

Some Physicochemical Properties and Fatty Acid Compositions of Different Originated Anatolian Water Buffaloes Milk Samples

Zeki Gürler¹, Senem Güner², Tuğba Dedebaş³, Teslime Ekiz Ünsal⁴

¹ Afyon Kocatepe University, Veterinary Faculty, Department of Food Hygiene and Technology, Afyonkarahisar.

^{2,4} Afyon Kocatepe University, Engineering Faculty, Department of Food Engineering, Afyonkarahisar.

³ Afyon Kocatepe University, Bolvadin Vocational School, Department of Food Technology, Afyonkarahisar.

e-posta¹: zgurler@aku.edu.tr

ORCID ID: <http://orcid.org/0000-0002-9037-2945>

Sorumlu yazar e-posta²: sguner@aku.edu.tr

ORCID ID: <http://orcid.org/0000-0002-6697-5535>

e-posta³: tugbadedebas@gmail.com

ORCID ID: <http://orcid.org/0000-0003-1663-0165>

e-posta⁴: tekiz@aku.edu.tr

ORCID ID: <http://orcid.org/0000-0001-7708-3420>

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Abstract

This study was carried out to determine the physicochemical properties and the fatty acid composition of milk samples collected from different originated Anatolian water buffaloes living in the same farm. The fat amounts in samples were ranged between 5.24 and 8.67%. The fatty acids with the highest ratios in buffalo milk fat belonged to C16:0 (palmitic acid), C18:1 (oleic acid), C18:0 (stearic acid) and C14:0 (myristic acid). Saturated fatty acids (SFA) (62.6 to 67.3 %) were found to be more dominant in buffalo milk fat than monounsaturated fatty acids (MUFA) (28.1 to 32.99 %) and polyunsaturated fatty acids (PUFA) (3.22 to 9.57%). Conjugated linoleic acid contents of the samples were determined between 1.46 % and 2.11 %. The conjugated linoleic acid ratios of the samples were similar. It was concluded that origin difference was effective on the physicochemical properties and the fatty acid composition of buffalo milk. It is thought that the results obtained from this study will contribute to obtaining information about the nutritional properties of Anatolian buffalo milk and to improve the nutritional and technological properties of products.

Keywords

Water buffalo; Milk fat; Milk composition; FAME analysis

Farklı Orijinli Anadolu Mandalarına Ait Süt Örneklerinin Bazı Fizikokimyasal Özellikleri ve Yağ Asidi Bileşimleri

Öz

Bu çalışma, aynı çiftlikte yaşayan farklı orijinli Anadolu mandalarından toplanan süt örneklerinin fizikokimyasal özelliklerini ve yağ asidi kompozisyonunu belirlemek amacıyla yapılmıştır. Örneklerdeki yağ miktarları % 5,24 ile % 8,67 arasında değişmiştir. Manda sütü yağında en yüksek oranlara sahip yağ asitleri C16:0 (palmitik asit), C18:1 (oleik asit), C18:0 (stearik asit) ve C14:0 (miristik asit) yağ asitlerine aittir. Doymuş yağ asitlerinin (SFA) (% 62,6 ila % 67,3) manda sütü yağında, tekli doymamış yağ asitlerinden (MUFA) (%28,1 - % 32,99) ve çoklu doymamış yağ asitlerinden (PUFA) (% 3,22 -% 9,57) daha baskın olduğu bulunmuştur. Bununla birlikte, örneklerin konjuge linoleik asit içerikleri % 1,46 ile % 2,11 arasında belirlenmiş ve konjuge linoleik asit oranları benzer bulunmuştur. Menşe farkının manda sütünün fizikokimyasal özellikleri ve yağ asidi bileşimi üzerinde etkili olduğu sonucuna varılmıştır. Sonuç olarak, bu çalışmadan elde edilen verilerin Anadolu manda sütünün besinsel özellikleri hakkında bilgi edinilmesine ve ürünlerin besinsel ve teknolojik özelliklerinin iyileştirilmesine katkı sağlayacağı düşünülmektedir.

