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Determination of the fatty and amino acid composition of camel milk, milk powder and shubat

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ABSTRACT

Camel milk is considered an essential source of nutrition and an effective remedy with healing properties in treating several diseases. Shubat, a fermented drink made from camel milk, contains easily digestible proteins, determining its nutritional value. Meanwhile, few studies have analysed the fatty and amino acid composition of Bactrian camel milk, milk powder and shubat in Kazakhstan. In this paper, we used the gas chromatography-mass spectrometry method to determine milk the fatty and amino acid composition of Kazakhstan camel milk and camel milk powder and submit samples. As a result, significant differences in the fatty acid and amino acid compositions were observed among samples of raw milk, milk powder and shubat. differences were found in all amino acids. The most representative fatty acids in the three groups were C16:0, C18:0, C18:1n9c, C14:0 FAs. In camel milk samples, lysine (29.64%) was the highest in concentration among indispensable amino acids, followed by methionine (25.68%). Some polyunsaturated fatty acids (PUFAs) such as C18:3n3c, C20:4n6, C18:3n3c, C20:3n3c 8,11,14 were found only in shubat samples. Furthermore, we revealed a significant decrease in both dispensable (DAA) and indispensable (IDAA) contents in camel milk powder. Meanwhile, an increase in the quantitative content of amino acids has been observed in shubat, especially in threonine (166.86%), aspargine (156.34%), alanine (114.48%), etc. The results provide a theoretical basis for additional studies of camel milk composition of Bactrian camel in Kazakhstan.

Keywords: camel milk, milk powder, shubat, fatty acids, amino acids.

INTRODUCTION

Kazakhstan is a country where horse and camel breeding are traditionally practised. Compared to other animals, camels have a peculiar peculiarity and adaptability to our harsh climatic conditions **[1]**. As most of our country has desert and semi-desert zones. Camels can tolerate high heat, low temperature, and temporary waterlessness well. As the raw material base is expanding in the country now, it allows the exporting of camel breeding products **[2]**. The main regions where camels are bred are Aktobe, Atyrau, Mangistau, Kyzylorda, South Kazakhstan and Almaty regions. Historically, the camel has played an important role, supplying Kazakhs with milk, meat, wool, and leather. In Kazakhstan's western and southern regions, camel milk is the primary foodstuff. Kazakhstan is the only country where one-humped and two-humped camels are kept, and hybrids are produced **[3]**. In Figure 1, it is shown that from 1990 to 2022, the camel population increased by 59%. Camel milk is of the albumin type, so it is well-digested in the human body **[4]**. It is recognised that camel milk is an effective remedy with healing properties in treating gastritis, diabetes, asthma, tuberculosis, skin diseases, urinary problems and hepatitis. Milk consumption does not cause allergic reactions or gastrointestinal irritation **[5]**. Camel milk contains 5 to 6 % fat, proteins and other components determining its nutritional value. Camel milk usually contains short-chain fatty acids (C4-C12) in very low amounts compared to other types **[6]**. In terms of vitamin C content, it is significantly higher in vitamin C than milk from cows **[7]**. Camel milk is consumed by the inhabitants of arid

regions of the world, mainly as a fermented milk product called shubat or kymyran (from two-humped camels) or chal (from one-humped camels). Shubat is a sour milk drink made from camel's milk that is a traditional drink of Kazakhs **[8]**. Scientists have also proved the positive effect of shubat in diabetes; treatment with these products leads to the normalisation of the intracellular function of the pancreas, increasing the number of patients with normal glycaemic curves **[9]**, **[10]**. Due to changes in the economic and social status of the elderly and old people, the caloric content of their diet is significantly decreasing, which makes energy and protein-calorie deficiency among the most important problems. The energy deficit is aggravated by deficient protein intake, vitamins and mineral elements and an imbalance of polyunsaturated fatty acids **[11]**. Nowadays, to produce high-quality products using modern processing technologies, high-quality requirements are imposed on raw milk, the leading controlled indicators of which are physical, chemical and technological properties **[12]**. Therefore, this study aimed to research the fatty acid and amino acid composition of camel milk, camel milk powder and shubat samples collected from Almaty region in Kazakhstan.

