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Comparative evaluation of quality composition, fatty acid profiles, and seasonal variability of kumys from mare's milk

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ABSTRACT
Kumys, a traditional fermented dairy product derived from mare's milk, possesses unique nutritional properties with significant potential in the functional food market. This study presents a comparative evaluation the chemical composition, and fatty acid profiles of kumys obtained from Kazakh mare's milk across two key regions in southern Kazakhstan (Almaty and Zhambyl) over five months of lactation (July to November). A total of 240 kumys samples were analyzed using MilkoScan FT1 for standard milk parameters and gas chromatography GC Shimadzu GC-2010 Plus for fatty acid profiling. Principal component analysis (PCA), ANOVA, and correlation analysis were applied to explore regional and seasonal variation. The results revealed significant differences in the composition between regions and across lactation months. Kumys from the Almaty region exhibited higher levels of protein (1.59% vs. 1.41%), lactose (5.68% vs. 5.12%), and solids-not-fat, while Zhambyl samples showed higher fat content (1.86% vs. 1.52%) and more favorable thrombogenic index (TI) values. Notably, linoleic acid (C18:2n6c) content increased by 28% from July (0.46 g/100g fat) to November (0.59 g/100g fat), and alpha-linolenic acid (C18:3n3c) increased by 21% over the same period.. The n-6/n-3 ratio improved significantly in autumn months, particularly in Zhambyl, suggesting enhanced health-promoting qualities. PCA distinguished samples based on both geographic and seasonal factors, with lactation stage having a more substantial impact on kumys composition than region. These findings underscore the importance of lactation month and regional pasture characteristics in shaping the nutritional value of kumys and provide a scientific basis for optimizing their production and valorization in the dairy sector.

Keywords: mare's milk, kumys, fatty acid, lactation, fermented dairy product

INTRODUCTION
Kumys, a traditional fermented dairy beverage made from mare's milk, is a culturally significant product in Central Asia and has been widely consumed for its nutritional and therapeutic properties for centuries. It is recognized for its probiotic content, high digestibility, and a favorable profile of essential nutrients and bioactive compounds that contribute to its potential health benefits, including immunomodulatory, antimicrobial, and antioxidant effects **[1]**, and **[2]**. The fermentation process enhances the bioavailability of fatty acids, resulting in a product that supports digestive health and may be beneficial in treating gastrointestinal and respiratory disorders **[3]**, and **[4]**. It is gaining renewed international interest as a functional food due to its probiotic microflora and potential health benefits—including antimicrobial, anti-inflammatory, and gut-modulating effects—supported by modern microbiome and metabolomics studies **[5]**.

Mare's milk differs markedly from the milk of other domestic species, particularly in its biochemical composition. It contains lower fat and protein but significantly higher lactose levels and is rich in polyunsaturated fatty acids (PUFAs), especially omega-3 and omega-6 fatty acids **[6]**, and **[7]**. Its fatty acid profile closely resembles that of human milk, making it highly digestible and suitable for dietary use in pediatric and geriatric nutrition **[8]**. These characteristics make kumys a promising functional food with potential applications in preventive and therapeutic nutrition **[9]**. Lipidomics analysis identified nearly 500 differential lipids in mare milk, highlighting unique molecular signatures that impact flavor and health-related bioactivity **[10]**. Seasonal and regional factors significantly influence the nutritional composition of kumys. A 2025 study in Kazakhstan demonstrated that mare age, foaling, and lactation stage under grazing management significantly influenced milk fat, protein, lactose, and total solids **[11]**. Similarly, analysis of fermented mare's milk in Northern China revealed how fermentation microflora such as Lactobacillus and Acetobacter were closely correlated with nutrient transformations and functional metabolite production **[12]**. Mituniewicz-Małek et al. evaluated the survivability of probiotic bacteria (*Lactobacillus acidophilus* LA-5 and *Bifidobacterium animalis* BB-12) in fermented and non-fermented mare's milk over cold storage, confirming the feasibility of developing kumys-based probiotic functional beverages **[13]**.

However, the composition of mare's milk and its fermented derivatives is susceptible to several factors, including breed, lactation stage, feeding regime, environmental conditions, and geographic origin **[14]**, and **[15]**. Seasonal variability, particularly in pasture availability and climate, can lead to marked differences in the milk's physicochemical and nutritional profile **[16]**. Regional studies are essential for characterizing such variability and ensuring the consistent quality of kumys, especially in countries like Kazakhstan, where traditional livestock practices are integrated with modern food production systems.

Despite the growing interest in kumys as a functional dairy product, comprehensive studies comparing its chemical composition across regions and lactation periods are limited. In Kazakhstan, two regions—Almaty and Zhambyl—represent major centers for mare milk production, yet comparative data from these regions remain scarce.

This study aims to evaluate the chemical composition and fatty acid profiles of kumys produced from the milk of Kazakh mares collected from the Almaty and Zhambyl regions over various months of lactation. Furthermore, multivariate statistical analyses, including principal component analysis (PCA), were employed to evaluate the influence of regional and temporal factors on kumys quality. The findings of this study will provide valuable insight into optimizing kumys production and standardization for use as a therapeutic and functional dairy product.

Scientific Hypothesis

We hypothesize that the chemical composition and fatty acid profiles of kumys derived from Kazakh mare's milk are significantly influenced by both regional (Almaty vs. Zhambyl) and seasonal (monthly lactation stage) factors. Specifically, we expect to observe statistically significant differences in nutrient profiles due to geographic variation in pasture quality and seasonal shifts in lactation-related physiology. These differences will be evident through multivariate statistical analyses, such as principal component analysis (PCA), one-way analysis of variance (ANOVA), and correlation assessments.

Objectives

The objective of this study is to assess seasonal changes in chemical composition and fatty acid profiles of kumys produced from mare's milk during spring, summer, and autumn in two key regions of Southern Kazakhstan—Almaty and Zhambyl. The study aims to identify statistically significant differences in nutritional indicators (such as fat, protein, lactose, and polyunsaturated fatty acids), evaluate regional variability, and determine how lactation stage affects kumys quality.

MATERIAL AND METHODS

Samples

Samples description: Mare's milk was obtained from 48 Kazakh mares of ages ranging from 3 to 7 years, which were housed at a horse dairy farm located in the Almaty (n=24) and Zhambyl (n=24) regions (Southern Kazakhstan).

Samples collection: Mares were individually milked during the six lactation months from July to November 2023. During lactation, mares were milked on a monthly basis. A total of 240 individual milk samples were collected. These mares had given birth between March and June. All mares grazed on pasture during the lactation period. When the availability of grass was low in October and November, mares were supplemented with hay or silage. The milking process was consistently conducted at the same time each morning, between 8:00 and 9:00 am. Following collection, the milk was stored in containers and preserved at −20°C in a deep freezer, pending further analysis in the laboratory. To minimize potential bias, milk sample collection was conducted according to a standardized schedule across both regions, with each mare milked individually at the same time of day. While complete randomization of animals was not feasible due to farm constraints, samples were collected from mares in a balanced manner across age groups and months. During analytical procedures, all samples were coded, and laboratory analysts were blinded to the region and lactation stage to prevent observer bias during chemical and chromatographic analysis.

