Functional foods containing probiotics and/or prebiotics are of scientific and practical importance. The method of pre-activating bifidobacteria before their use in the production of fermented milk products has a profound impact on enhancing the quality of the resulting products. Finding ways to shorten the activation time of bifidobacteria in the production of functional foods with probiotics is an urgent task.

Shortening the activation time and optimizing enzyme systems of bifidobacteria with antioxidants are crucial for innovative probiotic fermented milk technology. The object of this research is the technology of fermented acidophilic products enriched with bifidobacteria activated using Prunus padus (bird cherry) extract, known for its antioxidant properties. The finished product showed a significant increase in bifidobacteria count, reaching 1×109 CFU, and a 25.7 % boost in L. acidophilus after 7 days.

Furthermore, the activation of bifidobacteria by Prunus padus extract resulted in a threefold increase in the histidine content and increased the content of oleic, eicosanoic, linoleic, arachidonic, and docosahexaenoic acids by 10.0 %, 26.4 %, 14.4 %, 22.6 %, and 66.6 % in the experimental sample compared to the control sample, respectively. Moreover, pentadecanoic, selacholeic, eicosatrienoic acids and tyrosine were present in the experimental but not in the control sample. Microbiological safety tests found no pathogenic microorganisms in the fermented acidophilic product, yet lactic acid microorganism levels exceeded norms in the experimental sample, confirming the product's probiotic properties and high physiological value. Thus, the developed product is characterized by better taste, a longer shelf life, and the preservation of bacterial titers

Keywords: lactobacillus acidophilus, bifidobacterium bifidum, prebiotics, bird cherry extract, dairy product

UDC 664. 637.1
DOI: 10.15587/1729-4061.2024.300373

EVALUATION OF QUALITY CHARACTERISTICS OF FERMENTED ACIDOPHILIC PRODUCT WITH B. BIFIDUM AND PRUNUS PADUS EXTRACT

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Received date 17.01.2024
Accepted date 25.03.2024
Published date 30.04.2024

How to Cite: *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*

1. Introduction

People of socially active stratum pay special attention to their own health. The first place is given to food products and beverages that have a positive effect on the human body. Production and strengthening of such foodstuffs consumption propaganda in our country is one of the tasks on the realization of the Concept of development of the Republic of Kazakhstan public health till 2026.

One of the directions in solving this problem is the enrichment of food products with ingredients from a range of functional ones. In this regard, probiotic microorganisms, bifidobacteria and lactic acid bacteria (LAB) are in demand in the food industry and the development of fermented milk functional products based on them, improving the adaptation of the organism to negative environmental factors, is relevant.

Unbalanced diet and daily stressful situations are the causes of the immune system weakening of the human body and chronic diseases. Fermented milk products with probiotics and antioxidants improve metabolic processes and normalize the gastrointestinal tract activity. Nowadays, the use of fermented milk products is very widespread. Regular uses of them allow you to normalize the intestinal tract, improve immunity, as well as the resistance of the body to pathogenic and opportunistic microorganisms. Of particular interest are extracts of plants and natural objects, which are a source of many biologically active substances.

The need to develop functional fermented milk products enriched with antioxidants is determined by the limited range of fermented milk products in Kazakhstan, with the addition of probiotics, in particular bifidobacteria. Thus, "Dairy Factory Natizhe" produces two types of fermented milk drinks with the addition of microorganism *L. acidophilus* "Acidophilous" and "Narine-L" for children.

In this regard, it becomes relevant to expand the range of dairy products, as well as to develop innovative bio-fermented milk products containing probiotics (bifidobacteria and lactobacilli) and prebiotics with antioxidant properties.

2. Literature review and problem statement

According to the definition proposed by the FAO and WHO expert committee, "probiotics are live microorganisms that, when consumed in adequate amounts, have a favorable effect on the health of the host organism" [1]. The known probiotics are bifidobacteria and LAB.

Bifidobacteria occupy a special place among the representatives of normal intestinal microflora. In the human large intestine, they are contained in the amount of 10^8–10^{11} CFU/g. In children, they occupy a dominant place and make up 99 % of the microflora. They have a number of useful properties. For example, anticarcinogenic effect, stimulation of the immune system, lowers blood cholesterol levels. They also contribute to the maintenance of homeostasis of the macroorganism [2, 3].

Bifidobacteria can digest casein only after their partial hydrolysis. The breakdown of casein produces such compounds as polypeptides, glycopeptides, amino sugars, which contribute to the stimulation of bifidobacteria growth [4].

Lactobacilli occupy a dominant position in the stomach, duodenum and small intestine, reaching an amount of 10^4–10^8 CFU/g [5]. *L. acidophilus* expresses antagonistic activity against pathogenic and opportunistic microorganisms. Also, [6] studied the antioxidant activity of *L. acidophilus*. Their ability to exhibit antioxidant activity is related to the work of such enzymes as superoxide dismutase, peroxidase and high intracellular concentration of Mn^{2+} ions. There is also evidence that exopolysaccharides isolated by lactobacilli exhibit antioxidant properties [7, 8].

According to literature data, lactobacilli and bifidobacteria are known to produce β-d-galactosidase, which improves lactose tolerance. The high microbial tolerance may be due to intraintestinal digestion of lactose by β-d-galactosidase released from cultures. Thus, Lactobacillus and Bifidobacterium may improve lactose digestion [9, 10].

Lactobacillus and Bifidobacterium exhibit cholesterol lowering effects [11]. *L. acidophilus* converts bile acids into free acids, which in turn are rapidly eliminated from the body. As free salts are eliminated from the body, the synthesis of new bile acids from cholesterol reduces its concentration in the body.

Also according to literature data, it is known that the interaction of *L. acidophilus* and *B. bifidum* promotes changes in the effect of lactobacilli with *E. coli* – increased suppression of *Escherichia coli* by 1.5 times, as well as with *K. pneumoniae* –decreased expression of Klebsiella by 1.4 times [12].

Thus, the use of *L. acidophilus* in vitro, i.e. their intake through food or medicinal preparations is relevant. One of the simplest ways of *L. acidophilus* intake into the body is the consumption of fermented dairy products. However, combining *L. acidophilus* with bifidobacteria in starter products helps to improve the therapeutic effect, increase resistance to unfavorable environmental factors.

L. acidophilus is a strong acidifier (maximum acidity up to 300 °T) in contrast to bifidobacteria (maximum acidity 130 °T). The method [13] of joint cultivation of *L. acidophilus* and Bifidobacterium is known in the literature.

In the production of dairy products, the development of bifidobacteria in milk is poor due to the presence of dissolved oxygen in it. Therefore, over time, their number begins to decrease. In research of scientists, it is shown that in the process of storage of fermented milk products with bifidobacteria, the number of viable bifidobacteria decreases: on the 5^{th} day by 3 %, and on the 7^{th} day – by 10.5 % [14]. To ensure high functional properties of the products produced, activation (adaptation) of bifidobacteria is carried out in order to activate enzyme systems and maintain their viability in the dairy product. This can be achieved by using *Prunus padus* extract.