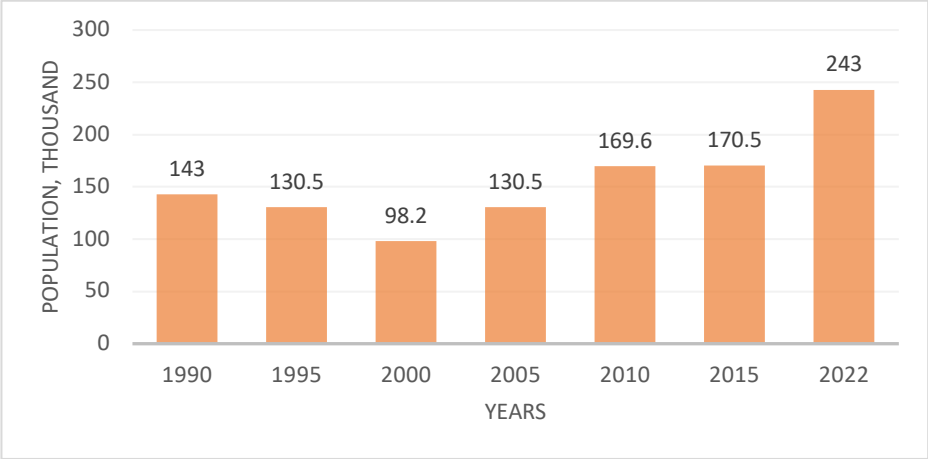

Figure 1 Number of camel population in Kazakhstan for 1990-2022.

Scientific Hypothesis

Camel milk provides nutrition through proteins, amino acids and fatty acids. There were significant differences in amino acid and fatty acid content between raw milk, dried milk and shubat samples from Kazakhstan.

MATERIAL AND METHODOLOGY

Samples

Fresh camel milk (n = 2), shubat (n = 2), milk powder (n = 2) samples were obtained from Bactrian camel breeding farm "Daulet-Beket" LLP, Akshi village, Almaty region, Kazakhstan November in 2022. Camels were pasture-fed. Samples were delivered in a thermos container to the laboratory.

Chemicals

All chemicals were purchased by “Laverna XXI century” (Moscow, Russia) and were of analytical grade quality. We used ethanol (purity ≥95%), isopropanol (purity ≥99.5%), sodium hydroxide (purity ≥99.5%), and methyl ether (purity ≥99%).

Instruments

A gas chromatograph “Shimadzu GC-2010 Plus” used for fatty acid analysis.

Laboratory Methods

Experiments were performed at the laboratory of the Kazakh-Japanese Innovation Centre of Kazakh National Agrarian Research University. Determination of fatty acid composition was carried out following GOST 32915-2014 “Milk and dairy products. Determination of fatty acid composition of the fat phase by gas chromatography method”. Briefly, the homogenisation was carried out with a blender for 3-5 min with maximum stirring. The separated hexane layer was transferred to a round bottom flask, then connected to a roundabout evaporator and the solvent was completely distilled off at a temperature of 70 ±2 °C. Methyl ether was added to the obtained fat fraction. 1 cm^3 of the fatty acid methyl ester solution was taken with a microsyringe and injected into a gas chromatograph “Shimadzu GC-2010 Plus”.

The following parameters were set for the chromatograph measurement: temperature of the flame ionisation detector – 260 °C; temperature parameters: 100 °C – 5 min, up to 210 °C – 8 min at a rate of 40 °C/min, up to 240 °C – 16.5 min at a rate of 10 °C/min; sample division flow 1/40. Analysis time – 60 min.

The qualitative and quantitative composition of amino acids was established by high-performance liquid chromatography (HPLC) **[13]**. To analyse the samples for total amino acid composition by HPLC, a precise sample of dry extract (~100 mg) was dissolved in 5 ml of 40% ethyl alcohol and incubated in an ultrasonic bath for 10 min. Aliquots (0.1-0.2 ml) were placed in a test tube. Then, they were dried in a water bath at 60 °C. The dried aliquots added 0.10 mL of 0.15 mol/l sodium hydroxide solution and then stirred. Next, 0.35 ml of phenylisothiocyanate solution in isopropyl alcohol and 0.05 ml of bidistilled water were added. The solution was mixed thoroughly again and left for 20 min at room temperature, then dried to dryness at 65 °C. The dry residue was dissolved in 1 ml of bidistilled water. The resulting solutions were subjected to chromatographic analysis.

