

Effect of Feeding Management and Seasonal Variation on Fatty Acid Composition and Tocopherol Content of Cows' Milk in Region of West Mediterranean, Turkey

Batı Akdeniz Bölgesindeki İnek Sütlerinin Yağ Asidi Dağılımı ve Tokoferol İçeriği Üzerine Beslenme ve Mevsimin Etkisi

Tülay Büyükoğlu¹, Hale Secilmis Canbay^{2*}, Ali Alkan Canbaz³, İsa Can Uyguralp³, Emine Tuncer³

¹Department of Biochemistry, Faculty of Veterinary Medicine, Mehmet Akif Ersoy University, Burdur, Turkey

²Department of Bioengineering, Faculty of Engineering and Architecture, Mehmet Akif Ersoy University, Burdur, Turkey

³Faculty of Veterinary Medicine, University of Mehmet Akif Ersoy, Burdur, Turkey

Abstract

Cow feeding plays a crucial role milk quality. The aim of the research was to examine the effects of two different feeding models and season variation (summer and winter) on fatty acid and tocopherol content of cow milk in two farms of West Mediterranean, Turkey. In one of examined farms, cows were fed pasture-based rations (n=12) and in another farm cows were fed commercial concentrate mix feed- based (n=12). Gross composition, fatty acids profile and tocopherol content of cows' milk were analyzed. The percentages of fat and dry matter of grazed cows' milk in summer season were higher than from cows' milk fed with commercial concentrate ration. Concentration of palmitic acid (C16:0), is higher in cows' milk fed pasture-based ration during summer period compared to that fed concentrate feed-based ration in summer and winter. In both seasons, the level of total tocopherols of cows' milk did not statistically differ between the groups. As a result, milk composition is affected by feeding management and seasonal variation. Likely, this change can be linked to grazing period and ration composition, which will influence potential nutritional qualities of the milk.

Keywords: Cow milk, Fatty acid, Grazing, Season, Tocopherol

Öz

İneklerin beslenmesi süt kalitesinde büyük bir öneme sahiptir. Araştırmanın amacı, Türkiye'nin Batı Akdeniz bölgesindeki iki çiftlikteki inek sütlerinin yağ asidi kompozisyonu ve tokoferol içeriği üzerine iki farklı beslenme modeli ve mevsim değişimlerinin (yaz ve kış) etkilerini incelemektir. İncelenen çiftliklerin birinde, inekler (n=12), çayır otu ağırlıklı rasyon ile diğer çiftlikteki inekler (n=12) ise ticari konsantre yem ağırlıklı rasyon ile beslendiler. Çalışmada, sütün bileşenleri, yağ asidi dağılımı ve tokoferol içeriği analiz edildi. Yaz sezonunda çayır otuyla beslenen ineklerin sütlerindeki yağ ve kuru madde yüzdelерinin, ticari konsantre yem ile beslenen inek sütlerindeki daha yüksek olduğu tespit edildi. Yaz döneminde çayır otuyla beslenen inek sütlerindeki palmitik asit konsantrasyonunun (C16:0), yaz ve kış aylarında konsantre yem ağırlıklı beslenen inek sütlerindeki göre daha yüksek olduğu belirlendi. Her iki mevsimde de, inek sütünün toplam tokoferol konsantrasyonları gruplar arasında istatistiksel olarak farklılık göstermedi. Sonuç olarak, sütün bileşimi besleme metotları ve iklim değişimlerinden etkilenmektedir. Muhtemelen, sütün potansiyel besinsel kalitesini etkileyecek bu değişiklikler merada otlama süresine ve rasyon bileşimine bağlı olabilir.

Anahtar Kelimeler: İnek sütü, Yağ asidi, Otlatma, İklim, Tokoferol

1. Introduction

Milk is one of the oldest foods known to man and is a complex mixture of fat, proteins, carbohydrates, minerals, vitamins and other miscellaneous constituents dispersed in water (Yasmin et al. 2012).

