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Seasonal changes in the fatty acid profile of Kyrgyz khainak milk

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Abstract:
Khainak milk is a traditional source of nutrition for people living in the highlands of Kyrgyzstan. It is consumed both in its natural form and in the form of butter, cheese, and cottage cheese. We aimed to determine the composition of fatty acids in khainak milk, as well as its seasonal changes, since such data is lacking in literature.
Fatty acids were determined by gas chromatography qualitatively and quantitatively in the milk from five lactating khainaks farm-bred in the Issyk-Kul region. The milk samples were collected and analyzed in the spring, summer, autumn, and winter seasons over three years (2019, 2020, and 2021).
Kyrgyz khainak milk fat was mostly represented by saturated fatty acids, with a maximum content of 73.10 ± 2.19 g/100 g in winter. C14:0, C16:0, and C18:0 dominated in their composition, exceeding 5 g/100 g, with C16:0 (palmitic acid) reaching almost 35 g/100 g in winter. The flora of mountain pastures favorably contributed to monounsaturated fatty acids in khainak milk, especially oleic acid, whose content reached 26.85 ± 0.81 g/100 g in spring and then gradually declined to 18.90 ± 0.56 g/100 g, following changes in vegetation. Polyunsaturated fatty acids were found in small quantities varying from 3.25 ± 0.09 g/100 g in winter to 4.28 ± 0.12 g/100 g in summer.
The seasonal changes in the fatty acid profile of Kyrgyz khainak milk are most likely due to differences in the animals' diet. Our data can be used to optimize the process parameters for the production of full-fat products from khainak milk (cheese, butter, sour cream, etc.).

Keywords: Milk, khainak, season, fatty acids, saturated fatty acids, monounsaturated fatty acids, polyunsaturated fatty acids, gas chromatography

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INTRODUCTION

Kyrgyzstan has large highland pastures at an altitude of 2500 m or more above sea level which are only suitable for yaks (*Bos grunniens* L.) or their hybrids to graze. Other types of domestic animals are almost impossible to keep under these climatic and forage conditions [1, 2]. There are currently about 50 000 yaks in Kyrgyzstan and they are the main source of meat and milk for local farmers. While yak meat can be bought in the city markets, there are very few products made from yak milk. Yaks are kept in plain-like areas in winter and moved to alpine meadows (syrts) to graze in the summer months. Such transhumance is determined mainly by the climate rather than a range of grass species, since lowlands with hot temperatures are uncomfortable for yaks [3].

In addition to breeding yaks, farmers are increasingly crossing them with cattle (*B. grunniens* × *Bos taurus*), and the resulting hybrids show clear signs of heterosis, or hybrid vigor [4]. The hybrids in the first generation are referred to as "khainags" in Mongolia and Buryatia, "khainaks" in Tajikistan, "zo" in Pakistan, and "dzo" in Tibet [5]. In Kyrgyzstan, khainaks are called "argyns" or "artyns" (Fig. 1), which means a "hybrid". In our

Figure 1 Cattle-yak hybrids bred in the Issyk-Kul region of Kyrgyzstan

study, we used the term "khainak" as the most common term, as well as the term "Kyrgyz khainak" to show regional affiliation.

Similarly to yaks, khainaks graze in mountain pastures and do not require any special care. However, unlike yaks, they can withstand higher temperatures (30–32°C) and survive in a wider range of mountain zones (2000–5000 m above sea level), not only alpine ones. Khainaks are important livestock species providing highlanders with meat, fur, fuel (manure), and other resources. They are just as good as cows in terms of milk yield, or even better. Yaks and their crossbreeds produce 300 and 470 kg of milk, respectively [6]. Yak milk is twice as rich in fat as cow milk (7.22 and 3.55 g/100 cm^3, respectively) [7]. The quality of khainak milk is also better than that of cows, with a higher content of fat, protein, calcium, and phosphorus [8–10]. Recently, there has been growing interest in ethnic foods and alternative dairy products with functional properties, for example, yak and khainak milk products in Kyrgyzstan [11].

Yak milk is rich in a variety of bioactive substances [6]. Although Tibetans live on the highest plateau in the world and therefore should be prone to altitude diseases such as premature aging, edema, atherosclerosis, or carcinogenesis, there have been very few reports of their health problems [12]. This may be due to their diet consisting mostly of yak milk products: butter, yogurt, cheese, and kula (skimmed, fermented, and powdered milk) [13, 14].

Due to grazing conditions, yak milk fat may contain some unique fatty acids lacking in the milk of other mammals [15]. Therefore, there is an urgent need for studying the milk of animals found in mountain regions. A lot of research has been done by Chinese scientists, since more than 13 million domestic yaks live on the Qinghai-Tibetan Plateau in China [16].

