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Research Article/Araştırma Makalesi

Functional koumiss from donkey's milk

Eşek sütünden yapılan fermente kımız

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ABSTRACT

The study aimed to expand the range of dairy products by developing a recipe and technology for a fermented milk drink made from donkey milk with unique properties. Additional functionality is provided by the introduced probiotic cultures and phytogenic raw materials. Two functional drinks were proposed as experimental products: Koumiss-1 - koumiss with added probiotics and peony tincture, and Koumiss-2 - probiotic koumiss with added dried burdock roots. The initial raw material, donkey milk, was collected in the farm of the Ala Too State Farm located at an altitude of 800 m above sea level, in the Chui Valley of the Kyrgyz Republic. The standard methods of physicochemical, microbiological and organoleptic analyses were used for the study. The fermentation intensity (Is) and fermentation coefficient (δ) were determined for each case based on the data on the change in active and titratable acidity during the fermentation of milk with koumiss ferment. In the sample of koumiss-1 with the addition of peony tincture δ appeared in the first 3 hours, and δ in koumiss-2 was closer to 6 hours. Based on organoleptic evaluations, the developed drinks exhibit a pleasant sour-milk aroma and taste. The consistency is liquid with a slightly foamy texture, while the drink with added burdock roots has a pleasant creamy flavor. Lactic acid bacteria and yeast are similar to the specifications found in natural koumiss made from mare's milk. Throughout the experiment, sour-milk beverages were successfully produced, and they can potentially to fill the market niche for functional products with medicinal properties.

Key Words: Donkey milk, probiotics, koumiss, functional foods

ÖZ

Bu çalışma, benzersiz özelliklere sahip eşek sütünden yapılan fermente bir süt içeceği üretimi için teknoloji geliştirerek süt ürünleri yelpazesini genişletmeyi amaçlamaktadır. Ek işlevsellik, eklenen probiyotik kültürler ve fitojenik hammaddeler tarafından sağlanmaktadır. Deneme ürünleri olarak iki farklı fonksiyonel içecek önerilmiştir: Koumiss-1 - probiyotik ve şakayık tentürü eklenmiş koumiss ve Koumiss-2 - probiyotik koumiss içine eklenmiş kurutulmuş dulavratotu kökü. İlk hammadde olan eşek sütü, Kırgız Cumhuriyeti'nin Çüy Vadisi'nde, deniz seviyesinden 800 metre yükseklikte bulunan Ala Too çiftliğinde toplanmıştır. Çalışma boyunca standart fizikokimyasal, mikrobiyolojik ve organoleptik analiz yöntemleri kullanılmıştır. Fermantasyon yoğunluğu (Is) ve fermantasyon katsayısı (δ), sütün kımız mayası ile fermantasyonu sırasındaki aktif ve titre asitlik değişikliklerine dayalı olarak her bir örnek için belirlenmiştir. Şakayık tentürü eklenmiş kımız-1 örneğinde δ ilk 3 saat içinde gözlenirken, kımız-2'de δ yaklaşık olarak 6 saat sürmüştür. Organoleptik değerlendirmelere göre, fermente edilen içecekler hoş bir ekşi süt aroması ve tadı sergilemektedir. Kıvamı hafif köpüklü bir dokuya sahip olan dulavratotu kökü eklenmiş içecek ise hoş bir kremsi tada sahiptir. Laktik asit bakterileri ve maya varlığı, doğal kımızdan üretilen kımızın özelliklerine benzemektedir. Deney süresince, ekşi süt içecekleri başarılı bir şekilde üretilmiştir ve tıbbi özelliklere sahip fonksiyonel ürünler için pazar boşluğunu doldurma potansiyeline sahiptir.