Bovine milk contains a large number of different FA (Fatty acid), some of which may be of potential benefit to human health, including CLA and PUFA (Polyunsaturated FA) of the n-3 FA group (Parodi 2004). It is recommended, therefore, that consumers increase their intake of these compounds. Many factors affect the FA composition of bovine milk, including breed, season, access to fresh grazing, cereal feeding and oil supplementation of feed. Conventional milk had higher contents of monounsaturated

*Corresponding Author: halecanbay@mehmetakif.edu.tr

Received / Geliş tarihi : 04.08.2016

Accepted / Kabul tarihi : 22.09.2016

FA (MUFA) and n-6 FA. However, organic milk had significantly higher contents of polyunsaturated FA (PUFA), conjugated linolenic acid (CLA), n-3 FA and branched FA (Mel'uchov' a et al. 2008). Significantly higher levels of grasses and lower levels of cereal concentrates in the fodder of organic farming could well explain these results.

Pasture-based dairying systems that provide fresh and conserved forages and occasional concentrate supplements leads to acceptable and sustainable productivity, as well as being a point of difference to concentrate-based milk production systems (Knowles et al. 2006).

An extensive production system with a high contribution from pasture in the diet of the dairy cows results in milk with a higher concentration of α -linolenic acid (ALA) and a lower concentration of linoleic acid (LA). Therefore, an extensive production form with a high level of pasture is recommended for production of milk with a high content of PUFA and high levels of potential antioxidants (Slots et al. 2010). Milk fat produced from pasture had a fatty acid (FA) profile that might be deemed more favourable by consumers, particularly the higher CLA and linolenic acid content and lower concentrations of saturated hypercholesterolemic fatty acids (Rego et al. 2004).

Along with other nutrients milk also contains vitamins that are essential organic compounds required in the diet. Most are not synthesized in the animal hence; milk levels can be influenced by dietary concentrations. These nutrients are essential for normal metabolism and well being of animals as well as man. Vitamin E content was increased in winter months than summer months in some localities (Yasmin et al. 2012). There are eight different forms of vitamin E (α -tocopherols), which are equally present in synthetic products, where as only RRR- α -tocopherol is naturally present in the feed plants. The naturally occurring RRR- α -tocopherol has the highest vitamin E activity of the eight forms and is thus the physiologically most important, meaning that animal requirements can be fulfilled from a lower amount of the natural form (e.g. from home-grown roughages) as compared to a synthetic source of α -tocopherols (Mogensen et al. 2012).

The objectives of the current study were to determine whether there is difference in FA composition and α -tocopherol content of milk from cows fed pasture-based and commercial concentrate rations and to compare the influence of seasons (summer and winter) on these parameters for two feeding management in region of West Mediterranean, Turkey.

2. Material and Methods

2.1. Reagents

Tocopherol standards, n-hexane, n-heptane, tetrahydrofuran, chloroform, methanol (HPLC grade), were obtained from Merck (Merck KGaA, Darmstadt, Germany); standards of fatty acid methyl esters (FAME) from Sigma-Aldrich (Supelco 37 Component FAME Mix Supelco, Bellefonte, PA); hydrochloric acid (HCl, % 37) were purchased from Sigma-Aldrich (Steinheim, Germany).

2.2. Study Area and Feeding Management

The study was conducted in two different areas of West Mediterranean, Turkey. The first of these regions is an intensive, semi-open system farm of Hacilar village, Burdur (A zone) and the second region is an extensive traditional family farm of the town of Babadag, Denizli (B zone). During the summer period (from March to August), cows in B zone grazed on a natural rangeland and during the winter period, same cows (from September to February) were fed on based dry grass silage and cereals (oat, barley, and straw) while in diets of cows in A zone were predominantly used a commercial concentrate mix feed and corn silage in both summer and winter periods.

2.3. Animals and Milk Sample Collection

The farm bulk tank milk samples were collected monthly from July 2012 to June 2013 from Holstein dairy cows in this two different regions. In this study, it was identified from September to February as the winter period and from March to August as summer period. A total of 500 ml of milk samples were collected in plastic bottles. A total of 24 milk samples were transported on ice boxes to the laboratory and all samples were frozen at -20°C until analysis.

