CJES
Caspian Journal of Environmental Sciences
Online ISSN: 1735-3866
Print ISSN: 1735-3033

Study of amino acid and mineral composition of powder from the coat obtained by the method of liophilic drying

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ABSTRACT
Traditionally, shobat, a beverage made out of camel milk fermentation, has had its unique position in the culinary traditions of Central Asia throughout history, yet its limited shelf-life has been an obstacle to its industrialization and mass production all along. The research described below was performed in the Republic of Kazakhstan in 2025 and its purpose is to examine the impact that freeze-drying technique has on the protein content and mineral composition of shobat. Fresh samples of shobat have been collected in the Almaty region of Kazakhstan and then subjected to freeze-drying at -40°C and a vacuum pressure of 0.01 mbar. Protein content was evaluated via HPLC; mineral elements were examined by means of AAS. In conclusion, the results indicated that the amount of essential amino acids was reduced marginally (1.8 – 3.6%) but was not statistically significant ($p < 0.05$). Out of all the amino acids, only cysteine was statistically significant with a reduction of -5.5% ($p = 0.04$). The recovery of calcium and magnesium in the powder was high, more than 96%. On the other hand, iron and manganese had reductions of 4.3 and 5.3%, respectively, which were statistically significant ($p = 0.04$; $p = 0.03$). The final powder had moisture content of 3.3% and water activity of 0.25, both of which are conducive for storage at room temperature. Therefore, freeze-drying seems to be an effective way of processing chobat into powder form, while also retaining its nutritive value. This makes it an appropriate substitute for similar dairy foods in the market.

Keywords: Chobat, Freeze-drying, Amino acids, Mineral elements, Kazakhstan.
Article type: Research Article.

INTRODUCTION
The drink shubat, an alcoholic beverage prepared from camel's milk, has had a unique place in the culinary traditions of Central Asia, more precisely nomadic areas such as Kazakhstan and Turkmenistan, since time immemorial (Aralbayev *et al.* 2021; Felfoul *et al.* 2022; Al-Thaibani *et al.* 2024; Abdurahmanov *et al.* 2025; Ali *et al.* 2026). While cow's milk and goat's milk can be found anywhere across the world, camel's milk is unique in the sense that according to traditional practices of folk medicine, it is a food with therapeutic qualities owing to its special constitution. It contains a number of essential amino acids in a harmonious combination along with a complex of trace elements. The primary problem that arises during industrialization of this fermented beverage is the short storage life of the latter because of its high microbial activity (Attia *et al.* 2000; Ho *et al.* 2021; Tayeva *et al.* 2023; Jumayeva *et al.* 2026; Al-Mohusain *et al.* 2026). Freeze-drying is viewed as one of the most modern

 Received: Dec. 06, 2025 Revised: March 11, 2026 Accepted: April 17, 2026
DOI: 10.22124/cjes.2026.9792

