International Journal of Agriculture, Environment and Food Sciences

JAEFS

e-ISSN : 2618-5946

DOI: 10.31015/jaefs.2020.1.2

www.jaefs.com

Research Article

Int J Agric Environ Food Sci 4 (1):7-13 (2020)

Determination of fatty acids profiles and volatile compounds of cows' and goats' butters

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Abstract

In this study, the fatty acid composition and volatile components of cows' and goats' yayik butters were analyzed. For this purpose, analyzes in cows' and goats' yayik butter obtained from local markets in Adıyaman province were performed. Saturated fatty acids (SFAs) were determined as dominant fatty acids in the butter samples. While the highest SFA content was detected in goats' butters, the highest unsaturated fatty acid (UNSFA) and monounsaturated fatty acid (MUFA) contents were in cow samples. Furthermore, some individual fatty acid concentrations (tridecanoic, behenic and α-linolenic) of cows' and goats' butters were significantly different. Additionally, the total 27 volatile compounds including alcohols, aldehydes, acids, esters, hydrocarbons, ketones and terpenes were quantified in samples. The most important volatile compounds in samples were oxylene, mxylene, butyric, caprylic and valeric acids.

Keywords: Yayik butter, Fatty acids, Volatile components, Cows' milk, Goats' milk

Introduction

Cream and yogurt are used as raw material in butter production and the butter made from yoghurt is called "yayik butter". Yayik butter is one of the popular dairy products in Turkey due to its unique flavor and aroma and it is traditionally produced by farmers in Anatolia for many centuries. "Yayik" is the name given to the churning of the yogurt during the production. Yogurt, which is raw material in the production of yayik butter, is obtained from different animal milk such as goats' and cows' milk (Sağdiç et al., 2002; Sağdıç et al., 2004). Goat breeding is still being maintened in southeastern region of Anatolia and goat's milk butter is produced in this region. It is well known that the butters produced from goats' milk have some characteristic features. Although, goat's butter is more preferred by some consumers than butter of other mammal milks (Hayaloglu and Karagul-Yuceer, 2011), it is not commercially produced in significant amount (Haenlein, 2004).

Milk fat contains more than 400 different fatty acids including saturated fatty acids, monounsaturated fatty acids and polyunsaturated fatty acids (Méndez-Cid et al., 2017). The certain fatty acids have benefical or potentially harmfull effects on human health (Pegolo et al., 2016). In the evaluation of the quality properties of butter, it is critical to define the content of fatty acids. Furthermore, milk fat is important in the formation of aroma components in butter because of the distinct flavor and low aroma thresholds of fat-derived compounds (Vagenas and Ioannis, 2012).

There has been some studies about butter and yayik butter produced from milk of different species of mammals (Sağdıç et al., 2004; Atamer et al., 2007; Şenel et al., 2011; Tahmas Kahyaoğlu, 2014). On the other hand, there has been investigating studies (Tosun, 2016; Haddar, 2017; Ergöz, 2017) examined the physical and chemical properties, fatty acid profiles and aroma compounds of the yayik butter. However, in our knowledge, there is little study investigating the volatile compounds and fatty acids of yayik butter produced from different animal milks. In this study, fatty acid compositions and volatile compounds of goats' and cows' yayik butter were investigated

Cite this article as:
Akgul, F.Y., Ceylan, H.G., Atasoy, A.F. (2020). Determination of fatty acids profiles and volatile compounds of cows' and goats' butters. Int. J. Agric. Environ. Food Sci., 4(1), 7-13.
DOI: https://dx.doi.org/10.31015/jaefs.2020.1.2
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Received: 21 November 2019 **Accepted:** 16 January 2020 **Published Online:** 14 February 2020
Year: 2020 **Volume:** 4 **Issue:** 1 (March) **Pages:** 7-13
Available online at : http://www.jaefs.com - http://dergipark.gov.tr/jaefs

for contribute to literature.

Materials and Methods

Material

Eight goats' and cows' yayik butter provided from Adıyaman province of Turkey was used as material.

Determination of fatty acids (FA) profile of yayik butter

Proportional FA compositions of butter were determined in their FA methyl esters (FAME) according to Bannon et al., (1982) and Ackman (1998). The FAME examined by a GC (Agilent 7697A, Agilent Technologies, USA) equiped with a flame ionization detector. The FAME separated with a capillary HP-FFAP column (J&W 19091F – 433, Agilent Technologies, USA; 30 m×0.25 mm i.d; 0.25 µm film thickness). Each sample was injected twice by the GC auto sampler.