2.4. Milk Basic Composition

Milk was analysed for fat, protein, lactose, dry matter (DM) and freezing point (FP) by a milk analyser (Bentley B150 Combi Milk Analyser (Minnesota, USA))

2.5. Extraction

Chloroform is widely used in defatting of biological materials. For milk fat extraction two solvent mixture methanol/chloroform ($\frac{1}{2}$, v/v) was used (Folch et al. 1957). 50 mL methanol/chloroform mixture was added to 10 ml of milk in a 100 mL glass flask. The flasks were introduced into an ultrasonic bath (60 min) (Bandelin, Berlin Germany) and extracts were filtered using vacuum filtration and collected into a clean container (Millipore, Merck, Darmstadt,

Germany). The milk fat was recovered by evaporation of the solvent mixture using rotary evaporator (Heidolph, Schwabach, Germany) at 40 °C.

2.6. Preparation of Milk Fatty Acid Methyl Esters (FAMEs)

A preparation step was necessary prior to introduction of the milk fat into the GC/MS for the individual determination of fatty acid composition. Milk FAMEs were obtained by trans esterification with 1.5 M HCl in metanol. 500 µL 1.5 M HCl in metanol and 100 µl milk fat were mixed and shaken vigorously for 15 min in Bandelin ultrasonic shaker (Berlin, Germany). Milk fat was methylated 2 h (70°C), and then 1 ml hexane was added to collect to the FAME in hexane as above and analysed by GC/MS (Yilmazer and Secilmis, 2006).

2.7. Analysis of FAME by GC/MS

A GC/MS (Agilent, 5975 C + 7890A GC, Santa Clara, USA) and an auto sampler (Agilent 7693, Santa Clara, USA). A 100 m * 0.25 mm ID * 0.20 µm film HP-88 capillary column (J&W Agilent, Santa Clara, USA), and GCMS Chemstation software system (Agilent, Santa Clara, USA) were used for analysing FAME. The split ratio was 1:20 and the flow-rate of carrier gas (helium) 2ml/min. The injector and detector temperatures were fixed at 250°C. The temperature programme for the column was: hold at 60°C for 1 min increase by 13°C/min to 175°C, increase at 4°C/min to 215°C, and then hold at 215°C for 35 min., total run was 86 min. The mass spectrometer was operated in EI mode at 70 eV scanning the range 30-500 m/z in a 1 s cycle, in a full scan acquisition mode. All mass spectra were also compared with the data system library (Wiley 275) (Secilmis Canbay et al. 2011).

2.8. Analysis of Tocopherols from Milk Fat

1 g milk fat sample was dissolved in 1 ml of mobil phase prior to injection into the liquid chromatography. Tocopherols (α -, β -, γ - and δ -tocopherol) were evaluated by HPLC with direct injection of samples in a mixture of heptane:tetrahydrofuran (95:5, v/v) solution. Detection and quantification were carried out with a Shimadzu Prominence System CBM 20A controller (Kyoto, Japan), SIL-20AC HTA prominence Autosampler, LC-20AT prominence pump and RF-10AXL Fluorescence Detector (Ex 295 nm, Em 330 nm) for tocopherols. The Luna Silica (250*4.6 mm, 5 µm, Phenomenex, Torrance, USA) column was used. The mobile phase was consisted of heptane/THF (95/5; v/v) at a

flow rate of 1.2 ml/min and the injection volume was 10 µL (Ulusoy et al. 2009).

2.9. Statistical Analysis

The results were analysed with a Completely Random Variance Analysis, to compare two year seasons (summer and winter) and two different feeding managements (grass and concentrate mix feed). Comparison of the means with a significant difference ($P < 0.05$) were determinate by Tukey's test. All data were analysed by ANOVA using Statistical Analyses System program (PASW, 2009). Descriptive statistics (mean, standard deviation) were computed to characterize the distribution of tocopherol concentrations in milk fat samples analyzed. We defined the LOD as three times the background noise of the chromatographic instrument. Samples with amount below the LOD were not detectable.