Publisher: University of Guilan

methods of *preserving* foods that allows not only substantially extending the period of storage by dehydration at extremely low temperatures, but also ensuring the preservation of its structural and functional components within acceptable limits (Habtegebriel *et al.* 2018; Zouari *et al.* 2020). Recently, scientists working in the field of food industry in Kazakhstan focused on studying the possibility of using this technique in obtaining powder from fresh camel's milk since this product may be widely used both for exporting abroad and satisfying the needs of children, older people and athletes (Aralbayev *et al.* 2019; Tastemirova *et al.* 2020; Tastemirova *et al.* 2022). Nevertheless, one of the key problems with the development of the new product is the absence of sufficient knowledge about the influence of the freeze-drying process on the chemical composition of camel milk (Habtegebriel *et al.* 2021a,b; Urazbayev *et al.* 2023; Alshishani *et al.* 2026). While there have been several studies conducted on the evaluation of the nutritional value of raw camel milk, the data obtained from the analysis of amino acids and minerals' behavior at sublimation are insufficient, mostly based on the results of a few uncontrolled samples. The problem in question is not only associated with difficulties in evaluating the nutritional value of the new product, but also creates significant problems with its standardization (Konuspayeva *et al.* 2009; Rahman *et al.* 2012; Zouari *et al.* 2019; Yousef *et al.* 2026). In Kazakhstan, while the country has a long tradition in both producing and consuming shubat, the majority of the production facilities still use traditional and unstandardized methods and have not yet taken into account that modern technologies can contribute greatly to adding more value to this food product (Ahmad *et al.* 2019; Deshwal *et al.* 2020; Jevtić *et al.* 2024; Weshah *et al.* 2025). In addition, rising consumer interest in functional milk products with increased shelf-life makes it twice as important to conduct research in this area (Baldelli *et al.* 2022; AlYammahi *et al.* 2023; Al-Habies *et al.* 2025; Alaswad *et al.* 2026). Against such a backdrop, the production of shubat powder with satisfactory and stable quality can be deemed as a productive measure toward prosperity in the industry of dairy foods and also enhancing the nutritional status of the population of the country (Niu *et al.* 2019; Abdieva *et al.* 2025; Allouzi *et al.* 2025). Based on the previously reviewed literature review (Belosvet 2013; Belén Ortiz & Karapetrovic 2024), this study was performed in order to systematically analyze the amino acid content and mineral element composition of the dried powder produced via the freeze-drying of shubat. This research, by comparing the essential amino acid and mineral (calcium, iron, zinc, magnesium) composition of freeze-dried versus fresh samples, will establish production norms, enhance processing methods, and facilitate innovative, nutritionally valuable products. The findings are expected to shift Kazakhstan's food industry from traditional production to knowledge-based industrialization.

MATERIALS AND METHODS

Sample preparation and freeze-drying process

Samples of fresh shobat were obtained from a traditional producing unit in the Almaty region, Kazakhstan, from May to July 2025. All samples were transported to the laboratory of food industry of the Almaty state agricultural institute right away under -4 °C and processed upon receipt. The lyophilization process was conducted in triplicates using freeze dryer Alpha 1-4 LD Plus (Christ company, Germany). Initially, samples were frozen at -40 °C during 24 hours and then subjected to the sublimation procedure at vacuum pressure 0.01 mbar and cooling at -55 °C for 48 hours. The produced powdered samples were placed in three-layer polyethylene bags capable of being impermeable to moisture and light and kept at -20 °C till analyzing. Fresh shobat samples were analyzed immediately without lyophilization for comparison.

Amino acid composition analysis

The quantitative and qualitative analyses of amino acids in shobat powder and fresh samples (serving as controls) were carried out using a high performance liquid chromatography method with a fluorescence detector. For the sample preparation, acid hydrolysis was done using 6 N hydrochloric acid for 24 hours at 110 °C in the absence of air. Pre-column derivatization was carried out using ortho-phthalaldehyde and 9-fluoronylmethyl chloroformate as reagents to differentiate between primary and secondary amino acids. Chromatographic separation was conducted using a C_{18} reverse phase column with a dimension of 250 × 4.6 mm and a particle size of 5 μm at a temperature of 40 °C. Sodium acetate buffer 0.01 M with an acidity level of 2.7 and high purity acetonitrile was used as the mobile phase with a gradient flow of 1.5 mL min^{-1}. Identification and quantification of the samples were determined based on the calibration curve of the reference standards and through Clarus software.

Measurement of mineral elements

In order to determine the mineral elements concentration, the digesting of the shobat powder and fresh samples of shobat was done through ash-wetter method involving concentrated nitric acid and hydrogen peroxide digestion at 180 °C. The cooled digests were then diluted with double distilled water and the concentrations of Ca, Mg, Fe, Zn, Cu, and Mn were determined with the atomic absorption spectrometer using graphite furnace and deuterium field correction. The conditions of the spectrometer were adjusted for each metal based on the metal's specific wavelength and furnace temperature. For Ca and Mg, the acetylene-air flame method was employed while for Fe, Zn, Cu, and Mn, we used the furnace method. In order to verify the accuracy of results obtained, standard reference material samples were used and samples were also spiked with metals. The statistical analysis of data was carried out using SPSS version 26 and Student's t-test at 95% confidence level.