Samples preparation: For kumys production, raw mare's milk samples were thawed at 4 °C and homogenized before fermentation. Fermentation was initiated by inoculating each sample with a 5% (v/v) starter culture consisting of a symbiotic mixture of lactic acid bacteria and yeast. Inoculated milk was incubated at 26–28 °C for 16–18 hours under aerobic conditions without agitation. The starter culture included *Lacticaseibacillus paracasei* (17K-6L12, GenBank: OR722737.1), *Lacticaseibacillus casei* (5K-9L1, GenBank: OR711014.1), *Lactiplantibacillus plantarum* (11S-23, GenBank: OR807502.1), and *Saccharomyces cerevisiae* (S430b, GenBank: MK649847.1).

During fermentation, microbial activity resulted in the acidification and slight carbonation of the product, leading to the development of the characteristic sour taste and slight effervescence of kumys. The endpoint of fermentation was determined by titratable acidity (reaching ~50–60 °D (degrees Dornic)) and a pH between 4.2 and 4.6. Upon completion, the fermented kumys was stored at 4 °C for no longer than 24 hours before compositional analysis, to minimize post-fermentation metabolic activity and preserve sample integrity.

All fermentation procedures were conducted under aseptic conditions to prevent contamination and ensure reproducibility. The same starter culture and fermentation parameters were used for all samples to ensure comparability across different regions and lactation months.

Number of samples analysed: 240

Chemicals

n-hexane (Sigma-Aldrich, St. Louis, MO, USA), sodium methylate powder (Sigma-Aldrich, St. Louis, MO, USA), methanol (Sigma Aldrich, St. Louis, MO, USA).

Animals, Plants, and Biological Materials

Animal: Horse (*equus ferus caballus*). Peasant farm "Sadygul" Zhambyl region, Peasant farm "Zhumabayev" Almaty region.

Instruments

Milko-Scan FT1 analyzer (Foss Electric, Denmark), Shimadzu GC 2010Plus gas chromatograph (Shimadzu, Kyoto, Japan), nitrogen gas generator (Parker Domnick Hunter G1110E, Hauppauge, NY, USA), CP-Sil 2560 high-polarity column (100 m × 0.250 mm × 0.20 µm, Agilent Technologies, Santa Clara, CA, USA).

Laboratory Methods

Milk samples were evaluated for the content of fat, protein, casein, lactose, solids-not-fat (SNF), total solids (TS), citric acid, urea, and pH contents by infrared spectroscopy using a Milko-Scan FT1 analyzer. The Milko-Scan FT1 analyzer requires a minimum of 15 ml of milk for duplicate analysis of each sample.

The fatty acid methyl esters were identified using the gas chromatograph. For gas chromatography, we prepared the samples as described by Toishimanov et al. (2023). Mare milk 500 mL samples were placed into 15 mL centrifuge tubes and subjected to centrifugation at 10,000 rpm for 20 minutes. Following the fractionation, the upper milk fat fraction was isolated into another 100 mL tube and then 50 mL n-hexane was added. This homogenized extract was evaporated to prepare the FAME. Next, 2.70 ± 0.01 g of sodium methylate powder was dissolved with 25 mL of absolute methanol in a 50 mL volumetric flask. The solution was mixed and cooled to ambient temperature, and then 0.10 ± 0.01 mL of the oil was weighed in a 15 mL Falcon tube, to which 2 mL of n-hexane was added. Then, 0.1 mL of a sodium methylate solution in methanol was added and vortexed for 1 min. After the methylation reaction mixture had settled for 5 min and was centrifuged at 3000 rpm for 5 min, 1 mL of the supernatant was transferred to a vial and injected for GC analysis **[17]**.

The methylated samples were then introduced into a Shimadzu GC 2010Plus gas chromatograph with flame ionization detector. Chromatographic separation was achieved on a CP-Sil 2560 high-polarity column. We used nitrogen as the carrier gas, with a purity of 99%, sourced from a nitrogen generator. The flow rates were set at 30 mL/min for hydrogen, 300 mL/min for air, and 30 mL/min for the make-up flow. The gas chromatograph was operated with an injector temperature of 250 °C, a detector temperature of 260 °C, a split ratio of 1:40, and a total flow rate of 95.5 mL/min. The temperature program began at 100 °C, held for 5 minutes, then ramped up by 4 °C/min to 210 °C, held for 8 minutes, and finally increased by 10 °C/min to 240 °C, where it was maintained for 16.5 minutes. The injection volume was 1.0 µL, and the total analysis time was 60 minutes. We used 37-component FAME Mix as the analytical standard to identify the FAMEs **[18]**.

Description of the Experiment

Study flow: In the first phase of the study, raw mare's milk samples were collected monthly from July to November 2024 from 48 Kazakh mares located in two different regions: Almaty (n = 24) and Zhambyl (n = 24).

In the second phase, collected milk samples were fermented under controlled laboratory conditions to produce kumys. The fermentation process was standardized using a consistent starter culture of lactic acid bacteria and yeast, which was incubated at 26–28°C for 16–18 hours. The endpoint was determined based on titratable acidity and pH measurements. Fermented kumys samples were then stored at 4 °C for no more than 24 hours before analysis.

In the third phase, samples were analyzed for chemical composition (fat, protein, lactose, SNF, TS, citric acid, urea, pH, etc.) using MilkoScan FT1 infrared spectroscopy. Fatty acid methyl esters (FAMEs) were prepared by transesterification and analyzed using a Shimadzu GC-2010Plus equipped with a CP-Sil 2560 column and a flame ionization detector.

A total of 240 kumys samples were evaluated. The results were subjected to one-way ANOVA with Tukey's HSD post hoc tests to identify statistically significant differences. PCA and Pearson correlation analysis were used to explore data structure and inter-variable relationships.

This multi-phase experimental design allowed for the comprehensive characterization of kumys variability related to both regional and seasonal factors.

Quality Assurance

Number of repeated analyses: 3

Number of experiment replication: 3

Reference materials: FAME Mix (37-component FAME Mix, Supelco, Merck, Darmstadt, Germany).

Calibration: A comprehensive quantitative assessment FAs was conducted, with calibration curves established over a range from 20.2 µg/mL to 612 µg/mL. These curves were derived from five distinct concentrations. The repeatability, expressed as the percentage relative standard deviation (%RSD) for retention times, was less than 0.5%. Similarly, the precision for peak areas remained below 1.0%, and for retention times, it was under 0.3% in identical conditions. The (LOD) ranged from 0.29 µg/mL to 1.95 µg/mL, while the LOQ varied between 2.06 µg/mL and 3.95 µg/mL, indicating the high sensitivity of the method. Based on these results, the analytical method is considered appropriate for the identification of fatty acids (FAs) in mare's milk. Milko-Scan FT1 analyzer calibrated for mare milk. As standard factory calibrations are typically optimized for bovine milk, a customized calibration curve was developed using reference samples of mare milk with known composition, which had been previously analyzed using classical chemical methods (e.g., Kjeldahl for protein and Gerber for fat).

Laboratory accreditation: The experiments were conducted in a laboratory accredited to the international standard ISO 17025:2019.

Data Access

Although the dataset supporting this study is not currently deposited in a public repository, it is available from the corresponding author upon reasonable request.