It is found that biologically active substances in plants can positively influence the growth parameters of probiotics due to the multicomponent nature of food sources [15]. For instance, various plant raw materials such as topinambur, licorice, burdock, and alfalfa have been incorporated into fermented milk products to stimulate the growth of lactic acid microorganisms [16–20]. However, despite these findings, unresolved questions persist regarding the application of these plant-based ingredients in dairy products.

The reasons for these gaps can be attributed to objective difficulties associated with understanding the underlying mechanisms, the fundamental impossibility of optimizing their incorporation, and the costly nature of further research, rendering corresponding investigations currently impractical. An option to overcome these challenges involves adopting a systematic approach similar to previous studies. For example, the paper by [16] focuses on the effects of licorice root feeding on the quality of cow milk and Stracciata cheese, suggesting it as a dietary supplement to enhance dairy product quality. Although positive effects on milk composition are observed, further research is needed to fully understand the mechanisms and potential health implications.

Similarly, the paper [17] explores the creation of licorice-flavored fermented milk, examining its sensory and nutritional qualities. While it adds diversity to dairy products and identifies consumer preferences, additional investigations into its long-term stability and shelf-life are essential for practical applications.

Furthermore, the paper [18] investigates the use of alfalfa in dairy production, emphasizing its potential to improve product quality and nutritional properties. This study highlights the broader potential of plant-based ingredients in enhancing dairy nutrition and fostering innovation in the industry.

The paper [19] explores the effects of burdock polysaccharides on the quality and antioxidative activity of fermented milk, showcasing their potential as a functional ingredient. However, further research is required to elucidate underlying mechanisms and optimize their incorporation into dairy formulations.

The paper [20] explores cedar pine oil's biofunctional properties, focusing on its antimicrobial and antioxidant effects for food safety and human health. While shedding light on its potential, more research is necessary to confirm its efficacy and safety in practical food applications.

Also, it is noted that the addition of cedar oil and polyunsaturated fatty acids of flaxseed oil stimulates the growth of bifidobacteria and enhances their cholesterol-metabolizing activity [21, 22], suggesting the potential health benefits of incorporating these natural ingredients into dairy products.

Recently, a new blend of vegetable and fruit juices was prepared by fermentation of topinambur, pineapple, pumpkin, spinach, apple and cucumber using the probiotic LAB, which showed increased LAB viability and acceptable sensory response [23]. Enrichment of various food products with polyphenols and their subsequent fermentation with

probiotic bacteria is another common way to enhance their functional properties. A functional milk drink enriched with olive polyphenols and fermented with the probiotic LAB was developed and showed consistent laboratory viability, improved amino acid profile and phenolic content [24].

Green tea yogurt, prepared by enriching traditional yogurt with different tea varieties, is gaining popularity as a new functional food [25]. Recent studies have shown that green tea extracts can be successfully incorporated into yogurt, inducing the synthesis of new phenolic compounds and enhancing antioxidant capacity while maintaining the flavor and viability of beneficial microorganisms [26–28]. The paper [26] explores the impact of green tea polyphenols addition on the physicochemical, microbiological, and bioactive characteristics of yogurt. This study provides valuable insights into the potential of green tea polyphenols as functional ingredients in yogurt formulations. Through comprehensive analysis, the authors assess the effects of polyphenol supplementation on yogurt properties, including texture, color, microbial viability, and bioactive compound content. While the study contributes to the understanding of the potential health benefits of green tea polyphenols in yogurt, further research is needed to evaluate the sensory attributes and consumer acceptance of polyphenol-enriched yogurt products. The paper [27] investigates the microbial, physiochemical, and functional properties of probiotic yogurt containing Lactobacillus acidophilus and Bifidobacterium bifidum enriched with green tea aqueous extract. This study examines the potential synergistic effects of probiotic bacteria and green tea extract on yogurt properties, including microbial viability, physicochemical characteristics, and functional attributes. While the study highlights the potential of combining probiotics and green tea extract to enhance the nutritional value of yogurt, further research is warranted to elucidate the underlying mechanisms and optimize the formulation for practical applications in the food industry. The paper [28] investigates changes in phenolic compound profiles in tea extracts and the composition of these compounds in yogurt. This study elucidates the impact of tea extract addition on the phenolic composition of yogurt and assesses the potential health-promoting properties of phenolic compounds in the final product. Through analytical methods, the authors characterize the phenolic profiles of tea extracts and their retention in yogurt during processing. While the study offers valuable insights, further research is required to understand the bioavailability and health effects of phenolic compounds in yogurt. It has been demonstrated that not only green tea but also fermentation with red ginger extract increases the bioactive potential of yogurt without negatively affecting the growth of probiotic microorganisms [29]. This evidence supports the argument for conducting a study focused on exploring the impact of various natural extracts, including plant-origin polyphenols, on the physicochemical, microbiological, and bioactive characteristics of fermented dairy product technologies.

Experimental data on the introduction of bird cherry into fermented dairy products are not sufficiently presented. Bird cherry is used as a medicinal remedy with a number of useful properties: anti-inflammatory, tonic, etc. It contains a sufficient amount of biologically active substances such as vitamins, flavonoids, fructosans, antioxidants, tannins, macro- and microelements, which equates it to prebiotics [30, 31].

Thus, the unresolved issue lies in the absence of a developed technology for fermented milk functional products that integrate *L. acidophilus* enriched with bifidobacteria activated by bird cherry extract, alongside a comprehensive analysis of their physicochemical parameters. This knowledge gap underscores the necessity for a thorough investigation into the technology of fermented acidophilic products with *B. bifidum* and *Prunus padus* extract, alongside an exploration of their physicochemical parameters, amino acid, fatty acid, vitamin, mineral, and antioxidant composition. All this allows us to assert that it is expedient to conduct a study on the aforementioned aspects.

3. The aim and objectives of the study

The aim of the study is to identify the qualitative parameters of a fermented milk functional product based on *L. acidophilus* enriched with *B. bifidum* activated by *Prunus padus* extract at a concentration of 10^{-5} g/cm^3.

To achieve this aim, the following objectives were accomplished:

– to develop the technology of fermented acidophilic products and study their physicochemical analysis;

– to study the amino acid and fatty acid composition of fermented acidophilic products;

– to study the vitamin and mineral composition of fermented acidophilic products;

– to study the microbiological parameters of fermented acidophilic products.

4. Materials and methods

4. 1. Object and hypothesis of the study

The object of the study was *Prunus padus* (bird cherry) extract obtained using microwave extraction.

The main hypothesis of the study posits that the integration of *L. acidophilus* enriched with bifidobacteria activated by *Prunus padus* (bird cherry) extract into fermented milk functional 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 and antioxidants in the final product.