The composition and content of fatty acids are two main factors that determine the quality of yak milk and its products, especially butter [15].

Since yaks and khainaks usually feed on natural pastures, the quantity and quality of their milk depend on the season and feeding conditions [17].

Landscape description. The Issyk-Kul region is located in the northeastern part of the Kyrgyz Republic and covers an area of 43 100 km^2. Its climate is affected by Lake Issyk-Kul, a large ice-free lake, and has some marine features such as mild winters, relatively warm summers, and smoothed annual temperatures. The mountain steppe accounts for 40.4% of the landscape, dominating in all of its subclasses (high-mountain, mid-mountain, low-mountain, inter-mountain basin, and mountain-valley). The landscape is formed by bunch-grass steppes: feather grass, ptilagrostis, white grass, and fescue. Intensive livestock grazing changes the vegetation of mountain steppes, reducing grass crops and forbs. The mountain steppe is dominated by turf grasses, including *Kobresia capilliformis*, *Ptilagrostis*, *Festúca valesiáca*, *Cárex*, and *Festuca tianschanica*. The upper parts of the slopes have higher moisture and therefore their meadow-steppes are rich in various forbs and tall-grass plants, particularly *Hordeum*, *Leucopoa albida*, and *Kobresia* [18].

The fat content in the milk of yaks found on the Qinghai-Tibetan Plateau is determined by the season, amounting to 6.2% during the vegetative stage of plants (May), 5.4% in the flowering stage (August), and 6.7% in the resting stage (December) [19]. Total fat in yak milk ranges from 5.6 to 8.6 g/100 g [20].

There are a number of studies on seasonal changes in fatty acids in the milk of different mammals, but little is known about the effect of the season on the fatty acid profile of khainak milk [21]. Liu *et al.* used gas chromatography to determine fatty acids in the milk of yaks found on the Qinghai-Tibet Plateau at different times of the year [15]. They found that the concentrations of *cis*-9 C18:1, *cis*-11 C18:1, *cis*-9, *trans*-11 C18:2, and C18:3n-3 were higher in summer (25.00, 26.00, 1.50, 1.46, and 0.33 g/100 g of total fatty acids, respectively) than in winter (22.00, 17.00, 0.77, 1.27, and 0.28 g/100 g of total fatty acids, respectively). Ding *et al.* confirmed that the summer season, when animals graze on green pastures, ensures the best fatty acid profile and highest milk yield. In another study, the contents of polyunsaturated fatty acids and conjugated linoleic acids in the milk of different species in India increased during the winter, which may be due to the seasonal availability of green feed [22, 23]. However, saturated fatty acids were by 13–14% higher in the summer. On the whole, the contents of monounsaturated fatty acids, polyunsaturated fatty acids, and conjugated linoleic acids were significantly altered by the season and the milk type. According to Marquardt *et al.*, the location of pastures at a certain altitude above sea level influences the fatty acid composition of milk [24]. In particular, the contents of oleic, linoleic, and linolenic acids in ghee were the lowest in the highest pasture (4500 m), with the highest concentrations of condensed tannins in feed.

We aimed to study seasonal changes in the fatty acid composition of milk from khainaks living in the highlands of Kyrgyzstan in order to identify the best period for obtaining a biologically complete product. We know of no studies to date that provide such information.

STUDY OBJECTS AND METHODS

Chemicals and reagents. Methanol and sodium methoxide for lipid transmethylation and hexane for chromatography were obtained from VWR International (USA). A standard solution of fatty acid methyl esters was purchased from Sigma-Aldrich (USA).

Milk collection. For research the milk of khainaks (first-generation hybrids) bred by farms in the Issyk-Kul region of the Kyrgyz Republic (N420432, E771827) was used. Milk was sampled in the Spring (May), Summer (August), Autumn (October), and Winter (December) of 2019, 2020, and 2021. The lactating khainaks (n = 5) had the same calving periods and were kept under pasture conditions. Milk samples (n = 3) were collected into clean containers. The samples were filtered through a fabric filter and poured into special sterile bags. They were frozen immediately after sampling and stored at −18°C. The frozen milk samples were delivered in insulated containers to the laboratory for analysis.

Sample preparation for fatty acid analysis. Milk samples were thawed at 4–6°C for at least 12 h. To extract total lipids, 100 cm^3 samples were placed in 50-cm^3 tubes and centrifuged at 10 000 min^{-1} for 15 min. The separated fat fraction was transferred to a 250-cm^3 glass and blended with 150 cm^3 of hexane for 1 min. The separated hexane layer with dissolved fat was transferred to a 25-cm^3 round-bottomed flask to distill the solvent off at 70 ± 2°C. The resulting fat fraction was used to prepare fatty acid methyl esters.

