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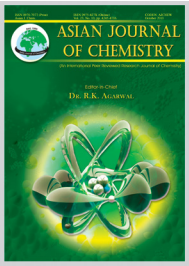

Determination of Fatty Acid Profiles Including Conjugated Linoleic Acids in Various Dairy Products

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The objective of the study was to determine conjugated linoleic acid (CLA) contents of milk and dairy products. Fatty acid profiles including conjugated linoleic acids of various dairy products were investigated. The amounts of fatty acids were determined in yogurt, butter, kaymak and variety of cheeses such as kasar made from ewe's milk, tulum, fresh kasar, old kashar and white brined cheese samples. Short chain fatty acids such as butyric acid, capric acid and caproic acid were also determined with this method as well as long chain fatty acids such as palmitic acid, oleic acid, miristic acid, linoleic acid and conjugated linoleic acid. Total conjugated linoleic acid amounts of aged kasar cheese, butter and kaymak were found as 2.06, 0.94 and 0.24 mg/g fat, respectively. It was observed that the higher the linoleic acid in the sample the higher concentration of conjugated linoleic acid. It was concluded that aging is an essential factor to obtain higher amounts of conjugated linoleic acid.

Key Words: Dairy products, Fatty acid profile, Conjugated linoleic acids.

INTRODUCTION

Milk and rumen fatty acid compositions are complex. Milk fat fatty acid chain lengths can vary from C:4 to C:26. Milk fat also has many positional and geometric isomers of mono-, di- and tri- unsaturated fatty acids[1]. Jensen *et al.*[1] estimated 400 fatty acids to be present in bovine milk. The overall role of dairy foods in the cancer issue is still unclear. Some studies suggested that high intake of dairy foods may increase the risk of cancer, probably due to saturated fat and cholesterol found in them. On the other hand, recent studies have shown that components of dairy foods such as calcium and vitamin D[2], bacterial cultures especially lactic acid bacteria[2-12], dairy proteins[7], dairy fat including sphingomyelin, ether lipids and fatty acids such as butyric acid, oleic acid, palmitic acid, palmitoleic and conjugated linoleic acids may have antimutagenic and anticarcinogenic properties.

Conjugated linoleic acids are a mixture of octadecadienoic acids that have been recognized as anticarcinogens. Conjugated linoleic acid are fatty acids primarily found in dairy products. Milk fat is also the most abundant source of conjugated linoleic acid, which refer to a group of geometrical and positional isomers of linoleic acid (LA 18:2 *cis*-9, *cis*-12) in which the double bound are conjugated (for instance position 9 and 11, or 10 and 12), either in *cis* or *trans* form[13]. Conjugated linoleic acid are synthesized in ruminants both from dietary linoleic acid (18:2 *cis*-9, *cis*-12) in rumen by the microbial flora and from vaccenic acid (18:1 *trans*-11) in mammary glands during de novo synthesis[14]. The nutritional benefits associated with the consumption of conjugated linoleic acid, as demonstrated *in vitro* and in animal studies, include activity as an anticarcinogen, antiatherosclerosis agent, immune system modulator, antidiabetic agent and lean body mass enhancer[8,9,15].

Present study focused on dairy products since milk and dairy products are the richest natural source of dietary conjugated linoleic acid. The objective of the present study is to determine the fatty acid profile and conjugated linoleic acid content of various dairy products.

EXPERIMENTAL

Petroleum ether (Merck, Darmstadt, Germany), *n*-hexane (Lab-scan, Dublin, Ireland), diethyl ether (J.T. Baker, Deventer, Holland) were analytical grade. Acetyl chloride solution and sodium methylate were obtained from Merck-Schuchardt (Germany). Potassium carbonate was obtained from Merck-Darmstadt (Germany). All fatty acids including conjugated linoleic acid methyl esters as GC standards were purchased from Sigma Aldrich Co. (Steinheim, Germany). Capillary columns: WCOT fused silica capillary column coated with Chrompack CP-Sil 88 for fatty acid methyl ester (100 m × 0.25 mm i.d. × 0.20 mm film thickness) (Agilent Technologies, Wilmington, DE).