Determination of volatile compounds of yayik butter

Injections of the samples into the gas chromatography system and characterization of aroma in the mass spectrometry (GC/MS), identification of the volatile components and determination of retention times were carried out according to Whetstine et al., (2003).

Extraction of volatile components

Extraction of volatile components in butter was performed by Solid Phase Micro Extraction (SPME) method (Stashenko and Martínez, 2007). 10 g samples were weighed into 40 mL amber vials and 1 g of NaCl (10% of the sample amount) and 10 µL internal standard (81 ppm, 2 methyl 3-heptanone + 2-methyl pentanoic acid) were added. The closed vials were stored in -25 ºC deep freezer until analysis. Prior to injection into the gas chromatographic system, the vials were allowed to equilibrate the volatile components for 30 minutes at 40 ºC in the SPME extraction system. After that, 50/30 µm Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS, Agilent, USA) was placed on fiber for 30 minutes with fiber for adsorption of volatile components.

Determination, identification and quantification of volatile components

Separation of volatile components was carried out with GC (Agilent 7890A, USA) fitted with flame ionization detector and mass spectrometer. Desorption of the volatile components to injection block was executed with SPME method, manual splitless mode and fiber at 250 ºC kept to desorption for 10 min. The volatile components separated in HP Innowax column (30 m x 0.25 mm x 0.25 µm) were determined by scanning in mass spectrometer (Agilent GC5975 C MSD, USA) at a range of 30-300 eV.

Statistical analysis

Descriptive statistics and independent sample t-test by using SPSS packet program were performed.

Result and Discussion

Fatty acid profiles of yayik butters

Fatty acid (FA) contents of cow and goat samples are shown in Table 1. Saturated fatty acids (SFA) were detected as dominant FAs in cow and goat samples.

It has been previously stated that saturated fatty acids were dominant fatty acids in cows' and goats' milks (Paszczyk et al., 2019). In addition, the total SFA content of goat samples (71.15%) was statistically higher ($P<0.05$) than cow samples (65.29%). Ruminant milks contain higher levels of SFA due to the biohydrogenation of unsaturated fatty acids which is responsible for reducing the toxic effects of unsaturated fatty acids on the development of rumen bacteria (Shingfield et al., 2008). Similarly, Bernard et al., (2018) stated milk of ruminants contains a high percentage of saturated FAs, which accounts for about 70 % of the total FAs. Furthermore, cow samples were characterized by significantly higher ($P<0.05$) contents of unsaturated fatty acids (UNSFA, 34.56%) and monounsaturated fatty acids (MUFA, 29.21%) compared to goat samples (UNSFA, 28.54%; MUFA, 24.11%). Moreover, the ratios of SFA/UNSFA and linoleic/α-linolenic acid (LA/ALA) in cow samples were found to be lower ($P<0.05$) than goat samples. Very high ratio of LA/ALA promotes many diseases such as cardiovascular disease, cancer and inflammatory diseases (Paszczyk et al., 2019) and the optimal ratio of LA/ALA can be ranges from 1:1 to 4:1 depending on the disease considered (Simopoulos, 2002).

Major individual FA in samples was identified as palmitic acid (C16; cow samples, 30.47; goat samples, 30.89%) and this is in agreement with Al-Khalifah and Al-Kahtani (1993) and Paszczyk et al., (2019). The second of the major FAs in cows' and goats' yayik butters was elaidic acid (C18:1n9t), which is monounsaturated trans FA. Trans unsaturated fatty acids in ruminants are synthesized in the rumen by microbial hydrogenation and they have been reported in butter to be between 4-11 % depending on region and season (Sommerfeld, 1983). However, it is seen that the elaidic acid content (cow samples, 24.19; goat samples, 19.91%) in our samples is considerably higher than this value. Furthermore, this can be attributed to the reduction of fiber content and the increase of grain content in animal feeding (Haenlein, 2004). Other FAs, which are determined at the highest amount, were myristic (C14) and stearic (C18) acids. The total amount of palmitic, elaidic, myristic and stearic FAs in the total FAs was 76.82% and 73.16% in cow and goat samples, respectively. The contents of individual dominant FAs were in agreement with to the results reported by Atasoy and Türkoğlu (2010) for Sanlıurfa butter oil. Furthermore, myristic, palmitic and stearic acids were accounted for approximately 53% of total FAs for cow and goat samples.