Anahtar kelimeler

Manda; Süt yağı; Süt kompozisyonu; FAME analizi

1. Introduction

Milk has high nutritional values and various health benefits and is an animal food that is heavily consumed by the society in all development and life stages (Nicolaou *et al.* 2011). Buffalo milk is a type of milk with a higher protein, fat and mineral (especially calcium and phosphorus) content than cow milk (Dame *et al.* 2010). Buffalo milk, which cannot be considered as drinking milk directly due to its intense aroma and taste, can be used in the productions of kaymak (similar to the clotted cream), butter, yoghurt and cheese (Kara *et al.* 2018, Aydın and Güneşer 2021, Salzano *et al.* 2021). Their intense aroma and taste are mainly occurred by high amounts of fats. Kaymak, which has a high milk fat content from these products, is a high nutritious food used as a breakfast food, alongside desserts, or as a condiment in some confectionery (Pamuk 2017).

Fat is one of the most important fractions of milk and also liable for the high energy and nutritional value of the milk. In addition, its high fat content makes buffalo milk extremely convenient for the milk product processes (Ménard *et al.* 2010). Within the same species, the circumferential conditions, including climate, feeding diet, number and stage of lactation, and genetic factors can significantly affect the chemical composition of the milk (Kanwal *et al.* 2004, Garau *et al.* 2021). Considering both intra- and inter-species differences, the most variable component of the milk is the lipid fraction (Aganga *et al.* 2002, Jensen *et al.* 2002). The fatty acid composition of the milk determines the texture, color and flavor which are some of its significant parameters (Gürsoy *et al.* 2021).

It has been reported that the fatty acid composition of buffalo milk varies as a function of breed, lactation stage, season, and animal diet (Arumughan and Narayanan 1981, Patiño *et al.* 2008, Talpur *et al.* 2007, Talpur *et al.* 2008). Conjugated linoleic acid is one of the important milk fatty acids. The main sources of CLA and its isomers are milk, meat and their products from ruminant animals (Fleck *et al.* 2021). About 80 to 90% of CLA

in milk fat is found as the isomer of cis-9, trans-11 (Zongo *et al.* 2021). It is stated that the conjugated linoleic acid has various physiological properties such as anticarcinogen, antidiabetogen, antiadipogen, and antiatherosclerotic agent etc. (Belury 2002, Jiao *et al.* 2021, Wang *et al.* 2022). The milk and milk products are quite important in worldwide. The milk composition and its attributes of common milks (such as cow, sheep, and goat) have been studied in detail. However there are few studies conducted for the buffalo milk and for the comparison of the origin differences. Thus, the Anatolian buffalo milk has not been studied in detail. The aim of this study was to compare the milk of different originated Anatolian water buffaloes living in the same farm in terms of the physicochemical and fatty acid (especially conjugated linoleic acid) content.

2. Material and Method

2.1 Material

Buffalo milk samples were collected from Anatolian water buffaloes belonged to four different regions (Afyonkarahisar, Çorum, Balıkesir, and Diyarbakır) in Turkey living in the same farm. The animals were located in Afyon Kocatepe University, Faculty of Veterinary Medicine, Education Research and Application Farm. Milk fats extracted from these milks were used to determine their fatty acid composition and conjugated linoleic acid (CLA) contents of different regions.

2.2 Milk fat extraction

The extraction of milk fat was conducted according to Güner *et al.* (2021) with some modifications. The 20 ml of milk was mixed with the 40 ml of methanol-chloroform solution (1:1, v/v). The mixture was shaken vigorously for a min and then kept at ambient temperature for 1 h. The lower phase was collected and dried under vacuum using a rotary evaporator. The obtained milk fat samples were stored at 4°C until further use (Güner *et al.* 2021).

2.3 Determination of physicochemical compositions of buffalo milk samples

Buffalo milk samples compositions (fat, protein, lactose, total dry matter and non-fat dry matter) were determined using the Milk Analyzer MID - Infrared (MIRIS).