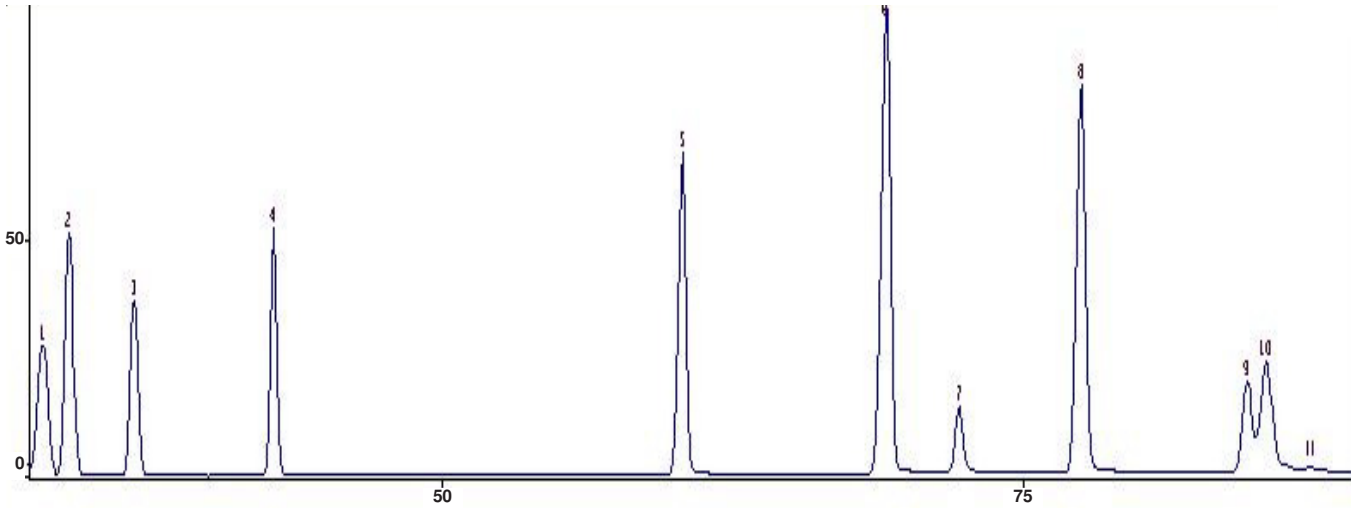

Fig. 1. Partial gas chromatograph of sigma GC standard; (1) Hexane: 32, 79; (2) Methyl butyrate: 33, 95; (3) Methyl caparate: 36, 74; (4) Methyl decanoate: 42, 72; (5) Methyl palmitate: 60, 36; (6) Methyl stearate: 69, 12; (7) Methyl oleate: 72, 27; (8) Methyl linoleate: 77, 55; (9) Conjugated linoleic acids first peak: 84, 69; (10) Conjugated linoleic acids second peak: 85, 51; (11) Conjugated linoleic acids third peak: 87, 35

Samples and extraction: Dairy products produced in various well-known big sized dairy plants were obtained from supermarkets in Isparta. Analyses were performed in duplicate for each sample.

Milk fat was extracted from milk and dairy products according to the Jiang *et al*.[16] with slight modifications. Briefly, the sample (10 g of cheese, butter and kaymak; 19 mL of yogurt, 38 mL of milk) was supplemented with 28 mL of a distilled water except from yogurt and milk sample. Yogurt sample was supplemented with 19 mL of distilled water. The sample was weighed into a 50 mL centrifuge tube. The sample was then homogenized at high speed using an Ultra Turrax, T25 homogenizer (Basic Ika Labortechnik, Germany) for 2 min. Before centrifugation, butter sample was warmed at 30 ºC. The homogenate was centrifugated using a refrigerated centrifugation (B. Braun Biotech International Biolab., Melsungen-Germany) at 12,000 rpm at 4 ºC for 0.5 h. After centrifugation, the upper layer (milk fat layer) was transferred into a centrifuge tube. 30 mL diethyl ether was added into the centrifuge tube. The mixture was allowed to stand for at least overnight. The solvent was removed using a rotational evaporator (Heidolph Laboratory, 4001) at 40 ºC under vacuum. The extract was then again centrifuged at 14,000 rpm for 2 min. (Hettich Universal, 32, Germany). After centrifugation, the extract was dried at 50 ºC under vacuum in drying oven with vacuum (Heraeus Vacutherm, Germany) for 12 h. The extract was applied two-step methylation with sodium methoxide.