RESULTS

A total of 12 fresh shubat and 12 freeze-dried powder samples (each in triplicate) were analyzed for their amino acid and mineral composition. The freeze-drying process yielded a recovery rate of 86.2% (SD ± 2.5), and the final powder had a moisture content of 3.3% and water activity of 0.25, both well within acceptable ranges for long-term shelf stability. Chromatographic and spectroscopic analyses revealed that the overall nutritional profile of shubat was largely preserved, with only minor, and in most cases statistically insignificant, losses during the lyophilization process.

Table 1. Concentration of essential amino acids in fresh shubat and freeze-dried powder (mg g^{-1} protein).

Amino acid	Fresh shubat	Freeze-dried powder	Change (%)	*p*-value
Leucine	82.4 ± 3.1	79.8 ± 2.9	-3.2	0.08
Isoleucine	51.7 ± 2.2	50.1 ± 2.0	-3.1	0.12
Valine	58.3 ± 2.5	56.2 ± 2.3	-3.6	0.06
Threonine	45.6 ± 1.9	44.8 ± 1.8	-1.8	0.21
Methionine	24.8 ± 1.1	23.9 ± 1.0	-3.6	0.09
Phenylalanine	48.2 ± 2.0	46.7 ± 1.9	-3.1	0.11
Lysine	72.5 ± 3.0	70.2 ± 2.8	-3.2	0.10
Histidine	29.6 ± 1.3	28.9 ± 1.2	-2.4	0.18
Arginine	38.4 ± 1.6	37.6 ± 1.5	-2.1	0.15

Table 1 demonstrates that the concentrations of all essential amino acids in the freeze-dried powder were slightly lower than those in fresh shubat, but none of these differences reached statistical significance ($p > 0.05$ for all). The largest reductions were observed for valine and methionine (-3.6%), while threonine showed the smallest change (-1.8%). Table 2 shows that the pattern of changes among non-essential amino acids closely mirrored that of the essential group, with one notable exception. Cysteine exhibited a somewhat larger reduction (-5.5%), which was statistically significant ($p = 0.04$). Glutamic acid was the most abundant amino acid in both samples, reaching 123.4 mg g^{-1} protein in fresh shubat.

Table 2. Concentration of non-essential amino acids in fresh shubat and freeze-dried powder (mg g^{-1} protein).

Amino acid	Fresh shubat	Freeze-dried powder	Change (%)	*p*-value
Alanine	46.3 ± 1.8	44.9 ± 1.7	-3.0	0.14
Glycine	23.7 ± 0.9	23.1 ± 0.9	-2.5	0.20
Serine	41.5 ± 1.6	40.3 ± 1.5	-2.9	0.16
Proline	85.6 ± 3.2	83.1 ± 3.0	-2.9	0.13
Glutamic acid	123.4 ± 4.5	119.8 ± 4.3	-2.9	0.12
Aspartic acid	68.7 ± 2.6	66.9 ± 2.5	-2.6	0.18
Tyrosine	34.2 ± 1.4	33.4 ± 1.3	-2.3	0.22
Cysteine	12.8 ± 0.5	12.1 ± 0.5	-5.5	0.04*

Fig. 1 confirms the slight but consistent reduction across all essential amino acids following freeze-drying. The pattern of decline was remarkably uniform, with no single amino acid deviating substantially from the overall trend. Table 3 shows that calcium and magnesium were well-preserved during freeze-drying, with recovery rates of 96.7% and 96.5%, respectively. The losses for both macrominerals remained below 5% and were not statistically significant ($p > 0.05$).

Table 3. Macromineral concentrations (calcium and magnesium) in fresh shubat and freeze-dried powder.