Anahtar Kelimeler: Eşek sütü, probiyotikler, kımız, fonksiyonel gıdalar

Introduction

Functional products, known as foodstuffs, are being actively introduced into the global food markets and are widely used in classical and traditional medicine. These products include ingredients of plant origin, prebiotics, probiotics, vitamins, etc., which possess therapeutic, health-improving, preventive, and functional properties. Dairy products are a technologically advanced group for developing new functional products and can be used purposefully as preventive and therapeutic products. Popular dairy products with antimicrobial, anti-tuberculosis and immunomodulatory properties are koumiss or airag (Mongolian), kymyz (Kyrgyz), qımız (Azerbaijani), kımız (Turkish), qimiz (Uzbek), and gymyz (Turkmen). The therapeutic properties of koumiss were mentioned in the writings of Abu Ali ibn Sina, who cured Vizier Suhailia (Kudayarova *et al., 2010).* Lactic acid streptococci produce an antibiotic substance called nisin, which has bactericidal and bacteriostatic properties against tubercle bacilli (Explanatory Dictionary, 2018; Afzaal *et al*., 2021). In addition to treating the respiratory system, koumiss has beneficial effects on the digestive system, improves metabolism, increases the body's immunity, modulates gut microbiota and is known to have antioxidant activity against coronary heart disease (Giosue *et al.,* 2008; Nikkhah, 2011; Gilmutdinova *et al.,* 2018; Hou *et al*., 2019). Traditionally, koumiss is made from mare's milk, but other variations include using cow's milk, a blend of camel's milk and cheese serum, and nontraditional milk types with anti-inflammatory, immunomodulatory, antitumor, and hypoallergenic properties. The utilization of non-traditional types of milk for producing dairy products with anti-inflammatory, immunomodulatory, antitumor, and hypoallergenic properties is a current topic of interest. According to many scientists, donkey milk possesses all of these properties. Both donkey and mare milk are classified as albumin milk, which is low in fat and cholesterol but high in PUFAs (polyunsaturated fatty acids) compared to cow's milk. It is known that these acids are not synthesized in the body and must be obtained through dietary sources (Lobanov, 2003). The percentage ratio of saturated and unsaturated fatty acids in donkey milk is 40% to 60%, respectively, and this ratio has high health benefits for humans (WHO, 2020). The protein composition of donkey milk differs significantly from cow's milk and is the closest to that of mare's and breast milk. Donkey milk is characterized by a high content of lysozyme, lactoferrin, and immunoglobulin which have an inhibitory effect on the development of pathogenic bacteria (Angela, 2011; Salimei and Fantuz, 2012). Recent studies have shown that α-lactalbumin possesses antiviral and antitumor properties. Additionally, it forms a complex with oleic acid called GAMLET, which has been inhibited in tumorigenesis (Mao *et al.,* 2009; Fantuz *et al*., 2001). The high lysozyme content of 21% in donkey milk indicates its antibacterial properties, while in mare's milk, the lysozyme content is 10,5%, and trace amounts have been found in cow's milk. Lysozyme interacts with a group of cytokines, helping to reduce proliferation and destroy tumors through the activation of lymphocytes and blood macrophages (Shidlovskaya, 2010).

Considering the unique properties of donkey milk, it is advisable to use it as a primary ingredient for creating a koumiss-like drink. Masses of cultured strains of lactobacilli and bifidobacteria were used as probiotic material, which can contribute to the activity of beneficial intestinal microflora (Kwoji *et al*., 2021).

Dried burdock root and peony root tincture were considered and studied as herbal supplements. Burdock root, also known as "lupan" in Asian cuisine, is popular due to its sweet taste and is used in preparing bread, soups, and casseroles. In scientific medicine, burdock roots of the first and second year of life are used, while in folk medicine, all plant parts are used (Masalova, 2018). In folk medicine, burdock roots, leaves, and petioles are used as diuretic, diaphoretic, antipyretic, antimicrobial, and antineoplastic agents. The antioxidant activity of burdock root is attributed to caffeoylquinic acid derivatives (Kolomiets, 2021; Derusheva, 2021). The root contains inulin (approximately 45%), polyacetylenes, arctic acids, volatile acids (acetic, propionic, oily, isovaleric, 3-hexene, 3-octene, etc.), fatty acids (such as lauric, myristic, stearic, palmitic, etc.), tannin, polyphenolic acids (such as caffeic and chlorogenic acids), rutin, quercetin, quercetin rhamnoside, coumarin, umbelliferon, scopoletin, esculin, esculetin, valine, leucine, tryptophan, threonine, lysine, histidine, proline, cysteine, glutamine, serine, ornithine, aspartic acid, and others (WHO, 2020; Khan, 2010; Belmer and Gasilina, 2010; Dos Santos, 2008). In England, the popular beverage Dandelion & Burdock is considered a unique product, combining burdock root and dandelion to impart a unique taste and aroma. Its special production technology and naturalness make it beneficial for health.