Fatty acid methyl esters were prepared using a 2 M solution of sodium methoxide in methanol. For this, 0.10 ± 0.02 g of the fat fraction was sampled into a test tube and dissolved in 2 cm^3 of hexane. Then, 0.1 cm^3 of sodium methoxide in methanol was added to the resulting solution. After vigorous stirring for 2 min, the reaction mixture was left for 5 min and then centrifuged. The resulting solution was used for analysis immediately after preparation.

Determination of fatty acids. The composition of fatty acids was determined by using a Cristallux 4000M gas chromatographer equipped with a flame ionization detector (Meta-Chrome, Russia). Fatty acid methyl esters were separated on a Supelco SP2560 capillary column (100 m long, 0.25 mm internal diameter, 0.20 μm film thickness) (Sigma-Aldrich, USA), with an HP 6890 injector. The parameters of gas chromatography were as follows:

– injected sample: 1 mm^3;
– flow division: 1:10;
– initial column temperature: 140°C;
– isothermal part: 5 min;
– temperature increase at 4°C/min to 240°C (analysis for 50 min);
– detector temperature: 260°C;
– carrier gas: nitrogen (constant flow of 1 cm^3/min).

Fatty acid methyl esters were identified by comparing the retention times of peaks obtained with a standard Supelco 37 Component FAME mix (Sigma-Aldrich, USA).

Method validation. Linearity and operating ranges were assessed by dividing working fatty acid methyl ester standards from 1 to 200 μg/cm^3. Response factors for quantifying individual fatty acids were determined by comparing the standard and the test solutions. Individual fatty acid percentages were calculated using the chromatograph's software.

The method was validated according to the guidelines for validating chromatographic methods (ICH 2014). For this, we calculated the response linearity, range, detection and quantification limits, as well as precision. The accuracy of the quantitative method was assessed through the repeatability (n = 2) and reproducibility (n = 1) of the experiment.

Statistical analysis. Microsoft Office Excel 2010 was used for statistical processing. An arithmetic mean of two measurements was taken as a measurement result. Statistical significance of differences between data samples was determined by confidence intervals. Differences between means were compared at a significance level of $p < 0.05$.

RESULTS AND DISCUSSION

Fatty acid methyl esters were identified by comparing the retention times of the peaks obtained by injecting a standard mix of methyl esters. Figure 2 presents a typical chromatogram for the separation of fatty acid methyl esters from khainak milk.

In particular, Fig. 2 shows the fatty acid methyl esters detected and identified with acceptable resolution. The mass fraction of fat in khainak milk was 4.50 ± 0.07, 5.00 ± 0.07, 5.20 ± 0.07, and 5.70 ± 0.14 g/100 g of milk in spring, summer, autumn, and winter, respectively.

The seasonal changes in the fatty acid composition of fat from khainak milk are presented in Table 1.

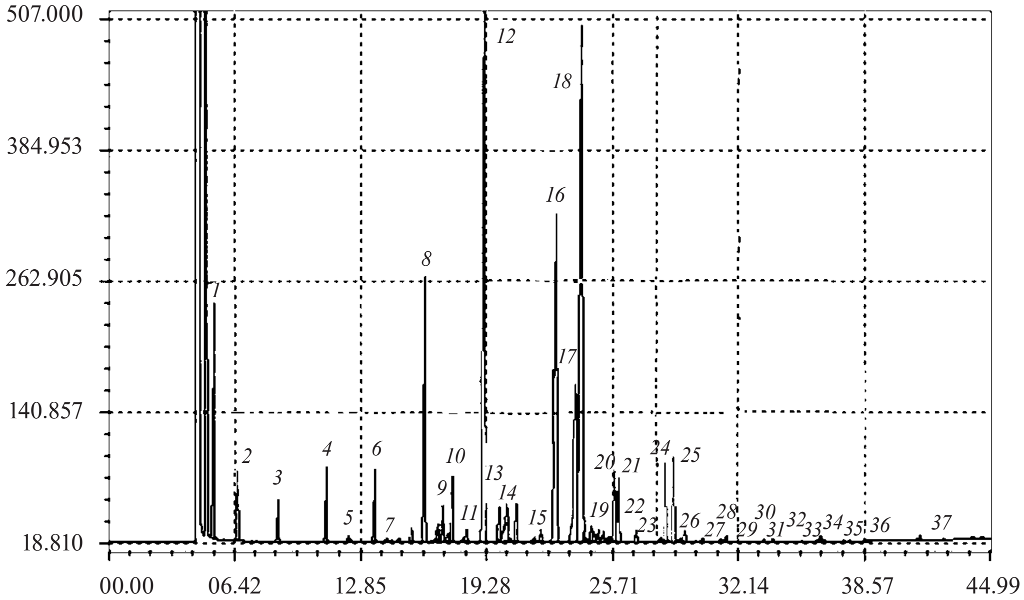
As can be seen from Table 1, khainak milk contained a significant amount (4.37%) of short-chain butyric acid ($p < 0.05$), which changed slightly in summer, autumn, and winter. According to [26], butyric acid (C4:0) is the most common short-chain fatty acid in yak, cow, sheep, and goat butter. One of its main functions is to stimulate the production of epithelial mucin-2. Also, butyric acid reduces inflammation and lowers the pH of the colon, promoting beneficial bacteria and increasing mineral absorption [27, 28]. Short-chain fatty acids improve glucose, lipid, and energy metabolism. They also exhibit antimicrobial properties and reduce the risk of gastrointestinal disorders, certain cancers, and cardiovascular diseases [29, 30].

