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FATTY ACID COMPOSITION WITH EMPHASIS ON CONJUGATED LINOLEIC ACID (CLA) AND CHOLESTEROL CONTENT OF PAKISTANI DAIRY PRODUCTS

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The fatty acid composition of principally conjugated linoleic isomers and cholesterol content of some Pakistani dairy products (butter, cheese, yoghurt and cream) are reported. The most abundant saturated fatty acids within the dairy products studied were palmitic (C16:0), stearic (C18:0) and myristic acid (C14:0), contributing 50-55 g/100 g of total fatty acids. The oleic acid (C18:1) content was considerably higher (21.20-24.12 g/100 g of total fatty acids) than the other unsaturated fatty acids in all dairy products examined. The mean cholesterol content ranged between 91-200 mg/100 g in total fat. A positive correlation between cholesterol and fat content ($r=0.6288$) of dairy products was found for pooled data. Among dairy products investigated, cheese contains higher mean values ($p<0.05$) of CLA (9.00 mg/g) as compared to butter (8.18 mg/g), yoghurt (8.27 mg/g) and cream (8.52 mg/g) samples. In order to understand the factors that affect the CLA levels in dairy products the whole production system needs to be carefully checked, with special attention to animal feeding patterns, characteristics of milk used and different stages in processing.

INTRODUCTION

Dairy products are generally known to increase the risk for cardiovascular diseases in humans because, in comparison to other lipid sources, they contain a higher proportion of lauric, myristic, and palmitic acids and a lower proportion of unsaturated fatty acids (FA) [Sacks & Katan, 2002]. A higher ratio of saturated FA's also contributes to the hardness and poor spreadability of butter at refrigeration temperature [Taylor & Norris, 1977; Ashes *et al.*, 1997]. However, recent studies have focused on the healthy components of ruminant milk and dairy products, including conjugated linoleic acid (CLA) [Gursoy *et al.,* 2003; Plourde *et al.,* 2007], which represents a mixture of positional and geometric isomers of octadecadienoic acid with conjugated double bonds. Theoretically, a number of CLA isomers that differ in the positions of the double bound pairs, *e.g*. 7–9, 8–10, 9–11, 10–12 and so forth, are possible. Additional differences can exist in the configuration of the double bond so that *cis–trans*, *trans–cis*, *cis–cis,* or *trans–trans* configurations are all possible [Bauman *et al*., 2001].

As shown in numerous studies on dairy cows, CLA results partially from linoleic acid biohydrogenation [Griinari & Bauman, 1999] and in more considerable quantity from endogenous synthesis in mammary gland. In fact, *cis*-9, *trans*-11 CLA is synthesized by Δ^9-desaturase from *trans*-11 octadecenoic acid (vaccenic acid), an intermediate product of rumen bacteria biohydrogenation of linoleic acid [Griinari *et al.*, 2000]. In addition to the ability of some rumen-derived bacteria to form CLA from dietary linoleic acid, it is shown that certain cultures used in food fermentations possess the ability to generate *cis*-9, *trans*-11 CLA. Much attention has been paid to dairy starters for CLA production; in fact, there have been studies on CLA production during lactic acid fermentation in milk: some strains of *Propionibacterium, Lactobacillus, Lactococcus, Streptococcus* and *Bifidobacterium* showed to posses this capability [Lin *et al.,* 1999; Coakley *et al*., 2003]. Few studies reported elevated CLA levels in fermented milk products: in dahi (yogurt), 26.5 mg/g fat compared to 5.5 mg/g fat in the raw material [Aneja & Murthy, 1990]; in yoghurt with 0.05% fat, 5.25 mg/g fat compared to unprocessed milk content of 4.40 mg/g fat [Shantha *et al.,* 1995]. Nevertheless, the CLA formation in fermented milk products can be affected by numerous factors such as bacterial strain, cell number, optimal substrate concentration and the period of incubation at neutral pH [Kim & Liu, 2002]. Beside this nutrition of the ruminants is also considered to be a primary factor influencing milk FA composition and consequently the dairy products [Jensen, 2002]. Factors such as genotype and stage of lactation are considered of less importance in influencing milk FA composition [Palmquist *et al.,* 1993; Jensen, 2002].

Dietary cholesterol is one of the factors reported to elevate serum cholesterol and dairy products are reported as the main sources of dietary cholesterol [Collins *et al.,* 2003]. On account of a significant role of dietary cholesterol in human health, the determination of cholesterol level and FA composition in animal food including milk and their products is of immense significance. Furthermore in several dairy products the CLA isomers are not specified on food composition label, so the CLA intakes by the population cannot be evaluated with precision.

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To this end it was advisable to update the food composition data banks in order to determine the CLA supplied by milk and dairy products and to evaluate the CLA intake by the Pakistani population. Previously we have determined cholesterol content and FA composition of Pakistani dairy cow breeds [Talpur & Bhanger, 2005; Talpur *et al*., 2006]. As a part of this update, we have investigated the CLA and cholesterol content of dairy products (butter, yoghurt cheese and cream) made with local Pakistani dairy herd milks. In Pakistan, butter is produced from either yoghurt or cream. The butter made from yoghurt is a popular product in most of the rural areas as well as urban population, due to its flavor and traditional concept of healthiness with its consumption. Butter and yoghurt is usually produced from local buffalo and cows dairy herds, while cheese (from buffalo milk) consumption is popular only in affluent society of Pakistan. The present study is focused on butter, yogurt, cheese and cream, since these are the most consumed animal fat in Pakistan [PARC, 2005] and are therefore representative of milk fat.

