Journal of
Food Chemistry & Nanotechnology

https://doi.org/10.17756/jfcn.2024-176

Research Article

Open Access

Synbiotic Nutritious Drink: Potential and Safety Evaluation

Serik Shaikhin[1], Zinigul Sarmurzina[2], Gulyaim Abitayeva[1], Gulmira Bissenova[3], Botagoz Mussabayeva[3], Yerassyl Naimanov[4], Zarina Shulgau[5], Aizhan Khalymbetova[2*], Arman Abilkhadirov[1], Kairtai Almagambetov[6], Aslan Temirkhanov[3] and Zhandarbek Bekshin[1]

[1]Laboratory of Genetics and Biochemistry of Microorganisms, Republican Collection of Microorganisms of the Committee of Science of the Ministry of Education and Science of the Republic of Kazakhstan, Astana, Republic of Kazakhstan

[2]Laboratory of Biotechnology, Republican Collection of Microorganisms of the Committee of Science of the Ministry of Education and Science of the Republic of Kazakhstan, Astana, Republic of Kazakhstan

[3] Laboratory of Microbiology, Republican Collection of Microorganisms, Kazakhstan

[4]JSC "Astana-Onim" Akmola region, Kazakhstan

[5]National Laboratory Astana, Nazarbayev University, Astana, Kazakhstan

[6]Biobank of Industrial Microorganisms in Republic Collection of Microorganisms of the Ministry of Healthcare of the Republic of Kazakhstan, Astana, Kazakhstan

[*]Correspondence to:

Aizhan Khalymbetova
Laboratory of Biotechnology,
Republican Collection of Microorganisms of the Committee of Science of the Ministry of Education and Science of the Republic of Kazakhstan, Astana, Republic of Kazakhstan.
E-mail: usensultanbakytzhanuly@gmail.com

Received: April 24, 2024
Accepted: May 24, 2024
Published: May 28, 2024

Citation: Shaikhin S, Sarmurzina Z, Abitayeva G, Bissenova G, Mussabayeva B, et al. 2024. Synbiotic Nutritious Drink: Potential and Safety Evaluation. *J Food Chem Nanotechnol* 10(2): 68-78.

Copyright: © 2024 Shaikhin et al. This is an Open Access article distributed under the terms of the Creative Commons Attribution 4.0 International License (CC-BY) (http://creativecommons.org/licenses/by/4.0/) which permits commercial use, including reproduction, adaptation, and distribution of the article provided the original author and source are credited.

Published by United Scientific Group

Abstract

The increasing concern for health and quality of life has motivated individuals to consume healthier food. Enriching food products with health-promoting additives represents a novel approach within the food industry of Kazakhstan, particularly pertinent due to the adverse environmental and radiation conditions in the Republic. The aim of this study was to develop a new synbiotic nutritional dairy drink (SN) fortified with vitamins, the prebiotic inulin, and the radioactive element blocker potassium iodide and evaluate its physicochemical and sensory properties, shelf life, and safety. In this study, original local strains of *Lactobacillus*, isolated from traditional Kazakh products, were utilized for the first time. The SN drink was obtained by fermenting natural cow's milk with probiotics: *Lactobacillus casei* 1A, *Lactobacillus paracasei* 2A, and *Lactobacillus brevis* 4 LB in the presence of the prebiotic inulin, vitamins A, B1, B2, C and the essential mineral potassium iodide. Titratable acidity (TA) was estimated according to the published technical specification. The viable number of lactobacilli was assessed by plate counting. Sensory evaluation was conducted using a 9-point hedonic scale. The overall viability of probiotic microorganisms of SN drink is maintained for 28 days within the permissible range for beverages according to scientific data. According to sensory analysis, the shelf life of SN drink is determined to be 14 days. The SN drink did not alter the behavior and general conditions of laboratory mice and did not reveal any signs of allergy on the skin and conjunctiva of guinea pigs and rabbits, respectively. The results of the study indicate that the SN drink is safe and suitable for human consumption.

Keywords

Drink, Probiotics, Synbiotics, Vitamins, Potassium iodide, Safety, Shelf life, *In vivo*

Introduction

Growing concern about health and quality of life prompted people to eat healthy foods, reduce the consumption of foods rich in sugar, salt, and fats, as well as consume probiotics, prebiotics, and organic products [1, 2]. The interaction between the human intestine and its microbiota, established by evolution, determines human health by responding to the penetration of pathogens into the gastrointestinal tract along with food. When an increase in the number of

pathogens occurs the balance of microorganisms is disturbed, and this leads to the development of an infectious process. The presence of live microorganisms, in particular lactic acid bacteria (LAB), in food has been traditionally associated with certain health benefits [3]. There is an assumption that beverages produced by LAB fermentation can suppress the growth of pathogens by restoring a useful set of microbes in the intestinal microbiota [4, 5].

LAB, which is characterized by a variety of metabolic processes, has innate abilities such as acid and aroma production, protein hydrolysis, exopolysaccharide production, and inhibition of the growth of harmful bacteria. Among LAB, lactobacilli are the most studied bacteria with probiotic potential [6]. The FAO/WHO definition of probiotics as "live microorganisms which, when administered in adequate quantities, confer a health benefit on the host" is still used today [7]. The process of fermentation of carbohydrates into organic acids, especially lactic acid, preserves the nutritional value of milk. Moreover, additional health benefits of LAB have since been discovered and their application in the food industry has increased [8]. Thus, maintaining the viability and ensuring the survival of probiotic bacteria during storage poses serious challenges to the food industry and is being intensively studied in the scientific world. These studies include prebiotics and synbiotics, the definition of which has recently been revised and clarified by FAO/WHO microbiology, nutrition, and clinical research experts. By definition, a prebiotic is "a substrate that is selectively used by host microorganisms to achieve significant health benefits" [9], while a synbiotic is "a mixture consisting of living microorganisms and substrate(s) selectively used by host microorganisms that confer health benefits to the host" [10]. Probiotics and prebiotics interact with the gastrointestinal tract, the immune system, and neurological systems, having a positive effect on the health of the host [11]. In a study of synbiotic yogurt containing the probiotic *L. brevis* PML1 and the prebiotic inulin, viable *L. brevis* cells reached 8 log CFU/g. The probiotic was resistant to artificial digestive juices, also it increased the production of antimicrobial compounds, and the viability of *L. brevis* increased when stored at 4 ^{0}C. The addition of 2.5% inulin not only improved the physical properties of the beverage but also maintained probiotic viability after 14 days of storage, with a viability rate above 6 log CFU/g of yogurt [5]. The physiological functions of inulin, due mainly to prebiotic activity, demonstrate the great nutritional and medicinal value of inulin. However, the mechanisms of some physiological functions are still unclear [12, 13].