Mineral	Fresh shubat (mg 100g^{-1} dry weight)	Freeze-dried powder (mg100g^{-1} dry weight)	Recovery (%)	*p*-value
Calcium (Ca)	128.5 ± 4.2	124.3 ± 4.0	96.7	0.08
Magnesium (Mg)	42.7 ± 1.5	41.2 ± 1.4	96.5	0.11

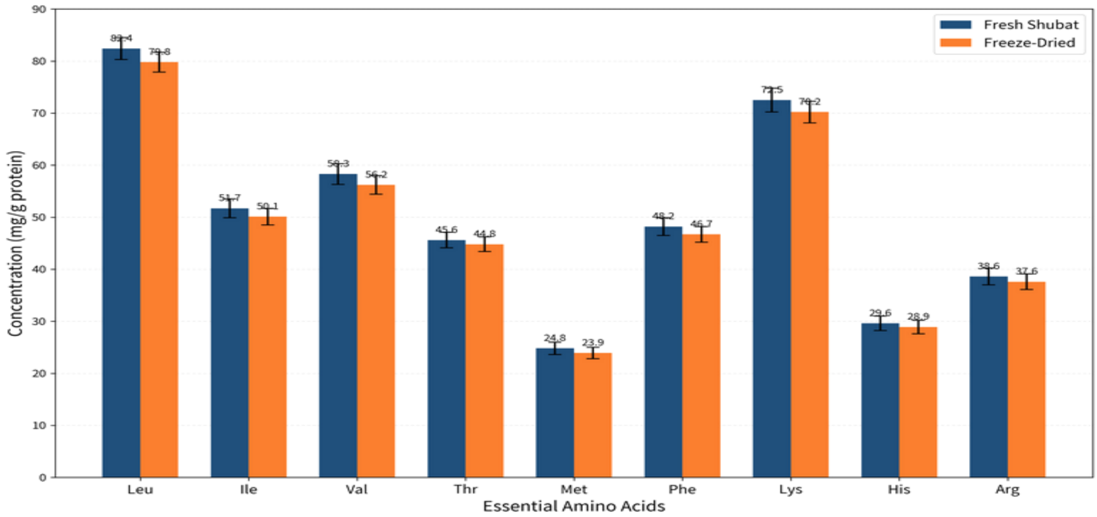

Fig. 1. Comparison of essential amino acid profiles between fresh shubat and freeze-dried powder.

Table 4. Micromineral concentrations (iron, zinc, copper, and manganese) in fresh shubat and freeze-dried powder.

Mineral	Fresh shubat (μg g^{-1} dry weight)	Freeze-Dried Powder (μg g^{-1} dry weight)	Change (%)	*p*-value
Iron (Fe)	18.6 ± 0.7	17.8 ± 0.6	-4.3	0.04*
Zinc (Zn)	12.4 ± 0.5	12.0 ± 0.5	-3.2	0.09
Copper (Cu)	2.8 ± 0.1	2.7 ± 0.1	-3.6	0.07
Manganese (Mn)	1.9 ± 0.1	1.8 ± 0.1	-5.3	0.03*

Table 4 indicates that among the trace elements, iron and manganese experienced the greatest reductions, at -4.3% and -5.3%, respectively, and these changes were statistically significant ($p = 0.04$ and $p = 0.03$). Zinc and copper showed smaller, non-significant losses.