MATERIAL AND METHODS

Sample collection

Total 136 dairy product samples were purchased at local retails of Hyderabad, Dadu, Jacobabad, Thatta, Shikarpur districts regions. Seven butter brands (fresh milk butter, commercial butter, n=49), five yoghurt (plain yoghurt, flavour yoghurt, n=35), six cheese (Cheddar cheese, proceed cheese, =42) and two brands of cream (n=14) were selected based on the amount of their production and consumption. All of the samples were selected from different manufacturers. At least seven sample of each brand were purchased for fatty acid analysis. The samples were immediately frozen at -18°C until that analysis was performed within one week. All analyses were done in triplicate. Buffalo milk with addition of cow milk or mostly buffalo milk only is used in most of the dairy products in Pakistan, further production characteristics of investigated dairy products are depicted in Table 1.

Fat determination

Fat determination of the samples (butter, yoghurt, cheese and cream) was performed according to AOAC method 989.04 [1990] using Babcock system for fat analysis.

Lipid extraction

Lipid extraction from yoghurt, butter and cheese was performed in cold conditions according to modified Folch's technique [Christie, 1989].

Portions of 25 g of the yoghurt sample or 10 g cheese/butter were mixed with 100 mL of a chloroform-methanol mixture (2:1 v/v); after homogenizing in a Ultra- Turrax T25 homogenizer (Janke & Kunkel, Gmbh & Co, Staufen, Germany), the mixture was agitated for 30 min and then was filtered into a separator funnel through filter paper (Albet folded circles, Ø 30 cm, extra rapid). Saturated NaCl solution (25 mL) was added to the filtrate; chloroform phase was subsequently recovered, dehydrated with anhydrous sodium sulphate (Na_2SO_4) and dried with rotary evaporator at 40°C under vacuum. The fatty acid methyl esters (FAME) were then prepared on the extracted fat.

TABLE 1. Characteristics of the analysed dairy products.

Product	Characteristics
Butter	Buffalo milk or buffalo milk with added cow milk. Fresh butter is produced by traditional way from yoghurt (dahi) at small scales almost every home in rural areas. Yoghurt is churned in a special round vessel of earthenware, in this process butter and yoghurt drink (lassi) is produced. For commercially butter ; milk fat is separated by separators at 60°C. Heating of cream to 73-85°C for 30 min. Physical maturation at 8°C for 3 h followed by neutralization. Standardization of cream to 40 g/100 g milk fat. Addition of starter culture (2 g /100 g), ripening at 8-19°C for up to 24 h. Churning at 6-10°C for 3-29 h, kneading.
Cheese	Buffalo milk. For cheddar cheese, milk is standardized (1: 0.7 casein fat ratio). Pasteurization at 71°C/5 min; inoculation of culture (0.04 % at 35-36°C). Addition of rennet 7.5 g /100 g and coagulation. Curd cutting and salt addition (2.5%). Whey drained and by curd cube filled in hoops. Curd draining under pressure followed by cutting (sillies); stored at 4-6°C. Processed cheese. Addition of ingredients (emulsifier salts, cream, powder, flavoring *etc*.). Standardization to at least 40 g/100 g milk fat. Melting at 90-120°C at 6-8 atm; no further aging.
Yoghurt	Buffalo milk or buffalo milk with added cow milk. Milk heated to 93°C for 10 min, cooled to 45°C. Inoculated with starter culture (10 g/2.5 kg), addition of flavour. Dispensed (in to plastic cups (200 cm^3), incubated at 45°C for 4-5 h. Transferred to a cold store 4-6°C.
Cream	Buffalo milk or buffalo milk with added cow milk. Heat treatment at 60°C followed by separation of cream from milk. Standardization to least 40 g/100 g milk fat. UHT pasteurization at 140°C for 1-4 sec. No further aging.

Preparation of methyl esters

The lipids were esterified in capped screw top tubes (Teflon liners) with 6 mL of 0.5N sodium methoxide heated at 50°C for 10 min [Kramer *et al*., 1997]. The fatty acid methyl esters (FAME) were then cooled to room temperature, and 2 mL of isooctane and 3 mL of 10% acetic acid were added. The tubes were recapped to prevent evaporation of the short chain FA's. The FAME were centrifuged (2000 × g) for 10 min, and a portion of the top layer was removed and placed in sealed gas chromatography vials and kept at -20°C until analysed.

GC-MS analysis

The GC-MS analysis for FAME's was carried out using Agilent Technologies gas chromatograph (GC-6890 N, Little Fall, New York, USA) equipped with an Agilent autosampler 7683-B injector, MS-5975 inert XL Mass selective detector and SP-2340 capillary column (60 m × 0.25 mm) was used for the separation of FA methyl esters. The initial temperature of 150°C was maintained for 2 min, raised to 230°C at the rate

of 4°C/min, and kept at 230°C for 5 min. The split ratio was 1:50, and helium was used as a carrier gas with the flow rate of 0.8 mL/min. The injector and detector temperatures were 240 and 260°C, respectively. The mass spectrometer was operated in the electron impact (EI) model at 70 eV in the scan range of 50–550 *m/z*.