Tryptophan was determined without derivatisation with phenylisothiocyanate by chromatographic analysis of a previously prepared solution of a suspension of the dry extract in ethyl alcohol 40%. To determine the sum of cysteine and cystine, the sample was pre-oxidised with supramuravic acid, and then cysteic acid was determined as a phenylthiocarbamate derivative. To obtain supramuric acid, one part of hydrogen peroxide and 9 parts of formic acid were mixed thoroughly in a 10 cm3 test tube. A suspension of the dry extract was placed in an evaporation cup, and 5 cm3 of oxidising agent was added and dried completely in a water bath at 60 °C. The dry residue was dissolved in 5 ml of 40% ethyl alcohol. An aliquot of the obtained solution was transferred into a test tube. Derivatisation with phenylisothiocyanate was carried out, filtered off, and the sample was injected into a chromatographic column.

Description of the Experiment

Sample preparation: Preparation of samples of camel milk, camel milk powder and shubat was carried out following GOST 32915-2014 “Milk and dairy products. Determination of the fatty acid composition of the fat phase by gas chromatography method”.

Number of samples analyzed: 6.

Number of repeated analyses: 2.

Number of experiment replication: 3.

Design of the experiment: In the experiment's first phase, we obtained the Bactrian camel raw milk, powder milk, and shubat samples from the "Daulet-Beket" LLP farm in the Almaty region. Then, we determined the fatty acid composition of raw milk, milk powder, and shubat. In the next phase, we detected the amino acid composition of these samples.

Statistical Analysis

The experiments were performed in three replications to get a true mean and support the hypothesis. Data were analyzed using the ANOVA in the SPSS software (Version 25.0, IBM Corporation, New York, USA). To reduce instance of a false positive, the threshold p-value for significance was adjusted after correction for multiple comparisons using the Bonferroni correction.

RESULTS AND DISCUSSION

Camel milk consumed by inhabitants of arid regions provides nutrition through proteins, amino acids and fatty acids **[14]**, **[15]**. Fatty acids are essential for the normal functioning of all body's systems: blood circulation and respiration to immunity and brain function. In addition, fatty acids are a membrane component of absolutely every cell in the organism **[16]**, **[17]**. Mass fractions of fatty acid (FA) composition of camel milk, camel milk powder and shubat are presented in Table 1. Significant differences (p <0.001; p <0.0001) were detected among all fatty acids after Bonforenni’s correction. Figure 2 shows the results of the milk fat chromatogram of camel milk, camel milk powder and shubat. The most representative fatty acids in the three groups were C16:0, C18:0, C18:1n9c, and C14:0 FAs (Table 1). Previous studies also observed the prevalence of C16:0, C18:0, and C14:0 in Mongolian Bacterian camel milk **[18]**. Of interest, we observed a higher amount of C18:1n9c in raw (28.9%), dry camel milk (31.14%) and shubat (27.51%) samples compared to those in Mongolian **[18]** and Turkish camel milk **[19]**. These differences may be due to the camel’s diet, breed and environmental factors **[20]**. The composition of fatty acids in dairy is mainly dependent on two processes: lipid metabolism in the rumen and the mammary gland. Recent studies have shown that dietary changes can affect the rumen and mammary gland microbiota. For instance, it has been reported that increasing the proportion of fresh forages, fibre and oilseeds in concentrates increases the level of fatty acids in raw milk **[21]**. Also, local breeds specific to certain areas demonstrate even higher levels of fatty acids than those traditionally considered the most productive species. In addition, genetics also influences the composition of the final raw material. Still, it is difficult to determine what the specific fatty acid content of milk will be without expensive and time-consuming analytical methods **[22]**.

Furthermore, significant differences were detected among all saturated fatty acids (SFAs), except for C14:0. Previously, **[23]** detected no statistically significant difference in the yield of SFAs in fermented and unfermented

milk. SFAs are fatty acids whose molecules are hydrogen-enriched. It is well known that excess saturated fatty acids increase blood cholesterol levels and contribute to obesity and the development of heart disease **[24]**.

Table 1 Mass fractions of fatty acid composition of camel milk, milk powder, and shubat % of total content.