Preparation of fatty acid methyl ester: Fatty acid methyl ester (FAME) of samples was prepared according to the Kramer *et al*.[17]. Approximately 100 mL of extract was added into a centrifuge tube with 15 mL. Then 1 mL sodium methoxide was added into extract and vortexed. The extract was incubated in a 50 ºC water bath for 10 min and removed from water bath and allowed cooling for 5 min. After 3 mL of 5 % methanolic HCl was added to extract, the mixture was vortexed and incubated in an 80 ºC water bath for 10 min and removed from water bath and cooled for 7 min. Then, 1 mL hexane and 7,5 mL 6 % K_2CO_3 was added to mixture and vortexed. The samples were centrifugated at 1200 rpm for 5 min. Samples were ready for GC analysis.

GC seperation of fatty acid methyl ester: Fatty acid methyl esters were analyzed using GC-FID (Perkin-Elmer Autosystem XL GC) gas chromatography equipped with flame ionization detection using a WCOT fused silica capillary column coated with CP-Sil 88 (100 m × 0.25 mm i.d. × 0.20 mm film thickness) (Agilent Technologies, Wilmington, DE). Helium was the carrier gas (15 cm/sec, inlet pressure 250 kpa). The column was operated at 60 ºC for 4 min and then temperature was programmed to increase at the rate of 13 ºC/min until 175 ºC. It was held at 175 ºC for 27 min and then temperature programmed to increase at the rate of 4 ºC/min until 215 ºC and held at that temperature for 35 min and then temperature programmed to increase at the rate of 5 ºC/min until 225 ºC and held at that temperature for 0.5 h. Starting at a temperature of 60 ºC allowed for the resolution of the short chain fatty acid methyl ester and maintaining the temperature at 225 ºC allowed for the resolution of the very long chain fatty acid methyl ester. Samples were injected using a splitter vent 2,6 mL/min. This method allowed detection of all short chain to long chain fatty acids including three isomers of conjugated linoleic acids determination[18] (Fig. 1).

RESULTS AND DISCUSSION

Fatty acid changes in various dairy products is presented in Table-1. Milk fat extracted from different dairy products had palmitic acid, oleic acid, myristic acid and stearic acid were dominated while it has low molecular weight butryic acid and caproic acid. Content of palmitic acid and stearic acid varied between 27.65-33.16 % and 9.01-13 %, respectively. Monounsaturated (omega 9) oleic acid had the second highest ratio and varied between 16.92-24.07 %. Oleic acid inhibited the mutagenic activity of food pyrolysate mutagens (Trp-P-1, Trp-P-2), polycyclic aromatic hydrocarbons and nitrosamines[19,20]. Hayatsu *et al*.[19,20] reported that the process of metabolic activation is blocked due to oleic acid micelles entrapping the mutagen or due to interactions between enzymes and oleic acid (Table-1).

TABLE-1
FATTY ACID COMPOSITIONS IN VARIOUS DAIRY PRODUCTS[a]

