

UDC 664. 637.1

DOI: 10.15587/1729-4061.2024.312708

DEVELOPMENT OF KEFIR PRODUCT WITH BIFIDOBACTERIUM ANIMALIS SUBSP. LACTIS (BB-12) ACTIVATED BY SANGUISORBA OFFICINALIS L. EXTRACT

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This study addresses the challenge of enhancing the activation of *Bifidobacterium animalis* subsp. *lactis* (BB-12) to improve the quality and functionality of kefir-based products. The objective was to develop a kefir product enriched with *Bifidobacterium animalis* subsp. *lactis*, activated using an extract of *Sanguisorba officinalis* L., a natural antioxidant, to reduce activation time and boost probiotic efficacy.

Two kefir samples were prepared: a control and an experimental product enriched with *Bifidobacterium animalis* subsp. *lactis* activated by *Sanguisorba officinalis* L. at 10^{-5} g/cm³ concentration. The experimental sample exhibited a significant increase in *Bifidobacterium animalis* subsp. *lactis* count, reaching $9.52 \lg(\text{CFU}/\text{cm}^3)$, within 30 minutes at 37 ± 1 °C, outperforming the control. This rapid activation led to improved physicochemical properties, including enhanced flavor, consistency, and a slower increase in titratable acidity over 14 days, indicating prolonged shelf life. The amino acid profile was enriched, with higher levels of essential amino acids such as valine, leucine, methionine, as well as increased water-soluble vitamins including riboflavin, ascorbic acid, and pantothenic acid. The slower decline in *Bifidobacterium animalis* subsp. *lactis* population during storage further highlighted the probiotic stability of the enriched kefir.

These findings demonstrate that activating *Bifidobacterium animalis* subsp. *lactis* with *Sanguisorba officinalis* L. extract enhances the nutritional and functional qualities of kefir. This product holds potential for commercial application in the functional food industry, offering consumers a probiotic-rich, nutritionally superior fermented dairy option.

Keywords: fermented milk product, *Bifidobacterium animalis* subsp. *lactis* (BB-12), extract of *Sanguisorba officinalis* L.

Received date 09.07.2024

Accepted date 24.09.2024

Published date 30.10.2024

How to Cite: Utebaeva, A., Yevlash, V., Gabrilyants, E., Abish, Z., Aitbayeva, A. (2024). Development of kefir product with *Bifidobacterium animalis* subsp. *Lactis* (BB-12) activated by *Sanguisorba officinalis* L. extract. Eastern-European Journal of Enterprise Technologies, 5 (11 (131)), 6–15. <https://doi.org/10.15587/1729-4061.2024.312708>

1. Introduction

Kefir is a fermented milk product traditionally associated with numerous health benefits, attributed largely to its rich microbiota and bioactive components. The growing consumer demand for functional foods with enhanced health benefits highlights the need for innovations in kefir product development with probiotics. *Bifidobacterium animalis* subsp. *lactis* (BB-12) is a well-documented probiotic known for its health-promoting properties, including gastrointestinal health, immune modulation, and improved lactose digestion. However, the incorporation of such probiotics into dairy products like kefir demands an understanding of how they interact with the matrix and other bioactive components, influencing the physicochemical characteristics of the final product.

Another area gaining attention is the use of plant-based bioactive compounds in kefir. Particularly, extracts derived from medicinal plants like *Sanguisorba officinalis* L., known for their antioxidant, anti-inflammatory, and antimicrobial properties, and offer promising potential. Recent advancements, such as microwave extraction, have emerged as efficient techniques for preserving the bioactivity of these plant compounds. The use of plant extracts, such as *Sanguisorba officinalis* L., in the activation of probiotics like *B. animalis* subsp. *lactis* can stimulate enzyme systems and enhance the viability of the probiotics. This may result in a synergistic effect, potentially improving not only the health benefits of kefir but also its physicochemical and sensory properties.

The growing interest in natural functional foods enriched with probiotics underlines the relevance of research aimed at

optimizing the production of such products. Studying the interaction between probiotics and bioactive compounds contained in *Sanguisorba officinalis* L. is crucial to activate the enzyme systems of *B. animalis* subsp. *lactis* (BB-12), as cow's milk is not their natural habitat. These investigations are key to ensuring the viability of probiotics throughout a product's shelf life and to maintaining or enhancing its organoleptic properties, which are critical for consumer acceptance.

Furthermore, with advances in extraction techniques like microwave extraction, which preserves the bioactivity of plant compounds, there is significant potential to create kefir products that deliver enhanced health benefits without compromising quality. Therefore, research into the development of kefir products enriched with *B. animalis* subsp. *lactis*, activated by *Sanguisorba officinalis* L. extract is highly relevant. This scientific field holds practical importance in meeting the growing demand for innovative, functional dairy products that align with health trends while ensuring product stability and quality.

2. Literature review and problem statement

The development of functional foods has attracted considerable attention in recent years, with probiotics and bioactive plant extracts at the forefront of research. Kefir, a fermented dairy product, has been widely studied for its health benefits primarily due to its diverse microbial community [1]. The addition of certain probiotic strains, such as *B. animalis* subsp. *lactis* (BB-12), to kefir, has been shown to increase its probiotic potential [2]. *B. animalis* subsp. *lactis* (BB-12) is a well-documented strain known for its ability to survive in the gastrointestinal tract, modulate the immune system, and improve gut health [3]. However, the effects *B. animalis* subsp. *lactis* on the physicochemical characteristics of kefir, such as texture, pH, and viscosity, require further investigation, especially in combination with other functional ingredients such as plant extracts.

Sanguisorba officinalis L. is a medicinal plant known for its antioxidant, anti-inflammatory, and antibacterial properties [4], however its integration into functional foods requires further critical evaluation. Its bioactive compounds, including tannins, flavonoids and phenolic acids make it an attractive candidate for incorporation into functional foods. Many studies have shown the strong antioxidant effect of the phenolic compounds known to be present in *S. officinalis*, such as catechins, quercetin and kaempferol, which also exhibit neuroprotective properties [5]. But there were unresolved issues related to their behavior and bioavailability in fermented dairy products.

Microwave-assisted extraction (MAE) has emerged as a preferred method for obtaining plant extracts due to its efficiency in preserving the bioactivity of the compounds [6]. This technique has been shown to enhance the extraction yield and quality of phenolic compounds, flavonoids, and other bioactives from various plants. [7]. However, the application of *Sanguisorba officinalis* L. extract in dairy products, particularly in kefir, remains largely unexplored.