Peak identification of the fatty acids in the analysed dairy products were carried out by the comparison with retention times and mass spectra of known standards. Authentic standards of FAME's obtained from Sigma Chemical Co and Matreya, Biotrend, Köln, Germany and High purity individual *cis*-9*, trans-11* CLA and *trans*-10, *cis*-12 (Matreya, Inc., Pleasant Gap, PA) were used for the confirmation of GC-MS libraries result. Additional CLA standard mixture (Sigma chemical co.) was used to identify CLA isomers in dairy products. The FA composition was reported as a relative percentage of the total peak area**.**

Determination of cholesterol content

The cholesterol content in all samples was determined according to the method of Fletouris *et al.* [1998]. For preparation of cholesterol standards the stock solution (2 mg /mL) was prepared by dissolving 20 mg of reference standard (Sigma Chemical company, St. Louis, MO, USA) with hexane into a 10 mL volumetric flask. Working solutions were prepared by appropriately diluting aliquots from the stock solution with hexane to obtain solutions in the range of 10-80 μg/mL.

GC conditions used for analysis were as follows: a fused silica capillary column (15 m x 0.32 mm), coated with SPB-1 (Supelco Inc., Bellefonte, PA, USA) with 1.0 mm film thickness. Oven temperature was set at 285°C; injection port temperature was set at 300°C, and flame ionization detector temperature at 300°C. The flow rates were 2 mL/min for helium, 30 mL/min for hydrogen, and 300 mL/min for air. The injection volume was 2 mL with a split ratio of 20:1.

Statistical analysis

The simple analysis of variance according to the SAS® technique (SAS/STAT, version 8, 2000, SAS Inc., Cary, NC) was carried out on the CLA levels and fatty acid composition found in categories of butter, yoghurt, cheese and cream.

RESULT AND DISCUSSION

The total fat, cholesterol and the concentration of conjugated linoleic acids (CLA) isomers in dairy product categories (fresh and commercial butter; cheddar and proceed cheese; natural and flavored yoghurt; cream) are depicted in Table 2.

The mean fat content was higher in butter averaging 82.24% (range 81.91-83.10%) followed by cheese 45.26% (44.10- 46.35%), cream 37.94% (36.25-39.63%) and yoghurt 3.45% (3.10-3.82%), comparable to earlier reported data for French butter (83.6%), Greek Manouri cheese (50.10%), Italian organic yoghurt (3.98%) and Turkish cream (35.00%) samples respectively [Ledoux *et al.,* 2005; Andrikopoulus *et al.,* 2003; Prandini *et al.,* 2001; Sagdic *et al.,* 2004].

TABLE 2. Fat, cholesterol and CLA content of some Pakistani dairy products[a].

Sample	Fat (g/100 g)	Cholesterol (mg/100 g fat)	CLA isomers (mg/100 g fat)			Total CLA (mg/100 g fat)
			cis-9, *trans*-11	*trans*-10, *cis*-12	Σ *trans- 11, cis- 13*; *trans* -7, *cis*- 9)	
FB1	83.00	195	7.72	0.28	0.33	8.33
FB2	82.00	164	6.67	0.26	0.38	7.31
FB3	82.50	171	8.02	0.34	0.39	8.75
CB4	81.20	176	7.49	0.30	0.33	8.12
CB5	82.00	190	7.89	0.27	0.31	8.47
CB6	83.10	200	6.98	0.29	0.35	7.62
CB7	81.90	152	7.95	0.32	0.36	8.63
CC1	45.18	148	8.07	0.29	0.32	8.68
CC2	46.35	119	8.80	0.30	0.30	9.40
CC3	44.10	135	7.78	0.31	0.35	8.44
CC4	44.50	128	7.81	0.29	0.33	8.43
PC1	46.21	179	9.02	0.31	0.32	9.65
PC2	45.24	138	8.82	0.30	0.35	9.47
PY1	3.82	136	8.01	0.32	0.33	8.66
PY2	3.50	114	7.59	0.32	0.38	8.29
PY3	3.10	140	6.92	0.33	0.35	7.60
FY4	3.20	91	7.78	0.30	0.32	8.40
FY5	3.65	130	7.81	0.30	0.29	8.40
C1	39.63	120	7.72	0.30	0.31	8.33
C2	36.25	135	7.56	0.29	0.31	8.16

[a] FB: fresh butter, CB: commercial butter, CC: cheddar cheese, PC: proceed cheese, PY: plain yoghurt, FY: flavored yoghurt, C: cream.

The cholesterol content ranged from 91-200 mg/100 g fat in all dairy products studied, with higher mean concentrations in butter 178.26 mg/100 g fat followed by cheese 141.17 mg/100 g, cream 127.5 mg/100 g, and yoghurt 122.2 mg/100 g. In previous studies, the cholesterol content of milk and milk products correlated with their fat contents, and positive correlation was found [Piironen *et al.,* 2002]. In the same way, we found a positive correlation (r=0.6288) between cholesterol and fat content of dairy products. However, the cholesterol content obtained for dairy products is lower than earlier reported data for Turkish dairy products (174-362 mg/100 g) made from cow milk. This is due to the fact that in Pakistan dairy products are made from buffalo milk, which is reported to have significantly lower cholesterol content (65 mg/100 g *vs*. 314 mg/100 g) when compared to cow milk [Zicarelli, 2004], since cholesterol content in dairy products results from milk properties used in production and during the processing conditions of dairy products.

The CLA isomers particularly in terms of biological activity *cis*-9 *trans*-11 octadecadienoic acid has been implicated as the most important CLA isomer, this isomeric derivative was the predominant isomer incorporated into the phospholipids of mouse forestomachs, rats livers, and mammary tumors [Werner *et al.,* 1992], therefore the *cis*-9 *trans*-11 was detected in the present work (Figure 1).