Fatty acid code	Classification	Camel milk, % ug/ml	Milk powder, % ug/ml	Shubat, % ug/ml
		Saturated fatty acids		
C14:0	Myristic	7.12 ±0.1	6.58 ±0	8.39 ±1.1
C15:0	Pentadecanoic	^{a}1.22 ±0.1	^{b}0.27 ±0	^{c}1.15 ±0.01
C16:0	Palmitic	^{a}28.48 ±0.1	^{b}7.44 ±0.01	^{c}26.18 ±0
C17:0	Margaric	^{a}1.71 ±0.1	^{b}0.39 ±0.01	^{c}0.94 ±0.01
C18:0	Stearic	^{a}17.65 ±0.09	^{b}17.35 ±0.01	^{c}14.6 ±0.01
C20:0	Arachidic	ND	ND	0.4 ±0.01
C21:0	Heneicosanoic	ND	^{a}1.50 ±0.01	^{c}0.63 ±0.01
		Monounsaturated fatty acids		
C14:1	Myristoleic, ω5	^{a}0.46 ±0.02	^{b}0.63 ±0.01	^{c}2.3 +0
C15:1	Pentadecenoic	^{a}0.55 ±0.02	^{b}25.03 ±0.01	^{c}0.31 ±0.03
C16:1	Palmitoleic, ω7	^{a}8.12 ±0.1	^{b}0.35 ±0	^{c}10 ±0
C17:1	Heptadecanoic acid	^{a}0.64 ±0.02	^{b}17.33 ±0.01	^{b}0.82 ±0.01
C18:1n9t	Elaidic	^{a}2.64 ±0.1	^{b}3.48 ±0.01	^{bc}2.29 ±0
C18:1n9c	Oleic	^{a}28.9 ±0.06	^{b}31.14 ±0.08	^{ac}27.51 ±0.6
		Polyunsaturated fatty acids		
C18:2n6t	Linolelaidic acid	^{a}1.31± 0.1	^{b}0.33 ±0.01	^{c}0.62 ±0.02
C18:2n6c	Linoleic, ω6	^{a}1.21 ±0.09	^{b}5.57 ±0.01	^{c}2.08 ±0.01
C18:3n3c	α-linolenic acid, ω3	ND	ND	0.08 ±0
C18:3n6c	γ-linolenic acid	0.29±0	ND	ND
C20:4n6	arachidonic	ND	ND	0.15 ±0.01
		Polyunsaturated fatty acids		
C18:3n3c	Linolenic	ND	ND	0.08±0.01
C20:3n3c 8,11,14	Eicosatetraenoic	ND	ND	0.08±0.01

Note: Data with different superscript letters display significant differences.

Unsaturated fatty acids (USFA) are monosaturated FAs (MUFAs) that have only one or polyunsaturated FAs (PUSFs) that have two or more double bonds between adjacent carbon atoms in their structure **[25]**. Shubat dominates in terms of increased content (C14:1 (2.3%), C16:1 (10%), C17:1 (0.82)) of MUFAs. Previous study has observed higher MUFA levels and low PUFA levels in camel milk **[18]**. MUFAs increase glucose absorption and thus prevent the development of diabetes and metabolic syndrome, prevent the development of breast cancer in women, and participate in strengthening the immune system. In addition, they reduce cholesterol levels in the blood and prevent the deposition of cholesterol plaques on the walls of blood vessels, thus reducing the risk of atherosclerosis **[26]**. One of the main monounsaturated fatty acids in camel milk is oleic acid – C18:1 (ω-9), which acts favorably on lipid metabolism, particularly cholesterol metabolism **[27]**.

The biological value of the lipid component of a product is characterized by its qualitative composition of fatty acids. The most significant biological significance of unsaturated fatty acids are PUFAs, the so-called essential FAs. The presence of polyunsaturated (essential) fatty acids in camel milk determines its usefulness and therapeutic effect. According to modern nutraceutical regulations, fats with a high content of PUFAs are considered biologically valuable **[28]**. The main benefit of PUFAs lies in their ability to strengthen the structure of cell membranes. They improve cellular activity, which naturally affects all organs and body systems **[28]**. PUFAs are primarily linoleic and linolenic acids. Importantly, a high amount (5.86%) of C18:2n6c is found in milk powder. It is well known that linoleic acid improves metabolism, regulates cholesterol levels and promotes muscle building **[29]**. Some polyunsaturated fatty acids (PUFAs) such as C18:3n3c, C20:4n6, C18:3n3c, C20:3n3c 8,11,14 were found only in shubat samples. These PUFAs promote estrogen production and increase immunity, affecting muscle tissue growth and repair **[30]**. Arachidonic acid has a high biological value in the nutrition of children. The absence or lack of it in the diet delays the physical development of the child **[31]**. During the fermentation process, complex biochemical processes take place in milk, as a result of which the chemical composition of the final product - shubat differs significantly from the chemical composition of the original raw milk. The content of beneficial fatty acids was higher in fermented milk than in unfermented milk **[23]**.