Several studies have explored the impact of adding probiotics and plant extracts, and prebiotics to dairy products, examining their effects on the physicochemical properties of these products. For instance, the paper [8] reported the enhancement of kefir's bioactive properties through the incorporation of various plant and agro-food waste extracts. The research highlights that the functional enrichment of

kefir with these extracts significantly boosts its antioxidant and antimicrobial properties. Specifically, extracts like grape pomace and olive leaves were identified as potent enhancers of kefir's bioactivity. The findings suggest that such functional enrichments could lead to the development of kefir products with improved health benefits, appealing to consumers looking for functional foods. However, while the research underscores the effectiveness of these extracts, further studies are needed to evaluate how the functional properties interact with probiotic strains in kefir and their long-term stability in fermented products.

The paper [9] presents the results of a study in which the quality characteristics of kefir fortified with olive leaf extract was evaluated. The study found that olive leaf extract significantly enhanced the antioxidant capacity of kefir, contributing to its health-promoting properties. Additionally, the fortified kefir exhibited improved microbial stability and extended shelf life. Sensory analysis indicated that the addition of olive leaf extract positively influenced the flavor, making it more appealing to consumers. This research supports the use of olive leaf extract as a functional ingredient in kefir, offering both nutritional and sensory benefits. However, while the sensory improvements are notable, particularly in flavor, further research is needed to investigate the impact of olive leaf bioactive compounds on the viability and activity of probiotic strains in kefir, as this aspect was not explored in the current study.

The paper [10] examined the effects of adding propolis extract to kefir on its physicochemical, microbiological, and sensory properties. The results showed that propolis extract enhanced the antioxidant activity and microbial stability of the kefir. Moreover, the addition of propolis influenced the sensory characteristics, imparting a distinctive flavor that was generally well-received in consumer evaluations. The study concludes that propolis extract can be a beneficial additive in kefir production, particularly for its health-promoting properties. However, higher concentrations of propolis extract were found to negatively impact the texture, making it less smooth. A way to overcome these difficulties can be further studies recommended to refine the concentration of propolis to maximize both functional and sensory properties without compromising texture.

The paper [11] investigated the impact of crude flaxseed extract on the growth of lactic acid bacteria (LAB) isolated from kefir. The research focused on the potential of flaxseed extract as a prebiotic enhancer for kefir fermentation. It was found that the addition of flaxseed extract significantly promoted the growth of LAB strains, particularly *Lactobacillus* and *Leuconostoc* species, which are crucial for the fermentation process in kefir. Flaxseed extract, rich in lignans and omega-3 fatty acids, not only enhanced microbial growth but also potentially increased the nutritional profile of the resulting kefir. While flaxseed extract boosts microbial activity and kefir's nutritional profile, further research is needed on its long-term effects on sensory attributes and probiotic viability.

The paper [12] explored the impact of *Clitoria ternatea* L. (butterfly pea) extract on the physicochemical, microbiological, and antioxidant properties of goat milk kefir. The study observed significant changes in pH, titratable acidity, and color, which are directly related to the inclusion of the butterfly pea extract. While these physicochemical changes are beneficial for product differentiation, further investigation is needed into how they influence sensory attributes and probi-

otic viability. Additionally, the effect of the extract in low concentration on kefir's microbial ecosystem was not explored.

The paper [13] investigated the anti-inflammatory effects of kefir fortified with Sangiovese cv pomace seeds extract, using an in vitro model of the intestinal epithelium (Caco-2 cells). The fortified kefir showed significant anti-inflammatory effects, reducing the expression of pro-inflammatory cytokines in Caco-2 cells. The pomace seed extract, rich in polyphenols, contributed to the antioxidant capacity of the kefir, which likely plays a role in its anti-inflammatory effects. The findings suggest that kefir fortified with grape pomace extracts could be developed as a functional food with potential benefits for gut health. While the research suggests the potential for gut health benefits, the direct application in human clinical trials remains unexplored. Moreover, the stability of these bioactives in kefir over time needs further analysis to assess their long-term functionality.

The paper [14] presents the results of a study in which Rosemary extracts significantly increased the antioxidant capacity of dairy products. However, the inclusion of these extracts altered the sensory profile, particularly in terms of taste and aroma, which could impact consumer acceptance. This finding underscores the need to carefully balance the health benefits of plant extracts with their impact on the physicochemical characteristics of dairy products.

Despite the growing body of research on kefir enriched with bifidobacteria and plant extracts, several gaps remain. First, while studies have shown the potential of bifidobacteria to enhance the health benefits of kefir, the impact of *Bifidobacterium animalis* subsp. *lactis* (BB-12) on the physicochemical properties of kefir, especially in combination with plant extracts, remains underexplored. Second, while the benefits of plant extracts like *Sanguisorba officinalis* L. are recognized, there is limited research on how these extracts interact with the kefir matrix and affect its sensory and physicochemical properties. Specifically, the potential synergistic effects between *Bifidobacterium animalis* subsp. *lactis* (BB-12) and plant extracts in kefir have not been thoroughly investigated. Literature on kefir production indicates that extracts are often added directly to enrich their beneficial properties. However, high concentrations of the above-mentioned plant-origin extracts can adversely affect the product's texture, flavor, and pH. Therefore, it is essential to investigate the technological, physicochemical, and biological parameters of kefir enriched with *B. animalis* subsp. *lactis* (BB-12) activated by low concentrations of *Sanguisorba officinalis* L. extract. This approach aims to activate the enzyme systems of *B. animalis* subsp. *lactis* (BB-12), potentially improving the product's quality, reducing the production process, and extending shelf life while maintaining the viability of beneficial microorganisms. Given these gaps, further research is required to explore the physicochemical characteristics of kefir products enriched with *Bifidobacterium animalis* subsp. *lactis* (BB-12) and activated by *Sanguisorba officinalis* L. microwave extract. This research will provide critical insights into the development of innovative functional dairy products that meet the growing demand for health-oriented food options while maintaining desirable sensory qualities.

3. The aim and objectives of the study

The aim of the study is to identify the qualitative parameters of a kefir product enriched with *Bifidobacterium*

animalis subsp. *lactis* (BB-12) activated by *Sanguisorba officinalis* L. extract. This will make it possible to evaluate the influence of bioactive compounds from the extract on the probiotic activation process, specifically regarding reduced activation time and enhanced probiotic efficacy. The expected outcome is a fermented dairy product that is rich in probiotics, nutritionally superior (with higher essential amino acid and vitamin content), and exhibits prolonged shelf life and consistent quality. These findings hold practical applications for the dairy industry, particularly in producing health-oriented fermented beverages with functional benefits.

To achieve this aim, the following tasks are solved:

- to develop the technology of kefir products and to study the activation process of bifidobacteria with *Sanguisorba officinalis* L. extract;
- to examine the organoleptic and physicochemical characteristics of kefir products;
- to analyze the nutritional value of kefir products.