3. Results

3.1. Milk Composition

Milk composition of cows are presented in Table 1. Fat and dry matter (DM) content are higher ($P < 0.01$) in grazed cows' milk, when compared to that fed concentrate diets in the summer period. Also, a lower proportion ($P < 0.05$) of lactose content characterizes milk from cows fed pasture in summer season (GS) when compared to cows in winter season (GW). However, milk protein and freezing point (FP) values were similar among groups. The main constituent of cow milk is dry matter amount which is important technologically. Nutritional value of milk and the quality of dairy products normally increase depending on dry matter amount of milk. In cows fed with the low roughage ration was observed increase in milk production, milk protein, and lactose content, but decrease in milk fat.

3.2. Milk Fat FAMEs and Tocopherols Composition

Our results suggest that palmitic acid (C16:0) concentration constitute majority of total lipids (Table 2). Also, Table 4 shows that concentration of palmitic acid (C16:0) which is monounsaturated fatty acid is higher in cows fed pasture-based ration (GS) during summer period compared to that fed concentrate feed-based ration in summer and winter (CS and CW). No significant differences in percentages of other analysed fatty acids (C18:0, C18:2, C18:3 and CLA) were detected. The chromatogram of a cow's milk fat sample is reported in Figure 1.

Dietary milk fats, on account of their higher content of saturated fatty acids, have long been associated with a variety of human diseases; however, recent studies have focussed on the healthy components of milk fats, including CLA (Mel'uchov'a et al. 2008, Tudisco et al. 2010). Conjugated linolenic acid is a group of positional and geometric fatty acid isomers derived from linoleic acid of which milk fat is the

richest dietary source. Fatty acid profile and CLA contents of milk yielded by goats raised according to the organic vs. conventional system. In particular, the nutritional quality of organic milk seems to be higher than that of conventional milk (Tudisco et al. 2010, Bergamo et al. 2003).

Significant differences were identified in fatty acid profiles between organic and conventional milk fat. Total SFA

Table 1. Milk composition of cows fed grass or concentrate diets in summer and winter seasons (mean± SD, %)

	GS	CS	GW	CW	P-value
Fat	4.41 ± 0.83 ^a	2.95 ± 1.06 ^b	4.26 ± 0.69 ^{ab}	3.39 ± 0.27 ^{ab}	*
Protein	2.53 ± 0.83	2.93 ± 0.09	2.66 ± 0.31	2.76 ± 0.36	ND
Lactose	4.02 ± 0.22 ^a	4.52 ± 0.10	4.25 ± 0.44 ^a	4.35 ± 0.38	*
DM	14.11 ± 1.92 ^a	12.66 ± 0.73 ^b	11.30 ± 1.77 ^{ab}	13.41 ± 1.57 ^{ab}	**
FP	0.54 ± 0.04	0.56 ± 0.01	0.54 ± 0.03	0.53 ± 0.03	ND

GS: Grass-Summer, **CS:** Concentrate-Summer, **GW:** Grass-Winter, **CW:** Concentrate-Winter, **DM:** Dry mater, **FP:** Freezing point, *: One each line, the values with letter are significantly different *= P<0.05 ** = P<0.01, **ND:** Not detectable.

Table 2. Milk fatty acid (% milk fat) and total tocopherols (mg/kg of milk fat) composition of cows fed grass or concentrate diets in summer and winter seasons