The fact that many individuals suffer from deficiencies in certain vitamins and other essential micronutrients justifies the consumption of fortified food products containing various supplements to maintain homeostasis [14, 15]. The addition of potassium iodide, serving as a blocker of radioactive elements, is crucial for inhabitants residing in regions of the Republic with unfavorable radiation conditions [16]. The novelty of this study lies, firstly, in enriching the SN drink with vitamins, the prebiotic inulin, and the radioactive element blocker potassium iodide, representing a novel approach within the food industry of Kazakhstan. This approach is particularly relevant due to the adverse environmental and radiation conditions prevailing in the Republic. Secondly, the novelty of this study stems from the application of a consortium of original indigenous Lactobacillus strains sourced from traditional Kazakh products as starter cultures, which have not been previously used for the preparation of the dairy beverages.

Probiotics have enormous potential for nutritional development, treatment, and prevention. According to World Health Organization (WHO) and Food and Drug Administration (FDA) regulations, although probiotics are predominantly members of the genera Lactococcus and Lactobacillus have GRAS status (generally considered safe), their safety must be assured and confirmed [17, 18]. The SN drink contains a consortium of strains that have not yet been included in new beverages, so the safety of the SN drink needs to be assessed.

The aim of this study is to develop a new SN fortified with vitamins, the prebiotic inulin, and the radioactive element blocker potassium iodide and evaluate its physicochemical and sensory properties, shelf life, and safety.

Materials and Method

Cultivation, storage, and preliminary evaluation of probiotic starter cultures

A consortium of starter cultures was assembled from lactic acid bacteria with probiotic potential, namely: *L. casei* 1A, *L. paracasei* 2A, and *L. brevis* 4 LB. Starters were isolated from Kazakh fermented dairy foods and identified in our early works [21, 22]. These strains have high acid-producing, antimicrobial and adhesive activity, acid (pH 3.0), and bile salts (0.4%) resistance. The biocompatibility of these strains was demonstrated by co-cultivation on MRS agar using the perpendicular streak method. Strains 2A, 4 LB, and 1A were stored in sterilized skim milk tubes. To grow Lactobacillus cultures, MRS broth and MRS agar produced by HiMedia Laboratories Pvt. Ltd. (India) were used. The microorganisms were incubated for 24 - 48 h at 37 °C. Cultures of Lactobacillus spp., grown abundantly under optimal conditions on a MRS agar medium, were washed off and transferred to a cryoprotective medium (60% nutrient medium, 10% sucrose (extra pure, TMMEDIA, India), 20% glycerol (Applichem Gmbh, Germany), autoclaved at 121 °C for 15 min). Aliquots of 1.0 ml of culture suspensions were transferred into plastic ampoules with screw caps and maintained as frozen stock at -80 °C in freezer (New Brunswick U570 - 86, Netherlands).

Preparation of the SN drink

Natural milk with a fat percentage of 3.8% from "Astana-Onim" dairy farm (Tselinograd district, Akmola region, RK) was sterilized at 0.5 atm for 20 min (112 °C), followed by immediate cooling to a temperature in the range of 37 - 40 °C. Then 3 (%) starter cultures, 5 (%) inulin and a vitamin-mineral premix (vitamins A, B1, B2, C and potassium iodide) were added to the reaction mixture (Table 1). After stirring for at least 15 min to distribute the culture evenly, the mixture was left alone for fermentation at 37 °C until a milky white clot with an acidity in the range of 70 - 105 (^{0}Th) or 0.63 - 0.95 (% l.a.) was formed. After fermentation (6 - 8 h), the SN drink

Table 1: Composition of the starter and additives in the SN drink.

The starter inoculum, additives, and drink matrix	Product manufacturers	Composition of the SN drink recipe	Acceptable quantities	Ref.
Consortium	Biobank of industrial microorganisms§	3.00%	1.0 - 3.0%	[19]
L. casei 1A*		10.0 g		
L paracasei 2A*		10.0 g		
L. brevis 4LB**		10.0 g		
Inulin from chicory root	Thermo scientific	5.00%	up to 5.0 %	[5]
Vitamin A	Sigma-aldrich	0.9 mg	0.5 - 1.0 mg	[20]
Vitamin B1		1.5 mg	1.0 - 1.5 mg	
Vitamin B2		1.9 mg	1.0 - 2.5 mg	
Vitamin C		85 mg	50- 120 mg	
Potassium iodide		0.14 mg	0.14 ± 0.03 mg	
Natural milk	Dairy plant "Astana-Onim"§§	1000 ml		-

Note: §= Biobank of industrial microorganisms in Republican Collection of Microorganisms of the Ministry of Healthcare of the Republic of Kazakhstan, Astana; §§= JSC "Astana-Onim," Krasnoyarka Village, Akmola Region, Kazakhstan; *= laboratory isolates; and **= B-RKM 0610 - collection depository number of biobank of industrial microorganisms in republican collection of microorganisms.

was cooled for 30 min, stirred for 8 - 12 min, and cooling continued to 25 ± 2 ˚C. The SN drink (100 ml) was aseptically transferred into 250 ml glass bottles (Duran lab bottles), tightly sealed with screw caps, and transferred to refrigerator (Samsung RL62ZBSH, Korea) at 4 ˚C [23]. Samples for physicochemical testing and sensory evaluation were taken 24 h after finishing fermentation (day 1) and then at weekly intervals (days 7, 14, 21, and 28).

Determination of the physicochemical properties of the SN drink

Physicochemical indicators were determined by colleagues in the accredited laboratory of the RSE "Center for Sanitary and Epidemiological Expertise" at the Medical Center of the Administration of the President of the Republic of Kazakhstan.

Determination of the pH value and TA of SN drink during refrigerated storage

The pH value was measured using a digital pH meter (Metrohm, Switzerland) model 827. TA was estimated according to the technical specification (ISO/TS 11869|IDF/RM 150:2012) [24]. TA "I" was calculated in the millimoles of sodium hydroxide per 100 g by the reference methodology. To quantify the test results in grams of lactic acid per 100 g of the product (% l.a.) the next formula was used:

$$W = I \times 0.09$$

Where "W" represents the percentage of lactic acid in TA, while "I" represents the amount of sodium hydroxide in millimoles per 100 g in TA [24].

Conversion between different reference systems for TA was carried out using an online resource [25]. Tests for pH and TA were performed in triplicate.