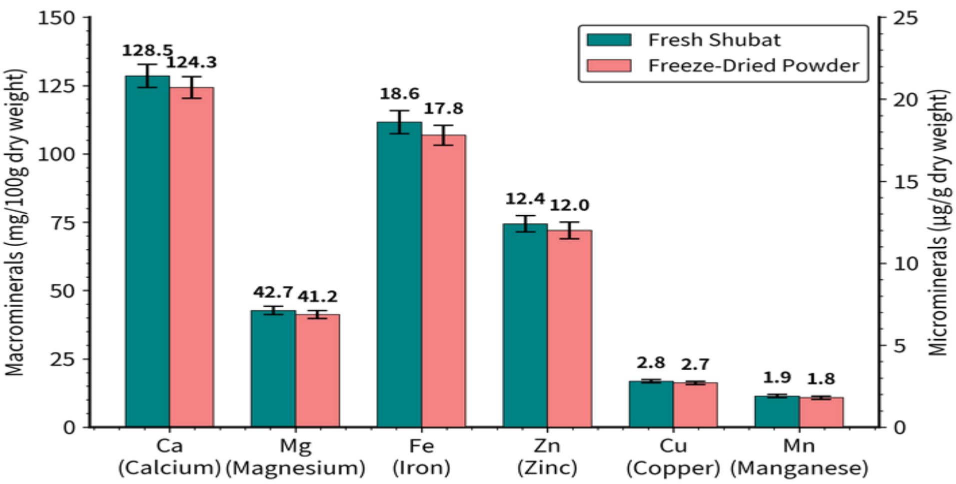

Fig. 2. Comparison of mineral concentrations between fresh shubat and freeze-dried powder.

Fig. 2 shows the high stability of all minerals during freeze-drying. Calcium was by far the most abundant mineral in both samples. Although minor reductions were observed across all elements, only those for iron and manganese reached statistical significance. Fig. 3 provides a comprehensive visual representation of the complete amino acid profiles. The near-perfect overlap between the two radar plots confirms that freeze-drying did not alter the overall amino acid pattern. Glutamic acid and proline were the most abundant, while cysteine and methionine were the least abundant in both samples. Table 5 summarizes the key quality parameters of the final freeze-dried product. The powder achieved a low moisture content (3.3%) and water activity (0.25), both favorable for extended shelf life at ambient temperature. Slight increases in lightness (L*)* and yellowness (b) alongside a small decrease in redness (a*) indicated acceptable color changes.