4. Materials and methods

4. 1. Object and hypothesis of the study

The object of the study was *Sanguisorba officinalis* L. extract obtained using microwave extraction.

The main hypothesis of the study posits that the integration of *Bifidobacterium animalis* subsp. *lactis* (BB-12) activated by *Sanguisorba officinalis* L. extract into kefir products enhances their quality and nutritional value. Specifically, it is hypothesized that this integration results in increased levels of beneficial microorganisms, improved physicochemical parameters, and enhanced content of essential nutrients in the final product.

The work assumes that the activation of bifidobacteria using *Sanguisorba officinalis* L. extract will effectively optimize enzyme systems, leading to a shortened activation time. Additionally, it assumes that the incorporation of bifidobacteria activated by *Sanguisorba officinalis* L. extract into fermented milk products will result in increased microbial count, improved vitamins and amino acid content. Furthermore, it assumes that the utilization of plant-based extracts, such as *Sanguisorba officinalis* L. extract, as prebiotics will positively influence the growth parameters of probiotics in dairy products, thereby enhancing their functional properties.

In the work, certain simplifications have been adopted to facilitate the research process and analysis. These include focusing primarily on the integration of bifidobacteria activated by *Sanguisorba officinalis* L. extract into kefir products, without considering other microorganisms or ingredients. Additionally, the work simplifies the investigation by concentrating on the physicochemical parameters, amino acid, and vitamin composition of the developed kefir products.

4. 2. Method of obtaining *Sanguisorba officinalis* L. extract by microwave treatment method

20 g of *Sanguisorba officinalis* L. powder purchased from a pharmacy chain (Zerde-Fito Pharmaceutical Company LLP, Shymkent, Republic of Kazakhstan) was added to 200 ml of distilled water and heated in the defrosting mode in a microwave oven for 4 minutes. The flasks were then placed in a desiccator at 70 °C for 3 hours. To the aqueous extract obtained, 2 ml of 25 % hydrochloric acid solution was added and allowed to stand for 12 hours until a precipitate

formed. The precipitate was filtered through a Bunsen flask and a Buechner funnel, passed through a paper filter and a Capron filter. The resulting particles were dried in atmospheric air for 12–14 hours [15].

4.3. Preparation of kefir product enriched with *Bifidobacterium animalis* subsp. *lactis* (BB-12)

To the prepared pasteurized milk with a fat content of 2.5 %, at temperature of 23 ± 1 °C introduced fermented milk starter – kefir fungi in the amount of 2.5 % according to method [16] (1 g kefir grain was added to 40 ml milk, and incubated for 23 h at 25 °C; the kefir grains were separated by filtration, and the fermentate was used as a starter culture) and pre-activated *Bifidobacterium animalis* subsp. *lactis* (BB-12) DSM strain No. 15954) obtained from Sandoz Pharmaceuticals (Holzkirchen, Germany) in the amount of 1 %. Activation of the biomass of bifidobacteria with a titre of 10^6 CFU/ml is carried out at 37 °C for 30 min *Sanguisorba officinalis* L. extract solution with a concentration of 10^{-5} g/cm³. Milk fermentation is carried out until the acidity reaches 75–80 °T, then the product is cooled to a temperature of 6–10 °C for maturation.

4.4. Determination of aroma-forming activity of starter (presence of diacetyl and acetoin)

The method is based on the ability of diacetyl and acetoin, produced by aroma-producing microorganisms in milk, to give colored compounds. The starter was heated in a water bath to 90 °C and cooled to room temperature. It was then filtered through a paper filter. 3 drops of filtrate on a white porcelain cup were mixed with 3 drops of 40 % KOH solution. The color change of the mixture was observed, noting the time of appearance of pink coloring. In the presence of the right amount of aromatic substances (acetoin+diacetyl) in 10–15 min a pronounced pink colouring appears.

4.5. Determination of the number of microorganisms (bifidobacteria)

Bifidobacterium animalis subsp. *lactis* (BB-12) was cultivated in Trypticase-Phytone-Yeast medium (TPY) media (Condalab, Madrid, Spain) at 37 °C for 24–72 h under anaerobic conditions. The number of microorganism cells in 1.0 cm^3 of the sample was counted by multiplying the number of grown colonies by the corresponding dilution. The arithmetic mean of the results obtained in 2 parallel cultures was taken as the final result of the analysis [17].

4.6. Microscopic examination

Microscopic examination of the obtained samples was carried out using Levenhuk D400 LCD Digital Microscope (Levenhuk, Shanghai, China) with magnification 100x. The samples were pre-stained with methylene blue dye.

4.7. Determination of amino acids

The content of amino acids, including arginine, lysine, tyrosine, phenylalanine, histidine, leucine-isoleucine (total), methionine, valine, proline, serine, alanine, and glycine, was determined according to method M-04-38-2009 (GOST 55569-2013). The technique involves acid hydrolysis of the samples to convert the amino acids into their free forms and form FTC derivatives. These were then separated and quantified by capillary electrophoresis, with detection at 254 nm in the UV spectrum. Tryptophan was quantified directly at 219 nm without forming an FTC derivative. The

analysis was performed using the “Kapel-105M” capillary electrophoresis system. The data was processed on a PC Windows 2000/XP/7.

4.8. Determination of water-soluble vitamins content

Water-soluble B vitamins were quantitatively determined using capillary electrophoresis on a “Kapel 105M” device. The method is based on the migration and separation of the free forms of these vitamins under an electric field, recording their electrophoretic mobility at 200 nm. Vitamins B1, B2, B3, B5, B6, and Bc were analyzed by capillary zone electrophoresis. The plant samples were crushed and the vitamins were extracted with an aqueous sodium tetraborate solution containing sulphite ions. The extract was centrifuged at 5000–6000 rpm for 5 minutes and filtered through a membrane. Detection was based on 200 nm and 267 nm absorbance with programmable wavelength switching. Separation conditions included a borate buffer (pH=8.9), a capillary L_{eff}/L_{comm} of 65/75 cm length and 50 µm diameter, a voltage of +25 kV and a temperature of +30 °C.

4.9. Analysis of experimental data

Statistical data processing is carried out by using «Excel 7.0» (MS Office, USA), and «Statistica 6.0» (StatSoft, USA) software. Values are expressed as means±standard deviations from three independent experiments ($n=3$) at 90 % confidence level ($P=0.90$).

5. Results of evaluation of quality indicators of kefir products technology

5.1. Results of development of kefir technology and activation of bifidobacteria with *Sanguisorba officinalis* L. extract

The kefir product was developed using the classical technological scheme shown in Fig. 1.