The work assumes that the activation of bifidobacteria using *Prunus padus* extract will effectively optimize enzyme systems, leading to a shortened activation time. Additionally, it assumes that the incorporation of bifidobacteria activated by bird cherry extract into fermented milk products will result in increased microbial count, improved amino acid and fatty acid profiles, and elevated antioxidant content. Furthermore, it assumes that the utilization of plant-based extracts, such as bird cherry 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 *L. acidophilus* and bifidobacteria activated by *Prunus padus* extract into fermented milk products, without considering other microorganisms or ingredients. Additionally, the work simplifies the investigation by concentrating on the physicochemical

parameters, amino acid, fatty acid, vitamin, mineral, and antioxidant composition of the developed fermented acidophilic products, without delving into broader aspects of food microbiology or processing.

Lyophilized cultures of microorganisms *Bifidobacterium bifidum* 1, not less than $5x10^8$ CFU, and *Lactobacillus acidophilus* n.v. Ep 317/402, not less than 1×10^9 CFU/g, were used as probiotics.

4. 2. Method of obtaining *Prunus padus* extract by microwave treatment method

20 g powder of bird cherry after sieving of particles of size 2–7 mm in a flask was poured with 200 ml of distilled water and heated in defrost mode in a microwave for 4 min until reaching a temperature of 70 °C. The flasks were then placed in a desiccator at 70 °C for 3 hours. To the obtained aqueous extract, 2 ml of 25 % hydrochloric acid solution was added and left for 12 hours until precipitate formation. The precipitate was filtered through a Bunsen flask and a Buchner funnel, passed through a paper filter and a capron filter. The obtained particles were dried in atmospheric air for 12–14 h [32].

4. 3. Dairy products samples preparation

L. acidophilus starter in the amount of 2.5 % and activated bifidobacteria with *Prunus padus* extract in the concentration of 10^{-5} g/cm^3 (a solution of 1 % *Prunus padus* extract was prepared and dilutions were made from this solution at 37±1 °C for 30 min) in the amount of 10 % were added to the prepared pasteurized milk with a fat content of 2.5 % and density of 1,028 g/cm^3. The initial concentration of the bifidobacterial population was 10^6 CFU/cm^3. The fermentation was carried out for 10 hours until the acidity reached 75 °T, then left to mature at 6–10 °C for 12 hours.

4. 4. Determination of the redox potential

The potentiometric method is based on the measurement of the potential difference between two electrodes immersed in the analyzed sample. Measurement of redox potential was carried out according to GOST 8.639-2014 (Federal Agency on Technical Regulating and Metrology, 2014) [33].

4. 5. Determination of water-soluble antioxidants content

Determination of antioxidants in fermented milk products was carried out on the "TsvetYauza-01- AA" device, based on the amperometric method of measuring the mass fraction of antioxidants by measuring the strength of the electric current arising from the oxidation of antioxidant molecules on the working electrode surface at a certain potential. The extraction was carried out with 70 % ethyl alcohol solution, shaking for one hour on a stirring device. Gallic acid solution was used to construct a graduation graph [34].

4. 6. Determination of the number of microorganisms (bifidobacteria, lactobacilli)

The method is based on the ability of bacteria to grow in nutrient media: hydrolysate milk medium (HMM), MRS, poured high column in tubes and thin layer in Petri dishes, at 37±1 °C and form characteristic colonies in them after 24–72 hours. The number of microorganisms 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 [35, 36].

4. 7. Microscopic examination

Microscopic examination of the obtained samples was carried out using a Levenhuk D400LCD electron microscope with a magnification of 100x. The samples were prestained with methylene blue dye.

4. 8. Determination of amino acids

According to the method M-04-38-2009 (GOST 55569-2013) (Federal Agency on Technical Regulating and Metrology, 2013) [14], the mass fraction of amino acids was determined: arginine (Arg), lysine (Lys), tyrosine (Tyr), phenylalanine (Phe), histidine (His), leucineisoleucine (Leu+Ile) (total), methionine (Met), valine (Val), proline (Pro), serine (Ser), alanine (Ala) and glycine (Gly) in the products. The measurement technique allowed the determination of the total amino acid content of the samples (total free and bound forms). Since leucine and isoleucine are not separated during sample decomposition, their total determination was envisaged.

The method was based on the decomposition of samples by acid hydrolysis with the conversion of amino acids into free forms, obtaining FTC derivatives, their further separation and quantification by capillary electrophoresis. Detection was carried out in the UV region of the spectrum at a wavelength of 254 nm. For direct quantification of tryptophan without obtaining the FTC derivative, absorbance was recorded at a wavelength of 219 nm. The following equipment and reagents were used for measurements: capillary electrophoresis system "Kapel-105 M" having a special cassette for amino acid analysis. Data collection, processing and output were carried out using a personal computer with Windows 2000/XP.7 operating system, on which the appropriate data collection and processing programme was installed.

4. 9. Determination of fatty acid composition

The fatty acid composition was determined by gas chromatography on a gas-liquid chromatograph "Crystal-4000" with a flame ionization detector and "NetChrom" programme during the transesterification of milk fat with methylatom sodium in methanol as described in [37]. The methyl esters were separated on a capillary column of 30 m length and 0.25 mm inner diameter, the carrier gas – hydrogen was flowed at a rate of 40 ml/h. The separation was carried out on the polar stationary phase SUPELCOQAX-10 by increasing the temperature from 60 °C to 180 °C at a rate of 20 °C per minute, the maximum temperature in the column was 230 °C.

4. 10. Determination of water-soluble vitamins content

Quantitative determination of the mass fraction of water-soluble vitamins of group B was carried out by capillary electrophoresis on the "Kapel 105M" device. The method of determination is based on the migration and separation of free forms of the analyzed water-soluble vitamins under the action of an electric field with registration of their electrophoretic mobility at a wavelength of 200 nm. The determination of vitamins B1, B2, B3, B5 (nicotinic acid), B6 and Bc was carried out in a variant of capillary zone electrophoresis. From the crushed plant samples, vitamins were

extracted with aqueous sodium tetraborate solution in the presence of sulfite ion. The extract was centrifuged (5,000–6,000 rpm for 5 minutes) and filtered through a membrane filter. The vitamins were detected by their intrinsic absorbance at wavelengths of 200 nm and 267 nm using programmable wavelength switching. Separation conditions: buffer: borate with pH=8.9. Capillary: L_{eff}/L_{comm}=65/75 cm, capillary diameter=50 μm. Voltage: +25 kV. Temperature: +30 °C.

4. 11. Determination of mineral content

The mineral content of fermented acidophilic products was determined by the scanning electron microscope JSM-6490LV with the energy dispersive microanalysis system INCA Energy 350 and the system of structural and textural analysis of polycrystalline samples HKL Basic.