Among dairy products investigated, cheese contains higher ($p<0.05$) mean values of CLA (9.00 mg/g) as compared to butter (8.18 mg/g), yoghurt (8.27 mg/g) and cream (8.52 mg/g) samples. Microorganisms, because of the differences in enzyme activity, have been identified that may positively contribute to differences in CLA contents of cheeses and other cultured dairy products [Lin *et al.,* 1995]. The presence of whey protein influences the CLA formation in fermented dairy products [Shantha *et al.,* 1995]. Lin [2000] has also reported that some fermented dairy products contain higher levels of CLA than non-fermented milk. The CLA content determined in the present study is comparable with that reported earlier for different dairy products [Prandini *et al.,* 2001; Kumar *et al.,* 2006]. However elevated CLA contents in cheese (17.3–25.4 mg/g) produced from sheep milk have been reported by several authors [Nudda *et al.,* 2005; Cabbidu *et al.,* 2006; Zhang *et al*., 2006] when compared with CLA concentrations in Pakistani cheese (8.2-9.00 mg/100 g).

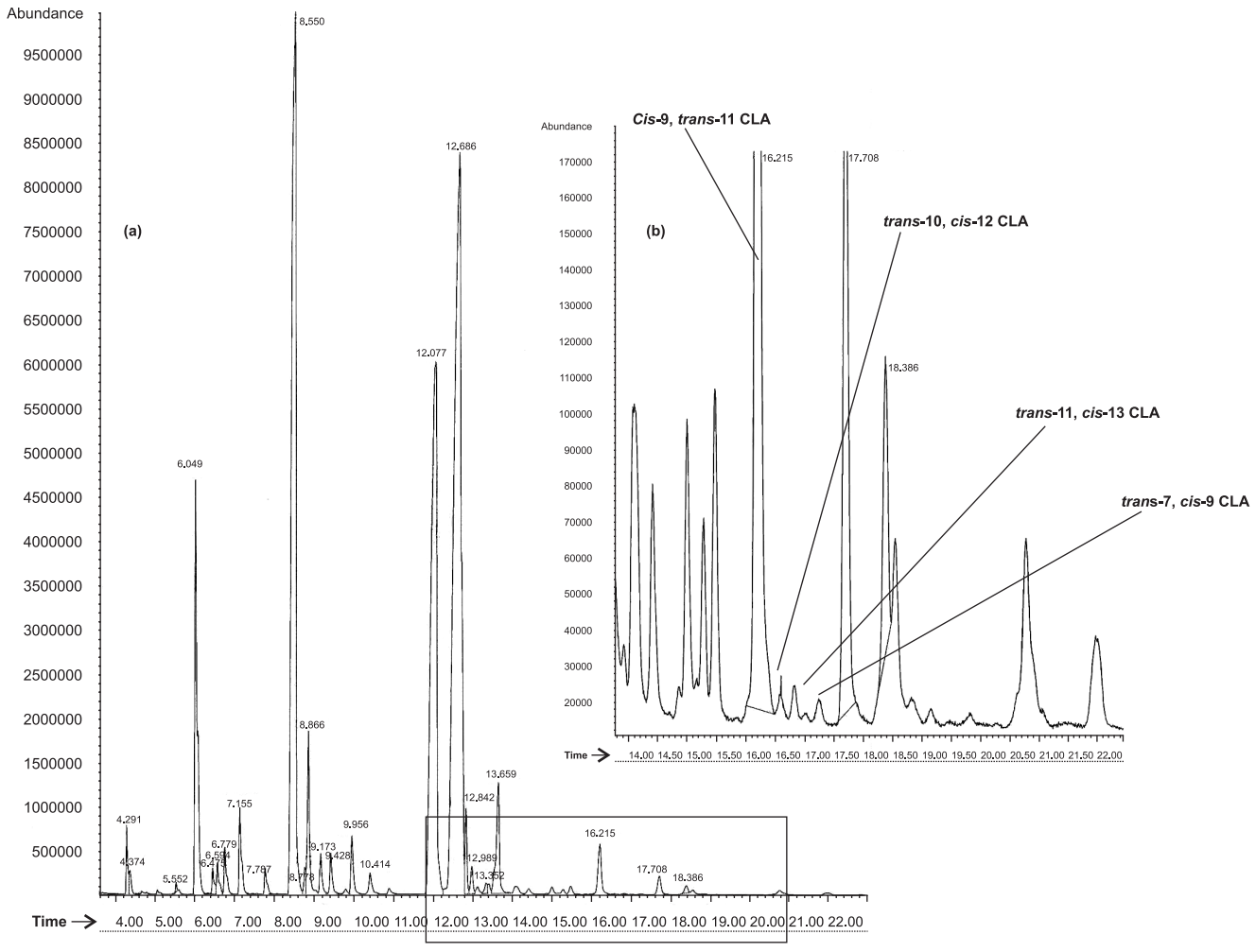

FIGURE 1. (a) Gas chromatogram of fatty acid methyl esters of a fresh butter fat sample separated on SP-2340 capillary column (60 m × 0.25 mm); see text for operating conditions. (b) Partially enlarged chromatogram showing the separation of *cis*- 9, *trans*-11; *trans*-10, *cis*-12; *trans*-11, *cis*-13; *trans*-7, *cis*-9 CLA isomers of the same fresh butter sample

The differences in CLA content of our samples may be explained by differences in origin, production technologies (Table 1) and initial CLA content of raw milk; since in Pakistan cheese are manufactured with buffalo milk as compared to Europe and USA where sheep or cow milk is used for dairy products manufacture. Furthermore several different mechanisms have been proposed for the formation of CLA in processed cheeses in the literature. In comparison to commercially available natural and proceed cheeses, Ha *et al.* [1989] attributed the higher CLA content in proceed cheeses to the additional heat treatment for the proceed cheeses. However Chin *et al.* [1992] who noted no differences in the CLA content of natural and proceed cheeses and suggested that processing parameters other than heat would contribute CLA formation in proceed cheeses. More recent studies [Van Nieuwenhove *et al.,* 2004; Luna *et al.,* 2005] support the idea that heating at high temperature does not raise CLA levels in milk fat. In addition Werner *et al.* [1992] and O'Shea *et al.* [1998] reported that CLA content of dairy products mostly depend on the geographical origin, seasonal variation and locally varying grass fodder of free-fed animals, initial concentration of raw milk, temperature, type of starter cultures, production and ripening process.