Peony (European peony, common peony) has been cultivated in Europe for many years. The root has been used in medicine for more than 2000 years, mainly to treat epilepsy and improve the menstrual cycle. It has been experimentally proven to have hypotensive, abortifacient, and antiulcer effects. The roots of this plant have significant therapeutic value in Unani and homeopathy. The roots contain asparagine, benzoic acid, flavonoids, paeoniflorin, peonin, peonol, protoanemonin, tannic acid, triterpenoids, and essential oil (Khan, 2010; Ahmad, 2012).

The study aims to develop a functional probiotic dairy product based on donkey milk with the introduction of phytogenic additives that possess immunomodulatory and antioxidant properties.

Material and Methods

Fresh donkey milk was collected from a private entrepreneur residing in the Ala-Too state farm in the Chui region, Kyrgyz Republic. Milk samples were taken from two donkeys, which were mixed to obtain a representative sample. After milking, the milk was filtered through a sieve and poured into sterile bags for storage and freezing. The protein, lactose and fat content in donkey milk was 1.7g 100 g^{-1}, 6.2 g 100 g^{-1} and 1,2 g 100 g^{-1}, respectively.

Starter cultures*: Lactobacillus acidophilus, Bifidobacterium animals ssp. lactis, Streptococcus thermophiles “Lyofast SAB 439 A” (*BioChem, Italy*); Lactobacillus delbrueckii subsp. bulgaricus* “Lyofast SAB 438B” (BioChem, Italy) for yogurt production; *yeasts Saccharomyces cerevisiae* (Saf Moment); cut dried burdock roots, 1 cm in average size, for an herbal tea drink from the manufacturer LLC "Biolit" Russia, corresponding to ISO 22000, TU 10.83.15-191-20680882-2020, and a peony tincture from the manufacturer LLC "Hippocrates" Russia, Samara were used.

Preparing the functional drink involves heating the milk to a temperature of 75°C for 5 minutes. This temperature regime is necessary due to the sensitivity of donkey milk proteins. Additionally, the high lysozyme content in donkey milk contributes to a longer preservation of its bactericidal effect (Ljubisa et al., 2014). Organoleptic evaluations of the raw donkey milk indicated a sweet taste and smell, homogeneous consistency, white color with a bluish tint, pH of 7.2 for acidity, and a titratable acidity of 70 °T.

The technological process for producing the sour milk drink was conducted as follows:

For the production of Koumiss-1, Donkey milk was heated to 75 °C for 5 minutes and then cooled to 25 °C. Subsequently, starter culture was introduced according to the manufacturer's recommendations Lyofast SAB 439A and Lyofast SAB 438B - 1 UC for 100 L of milk, yeasts (5 g for 1250 L milk) and peony tincture (1%) were added. The mixture underwent fermentation at 25°C until reaching an acidity of 75 °Th. Throughout the fermentation process, the drink was aerated for 5 minutes every hour.

For the production of Koumiss-2, 2.8% of dried burdock roots were rinsed in cold running water and added to the tested milk. The mixture was then heated to 75 °C for 5 minutes, filtered, and cooled to 25 °C. Subsequently, starter culture was introduced according to the manufacturer's recommendations Lyofast SAB 439A and Lyofast SAB 438B - 1 UC for 100 l for milk, yeasts (5 g for 1250 l milk). Fermentation was carried out at 25 °C until reaching an acidity of 75 °Th. Throughout the fermentation process, the drink was aerated for 5 minutes every hour. Figure 1 and 2 display the titratable and active acidity of the investigated drinks.