Determination of the viability of lactobacilli in the SN drink

The viable number of lactobacilli was assessed by plate counting. Briefly, 10 ml of the sample was measured aseptically and homogenized with 90 ml of sterile saline (0.85%, w/v) to obtain a dilution series. The appropriate dilution factor was determined, and serial dilutions of the culture were plated in duplicate on MRS agar, then incubated under microaerophilic conditions for 48 h at 37 ˚C. After incubation, bacterial colonies were counted, and the number of lactobacilli was calculated according to IS 16068 [26]. Results were presented as log CFU/ml. Three replicates were performed for each sample.

Sensory evaluation

Sensory analysis of SN samples on days 1, 7, 14, and 21 after refrigerated storage was conducted according to Hekmat et al. [27] with minor modifications by a commission consisting of 14 selected participants and specially trained employees of JSC "Astana-Onim" and students from L.N. Gumilyov Eurasian National University, Astana, Kazakhstan. Experts aged 18 - 23 years (7 males and 7 females) were accommodated in the room. Participants did not have explicit taste preferences. All samples were numbered and randomly presented to the experts. Clean water was provided for rinsing the mouth between tastings. The following evaluation criteria were established: consistency, color, odor, taste, and sensory experts were asked to assess the overall acceptability of each sample on a 9-point hedonic scale, where 1 - dislike, 5 - neutral, and 9 - like very much.

Evaluation of the acute toxicity of the SN drink

This study was carried out in strict accordance with the recommendations of preclinical drug studies of Kazakhstan. The protocol was approved by the local ethics committee of the RSE "National Biotechnology Center" (Permit Number: protocol 1, 19.01.2021). All efforts were made to minimize suffering.

Effect of the SN drink consumption at the maximum achievable experimental dose on the body weight of laboratory mice

The potential intragastric toxicity of the SN drink was studied and analyzed, just like it was by colleagues from oth-

er laboratories [28-30]. Mice were orally gavage with the SN drink before feeding using a sterile tube leading through the throat into the stomach. Unanesthetized laboratory white outbred CD-1 mice of both sexes, aged 8 weeks and weighing between 28 - 32 g, were administered the SN drink once a day for 2 weeks. The experimentally achievable dose was 50 ml per kg of animal body weight. For a mouse weighing 30 g, the volume of the administered drink was 1.5 ml (50 ml divided by 33.3), which is equivalent to 2.0 × 8 log (CFU) per mouse. Since the SN drink action may depend on the physiological condition of the animals, which can change under the influence of several external factors, all studies were conducted at the same time in the morning before noon. The number of mice consisted of 12 female mice and 12 male mice. Groups 1 and 3 are the model samples with 6 male mice and 6 female mice, respectively, that consumed the SN drink. Groups 2 and 4 are control samples with 6 male and 6 female mice consumed drinking water. The animal body weight was measured before the SN drink consumption, one week, and two weeks after the SN drink consumption.

Assessment of the effect of the SN drink consumption at the maximum achievable experimental dose on the behavior and general condition of mice

The general condition of mice and behavioral features in motor activity or lethargy, sleep, and somatomotor activity, as well as changes in fur, eyes, skin and mucous membranes, tremors, behavior patterns, convulsions, salivation, diarrhea, local damage, presence and nature of seizures, frequency and respiration depth and fecal quantity and consistency, were assessed daily for 2 weeks.

Evaluation of the SN drink allergic effect

Allergy test of the SN drink on the skin in guinea pigs

Five albino guinea pigs weighing 550 - 600 g were taken to the experiments. Three drops of the tested SN drink were applied to a 2 × 2 cm shaved area of the skin on the lateral surface of the albino guinea pigs' torso (Figure 1a). This application occurred 5 times per week for 2 weeks, resulting in a total of 10 applications. Skin reaction was assessed daily using a skin test scale. The sites are graded as 1 + (erythema), 2 + (edema or vesiculation < 50% of the test site), 3 + (edema or vesiculation > 50% of the test site), (questionable), or Ir (irritant) [31].

Allergy test of the SN drink on the rabbit conjunctiva

The skin application method (a) and conjunctival test (b) staging for the SN drink treatments. (a) Five albino guinea pigs weighing 550 - 600 g were taken to the experiments (Figure 1b). Three drops of the tested SN drink were applied to a 2 × 2 cm shaved area of the skin on the lateral surface of the albino guinea pigs' torso. (b) Three male New Zealand rabbits weighing 3.0 - 3.5 kg were used in the experiments. One drop of the SN drink was applied under the upper eyelid of the rabbit, and one drop of water was applied into the second eye as a control. The response was observed after 15 min (rapid reaction) and after 24 - 48 h (delayed hypersensitivity).



Figure 1: The skin application method **(a)** and conjunctival test **(b)** staging for the SN drink treatments. Five albino guinea pigs weighing 550 - 600 g were taken to the experiments. Three drops of the tested SN drink were applied to a 2 × 2 cm shaved area of the skin on the lateral surface of the albino guinea pigs' torso. B: Three male New Zealand rabbits weighing 3.0 - 3.5 kg were used in the experiments. One drop of the SN drink was applied under the upper eyelid of the rabbit, and one drop of water was applied into the second eye as a control. The response was observed after 15 min (rapid reaction) and after 24 - 48 h (delayed hypersensitivity)

Ethics approval of research

Research work with laboratory animals was performed in accordance with generally accepted ethical standards for the treatment of animals, based on standard operating procedures that comply with the rules adopted by the European convention for the protection of vertebrate animals used for research and other scientific purposes [33].

Methods of statistical analysis

Statistical analysis of the TA and pH values of the SN drink and the survival of lactobacilli in the SN drink was carried out using a t-test and microsoft excel 97. The average values of the 3 measurements and their standard deviations (SD) were calculated. Confidence intervals (CI) for the measurements at 95% were used. If the CI of two measurements overlapped in the diagrams, the difference was considered statistically insignificant, and hypothesis H_0 was accepted. Intergroup differences in body weight of mice were assessed using the nonparametric Mann-Whitney U test. To determine whether the difference between the rank sums in the control and experimental groups was statistically significant, the p - value was compared with a significance level of $\alpha = 0.05$. The difference in the weight of mice was considered statistically insignificant and the hypothesis H_0 was accepted if the p value $\geq \alpha$.

Results and Discussion

Recently, a series of *in vivo* studies have been conducted showing that bacteria associated with fermented milk products behave as probiotics with positive effects in the prevention and treatment of various diseases [34, 35]. In this study, the SN drink (Table 2) was prepared and its physicochemical and sensory properties during the refrigerated storage, shelf life and safety was studied.