Caproic acid C6:0 dominated among the middle-chain fatty acids found in the milk of the Kyrgyz khainak. In the autumn, its content exceeded 3 g/100 g of total fatty acids. Like short-chain fatty acids, middle-chain fatty acids are involved in several regulatory and signaling functions of the human body.

According to Table 1, saturated fatty acids C14:0, C16:0, and C18:0 dominated in khainak milk, with a content of over 5 g/100 g. Palmitic acid (C16:0) reached almost 35 g/100 g of total fatty acids in the winter time.

The content of very-long-chain fatty acids was slightly higher than 1 g/100 g of total fatty acids. It hardly changed throughout the year.

Saturated fatty acids had a higher content in winter (73.10 ± 2.19 g/100 g of total fatty acids) compared to the other seasons. Their increase is mainly associated with changes in myristic (C14:0) and palmitic (C16:0) acids (8.50 ± 0.25 and 33.57 ± 0.99%, respectively). Khainak spring milk had a lower content of saturated fatty acids (59.32 ± 1.77%) than cow milk (62.60 ± 0.78%) [31]. The



1	Methyl butyrate (C4:0)	20	Methyl linoleate (18:2n-6c)
2	Methyl hexonoate (C6:0)	21	Methyl arachidate (C20:0)
3	Methyl octonate (C8:0)	22	Methyl-gamma-linoleate (18:3n-6)
4	Methyl decanoate (C10:0)	23	Methyl *cis*-11-eicosenoate (C20:1)
5	Methyl undecanoate (C11:0)	24	Methyl linolenate (C18:3n-3)
6	Methyl dodecanoate (C12:0)	25	Methyl heneicosanoate (C21:0)
7	Methyl tridecanoate (C13:0)	26	Methyl *cis*-11,14-eicosadienoate (C20:2)
8	Methyl myristate (C14:0)	27	Methyl behenate (C22:0)
9	Methyl myristoleate (C14:1)	28	Methyl *cis*-8,11,14-eicosatrienoate (C20:3n-6)
10	Methyl pentadecanoate (C15:0)	29	Methyl erucate (C22:1n-9)
11	Methyl *cis*-10 pentadecanoate (C15:1)	30	Methyl *cis*-11,14,17-eicosatrienoate (C20:3n-3)
12	Methyl palmitate (C16:0)	31	Methyl *cis*-5,811,14-eicosatetraenoate (C20:4n-6)
13	Methyl palmitoleate (C16:1)	32	Methyl tricosanoate (C23:0)
14	Methyl heptadecanoate (C17:0)	33	Methyl *cis*-13,16-docosadienoate (C22:2)
15	Methyl *cis*-10 heptadecanoate (C17:1)	34	Methyl *cis*-5,8,11,14,17-eicosapentaenoate (C20:5n-3)
16	Methyl stearate (C18:0)	35	Methyl lignocerate (C24:0)
17	Methyl *trans*-9 eladiate (C18:1n-9t)	36	Methyl nervonate (C24:1)
18	Methyl *cis*-9 oleate (C18:1n-9c)	37	Methyl *cis*-4,7,10,13,16,19-docosahexaenoate (C22:6n-3)
19	Methyl linolelaidate (18:2n-6t)		

Figure 2 Chromatogram for separation of fatty acid methyl esters lipid fraction from Kyrgyz khainak milk (4.5% fat)