Ruminant milk fats contain a wide range of FA's and 437 distinct have been identified in bovine milk fats [Collins *et al.,* 2003]. This situation is reflected to dairy products. Results of fatty acid composition of the dairy products are presented in Table 3 and 4. The FA composition of all samples is quite similar. For the saturated fatty acids SFA's, the most abundant in the dairy products investigated were palmitic acid (C16:0), stearic acid (C18:0) and myristic acid (C14:0). Palmitic is one of the major SFA's; it raises serum cholesterol while stearic acid does not [Grundy, 1997]. Palmitic acid was found to have the highest level SFA's in all samples. Generally butter and cream had higher amount of palmitic acid. The short (C4:0-C10:0) and medium chain FA's (C12:0, C14:0) constituted 10% and 12% of total FA's, respectively, while long-chain FA's represented the reminder (78%).

Comparable results for short (6-14%) and medium chain FA's (13-15%) have been reported by Sagdic *et al.* [2004] for Turkish Yayik butters produced from goats, ewes or cows milk. As seen in Table 4, oleic acid (C18:1 *cis*) was the predominant FA and accounted for higher concentration (21.20-24.12 g/100 g of total FA's) among *cis* monoenes in all Pakistani dairy products. Donmez *et al.* [2005] and Molkentin [2006] have also reported similar values (20-24 g/100 g) for Turkish and German dairy products made within different processing conditions.

Total *trans*-isomers ranged from 2.80 g/100 g to 3.61 g/100 g for all dairy products with mean value of 3.14 g/100 g of total FA's. Among *trans* fatty acids (TFA) the C18:1 comprised 68-82% of total TFA (73.84% average), followed by the C18:2 *trans* (average: 9.95%, 6-14% of total) and C16:1 *trans* isomers (average:16.21%, 10-21% of total). These values are comparable with earlier reported data for dairy products from 14 European countries, independent of the serious factors such as properties of raw milk, origin, feeding, and season [Aro *et al.,* 1998].

The long chain omega-3 polyunsaturated FA's, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) accounted for 0.044 and 0.034 g/100 g of total FA's comparable

TABLE 3. Composition of saturated fatty acids in some Pakistani dairy products[a] (g/100 g fatty acids).

Code	C4:0	C6:0	C8:0	C10:0	C12:0	C14:0	C16:0	C17:0	C18:0	C20:0
FB1	4.01	2.69	2.19	1.80	1.88	8.37	32.4	0.66	11.9	0.26
FB2	4.12	1.81	2.21	1.75	1.97	10.01	32.20	0.58	12.15	0.22
FB3	3.98	2.49	1.96	2.00	1.89	9.87	32.58	0.52	11.68	0.21
CB4	4.11	2.52	1.87	1.67	2.03	9.45	32.01	0.63	11.06	0.27
CB5	3.69	2.72	1.92	1.68	1.92	9.82	31.65	0.54	10.94	0.22
CB6	3.94	2.59	1.79	1.73	2.02	9.14	32.48	0.60	11.62	0.24
CB7	4.10	2.46	2.00	1.57	2.02	9.54	31.25	0.57	12.87	0.27
CC1	4.12	2.41	2.06	1.46	2.32	10.08	29.03	0.66	13.01	0.33
CC2	4.00	2.27	1.97	1.74	1.76	10.28	30.25	0.57	12.57	0.25
CC3	3.78	2.39	2.01	1.68	1.68	10.88	29.57	0.63	11.52	0.24
CC4	3.82	2.31	1.87	1.56	1.83	9.46	30.20	0.59	12.09	0.23
PC1	4.11	2.48	1.90	1.84	2.10	10.53	30.04	0.61	10.96	0.26
PC2	4.02	2.53	2.00	1.71	2.00	11.32	28.97	0.71	11.23	0.27
PY1	4.2	2.68	2.58	1.68	2.29	9.53	29.24	1.00	11.43	0.32
PY2	3.69	2.54	1.68	1.80	2.00	10.58	30.25	0.69	12.82	0.29
PY3	4.58	2.34	1.96	1.56	1.94	9.68	30.48	0.92	11.64	0.24
FY4	3.95	2.51	2.13	1.92	1.88	9.95	30.91	0.53	11.69	0.28
FY5	4.03	2.62	1.78	1.86	1.72	10.21	31.05	0.62	10.33	0.3
C1	4.14	1.89	1.94	1.71	1.63	10.88	32.62	0.74	9.97	0.25
C2	4.10	1.93	2.2	1.84	1.65	10.95	32.2	0.61	10.25	0.26

[a] FB: fresh butter, CB: commercial butter, CC: cheddar cheese, PC: proceed cheese, PY: plain yoghurt, FY: flavored yoghurt, C: cream.

TABLE 4. Composition of unsaturated fatty acids in some Pakistani dairy products[a] (g /100 g fatty acids).