Statistical Analysis

All statistical analyses were carried out using JMP 17 Pro (JMP Statistical Discovery LLC, Cary, NC, USA).

The influence of physical chemical indicators on the milk was evaluated by one-way ANOVA followed by Tukey's HSD post hoc test for multiple comparisons, when significant differences ($p < 0.05$) between the mean values were found. Prior to ANOVA, the assumptions of normality and homogeneity of variance were verified. Principal component analysis was used to assess the correlation between the parameters studied (PCA). Pearson's correlation coefficients were used to examine the relationships among the variables.

RESULTS AND DISCUSSION

The chemical composition of kumys derived from Kazakh mare's milk across two regions (Almaty and Zhambyl) and over a five-month lactation period is presented in Table 1.

Table 1 Chemical composition of Almaty and Zhambyl regions, Kazakh mare milks' kumys by month and region.

Parameter	Region		Lactation period by month					p-Value	
	Almaty	Zhambyl	July	August	September	October	November	By region	By month
Fat (%)	1.45±0.02	2.04±0.03	1.65±0.02	1.69±0.06	1.58±0.01	2.00±0.06	1.91±0.01	0.0001	0.02
Protein (%)	1.59±0.01	1.41±0.02	1.72±0.01	1.69±0.04	1.30±0.03	1.58±0.01	1.08±0.04	0.043	0.0001
SNF (%)	8.12±0.08	4.90±0.04	5.86±0.08	5.86±0.04	7.25±0.07	7.27±0.04	6.08±0.03	0.0001	NS
TS (%)	8.76±0.03	7.01±0.09	7.49±0.08	7.57±0.05	7.92±0.06	8.04±0.04	8.47±0.05	0.0001	NS
Lactose (%)	5.68±0.01	3.75±0.03	4.66±0.07	4.50±0.02	4.98±0.14	4.62±0.03	4.67±0.04	0.0001	NS
Freezing Point (°C)	-0.658±0.07	-0.637±0.09	-0.448±0.12	-0.541±0.08	-0.663±0.12	-0.767±0.04	-0.895±0.08	NS	0.001
Acidity °Dornic (°D)	18.35±1.54	20.15±1.11	10.05±2.01	5.02±0.99	29.26±1.36	38.03±2.36	15.38±1.2	NS	0.001
Density (g/L)	1024.81±2.94	1004.13±9.55	1026.28±5.74	1014.48±2.44	1014.46±6.52	1005.71±10.36	1007.2±21.54	0.0001	0.0024
Citric acid (%)	0.16±0.001	0.12±0.01	0.14±0.00	0.12±0.01	0.05±0.00	0.31±0.01	0.09±0.00	NS	0.007
Urea (mg/dL)	3.07±0.02	2.16±0.03	4.65±0.01	3.20±0.05	2.10±0.01	1.27±0.02	1.26±0.01	0.0459	0.0001
Casein (%)	0.89±0.001	1.04±0.01	1.51±0.03	1.43±0.04	0.76±0.00	0.59±0.05	0.36±0.00	NS	0.0001

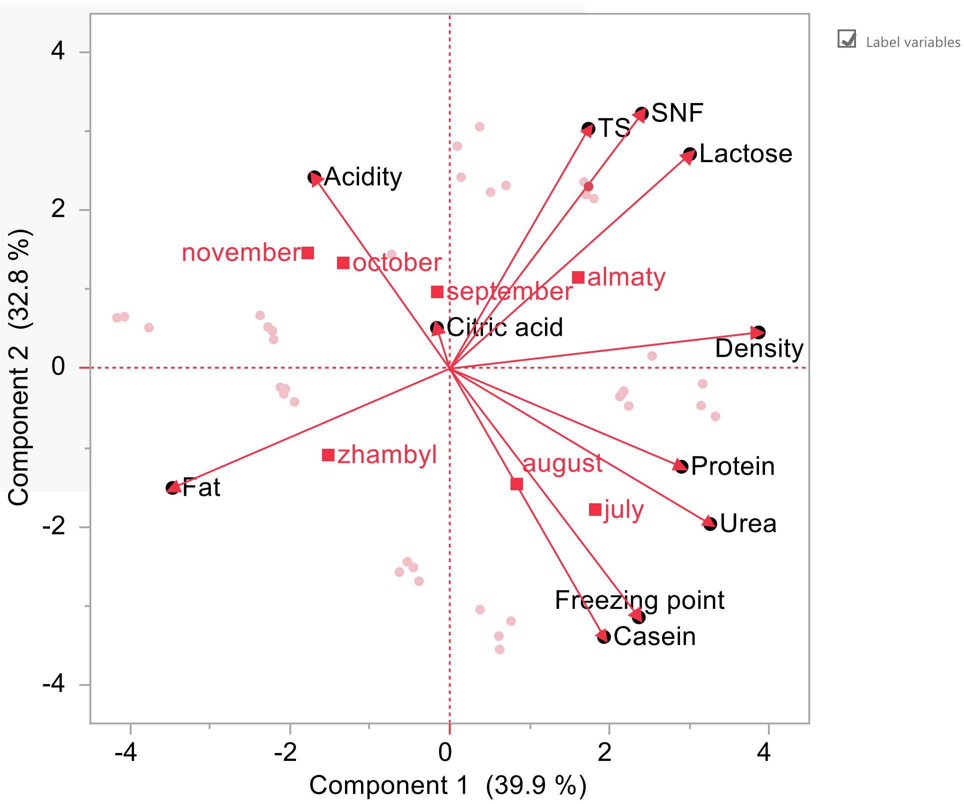

Figure 1 PCA biplot of the kumys quality indicators.

Statistically significant regional differences ($p < 0.05$) were observed in fat, protein, solids-not-fat (SNF), total solids (TS), lactose, density, and urea content. On average, the fat content was higher in kumys from the Zhambyl region (2.04%) compared to those from Almaty (1.45%), with a notable monthly variation ($p = 0.02$). Protein content showed an inverse trend, being higher in Almaty (1.59%) than in Zhambyl (1.41%), and exhibited strong monthly fluctuations (p < 0.0001). SNF and TS levels were both significantly higher in Almaty samples (8.12% and 8.76%, respectively) compared to Zhambyl (4.90% and 7.01%, respectively), although variation by month was non-significant. Lactose concentrations followed a similar pattern, being significantly elevated in Almaty kumys (5.68%) ($p < 0.0001$). Monthly changes in freezing point and acidity were significant ($p = 0.001$), while regional influence was not. Citric acid and casein contents varied significantly by month ($p = 0.007$ and $p < 0.0001$, respectively), with casein showing a decreasing trend from July to November. Urea content was significantly influenced by both region and month ($p = 0.0459$ and $p < 0.0001$, respectively), with higher levels in Almaty.

PCA was conducted to assess the underlying structure of the variation in the chemical composition of kumys samples collected across different months and regions. The first two principal components (PC1 and PC2) explained 39.9% and 32.8% of the total variance, respectively, accounting for a cumulative 72.7% of the variation in the dataset. The PCA biplot discriminated samples by month and region. Almaty samples clustered closer to later months (October and November), while Zhambyl samples were primarily associated with earlier lactation months (July and August). Variables such as SNF, TS, and lactose were positively associated with PC1, indicating their contribution to the separation of samples along this axis. In contrast, fat content was negatively correlated with PC1, suggesting an inverse relationship with the components. PC2 was primarily influenced by acidity and citric acid, allowing for the separation of samples collected in November from those from earlier months. Casein and protein contributed significantly along the negative direction of PC2.