Two products for comparison were prepared according to Fig. 1:

- 1) control sample – kefir product;
- 2) experimental sample – kefir product enriched with *B. animalis* subsp. *lactis* (BB-12), activated with *Sanguisorba officinalis* L. extract at a concentration of 10^{-5} g/cm³.

Before the experiment, tests for the presence of bacteriophage in the starter were carried out by traditional method [18], and their ability to produce aroma-forming substances was determined (Table 1).

Table 1

Determination of starter quality

Object	Test for the presence of bacteriophage	Aroma-forming activity (presence of diacetyl and acetoin)
Kefir fungus starter	–	+

Note: “–” – negative; “++” – strong staining, “+” – weak staining.

The kefir starter used in both the control and the experimental samples was free of bacteriophages, which is crucial for maintaining the integrity and consistency of the fermentation process. The starter demonstrated limited but positive aroma-forming activity.

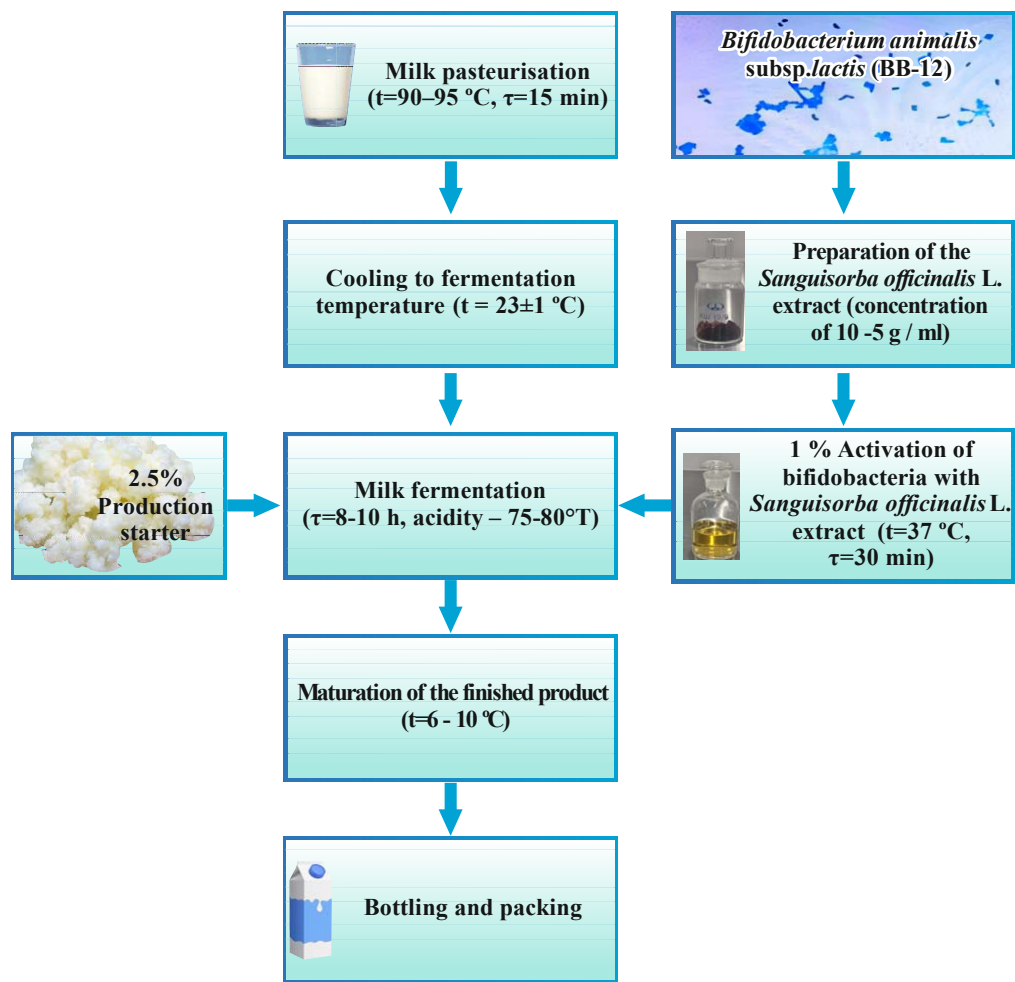


Fig. 1. Scheme of kefir product

At the first stage of the research, an activation of *B. animalis* subsp. *lactis* (BB-12) with *Sanguisorba officinalis* L. extract at a concentration of 10⁻⁵ g/cm³ was developed. Activation was carried out at two temperatures: 23±1 °C optimal for the growth of kefir fungi and 37±1 °C optimal for the growth of bifidobacteria (Table 2).

Table 2

Bifidobacteria activation conditions

Time	Activation temperature, 23±1 °C		Activation temperature, 37±1 °C	
	lg (CFU/cm ³)		lg (CFU/cm ³)	
	0 h	24 h	0 h	24 h
Control	6.10±0.24	7.20±0.18	6.10±0.18	7.48±0.55
30 min	6.10±0.22	7.45±0.22	6.10±0.18	9.52±0.35
1 h	6.10±0.18	7.55±0.33	6.10±0.20	9.18±0.40
3 h	6.10±0.18	7.44±0.40	6.10±0.22	8.74±0.44

According to the results, it was found that the optimal conditions for the activation of *B. animalis* subsp. *lactis* (BB-12) are 37±1 °C with an extract of *Sanguisorba officinalis* L. At this temperature, the bifidobacteria showed the most significant increase in CFU, especially after 30 minutes, indicating rapid and effective activation.

The higher temperature (37±1 °C) is more conducive to bifidobacteria growth compared to 23±1 °C, which aligns with the temperature preference of bifidobacteria over kefir fungi.

5. 2. Results of organoleptic and physicochemical evaluation of kefir products

Fig. 2 shows organoleptic characteristics of the finished kefir products.

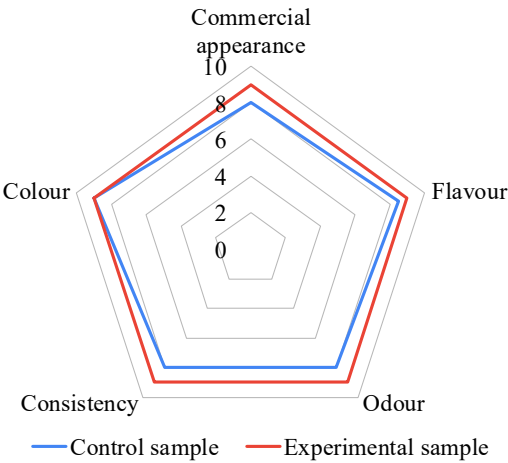


Fig. 2. Organoleptic characteristics of the finished kefir products

Organoleptic analysis of finished products showed that the experimental sample does not differ from the control in color. The experimental product enriched with *B. animalis*

subsp. *lactis* (BB-12), activated with *Sanguisorba officinalis* L. extract at a concentration of 10^{-5} g/cm³ has better flavor, consistency and commercial appearance.