Code	C14:1	C16:1 *cis*	C16:1 *trans*	C18:1 *cis*	C18:1 *trans*-11	C18:2 *cis*-9,12	C18:2 *trans-9 ,12*	C18:3 n- 3	C20:5 n-3 EPA	C 22:6 n-3 DHA
FB1	1.27	2.21	0.58	22.05	2.07	2.01	0.33	0.46	0.06	0.04
FB2	1.33	2.08	0.62	22.14	1.97	1.75	0.24	0.39	0.05	0.05
FB3	1.19	2.32	0.51	21.20	2.24	1.88	0.34	0.46	0.06	0.04
CB4	0.98	2.19	0.39	23.10	2.36	1.49	0.39	0.33	0.05	0.05
CB5	1.13	1.95	0.65	22.85	2.45	1.9	0.28	0.34	0.05	0.03
CB6	1.2	2.23	0.48	22.28	2.39	1.38	0.33	0.45	0.03	ND
CB7	1.09	1.9	0.57	22.71	2.14	1.45	0.29	0.52	ND	0.02
CC1	1.1	1.62	0.58	23.01	2.72	1.5	0.27	0.62	0.06	0.03
CC2	1.28	2.11	0.44	23.32	2.31	1.93	0.36	0.47	0.05	0.03
CC3	1.32	2.25	0.32	23.55	2.41	1.75	0.3	0.55	0.04	0.04
CC4	1.45	2.14	0.48	24.05	2.25	1.65	0.48	0.58	ND	0.03
PC1	1.2	2.22	0.52	23.42	2.31	1.87	0.37	0.44	0.05	ND
PC2	1.36	2.14	0.49	23.20	2.18	1.77	0.44	0.62	0.03	ND
PY1	1.04	2.24	0.37	22.31	2.5	2.05	0.26	0.48	0.04	0.03
PY2	1.11	2.1	0.4	23.10	2.98	2.1	0.23	0.28	ND	0.02
PY3	0.97	1.85	0.54	22.25	2.54	1.95	0.25	0.3	0.03	ND
FY4	0.87	1.92	0.49	22.85	2.13	1.74	0.18	0.27	0.02	0.03
FY5	1.07	1.58	0.61	24.12	2	1.65	0.22	0.43	0.04	0.04
C1	1.12	1.96	0.6	22.88	2.01	1.57	0.32	0.54	0.05	0.04
C2	1.2	1.8	0.47	23.14	2.41	1.8	0.36	0.4	ND	0.02

[a] FB: fresh butter, CB: commercial butter, CC: cheddar cheese, PC: proceed cheese, PY: plain yoghurt, FY: flavored yoghurt, C: cream; ND: not detected.

TABLE 5. Fatty acid ratios of some Pakistani dairy products[ab].

Fatty acids	Butter (n=49)	Cheese (n=42)	Yoghurt (n=35)	Cream (n=10)
SFA	66.27 ± 2.25	64.85 ± 1.38	65.38 ± 1.62	65.88 ± 2.10
MUFA	25.63 ± 1.03	26.79 ± 0.98	25.90 ± 1.01	26.05 ± 0.87
PUFA	3.01 ± 0.41	3.25 ± 0.60	3.12 ± 0.38	3.03 ± 0.62
TFA	3.10 ± 0.26	3.21 ± 0.32	3.14 ± 0.40	3.10 ± 0.31
PUFA / SFA	0.045 ± 0.001	0.050 ± 0.002	0.047 ± 0.001	0.046 ± 0.001

[a] SFA: Saturated fatty acids, MUFA: Monounsaturated fatty acids, PUFA: Polyunsaturated fatty acids, TFA: *trans* fatty acids; [b] seven samples for each brand of dairy products were analysed in triplicate.

to earlier reported data for Fulani cow milk [Robert *et al.*, 1999]. Milk fat contents of EPA and DHA are of interest because of their potential benefits in reducing the risk of cardiovascular disease, type two diabetes, hypertension, cancer, and certain disruptive neurological functions [Connor, 2000]. Milk and dairy products normally contain very low amounts of EPA and DHA, and increasing their content is limited primarily because their biohydrogenation in the rumen is extensive and secondarily because they circulate in specific plasma fractions that contribute minimally to mammary supply of these FA's [Lock & Bauman, 2004].

In Table 5, the results for the fatty acid ratios of dairy products (percentage of methyl esters) are categorized as saturated (SFA), monounsaturated (MUFA), polyunsaturated (PUFA), and *trans* fatty acids (TFA). These results are not much different from those found in the literature for several dairy varieties of different origin [Andrikopoulos *et al.,* 2003; Gursoy *et al.,* 2003; Sagdic *et al.,* 2004]. Among the FA's classes, saturated were predominating, following by the *cis*-MUFA, and PUFA (Table 5). The ratio of PUFA/SFA ranged between 0.045-0.50, within range (0.043-0.058) reported for Greek and Turkish dairy products [Andrikopoulos *et al.,* 2003; Donmez *et al.,* 2005]. The abundance of FA's decreased, in average, in order: 16:0 > 18:1 *cis* >18:0 > 14:0 > 4:0 >6:0 >18:1 *trans*-11> 16:1 *cis* > 8:0 >12:0 > 18:2 *cis* -9,12 > 10:0 >14:1 > 17:0 >16:1 *trans* > 18:3 *cis*-6,9,12 >1 8:2 *trans*-9,12 15:1 > >20:5 > 22:6.