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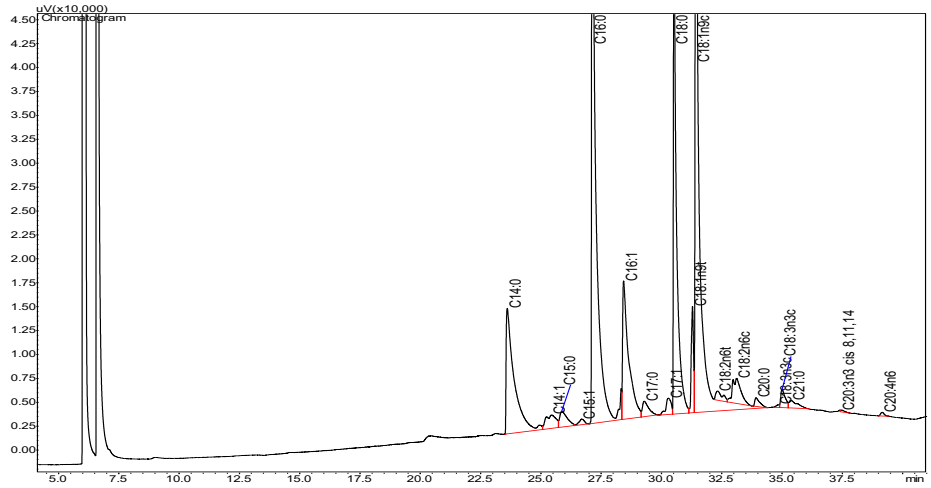

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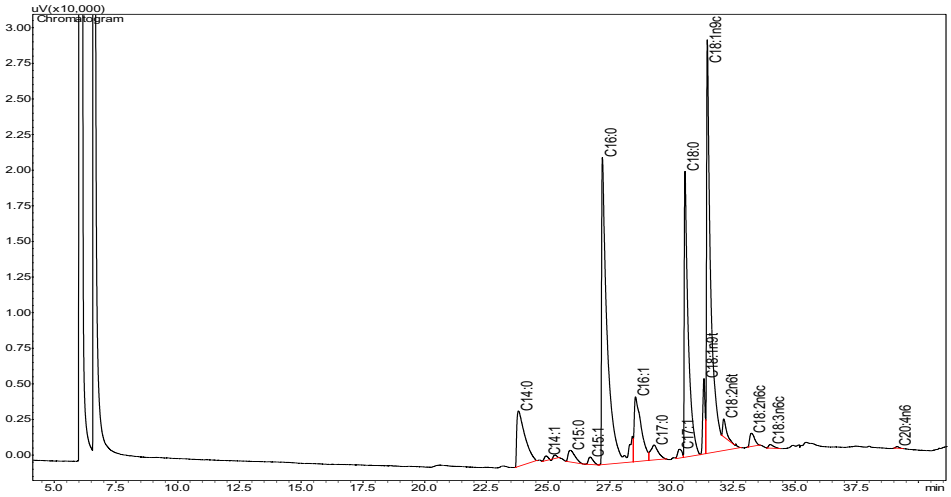