Table 1 Fatty acid composition (g/100 g of total fatty acids) of milk from khainaks, cows, and yaks by season

Fatty acids	Khainak raw milk (own data)					Cow milk [25]	Yak milk [25]
	Spring (May)	Summer (August)	Autumn (October)	Winter (December)	Mean		
C4:0	4.25 ± 0.12^{a}	3.63 ± 0.11^{a}	3.74 ± 0.11^{a}	3.57 ± 0.11^{b}	3.79 ± 0.11	1.23 ± 0.02	2.05 ± 0.03
C6:0	2.03 ± 0.06^{b}	2.36 ± 0.07^{b}	3.09 ± 0.09^{b}	2.10 ± 0.06^{b}	2.39 ± 0.07	1.75 ± 0.03	3.42 ± 0.05
C8:0	0.79 ± 0.02^{a}	1.10 ± 0.03^{b}	1.69 ± 0.05^{c}	0.94 ± 0.03^{a}	1.13 ± 0.03	1.20 ± 0.02	1.61 ± 0.01
C10:0	0.96 ± 0.03^{a}	1.62 ± 0.05^{a}	2.29 ± 0.07^{a}	1.78 ± 0.05^{a}	1.91 ± 0.05	2.15 ± 0.02	2.03 ± 0.04
C12:0	1.07 ± 0.03^{a}	1.85 ± 0.05^{b}	1.24 ± 0.04^{a}	1.67 ± 0.05^{a}	1.45 ± 0.04	2.42 ± 0.03	1.34 ± 0.01
C13:0	0.070 ± 0.002^{a}	0.210 ± 0.006^{a}	0.120 ± 0.003^{a}	0.060 ± 0.002^{a}	0.110 ± 0.003	0.14 ± 0.01	0.12 ± 0.01
C14:0	5.11 ± 0.15^{b}	7.75 ± 0.23^{b}	7.82 ± 0.23^{c}	8.50 ± 0.25^{a}	7.29 ± 0.21	10.00 ± 0.19	8.10 ± 0.13
C15:0	1.26 ± 0.03^{a}	1.43 ± 0.04^{a}	1.27 ± 0.03^{a}	1.83 ± 0.05^{a}	1.45 ± 0.04	1.19 ± 0.01	1.76 ± 0.02
C16:0	21.64 ± 0.64^{b}	24.37 ± 0.73^{c}	27.48 ± 0.82^{a}	33.57 ± 0.99^{a}	26.76 ± 0.80	27.80 ± 0.28	25.50 ± 0.17
C17:0	0.98 ± 0.02^{b}	0.87 ± 0.02^{b}	1.32 ± 0.04^{b}	1.13 ± 0.03^{b}	1.07 ± 0.03	0.65 ± 0.02	0.89 ± 0.02
C18:0	17.47 ± 0.52^{a}	16.77 ± 0.50^{a}	15.05 ± 0.45^{a}	15.96 ± 0.47^{a}	16.31 ± 0.48	13.50 ± 0.13	15.00 ± 0.26
C20:0	0.320 ± 0.009^{a}	0.180 ± 0.005^{b}	0.63 ± 0.02^{a}	0.280 ± 0.008^{a}	0.35 ± 0.01	0.17 ± 0.01	0.40 ± 0.00
C21:0	1.98 ± 0.06^{c}	2.21 ± 0.06^{c}	1.96 ± 0.06^{c}	1.45 ± 0.04^{c}	1.90 ± 0.06	0.03 ± 0.00	0.06 ± 0.00
C22:0	1.18 ± 0.03^{b}	0.090 ± 0.003^{b}	0.190 ± 0.006^{a}	0.110 ± 0.003^{a}	0.39 ± 0.01	0.08 ± 0.00	0.27 ± 0.01
C23:0	0.090 ± 0.002^{a}	0.080 ± 0.002^{a}	0.050 ± 0.001^{a}	0.050 ± 0.001^{a}	0.070 ± 0.002	0.02 ± 0.00	0.12 ± 0.00
C24:0	0.120 ± 0.003^{a}	0.100 ± 0.003^{a}	0.200 ± 0.006^{a}	0.100 ± 0.003^{a}	0.130 ± 0.004	0.04 ± 0.00	0.11 ± 0.00
Total SFAs*	59.32 ± 1.77	64.62 ± 1.93	68.14 ± 2.04	73.10 ± 2.19	66.28 ± 1.98	62.60 ± 0.78	65.90 ± 0.77
C14:1	0.180 ± 0.005^{b}	0.41 ± 0.01^{b}	0.59 ± 0.02^{a}	0.77 ± 0.02^{a}	0.48 ± 0.01	–	–