2.4 Determination of fatty acid compositions of milk fats

The fatty acid compositions of the milk fat samples were obtained using fatty acid methyl esters (FAME) analysis (Güner *et al.* 2021). For the FAME analysis, the milk fat samples were diluted using hexane and following that mixed with potassium hydroxide. The methylated fatty acids of samples were analyzed with a GC HP 6890N system, assembled with a flame ionization detector (FID) and a HP-88 capillary column (Agilent J&W Scientific, Santa Clara, CA, USA; 60 m x 0.25 mm). The split rate was 70:1 and the carrier gas (hydrogen) was 0.4 ml/min. The injection and detector temperatures were 270°C and 290°C, respectively. The initial oven temperature was 60°C, gradually heated to 103°C at a rate of 10°C /min, gradually heated to 170°C at a rate of 6.5°C /min, gradually heated to 215°C at a rate of 3°C /min and held for 10 min. Later the oven was heated to 235°C at a rate of 5°C /min and held at this temperature for 5 min. The peaks of the compounds were identified using the FAME Mix 37 standards (Supelco, Sigma Aldrich, St. Louis, MO, USA).

2.5 Statistical analysis

Data were fabricated as the averages of duplicates and expressed with the standard deviations. The One-way ANOVA (analysis of variance) was used to compare the groups ($P < 0.5$) and Tukey's studentized range test was done to observe the differences in the groups using JMP Pro 11 statistical analysis system (SAS Institute Inc., Cary, NC, USA).

3. Results and Discussion

3.1. Physicochemical compositions of buffalo milk samples

The physicochemical properties (fat, protein, lactose, total dry matter, non-fat dry matter) of the

different originated Anatolian water buffaloes milk samples were shown in Table 1.

The fat level in samples was in the range of 5.24 and 8.67%. In the present experiment, the lowest fat content was identified for the OR2 (5.24%) while for the sample OR3 showed the highest value (8.67%) (Table 1). The fat amount in the sample OR2 was statistically different ($P < 0.05$) from the others ($P < 0.05$). Similar fat amounts were found for the buffalo milk samples (Varricchio *et al.* 2007, Han *et al.* 2012, Kashwa 2016, Çinar *et al.* 2019, Gürler *et al.* 2021). The results show that, the fat amount in the samples other than the sample OR2, are in agreement with the reported literature.

For the protein, the lowest level was obtained for the OR4 (3.38%) and the highest level belonged to the OR3 (4.73%). In the literature, the amount of protein of the buffalo milk was found as 5.53% (Gürler *et al.* 2021), 4.70% (Han *et al.* 2012), 4.71% (Kashwa 2016) and 4.5% (Varricchio *et al.* 2007). Compared to the literature, the protein content of the samples of the OR2 was lower, while the others are compatible with previously recorded data. In this study, the protein amounts of the provinces OR1/OR2 and others showed significantly difference.

In all investigated buffalo milk samples, the OR4 contained the lowest and the OR1 highest amounts of lactose. In the studies of (Han *et al.* 2012, Kashwa 2016, Çinar *et al.* 2019, Gürler *et al.* 2021) reported that the lactose in the milk was 4.60%, 4.84%, 4.84% and 4.97%, respectively. In the current study, amount of lactose of the sample OR4 was found to be much lower compared to the previous studies. All province sample groups exposed similar lactose amounts, except for the OR4, which was the lowest. The concentration range of total solids was from 13.55 to 18.46% as given in Table 1. The lowest value (13.55%) was found for the OR4 and the highest value (18.46%) was found for the OR3. Han *et al.* (2012), Kashwa (2016) and Gürler *et al.* (2021)] reported that the total solids in the milk was ranged from 17.11 to 18.83% which was higher than the OR4 and OR2 province samples in the current study. In general, the milk samples of OR3 and OR1 were in accordance with the literature.

Among all samples, the OR4 had the lowest amount of non-fat dry matter (6.73%), followed by the OR2 (8.86%) and the highest value was found in the OR1 (9.82%). In the current study, the amount of non-fat dry matter of the sample OR4 was found to be lower than reported values of Çınar *et al.* (2019) and Gürler *et al.* (2021), while the others were compatible with the literature.

3.2. Fatty acid compositions of milk fats

The fatty acid profiles of milk fat samples obtained from different buffalo origin were given in Table 2. Regarding the short chain fatty acids (C4-C10), no significant differences were observed between origins (Table 2). The sample OR2 had the highest contents of both FAs (4.99 % of butyric acid and 2.40 % of caproic acid), whereas the OR4 had the lowest level (3.58 % and 1.67 %, respectively). The highest caprilic (C8:0) and capric (C10:0) fatty acid amounts were determined in the OR1, while the lowest values of these fatty acids were determined in the OR4. These results were in accordance with the literature (Qureshi *et al.* 2010, Saroha *et al.* 2014). Insignificant differences may be caused mainly by the environmental and seasonal differences between studies.