Experimental studies and microbiological analysis were conducted in the Faculty of Food Engineering Laboratory, Kyrgyz-Turkish Manas University, Bishkek, Kyrgyzstan.

Standard, well-known methods of physico-chemical, microbiological and organoleptic analysis were applied for the research. The samples titratable acidity (TA) was measured every 60 min during fermentation with the titrimetric method according to AOAC method no. 947.05 (AOAC, 2005e), and pH values were measured using a digital pH meter (Model 220, Denver Instrument, USA) hourly.

Based on the data on the change pH and titratable acidity (TA) during the fermentation of milk with koumiss ferment, for each case, the values of the fermentation intensity (Is) calculated by Eq. 1 and the fermentation coefficient (δ) are calculated by Eq. 2 (Tepel, 1979) were determined, expressing the biochemical nature of the process of mixed lactic acid fermentation. The intensity of fermentation refers to the increase in lactic acid concentration, expressed in °Th, per time unit.

$$I_s = TA_{C2} - TA_{C1} \qquad (1)$$

where TA_{C1} – titratable acidity at the beginning of the observation (ºTh), and TA_{C2} – titratable acidity at the end of observation (ºTh).

The fermentation coefficient is defined as an increase in the concentration of lactic acid, expressed in ºThorner (Th), per unit time Δt. The fermentation coefficient is calculated by Eq. (2).

$$\delta = \frac{\Delta TA_{C2}}{\Delta TA_{C1}}, \qquad (2)$$

where ΔTA_{C2}, difference between the initial acidity and the acidity measured at the end of the observation period, °Th; ΔTA_{C1}, difference between the initial acidity and the acidity measured at the beginning of the observation period, Th. Based on the acidity data in the process of milk fermentation for samples 1 and 2, the values of fermentation intensity (I_s) and fermentation rate (δ) were determined, and the results are shown in Figure 3 and Figure 4, respectively.

Three female and one male participant took part in the organoleptic analysis of the products. The participants were aged between 40 - 50 years. The evaluation was carried out according to SS 22935- 2- 2011 (SS, 2011) organoleptic analysis of milk and milk products. Trained panelists evaluated sensory properties: color, texture, odor, taste, mouthfeel, and overall acceptance of the fermented donkey samples. Participants evaluated the samples using a five-point hedonic scale: liked very much (5), liked moderately (4), generally liked (3), neutral (2), disliked moderately (1), and disliked very much (0) (Smanalieva, Iskakova, & Fischer, 2021).

Results and Discussion

As a result of the action of the microflora present in the koumiss microflora lactose is partially digested into lactic acid, as evidenced by an increase in the acidity of the product during ripening. Lactic acid formed during fermentation plays a crucial role in the formation of a protein gel in milk. This gel has a significant effect on the viscosity of milk products and contributes to the quality of fermented milk products. During milk fermentation, pH reaches values for koumiss-1 of 4.51 and koumiss-2 of 4.61. The duration of donkey milk fermentation was 7 hours, at which the acidity reached a level for koumiss-1 of 65 °Th and for koumiss-2 of 75 °Th. For koumiss from mare's milk, these values correspond to koumiss of weak strength (SSK, 2019). The analysis results indicate noticeable differences in the titratable and active acidity between the Koumiss-1 and Koumiss-2 samples, which were determined simultaneously.