The tridecylic (tridecanoic; C13), behenic (docosanoic; C22) and α-linolenic acid (C18:3n3) concentrations of cow and goat samples were significantly different ($P<0.05$). Goat samples (0.99%) were characterized by statistically higher ($P<0.05$) contents of tridecylic acid compared to cow samples (0.23%). In addition to these, behenic acid (0.10%) and α-linolenic acid (0.42%) contents in goat samples were found to be lower ($P<0.05$) than cow samples (behenic, 0.29%; α-linolenic, 0.86%). The difference of FAs concentrations may be explained by variations of physiological and anatomical between cow and goat (Haenlein, 2004). Short and medium FAs originated from *de novo* synthesis in the mammary gland, while long FAs arising from either body fat mobilization or dietary fat (Lucas et al., 2008).

In order to better characterize the health effects of fatty acids, indices such as atherogenicity index (AI) and desaturation index (DI) have been proposed (Ulbricht and Southgate, 1991). The low AI is desirable in milk and dairy products to provide protection against coronary heart disease (Paszczyk et al., 2019). AI was found to be 2.24±0.20 and 2.82±0.26 in cow and goat samples, respectively. Similar results were observed by Paszczyk et al., (2019) and Blasko et al., (2010) for butters. Also, the DI expresses the concentration of the unsaturated product proportional to the sum of the unsaturated product and the saturated substrate (Schennink et al., 2008). DI-14, DI-16 and DI-18 were calculated as average 0.08 and 0.078, 0.046 and 0.034, 0.129 and 0.112 in cow and goat samples, respectively.

Volatile Compounds of Yayik Butters

Total concentrations of volatile compounds are shown in Table 2. Total 27 components including alcohols (4), aldehydes (1), acids (9), esters (2), hydrocarbons (6), ketones (3) and terpens (2) were identified in all samples. In a study (Haddar, 2017), 35 aroma components containing acid, alcohol, ketone, aldehyde, ester, alkane, alkene and various compounds were detected in yayik butter produced with different starter cultures. Major components were determined as oxylene, mxylene, valeric, caprylic and butyric acid in cows' yayik butters. In goats' yayik butters, dominant volatile components were quantified as butyric, caprylic, valeric acid, oxylene, mxylene, capric and acetic acid, respectively. Ergöz (2017) reported the major volatile components in buffalo yayik butter were butyric acid, 2-methylbutanoic, 2-nonanone, butyl alcohol, gamma-terpinene, hexanoic acid, n-hexanol, styrene. Furthermore, components such as acetic acid, butyric acid, caprylic acid, 2-methylbutanoic acid, ethylbenzene, styrene, 2-nonanone, 2-undecanone, which are detected by Ergöz (2017) in buffalo yayik butter, were common to our results. Some volatile components identified in our examples such as acetic acid, butyric acid, valeric acid, lauric acid, ethanol, 2-propanol, benzaldehyde, ethylbenzene, limonene were also determined by Senel et al., (2016) for yayik butter.

Alcohols including ethanol, 2-propanol and 3-methyl-2-butanol in yayik butter samples were detected. Furthermore, 2,3 butanediol was only detected in cow samples. Alcohols in dairy products are produced by many metabolic pathways such as lactose metabolism, methyl ketone degradation, amino acids metabolism (Molimard and Spinnler, 1996). Moreover, the native flora of milk plays an important role in the formation of alcohol in dairy products. Despite the ethanol is the precursor of ethyl esters, it has a limited aromatic role in dairy products (Molimard and Spinnler, 1996). In addition, branched-chain alcohols, such as 3-methyl-2-butanol, have been generally accepted as off-flavours components in milk (Centeno et al., 2003). Only benzaldehyde from aldehydes has been identified in cow and goat samples. This can be thought to result from the conversion of reactive aldehydes to alcohol or acid components (Molimard and Spinnler, 1996).