Compound	R _T	GS	CS	GW	CW
C4:0	14.7	0.94 ± 0.41	1.35 ± 0.59	0,60 ± 0.39	0.96 ± 0.36
C6:0	17.5	1.09 ± 0.31	1.34 ± 0.20	0,91 ± 0.31	1.19 ± 0.24
C8:0	19.6	0.95 ± 0.16	1.11 ± 0.12	0.85 ± 0.16	1.12 ± 0.16
C10:0	21.5	2.34 ± 0.42	2.54 ± 0.22	2.18 ± 0.28	2.65 ± 0.40
C12:0	23.7	3.55 ± 0.79	4.11 ± 0.89	3.40 ± 0.85	3.55 ± 0.28
C14:0	26.8	12.19 ± 1.82	11.98 ± 1.47	11.64 ± 1.71	12.03 ± 0.81
C14:1	28.3	1.07 ± 0.43	1.10 ± 0.33	1.36 ± 0.29	0.87 ± 0.20
C15:0	28.7	1.14 ± 0.32	1.05 ± 0.13	1.3 ± 0.22	0.98 ± 0.18
C16:0	31.0	34.24 ± 2.82 ^a	28.46 ± 0.66 ^b	31.99 ± 4.49 ^{ab}	29.01 ± 1.87 ^b
C16:1	32.5	2.04 ± 0.48	1.81 ± 0.13	1.91 ± 0.68	1.55 ± 0.27
C17:0	33.3	0.52 ± 0.11	0.61 ± 0.31	0.59 ± 0.25	0.49 ± 0.06
C18:0	35.8	9.60 ± 2.40	10.87 ± 1.97	9.84 ± 2.30	11.98 ± 1.43
C18:1	37.5	22.62 ± 3.39	22.56 ± 1.70	23.49 ± 3.79	23.01 ± 1.56
C18:2	40.0	1.67 ± 0.49	2.29 ± 0.74	1.85 ± 0.71	2.47 ± 0.47
C18:3	42.1	0.14 ± 0.09	0.19 ± 0.06	0.11 ± 0.03	0.11 ± 0.05
CLA	53.8	0.56 ± 0.18	0.56 ± 0.22	0.75 ± 0.42	0.59 ± 0.25
Total SFA		66.56 ± 10.50	63.42 ± 8.81	63.3 ± 9.86	63.96 ± 9.10
Total MUFA		25.73 ± 12.17	25.47 ± 12.19	26.76 ± 12.62	25.43 ± 12.59
Total PUFA		2.37 ± 0.79	3.04 ± 1.12	2.71 ± 0.88	3.17 ± 1.25
Total tocopherols		15.1 ± 8.22	67.21 ± 70.87	29.54 ± 26.74	14.68 ± 17.29

^{ab}: On each line, the values with different letters are significantly different(P<0.05); **R_T:** Retention time; **CLA:** Conjugated linoleic acid (cis 9, trans 11-C18:2 isomer) **GS:** Grass-Summer, **CS:** Concentrate-Summer, **GW:** Grass-Winter, **CW:** Concentrate-Winter.

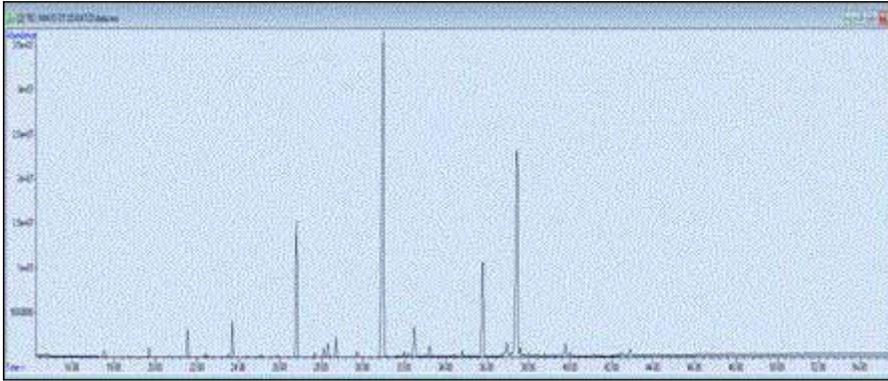


Figure 1. Chromatogram of a cow's milk oil sample (FAMES).

concentration was not influenced by the system of production. It was found significantly higher concentrations of LA (15%), CLA9 (32%), α -LN (57%), EPA (62%), n-3 (60%), n-6 (12%) and total PUFA (24%) in organic compared with conventional milk fat. Also, milk purchased in summer was found to have significantly higher fat content (5%) and a similar protein content compared with milk purchased in winter. Total SFA were significantly lower (6%) during the summer, whereas significantly lower concentrations of MUFA and PUFA (both by 15%) were found in winter milk fat. Concentrations of total n-3 FA were higher (37%) in summer, concentrations of n-6 FA were significantly higher (4%) in winter (Butler et al. 2011).