C16:1	1.37 ± 0.04^{c}	1.26 ± 0.04^{a}	2.27 ± 0.07^{a}	0.070 ± 0.002^{a}	1.24 ± 0.03	0.94 ± 0.03	1.07 ± 0.03
C18:1n-9, *trans*	7.89 ± 0.23^{b}	6.86 ± 0.21^{b}	4.52 ± 0.13^{b}	3.74 ± 0.11^{c}	5.75 ± 0.17	1.09 ± 0.01	3.06 ± 0.03
C18:1n-9, *cis*	26.85 ± 0.81^{a}	23.32 ± 0.69^{a}	20.82 ± 0.62^{a}	18.90 ± 0.56^{a}	24.47 ± 0.67	28.7 ± 0.18	20.81 ± 0.21
C20:1n-9, *cis*-11	0.180 ± 0.005^{a}	0.200 ± 0.005^{b}	0.370 ± 0.011^{a}	0.080 ± 0.002^{b}	0.210 ± 0.006	0.03 ± 0.00	0.03 ± 0.00
C22:1n-9	0.010 ± 0.001^{c}	0.030 ± 0.001^{c}	0.080 ± 0.002^{c}	0.080 ± 0.002^{c}	0.050 ± 0.001	0.01 ± 0.00	0.03 ± 0.00
C24:1n-9	< 0.001^{a}	0.001 ± 0.000^{a}	0.001 ± 0.000^{b}	0.001 ± 0.000^{c}	0.001 ± 0.000	0.01 ± 0.00	0.03 ± 0.00
Total MUFAs*	36.48 ± 1.09^{a}	32.08 ± 0.96^{a}	28.65 ± 0.85^{a}	23.64 ± 0.71^{a}	30.21 ± 0.90	33.00 ± 0.30	27.00 ± 0.32
C18:2n-6, *trans*	0.33 ± 0.01^{b}	0.33 ± 0.01^{b}	0.36 ± 0.01^{b}	0.25 ± 0.01^{c}	0.31 ± 0.01	0.13 ± 0.01	0.18 ± 0.01
C18:2n-6, *cis*	1.90 ± 0.05^{b}	1.49 ± 0.04^{b}	1.42 ± 0.04^{b}	1.62 ± 0.05^{b}	1.61 ± 0.05	1.73 ± 0.01	1.62 ± 0.03
C18:3n-3 (ALA)	1.78 ± 0.05^{a}	1.36 ± 0.04^{a}	1.32 ± 0.04^{a}	1.25 ± 0.04^{a}	1.42 ± 0.04	0.16 ± 0.01	1.37 ± 0.01
C20:3n-3, *cis*-11,14,17	0.020 ± 0.001^{c}	0.010 ± 0.001^{c}	0.010 ± 0.001^{c}	0.020 ± 0.001^{c}	0.015 ± 0.001	0.01 ± 0.00	0.02 ± 0.00
C20:3n-6, *cis*-8,11,14	0.020 ± 0.001^{a}	0.010 ± 0.001^{b}	0.020 ± 0.001^{b}	0.020 ± 0.001^{a}	0.017 ± 0.001	0.09 ± 0.00	0.02 ± 0.00
C20:4n-6 (ARA)	0.120 ± 0.003^{a}	0.040 ± 0.001^{a}	0.040 ± 0.001^{b}	0.030 ± 0.001^{b}	0.060 ± 0.001	0.19 ± 0.01	0.16 ± 0.01
C20:5n-3 (EPA)	0.070 ± 0.002^{b}	0.040 ± 0.001^{b}	0.030 ± 0.001^{c}	0.050 ± 0.001^{a}	0.050 ± 0.001	0.01 ± 0.00	0.08 ± 0.00
C22:6n-3 (DHA)	0.030 ± 0.001^{a}	0.010 ± 0.001^{a}	0.030 ± 0.001^{c}	0.010 ± 0.001^{a}	0.020 ± 0.001	0.01 ± 0.00	0.04 ± 0.00
Total PUFAs*	4.28 ± 0.12	3.29 ± 0.09	3.23 ± 0.09	3.25 ± 0.09	3.51 ± 0.10	3.05 ± 0.06	4.91 ± 0.09
SCFAs	8.03 ± 0.24	8.71 ± 0.26	10.81 ± 0.32	8.39 ± 0.25	8.98 ± 0.26	–	–
MCFAs*	30.62 ± 0.92	37.28 ± 1.11	40.76 ± 1.22	46.47 ± 1.39	38.81 ± 1.16	–	–
LCFAs*	59.92 ± 1.84	53.69 ± 1.62	47.87 ± 1.45	44.78 ± 1.35	52.22 ± 1.56	–	–
VLCFAs*	1.42 ± 0.04	0.31 ± 0.01	0.55 ± 0.02	0.35 ± 0.01	0.66 ± 0.02	–	–

