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Detection of biologically active isomers of conjugated linoleic acid in kaymak*

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RESUMEN

Detección de isómeros biológicamente activos del ácido linoleico conjugado en kaymak.

Numerosos efectos fisiológicos se atribuyen a los ácidos linoleico conjugados (CLA). Así los isómeros biológicamente activos (*cis*-9, *trans*-11 y *trans*-10, *cis*-12) del ácido linoleico han sido descritos con propiedades anticarcinogénicas, antioxidantes y antiarteriosclerótica. Fuentes relativamente ricas de CLA incluyen alimentos con grasas lácteas tales como el kaymak. El kaymak es una crema concentrada elaborada de leche de búfalo o vaca principalmente en Turquía. El objetivo de este estudio fue la determinación de la concentración de CLA durante la producción de kaymak. El kaymak objeto de estudio fue elaborado a partir de leche de vaca que fue enriquecida con crema no fermentada. Los isómeros biológicamente activos del CLA fueron analizados por cromatografía gaseosa en leche cruda, crema y kaymak. El método empleado fue rápido, reproducible y sensible. Se encontraron diferencias significativas en las concentraciones de ambos isómeros y de CLA total durante el proceso de producción ($p < 0.01$). Los contenidos totales de CLA para leche cruda, crema y kaymak fueron 0.08 ± 0.02, 0.234 ± 0.04 y 0.091 ± 0.08 g/100 g de lípido, respectivamente.

PALABRAS-CLAVE: Grasa láctea - Isómeros del ácido linoleico conjugado - Kaymak.

SUMMARY

Detection of biologically active isomers of conjugated linoleic acid in kaymak.

Numerous physiological effects are attributed to conjugated linoleic acids (CLA). Biologically active isomers of CLA (*cis*-9, *trans*-11 (C18:2) and *trans*-10, *cis*-12 (C18:2)) have been reported to have anticarcinogenic, antioxidative and antiatherosclerotic properties. Relatively rich sources of CLA include milk fat-containing foods such as kaymak. Kaymak is a kind of concentrated cream which is traditionally manufactured from buffalo or cow's milk mainly in Turkey, . The objective of this study was to determine CLA concentrations during kaymak production. Kaymak was manufactured from cow's milk which was enriched with unfermented cream. Biologically active isomers of CLA in raw milk, cream and kaymak were analyzed using gas chromatography. The method was quick, repeatable and sensitive for the CLA determination of samples. Significant differences were found among the concentrations of both isomer and total CLA during the production process ($p<0.01$). The total CLA contents of raw milk, cream and kaymak were determined as 0.08 ± 0.02, 0.234 ± 0.04 and 0.091 ± 0.08 g/ 100 g lipid, respectively.

KEY-WORDS: CLA isomers - Kaymak - Milk fat.

1. INTRODUCTION

Conjugated Linoleic Acid (CLA) is a descriptor for all positional and geometric conjugated dienoic isomers of linoleic acid. The two isomers known to possess biological activity are *cis*-9, *trans*-11 and *trans*-10, *cis*-12. A number of health benefits are associated with these isomers of CLA including anticarcinogenic, antiatherogenic and antidiabetogenic activities (Rainer *et al.*, 2004). The intake of CLA-enriched milk fat led to alterations in mammary gland morphogenesis and a reduction in mammary cancer risk in rats (Ip *et al.*, 1999). It is proposed that the *cis*-9, *trans*-11 CLA isomer enhances growth and probably feed efficiency in young rodents (Pariza *et al.*, 2001). The *trans*-10, *cis*-12 CLA isomer is reported to be responsible for the re-partitioning of fat to muscle (Roach *et al.*, 2001). It is also likely that some biological effects are induced and/or enhanced by these isomers acting sinergistically (Pariza *et al.*, 2001).

The contents of CLA in animal products is much higher than in plant oils. Among the animal products, CLA contents are generally higher in ruminant tissues, and dairy products are recognized as major dietary sources of CLA (Chin *et al.*, 1992). Milk fat is probably the most complex of all edible fats and the most abundant source of CLA (Christie, 1997). Therefore, milk-fat containing foods such as kaymak, butter and cheese are rich dietary sources for CLA intake.