The eigenvalues for PC1 and PC2 were 4.384 and 3.612, respectively, indicating that these components made strong contributions to the data structure. The biplot of the PCA demonstrated clear separation of kumys samples by both lactation month and region. Samples from the Almaty region and later lactation months (October, November) were positioned in the positive quadrant of PC1 and PC2, indicating a higher association with SNF, TS, lactose, citric acid, and density. In contrast, samples from Zhambyl and the earlier months (July and August) clustered on the negative side of PC1, corresponding to higher fat content and lower protein and casein levels.

Regional Differences

Statistical comparisons using unpaired t-tests revealed that most fatty acids did not exhibit significant differences between the Almaty and Zhambyl regions (p > 0.05), indicating a broadly similar baseline composition (Table 2).

Table 2 Fatty acid composition in kumys from Almaty and Zhambyl regions milk across lactation months.

	Almaty region					Zhambyl region					p-Value	
	July	August	September	October	November	July	August	Septem-ber	October	November	By region	By lactation
C8:0	0.81	1.26	0.35	2.59	2.59	1.64	1.26	1.22	2.17	2.60	NS	0.0064
C10:0	1.96	3.51	2.93	6.22	5.78	3.49	3.91	3.82	4.14	4.91	NS	0.0353
C12:0	2.26	4.47	3.76	7.54	7.04	5.67	5.81	6.37	5.67	6.59	NS	NS
C13:0	0.00	0.07	0.00	0.13	0.02	0.12	0.00	0.00	0.03	0.03	NS	NS
C14:0	7.18	5.20	4.78	7.54	6.88	6.50	7.26	7.09	6.75	7.10	NS	NS
C14:1	0.00	0.33	0.31	0.89	1.07	0.52	0.67	0.77	1.07	0.00	NS	NS
C15:0	0.84	0.30	0.25	0.49	0.06	0.39	0.44	0.37	0.06	0.05	NS	0.0414
C16:0	25.96	24.00	20.07	20.20	19.61	24.60	24.33	20.55	20.50	17.99	NS	0.0002
C16:1	5.88	6.44	7.02	6.34	6.09	10.53	9.84	7.55	9.38	8.15	0.0015	NS
C17:0	0.85	0.12	0.08	0.47	0.63	0.14	0.13	0.06	0.50	0.51	NS	NS
C17:1	0.50	0.57	0.62	0.72	0.56	0.57	0.82	0.65	0.84	0.07	NS	0.0489
C18:0	6.56	3.41	1.18	0.03	0.09	1.24	1.03	1.68	0.21	0.00	NS	0.0417
C18:1n9c	26.85	26.23	20.90	18.56	19.08	24.93	24.03	18.46	20.97	20.37	0.0455	0.0086
C18:2n6c	11.76	12.92	12.98	17.95	19.79	6.83	9.69	13.73	15.42	18.15	NS	0.0059
C20:0	0.00	0.02	0.00	0.00	0.00	0.03	0.00	0.08	0.00	0.00	NS	0.0009
C18:3n3c	4.10	3.12	2.10	2.31	3.85	0.19	0.11	0.09	5.52	4.72	NS	NS
C18:3n6c	2.52	6.32	8.19	0.37	0.07	10.67	9.63	5.62	0.10	0.04	NS	0.0412
C21:0	0.82	0.00	0.00	0.02	0.45	0.03	0.00	0.00	0.59	0.42	NS	NS

However, a few specific fatty acids showed statistically significant regional variation: palmitoleic acid (C16:1) concentrations were significantly higher in samples from Zhambyl (p = 0.0015), potentially due to greater Δ9-desaturase activity or differences in feeding systems, forage availability, or breed-specific metabolic traits. Oleic acid (C18:1n9c) was also elevated in Almaty compared to Zhambyl (p = 0.0455), reflecting region-specific lipid metabolism or dietary input of oleic acid precursors.

Although not statistically significant, trends toward higher levels of stearic acid (C18:0) and capric acid (C10:0) in Almaty, and higher levels of gamma-linolenic acid (C18:3n6c) and alpha-linolenic acid (C18:3n3c) in Zhambyl were observed. They may merit further investigation with larger sample sizes.

Seasonal Differences Across Lactation Stages

ANOVA revealed statistically significant seasonal shifts for numerous fatty acids, indicating a strong effect of lactation stage and possibly associated metabolic and dietary changes over time: caprylic acid (C8:0) showed a sharp increase toward the end of lactation (October–November), with p = 0.0064, consistent with enhanced de novo fatty acid synthesis during late lactation. Capric acid (C10:0) also increased significantly over time (p = 0.0353), supporting a similar mechanism. Palmitic acid (C16:0), the predominant saturated fatty acid, showed a highly significant decrease across the lactation months (p = 0.0002), potentially linked to physiological adaptations in the mammary gland lipid biosynthesis pathway. Oleic acid (C18:1n9c) declined significantly over time (p = 0.0086), possibly reflecting reduced desaturation activity or changes in substrate availability in later lactation.

Gamma-linolenic acid (C18:3n6c) exhibited a notable drop from peak levels in July–August to minimal values in October–November (p = 0.0412), likely linked to seasonal variations in pasture composition and dietary fatty acid precursors. Linoleic acid (C18:2n6c) and alpha-linolenic acid (C18:3n3c) also exhibited non-significant but observable trends of increase in late lactation. Other minor fatty acids such as pentadecanoic acid (C15:0, p = 0.0414), heptadecenoic acid (C17:1, p = 0.0489), and arachidic acid (C20:0, p = 0.0009) also demonstrated statistically significant shifts across months, despite their lower concentrations.

These observations emphasize that seasonal (lactation stage-dependent) factors exert a more substantial influence on fatty acid composition than geographic origin, particularly affecting medium-chain and desaturation-dependent fatty acids (Table 3).

Table 3 Monthly dynamics of fatty acid indices in kumys from Almaty and Zhambyl regions.

	Almaty region					Zhambyl region				
Parameter	**July**	**August**	**September**	**October**	**November**	**July**	**August**	**September**	**October**	**November**
SFA	48.13	43.51	33.99	46.13	43.83	43.85	44.23	41.23	41.48	40.87
MUFA	33.46	33.71	28.84	27.21	27.85	36.54	35.36	27.71	33.28	29.42
PUFA	18.38	22.79	23.62	22.25	24.43	18.12	19.75	19.77	21.72	23.92
USFA	51.84	56.49	52.46	49.46	52.28	54.67	55.10	47.49	55.00	53.33
n-6	14.28	19.23	21.17	18.34	19.90	17.55	19.32	19.35	15.59	18.22
n-3	4.10	3.18	2.21	2.40	4.05	0.46	0.28	0.17	5.65	5.41
n-6/n-3	3.48	6.05	9.57	7.65	4.92	37.98	68.75	113.83	2.76	3.37
AI	1.10	0.87	0.82	1.17	1.04	1.03	1.07	1.16	0.97	0.99
TI	1.00	0.78	0.63	0.74	0.65	0.49	0.34	0.21	0.62	0.58

Saturated and Unsaturated Fatty Acids (SFA, MUFA, PUFA, USFA)

In both regions, SFA values exhibited a decreasing trend from July to September, with a partial rebound in October and November. The lowest SFA level was observed in Almaty in September (33.99%), suggesting a mid-lactation shift toward higher unsaturation in milk fat.