* SFAs – saturated fatty acids; MUFAs – monounsaturated fatty acids; PUFAs – polyunsaturated fatty acids; SCFAs – short-chain fatty acids; MCFAs – middle-chain fatty acids; LCFAs – long-chain fatty acids; VLCFAs – very-long-chain fatty acids

Means ± SD with the same letter are not significantly different at $p = 0.05$

variations in the saturated fatty acid contents in Kyrgyz khainak milk (59.32–73.10%) were similar to those reported by Liu *et al.* for yak milk (67.78–70.67%) [15]. The total saturated fatty acids in khainak milk were higher than in cow and yak milk. Saturated fatty acids have long been associated with cardiovascular disease, leading to reduced consumption of saturated fatty acid-rich foods such as butter [17]. However, many authors have reported a lack of strong evidence to support the association of saturated fatty acids with cardiovascular disease and found their protective effects against stroke [19]. Therefore, moderate consumption of foods rich in saturated fatty acids is essential for human health. Milk fat is the main dietary source of saturated fatty acids for humans.

Oleic acid (C18:1n-9, *cis*) is the most abundant monounsaturated fatty acid in the milk of ruminants, including yak [25, 27]. In our study, the content of oleic acid reached 26.85 ± 0.81 g/100 g of total fatty acids in spring, gradually decreasing to 18.90 ± 0.56 g/100 g as the pasture vegetation changed, which was consistent with the general pattern [32]. Oleic acid is one of the most essential fatty acids for human metabolism. It regulates blood pressure, prevents diabetes, and has antioxidant properties [33, 34].

Evidence suggests that the fatty acid composition of milk is independent of the stage of lactation, so its differences may depend on the geographic region and a variety of grasses growing there [35]. We found that in the spring and summer periods, fresh subalpine grasses contributed to high concentrations of oleic (C18:1 *cis* – 26.85 and 23.32%, respectively) and vaccenic (C18:1 *trans* – 7.89 and 6.86%, respectively) acids in the milk of the Kyrgyz khainak.

Polyunsaturated fatty acids are vital components for normal body growth, the development of the nervous system and immune functions, as well as for preventing cardiovascular diseases [36, 37]. Of particularly high biological value are n6 polyunsaturated fatty acids (linoleic – 18:2n-6, gamma-linolenic – 18:3n-6, and arachidonic – 20:4n-6) and essential n-3 polyunsaturated fatty acids (alpha-linolenic – C18:3n-3, eicosapentaenoic – C20:5n-3, and docosahexaenoic – C22:6n-3), which contain isolated double bonds and are less susceptible to oxidation. Many studies have shown the potential benefits of conjugated linoleic acid and its isomers, which exhibit antidiabetic and anticarcinogenic action and have a positive effect on the immune function [38–40]. Eicosapentaenoic and docosahexaenoic acids are especially important for the proper functioning of the brain, heart, and retina [41–43].

Yak milk fat contains about 3.2 times more polyunsaturated fatty acids than cow milk fat [6, 44]. According to [32], spring milk of a cattle-yak hybrid contains abundant polyunsaturated fatty acids, but their content decreases towards the end of lactation in line with changes in the phenological phase of pasture grass.

Of all polyunsaturated fatty acids, conjugated linoleic acid (C18:2n-6, *trans* and *cis*) has the strongest positive effect on human health, exhibiting anticarcinogenic (breast, stomach, skin cancer), antiatherogenic, antidiabetic, and immunomodulatory properties, as well as suppressing osteoporosis [25, 27, 45–48].

Conjugated linoleic acid is a powerful antioxidant that protects structural lipids from free radicals [49]. Its antioxidant activity is higher than that of other essential fatty acids and alpha-tocopherol. The content of this acid in khainak milk varied from 0.24 to 1.85 g/100 g of total fatty acids, reaching a maximum in spring.