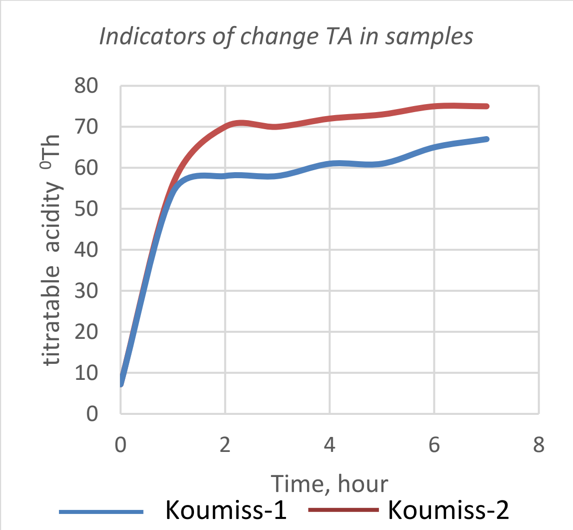

Figure 1*: Indicators of change in titratable acidity (TA) in the studied samples*

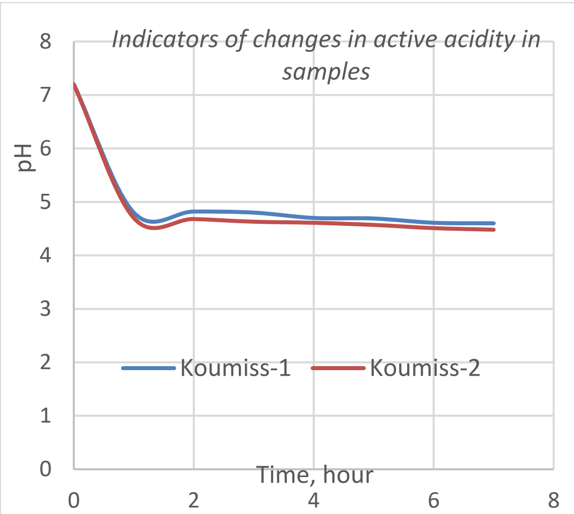

Figure 2: *Indicators of changes pH in the studied samples*

According to the findings depicted in Figure 1 and Figure 2, the titratable acidity in the Koumiss-2 sample is higher than that in the Koumiss-1 sample. This difference could be attributed to carbohydrates in burdock roots, which may create a favorable environment for the vigorous activity of microorganisms.

Following this, the drinks undergo further maturation at 4°C for 5 days to observe the changes in acidity levels over time. According to the results of titratable acidity, the koumiss 1 and koumiss 2 reached values of 89-94 °Th on the fifth day, respectively, indicating a degree of acidity characteristic of medium-strength koumiss (SSK, 2019).

Based on the fermentation intensity graph of both drinks, we can observe significant activity during the first hour of fermentation, indicating a favorable environment in the milk. Subsequently, fermentation progressed steadily with a moderate increase in beverage acidity.

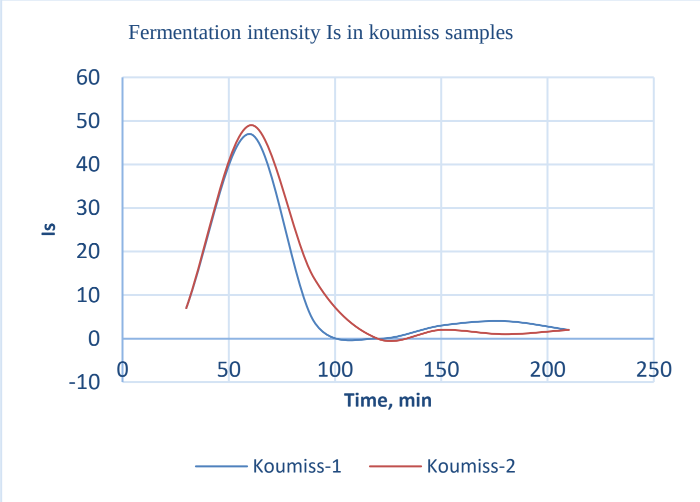

Figure 3: The dynamics of the intensity of the fermentation of the drink in the studied samples