Sample	Butyric acid (%)	Caproic acid (%)	Capric acid (%)	Palmitic acid (%)	Stearic acid (%)	Oleic acid (%)	Linoleic acid (%)	CLA (%)	Myristic acid (%)	Tridecanoic acid (%)
Kashar cheese	1.12±0.22	1.50±0.39	2.98±0.61	32.32±3.76	10.50±1.98	20.28±2.78	2.03±0.38	1.05±0.72	12.07±0.92	3.42±0.43
Ewe's milk old kashar cheese	0.95±0.00	1.19±0.00	2.51±0.00	28.48±0.00	12.56±0.00	19.53±0.00	1.56±0.00	2.59±0.00	11.24±0.00	3.20±0.00
Kars old kashar cheese	1.37±0.00	1.81±0.00	3.38±0.00	27.65±0.00	13.00±0.00	18.22±0.00	1.99±0.00	2.87±0.00	11.85±0.00	3.54±0.00
Tulum cheese	1.20±0.27	1.61±0.14	5.93±4.72	31.21±0.01	9.01±0.88	16.92±2.30	2.26±0.11	0.66±0.26	12.47±3.09	4.62±2.33
Butter	1.37±0.33	2.29±0.44	3.66±0.34	33.16±2.96	10.02±1.71	23.21±1.16	1.90±0.54	0.79±0.27	12.18±0.56	3.71±0.64
Kaymak	2.30±0.00	2.90±0.00	3.75±0.00	32.37±0.00	9.53±0.00	21.33±0.00	1.99±0.00	0.63±0.00	12.30±0.00	3.72±0.00
Full fat yoghurt	1.03±0.48	1.22±0.50	2.55±0.74	34.90±0.83	10.96±1.87	20.22±3.30	2.19±0.13	0.62±0.16	12.28±0.53	3.29±0.62
White brined cheese	1.01±0.47	1.66±0.42	3.03±0.35	33.56±1.20	10.23±1.04	24.07±4.35	2.13±0.28	0.70±0.12	12.01±1.31	3.38±0.24
Ewe's milk Cheese	1.24±0.34	2.51±0.82	7.77±7.11	28.05±1.85	12.00±2.76	22.05±8.11	1.94±0.08	0.97±0.35	11.19±0.69	4.26±2.13
Raw Ewe's milk	1.50±0.00	2.83±0.00	10.81±0.00	29.26±0.00	6.40±0.00	16.86±0.00	2.00±0.00	0.28±0.00	12.72±0.00	5.78±0.00

[a]Mean value ± standard deviation (SD); CLA = Conjugated linoleic acid.

Even though the content of low molecular weight saturated fatty acids such as butryic acid and caproic acid are low they are very important and unique to milk fat. Butyric acid is a short chain, 4-carbon fatty acid that occurs naturally in the body. *In vitro* studies suggest that butyric acid can be used as an anticancer agent. Sodium butyrate causes reversible growth inhibition in a variety of mammalian tumor cells, probably due to an inhibition of anaerobic glycolysis since sodium butyrate inhibits the lactate dehyrogenase enzyme[11]. Butyric acid is a potent inhibitor of proliferation and an inducer of differentiation and apoptosis in a number of cancer cell lines[5,6,21,22].

In various dairy products conjugated linoleic acid values significantly changed from 0,28 to 2,87 mg/g fat. Also, there is a difference in concentrations of conjugated linoleic acid of milks between the different type of animal; for example, quantity of conjugated diene fatty acids was higher in sheep milk than cow milk. Seasonal changes also are reported to affect of conjugated linoleic acid contents of milks. Higher conjugated linoleic acid values were recorded during cold season[23].

Kars aged kashar and aged kashar cheese made from ewe's milk were richest in total conjugated linoleic acid content (2, 87 mg/g fat and 2.59 mg/g, respectively), which implicated that aging would be a significant factor to increase conjugated linoleic acid content. Lin *et al.*[4] reported that conjugated linoleic acid content of cheeses ranged from 3, 59 to 7.96 mg/g of lipid; Blue, Brie, Edam and Swiss cheeses had significantly higher conjugated linoleic acid content than the other cheeses. In another research, the average conjugated linoleic acid levels in butter were 0.45 g conjugated linoleic acid/100 g butter in winter, 0.58 in spring and 0.80 in summer according to the Ledoux *et al.*[24]. Jiang *et al.*[16] showed that the concentration of conjugated linoleic acid (*cis*-9, *trans*-11 isomer) was 5.8-5.9 mg/g fat in pasteurized in milk, 4.6-6.2 in fermented milk, 6.1-6.2 in cream products and 5.0-7.1 in cheeses. Also Prandini *et al.*[10] showed that the concentration of conjugated linoleic acid was 6.15 mg/g fat in fermented milk, 6.06 mg/g fat in yoghurt of mountain pasture and 6.05 mg/g fat in organic yoghurt.

Among the various types of dairy products, different conjugated linoleic acid contents could also be associated with specific features of the milk used in manufacturing, with special reference to the species and the conjugated linoleic acid content of the milk and the length of aging. In any case, in order to understand the factors that might affect the conjugated linoleic acid levels in dairy products the whole production system should be carefully checked, paying special attention to the animal feeding patterns, the characteristics of the milk used and the different stages in processing.

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