Of significant interest are also some other polyunsaturated fatty acids with three or more double bonds in the molecule. Spring milk of the Kyrgyz khainak ($p < 0.05$) contained more α-linolenic (1.78 ± 0.05 g/100 g of total fatty acids), arachidonic (0.120 ± 0.003 g/100 g of total fatty acids), and eicosapentaenoic (0.070 ± 0.002 g/100 g of total fatty acids) acids than in the other seasons. According to [15], yak milk had a higher content of α-linolenic acid in August (0.34 g) than in the autumn months. This was probably due to abundant spring forbs on mountain pastures and a higher content of linoleic acid in fresh grass compared to fading grass. High levels of linoleic acid are associated with the synthesis of long-chain unsaturated fatty acids [49–51]. In another study, the milk from mountain and alpine pastures in the Aosta Valley (northwestern Italy) had a higher content of α-linolenic, vaccenic, and conjugated linoleic fatty acids than the milk from lowland pastures, which was associated with the presence of terpenoids in the grass and the grazing altitude [52].

Increasing the content of α-linolenic acid in the diet has been reported to reduce the risk of cardiovascular diseases [53]. However, reliable evidence is needed to confirm this conclusion and recommendations need to be revised before α-linolenic acid can be taken to prevent this pathology. In our study, the content of α-linolenic acid in khainak milk gradually decreased from a maximum in spring (1.78 ± 0.05 g/100 g of total fatty acids) to a minimum in winter (1.25 ± 0.04 g/100 g).

Arachidonic acid (C20:4n-6) is mainly obtained from food or synthesized from linoleic acid. It is involved in biological functions and significantly influences the electrical activity of ion channels in excitable tissues such as those of the brain, heart, and muscles. Also, arachidonic acid controls the central nervous system, as well as visual and hearing impairment in premature infants [54, 55]. In our study, khainak milk contained a small amount of this acid (less than 1 g/100 g of fatty acids).

Studies have generally confirmed the beneficial effects of n-3 polyunsaturated fatty acids on depression and cardiovascular health, as well as on the risk of preterm birth [56]. According to [57], the content of eicosapentaenoic acid in yak milk reached 4.91 g/100 g. However, the milk of the Kyrgyz khainak in our study contained negligible amounts of this fatty acid (0.07 g/100 g of fatty acids), which needs further research.

The qualitative and quantitative differences in the fatty-acid compositions of cow and yak milk may be associated with the animals' feed, breed, breeding methods, lactation stage, season, habitat, altitude, and other factors [15, 58]. Compared to the milk from penned cows, the milk from grazing cows contained more functional fatty acids and lipophilic antioxidants (tocopherols, retinol, and carotenoids), as well as smaller amounts of saturated fatty acids [31].

The ratio of unsaturated to saturated fatty acids is an important criterion for the quality of milk fat. This ratio for yak milk is 0.78 [57]. The higher the unsaturated to saturated fatty acids ratio, the more health benefits in fat. The oil of oceanic fish is commonly rich in health-benefitting fatty acids such as oleic (C18:1), linoleic (C18:2), conjugated linoleic (C18:2 *cis*-9, *trans*-11), arachidonic (C20:4), and eicosapentaenoic acids [59]. Khainak milk fat, like any fat of animal origin, has a far lower content of these fatty acids. However, the unsaturated to saturated fatty acids ratio in the spring khainak milk was about 0.69, which is comparable to yak milk. In cow milk, this ratio is only 0.57 [25].

The biological value of khainak milk fat can also be assessed by the efficiency of metabolism of essential fatty acids – the ratio of arachidonic acid (the main polyunsaturated fatty acid in membrane lipids) to all other polyunsaturated fatty acids with 20 and 22 carbon atoms. In khainak milk fat, this ratio reaches its maximum in spring (0.77), gradually decreasing to its minimum in winter (0.29), with 0.57 in summer and 0.42 in autumn.

The determination of fatty acids in khainak milk may stimulate the development of food additives, alternative dietary sources of fat, and balanced dairy products.

CONCLUSION

Our study was the first attempt to develop a scientific basis for processing the milk of khainaks, the first-generation cattle-yak hybrids living in the highlands of Kyrgyzstan. We described seasonal changes in the composition of fatty acids in hybrid milk and found that spring was the most favorable period for obtaining a biologically complete product with a high content of physiologically functional ingredients. In this period, khainak milk had the minimum content of saturated fatty acids (59.32 ± 1.77 g/100 g of total fatty acids) and the maximum content of monounsaturated (36.48 ± 1.09 g/100 g of total fatty acids) and polyunsaturated (4.28 ± 0.12 g/100 g of total fatty acids) fatty acids. Thus, khainak milk can become an alternative source of high-quality animal fat for a wide range of food products.

CONTRIBUTION

R.Sh. Elemanova prepared the original draft and edited the manuscript. M.M. Musulmanova reviewed and edited the original manuscript. T.Sh. Dzhunushalieva and E.A. Yurova reviewed the manuscript. All the authors made a substantial contribution to the concept and design of the manuscript, as well as approved its final version for publication.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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