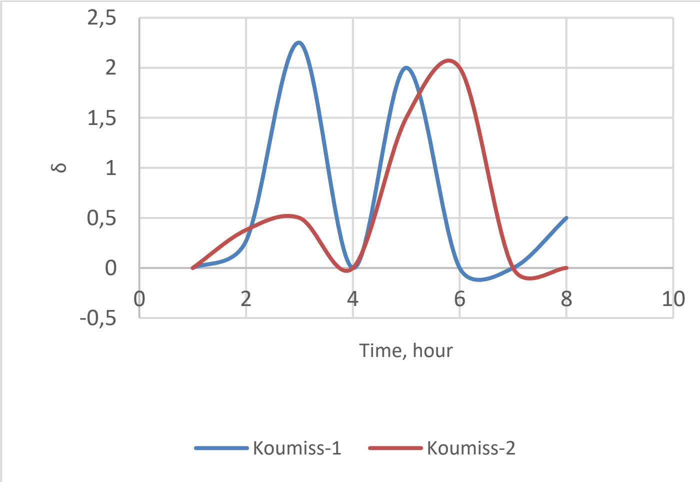

Figure 4: The dynamics of changes in the coefficient of ripening in the samples

The fermentation intensity (I_s), calculated according to Eq.1, better expresses the accumulation rate of lactic acid (Fig.3). The fermentation intensity curves indicate that the rate of lactic acid accumulation in donkey milk with propion and burdock root did not differ significantly. Already at 60 min in both samples there is an increased accumulation of lactic acid. At the same time, in koumiss-2, the accumulation of lactic acid was more significant. Perhaps this is due to the high content of carbohydrates in burdock, which favorably affects the growth of microorganisms.

As depicted in Fig 4, the maximum fermentation coefficients were observed at different time intervals in the tested samples. In the case of Koumiss-1, with the addition of peony tincture, the fermentation coefficient reached its peak within the first 3 hours. On the other hand, in Koumiss-2, the peak fermentation coefficient was observed closer to the 6-hour mark.

The sensory properties of fermented samples Koumiss 1 and Koumiss 2 were evaluated by reaching 75 ºTh at storage 4 °C. Organoleptic evaluation of experimental samples of milk product are represented in Figure 5.

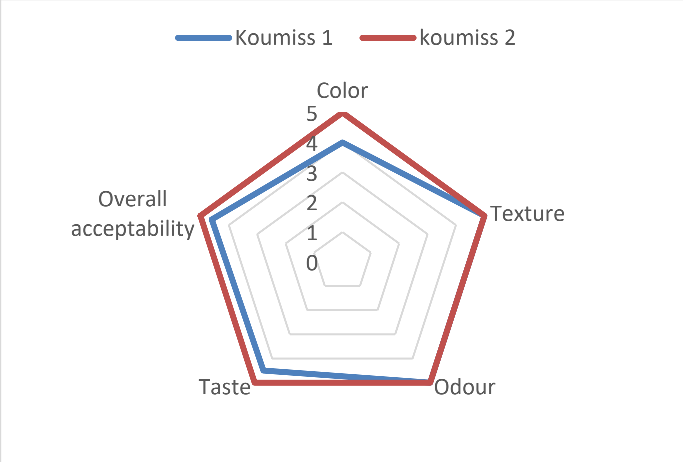

Figure 5: Sensory properties of fermented samples Koumiss 1 and Koumiss

The organoleptic evaluation of koumiss-1 reveals it an opaque liquid with a pure sour-milk taste, devoid of any extraneous flavors and odors. The consistency is liquid, homogeneous, slightly foamy, and free from flakes or clumped lumps of fat. The color is milky-white and uniform throughout the mass. The participants of the organoleptic analysis gave high marks and noted that the sour-milk product made from donkey milk with burdock roots had a pleasant creamy color, differed from the drink with peony tincture by a sweeter taste. It is an opaque liquid with a clear sour-milk taste, without extraneous taste and odor. The consistency is liquid, homogeneous, slightly frothy, without flakes and clumped lumps of fat.

Microbiological analysis of experimental donkey milk product samples results were shown in Table 1.