All physico-chemical parameters of the obtained kefir products met the requirements of GOSTs 32923-2014 and 33491-2015 (Federal Agency on Technical Regulating and Metrology, 2014, 2015).

Table 3 shows that the increase in titratable acidity over 14 days was slower in the experimental sample, which was enriched with *B. animalis* subsp. *lactis* activated by *Sanguisorba officinalis* L. extract, compared to the control sample. On day 7, titratable acidity in the control sample increased by 10 % compared to the day 1 value, while in the experimental sample, it increased by 7.7 %. By day 14, titratable acidity in the control sample increased by 18.8 %, while in the experimental sample, it increased by 15.4 % compared to the day 1.

Physicochemical parameters of the finished products

Days	Products	Titratable acidity, °T	Number of <i>B. animalis</i> subsp. <i>lactis</i> , lg CFU/cm ³	Viscosity, sec	Water-holding capacity, %
1	Control sample	80.00±0.04	8.10±0.01	9.8	30
	Experimental sample	78.00±0.03	9.30±0.01	10.6	30
7	Control sample	88.00±0.08	8.20±0.02	11.4	30
	Experimental sample	84.00±0.08	10.00±0.01	12.2	30
14	Control sample	95.00±0.10	7.40±0.01	14.2	35
	Experimental sample	90.00±0.08	9.50±0.01	16.3	32

On the 7th day, the control sample showed a slight increase in the number of *B. animalis* subsp. *lactis*, with a rise of 1.2 % compared to the initial count on day 1. In contrast, the experimental sample exhibited a significant increase of 7.5 % in the number of *B. animalis* subsp. *lactis* compared to its count on day 1. When comparing the experimental sample to the control sample, the increase was 14.8 % on day

1 and 21.9 % on day 7. By the 14th day, a decrease in the population of *B. animalis* subsp. *lactis* was observed in both the control and experimental samples, with reductions of 9.8 % and 5 % respectively, compared to the 7-day counts.

The amino acid composition of the fermented kefir product is shown in Fig. 3.

The data analysis in Fig. 3 indicates significant differences between the control and experimental samples. These differences highlight the impact of the experimental conditions on the protein profile of the kefir. The kefir products contain such essential amino acids as valine, leucine+isoleucine, threonine, methionine, lysine, and phenylalanine, as well as substituted amino acids: arginine, tyrosine, histidine, proline, serine, alanine and glycine. The content of arginine in the experimental sample (0.342 ± 0.116 %) was slightly higher compared to the control sample (0.328 ± 0.130 %). The experimental sample showed a slight increase in tyrosine content (0.187 ± 0.056 %) compared to the control (0.163 ± 0.049 %). There was an increase in leucine and isoleucine content in the experimental sample (0.342 ± 0.089 %) compared to the control (0.292 ± 0.076 %). Methionine content was higher in the experimental sample (0.152 ± 0.052 %) than in the control (0.128 ± 0.044 %). Valine content showed a significant increase in the experimental sample (0.364 ± 0.140 %) compared to the control (0.224 ± 0.033 %). Proline content also increased in the experimental sample (0.515 ± 0.150 %) compared to the control (0.423 ± 0.103 %). Threonine levels were higher in the experimental sample (0.265 ± 0.105 %) than in the control (0.164 ± 0.064 %). Glycine content also increased in the experimental sample (0.089 ± 0.023 %) compared to the control (0.062 ± 0.020 %). Thus these amino acids are crucial for human health, for instance, muscle protein synthesis and metabolism, for collagen synthesis.

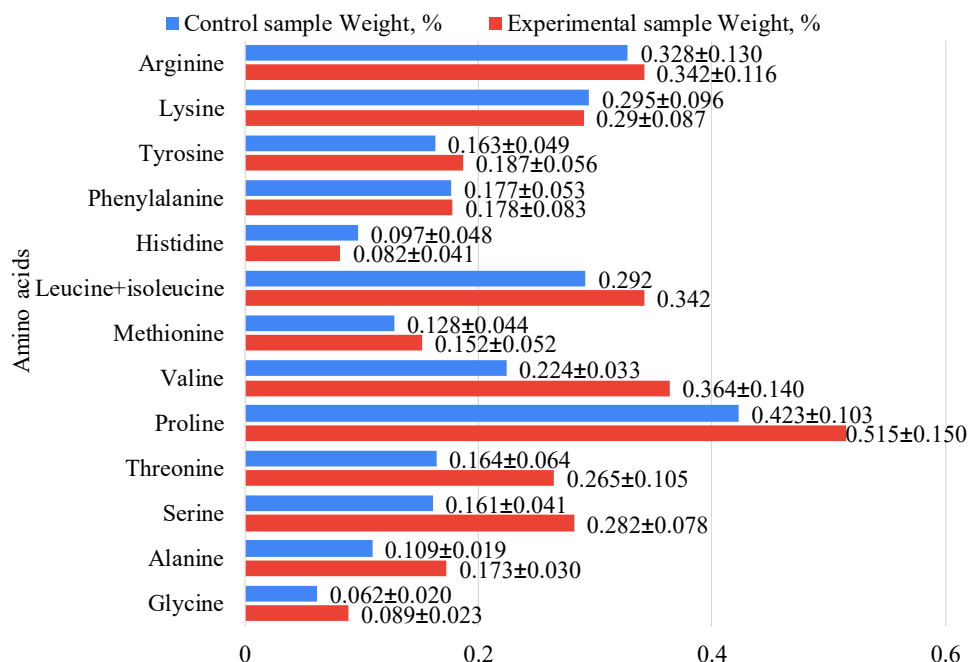


Fig. 3. Amino acid composition of the kefir product

The composition of water-soluble B vitamins of the control sample and the experimental sample of kefir product are presented in Fig. 4.