Table 1. Fatty acid acid profiles of cows' and goats' yayik butters ((w/w) %, mean±std error)

Fatty acids	Cows' Yayik Butter			Goats' Yayik Butter			p-value
	Mean	Minimum	Maximum	Mean	Minimum	Maximum	
C4	2.30±0.25	1.45	3.53	2.33±0.24	1.38	3.13	0.93
C6	1.35±0.13	0.57	1.90	1.40±0.28	0.16	2.55	0.88
C8	1.00±0.08	0.52	1.32	1.66±0.44	0.23	3.53	0.18
C10	2.22±0.12	1.60	2.57	5.38±1.55	1.28	12.07	0.08
C11	nd	nd	nd	nd	nd	nd	-
C12	2.71±0.12	2.31	3.32	3.70±0.37	2.50	5.10	0.03
C13	0.23±0.05	nd	0.49	0.99±0.32	nd	2.36	0.05
C14	10.53±0.53	8.47	13.02	10.70±0.55	9.12	14.10	0.83
C14:1	0.96±0.14	0.57	1.54	0.94±0.19	0.18	1.69	0.93
C15	1.35±0.09	0.87	1.80	1.32±0.14	0.79	1.93	0.86
C15:1	nd	nd	nd	nd	nd	nd	-
C16	30.47±1.30	25.56	38.22	30.89±1.40	24.60	36.58	0.83
C16:1	1.51±0.23	0.92	2.99	1.15±0.19	0.44	2.10	0.25
C17	0.75±0.03	0.58	0.91	0.69±0.11	0.16	1.21	0.62
C17:1	0.42±0.04	0.25	0.63	0.38±0.07	0.18	0.73	0.61
C18	11.63±0.86	6.54	13.91	11.66±0.50	8.73	13.32	0.98
C18:1n9c	1.80±0.26	0.65	2.86	1.47±0.18	0.56	2.20	0.32
C18:1n9t	24.19±1.00	19.18	28.91	19.91±1.79	14.73	29.68	0.06
C18:2n6c	0.58±0.18	0.20	1.66	0.47±0.08	0.22	0.90	0.61
C18:2n6t	2.19±0.14	1.41	2.65	1.95±0.14	1.40	2.67	0.26
CLA (C18:2 cis 9, trans 11)	0.28±0.03	0.17	0.44	0.24±0.03	0.11	0.36	0.38

CLA (C18:2 trans 9, cis 11)	0.30±0.03	0.20	0.43	0.32±0.05	0.13	0.51	0.72
C18:3n6	0.41±0.04	0.27	0.64	0.37±0.08	0.16	0.75	0.62
C18:3n3	0.86±0.07	0.59	1.12	0.42±0.06	0.19	0.71	0.001
C20	0.33±0.02	0.25	0.41	0.31±0.03	0.18	0.44	0.56
C20:1	nd	nd	nd	nd	nd	nd	-
C20:2	nd	nd	nd	nd	nd	nd	-
C20:3n6	nd	nd	nd	nd	nd	nd	-
C20:3n3	0.05±0.04	nd	0.29	0.14±0.10	nd	0.80	0.43
C20:4n6	0.32±0.06	0.11	0.53	0.22±0.04	nd	0.35	0.18
C20:5n3	0.32±0.05	nd	.49	0.22±0.06	nd	0.50	0.25
C21	nd	nd	nd	0.02±0.02	nd	0.14	0.35
C22	0.29±0.07	nd	0.61	0.10±0.05	nd	0.32	0.05
C22:1n9	0.35±0.08	nd	0.80	0.27±0.07	nd	0.44	0.51
C22:1	nd	nd	nd	nd	nd	nd	-
C22:2	nd	nd	nd	nd	nd	nd	-
C22:6n3	0.05±0.05	nd	0.37	0.09±0.09	nd	0.73	0.67
C23	nd	nd	nd	nd	nd	nd	-
C23:1	nd	nd	nd	nd	nd	nd	-
C24	nd	nd	nd	nd	nd	nd	-
C24:1	nd	nd	nd	nd	nd	nd	-
SFA	65.29±1.31	59.67	71.59	71.15±2.06	62.54	78.05	0.03
UNSFA	34.56±1.34	28.02	40.25	28.54±2.03	21.82	37.46	0.03
MUFA	29.21±1.03	24.30	33.00	24.11±1.96	18.04	33.57	0.04
PUFA	5.35±0.43	3.72	7.25	4.44±0.27	3.38	5.33	0.10
SFA/UNSFA	1.92±0.12	1.48	2.55	2.62±0.26	1.67	3.58	0.03
LA/ALA	2.33±0.20	1.37	3.16	3.77±0.60	1.40	6.75	0.04
AI	2.24±0.20	1.53	3.34	2.82±0.26	2.09	4.24	0.10
Total ω-3	1.27±0.13	0.92	2.00	0.87±0.16	0.49	1.67	0.07
Total ω-6	2.90±0.31	1.73	4.67	2.71±0.18	2.01	3.51	0.61
Total ω-9	2.14±0.31	0.65	3.35	1.74±0.23	0.56	2.58	0.32
DI-14	0.084±0.013	0.051	0.150	0.078±0.013	0.018	0.126	0.74
DI-16	0.046±0.004	0.032	0.073	0.034±0.004	0.016	0.056	0.12
DI-18	0.129±0.012	0.090	0.179	0.112±0.014	0.047	0.171	0.39