The scanning electron microscope allows the observation of fine features using a microanalyzer of chemical composition, and details of the structure of micro-objects at the atomic-molecular level. It also allows obtaining images of the object surface with high (up to 0.4 nm) spatial resolution, as well as information on the composition, structure and some other properties of the near-surface layers. SEM has a large depth of focus, which allows observing a volumetric image of the structure with the possibility of its quantitative evaluation.

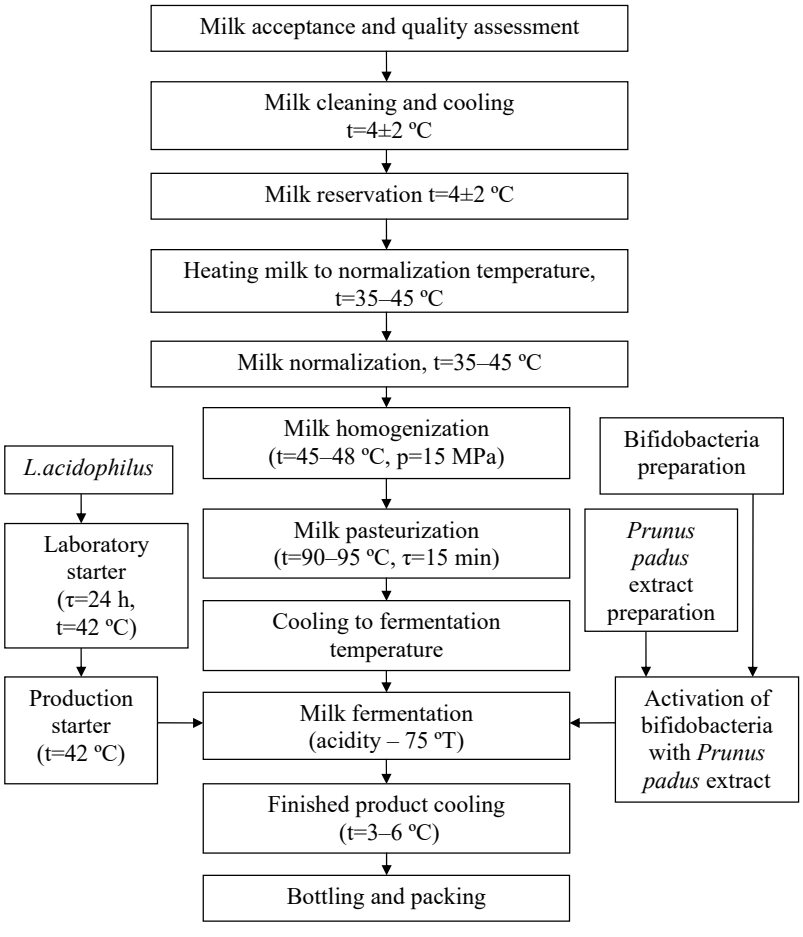


Fig. 1. Scheme of fermented milk product production based on *L. acidophilus*

4. 12. Analysis of experimental data

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

5. Results of evaluation of fermented acidophilic products technology quality indicators

5. 1. Results of the development of fermented acidophilic products technology and evaluation of their physicochemical analysis

The co-cultivation of *L. acidophilus* and *B. bifidum* was investigated. For this purpose, an *L. acidophilus* based fermented milk product was prepared.

The block diagram of the fermented acidophilic product production is presented in Fig. 1.

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

1) control sample – fermented acidophilic product;

2) experimental sample – fermented acidophilic product enriched with *B. bifidum* 1, activated with *Prunus padus* extract at a concentration of 10^{-5} g/cm^3.

Bifidobacteria in the amount of 1×10^9 CFU were recorded in the finished fermented milk product based on *L. acidophilus* enriched with bifidobacteria, activated by *Prunus padus* extract.

Table 1 shows the organoleptic characteristics of the finished fermented acidophilic products.

Table 1

Organoleptic characteristics of the finished product

Indicator name	Characterization	
	Control sample	Experimental sample
Taste and smell	Pure, sour, without extraneous flavors and odors	
Color	Milky-white, uniform throughout the mass	
Appearance and consistency	Homogeneous, moderately viscous, slightly sticky	

Organoleptic analysis of finished products showed that the experimental sample does not differ from the control in color, smell and commercial appearance. The experimental product enriched with *B. bifidum* activated with *Prunus padus* extract has better consistency and flavor.

The physicochemical parameters of the finished product are presented in Table 2.

The indicators of the obtained products during storage were analyzed. All products meet the requirements of GOSTs 33491-2015 and 54340-2011 [35, 38] (Federal Agency on Technical Regulating and Metrology, 2015, 2011). From the obtained data we can see that on the 7th day in the control, the number of *L. acidophilus* increases by 20.2 % in comparison with the finished product after 24 hours. In the experimental sample, the number of *L. acidophilus* increases

by 25.7 % compared to the finished product after 24 hours, and compared to the control in the finished product after 24 hours by 1.8 % and by 6.5 % after 7 days, respectively.

Table 2

Physicochemical parameters of the finished product

Indicator name	Characterization			
	24 h		7 days	
	Control sample	Experimental sample	Control sample	Experimental sample
Titratable acidity, °T	106.5±0.22	105.7±0.49	134.2±0.60	130.8±0.74
Number of *L. acidophilus*, lg CFU/cm^3	9.04±0.04	9.21±0.06	10.87±0.05	11.58±0.04
Viscosity, sec	10.5	11.2	10.8	11.6
Water-holding capacity, %	35	20	35	15

Viscosity is an important indicator for assessing the consistency of fermented milk products. It is shown that the viscosity of the fermented acidophilic product enriched with bifidobacteria, activated with *Prunus padus* extract is 6.6 % higher after 24 hours and 7.4 % higher after 7 days of storage compared to the control sample.

To characterize the product in terms of synergistic properties, the water-holding capacity of the clot was determined in dynamics. In the finished product, the most stable clot was obtained in the experimental sample. In the control, an increase in the amount of whey released over time was observed. In the experimental sample, the amount of whey released decreases compared to the control sample by 25 %. This indicates a decrease in syneresis.

The content of antioxidants in finished products is presented in Table 3.

Table 3

Content of antioxidant substances

Indicators name, measurement units	Control sample	Experimental sample
Flavonoids, mg/g	0.15±0.001	0.18±0.001
Content of water-soluble antioxidants, mg/g	0.70±0.0024	0.67±0.0053

The data in Table 3 show an increase in flavonoid content in the experimental sample by 20.0 % compared to the control sample.

5. 2. Results of a study of the amino acid and fatty acid compositions of fermented acidophilic products

The amino acid composition of the fermented acidophilic product is shown in Table 4 and their chromatograms are shown in Fig. 2, 3.

Analysis of the data in Table 4 shows that the fermented acidophilic 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. Tyrosine was detected in the experimental sample, while it was not detected in the control. The content of amino acid histidine is 3 times higher than in the control sample.

The fatty acid composition of the control and experimental samples of fermented acidophilic products is presented in Table 5.