MUFA levels were generally higher in Zhambyl compared to Almaty, particularly in the early months (July and August). Zhambyl peaked at 36.54% in July, while Almaty showed a gradual decline in MUFA from July (33.46%) to October (27.21%). This pattern may reflect differences in feed composition or metabolic regulation of desaturase enzymes.

PUFA content increased in both regions over time, with Almaty reaching its highest value in November (24.43%), indicating a possible cumulative effect of forage-derived essential fatty acids during the grazing season. Zhambyl followed a similar trend, albeit with slightly lower absolute PUFA values in mid-lactation.

The total unsaturated fatty acid fraction (USFA) was consistently higher than SFA in all months and both regions, reflecting the unsaturated nature of equine milk lipids. Peak occurrences in the USFA were observed in August in Almaty (56.49%) and in October in Zhambyl (55.00%).

Omega-6 and Omega-3 Polyunsaturated Fatty Acids (n-6, n-3) and Their Ratio

Omega-6 (n-6) fatty acids were present in greater quantities than omega-3 (n-3) in all samples. In Almaty, n-6 values increased steadily from July (14.28%) to September (21.17%), followed by a slight decline. Zhambyl exhibited a more moderate fluctuation, with relatively stable n-6 levels across the months.

A distinct contrast was observed in n-3 fatty acids between the regions. Almaty showed a mid-lactation dip in n-3 (2.21% in September), while Zhambyl exhibited a dramatic rise in n-3 levels from September to October (0.17% to 5.65%). This increase in Zhambyl may be linked to seasonal access to fresh pasture rich in α-linolenic acid or variability in animal response to forage intake.

As a result, the n-6/n-3 ratio varied widely: in Almaty, it peaked in September (9.57) before dropping to 4.92 in November. In Zhambyl, extremely high ratios were observed from July to September (up to 113.83), followed by a sharp correction in October and November (2.76 and 3.37, respectively). These dynamics suggest strong seasonal influence and a potentially more favorable omega-6/omega-3 balance in late lactation.

Atherogenic and Thrombogenic Indices (AI, TI)

The atherogenic index (AI) remained relatively stable across regions and months, ranging from 0.82 to 1.17 in Almaty and 0.97 to 1.16 in Zhambyl. A slight peak in AI was observed in Almaty in October, possibly reflecting a transient increase in lauric and palmitic acid content.

In contrast, the thrombogenic index (TI) displayed a more distinct difference between regions: Almaty ranged from 0.63 to 1.00, with the lowest values in September. Zhambyl exhibited notably lower TI values throughout, with a minimum of 0.21 in September, suggesting a more favorable lipid profile in terms of cardiovascular health impact.

Overall, Almaty demonstrated greater seasonal fluctuation in PUFA and USFA, while Zhambyl showed more pronounced variation in MUFA and n-3 content. The sharp correction of the n-6/n-3 ratio in Zhambyl during late lactation highlights the importance of seasonal pasture composition. Meanwhile, the consistently lower TI values in Zhambyl suggest that regional forage and animal management conditions may positively influence the health value of the milk fat.

The findings of this study confirm that both regional and seasonal factors significantly influence the chemical composition and fatty acid profiles of kumys derived from Kazakh mare's milk. Our results indicate that kumys from the Almaty region exhibited higher protein, lactose, SNF, and TS contents compared to samples from the Zhambyl region. This observation is consistent with previous studies, where milk composition in mares was shown to vary depending on regional differences in seasonal climate shifts **[19]**, pasture vegetation **[20]**, and management practices such as feeding systems or housing **[21]**, and **[22]**.

In contrast, protein content was significantly higher in Almaty samples (1.59%) than in those from Zhambyl (1.41%), showing strong temporal variability ($p < 0.0001$). This is consistent with results by Boranbayeva et al. **[6]**, who documented increased casein and whey protein fractions in mare's milk from southern regions of Kazakhstan, possibly due to differences in pasture flora and mare physiological condition. On average, fat content was higher in kumys from the Zhambyl region (2.04%) compared to Almaty (1.45%), with notable seasonal variation ($p = 0.02$). This value is comparable to findings in kumys from West Kazakhstan's Adaev horses, which range from 1.8% to 2.2% fat depending on the diet and pasture quality **[23]**. However, it remains slightly lower than fat levels reported in Kyrgyz kumys, where traditional fermentation practices can elevate fat concentration up to 2.05% **[24]**, likely due to fermentation duration and regional feeding systems.

Conversely, protein levels were higher in Almaty kumys (1.59%) than in Zhambyl (1.41%, $p < 0.0001$). This protein range aligns with reports from Mongolian mare's milk, where protein levels between 1.86%–2.22% are typical **[25]**, often attributed to cooler climates and shorter lactation periods. The higher protein values in Almaty may reflect similar ecological influences, including alpine pasture diversity and increased physiological demands in early lactation.

SNF and TS were also significantly greater in Almaty kumys (8.12% and 8.76%) compared to Zhambyl (4.90% and 7.01%), highlighting regional contrasts in nutrient density. These findings exceed values reported in kumys from parts of Inner Mongolia, where SNF values can drop below 7.5% in extensive systems **[26]**, suggesting better mineral and nitrogen supply in Almaty's grazing conditions.

Lactose concentration was significantly higher in Almaty samples (5.68%, $p < 0.0001$), which is within the typical mare milk range of 5.0%–6.0% but on the upper spectrum. Afzaal et al. reported lower lactose levels than those observed in this research **[2]**, potentially reflecting colder regional climates.

While freezing point and acidity varied significantly over months ($p = 0.001$), regional differences were not significant. This is consistent with previous observations that fermentation-related properties, such as titratable acidity, are influenced more by microbial populations and processing traditions than by geography or breed. In the case of Kyrgyzstan, kumys is often produced as part of mixed milk-and-cereal fermented systems, which alters both acidity and texture characteristics **[27]**, highlighting the role of cultural practices in shaping product properties beyond raw milk composition.

Citric acid and casein levels fluctuated significantly over time ($p = 0.007$ and $p < 0.0001$), with casein declining through the lactation season. Similar seasonal drops in casein content were reported in mare's milk from France and Germany, attributed to mammary involution and decreased secretory activity **[28]**, and **[29]**.

The study by Pecka et al. (2012) provides valuable insights into the urea content in mare colostrum and milk. Their research observed that urea levels increased as lactation progressed, suggesting a correlation between lactation stage and urea concentration. This finding aligns with the understanding that as lactation advances, changes in protein metabolism and nitrogen utilization occur, influencing urea levels in milk. In our current study, we observed that urea content was significantly influenced by both region and month ($p = 0.0459$ and $p < 0.0001$, respectively), with higher levels noted in Almaty. This regional variation may reflect differences in forage quality, mare diet, and environmental factors affecting nitrogen metabolism **[30]**.