CONCLUSIONS

The present study suggests that dairy products in general, illustrate rather similar fatty acids composition. Palmitic

(C16:0) was predominant, whereas oleic (C18:1, *cis* 9) was the major monounsaturated fatty acid. Comparable results for mean fatty acids among dairy products indicate that manufacturing practices had negligible effects on the total CLA concentrations as well as on CLA isomer distribution in commercial processed dairy products except the cheese samples, which showed elevated CLA concentrations. The higher CLA concentration in cheese samples products could be associated to the composition of microbial cultures and the length of aging during cheese processing.

In any case, in order to understand the factors that might affect the CLA levels in dairy products the whole production system should be carefully checked, paying special attention to the animal feeding patterns, the characteristics of milk used and different stages in processing.

REFERENCES

1. Andrikopoulus N.N., Kalogeroopoulu N., Zerva A., Zerva U., Hassapidou M., Kapoulas M.V., Evaluation of cholesterol and other nutrient parameters of Greek cheese varieties. J. Food Compos. Anal., 2003, 16, 155–167.
2. Aneja R.P., Murthy T.N., Conjugated linoleic acid contents of Indian curds and ghee. Indian J. Dairy Sci., 1990, 43, 231–238.
3. AOAC, Association of Official Analytical Chemists. 1990, Official Methods of Analysis. Vol. 1. 15th ed. AOAC, Arlington: VA.
4. Aro A., Antoine J.M., Pizzoferrato L., Reykadal O., Van Poppel G., Trans fatty acids in dairy and meat products from 14 European countries: the transfer study. J. Food Compos. Anal., 1998, 11, 150–160.
5. Ashes J.R., Gulati S.K., Scott T.W., Potential to alter the content and composition of milk fat through nutrition. J. Dairy Sci., 1997, 80, 2204–2212.
6. Bauman D.E., Corl B.A., Baumangard L.H., Conjugated linoleic acid in ruminants, 2001, *in*: Proceedings of American Society of Animal Sciences, vol. 77, pp. 1–15.
7. Cabiddu A., Addis M., Pinna G., Decandia M., Sitzia M., Piredda G., Pirisi A., Molle G., Effect of corn and beet pulp based concentrates on sheep milk and cheese fatty acid composition when fed Mediterranean fresh forages with particular reference to conjugated linoleic acid *cis*-9, *trans*-11. Anim. Feed Sci. Technol., 2006, 131, 292–311.
8. Chin S.F., Liu W.L., Storkson J.M., Ha Y.L., Pariza M.W., Dietary sources of conjugated dienoic isomers of linoleic acid a newly recognized class of anticarcinogen. J. Food Compos. Anal., 1992, 5, 185–197.
9. Christie W.W., Gas Chromatography and Lipids. 1989, Oily Press, Alloway, Scotland, pp. 64–84.
10. Coakley M., Ross R.P., Nordgen M., Fitzgerald G., Devery R., Stanton C., Conjugated linoleic acid biosynthesis by human--derived *Bifidobacterium* species. J. Appl. Microbiol., 2003, 94, 138–145.
11. Collins Y.F., Mcsweeney P.L.H., Wilkinson M.G., Lipolysis and free fatty acid catabolism in cheese: a review of current knowledge. Int. Dairy J., 2003, 13, 841–866.
12. Connor W.E., Importance of n–3 fatty acids in health and disease. Am. J. Clin. Nutr., 2000, 71, 171–175.
13. Donmez M., Seckin K.A., Sagdic O., Simsek B., Chemical characteristics, fatty acid compositions, conjugated linoleic acid contents and cholesterol levels of some traditional Turkish cheeses. Int. J. Food Sci. Nutr., 2005, 56, 157–163.
14. Fletouris D.J., Botsoglou N.A., Psomas I.E., Mantis A.I., Rapid determination of cholesterol in milk and milk products by direct saponification and capillary gas chromatography. J. Dairy Sci., 1998, 81, 2833–2840.
15. Griinari J.M., Bauman D.E., Biosynthesis of conjugated linoleic acid and its incorporation into meat and milk in ruminants. 1999, *in*: Advances in Conjugated Linoleic Acid Research vol. 1 (eds. Yurawecz M.M., Mossoba M.M., Kramer J.K.G., Pariza M.W., Nelson J.G). American Oil and Chemical society Press, Champaign IL, pp. 180–200.
16. Griinari J.M., Corl B.A., Lacy C.A., Chouinard P.Y., Nurmela K.V.V, Bauman D.E., Conjugated linoleic acid is synthesized endogenously in lactating dairy cows by Δ^9- desaturase. J. Nutr., 2000, 130, 2285–2291.
17. Grundy S.M., What is the desirable ratio of saturated, polyunsaturated, and monounsaturated fatty acids in the diet?. Am. J. Clin. Nutr., 1997, 66, 988–990.
18. Gursoy O., Seckin A.K., Kinik O., Metin M., Conjugated linoleic acid (CLA) content of most popular Turkish hard and soft cheeses. Milchwissenschaft, 2003, 58, 622–623.
19. Ha Y.L., Grimm N.K., Pariza M.W., Newly recognized anticarcinogenic fatty acids: identification and quantitation in natural and proceed cheeses. J. Agric. Food Chem., 1989, 37, 75–81.
20. Jensen R.G., The composition of bovine milk lipids: January 1995 to December 2000. J. Dairy Sci., 2002, 85, 295–350.
21. Kim Y.J., Liu R.H., Increase in conjugated linoleic acid content in milk by fermentation with lactic acid bacteria. J. Food Sci., 2002, 67, 1731–1737.
22. Kramer J.K.G., Fellner, Dugan M.E.R., Sauer F.D., Mossoba M.M., Yurawecz M.P., Evaluating acid and base catalysts in the methylation of milk and rumen fatty acids with special emphasis on conjugated dienes and total *trans* fatty acids. Lipids, 1997, 32, 1219–1228.
23. Kumar V.V., Sharma V., Bector B.S., Effect of repining on total conjugated linoleic acid and its isomers in buffalo Cheddar cheese. Int. J. Dairy Tech., 2006, 59, 256–260.
24. Ledoux M., Chardigny J., Darbois M., Soustre Y., Sebedio J., Laloux L., Fatty acid composition of French butters, with special emphasis on conjugated linoleic acid (CLA) isomers. J. Food Compos. Anal., 2005, 18, 409–425.
25. Lin H., Boylston T.D., Chang M.J., Luedecke O., Shultz T.D., Survey of conjugated linoleic acid contents of dairy products. J. Dairy Sci., 1995, 78, 2358–2365.
26. Lin T.Y., Lin C.W., Lee C.H., Conjugated linoleic acid concentration as affected by lactic cultures and added linoleic acid. Food Chem., 1999, 67, 1–5.
27. Lin T.Y., Conjugated linoleic acid concentration as affected by lactic cultures and additives. Food Chem., 2000, 69, 27–31.
28. Lock A.D., Bauman D.E., Modifying milk fat composition of dairy cows to enhance fatty acids beneficial to human health. Lipids, 2004, 39, 1197–1206.
29. Luna P., Fuente D.M., Juarez M., Conjugated linoleic acid in processed cheeses during the manufacturing stages. J. Agric. Food Chem., 2005, 53, 2690–2695.
30. Molkentin J., Cholesterol content and lipid composition of low fat dairy products. Eur. J. Lipid Sci. Technol., 2006, 223, 253–260.
31. Nudda A., McGuire M.A., Battacone G., Pulina G., Seasonal