Table 4

Amino acid composition of fermented acidophilic products

Indicators name, measurement units	Name of samples			
	Control sample		Experimental sample	
	Conc., mg/l	Weight, %	Conc., mg/l	Weight, %
Arginine	31.0	0.360±0.144	27.0	0.319±0.127
Lysine	33.0	0.383±0.130	29.0	0.342±0.116
Tyrosine	0	0	18.0	0.212±0.064
Phenylalanine	17.0	0.197±0.059	15.0	0.177±0.053
Histidine	2.70	0.031±0.016	8.20	0.097±0.048
Leucine+isoleucine	34.0	0.395±0.103	29.0	0.342±0.089
Methionine	14.0	0.163±0.055	12.0	0.142±0.048
Valine	39.0	0.453±0.181	23.0	0.271±0.109
Proline	42.0	0.488±0.127	39.0	0.460±0.120
Threonine	17.0	0.197±0.079	17.0	0.201±0.080
Serine	20.0	0.232±0.060	17.0	0.201±0.052
Alanine	15.0	0.174±0.045	14.0	0.165±0.043
Glycine	7.60	0.088±0.030	5.90	0.070±0.024

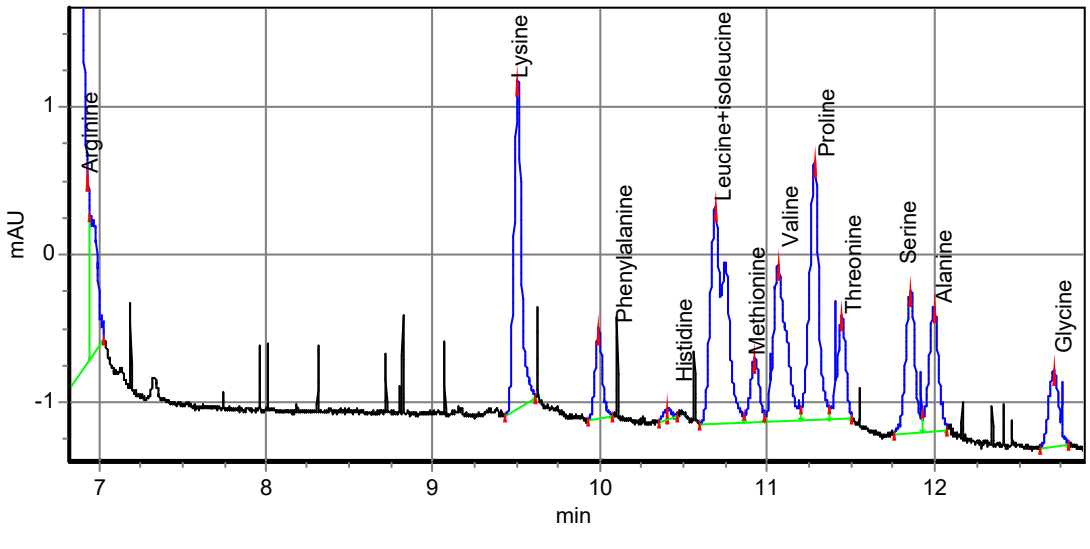


Fig. 2. Chromatogram of the amino acid composition of the control sample

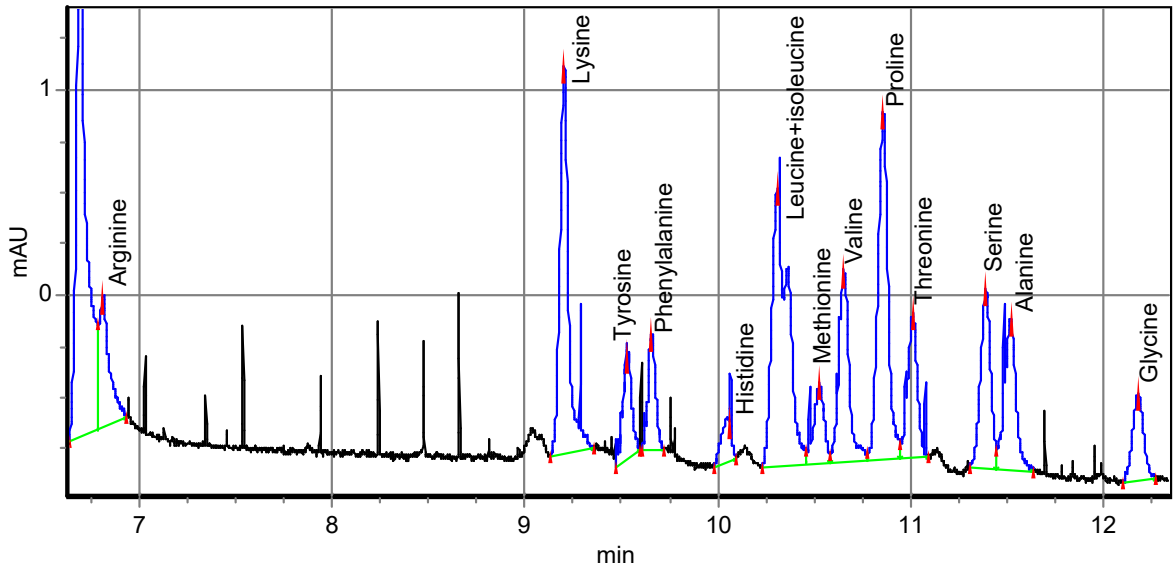


Fig. 3. Chromatogram of the amino acid composition of the experimental sample

Table 5

Fatty acid composition of fermented acidophilic products

Fatty acid composition, %	Control sample	Experimental sample
Saturated fatty acids, %		
C4:0 Butyric acid	3.019±0.302	2.837±0.284
C60 Caproic acid	2.358±0.236	2.117±0.211
C8:0 Caprylic acid	1.633±0.163	1.428±0.143
C10:0 Decanoic acid	4.140±0.414	3.430±0.343
C11:0 Undecanoic acid	0.081±0.008	0.076±0.008
C12:0 Lauric acid	4.928±0.493	4.157±0.416
C13:0 Tridecanoic acid	0.146±0.014	0.125±0.013
C14:0 Myristic acid	14.594±1.459	13.244±1.324
C15:0 Pentadecylic acid	1.351±0.135	1.264±0.126
C16:0 Palmitic acid	34.775±3.477	35.927±3.593
C17:0 Margaric acid	0.619±0.062	0.654±0.065
C18:0 Stearic acid	7.035±0.703	8.513±0.851
C20:0 Arachidic acid	0.065±0.006	0.091±0.009
C22:0 Behenic acid	0.059±0.006	0.059±0.006
C21:0 Heneicosylic acid	0.259±0.026	0.048±0.005
C24:0 Lysergic acid	0.032±0.003	0.08±0.008
Monounsaturated fatty acids, %		
C14:1 (cis-9) Myristoleic acid	1.334±0.133	1.119±0.112
C15:1 (cis-10) Pentadecanoic acid	–	0.011±0.001
C16:1 (cis-9) Palmitoleic acid	1.906±0.191	1.879±0.188
C17:1 (cis-10) Margarineoleic acid	0.267±0.027	0.262±0.026
C18:1 (cis-9) Oleic acid	17.675±1.768	19.427±1.943
C20:1 (cis-11) Eicosanoic acid	0.121±0.012	0.153±0.015
C24:1 (cis-15) Selacholeic acid	–	0.008±0.0008
Polyunsaturated fatty acids, %		
C18:2n6t α-Linolenic acid	0.341±0.034	0.390±0.039
C18:2n6c Linoleic acid	2.364±0.024	1.953±0.195
C18:3n3 Linolenic acid	0.382±0.038	0.380±0.038
C20:2 Eicosadienoic acid	0.114±0.011	0.058±0.006
C20:3n6c (cis-8,11,14) Eicosatrienoic acid	–	0.042±0.004
C20:3n3c (cis-11,14,17) Eicosatrienoic acid	0.267±0.027	0.101±0.010
C20:4n6 Arachidonic acid	0.075±0.007	0.092±0.009
C20:5n3 Eicosapentaenoic acid	0.034±0.003	0.025±0.003
C22:6n3 Docosahexaenoic acid	0.027±0.003	0.045±0.005

Analysis of the data in Table 5 shows that saturated, monounsaturated and polyunsaturated fatty acids were found in the composition of fermented acidophilic products. Comparative analysis of the fatty acid content of the control and experimental samples of the fermented acidophilic product shows that when enriching the composition of the experimental sample with bifidobacteria activated with *Prunus padus* extract increases the content of oleic, eicosanoic, linoleic, arachidonic and docosahexaenoic acids by 10.0 %, 26.4 %, 14.4 %, 22.6 % and 66.6 %, respectively. Also, pentadecenoic, selacholeic and eicosotrienoic monounsaturated and polyunsaturated fatty acids were found in the composition of the experimental sample with *B. bifidum*, activated with *Prunus padus* extract.

5. 3. Results of a study of the vitamin and mineral compositions of fermented acidophilic products

The composition of water-soluble B vitamins of the control sample and the experimental sample of the fermented acidophilic product are presented in Fig. 4, 5. In addition, the processed chromatogram data are presented in Table 6.

Table 6

Vitamin content

Water-soluble vitamins, mg/100 g	Name of samples	
	Control sample	Experimental sample
B2 (Riboflavin)	0.047±0.020	0.050±0.021
B6 (Pyridoxine)	0.019±0.004	0.022±0.004
C (Ascorbic acid)	4.993±1.698	4.989±1.696
B5 (Pantothenic acid)	0.649±0.117	0.898±0.162
B3 (Nicotinic acid)	0.160±0.032	0.274±0.055
B9 (Folic acid)	0.047±0.020	0.003±0.001

Analysis of Table 5 shows that in terms of water-soluble vitamin content, the experimental sample was further enriched with the following water-soluble vitamins: pyridoxine (B6) – 15.7 % higher, nicotinic acid (B3) – 38.4 % higher and pantothenic acid (B5) – 71.2 % higher compared to the control sample.

The mineral content is presented in Table 7 and Fig. 6.