Physicochemical properties of the SN drink

In partnership with employees of JSC "Astana-Onim" we assessed the physicochemical properties of the SN drink. According to the data provided (Table 2), the SN drink complies with regulatory documents of Eurasian Customs Union [36, 37].

The SN drink contains the types of lactobacilli that are found in drinks and other products that have proven to be healthy probiotics [38-41]. Due to the facultative heterofermentative nature of *L. casei*, hexose sugars in the food matrix are almost wholly fermented to lactic acid and acetic acid. Therefore, strains of the *L. casei* group show a natural tolerance for acid stress [38]. There are reports that *L. paracasei* inhibits well-known pathogens and suppresses the colonization of *C. albicans* in the intestine [39]. *L. brevis* acts as a functional milk starter with high production of γ-aminobutyric acid [40, 41].

It was found that the physicochemical properties of the SN drink, including the concentration of fat, protein, solids milk solids non-fat, acidity and conductivity meet the established standards of the Republic of Kazakhstan [36] and Eurasian Customs Union [37].

TA, pH value, and viability of lactobacilli of the SN drink during storage

The minimum concentration of probiotics in a product that serves as a delivery system to produce health benefits varies in scientific journals [42-46]. In general, probiotic concentrations in supplemented media should be above the minimum recommended therapeutic level of 6 log CFU/ml and within the minimum recommended daily dose of 1 × 8 log to 1 × 9 log cells. The main obstacle to achieving and maintaining the required levels of probiotic concentrations is the dependence on the strain properties of probiotic organisms and poor survival due to increased acidity, oxygen tension, high redox potential of the environment, and nutrient depletion [47]. To overcome these obstacles, three strains with probiotic properties and inulin with prebiotic activity were used (Table 1). In the work of Parhi et al. [48] the addition of inulin (3%, 4%) to sucrose and lactose systems significantly increased the stability of *L. plantarum* during refrigerated storage. The survival of probiotics during storage is significantly affected by the pH and TA of foods [49]. Low pH values increase the concentration of undissociated organic acids in fermentation products, thereby enhancing the bactericidal effect of these acids. When stored in a refrigerator at a temperature of 4 °C, metabolism slows down, which prevents increased accumulation of organic acids and complete depletion of the environment in carbohydrates for 4 weeks. Figure 2 shows a gradual increase in TA and the same moderate decrease in pH values during storage. Thus,

Table 2: Physicochemical properties of the SN drink¥.

Name of indicators	Study results	Main indicators	Regulatory documents
Mass fraction of fat not less (%)	3.74	0.10 - 9.90	[36]
Mass fraction of milk solids non-fat§ (%)	8.37	≥ 7.80	
Acidity (°Th)	96.7	70 - 140	
Mass fraction of protein (%)	3.88	2.60 - 4.00	
pH	4.4	-	-
Density (kg/m³)	1057	-	-
Mass fraction of lactose (%)	3.2	-	-
Mass fraction of salts (%)	0.96	-	-
Conductivity (mS/cm)	6.78	-	[37]
Sample temperature (°C)	24.63	-	-

Note: ¥ = The number of lactobacilli, inulin, and vitamin-mineral mixture is presented in table 1. § = Milk solids not fat (MSNF).

TA increases and pH decreases during storage due to the persistence of metabolic and enzymatic activity of mixed cultures during low-temperature storage, which is in good agreement with the other research [4, 5, 48-50].

The increase of organic acids is an important marker of bacterial activity in a drink. The SN drink, containing the prebiotic inulin and a vitamin and mineral supplement, showed an increase in TA by 0.37% from 0.87 (day 1) to 1.24 (day 28) and a decrease in pH by 0.24 units from 4.4 (1st day) to 4.16 (28th day) at the end of storage (Figure 2). Thus, a moderate drop in pH and an increase in TA may be a consequence of moderate activity of lactobacilli during storage of the drink. Natural milk proteins denatured after sterilization may also indirectly promote lactobacilli activity/viability by acting as a buffer. Some studies support the prebiotic activity of peptides and amino acids resulting from protein hydrolysis [47]. The SN drink contains the prebiotic inulin, which may also stimulate the growth of lactobacilli in the SN drink [48, 50]. Thus, the process of storing the SN drink in figure 2 and figure 3 can hypothetically be represented as follows. Metabolism of lactose in the milk matrix by a consortium of lactobacilli leads to the formation of lactic acid and other secondary metabolites, which lower the pH and have a bactericidal effect. The buffer environment created by denatured milk proteins, peptides, and amino acids resulting from milk sterilization and possible extracellular proteolysis of lactobacilli [47, 49, 51-55] weakens the effects associated with acidification and thereby helps save lactobacilli from death. The added inulin and the resulting peptides and amino acids activate the metabolism of probiotics. As a result, the expected decrease in the survival rate of lactobacilli due to depletion of the main growth factors or their limited availability, acidification of the drink, and other unfavorable environmental conditions in the milk matrix during storage on the 21st day of storage does not occur. Only on the 28th day, a decrease in cell concentration by 0.6 logarithmic units is observed from 8.1 to 7.5 logarithmic units (Figure 3).

Overall, the total lactobacilli count in the SN drink remained at 8.1 log CFU/ml (1.3 × 8 log CFU/ml) live microorganisms during the 21 day storage period and decreased to 3.2 × 7 log CFU/ml by 28 day storage of the drink, i.e., 4.1 times less (Figure 3). Both survival parameters are in good agreement with the maximum permissible concentrations of probiotics in foods [42-46]. The fact that the overall viability of lactobacilli was statistically unchanged over 21 days of refrigerated storage indicates that our beverage formulation provides a suitable nutritional matrix for the growth and viability of the selected probiotic starter cultures [23]. Since the total number of lactobacilli was measured during storage, it is unknown which of the three strains *L. casei* 1A, *L. paracasei* 2A, and *L. brevis* 4LB is more stable under storage conditions, but in scientific periodicals, one can find similar studies on the storage of synbiotic drinks with related species of lactobacilli in a single species design [4, 5, 50]. In the work of Rizal et al., [4] the viability of *L. casei* in green grass jelly synbiotic drink decreased by 0.59 log units when stored at 10 °C, from 10.10 to 9.51 (log CFU/ml) or 4 times less from 1.26 × 10 log to