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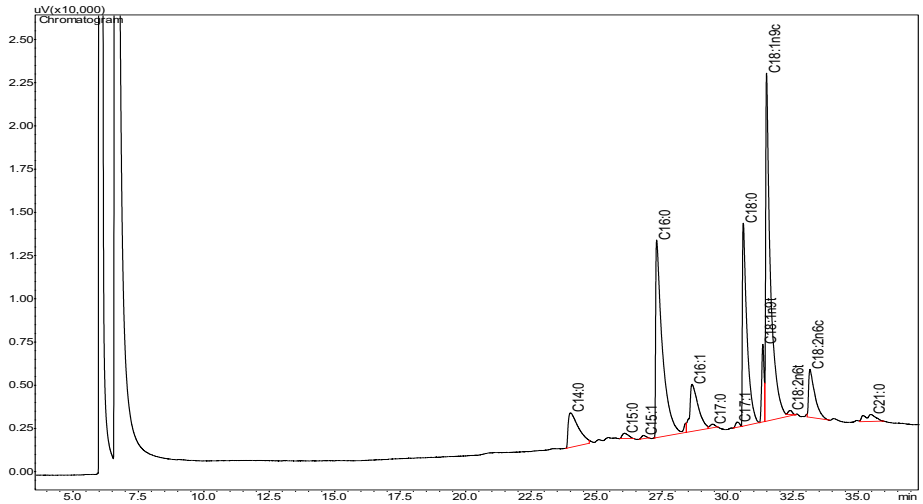

Figure 1 Mass fractions of fatty acid composition of camel milk, milk powder, and shubat % of total content. x-axis is a retention time (min), y-axis represents abundance.

Amino acids directly affect the nervous system, regulating mental performance, mood and sleep **[32]**, **[33]**, **[34]**. These components are essential for the formation of muscles, tendons and ligaments, as well as hair and skin. Without a sufficient amount of amino acids, active muscle growth is impossible **[35]**. Table 2 shows the concentrations of amino acids in the camel milk, milk powder and shubat samples. Figure 3 displays the results of a chromatogram study of the amino acid composition of camel milk, camel milk powder and shubat.

Table 2 Amino acid composition of camel milk, dry milk and shubat.

Amino acids	Camel milk	Camel milk powder	Shubat
Indispensable			
Valine	^{a}10.48 ±0.1	^{b}13.32 ±0.07	^{c}43.86 ±0.02
Isoleucine	^{a}13.98 ±0.09	^{b}2.14 ±0.1	^{c}25.34 ±0.1
Leucine	^{a}14.79 ±0.1	^{b}0.35 ±0.2	^{a}15.35 ±0.03
Lysine	^{a}29.64 ±0.1	^{b}0.91 ±0.04	^{c}44.86 ±0.05
Methionine	^{a}25.68 ±0.06	^{b}0.47 ±0.2	^{a}25.45 ±0.1
Threonine	^{a}20.88 ±0.06	^{b}1.3 ±0.06	^{c}166.86 ±0.07
Phenylalanine	^{a}20.65 ±0.06	^{b}1.15 ±0.03	^{c}59.92 ±0.03
Dispensable			
Alanine	^{a}88.96 ±0.02	^{b}2.88 ±0.02	^{c}114.48 ±0.01
Arginine	^{a}42.75 ±0.06	^{b}18.51 ±0.04	^{c}49.89 ±0.08
Asparagine	^{a}15.82 ±0.1	^{a}15.14 ±0.05	^{b}156.34 ±0.1
Aspartic acid	^{a}57.62 ±0.04	^{b}9.75 ±0.03	^{c}56.52 ±0.2
Glutamic acid	^{a}16.59 ±0.09	^{b}0.4 ±0.2	^{c}25.77 ±0.1
Histidine	^{a}13.74 ±0.04	^{b}10.98 ±0.01	^{c}11.62 ±0.2
Proline	^{a}0.94 ±0.03	^{a}0.34 ±0.2	^{b}1.36 ±0
Serine	^{a}13.68 ±0.2	^{b}2.3 ±0.04	^{a}14.4 ±0.1
Tyrosine	^{a}31.83 ±0	^{c}1.33 ±0.04	^{b}29.30 ±0.05
Cysteine	^{a}0.4 ±0.06	^{b}12.85 ±0.03	^{c}34.29 ±0.01
Cystine	^{a}16.64 ±0.07	^{b}20.54 ±0.04	^{c}41.80 ±0.01

Note: Data with different superscript letters display significant differences.

Significant differences were found in all amino acids. In camel milk samples, among indispensable amino acids, lysine (29.64%) was the highest in concentration, followed by methionine (25.68%). The results of our study indicate a higher amino acid content than those obtained on Saudi Arabian camels **[36]**. Among dispensable amino acids, cysteine was the least abundant (0.4%), consistent with the previous results **[18]**. Raw camel milk and shubat contain more essential amino acids than powdered milk. As is known, animal proteins play a crucial role in rational nutrition. Camel milk proteins are biologically valuable in digestibility and balanced amino acid composition.