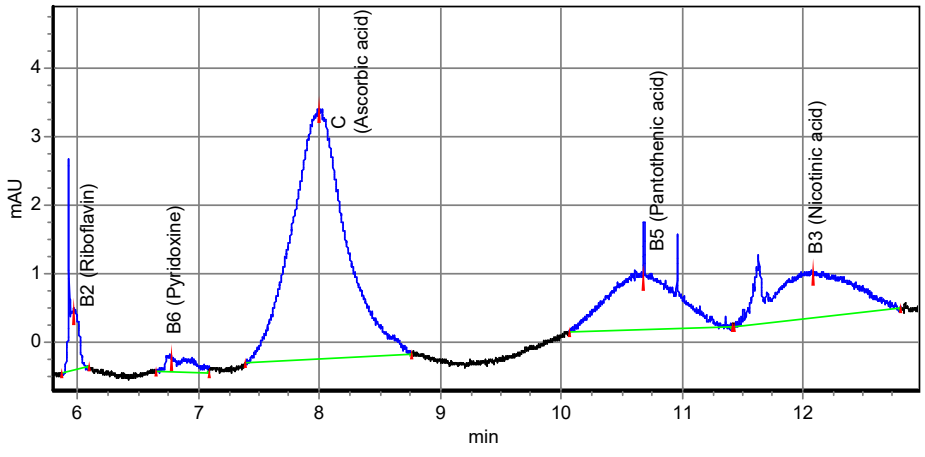


Fig. 4. Chromatogram of the vitamin content of the control sample

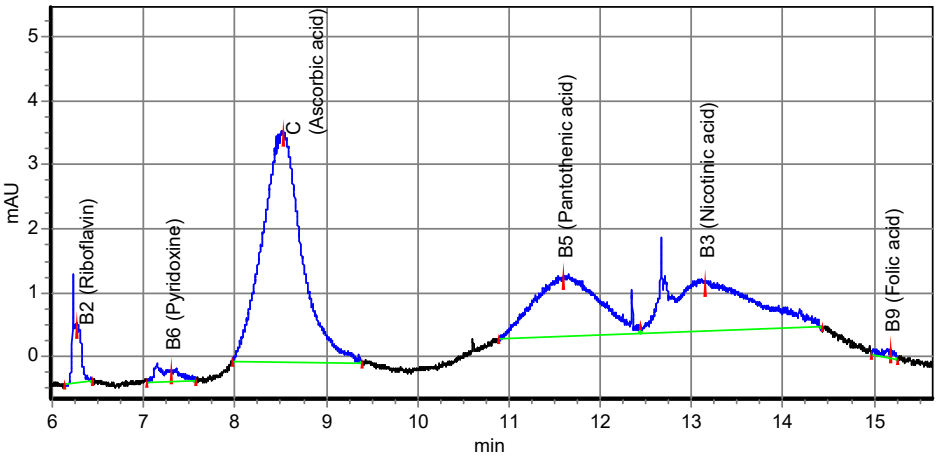


Fig. 5. Chromatogram of the vitamin content of the experimental sample

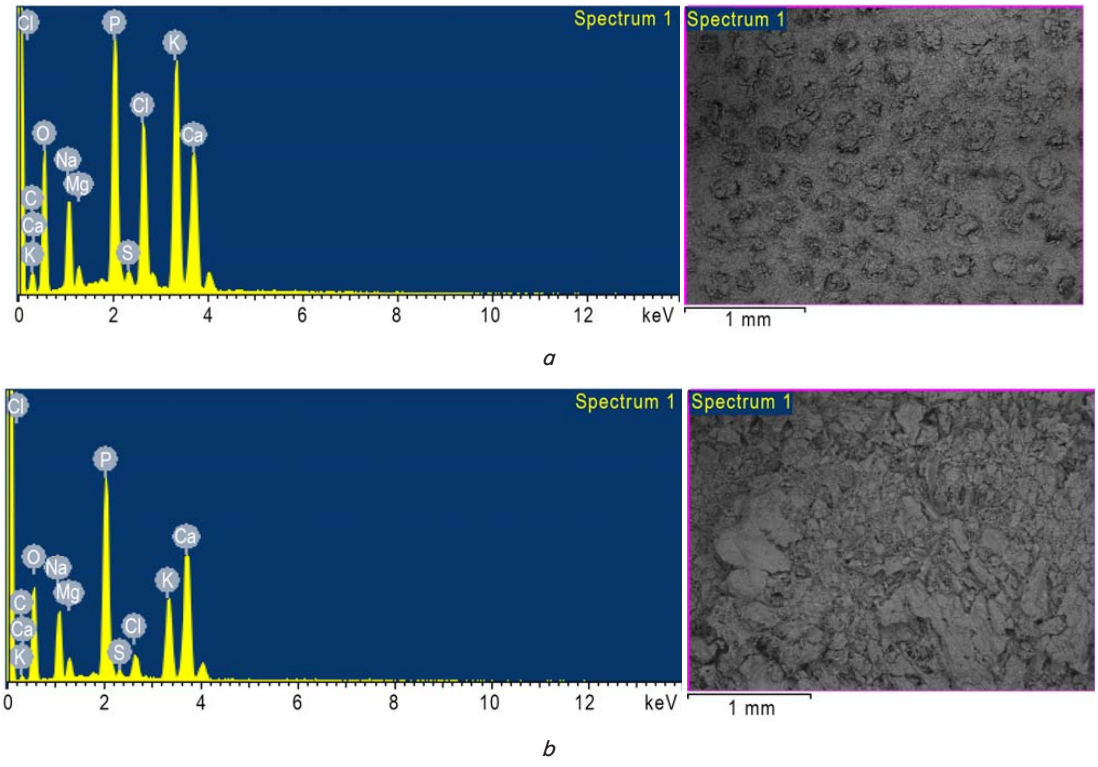


Fig. 6. Spectra of the microelement composition of fermented acidophilic products: *a* — control sample; *b* — experimental sample

Table 7

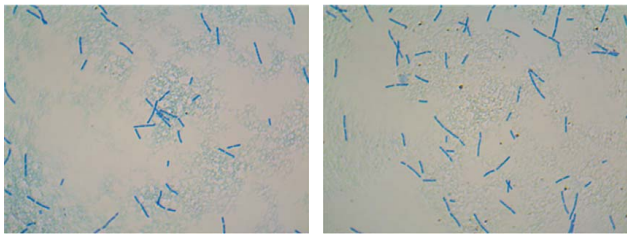
Elemental composition of fermented acidophilic products

Element names, weight %	Control sample	Experimental sample
C	14.97	6.95
O	34.61	38.97
Na	6.60	8.95
Mg	0.89	1.74
P	10.72	15.95
S	0.68	0.61
Cl	8.09	2.21
K	14.13	8.92
Ca	9.32	15.69

The study of the trace elements content shows that in the experimental sample of fermented acidophilic products the content of sodium by 35.6 %, magnesium by 95.5 %, phosphorus by 48.8 % and calcium by 68.3 % was higher than in the control sample.

5. 4. Results of the microbiological parameters study of fermented acidophilic products

The microscopic patterns of finished fermented milk products based on *L. acidophilus* are shown in Fig. 7.



a *b*

Fig. 7. Microscopic picture of the finished fermented acidophilic products ×100: *a* — control sample; *b* — experimental sample

Microbiological indicators of fermented acidophilic products were investigated. The results are presented in Table 8.

Table 8

Microbiological parameters of fermented acidophilic products

Indicator names		Indicator value according to TR CU 033/2013	Actual value	
			Control sample	Experimental sample
Lactic acid microorganisms, CFU/cm^3 (g), not less (at the end of shelf life)		1×10^7	$1.6\cdot10^7$	$2\cdot10^8$
Weight of product cm^3 (g) in which the following is not allowed	Coliforms	0.1	not found	not found
	Staphylococcus aureus	1.0		
	Listeria monocytogenes	–		
	Pathogenic (including salmonella)	25		
Yeast, CFU/cm^3 (g), not more than		50	25	15
Molds, CFU/cm^3 (g), not more than		50	20	20

From Table 8, it can be interpreted that the experimental sample generally exhibited higher counts of lactic acid microorganisms compared to the control sample. Regarding hygiene indicators, both the control and experimental samples met the requirements by the Technical Regulations of the Customs Union "On the Safety of Milk and Dairy Products" as no coliforms, *Staphylococcus aureus*, *Listeria monocytogenes*, or pathogenic microorganisms were found within the specified limits. Overall, Table 8 shows that the experimental acidophilic product exhibited favorable microbiological parameters, particularly in terms of lactic acid microorganisms and yeast counts, indicating its potential as a high-quality fermented product with improved microbiological stability.