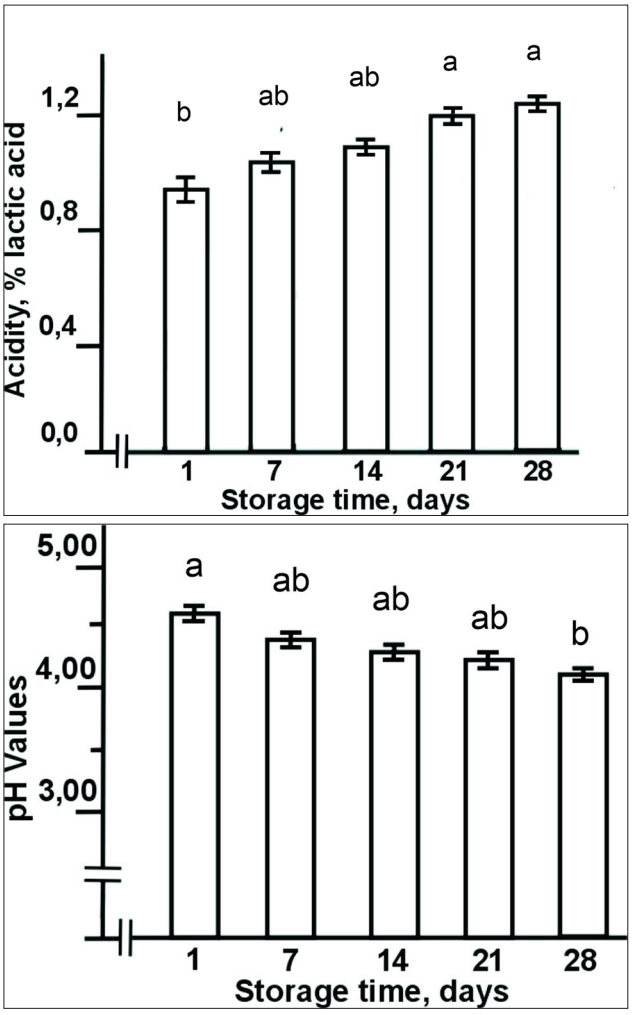

Figure 2: Change in the titratable acidity **(a)** and pH; and **(b)** of the SN drink during storage at 4 °C. Error bars represent SD (n = 3). The same letters indicate no statistically significant differences (p > 0.05).

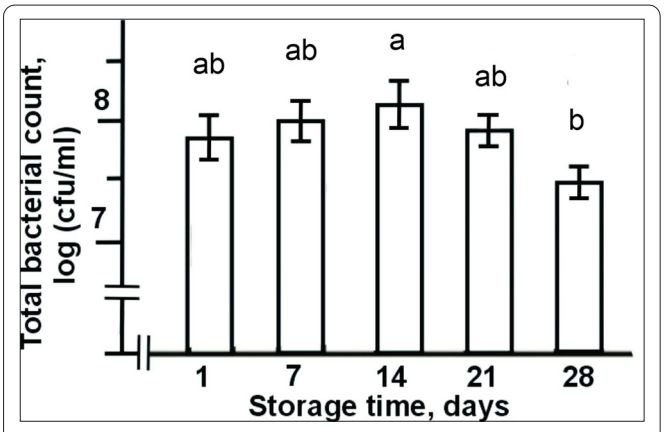

Figure 3: Measuring the concentration of lactobacilli in the SN drink during storage at 4 °C. Error bars represent SD (n = 3). The same letters indicate no statistically significant differences (p > 0.05).

3.24 × 9 log (CFU/ml). The total lactic acid content increased by 0.39% (l.a.), i.e., from 0.48% to 0.87% and the pH value decreased by 0.3 units, i.e., from 4.08 to 3.78. In contrast, Paseephol et al. [50] documented that the viability of *L. casei* LC-01 strain increased during 28 days of yogurt storage at

4 °C after the addition of inulin. The survival of probiotic in yogurt with inulin was approximately one log unit higher than in yogurt without inulin at the end of 28 days of storage. In the work of Falah et al. [5] synbiotic yogurt contained both the native probiotic strain *L. brevis* PML1 and inulin. The authors found growth of the strain when stored at 4 °C in the presence of inulin and suggested that this probiotic with α-galactosidase activity, in addition to consuming simple sugars and proteins, additionally metabolizes the prebiotic inulin by hydrolyzing oligosaccharides. The probiotic concentration is replenished through the metabolism of inulin and proteolytic products in the milk matrix [5, 48, 50-55] and the consistency of the lactobacilli concentrations in the SN drink in figure 3 could be the result of simultaneous increase in the survival rate of one (two) strain and an equivalent decrease in the survival rates of the other two (one) strains, as discussed earlier. The overall viability of probiotic microorganisms in the SN drink is maintained during 21 days of storage at + 4 °C at a level of 1.3 × 8 log CFU/ml and decreases by 0.6 log to a probiotic concentration of 3.2 × 7 log CFU/ml per 28 days of storage, remaining within the permissible limits for probiotic concentrations for drinks according to the scientific reports [42-46]. Therefore, the viability of the starter cultures of the SN drink, due to the kinetics of survival of probiotic lactobacilli and their acidifying activity during storage in a refrigerator at 4 °C, is determined by a time interval of 21 - 28 days (Figure 3).

Sensory evaluation

Table 3 shows the results of the sensory evaluation of SN drink. According to the total evaluation scores, the SN drink received the highest total scores after 1 day of storage. SN drink had a slightly viscous consistency, homogeneous mass with grit. It had a good taste, aroma, and a creamy color.

Ertekin et al. [56] found that the odor and taste evaluation of kefir samples with and without inulin did not differ. In our study, the color of the SN drink with inulin and vitamin and mineral supplementation did not change, while the sensory response regarding smell and taste changed on the seventh and 14th day of storage (Table 3). Apparently, the addition of vitamins, minerals, and inulin resulted in a change in the mean consistency score. The scores characterizing odor and taste changed, so that by the 14th day of storage in the refrigerator with hermetically sealed packaging of the samples, the quality of the SN drink approached neutral scores on the hedonic scale, and the drink acquired a sour taste. Based on this observation, we conclude that the shelf life of the SN drink extends up to 14 days of refrigerated storage. Therefore, we estimate that the shelf life of the SN drink is 14 days, and additional studies are required to increase it.

Study of the acute toxicity of the SN drink

Effect of the SN drink consumption at the maximum achievable experimental dose on the body weight of laboratory mice

In an experiment on the consumption of the maximum possible dose of the beverage by laboratory animals, the aim was to evaluate the product's toxicity. The toxicity of the beverage was determined by the potential change in body weight of the test animals. When consuming a toxic beverage, the body weight of the animals in the experimental group is likely to decrease compared to the control group that did not receive the beverage. To answer this question, a statistical analysis was conducted using the Mann-Whitney U-test at a significance level of α = 0.05 (Table 4). The final result showed no effect of the beverage on the body weight of the test animals. Thus, the SN drink at a dose of up to 8 log CFU per animal does not

Table 3: Sensory properties of the SN drink.