In all investigated buffalo milk fats, the highest concentrations were belonged to C14:0 (myristic acid), C16:0 (palmitic acid), C18:0 (stearic acid), and C18:1 (oleic acid), shown in Table 2 and were in parallel with the literature (Penchev *et al.* 2016, Garau *et al.* 2021). Commonly, the palmitic acid (C16:0) was the most abundant long fatty acid (from 29.14 % of OR2 to 30.83 % of OR1) ($P>0.05$). In addition, the oleic acid (C18:1), was the most abundant MUFA, with a very similar levels in all the samples (ranged from 25.49 to 29.34 %); the OR3 had the highest palmitoleic acid content (2.17 %, C16:1), when compared to the other origin samples. The total levels of SFAs, MUFAs and PUFAs were shown in Table 2. As expected, the SFA levels were the highest in all tested milk samples. The PUFAs of the milk samples were low as expected and parallel with the literature (Garau *et al.* 2021). Among all samples, the OR2 had the highest content of PUFAs. The PUFAs results showed that, the OR1 had the

highest amount of linoleic (C18:2) and the OR2 had the highest amount of linolenic (C18:3n-3) acids. These differences may be caused by the genetic differences of the sampled animals regardless of the similar environmental conditions.

The conjugated linoleic acid isomers are gained some attention due to their promising health gains such as anticarcinogenic, antiatherogenic, antidiabetic, and immune modulating (Talpur *et al.* 2007). They are believed to be positively effective on the reduction of the body fat mass. The CLA are produced from oleic acid through the ruminal biohydrogenation (Han *et al.* 2012). The higher levels of CLA of this study may be basically related to the diet of the animals since the variety, concentration, and intake levels of unsaturated FAs can dramatically affect the final levels of produced CLA. The presence of high levels of linoleic acid in plant oils can favor the production of CLA by ruminant animals (Talpur *et al.* 2007). Table 2 shows the levels of isomer c9, t11 linoleic acid (conjugated linoleic acid, CLA) obtained from milk samples. CLA in the OR2 were higher (2.11%) than the other origin samples had ($P>0.05$). As for the CLA content in the milk samples, it resulted in higher CLA contents than studies in buffalo milk (Miyavlova and Peeva 2007, Talpur *et al.* 2007, Varricchio *et al.* 2007, Çınar *et al.* 2019, Garau *et al.* 2021). The CLA levels of each origin sample were insignificantly different from each other and found as approximately 2%. These levels show the importance of the buffalo milk as a source for CLA.

The physicochemical and fatty acid compositions greatly determine the biological and nutritive values of milk. Milk fat mainly consists of triacylglycerols, containing fatty acids mixed as short, medium and long chain (Alonso *et al.* 1999). Of the approximately 400 different fatty acids, identified in milk fat, which play a major role in maintaining good health, only 10 of them are present at concentrations greater than 1% (Creamer and Macgibbon 1996).

The lactation stage and age are some factors that significantly affect the milk composition and fatty acid concentration in buffaloes (Qureshi *et al.* 2015). In our study, there are differences in the milk composition and fatty acid profiles of samples belonging to the different origins. It is thought that

the reason for this is mostly due to the differences in age and lactation stages, since we obtained from breeds living in the same farm. Although the fat levels were significantly different in each origin, the

fatty acid compositions were not significantly different from each other.

Table 1. Physicochemical parameters of different originated Anatolian water buffaloes milk samples*

Origin	n	Fat (%)	Protein (%)	Lactose (%)	Total dry matter (%)	Non-fat dry matter (%)
OR1	8	7.81±0.99 ^a	4.27±0.28 ^b	4.90±0.39 ^a	17.61±0.90 ^a	9.82±0.76 ^a
OR2	8	5.24±2.70 ^b	3.90±0.34 ^b	4.27±0.74 ^a	14.10±1.99 ^b	8.86±0.95 ^a
OR3	8	8.67±2.12 ^a	4.73±0.65 ^a	4.36±1.13 ^a	18.46±2.89 ^a	9.79±1.58 ^a
OR4	8	7.41±2.19 ^a	3.38±0.63 ^c	2.82±1.61 ^b	13.55±4.25 ^b	6.73±2.28 ^b

Note: Means with different letters are significantly different ($P \leq 0.05$).