We also revealed decreased dispensable (DAA) and indispensable (IDAA) contents in camel milk powder. It might be explained by denaturation changes of thermolabile protein substances of milk are possible during the drying process **[37]**. Milk processing can lead to denaturation, aggregation, and chemical alterations of amino acids. Prolonged heating causes a change in the charge and degree of hydration of protein molecules, as well as the release of active sites capable of interaction on their surface. The decrease in some amino acids may be due either to their degradation by heat or to their combination with other components **[38]**. Meanwhile, an increase in the quantitative content of amino acids has been observed in shubat, especially in threonine (166.86%), aspargine (156.34%), alanine (114.48%), etc. Threonine is essential for the synthesis of amino acids such as serine and glycine, which in turn are involved in the synthesis of collagen and elastin – proteins of connective and muscle tissue **[39]**. Thus, in fermentation and maturation, the amount of milk sugar is significantly reduced, and lactic acid, ethyl alcohol and carbon dioxide accumulate in shut **[40]**, **[41]**. In addition, proteinase enzymes of lactic acid bacteria and yeast hydrolyse casein and whey proteins of milk, turning part of them into polypeptides, peptides and free amino acids. This is how camel milk proteins are converted into easily digestible nitrogen-containing compounds. **[42]**. The essential amino acid content of shubat makes it a valuable source of protein while being low in fat and cholesterol. Moreover, the study by Bai et al. reported that the ratio of amino acids in shut was higher than that of camel milk **[43]**. Several studies describe the therapeutic and dietary value of preserved and reconstituted fermented milk products in the diet of the population in terms of their suppression of putrefactive processes in the intestine **[44]**. Of interest, traditional sour milk products such as koumis and shut were suggested as a remedy for intestinal diseases **[45]**.

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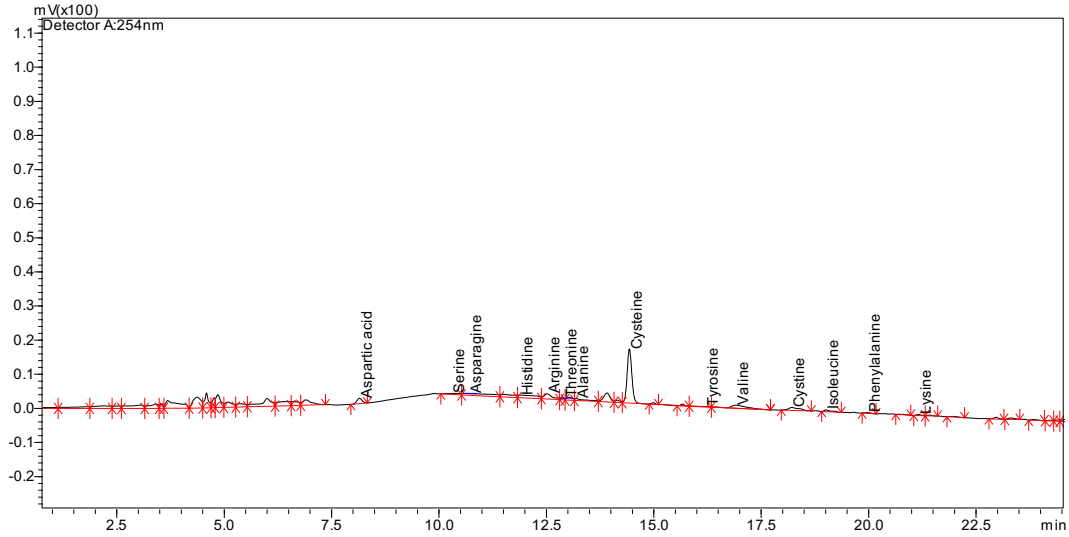