Attribute	SN drink							
	Day 1	Score	Day 7	Score	Day 14	Score	Day 21	Score
Consistency	Slightly viscous	8.4 ± 0.6	Viscous	7.9 ± 0.7	Viscous	7.3 ± 0.6	Slight syneresis and viscous	5.7 ± 0.6
Color	Creamy	9	Creamy	9	Creamy	9	Creamy	9
Odor	Good aroma	7.8 ± 0.7	Fermented	7.0 ± 0.5	Fermented	5.4 ± 0.8	Fermented	5.1 ± 0.8
Taste	Good taste	8.5 ± 0.5	Sweet and sour	6.1 ± 0.8	Acidic	4 ± 0.7	Highly acidic	3.1 ± 0.9
Total score	-	8.4 ± 0.4	-	7.6 ± 0.8	-	6.4 ± 1.8	-	5.7 ± 2.1

Table 4: The SN drink effect on the body weight of mice after consumption.

Group number	Tested beverage	Body weight of mice at baseline (g)	Body weight of mice after one week of SN drink consumption (g)	Body weight of mice after two weeks of SN drink consumption (g)
Males (n = 6)§				
1	SN drink¥	32.0 ± 0.9 p = 0.9564§§	31.1 ± 0.7 p = 0.6608	32.8 ± 0.9 p = 0.3176
2	Water	32.1 ± 1.2	31.8 ± 1.5	31.1 ± 1.4
Females (n = 6)				
3	SN drink¥	28.5 ± 0.9 p = 0.9509	26.7 ± 1.3 p = 0.7133	28.4 ± 1.3 p = 0.5402
4	Water	28.4 ± 0.4	27.4 ± 1.5	29.7 ± 1.5

Note: § = n number of animals in the group; §§ = p (p – values) which are compared with a significance level of α = 0.05; and ¥ = The maximum achievable experimental dose, which was 50 ml/kg of animal body weight or ~2.0 × 8 log (CFU) per mouse.

affect body weight and is therefore considered safe after 7 and 14 days of oral administration to animals.

Effect of the SN drink consumption at the maximum achievable experimental dose on the behavior and general condition of mice

During the initial 24 h after SN drink consumption and throughout the entire two-week experiment, there were no noticeable alterations in the behavior, appearance, or motor activity of the laboratory mice. The mice did not show any significant changes in behavior and general conditions after administration of the SN drink (Table 5).

Evaluation of the SN drink allergic effect on the skin and conjunctiva of animal models

The allergic effects of the SN drink on the animals were assessed by using the skin application method on guinea pigs (Figure 1a) and the conjunctival test on rabbits (Figure 1b).

Test for allergy to the SN drink after application to the skin in guinea pigs

The results of assessing the allergic effect of the SN drink using the skin application method are presented in table 6.

Based on the data in table 6, it can be concluded that a skin application of the SN drink to guinea pigs does not damage their skin in the form of erythema or edema.

Test for allergy to the SN drink after application to the rabbit conjunctiva

The test results confirm the absence of a conjunctival reaction in rabbits after 15 min and 24 - 48 h after the instillation of the SN drink (Table 7). After a visual assessment of the condition of the conjunctiva, cornea, and eyelids of the animals' eyes, it was found that there were no differences between the control eye and the experimental eye. There was no hyperemia throughout the study, swelling of the eyelids and discharge from the eyes, redness and swelling of the lacrimal ducts, conjunctiva, and sclera. There were also no cases of corneal opacity identified.

From the data presented in table 7, it is evident that the SN drink does not have any allergic effect as determined in the conjunctival test.

Consequently, studies of acute toxicity and allergenic effects on animals (Table 3, table 4, table 5 and table 6) have demonstrated that the SN drink does not cause harm to the bodies of laboratory animals, and there is no allergic effect observed in animals. Based on the results of the study, the SN drink is classified as a low-hazard substance, belonging to class 4 in terms of danger [57].

Table 5: Effect of the SN drink on the behavior and general condition of mice for 2 weeks of consumption.

Behavior and general conditions of animals¥	Animal group options			
	males (n = 6)§		females (n = 6)§	
	Group 1 (Consumed the SN drink¥)	Group 2 (Consumed water)	Group 3 (Consumed the SN drink¥)	Group 4 (Consumed water)
Intensity and nature of motor activity	Mice were active, coordination of movements was not impaired			
Presence and nature of seizures	Absent			
Condition of hair and skin	No changes were detected (fur was white, clean, and smooth)			
Condition and color of mucous membranes	No changes were detected			
Reaction to sound and pain stimuli	The animals always responded			
Animal death	Did not occur			
Urination (urine color)	No changes detected			
Defecation (consistency, color)	No changes detected			

Note: § = Number of animals in the group; and ¥ = The maximum experimental dose, equal to 50 ml per kg of animal body weight or 2.0 × 8 log (CFU) per mouse.

Table 6: Allergic effect of the SN drink in the cutaneous application test on guinea pigs.

Variant	Number of applications	Number of animals with a positive reaction, namely the presence of erythema/edema (males (n = 5)§)	Irritant effect
+ SN drink	10	0/0	Missing

Note: § = Number of animals in the group.

Table 7: Allergic effect of the SN drink in the conjunctival test on rabbits.

Variant	Number of animals with a positive reaction in the conjunctival test (males (n = 3)§)		
	After 15 min	After 24 h	After 48 h
Experimental + SN drink	No reaction	No reaction	No reaction
Control + water	No reaction	No reaction	No reaction

Note: § = Number of animals in the group.

Conclusions

- The new SN fortified with vitamins, prebiotic inulin, and the radioactive element blocker potassium iodide was developed.
- The physicochemical properties of the SN drink, including the mass fraction of fat, milk solids non-fat (MSNF), protein content, as well as acidity and electrical conductivity, complies with the parameters of fermented milk products in the Eurasian Customs Union countries.
- The overall viability of probiotic microorganisms of SN drink is maintained for 28 days within the permissible range for beverages according to scientific data.
- According to sensory analysis, the shelf life of the SN drink is determined to be 14 days.
- *In vivo* acute toxicity studies in mice have shown that the SN drink does not cause harm to the bodies of laboratory animals and does not alter the behavior, appearance, or motor activity of laboratory mice. Trials conducted on guinea pigs and rabbits to assess the allergic reaction to the SN drink did not reveal any signs of allergy.
- The results of the study indicate that the new fermented milk drink, namely SN drink is safe and suitable for human consumption.

Acknowledgements

This research was funded by the Science Committee of the Ministry of Science and Higher Education of the Republic of Kazakhstan within the framework of Targeted funding of the Program BR18574066 "Creation of a biobank of industrially significant microorganisms in the field of biotechnology, ecology, and agriculture".