SFA: sum of saturated fatty acids, UNSFA: sum of unsaturated fatty acids, MUFA: sum of monounsaturated fatty acids, PUFA: sum of polyunsaturated fatty acids, CLA: Conjugated linolenic acid, LA: linoleic acid, ALA: α- linolenic acid, AI: atherogenicity index (sum of lauric (C12:0), palmitic (C16:0), and 4 times myristic acid (C14:0) contents divided by the unsaturated fatty acids content), **ω:** omega, DI: desaturation index (DI-14: C14:1/(C14:0 + C14:1); DI-16: C16:1/(C16:0 + C16:1); DI-18:C18:1n9c/(C18:0 + C18:1n9c)), nd: not detected.

Butyric, valeric, caprylic, capric, lauric, benzoic, acetic, propionic and 2-methylbutanoic acids were quantified in all samples. In cow samples, valeric, caprylic, butyric and capric free fatty acids were dominating, respectively. Butyric, caprylic, valeric and capric free fatty acids in goat samples were the major free fatty acids. Most of the detected major free FAs have previously been reported as dominant free fatty acids in butters (Şenel et al., 2011; Méndez-Cid et al., 2017; Iradukunda et al., 2018). Lipase or esterase enzymes are responsible for lipolysis, which is responsible for the formation of free FAs. The source of these enzymes is milk itself (lipoprotein lipase) or psychrotrophic microorganisms (Vagenas and Ioannis, 2012). The short and medium chain free fatty acids are mostly formed by lipoprotein lipase (Collins et al., 2003). Goat samples had a higher amount of short and medium chain free fatty acids than cow samples. This may be related to the higher concentration of lipoprotein lipase in the cream phase in goat milk compared to cow's milk (Chilliard et al., 1984). Similarly, Şenel et al., (2011) reported that level of total free fatty acids was higher in goats' yayik butter. Although the presence of short-chain free fatty acids plays an important role in the characteristic flavor of goats' milk products, the high amount of free fatty acids is associated with hydrolytic rancidity (Chilliard et al., 1984). The long-chain free fatty acids have little effect on flavor due to their high detection thresholds. They also serve as precursors to other aroma components such as esters, ketones, aldehydes (Vagenas and Ioannis, 2012).

Table 2. Volatile compounds of cows' and goats' yayik butters (ppm, mean±std error)

	Cows' Yayik Butter			Goats' Yayik Butter			
	Mean	Minimum	Maximum	Mean	Minimum	Maximum	p-value
Alcohols							
Ethanol	24.92±9.98	nd	75.61	5.08±4.12	0.00	33.18	0.10
2,3 butanediol	1.36±0.89	nd	5.73	nd	nd	nd	0.17
3-methyl-2-butanol	17.22±10.51	nd	78.10	4.21±4.21	nd	33.67	0.28
2-propanol	68.06±36.53	nd	246.81	38.36±29.12	nd	231.42	0.54
Aldehydes							
Benzaldehyde	2.53±1.72	nd	12.56	15.76±12.13	nd	99.99	0.30
Acids							
Acetic acid	78.90±25.27	13.84	230.32	190.62±107.50	10.79	897.19	0.33
Propionic acid	5.34±3.51	nd	23.12	nd	nd	nd	0.17
Butyric acid	106.96±44.95	nd	401.73	527.11±337.50	17.43	2781.340	0.26
Valeric acid	183.73±40.59	48.88	351.23	441.95±292.51	0.00	2393.53	0.41
Caprylic acid	143.89±33.04	57.56	284.98	461.75±238.76	31.15	2000.56	0.23
Capric acid	88.95±18.85	28.100	157.69	202.47±64.16	25.42	481.14	0.13
Lauric acid	10.07±8.47	nd	67.97	69.79±29.83	nd	259.01	0.09
Benzoic acid	8.43±7.20	nd	58.31	37.51±20.86	nd	158.84	0.22
2-methylbutanoic acid	2.71±2.71	nd	21.68	3.67±2.43	nd	16.45	0.80
Esters							
Methyl caproate	nd	nd	nd	7.84±4.90	nd	13.88	0.15
Ethyl oleate	62.99±34.21	nd	256.81	20.30±20.30	nd	162.36	0.30
Hydrocarbons							
Hexane	14.01±12.43	nd	100.40	71.62±62.00	nd	501.14	0.38
Heptane	23.94±13.28	nd	99.03	68.94±26.92	nd	179.11	0.16
Ethylbenzene	19.42±4.80	nd	38.26	21.74±3.31	10.10	40.63	0.70
Mxylene	157.94±29.39	43.05	294.40	238.61±52.78	50.46	515.94	0.20
Oxylene	230.06±66.49	41.55	658.64	244.67±44.36	122.82	500.38	0.86
Styrene	51.13±8.22	12.43	95.56	68.34±9.40	29.80	103.44	0.19
Ketones							
3-hydroxy-2-butanone	2.23±1.46	nd	9.38	5.33±3.02	nd	23.50	0.37
2-Nonanone	6.91±2.96	nd	22.22	13.20±3.58	nd	25.65	0.20
2-undecanone	3.82±1.63	nd	11.48	2.86±2.02	nd	21.68	0.72
Terpens							
Limonene	2.45±1.24	nd	8.35	3.20±1.24	nd	7.67	0.67
Beta-Caryophyllene	1.23±0.81	nd	5.15	3.76±1.70	nd	13.44	0.20