6. Discussion of the study results of the addition of *B. bifidum* 1 activated with *Prunus padus* extract to the fermented acidophilic product

Interest in the benefits of probiotic Bifidobacterium species for human health continues to grow. Bifidobacterium species living in the large intestine help to maintain human health, and the disappearance or reduction of the bifidobacterium population signals a problematic state of health.

The data presented in Table 2 show that in the fermented acidophilic product enriched with *B. bifidum* activated by *Prunus padus* extract at a concentration of 10^{-5} g/cm^3, during the shelf life (7 days) the number of *L. acidophilus* increases and remains at a stable level up to 7 days. The experimental sample consistently demonstrates a higher number of *Lactobacillus acidophilus* compared to the control sample at both time points, indicating a potential beneficial effect of the experimental conditions on probiotic growth. These findings are consistent with research indicating that certain plant extracts can enhance the growth of beneficial bacteria during fermentation [39]. These data correlate well with the data obtained from the analysis of titratable acidity, viscosity and water-holding capacity of the product. Both control and experimental samples show a decrease in titratable acidity from 24 hours to 7 days, indicating a fermentation process. The experimental sample consistently exhibits slightly lower acidity compared to the control sample at both time points, suggesting a potential effect of the experimental conditions on acidity. Viscosity slightly increases from 24 hours to 7 days for both samples, suggesting a thickening of the product over time. The experimental sample consistently exhibits slightly higher viscosity compared to the control sample at both time points, indicating a potential impact of the experimental conditions on product texture. These findings could be discussed in the context of the potential effects of the experimental conditions on product quality and shelf-life.