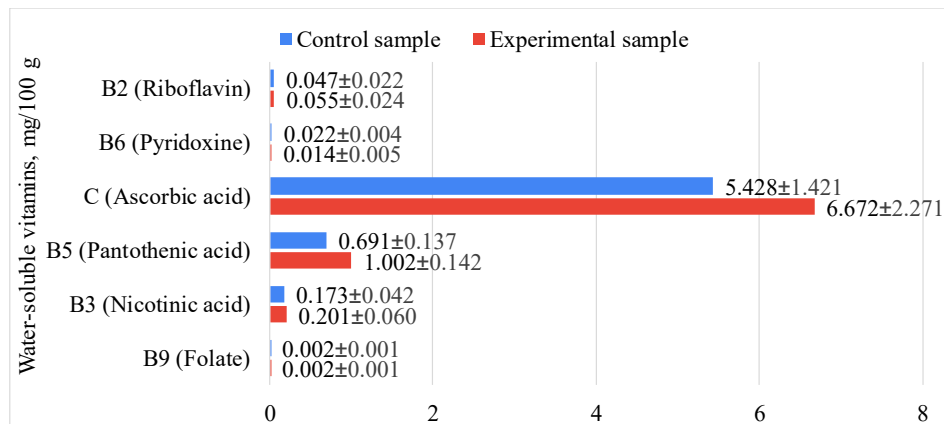


Fig. 4. Vitamin content of fermented kefir products

The analysis of the data in Fig. 4 shows that the experimental sample was further enriched with water-soluble vitamins, including riboflavin, ascorbic acid, pantothenic acid and nicotinic acid. The riboflavin content in the experimental sample (0.055 ± 0.024 mg/100 g) was slightly higher compared to the control sample (0.047 ± 0.022 mg/100 g). Riboflavin is essential for energy metabolism and cellular function. The ascorbic acid content was higher in the experimental sample (6.672 ± 2.271 mg/100 g) compared to the control sample (5.428 ± 1.421 mg/100 g). Vitamin C is an important antioxidant and immune booster. The pantothenic acid content showed a notable increase in the experimental sample (1.002 ± 0.142 mg/100 g) compared to the control (0.691 ± 0.137 mg/100 g). Pantothenic acid is vital for coenzyme A synthesis and energy metabolism. Nicotinic acid content in the experimental sample (0.201 ± 0.060 mg/100 g) was higher compared to the control sample (0.173 ± 0.042 mg/100 g). Vitamin B3 is essential for DNA repair and metabolic processes.

5.3. Results of the nutritional value evaluation of kefir products

The nutritional values of kefir products enriched with *B. animalis* subsp. *lactis* activated with *Sanguisorba officinalis* L. extract compared to the control are presented in Table 4.

Table 4

Nutritional value of the fermented kefir products. Values are means \pm SD at significant differences at $p < 0.05$, $n = 3$

Indicators	Control sample	Experimental sample
Protein, %	4.43±0.27	4.54±0.27
Fats, %	2.40±0.16	2.42±0.19
Carbohydrates, %	3.31±0.19	3.61±0.13*
Moisture, %	89.87±4.37	90.03±4.5
Ash, %	0.44±0.02	0.44±0.02
Energy value, kcal/100 g	52.17	53.93*

Note: Values marked with an asterisk (*) are significantly different ($p < 0.05$).

According to the result of the nutritional evaluation, it was found that the experimental kefir product enriched with *B. animalis* subsp. *lactis* and activated with *Sanguisorba officinalis* L. extract showed slight improvements in nutritional value, especially protein and carbohydrate content, without affecting the overall fat, moisture, and mineral content. There was also a slight increase in energy value.

6. Discussion of the study results of the kefir product development, enriched with *B. animalis* subsp. *lactis*, activated with *Sanguisorba officinalis* L. extract

The most important results of this study highlight the significant impact of enriching kefir with *Bifidobacterium animalis* subsp. *lactis* (BB-12) and activating it with *Sanguisorba officinalis* L. extract on the organoleptic, physicochemical, nutritional, and functional properties of the final product. According to the results given in Table 2 it was found that the optimal conditions for activation of bifidobacteria are incubation in *Sanguisorba officinalis* L. extracts at a concentration of 10^{-5} g/cm³ at 37 ± 1 °C for 30 min with subsequent addition to milk with starter, which helps reduce the negative effect of oxygen on their vital activity. As shown in study [19], the activation of bifidobacteria by the proposed method allows to reduce the lag-phase by 2 times, which will reduce the production cycle by 2–2.5 hours compared to their activation on milk medium, and will also contribute to the preservation of the titre of bifidobacteria at a high level.

The results presented in Table 3 suggests that the slower increase in acidity in the experimental kefir product enriched with *B. animalis* subsp. *lactis* (BB-12) and activated by *Sanguisorba officinalis* L. extract indicates better preservation and slower spoilage, potentially preserving more favorable sensory properties. These results are consistent with previous studies [20], which demonstrated that the addition of natural extracts can modulate the rate of acidification in fermented milk products. Similar trends were observed in other research [21], where the addition of plant extracts resulted in a slower increase in titratable acidity, which improved the shelf life and sensory quality of the fermented milk products.

Viscosity is an important parameter for assessing the consistency of fermented milk products. The data (Table 3) demonstrate that the viscosity of the experimental sample, enriched with *B. animalis* subsp. *lactis* and activated with *Sanguisorba officinalis* L. extract, was higher compared to the control sample. Specifically, the viscosity was 8.2 % higher on day 1 and 7 % higher on the 7th day of storage, and 14.8 % on the 14th day of storage in the experimental sample compared to the control sample. The higher viscosity of the enriched products indicates an improved texture and mouthfeel, which enhances the sensory experience. These results are consistent with previous studies [9, 10, 21], who found that the addition of natural extracts to kefir formulations significantly improved viscosity and texture.

The water-holding capacity (WHC) of the clot is an important characteristic for the evaluation of the synergistic properties of fermented milk products [22]. During the 7-day storage period (Table 3), both products showed stable clotting. On day 14, whey release increased by 9 % in the control sample compared to the experimental sample. This indicates a decrease in syneresis, suggesting that the experimental sample has a better water-holding capacity and therefore better structural properties. Thus, it maintained higher number of probiotics, lower acidity, increased viscosity and stable water holding capacity, making it a potentially more desirable product from both a nutritional and sensory perspective.

Our results (Table 3) indicate that the population of *B. animalis* subsp. *lactis* increased by 14.8 % on day 1 and 21.9 % on day 7 in the experimental sample compared to the control. By day 14, a decrease in *B. animalis* subsp. *lactis* numbers was observed in both control and experimental samples, with reductions of 9.8 % and 5 %, respectively, compared to the counts on day 7. This may be caused by nutrient depletion or by metabolic by-products accumulating.

Proteins, particularly essential amino acids, play a critical role in various physiological functions, including muscle protein synthesis, enzyme function, and immune responses. The observed increase in essential amino acids, such as arginine, methionine, leucine, valine, proline, threonine, serine, alanine, and glycine (Fig. 3) suggests that the experimental kefir could offer enhanced benefits for muscle recovery and overall protein metabolism [23]. Obtained results consistent with the research results where kefir had higher amounts of threonine, serine, alanine, lysine, etc. are important for anticarcinogenicity [24].