Kaymak is a kind of concentrated cream traditionally manufactured from water buffalo or cow's milk mainly in Turkey It is especially manufactured in the Afyon, Edirne, Kocaeli, Istanbul, Bursa, Ankara and Izmir regions of Turkey. Kaymaks which are manufactured from water buffalo milk are prefered by consumers because of its high fat content (9.3%) and white color (Eralp, 1969). However since the number of water buffalos is decreasing recently in Turkey, cow's milk is much

more frequent in kaymak production. Kaymak is generally consumed with honey at breakfast and traditional Turkish desserts.

The objective of this study was to determine whether production practices in the manufacturing of kaymak affect concentrations of biologically active isomers of CLA in the finished product.

2. EXPERIMENTAL PART

2.1. Materials

The CLA isomer standard was purchased from Sigma Chemical Company (St Louis, MO). All reagents and organic solvents used were of the analytical or HPLC grade.

2.2. Manufacture of kaymak

Kaymak has a special manufacturing process as a traditional product. This process was carried out in Akgül dairy products plant (Çigli, Izmir, TR). Firstly, raw milk (100 L) containing 4 % fat was heated at 90 ^{o}C for 8-10 min. After addition of cream (13 kg) containing 70 % fat at 85 ^{o}C, the mixture was stirred and heated to 87 ^{o}C. The mixture was poured to stainless steal pans and reheated until the foam would disappear. Then, they were cooled to 20-25 ^{o}C by itself and taken to cold storage at 4 ^{o}C for 8-12 h in order to ripening. Finally, the kaymak layer formed at the upper part was cut and separated from skim milk layer. They were shaped like small rolls and packaged into plastic cups. Two trials were performed. Three replicates of each analysis were carried out in raw milk, cream and kaymak for each trial.

2.3. Methods

Total Dry matter content. Duplicate samples were dried in an electric oven at 105 ± 2 ^{o}C to a constant weight (AOAC, 1999).

Total Ash content. Total ash was determined by incineration of a represantative 1.0 g sample at 450 ^{o}C for 8 h.

Lipid extraction and CLA analysis. Total lipid analysis was modified by a described method from Tokusoglu *et al*. (2003). 2 g of the sample was homogenized with 8 mL of chloroform/ methanol (2:1 v/v) and then mixed using vortex for 30s. Homogenized samples were centrifuged at 4000x*g* for 10 min. The chloroform layer including the extracted lipids was transferred to another tube and the residue was extracted with the same procedure 3 more times. The combined filtrates were concentrated in a rotary evaporator at 30 ^{o}C and dried using N_2 flow to dryness. The total lipid obtained was calculated gravimetrically by the AOAC (1999) method.

Fatty Acid Methyl Esters (FAME) of Samples. The fatty acid methyl esters were prepared from extracted lipids of samples by transesterification reaction with 14% Boron trifluoride (BF3)- methanol complex. Extracted lipids (2 mL) of samples was refluxed with 2 ml of 0.05 N NaOH with methanole for up to 5 min and then reacted with 2 ml of BF_3 solution for 15 min. Then solutions were equilibrated to room temperature (25 ^{o}C) and treated with 2 ml of hexane and 2 ml of satiated salt, respectively and fractionated in a separatory funnel. The separated methyl ester phase was transferred to GC vials and dried. Anhydrous sodium sulfate (Na_2SO_4) was added and then injected to GC. GC analysis was carried out in a Hewlett-Packard (HP) gas chromatograph using a capillary HP-1 crosslinked methyl siloxane (25 m; 0.17 μm film thickness; 0.32 mm ID) capillary column and a flame ionization detector (FID). The gas chromatograph was temperature-programmed to start at 170 ^{o}C for 0 min isotherm (initial temperature) and increased at 1 ^{o}C/min to 205 ^{o}C, then held for 25 min isotherm (Ramp 1), and increased at 15 ^{o}C/min to 300 ^{o}C, and, finally, held for 10 min isotherm (Ramp 2). Both injector and detector temperatures were set at 250 ^{o}C. Elution time was 35 min. Carrier gas was helium (He) at a flow rate of 1.5 mL/min, pressure was 10 psi, and split ratio was 33:1. The injection amount was 1 μL.