* Data are expressed as the mean ± standard deviation (SD); n: sample size.

OR1: Afyonkarahisar originated Anatolian water buffaloes milk samples, OR2: Balıkesir originated Anatolian water buffaloes milk samples, OR3: Çorum originated Anatolian water buffaloes milk samples, OR4: Diyarbakır originated Anatolian water buffaloes milk samples.

Table 2. Fatty acid compositions of different originated Anatolian water buffaloes milk samples*

Fatty acids (%) (n:8)	Origin			
	OR1	OR2	OR3	OR4
C4:0	3.87±0.34 ^b	4.99±0.97 ^a	3.77±0.42 ^b	3.8±0.53 ^b
C6:0	2.31±0.24 ^a	2.40±0.65 ^a	2.20±0.24 ^{ab}	1.67±0.55 ^b
C8:0	1.34±0.25 ^a	1.25±0.41 ^{ab}	1.21±0.24 ^{ab}	0.84±0.35 ^b
C10:0	2.69±0.61 ^a	2.47±0.72 ^a	2.46±0.35 ^a	1.56±0.65 ^b
C12:0	3.25±0.67 ^a	2.85±0.64 ^{ab}	3.04±0.37 ^a	2.06±0.71 ^b
C14:0	12.14±0.96 ^a	11.02±3.32 ^a	11.39±1.36 ^a	9.71±2.64 ^a
C14:1	1.42±0.12 ^b	1.59±0.51 ^{ab}	1.83±0.21 ^a	1.82±0.17 ^a
C15:0	1.40±0.11 ^b	1.50±0.41 ^{ab}	1.76±0.25 ^{ab}	1.85±0.35 ^a
C16:0	30.83±4.24 ^a	29.14±7.13 ^a	30.24±2.31 ^a	30.37±3.95 ^a
C16:1	1.40±0.19 ^a	1.02±0.48 ^a	2.17±1.64 ^a	1.83±0
C18:0	9.52±1.65 ^a	8.80±3.43 ^a	9.45±1.38 ^a	10.99±4.06 ^a
C18:1	26.86±2.60 ^a	25.49±9.79 ^a	27.38±2.72 ^a	29.34±4.89 ^a
C18:2, 9c 11 t (CLA)	1.69±0.39 ^a	2.11±0.98 ^a	1.46±0.43 ^a	2.02±0.30 ^a
C18:2n6	0.26±0.06	ND	ND	ND
C18:3n-3	0.75±0.37 ^b	1.45±0.57 ^a	0.57±0.26 ^b	0.90±0.26 ^{ab}
C18:3n-6	ND	1.70±1.48	1.99	ND
C20:4	0.52±0.29 ^b	4.31±4.08 ^a	0.77±0.57 ^b	0.39±0.21 ^b
ΣSFA	67.35	64.42	65.52	62.63
ΣMUFA	29.68	28.1	31.38	32.99
ΣPUFA	3.22	9.57	4.02	3.31

Note: Means with different letters are significantly different ($P \leq 0.05$).

ND: Non detectable; n: sample size.

* Data are expressed as the mean ± standard deviation (SD).

OR1: Afyonkarahisar originated Anatolian water buffaloes milk samples, OR2: Balıkesir originated Anatolian water buffaloes milk samples, OR3:Çorum originated Anatolian water buffaloes milk samples, OR4: Diyarbakır originated Anatolian water buffaloes milk samples.

4. Conclusion

In this study, the physicochemical and fatty acid compositions of the milk obtained on different days from different originated buffaloes living in the same farm were investigated. The physicochemical properties and FA compositions of buffalo milk samples were generally compatible with the previous literature. It is thought that, the reasons for the differences with other study results may have occurred due to the origin, age, lactation period, seasonal and climatic characteristics, feed, etc. of the animal from which the milk was obtained. Studies on the determination of the composition of buffalo milk are fewer when compared to the studies conducted with milk of other species. It is thought that, our results may assist in selecting an appropriate feed source with a desirable fatty acid profile (especially CLA) and contribute in obtaining a general data, determining and improving the current situation, and preparing for future studies.

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