The PCA analysis clearly separated samples by both month and region, with late lactation samples from Almaty clustering around high SNF and TS values. This seasonal trend reflects known physiological changes in lactating mares, where nutrient partitioning adapts to the declining milk volume while maintaining or increasing nutrient density **[31]**. Similarly, the increase in acidity and decrease in casein content over time may be attributed to ongoing proteolytic activity and the shift in protein fractions with lactation progress **[32]**.

Fatty acid composition revealed strong temporal dynamics. The significant increase in medium-chain fatty acids (MCFAs) such as C8:0 and C10:0 during late lactation supports enhanced de novo synthesis in mammary epithelial cells under reduced milk volume conditions **[33]**. Concurrently, the observed reduction in C16:0 and C18:1n9c content across months suggests a modulation in lipogenic enzyme activity, such as Δ9-desaturase, influenced by dietary substrate availability and hormonal regulation **[34]** and **[35]**. Interestingly, Zhambyl samples exhibited significantly higher palmitoleic acid (C16:1) concentrations, potentially indicating a more active lipogenic pathway or higher forage intake during early lactation months. In contrast, Almaty samples demonstrated elevated oleic acid levels (C18:1n9c), which could be attributed to the different botanical composition of pastures or metabolic differences in the mares. The differences observed in specific fatty acids between regions may carry important nutritional implications. For instance, palmitoleic acid (C16:1), which was significantly higher in Zhambyl kumys, has been associated with beneficial effects on lipid metabolism and insulin sensitivity. C16:1 may play a role in modulating inflammation and reducing the risk of metabolic syndrome when consumed in moderate amounts. Similarly, oleic acid (C18:1n9c), which was more abundant in Almaty samples, is the principal monounsaturated fatty acid in the Mediterranean diet and has been extensively studied for its cardio-protective properties, including its ability to improve plasma lipid profiles, reduce oxidative stress, and decrease low-density lipoprotein cholesterol levels. **[36]**. PUFAs, including linoleic (C18:2n6c) and alpha-linolenic acid (C18:3n3c), showed increasing trends toward late lactation, particularly in Almaty. These shifts may be driven by the higher intake of fresh forage in autumn, rich in essential fatty acid precursors **[37]**. Notably, the n-6/n-3 ratio sharply improved in Zhambyl in October–November, indicating a more favorable balance for human health during this period, consistent with recommendations emphasizing a lower omega-6 to omega-3 ratio for anti-inflammatory benefits **[38]**.

A key finding was the seasonal and regional variation in omega-6 (n-6) and omega-3 (n-3) fatty acids, which directly affected the n-6/n-3 ratio, a crucial nutritional marker. The n-6/n-3 ratio in Zhambyl kumys was exceptionally high during July to September, peaking at 113.83 in September, far above the recommended nutritional threshold (<4:1) for human health **[39]**. This indicates an imbalance likely due to low alpha-linolenic acid (C18:3n3) intake or limited biosynthesis. However, a dramatic reduction in the n-6/n-3 ratio was observed in October and November, dropping to 2.76 and 3.37 in Zhambyl, corresponding to a sharp rise in n-3 levels, possibly reflecting seasonal forage shifts rich in omega-3-rich herbaceous species. These findings are supported by Polidori et al. (2022), who reported that equid milk—particularly that from mares—has a naturally favorable balance of PUFAs, and that the n-6/n-3 ratio can be strongly influenced by regional pasture composition, animal metabolism, and the stage of lactation **[40]**.

The observed seasonal variations in the chemical and fatty acid composition of kumys can be attributed to multiple interacting factors, including pasture availability, plant biodiversity, and the physiological state of the mares during different stages of lactation. In spring and summer, mares graze on fresh, diverse forage rich in α-linolenic acid, a precursor to omega-3 fatty acids, which likely contributes to the higher levels of unsaturated fatty acids during these months. In contrast, the decline in nutritional quality of forage in autumn may reduce lipid precursors in the diet, thus influencing milk composition. Moreover, advancing lactation is known to alter the hormonal profile and mobilization of body reserves, affecting the synthesis and secretion of milk components **[41]** and **[42]**. In a recent study, Boranbayeva et al. (2025) demonstrated that both seasonal and regional factors significantly influence the bacterial and fungal biodiversity of raw mare's milk and koumiss in the Almaty and Zhambyl regions, emphasizing the complex interplay between environmental conditions and microbial dynamics in traditionally fermented milk products. Our study complements these findings by characterizing not only the microbial but also the chemical and fatty acid profiles of kumys, reinforcing its role as a functional fermented food shaped by natural ecological conditions **[43]**.

From a health perspective, the AI and TI indices remained within favorable ranges throughout the study period. TI values were consistently lower in Zhambyl kumys, suggesting potential cardiovascular benefits of milk fat produced in this region. This observation agrees with earlier reports that emphasize the health-promoting profile of fermented equine milk fats when properly managed **[44]**, and **[45]**. Overall, our study demonstrates that seasonal lactation progression exerts a more pronounced effect on kumys composition than regional factors. Nevertheless, regional influences remain important, particularly in shaping early lactation profiles and lipid health indices. These insights are crucial for standardizing kumys as a functional dairy product and optimizing its nutritional profile through controlled pasture management and targeted harvesting at specific lactation stages. While this study provides a comprehensive assessment of the chemical and fatty acid composition of kumys, it does not include a sensory evaluation component. Given that kumys is a fermented dairy product, organoleptic properties such as taste, aroma, and texture play a critical role in consumer acceptance and product quality. Future studies should incorporate structured sensory analysis using trained panels to evaluate how regional and seasonal differences affect the sensory profile of kumys. Including such data would help bridge the gap between compositional quality and consumer perception.

CONCLUSION

This study provides a comprehensive assessment of the regional and seasonal variability in the chemical composition and fatty acid profile of kumys produced from Kazakh mare's milk. Significant differences were observed between samples from Almaty and Zhambyl regions. Kumys from Almaty exhibited higher protein (1.59%), lactose (5.68%), SNF (8.12%), and TS (8.76%), while Zhambyl samples showed higher fat content (2.04%) and more favorable thrombogenic index (TI), with values as low as 0.21 in September. The lactation stage had a pronounced impact on the composition of kumys, especially fatty acid dynamics. Medium-chain fatty acids such as caprylic (C8:0) and capric (C10:0) increased significantly during late lactation, while palmitic acid (C16:0) decreased from 25.96% in July to 19.61% in November ($p = 0.0002$), and oleic acid (C18:1n9c) declined over time ($p = 0.0086$). Omega-3 content in Zhambyl samples increased from 0.17% in September to 5.65% in October, improving the n-6/n-3 ratio from a peak of 113.83 in September to 2.76 in October, aligning with nutritional guidelines. PCA confirmed that seasonal effects (explaining 72.7% of variance) outweighed regional differences in shaping kumys quality. These compositional shifts indicate dynamic metabolic adaptations in mares influenced by forage availability and lactation physiology. From a health perspective, the AI ranged from 0.82 to 1.17, while the TI remained consistently low in Zhambyl kumys, suggesting cardiovascular benefits. Overall, these findings support the valorization of kumys as a functional dairy product, with the potential to optimize its nutritional profile through targeted harvesting during late lactation and strategic pasture management. Future research should expand into other ecological zones, assess fermentation microbiota and bioavailability, and include structured sensory evaluation to align compositional quality with consumer acceptability..