Conflict of Interest

None.

References

1. Leddomado LS, Silva R, Guimarães JT, Balthazar CF, Ramos GL, et al. 2021. Technological benefits of using inulin and xylooligosaccharide in dulce de leche. *Food Hydrocoll* 110: 106158. https://doi.org/10.1016/j.foodhyd.2020.106158
2. Alhamdan AM, Al Juhaimi FY, Hassan BH, Ehmed KA, Ahmed IAM. 2021. Physicochemical, microbiological, and sensorial quality attributes of a fermented milk drink (laban) fortified with date syrup (dibs) during cold storage. *Foods* 10: 3157. https://doi.org/10.3390/foods10123157
3. Yu J, Sun M, Jiang S, Jiang C, Mu G, et al. 2024. Oral administration of fermented milk from co-starter containing *Lactobacillus plantarum* Y44 shows an ameliorating effect on hypertension in spontaneously hypertensive rats. *Foods* 13: 641. https://doi.org/10.3390/foods13050641
4. Rizal S, Nurainy FS, Amelia JR. 2020. The effects of low-temperature storage on the viability of *Lactobacillus casei* and the stability of antibacterial activity in green grass jelly synbiotic drinks. *Biodiversitas* 21(8): 3826-3831. https://doi.org/10.13057/biodiv/d210851
5. Falah F, Vasiee A, Yazdi FT, Behbahani BA. 2021. Preparation and functional properties of synbiotic yogurt fermented with *Lactobacillus brevis* pml1 derived from a fermented cereal-dairy product. *BioMed Res Int* 2021: 1-9. https://doi.org/10.1155/2021/1057531
6. Zheng J, Wittouck S, Salvetti E, et al. 2020. A taxonomic note on the genus *Lactobacillus*: Description of 23 novel genera, emended description of the genus *Lactobacillus Beijerinck* 1901, and union of *Lactobacillaceae* and *Leuconostocaceae*. *Int J Syst Evol Microbiol* 70(4): 2782-2858. https://doi.org/10.1099/ijsem.0.004107
7. Hill C, Guarner F, Reid G, Gibson GR, Merenstein DJ, et al. 2014. Expert consensus document. The international scientific association for probiotics and prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nat Rev Gastroenterol Hepatol* 11(8): 506-514. https://doi.org/10.1038/nrgastro.2014.66
8. Wang Y, Wu J, Lv M, Shao Z, Hungwe M, et al. 2021. Metabolism characteristics of lactic acid bacteria and the expanding applications in food industry. *Front Bioeng Biotechnol* 9: 612285. https://doi.org/10.3389/fbioe.2021.612285
9. Gibson GR, Hutkins R, Sanders ME, Prescott SL, Reimer RA, et al. 2017. Expert consensus document: the international scientific association for probiotics and prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. *Nat Rev Gastroenterol Hepatol* 14(8): 491-502. https://doi.org/10.1038/nrgastro.2017.75
10. Swanson KS, Gibson GR, Hutkins R, Reimer RA, Reid G, et al. 2020. The international scientific association for probiotics and prebiotics (ISAPP) consensus statement on the definition and scope of synbiotics. *Nat Rev Gastroenterol Hepatol* 17(11): 687-701. https://doi.org/10.1038/s41575-020-0344-2
11. Manzoor S, Wani SM, Mir SA, Rizwan D. 2022. Role of probiotics and prebiotics in mitigation of different diseases. *Nutrition* 96: 111602. https://doi.org/10.1016/j.nut.2022.111602
12. Wan X, Guo H, Liang Y, Zhou C, Liu Z, et al. 2020. The physiological functions and pharmaceutical applications of inulin: a review. *Carbohydr Polym* 246: 116589. https://doi.org/10.1016/j.carbpol.2020.116589
13. Hughes RL, Alvarado DA, Swanson KS, Holscher HD. 2022. The prebiotic potential of inulin-type fructans: a systematic review. *Adv Nutr* 13(2): 492-529. https://doi.org/10.1093/advances/nmab119
14. Vitamin A and Carotenoids. [https://ods.od.nih.gov/factsheets/VitaminA-HealthProfessional/] [Accessed May 20, 2024]
15. Roa FJ, Peña E, Gatica M, Escobar-Acuña K, Saavedra P, et al. 2020. Therapeutic use of vitamin C in cancer: physiological considerations. *Front Pharmacol* 11: 211. https://doi.org/10.3389/fphar.2020.00211
16. Ibrayeva D, Bakhtin M, Kashkinbayev Y, Kazymbet P, Zhumadilov K, et al. 2020. Radiation situation in the territories affected by mining activities in stepnogorsk areas, Republic of Kazakhstan: pilot study. *Radiat Prot Dosimetry* 189(4): 517-526. https://doi.org/10.1093/rpd/ncaa068
17. Salminen S, von Wright A, Morelli L, Marteau P, Brassart D, et al. 1998. Demonstration of safety of probiotics – a review. *Int J Food Microbiol* 44: 93-106. https://doi.org/10.1016/S0168-1605(98)00128-7
18. Marteau P, Seksik P, Jian R. 2002. Probiotics and health: new facts and ideas. *Curr Opin Biotechnol* 13(5): 486-489. https://doi.org/10.1016/S0958-1669(02)00368-3
19. CXS 243-2003; CODEX Standard for Fermented Milks. FAO: Rome, Italy; WHO: Geneva, Switzerland. [https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXS%2B243-2003%252FCXS_243e.pdf] [Accessed May 20, 2024]
20. GOST 32252-2013 Drinking Milk for the Nutrition of Preschool and School-age Children. Technical Conditions, Moscow. [https://files.stroyinf.ru/Data/558/55861.pdf] [Accessed May 20, 2024]
21. Nagyzbekkyzy E, Abitayeva G, Anuarbekova S, Shaikhina D, Li K, et al. 2016. Investigation of acid and bile tolerance, antimicrobial activity and antibiotic resistance of *Lactobacillus* strains isolated from Kazakh dairy foods. *Asian J Appl Sci* 9(4): 143-158. https://doi.org/10.3923/ajaps.2016.143.158