2.4. Statistical analysis

Data were subjected to analysis of variance using Statistica, *version 6.0, StatSoft*, OK, USA. Significance of the differences between means was evaluated by Duncan's Multiple Range Test taking $p<0.01$ as significant.

3. RESULTS AND DISCUSSION

GC chromatograms of the CLA region of fatty acid methyl esters from raw milk and kaymak samples are presented in Fig.1 and Fig. 2.

Analytical parameters for CLA isomers are given in Table I.

Table II shows the composition of raw milk, cream and kaymak.

As expected, the dry matter and total lipid contents of cream and kaymak were higher than those found in raw milk. Kaymak had lower dry matter and lipid concentrations, but higher ash content in comparison to cream (Table II). According to Turkish Food Codex (2003) kaymak should contain at least 60 % fat and our sample was in accordance with this regulation

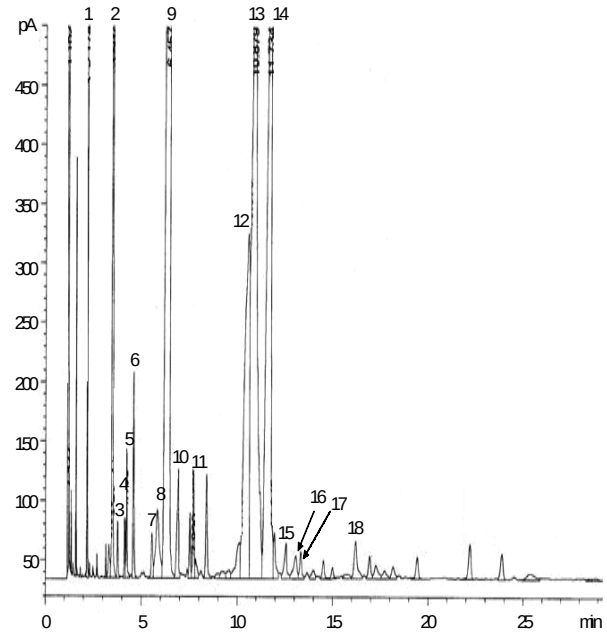

Figure 1
GC Chromatogram of CLA area of fatty acid methyl esters from a raw milk sample. 1.Butryic ; 2. Caproic ; 3. Caprylic; 4. Capric; 5. Lauric; 6. Myristic; 7. Myristoleic; 8. Pentadecanoic; 9. Palmitic; 10. Palmitoleic; 11. Stearic; 12. Elaidic; 13. Oleic; 14. Linoleic; 15. Linolenic ; 16. CLA isomer (1) (*t*10,*c*12 CLA) ; 17. CLA isomer (2) (*c*9,*t*11 CLA) ; 18. Arachidic

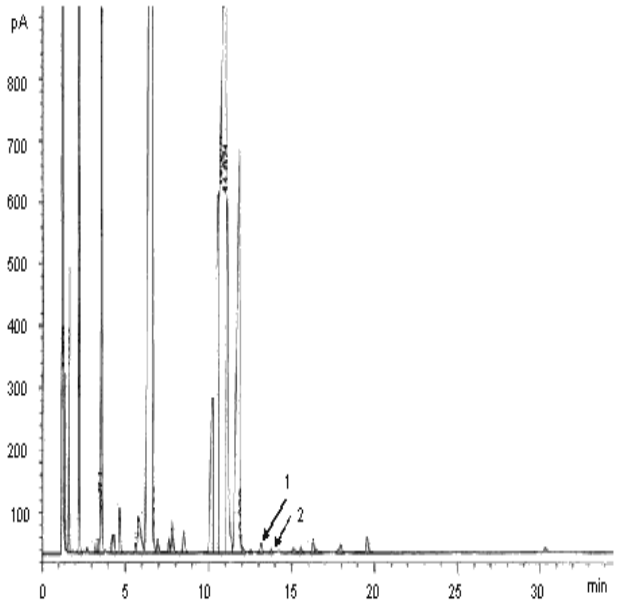

Figure 2
GC Chromatogram of CLA area of fatty acid methyl esters from a kaymak sample. 1. CLA isomer (1) (*t*10,*c*12 CLA) ; 2. CLA isomer (2) (*c*9,*t*11 CLA)