REFERENCES

1. Kondybayev, A., Loiseau, G., Achir, N., Mestres, C., & Konuspayeva, G. (2021). Fermented mare milk product (Qymyz, Koumiss). International Dairy Journal, 119, 105065. https://doi.org/10.1016/j.idairyj.2021.105065
2. Afzaal, M., Saeed, F., Anjum, F., Waris, N., Husaain, M., Ikram, A., Ateeq, H., Muhammad Anjum, F., & Suleria, H. (2021). Nutritional and ethnomedicinal scenario of koumiss: A concurrent review. Food Science & Nutrition, 9(11), 6421–6428. https://doi.org/10.1002/fsn3.2595
3. D'Este, M., Alvarado-Morales, M., & Angelidaki, I. (2018). Amino acids production focusing on fermentation technologies–A review. Biotechnology advances, 36(1), 14-25. https://doi.org/10.1016/j.biotechadv.2017.09.001
4. Siddiqui, S. A., Erol, Z., Rugji, J., Taşçı, F., Kahraman, H. A., Toppi, V., Musa, L., Giacinto, G.D., Bahmid, N. A., Mehdizadeh, M. & Castro-Muñoz, R. (2023). An overview of fermentation in the food industry-looking back from a new perspective. Bioresources and Bioprocessing, 10(1), 85. https://doi.org/10.1186/s40643-023-00702-y
5. Li, Q., Zhao, Y., Siqin, B., Xilin, T., Zhang, N., & Li, M. (2022). Changes in microbial diversity and nutritional components of mare milk before and after traditional fermentation. Frontiers in Sustainable Food Systems, 6, Article 913763. https://doi.org/10.3389/fsufs.2022.913763
6. Boranbayeva, T., Dossimova, Z., Zhalelov D., Zhunisbek, A., Bolat, A., Abzhaliyeva, A., & Toishimanov, M. (2024). Influence of stage lactation on quality and protein compositions of Kazakh mare milk and koumiss. Potravinarstvo Slovak Journal of Food Sciences, 18, 964–976. https://doi.org/10.5219/2026
7. Polidori, P., Cammertoni, N., Santini, G., Klimanova, Y., Zhang, J. J., & Vincenzetti, S. (2021). Nutritional properties of camelids and equids fresh and fermented milk. Dairy, 2(2), 288-302. https://doi.org/10.3390/dairy2020024
8. Barreto, Í. M. L. G., Rangel, A. H. D. N., Urbano, S. A., Bezerra, J. D. S., & Oliveira, C. A. D. A. (2019). Equine milk and its potential use in the human diet. Food science and technology, 39, 1-7. https://doi.org/10.1590/fst.11218
9. Saleem, G. N., Gu, R., Qu, H., Bahar Khaskheli, G., Rashid Rajput, I., Qasim, M., & Chen, X. (2024). Therapeutic potential of popular fermented dairy products and its benefits on human health. Frontiers in Nutrition, 11, 1328620. https://doi.org/10.3389/fnut.2024.1328620
10. Wei, T., Zhou, T., Zhang, S., Quan, Z., & Liu, Y. (2025). Non-Targeted Lipidomics Analysis of Characteristic Milk Using High-Resolution Mass Spectrometry (UHPLC-HRMS). Foods, 14(12), 2068. https://doi.org/10.3390/foods14122068
11. Toishimanov, M., Zhanten, O., Kanat, R., Beishova, I., Ulyanov, V., Assanbayev, T., Sharapatov, T., Daurov, D., Daurova, A., Sapakhova, Z., Nametov, A., & Shamekova, M. (2025). The effects of the lactation period, mare age, and foaling on the chemical and physical composition of milk from Kazakh mares kept under natural pasture conditions. Animals, 15(12), 1817. https://doi.org/10.3390/ani15121817
12. Kong, F., Zhao, Q., Wang, S., Mu, G., & Wu, X. (2025). Comparative study on the physical and chemical properties influenced by variations in fermentation bacteria groups: Inoculating different fermented mare's milk into cow's milk. Foods, 14(8), 1328. https://doi.org/10.3390/foods14081328
13. Mituniewicz-Małek, A., Ziarno, M., Dmytrów, I., & Szkolnicka, K. (2025). Survivability of probiotic microflora in fermented and non-fermented mare's milk: A comparative study. Applied Sciences, 15(2), 862. https://doi.org/10.3390/app15020862
14. Rivero, M. J., Cooke, A. S., Gandarillas, M., Leon, R., Merino, V. M., & Velásquez, A. (2024). Nutritional composition, fatty acids profile and immunoglobulin G concentrations of mare milk of the Chilean Corralero horse breed. PloS one, 19(9), e0310693. https://doi.org/10.1371/journal.pone.0310693
15. Barłowska, J., Polak, G., Janczarek, I., & Tkaczyk, E. (2023). The influence of selected factors on the nutritional value of the milk of cold-blooded mares: The example of the Sokólski breed. Animals, 13(7), 1152. https://doi.org/10.3390/ani13071152
16. Parmar, P., Lopez-Villalobos, N., Tobin, J. T., Murphy, E., McDonagh, A., Crowley, S. V., Kelly, A.L. & Shalloo, L. (2020). The effect of compositional changes due to seasonal variation on milk density and the determination of season-based density conversion factors for use in the dairy industry. Foods, 9(8), 1004. https://doi.org/10.3390/foods9081004
17. Kurmanbayeva, M., Rašeta, M., Sarsenbek, B., Kusmangazinov, A., Zhumagul, M., Karabalayeva, D., ... & Toishimanov, M. (2024). Comparison of fatty acids and amino acids profiles of the selected perennial and annual wheat varieties from Kazakhstan. Natural product research, 1-6. https://doi.org/10.1080/14786419.2024.2305654

18. Toishimanov, M., Nurgaliyeva, M., Serikbayeva, A., Suleimenova, Z., Myrzabek, K., Shokan, A., & Myrzabayeva, N. (2023). Comparative analysis and determination of the fatty acid composition of Kazakhstan's commercial vegetable oils by GC-FID. Applied Sciences, 13(13), 7910. https://doi.org/10.3390/app13137910

19. Jastrzębska, A., Zochowska-Kujawska, J., Brodziak, A., Krzyżewski, J., & Wierzbicka, A. (2017). Changes in chemical composition and fatty acid profile of mares' milk during lactation. Journal of the Science of Food and Agriculture, 97(10), 3355–3360. https://doi.org/10.1002/jsfa.8202

20. Popova, T., Ignatova, M., & Petkov, E. (2019). Effect of pasture vegetation and feeding season on milk composition in grazing mares. Emirates Journal of Food and Agriculture, 31(8), 634–641. https://doi.org/10.9755/ejfa.2019.v31.i8.1995

21. Rakhmanova, A., Wang, T., Xing, G., Ma, L., Hong, Y., Lu, Y., Xin, L., Xin, W., Zhu, Q., & Lü, X. (2021). Isolation and identification of microorganisms in Kazakhstan koumiss and their application in preparing cow-milk koumiss. Journal of dairy science, 104(1), 151-166. https://doi.org/10.3168/jds.2020-18527