Moreover, the experimental sample enriched with water-soluble vitamins, such as riboflavin, ascorbic acid, pantothenic acid, and nicotinic acid, contributes to the product's overall nutritional profile (Fig. 4). These vitamins are crucial for energy metabolism, antioxidant defense, and cellular repair, making the experimental kefir a functional food with added health benefits. This finding parallels the growing interest in functional foods, where the focus is on enhancing the nutritional value of traditional food products to meet the specific health needs of consumers.

From a nutritional perspective, the slight increase in protein and carbohydrate content in the experimental sample is notable (Table 4). Although the difference was modest, including *B. animalis* subsp. *lactis* and *Sanguisorba officinalis* L. extract could potentially enhance the protein content of the kefir.

The fat content was almost identical in both samples, with only a negligible increase in the experimental sample. This indicates that the addition of *B. animalis* subsp. *lactis* and *Sanguisorba officinalis* L. extract does not significantly alter the fat composition of kefir. This result is consistent with research [25], in which enrichment with probiotics had no major effect on the fat content of fermented milk products.

The carbohydrate content in the experimental sample increased significantly. This could be due to the metabolic activity of *B. animalis* subsp. *lactis*, which may consume lactose (a primary carbohydrate in milk) and produce other metabolites that contribute to the total carbohydrate content. In addition, *Sanguisorba officinalis* L. extract contains certain sugars [5, 6] that contribute to the total carbohydrate content of kefir. The slight increase in energy value also suggests a potentially more nutritious product, which could appeal to consumers seeking higher nutritional value in fermented dairy products.

The proposed technology will allow to obtain a functional kefir product enriched with *B. animalis* subsp. *lactis*, activated

with *Sanguisorba officinalis* L. extract, has better organoleptic properties, enriched with essential amino acids and vitamins.

The limitations of this study include constraints linked to the concentration of *Sanguisorba officinalis* L. extract employed for the activation of *B. animalis* subsp. *lactis*. The incorporation of concentrations of *Sanguisorba officinalis* L. extract exceeding or falling below 10^{-5} g/cm³ detrimentally impacts both the growth kinetics of bacteria and the microbial population number during *B. animalis* subsp. *lactis* activation in the technology of kefir products.

Further research will be directed to the investigation of new sources of plant origin antioxidants and their effects on probiotic microorganisms further advancing the development of functional fermented milk products.

7. Conclusions

1. The study presents a novel approach to improve the quality and nutritional value of kefir products by activating *Bifidobacterium animalis* subsp. *lactis* (BB-12) with *Sanguisorba officinalis* L. extract. The research demonstrated that the optimal conditions for the activation of bifidobacteria were incubation at 37 ± 1 °C with *Sanguisorba officinalis* L. extract at a concentration of 10^{-5} g/cm³ for 30 minutes, leading to a significant increase in the probiotic count and improved fermentation kinetics.

2. The experimental kefir enriched with activated bifidobacteria showed better organoleptic properties than the control sample, including better flavor, consistency, and appearance. The slower increase in titratable acidity in the experimental sample suggests a longer shelf life and better preservation of sensory properties. Additionally, the experimental kefir showed a higher viscosity and better water-holding capacity, indicating better textural properties and a reduced syneresis. It was found that the number of *B. animalis* subsp. *lactis* increased by 14.8 % on day 1 and 21.9 % on day 7 in the experimental sample compared to the control sample. Also the experimental sample had a higher concentration of essential amino acids such as arginine, methionine, leucine and valine, which are crucial for muscle protein synthesis and other physiological functions. The enriched kefir with *Bifidobacterium animalis* subsp. *lactis* (BB-12) with *Sanguisorba officinalis* L. extract also contained higher levels of water-soluble vitamins, including riboflavin, ascorbic acid, pantothenic acid, and nicotinic acid, which improved its overall nutritional profile and functional properties.

3. Nutritional analysis revealed that the experimental kefir had a slightly higher protein and carbohydrate content. The established slight increase in energy value also suggests a potentially more nutritious product, which could appeal to consumers seeking higher nutritional value in fermented dairy products. Thus, this innovative technology offers a functional kefir product with improved organoleptic and nutritional properties, providing potential health benefits beyond traditional kefir.

Conflict of interest

The authors declare that they have no conflicts of interest in relation to the current research, including financial, personal, copyright or any other that could affect the research and the results presented in this article.

Financing	Use of artificial intelligence
The authors extend their appreciation to the Ministry of Science and Higher Education of the Republic of Kazakhstan for funding this work through the research project No: AP13068387 “Development of technology for the production of innovative functional bio-fermented milk products with probiotics and antioxidants”.	The authors confirm that they did not use artificial intelligence technologies when creating the current work.
Data availability	Acknowledgements
All data is available in the main text of the manuscript.	The authors extend their appreciation to the Ministry of Science and Higher Education of the Republic of Kazakhstan for funding this work through the research project No:AP13068387 “Development of technology for the production of innovative functional bio-fermented milk products with probiotics and antioxidants”.