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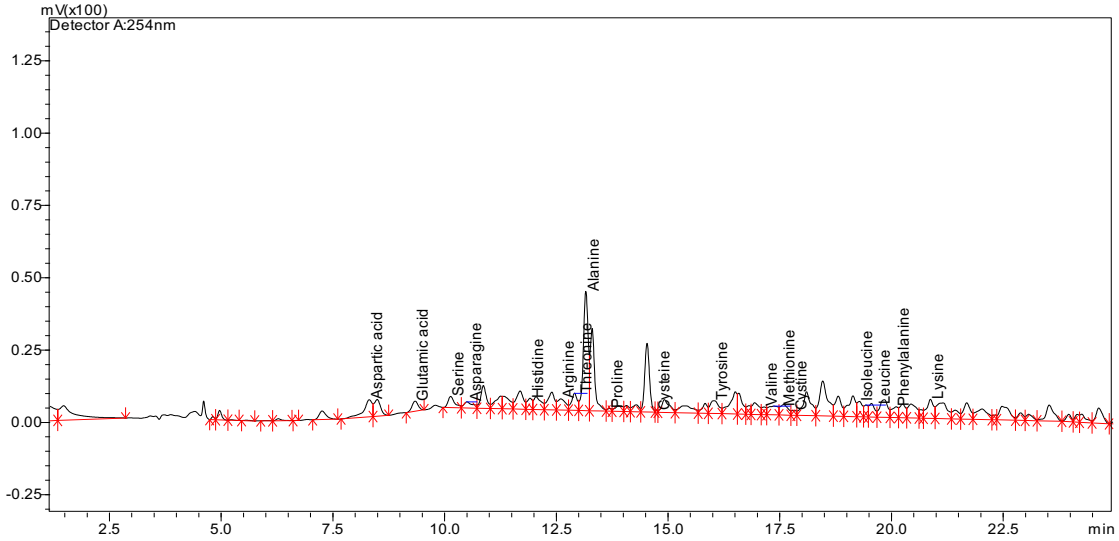

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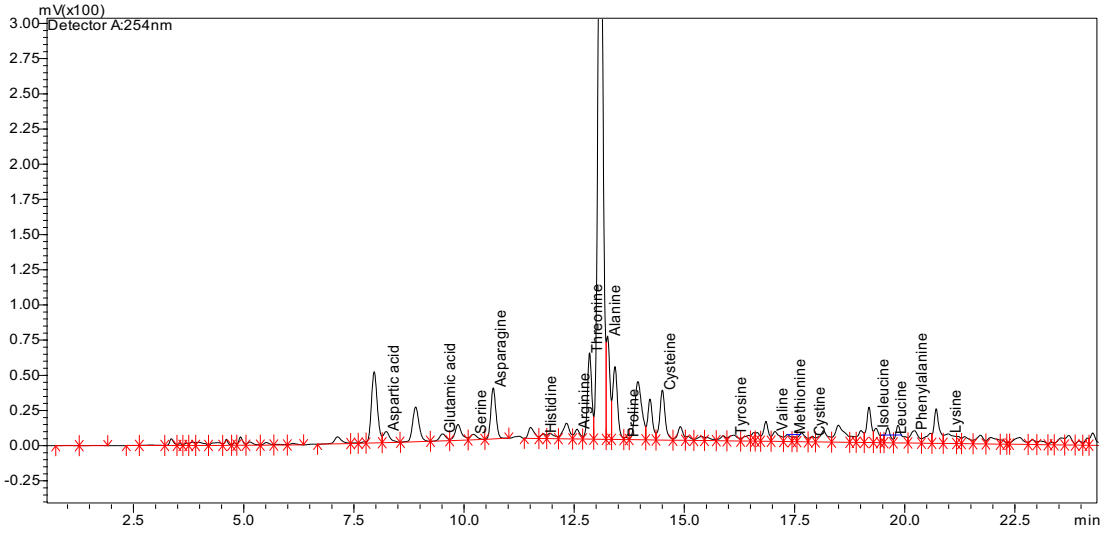

Figure 3 Chromatograms of amino acid composition of a) camel milk; b) camel milk powder; c) shubat. Note: x-axis is a retention time (min), y-axis represents abundance.

CONCLUSION

This study analysed amino acid and fatty acid compositions to differentiate between camel milk, milk powder and shubat. As a result, there were significant differences in amino acid and fatty acid content between raw milk, dried milk and shubat. Higher MUFA and low PUFA levels in our milk, dry milk and shubat were observed. Moreover, raw camel milk and shubat samples contained more essential amino acids than dried milk, indicating their biological value in digestibility and balanced amino acid composition. In addition, a higher content of essential amino acids was found in shubat samples compared to raw and dried milk. The results provide a theoretical basis for additional studies of camel milk composition of Bactrian camel in Kazakhstan.

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