22. Pacheco-Pappenheim, S., Yener, S., Heck, J. M., Dijkstra, J., & van Valenberg, H. J. (2021). Seasonal variation in fatty acid and triacylglycerol composition of bovine milk fat. Journal of dairy science, 104(8), 8479-8492. https://doi.org/10.3168/jds.2020-19856

23. Kossaliyeva, G., Rysbekuly, K., Zhaparkulova, K., Kozykan, S., Li, J., Serikbayeva, A., Shynykul, Z., Zhaparkulova, M., & Yessimsiitova, Z. (2025). Chemical composition, physical properties, and immunomodulating study of mare's milk of the Adaev horse breed from Kazakhstan. Frontiers in Nutrition, 12. https://doi.org/10.3389/fnut.2025.1443031

24. Ibrayeva, A., Mukasheva, B., & Mursalykova, M. (2024). Development of technologies for improving the quality of mare's milk-based products: composition analysis, fermentation, and drying. Endless light in science, 31, 82-87. Retrieved from: https://cyberleninka.ru/article/n/development-of-technologies-for-improving-the-quality-of-mare-s-milk-based-products-composition-analysis-fermentation-and-drying

25. Liu, W., Wang, J., Zhang, J., Mi, Z., Gesudu, Q., & Sun, T. (2019). Dynamic evaluation of the nutritional composition of homemade koumiss from Inner Mongolia during the fermentation process. Journal of Food Processing and Preservation, 43(8). https://doi.org/10.1111/jfpp.14022

26. Doreau, M., & Martuzzi, F. (2006). Milk yield of nursing and dairy mares. In Nutrition and feeding of the broodmare (pp. 57–64). Brill | Wageningen Academic. https://doi.org/10.3920/9789086865840_006

27. Smanalieva, J., Iskakova, J., & Musulmanova, M. (2022). Milk-and cereal-based Kyrgyz ethnic foods. International Journal of Gastronomy and Food Science, 29, 100507. https://doi.org/10.1016/j.ijgfs.2022.100507

28. Doreau, M., & Martuzzi, F. (2006). Milk yield of nursing and dairy mares. Nutrition and feeding of the broodmare, 57-64. https://doi.org/10.3920/9789086865840_006

29. Doreau, M., & Martuzzi, F. (2006). Fat content and composition of mare's milk. In Nutrition and Feeding of the Broodmare (pp. 77-87). Wageningen Academic. https://doi.org/10.3920/9789086865840_008

30. Pecka, E., Dobrzański, Z., Zachwieja, A., Szulc, T., & Czyż, K. (2012). Studies of composition and major protein level in milk and colostrum of mares. Animal Science Journal, 83(2), 162–168. https://doi.org/10.1111/j.1740-0929.2011.00930.x

31. Blanco-Doval, A., Barron, L. J. R., & Aldai, N. (2024). Nutritional Quality and Socio-Ecological Benefits of Mare Milk Produced under Grazing Management. Foods, 13(9), 1412. https://doi.org/10.3390/foods13091412

32. Faye, B., & Konuspayeva, G. (2012). The sustainability challenge to the dairy sector–The growing importance of non-cattle milk production worldwide. International Dairy Journal, 24(2), 50–56. https://doi.org/10.1016/j.idairyj.2011.12.011

33. Cabiddu, A., Addis, M., Pinna, G., Decandia, M., & Molle, G. (2021). Seasonal variation of milk fatty acid profile in sheep and goats under pastoral systems. Animals, 11(1), 1–19. https://doi.org/10.3390/ani11010206

34. Chilliard, Y., Ferlay, A., & Doreau, M. (2001). Effect of different types of forages, animal fat or marine oils in cow's diet on milk fat secretion and composition, especially conjugated linoleic acid (CLA) and polyunsaturated fatty acids. Livestock production science, 70(1-2), 31-48. https://doi.org/10.1016/S0301-6226(01)00196-8

35. Minjigdorj, N., Haug, A., & Austbø, D. (2012). Fatty acid composition of Mongolian mare milk. Acta Agriculturae Scandinavica, Section A–Animal Science, 62(2), 73-80. https://doi.org/10.1080/09064702.2012.721000

36. Kouba, M., & Mourot, J. (2011). A review of nutritional effects on fat composition of animal products with special emphasis on n-3 polyunsaturated fatty acids. Biochimie, 93(1), 13-17. https://doi.org/10.1016/j.biochi.2010.02.027

37. Markiewicz-Kęszycka, M., Czyżak-Runowska, G., Lipińska, P., & Wójtowski, J. (2013). Fatty acid profile of milk-a review. Bull. Vet. Inst. Pulawy, 57(2), 135-139. https://doi.org/10.2478/bvip-2013-0026

38. Simopoulos, A. P. (2002). Omega-3 fatty acids in inflammation and autoimmune diseases. Journal of the American College of Nutrition, 21(6), 495–505. https://doi.org/10.1080/07315724.2002.10719248

39. Simopoulos, A. P. (2004). Omega-6/omega-3 essential fatty acid ratio and chronic diseases. Food reviews international, 20(1), 77-90. https://doi.org/10.1081/FRI-120028831

40. Polidori, P., Cammertoni, N., Santini, G., Klimanova, Y., Zhang, J. J., & Vincenzetti, S. (2021). Nutritional properties of camelids and equids fresh and fermented milk. Dairy, 2(2), 288-302. https://doi.org/10.3390/dairy2020024

41. Barłowska, J., Polak, G., Janczarek, I., & Tkaczyk, E. (2023). The influence of selected factors on the nutritional value of the milk of cold-blooded mares: The example of the Sokólski breed. Animals, 13(7), 1152. https://doi.org/10.3390/ani13071152

42. Gebreyowhans, S., Lu, J., Zhang, S., Pang, X., & Lv, J. (2019). Dietary enrichment of milk and dairy products with n-3 fatty acids: A review. International Dairy Journal, 95, 82–96. https://doi.org/10.1016/j.idairyj.2019.05.011

43. Boranbayeva, T., Karahan, A. G., Toishimanov, M., Zhalelov, D., & Bolat, A. (2025). Effects of seasonal and regional variations on the bacterial and fungal biodiversity of mares' milk and koumiss in the Almaty and Zhambyl regions of Kazakhstan. International Dairy Journal, 145, 106331. https://doi.org/10.1016/j.idairyj.2025.106331

44. Jastrzębska, E., Wadas, E., Daszkiewicz, T., & Pietrzak-Fiećko, R. (2017). Nutritional value and health-promoting properties of mare's milk - a review. Czech Journal of Animal Science, 62(12), 511–518. https://doi.org/10.17221/61/2016-cjas

45. Ulbricht, T. L. V., & Southgate, D. A. T. (1991). Coronary heart disease: seven dietary factors. The Lancet, 338(8773), 985–992. https://doi.org/10.1016/0140-6736(91)91846-M

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The authors reported no potential conflict of interest.

Ethical Statement:

This study did not involve any experimental procedures on animals that would require formal ethical approval under national or institutional guidelines. Milk samples were collected as part of routine milking practices from mares raised on local farms under natural pasture conditions. All handling of animals was performed by trained personnel in accordance with standard animal welfare protocols, ensuring minimal stress and no harm to the animals. No invasive procedures, treatments, or experimental interventions were conducted. .

AI Statement:

AI tools were not used in the preparation of the manuscript.

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