variation in conjugated linoleic acid and vaccenic acid milk fat of sheep and its transfer to cheese and rocota. J. Dairy Sci., 2005, 88, 131–1319.
32. O'Shea M., Lawless F., Stanton C., Devery R., Conjugated linoleic acid in bovine milk fat: a food-based approach to cancer chemoprevention. Trends Food Sci. Technol., 1998, 9, 192–196.
33. PARC, Pakistan Agriculture Research Council., Dairy Technology research Report. 2005. Assessed on 12–2-2007. Available online [http://www.parc.gov.pk/index.htm].
34. Palmquist D.L., Beaulie A.D., Barbano D.M., Feed and animal factors influencing milk fat composition. J. Dairy Sci., 1993, 76, 1753–1771.
35. Piironen V., Toivo J., Lampi A.M., New data for cholesterol contents in meat, fish, milk, egg and their products consumed in Finland. J. Food Compos. Anal., 2002, 15, 705–713.
36. Plourde M., Destaillats F., Chouinard P.Y., Angers P., Conjugated α-linolenic acid isomers in bovine milk and muscle. J. Dairy Sci., 2007, 90, 5269–5275.
37. Prandini A., Geromin D., Conti F., Masoero F., Piva A., Piva G., Survey on the level of conjugated linoleic acid in dairy products. Ital. J. Food Sci., 2001, 13, 243–253.
38. Robert H.G., Seline N.O., Lu-Te C., Yung-Sheng H., Dorothy J.V., Fatty acid composition of Fulani "butter oil" made from cow's milk. J. Food Compos. Anal., 1999, 12, 235–240.
39. Sacks F.M., Katan M., Randomized clinical trails on the effects of dietary fat and carbohydrate on plasma lipoproteins and cardiovascular disease. Am. J. Med., 2002, 113, 13S-24S.
40. Sagdic O., Donmez M., Demirci M., Comparison of characteristics and fatty acid profiles of traditional Turkish yayik butters produced from goats', ewes', or cows' milk. Food Contr., 2004, 15, 485–490.
41. Shantha N.C., Ram L.N., O'Leary J., Hicks C.L., Decker E.A., Conjugated linoleic acid concentrations in dairy products as affected by processing and storage. J. Food Sci., 1995, 60, 695–697.
42. Talpur F.N., Bhanger M.I., Conjugated linoleic acid: A mixture of bio-active fatty acids in milk fat of ruminants. Pak. J. Anal. Chem., 2005, 6, 22–27.
43. Talpur F.N., Bhanger M.I., Khuhawar M.Y., Comparison of fatty acids and cholesterol content in the milk of Pakistani cow breeds. J. Food Compos. Anal., 2006, 19, 698–703.
44. Taylor M.W., Norris R., The physical properties of dairy spreads. N. Z. J. Sci. Tech., 1997, 12, 166–170.
45. Van Nieuwenhove C., Gonzalez S., Perez-Chaia A., Ruiz-Holgado A.P., Conjugated linoleic acid in buffalo (*Bubalus bubalis*) milk from Argentina. Milchwissenshaft, 2004, 59, 506–509.
46. Werner S., Luedecke L.O., Shultz T.D., Determination of conjugated linoleic acid content and isomer distribution in three Cheddar-type cheeses: effects of cheese culture, processing, and aging. J. Agric. Food Chem., 1992, 40, 1817–1821.
47. Zicarelli L., Buffalo milk: Its properties, dairy yield and Mozzarella production. Vet. Res. Commun., 2004, 28, 127–135.
48. Zhang R., Mustafa A.F, Zhao X., Effects of flaxseed supplementation to lactating ewes n milk composition, cheese yield, and fatty acid composition of milk and cheese. Small Rumin. Res., 2006, 63, 233–241.

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