The safety and quality of milk products is reliant on milk composition that varies with locality, stage of lactation, breed and species, milking system, age and size of the cow, environment, climate, temperature, dietary composition and season. The effect of season in the wider sense is the result of interaction of different physiological, climatic and feeding factors that interact with erratic intensity throughout the year. The general seasonal variation in the chemical composition of milk is well understood. Milk fat is most sensitive to dietary changes and can vary over a range of nearly 3.0 percentage units. Dietary manipulations result in milk protein concentration varying approximately 0.60 percentage units. Milk fat and protein percentages are higher during autumn and winter seasons and lower in spring and summer.

The biochemical characteristics and content of the main nutrients of herbages offered to dairy ruminants influence the milk quality, in particular to the fat and fatty acid composition. Besides their quantitative contribution to dietary energy, the different fatty acids: short- and medium-chains, branched, cis- and trans- isomers, saturated, mono and polyunsaturated are potentially defined as positive

or negative (in some cases) factors for the consumer health (Tsvetkova et al. 2010). α -linolenic, linoleic and palmitic acids were predominant lipids in plant samples, and α -Linolenic acid was the most abundant fatty acid in all-botanical species analyzed. The content of α -linolenic acid significantly decreased from 62% to 39% between May and August, and subsequently it significantly rose to 57% from August to September. The predominant saturated fatty acid in all investigated plant species was palmitic acid and, it increased from May (13%) to June (19%), and subsequently it decreased from August (17%) to September (14%). The content of polyunsaturated FA in an average pasture forage sample during pasture season decreased from 77% in May to 59% in August, and subsequently it increased to 74% in September. The fat content in analyzed average samples of pasture plants increased from 3.5% in May to 3.8% in June, decreased to 3.2% in August, and subsequently it increased to 4.2% in September. The CLA content in ewe milk fat during pasture season decreased from May to August, and subsequently it increased in September to the value similar to that at the beginning of pasture season (Mel'uchov'a et al. 2008).

Nutritional manipulation of the rumen ecosystem provides as strategy to alter the content and composition of milk fat. In addition, change is influenced by the transfer of dietary fat into milk, which is related to fatty acid composition, degree of ruminal metabolism, and efficiency of digestion (Ashes et al. 1997).

Ruminants are generally supplied with unsaturated fatty acid (UFA) from the forage portion of their diet and animals consuming fresh pasture will have a higher content of UFA in their milk than those receiving a cereal-based concentrate diet (Woods and Fearon, 2009). The C6:0 to C16:0 content of milk fat is typically reduced when roughage diets are fed and the proportion of C18:1 and C18:2 is increased

(Grummer, 1991). In our study, palmitic acid (C 16:0) content of milk fat is increased in cows fed pasture-based ration (GS) during summer period compared to that fed concentrate feed-based ration in summer and winter (CS and CW). The increase of palmitic acid in milk fat may be because of the predominant palmitic acid in plant species (Mel'uchov'a et al. 2008).

Bergamo et al. (2003) were determined that organic buffalo milk and mozzarella cheese contained significantly higher *cis-9 trans-11*C_{18:2} (CLA), *trans-11* C_{18:1} (TVA), linolenic acid (LNA), α-tocopherol (TH) and β-carotene concentrations than did conventional dairy foods. However, Slots et al. (2009) were suggested that the concentration of α-tocopherol and β-carotene in milk was not affected by the proportion of pasture used in the organic and conventional milk production systems. In the present study, we also could not determine the effect of pasture feeding management on the total tocopherols in cow milk (P>0.05).

Table 3 shows the retention time (Rt), correlation coefficients (r²), limits of detection (LOD, S/N = 3.3), limits of quantification (LOQ, S/N = 10), relative standard deviations (RSDs) and recovery (R%) by HPLC-RF of

studied tocopherols. In both seasons, concentration of total tocopherol of cows'milk did not statistically differ between the groups (Table 2). Figures 2 show chromatograms of tocopherol in standard solutions.