References

1. Apalowo, O. E., Adegoye, G. A., Mbogori, T., Kandiah, J., Obuotor, T. M. (2024). Nutritional Characteristics, Health Impact, and Applications of Kefir. *Foods*, 13 (7), 1026. <https://doi.org/10.3390/foods13071026>
2. Moradi, S., Nouri, M. (2023). Production of functional kefir supplemented by *Portulaca oleracea* L. seed oil. *Journal of Food Measurement and Characterization*, 17 (5), 5000–5011. <https://doi.org/10.1007/s11694-023-01993-7>
3. Yerlikaya, O., Saygili, D., Akpinar, A. (2021). An application of selected enterococci using *Bifidobacterium animalis* subsp. *lactis* BB-12 in set-style probiotic yoghurt-like products. *Food Bioscience*, 41, 101096. <https://doi.org/10.1016/j.fbio.2021.101096>
4. Guo, D.-L., Chen, J.-F., Tan, L., Jin, M.-Y., Ju, F., Cao, Z.-X. et al. (2019). Terpene Glycosides from *Sanguisorba officinalis* and Their Anti-Inflammatory Effects. *Molecules*, 24 (16), 2906. <https://doi.org/10.3390/molecules24162906>
5. Heidari, B., Saeedi, M., Khanavi, M. (2024). Phytochemistry and Biological Properties of *Sanguisorba* spp.; an Updated Review. *Research Journal of Pharmacognosy*, 11 (3). <https://doi.org/10.22127/rjp.2024.441696.2351>
6. Nonglait, D. L., Gokhale, J. S. (2023). Review Insights on the Demand for Natural Pigments and Their Recovery by Emerging Microwave-Assisted Extraction (MAE). *Food and Bioprocess Technology*, 17 (7), 1681–1705. <https://doi.org/10.1007/s11947-023-03192-0>
7. Cassani, L., Silva, A., Carpena, M., Pellegrini, M. C., García-Pérez, P., Grosso, C. et al. (2024). Phytochemical compounds with promising biological activities from *Ascophyllum nodosum* extracts using microwave-assisted extraction. *Food Chemistry*, 438, 138037. <https://doi.org/10.1016/j.foodchem.2023.138037>
8. Aiello, F., Restuccia, D., Spizzirri, U. G., Carullo, G., Leporini, M., Loizzo, M. R. (2020). Improving Kefir Bioactive Properties by Functional Enrichment with Plant and Agro-Food Waste Extracts. *Fermentation*, 6 (3), 83. <https://doi.org/10.3390/fermentation6030083>
9. Okur, Ö. D. (2021). An evaluation of the quality characteristics of kefir fortified with olive (*Olea europaea*) leaf extract. *British Food Journal*, 124 (5), 1727–1736. <https://doi.org/10.1108/bfj-07-2021-0721>
10. Bengi, S., Gursay, O., Güler Dal, H. Ö., Yilmaz, Y. (2023). Effect of propolis extract addition on some physicochemical, microbiological, and sensory properties of kefir drinks. *Food Science & Nutrition*, 11 (11), 7407–7417. <https://doi.org/10.1002/fsn3.3671>
11. Kim, D.-H., Jeong, D., Oh, Y.-T., Song, K.-Y., Kim, H.-S., Chon, J.-W. et al. (2017). Stimulating the Growth of Kefir-isolated Lactic Acid Bacteria using Addition of Crude Flaxseed (*Linum usitatissimum* L.) Extract. *Journal of Milk Science and Biotechnology*, 35 (2), 93–97. <https://doi.org/10.22424/jmsb.2017.35.2.93>
12. Melia, S., Juliyarsi, I., Oktania, M., Putri Deavy, D. D. (2024). Physicochemical Characteristics, Microbiology, and Antioxidants of Goat Milk Kefir Supplemented with *Clitoria ternatea* L. Extract. *Current Nutrition & Food Science*, 20 (3), 369–373. <https://doi.org/10.2174/1573401319666230518153436>
13. Carullo, G., Governa, P., Spizzirri, U. G., Biagi, M., Sciubba, F., Giorgi, G. et al. (2020). Sangiovese cv Pomace Seeds Extract-Fortified Kefir Exerts Anti-Inflammatory Activity in an In Vitro Model of Intestinal Epithelium Using Caco-2 Cells. *Antioxidants*, 9 (1), 54. <https://doi.org/10.3390/antiox9010054>
14. Gad, A. S., Sayd, A. F. (2015). Antioxidant Properties of Rosemary and Its Potential Uses as Natural Antioxidant in Dairy Products – A Review. *Food and Nutrition Sciences*, 06 (01), 179–193. <https://doi.org/10.4236/fns.2015.61019>
15. Utebaeva, A., Gabrilyants, E., Abish, Z., Yevlash, V. (2024). Evaluation of quality characteristics of fermented acidophilic product with *B. Bifidum* and *Prunus padus* extract. *Eastern-European Journal of Enterprise Technologies*, 2 (11 (128)), 13–25. <https://doi.org/10.15587/1729-4061.2024.300373>
16. Tratnik, L., Božanić, R., Herceg, Z., Drgalić, I. (2006). The quality of plain and supplemented kefir from goat’s and cow’s milk. *International Journal of Dairy Technology*, 59 (1), 40–46. <https://doi.org/10.1111/j.1471-0307.2006.00236.x>
17. Nguyen, B. T., Bujna, E., Fekete, N., Tran, A. T. M., Rezessy-Szabo, J. M., Prasad, R., Nguyen, Q. D. (2019). Probiotic Beverage From Pineapple Juice Fermented With *Lactobacillus* and *Bifidobacterium* Strains. *Frontiers in Nutrition*, 6. <https://doi.org/10.3389/fnut.2019.00054>

18. Oluchi, M., Augusta, Agorye, U., Augustine, Ulekwu, O., Chinyere et al. (2022). Concordance of methylene blue reduction and microbiological techniques in the analysis of raw and processed milk quality: a comparative study. *Asian Journal of Microbiology and Biotechnology*, 7 (1), 28–36. <https://doi.org/10.56557/ajmab/2022/v7i17801>
19. Ervolder, T. M., Bolotov, V. D., Vainshtok, I. I. (2002). Pat. No. 2242133 C2 RF. Method of preparation of fermented milk product such as ryazhenka. No. 2002134996/13; declared: 25.12.2002; published: 20.12.2004.
20. Vanaki, E., Kamkar, A., Noori, N., Azizian, A., Mohammadkhan, F. (2023). The effect of aqueous extract of *Arctium lappa* root on the survival of *Lactobacillus acidophilus* La-5 and *Bifidobacterium bifidum* Bb-12 and sensorial and physicochemical properties of synbiotic yogurt. *Food Science & Nutrition*, 12 (3), 2182–2191. <https://doi.org/10.1002/fsn3.3919>
21. Utebaeva, A., Gabrilyants, E., Abish, Z. (2024). Developing a Symbiotic Fermented Milk Product with Microwave-Treated Hawthorn Extract. *Fermentation*, 10 (8), 377. <https://doi.org/10.3390/fermentation10080377>
22. Ziarno, M., Zaręba, D., Ścibisz, I., Kozłowska, M. (2023). Comprehensive studies on the stability of yogurt-type fermented soy beverages during refrigerated storage using dairy starter cultures. *Frontiers in Microbiology*, 14. <https://doi.org/10.3389/fmicb.2023.1230025>
23. Hou, Y., Wu, G. (2018). Nutritionally Essential Amino Acids. *Advances in Nutrition*, 9 (6), 849–851. <https://doi.org/10.1093/advances/nmy054>
24. Guzel-Seydim, Z. B., Seydim, A. C., Greene, A. K. (2003). Comparison of amino acid profiles of milk, yogurt and Turkish kefir. *Milchwissenschaft*, 58, 158–160.
25. Tavakoli, M., Habibi Najafi, M. B., Mohebbi, M. (2019). Effect of the milk fat content and starter culture selection on proteolysis and antioxidant activity of probiotic yogurt. *Heliyon*, 5 (2), e01204. <https://doi.org/10.1016/j.heliyon.2019.e01204>