Enriching the composition of the fermented acidophilic product with bifidobacteria activated for 30 min in aqueous *Prunus padus* extract obtained by microwave treatment not only increased the concentration of biologically active components but also reduced the activation time of bifidobacteria. Activation or adaptation of bifidobacteria with *Prunus padus* extract also provided high functional properties of the produced products due to the activation of enzyme systems and maintenance of their vital activity in the dairy product. Consequently, multiple

inoculations of bifidobacteria culture and contaminations by foreign microflora were excluded. Due to enriching the composition of the experimental sample of the fermented acidophilic product with *B. bifidum* activated by *Prunus padus* extract (Table 3), the content of antioxidant substances (flavonoids) in the product increased. The obtained results are confirmed by the studies [27, 40] that enriching fermented milk products with *Bifidobacterium bifidum* and plant extracts can lead to an increase in the content of antioxidant substances, such as flavonoids, in the final product.

The proposed technology will allow to obtain a functional fermented acidophilic product enriched with *B. bifidum* activated by *Prunus padus* extract, having better organoleptic properties, enriched with minerals and vitamins. Fermentation conditions and the presence of plant extracts may influence the synthesis of organic acids, phenolic compounds, and other metabolites with potential health-promoting properties. According to the results of studies [41], some bacterial strains belonging to the genera *Lactobacillus* and *Bifidobacterium* can serve as an additional source of B vitamins in the fermentation of dairy products. Thus, B vitamins such as pyridoxine, nicotinic acid and pantothenic acid were synthesized in the prototype of the developed fermented acidophilic product with bifidobacteria activated by *Prunus padus* extract (Fig. 4, 5). As well as in the experimental sample of the fermented acidophilic product, high contents of vital minerals were found: sodium – 35.6 %, magnesium – 95.5 %, phosphorus – 48.8 % and calcium – 68.3 % more than in the control sample.

Monounsaturated fatty acids are considered beneficial for health, as they help to reduce the level of "bad" cholesterol and increase the level of "good" cholesterol, which contributes to the prevention of cardiovascular diseases. The content of oleic and eicosanoic acids in the composition of the experimental sample of the fermented acidophilic product enriched with *B. bifidum* activated by *Prunus padus* extract was many times higher compared to the control. At the same time, in the experimental sample such monounsaturated fatty acids as pentadecanoic, selacholic acids were found, while in the control they were not found. Polyunsaturated fatty acids have anti-inflammatory properties and reduce the risk of cardiovascular diseases. The contents of linoleic, arachidonic and docosahexaenoic acids were 14.4 %, 22.6 % and 66.6 %, respectively, higher in the composition of the experimental sample compared to the control sample of the fermented acidophilic product. The amount of eicosotrienoic acids in the experimental sample was 0.042±0.004 %. This is important because polyunsaturated fatty acids are one of the very important basic elements for human health and are essential nutritional factors. They are not formed in the body and must be supplied by food.

In terms of amino acid content, tyrosine was detected in the experimental sample, while it was not detected in the control sample of the fermented acidophilic product. Tyrosine improves mood, attentiveness, gives courage. The content of the amino acid histidine in the experimental sample was 3 times higher than in the control sample. Histidine regulates blood acidity, promotes wound healing, and accelerates the growth and recovery of the organism. The results obtained are consistent with the research results [42] suggesting that fermented milk products enriched with probiotics and plant extracts could serve as valuable sources of high-quality protein.

Microbiological criteria of safety of the developed fermented acidophilic product enriched with *B. bifidum* activated by *Prunus padus* extract, from the conducted analyses and experimental data, determine the acceptability of this product based on the absence of microorganisms groups (coliforms, *Staphylococcus aureus,* pathogenic bacteria including salmonella*, Listeria monocytogenes*), which meets the requirements and safety for consumption. The number of lactic acid microorganisms in the experimental sample was an order of magnitude higher than both the normative indicators and the values of the control sample, which proves the probiotic properties of the product and its high physiological value.

The limitations of this study include constraints linked to the concentration of *Prunus padus* extract employed for the activation of bifidobacteria. The incorporation of concentrations of *Prunus padus* extract exceeding or falling below 10^{-5} g/cm^3 detrimentally impacts both the growth kinetics of bacteria and the microbial population number during bifidobacteria activation in the technology of fermented acidophilic products.

Further research will be directed to the study of new sources of plant-origin antioxidants on the growth and development of probiotic microorganisms and their application in the technology of development of functional fermented milk products.

7. Conclusions

1. The technology of the fermented acidophilic product with bifidobacteria, using *Prunus padus* extract obtained by microwave treatment as prebiotics, was investigated. It was found that in the finished fermented milk product based on *L. acidophilus*, enriched with bifidobacteria, activated by *Prunus padus* extract, bifidobacteria in the amount of 1×10^9 CFU were recorded. In the experimental sample, the number of *L. acidophilus* increases by 25.7 % in comparison with the finished product after 24 hours, and in comparison with the control in the finished product after 24 hours by 1.8 % and by 6.5 % on the 7th day, respectively. Additionally, the viscosity of the fermented acidophilic product enriched with bifidobacteria, activated by *Prunus padus* extract, was found to be 6.6 % higher after 24 hours and 7.4 % higher on the 7th day of storage compared to the control. Moreover, there was a 20.0 % increase in flavonoid content in the experimental sample.

2. The amino acid and fatty acid composition of fermented acidophilic products were studied. The histidine content in the experimental sample was three times higher than in the control sample. Notably, only tyrosine was detected in the experimental sample, whereas it was not detected in the control sample. Regarding fatty acid content, enriching the experimental sample with bifidobacteria activated with *Prunus padus* extract led to a 10.0 % increase in oleic acid, 26.4 % increase in eicosanoic acid, 14.4 % increase in linoleic acid, 22.6 % increase in arachidonic acid, and 66.6 % increase in docosahexaenoic acid. Additionally, pentadecanoic, selacholeic, and eicosatrienoic acids were only found in the experimental sample.

3. It is proved that the acidophilic fermented milk product enriched with bifidobacteria activated with *Prunus padus* extract contains more minerals: sodium, magnesium, phosphorus and calcium compared to the control sample. According to the content of the vitamin composition, there

were positive dynamics in the experimental sample, relative to vitamins B2 (Riboflavin), B6 (Pyridoxine), B5 (Pantothenic acid), and B3 (Nicotinic acid) compared to the control.

4. As a result of the microbiological analysis, pathogenic microorganisms were not found in the developed fermented acidophilic product: *E. coli, Staphylococcus aureus, Listeria monocytogenes*. However, the number of lactic acid microorganisms in the experimental sample was an order of magnitude higher than both the normative indicators and the values of the control sample, which proves the probiotic properties of the product and its high physiological value.

Conflict of interest

The authors declare that they have no conflicts of interest in relation to the current research, whether financial, personal, authorship or otherwise, that could affect the research and the results presented in this paper.

Financing

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".

Data availability

All data is available in the main text of the manuscript.

Use of artificial intelligence

The authors confirm that they did not use artificial intelligence technologies when creating the current work.

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