4. Discussion

In conclusion, results of this study indicated that fat content in cow's milk were affected by seasons and feeding. During the summer period, pasture based diets increased monounsaturated fatty acid (C16:0, palmitate) concentration. However, it could not be determined the effect pasture feeding management on the concentration of total tocopherols in cow milk. Further studies to investigate the effects on cows' milk composition of the pasture-based feeding could improve potential nutritional qualities of the milk.

5. Acknowledgments

The research was carried out with resources from two projects entitled " Assesment of Fat Soluable Vitamin Changes in the HPLC Composition of Cow Milks in the Lake District According to Different Nutrition Ways

Table 3. Retention time (Rt), correlation coefficients (r²), limits of detection (LOD, S/N = 3.3), limits of quantification (LOQ, S/N = 10), relative standard deviations (RSDs, %) and recovery (R, %) by HPLC-RF.

Compound	Rt	r ²	LOD (mg/kg)	LOQ (mg/kg)	RSDs (%)	R (%)
alpha-tocopherol	2.9	0.999	0.03	0.10	2.11	98
beta-tocopherol	5.8	0.999	0.03	0.10	2.64	96
gamma-tocopherol	6.4	0.999	0.03	0.10	2.53	95
delta-tocopherol	11.0	0.999	0.03	0.10	2.90	95

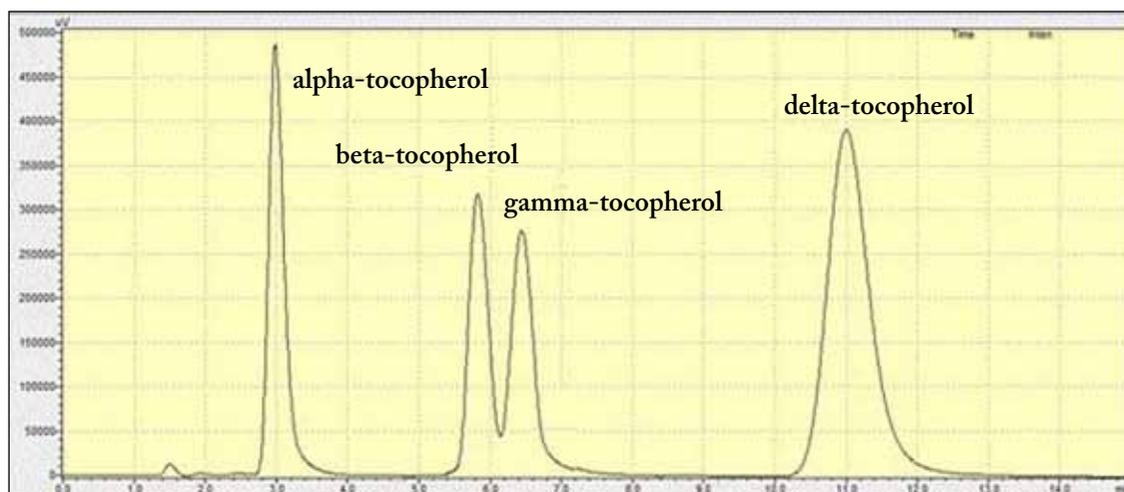


Figure 2. Chromatogram of tocopherol standards.

and Seasons” and “How Nutrition and Seasons Effect The Fatty Acid Composition of the Cows’Milk which Live in The Region of Lakes” and these projects were supported by the Domestic Research Projects Support Program for University Students (TUBITAK 2209-A). Also, these two projects were presented as poster in İstanbul University Faculty of Veterinary Medicine, 16th Veterinary Medicine Student Scientific Research Congress, May 8-10, 2014, İstanbul/TURKEY.