Table 1. Microbiological analysis of experimental donkey milk product samples

Significative	Koumiss-1		Koumiss-2	
	1 day	2 day	1 day	2 day
Total microbial count	$1.1x10^7$ CFU/cm^3	$2.8x10^7$ CFU/cm^3	$1.5x10^7$ CFU/cm^3	$3.9x10^7$ CFU/cm^3
Lactic acid bacteria	$1.0x10^7$ CFU/cm^3	$1.4x10^7$ CFU/cm^3	$1.2x10^7$ CFU/cm^3	$1.5x10^7$ CFU/cm^3
Yeast	$<1.0x10^6$ CFU/cm^3	$1.0x10^6$ CFU/cm^3	$1.0x10^7$ CFU/cm^3	$<1.0x10^6$ CFU/cm^3

It is known that the composition of donkey milk is closest to mare's milk. Therefore, the standards for mare's milk koumiss can be used as a basis for compliance. In accordance with SS 52974-2008 (SS, 2008), the number of lactic acid microorganisms in koumiss from mare's milk at the end of shelf life should be at least 1×10^7 CFU/cm^3, and yeast - at least 1×10^5 CFU/cm^3. Microbiological parameters of koumiss from donkey milk during the 48-hour maturation period indicate that the composition of donkey milk is favorable for microflora growth. The total number of microorganisms in koumiss-2 on the second day is significantly higher than in koumiss with peony tincture. When conducting microbiological analysis of the product under study, it was found that the number of microorganisms corresponds to the indicators of SSK 720:2019 (State Standard of the Kyrgyz Republic) technical conditions for koumiss natural from mare's milk (SSK, 2019). According to the standard, the recommended shelf life for koumiss made from mare's milk is not exceeding 5 days when stored at a temperature of 4°C. Given the absence of specific state standards for donkey milk koumiss, it is advisable to adhere to these established standards. This approach is recommended due to the close similarity in composition and properties between mare's milk and donkey milk, providing a reliable reference for ensuring the quality and safety of the product (SS, 2008).

Conclusion

Drawing from both theoretical insights and experimental findings, it becomes apparent that donkey milk boasts a remarkable composition of biologically active substances. This revelation undeniably prompts the consideration of its potential utilization in the creation of a functional koumiss-like beverage. By introducing bifidobacteria and lactobacilli, known for their antagonistic activity against intestinal diseases and their capacity to enhance the biological activity of the drink, and incorporating dried burdock root and peony tincture, we have successfully enriched the beverage. The active growth of bifidobacteria is known to benefit from the presence of prebiotic cultures, such as inulin, in products. Burdock roots, with an inulin content of 45%, inherently contribute to the functionality of the experimental drink. The organoleptic assessment of Koumiss-1 revealed it to be a cloudy liquid with a distinct sour-milk flavor, devoid of any unwanted tastes or aromas. The texture is fluid, uniform, with a slight frothiness and an absence of clumps or fatty deposits. The color is a consistent milky-white throughout the entirety of the beverage. Evaluators in the organoleptic analysis bestowed high ratings, emphasizing that the sour-milk product derived from donkey milk infused with burdock roots possesses an appealing creamy hue. Notably, they observed that this variant, when compared to the version containing peony tincture, exhibits a sweeter and milder taste. The liquid maintains its opacity, delivering a clear sour-milk taste without any unwanted sensory elements. The overall consistency remains liquid, uniform, and mildly frothy, with no presence of flakes or clustered fat particles.

Employing gentle temperature regimes during the processing of donkey milk has been instrumental in safeguarding its inherent biologically active substances. The results of thorough organoleptic evaluations have consistently reflected positive outcomes. Through our experimentation, we have positioned the resulting sour-milk beverages to occupy a unique niche among functional products endowed with therapeutic properties.

In conclusion, our findings strongly suggest that sour-milk beverages based on donkey milk hold substantial promise as functional products, offering therapeutic benefits. This exploration opens avenues for innovative and health-enhancing dairy products, show casing the potential of donkey milk in the realm of functional beverages.

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Conflict of Interest: The authors declare no conflict of interest.

Authors' Contribution: Nadira T. writing, editing, and submitting the manuscript; Zhyldyzai O. and Ruslan Adil A.T. data collection and analysis.

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