oxidation by microwaves or production of pro-oxidants (Ha et al., 1989; Lin et al., 1998).

The results of reheating Nabulsi cheese stored in cans for 1 mo at room temperature ($18 \pm 1^\circ\text{C}$) indicated that storage has no significant effect on *trans* fatty acid isomers or CLA content compared with the results before storage. The insignificant effect of storage on CLA contents is in agreement with the result found by Lin et al. (1998), which demonstrated that processing steps

and storage had a minor influence on the formation of CLA in Cheddar cheese.

CONCLUSIONS

With the exception of microwave heating, heat treatments and refrigerated storage of milk and dairy products did not cause significant changes in the *trans* fatty acid isomer content. Microwave heating caused an increase in *trans* fatty acid from 1.69% in raw milk to 2.22% in microwave-heated milk (an increase of 31%) and a ~19% increase in milk pasteurized at $63 \pm 1.0^\circ\text{C}$ for 30 min. This increase could have an adverse effect on health. Likewise, with the exception of microwaving, none of the heat or storage treatments used caused significant changes in CLA content. Microwaving caused a significant decrease in CLA content in all products compared with freshly boiled cheese. The percentage decrease of CLA in cheese microwaved when immersed in water for 5 min was 21%, whereas CLA content was decreased by only 1% after boiling of cheese in water on a stovetop. Microwaving of cheese directly for 10 min decreased the CLA content by 53%. This means that the microwave-heated cheese has lost one of the most valuable anticarcinogenic components.

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