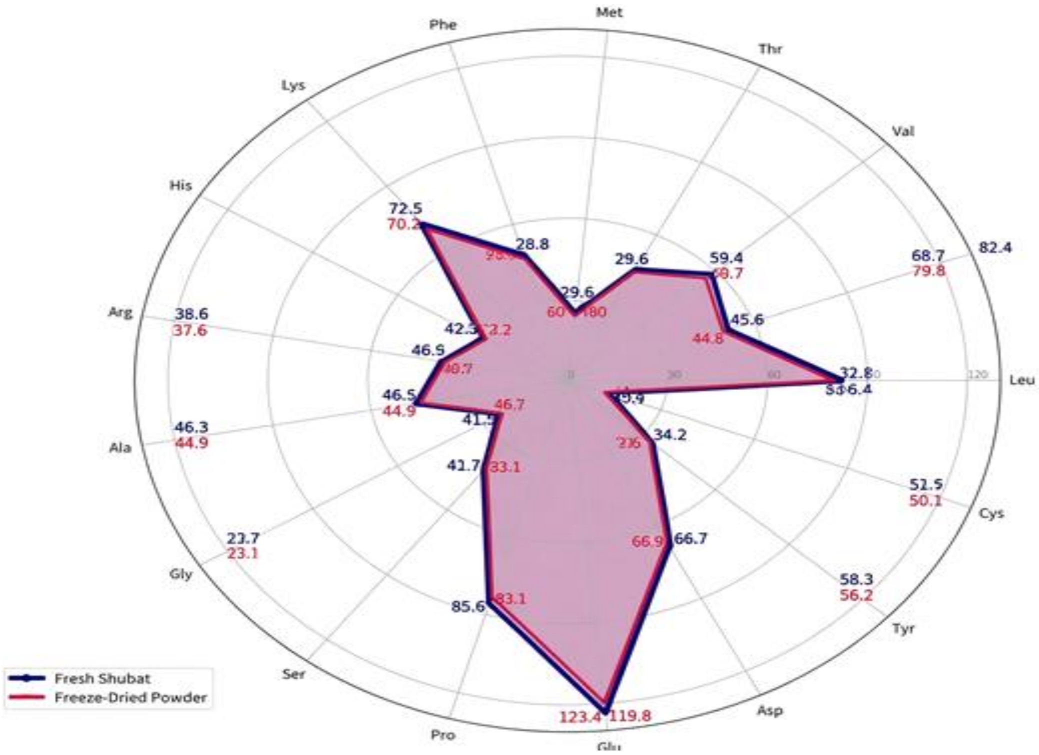

Fig. 3. Radar chart comparing complete amino acid profiles of fresh shubat and freeze-dried powder.

Table 5. Physicochemical quality indices of freeze-dried shubat powder.

Parameter	Fresh shubat	Freeze-dried powder
Moisture content (%)	88.2 ± 1.5	3.3 ± 0.2
Water activity (aw)	0.98 ± 0.01	0.25 ± 0.02
Dry matter recovery (%)	-	86.2 ± 2.5
Color L* (lightness)	78.4 ± 1.2	82.6 ± 1.4
Color a* (redness)	2.3 ± 0.3	1.8 ± 0.2
Color b* (yellowness)	12.5 ± 0.8	14.2 ± 0.9

The dry matter recovery of 86.2% confirmed the overall efficiency of the lyophilization process. Fig. 4 shows associations between amino acids and minerals in the freeze-dried powder. Strong positive correlations were observed between branched-chain amino acids (leucine, isoleucine, and valine) and calcium (r = 0.75–0.82), whereas cysteine showed a negative correlation with iron (r = -0.42) and methionine with zinc (r = -0.38). These patterns may inform the design of targeted nutritional products.

DISCUSSION

Clearly, the results of the present study confirm that regardless of the considerable transformation caused by the freeze drying method in the product, there is no noticeable effect of this technique on the amino acid and mineral profile of the schobat. Though a reduction in the concentration of essential amino acids in the obtained powder was observed in all cases, the amount of reduction never exceeded 3.6%, which was not statistically significant (p < 0.05). This surprising resistance of schobat to the adverse effects of freezing and drying is quite similar to other studies carried out in the field of freeze-dried camels' milk and fermented dairy products. It could be attributed to the low temperatures used in freeze drying that help prevent the degradation of amino acids by both thermal and oxidative degradation. The only amino acid affected by the freezing process was cysteine with a decrease of -5.5% (p = 0.04). The same situation was observed concerning the content of mineral components, where the content of such important minerals as calcium and magnesium remained at a very high level with a recovery above 96%, and the reduction in the amount of these components was not statistically significant.