nd: not detected

Ethyl esters of fatty acids contribute to the aroma of dairy products at low levels, while at higher levels it causes a flavor defect called "fruity". The presence of high amounts of ethyl esters of long chain fatty acids (C12 or above) can give the product an undesirable soapy, tallowy odor (Liu et al., 2004). Ethyl oleate which is one of the long chain free fatty acid ethyl esters was detected in cow and goat samples. Particularly high amounts were detected in goat samples. Methyl caproate was found only in goat samples. 3-hydroxy-2-butanone, 2-nonanone and 2-undecanone from ketones in samples were quantified at low amount. Ketones can be perceived at low levels and cause typical odors such as fruity, floral, and musty (Vagenas and Ioannis, 2012). Moreover, ketones such as 2-nonanone and 2-undecanone are known to contribute to the aroma of dairy products (Vagenas and Ioannis, 2012).

Hydrocarbons including hexane, heptane, ethylbenzene, mxylene, oxylene and styrene were identified in the butter samples. The concentrations of hydrocarbons, particularly xylene and mxylene, were quite high in butter samples. Hydrocarbons, the secondary products of lipid autoxidation, do not directly affect the aroma, but lead to the formation of other aroma components (Bintsis and Robinson, 2004). Limonene and beta-caryophyllene were determined at low levels in yayik butter samples. Terpenes, which are found as secondary metabolites in plants, are transferred directly to the milk with feeding and they are important for determining the geographical origin (Bontinis et al., 2012). Particularly, terpenes such as limonene are a known component of citrus essential oils (Condurso et al., 2008).

Conclusion

The fatty acid compositions and volatile compounds of goats' and cows' yayik butters were investigated in this study. About 60-70% of the identified fatty acids in samples were saturated fatty acids and saturated fatty acids in goats' yayik butters were found higher than cows' butters. Myristic, palmitic and stearic acids were major saturated fatty acids in all samples. Furthermore, goats' and cows' yayik butter samples were different in tridecylic, behenic and α-linolenic acid contents. Total 27 volatile components including alcohols, aldehydes, acids, esters, hydrocarbons, ketones and terpens were quantified in samples. In general, acids and hydrocarbons were the most important volatile components in all samples. Furthermore, dominant volatile compounds in samples were detected as oxylene, mxylene, butyric, caprylic and valeric acids.

In addition to these, it has been found that the fatty acids and volatile compounds of the samples changed within a very wide margin. These can be explained by the fact that production is mostly done by local farmers at home and there is no standard production technique. Further studies require to investigation of standard yayik butter production. Moreover, future studies should be focused to investigate the fatty acid profiles and flavor components in yayik butters from a wider region at different time intervals.

Compliance with Ethical Standards

Conflict of interest

The authors declare that for this article they have no actual, potential or perceived the conflict of interests.

Author contribution

The contribution of the authors is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethics committee approval

Ethics committee approval is not required.

Funding

This work was financed by Harran University Scientific Research Projects Authority (HUBAK Project No: 18058).

Data availability

Not applicable.

Consent for publication

Not applicable.

Acknowledgements

Authors are thankful to Harran University Scientific Research Projects Authority about their financial supports.

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