6. References

- Ashes, J.R., Gulati, S.K., Scott, T.W. 1997. Potential to alter the content and composition of milk fat through nutrition. *J Dairy Sci.*, 80: 2204-2212.
- Bergamo, P., Fedele, E., Iannibelli, L., Marzillo, G. 2003. Fat-soluble vitamin contents and fatty acid composition in organic and conventional Italian dairy products. *Food Chem.*, 82: 625-631.
- Butler, G., Stergiadis, S., Seal, C., Eyre, M., Leifert, C. 2011. Fat composition of organic and conventional retail milk in North east England. *J Dairy Sci.*, 94: 24-36.
- Collomb, M., Bisig, W., Butikofer, U., Sieber, R., Bregy, M., Etter, L. 2008. Fatty acid composition of mountain milk from Switzerland: Comparison of organic and integrated farming systems. *Int Dairy J.*, 18: 976-982.
- Folch, J., Lees, M., Sloane Stanley, G.H. 1957. A simple method for isolation and purification of total lipids from animal tissues. *J Biol Chem.*, 226: 497-509.
- Grummer, R.R. 1991. Effect of feed on the composition of milk fat. *J Dairy Sci.*, 74: 3244-3257.
- Knowles, S.O., Grace, N.D., Knight, T.W., McNabb, W.C., Lee, J. 2006. Reasons and means for manipulating the micronutrient composition of milk from grazing dairy cattle. *Anim Feed Sci Tech.*, 131: 154-167.
- Melúchová, B., Blasko, J., Kubinec, R., Górová, R., Dubravská, J., Margetin, M., Soják, L. 2008. Seasonal variations in fatty acid composition of pasture for age plants and CLA content in ewe milk fat. *Small Ruminant Res.*, 78: 56-65.
- Mogensen, L., Kristensen, T., Søgaard, K., Jensen, S.K., Sehested, J. 2002. Alfa-tocopherol and beta-carotene in rough ages and milk inorganic dairy herds. *Livest Sci.*, 145: 44-54.
- Parodi, P.W. 2004. Milk fat in human nutrition. *Aust J Dairy Technol.*, 59: 3-59.
- Rego, O.A., Portugal, P.V., Sousa, M.B., Rosa, H.J.D., Vouzela, C.M., Borba, A.E.S, Bessa, R.B. 2004. Effect of diet on the fatty acid pattern of milk from dairy cows. *Anim Res.*, 53: 213-220.
- Secilmis Canbay, H., Atay, E., Ogut, S. 2011. Determination of fruit characteristics, fatty acid and total antioxidant capacity of İstanbul Medlar Variety (*Mespilus germanica* L.). *Curr Opin Biotech.*, 22: 142.
- Slots, T., Butler, G., Leifert, C., Kristensen, T., Skibsted, L.H., Nielsen, J.H. 2009. Potentials to differentiate milk composition by different feeding strategies. *J Dairy Sci.*, 92: 2057-2066.
- Tsvetkova, V., Angelov, L. 2010. Influence of the season on the total lipids and fatty acid composition of grasses at the different altitudes in the region of the middle Rhodopes. *Bulg J Agric Sci.*, 16: 748-753.
- Tudisco, R., Cutrignelli, M.I., Calabrò, S., Piccolo, G., Bovera, F., Guglielmelli, A., Moniello, G., Infascell, F. 2010. Influence of organic systems on milk fatty acid profile and CLA in goats. *Small Ruminant Res.*, 88: 151-155.
- Ulusoy, S., Boşgelmez Tınaz, G., Seçilmiş Canbay, H. 2009. Tocopherol, Carotene, Phenolic Contents and Antibacterial Properties of Rose Essential Oil, Hydrosol and Absolute. *Curr Microbiol.*, 554-558.
- Woods, V.B., Fearon, A.M. 2009. Dietary sources of unsaturated fatty acids for animals and their transfer into meat, milk and eggs: A review. *Livest Sci.*, 126: 1-20.
- Yasmin, A., Huma, N., Sadiq Butt, M., Zahoor, T., Yasin, M. 2012. Seasonal variation in milk vitamin contents available for processing in Punjab, Pakistan. *J Saudi Soc Agricul Sci.*, 11: 99-105.
- Yılmaz, M., Secilmis, H. 2006. Investigation of derivatization conditions of Free Fatty Acids in Methanolic HCl. III National Congress of Analytical Chemistry. July 5-8. Çanakkale, Turkey, pp: 90.