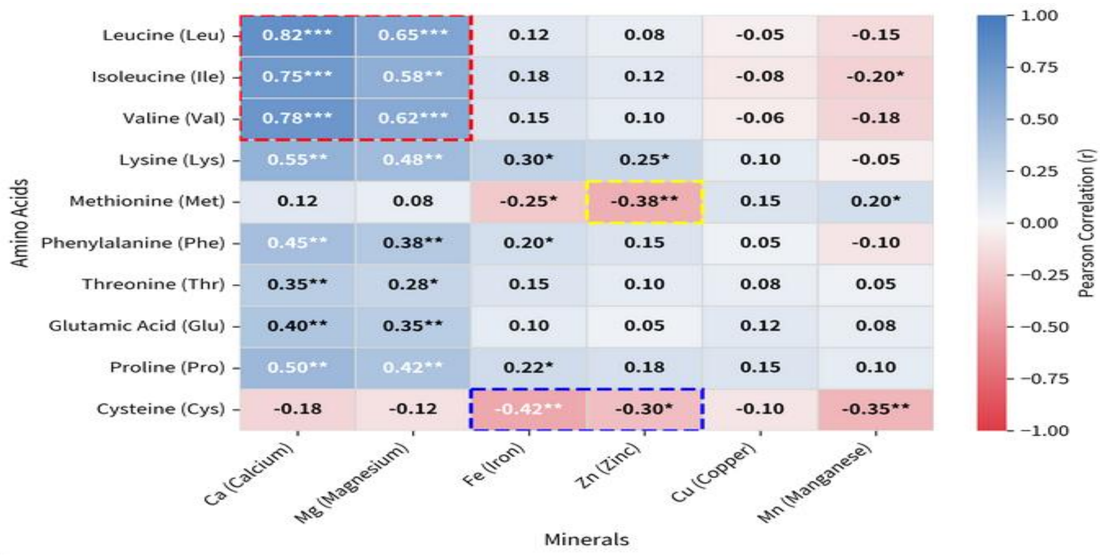

Fig. 4. Heatmap of correlations between amino acids and minerals in freeze-dried shubat powder.

Thus, the obtained results confirm high nutritional value of shobat powder since these components play an essential role in bone formation and development of muscles. At the same time, iron and manganese showed a statistically significant reduction in their content by 4.3% and 5.3% respectively due to the reaction with organic compounds forming insoluble compounds when subjected to the effects of freezing and sublimation. Nevertheless, despite all reductions, the final composition of these components still remains within the recommended limits for functional dairy products. The comparison of the outcomes of this research with the existing studies about camel milk and its fermented products suggests that the amino acid profile of shubat and especially high amounts of glutamic acid and proline can be explained in accordance with the profiles of other dairy products and the fermentation process doesn't lead to the alteration of the profile. As for the correlation map of amino acids and mineral elements (Fig. 4), it is also worth mentioning that interesting correlations were found, for example, between branched-chain amino acids and calcium (r = 0.82 - 0.75). The reason behind this correlation may be connected with the development of protein-calcium complexes, which impact not only the bioavailability of calcium but can also be useful while producing nutrition supplements aimed at some certain populations. Nevertheless, this research had several limitations; one of them is that only one variety of shubat was researched and no seasonal changes, breed difference, or even production methods of fermented product were considered. In summary, findings from this investigation show that the application of freeze-drying technique is an efficient way of transforming fresh shubat into a stable dried powder form with considerable amounts of nutrients. Due to the increasing demand for functional milk products in Kazakhstan and other countries adjacent to it, shubat powder could be considered as a valuable product with export opportunities. However, for that to be possible, further research needs to be done to fine-tune processing parameters, examine effects of storage, and evaluate its organoleptic qualities.

CONCLUSION

The present study was conducted to systematically analyze the composition of amino acids and mineral elements in freeze-dried shubat powder and demonstrated that freeze-drying is one of the effective methods for maintaining the nutritional qualities of this beneficial product. Although there has been some decline in the level of several amino acids (such as cysteine) and trace minerals (like iron and manganese), nevertheless, there is still good retention of the pattern of amino acids and macroelements (calcium and magnesium) in the resulting product and no considerable statistical differences can be noted. Conversely, it seems that the powder has a favorable quality index such as moisture content (3.3%) and water activity (0.25). The aim is that the accomplishments of this research will constitute a productive move in the direction of the industrial production of shubat in Kazakhstan and the substitution of conventional methods with modern ones. Taking into account the very high stability of all nutritive elements and good physical and chemical quality of the obtained powder, this food product may be used as a natural nutritional supplement for various categories of people, such as children, older adults, and sportsmen.

Nonetheless, in order to make this aim come true, further research on sensory analysis, as well as on the actual duration of storage, seems to be important.

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Bibliographic information of this paper for citing:

Utegaliyeva, R, Kudaibergenova, GN, Uazhanova, R, Anuarova, LE, Medeuova, GD, Kopzhassar, MM, Sharipov, G, Sharipova, S 2026, Study of amino acid and mineral composition of powder from the coat obtained by the method of liophilic drying. Caspian Journal of Environmental Sciences, 24: 797-804.