Table I
Analytical parameters of CLA isomers

Analytical Parameters		**CLA *cis*9,*trans*11**	**CLA *trans*10,*cis*12**
Interday precision *n=6*	g $100g^{-1}$ lipid	0.048 ± 0.003	0.030 ± 0.001
Relat.Std.Deviation	(%)	0.71	0.34
Recovery *n=6*	(%)	%99.998	%99.997

Table II
Composition (%) of samples during kaymak manufacture*

Sample	**Dry matter (%)**	**Lipid (%)**	**Ash (%)**
Raw milk	**11.04 ±0.02**	**4.12 ±0.03**	**0.41 ±0.07**
Cream	**72.61 ±0.05**	**70.87±0.05**	**0.01 ±0.01**
Kaymak	**67.75 ±0.04**	**65.34±0.06**	**1.77 ±0.04**

*Means of two trials, and each trial was examined in triplicate.

The concentrations of CLA isomers in the samples during the manufacturing of kaymak are given in Table III.

As shown in Table III, significant differences were found among the concentrations of *cis*-9, *trans*-11 and *trans*-10, *cis*-12 isomers and total CLA of

Table III
CLA concentrations of samples during manufacture of kaymak*

	CLA *cis*9,*trans*11 g/100g lipid	CLA *trans*10,*cis*12 g/100g lipid	Total CLA g/100g lipid
Raw milk	0.050^{b}±0.01	0.031^{c}±0.01	0.081^{c}±0.02
Cream	0.092^{a}±0.03	0.142^{a}±0.01	0.234^{a} ±0.04
Kaymak	0.039^{c}±0.05	0.052^{b}±0.03	0.091^{b} ±0.08

Means of two trials, and each trial was examined in triplicate. Means with the same superscript (a-d) in a column are not significantly different (p>0.01).

samples. Cream contained the highest amounts of both isomers and total CLA while the lowest values were determined in raw milk except *cis*-9, *trans*-11 isomer (p<0.01). The total CLA content of raw milk was lower than those found in kaymak in the production (p<0.01). Therefore, the processing of milk and cream into kaymak had a significant effect on the increase of CLA content. On the other hand, the CLA level of kaymak may have been affected by the relatively high contents of CLA isomers in cream.

In our study, the *trans*-10, *cis*-12 CLA isomer level was unexpectedly higher than the content of *cis*-9, *trans*-11 isomer in cream and kaymak. The formation of CLA in ruminant milks can be explained by the conversion of dietary linoleic acid through ruminal bacteria and mainly by vaccenic acid in the mammary gland. While some ruminal bacteria such as *Butyrivibrio fibrisolvens* produce *cis*-9, *trans*-11 CLA, others are able to produce significant amounts of *trans*-10, *cis*-12 CLA (Kim *et al*., 2002). The concentrations of *trans*-10, *cis*-12 CLA in our cream and kaymak samples were found to be higher than data obtained by Zlatanos *et al*. (2002) and similar to that reported by Prandini *et al*. (2001) for butters.

No study could be found on the CLA content of kaymak. In our study, the concentrations of *cis*-9, *trans*-11 and total CLA in raw milk, cream and kaymak were generally found to be lower than data from previous studies including raw milk, cream and butter (Lin *et al*., 1995; Shantha *et al*., 1995; Jiang *et al*., 1997; Prandini *et al*., 2001; Gonzales *et al*., 2003; Bergamo *et al*., 2003, Ledoux *et al*., in press). Relatively low CLA levels in raw milk and cream may be caused by several factors. The factors that have been shown to affect the CLA concentration in milk fat include animal diet, seasonal variation, endogenous synthesis or free-radical oxidation of linoleic acid during processing (Pariza *et al.,* 2001). Diet plays an important role in the CLA content of milk. However, previous investigations have determined substantial variations in milk fat content of CLA among indivudial cows which were fed the same diet (Kelly *et al.,* 1998; Peterson *et al*., 2002).

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