22. Abitayeva G, Sarmurzina Z, Bissenova G, Mussabayeva B, Tultabayeva T. 2022. Characteristics of probiotic strains for the development of preventive drinks. *Microbiol Virol* 4(39): 141-151. https://doi.org/10.53729/MV-AS.2022.04.11
23. Bissenova GN, Sarmurzina ZS, Abitayeva GK, et al. 2023. Development of recipes for prophylactic milk and whey-based drinks and determination of their properties. *Microbiol Virol* 3(42): 158-175. https://doi.org/10.53729/mv-as.2023.03.10
24. ISO/TS 11869:2012 - Fermented Milks — Determination of Titratable Acidity — Potentiometric Method. [https://www.iso.org/standard/56875.html] [Accessed May 20, 2024]
25. Mullan, W.M.A. Calculator for Convert between Soxhlet Henkel degrees, Thörner Degrees, Dornic Degrees and Percentage Lactic Acid Milk Titratable Acidity Measurements [https://www.dairyscience.info/newcalculators/acidity/] [Accessed May 20, 2024]
26. IS 16068. 2013. Microbiology of Food and Animal Feed Stuffs - Horizontal Method for the Enumeration of Mesophilic Lactic Acid Bacteria - Colony-Count Technique.
27. Hekmat S, Morgan K, Soltani M, Gough R. 2015. Sensory evaluation of locally-grown fruit purees and inulin fibre on probiotic yogurt in Mwanza, Tanzania and the microbial analysis of probiotic yogurt fortified with Moringa oleifera. *J Health Popul Nutr 33*(1): 60-67.
28. Lazarenko LM, Babenko LP, Gichka SG, Sakhno LO, Demchenko OM, Bubnov RV, Spivak MY. 2021. Assessment of the safety of *Lactobacillus casei* IMV B-7280 probiotic strain on a mouse model. *Probiotics Antimicrob Proteins* 13(6): 1644-1657. https://doi.org/10.1007/s12602-021-09789-1
29. Zhou JS, Shu Q, Rutherfurd KJ, Prasad J, Birtles MJ, Gopal PK, Gill HS. 2000. Safety assessment of potential probiotic lactic acid bacterial strains *Lactobacillus rhamnosus* HN001, *Lb. acidophilus* HN017, and *Bifidobacterium lactis* HN019 in BALB/c mice. *Int J Food Microbiol* 56(1): 87-96. https://doi.org/10.1016/S0168-1605(00)00219-1
30. Pradhan D, Mallappa RH, Grover S. 2020. Comprehensive approaches for assessing the safety of probiotic bacteria. *Food Control* 108: 106872. https://doi.org/10.1016/j.foodcont.2019.106872
31. Ahmadullin RM, Muhammadiev RS, Valiullin LR. 2020. Experimental evaluation of allergenic properties of Bisphenol-5. In Proceedings of the BIO Web Conferences, pp 1-6.
32. Grammer LC, Greenberger PA. 2018. Patterson's Allergic Diseases. Wolters Kluwer Health: Philadelphia, USA.
33. European Union. 2010. Directive 2010/63/EU of the European parliament and of the council of 22 September 2010 on the protection of animals used for scientific purposes. *Off J Eur Union* 276: 33-79.
34. Aprea G, Del Matto I, Tucci P, Marino L, Scattolini S, et al. 2023. *In vivo* functional properties of dairy bacteria. *Microorganisms* 11(7): 1787. https://doi.org/10.3390/microorganisms11071787
35. Yamase Y, Huang H, Mitoh Y, Egusa M, Miyawaki T, et al. 2023. Taste responses and ingestive behaviors to ingredients of fermented milk in mice. *Foods* 12: 1150. https://doi.org/10.3390/foods12061150
36. Government standard: ST RK 1733-2015 Milk and Milk Products. General Technical Conditions. Astana. [https://files.stroyinf.ru/Data2/1/4293740/4293740673.pdf] [Accessed May 20, 2024]
37. Podorozhnyaya IV, Vetokhin SS. 2020. The comparative analysis of some physical-chemical indices of sour milk prepared on the industrial scale and in laboratory. Act Issu Dairy Ind Int Technol Quality Manag Syst 2: 422-431. https://doi.org/10.37442/978-5-6043854-1-8-2020-1-422-431
38. Qin D, Ma Y, Wang Y, Hou X, Yu L. 2022. Contribution of lactobacilli on intestinal mucosal barrier and diseases: perspectives and challenges of *Lactobacillus casei. Life* 12: 1910. https://doi.org/10.3390/life12111910
39. Deng K, Chen T, Wu Q, Xin H, Wei Q, et al. 2015. *In vitro* and *in vivo* examination of anticolonization of pathogens by *Lactobacillus paracasei* FJ861111.1. *J Dairy Sci* 98(10): 6759-6766. https://doi.org/10.3168/jds.2015-9761
40. Cataldo PG, Villegas JM, de Giori GS, Saavedra L, Hebert EM. 2020. Enhancement of γ-aminobutyric acid (GABA) production by *Lactobacillus brevis* CRL 2013 based on carbohydrate fermentation. *Int J Food Microbiol* 333: 108792. https://doi.org/10.1016/j.ijfoodmicro.2020.108792
41. Chang VHS, Chiu TH, Fu SC. 2016. *In vitro* anti-inflammatory properties of fermented pepino (*Solanum muricatum*) milk by γ-aminobutyric acid-producing *Lactobacillus brevis* and an *in vivo* animal model for evaluating its effects on hypertension. *J Sci Food Agric* 96(1): 192-198. https://doi.org/10.1002/jsfa.7081
42. Cunningham M, Vinderola G, Charalampopoulos D, Lebeer S, Sanders ME, et al. 2021. Applying probiotics and prebiotics in new delivery formats – is the clinical evidence transferable? *Trends Food Sci Technol* 112: 495-506. https://doi.org/10.1016/j.tifs.2021.04.009
43. Marco ML, Hill C, Hutkins R, Slavin J, Tancredi DJ, et al. 2020. Should there be a recommended daily intake of microbes? *J of Nutr* 150(12): 3061-3067. https://doi.org/10.1093/jn/nxaa323
44. Terpou A, Papadaki A, Lappa IK, Kachrimanidou V, Bosnea LA, Kopsahelis N. 2019. Probiotics in food systems: significance and emerging strategies towards improved viability and delivery of enhanced beneficial value. *Nutrients* 11(7): 1591. https://doi.org/10.3390/nu11071591
45. Martín I, Barbosa J, Pereira SI, Rodríguez A, Córdoba JJ, et al. 2023. Study of lactic acid bacteria isolated from traditional ripened foods and partial characterization of their bacteriocins. *LWT* 173: 114300. https://doi.org/10.1016/j.lwt.2022.114300
46. González-González F, Delgado S, Ruiz L, Margolles A, Ruas-Madiedo P. 2022. Functional bacterial cultures for dairy applications: towards improving safety, quality, nutritional and health benefit aspects. *J Appl Microbiol* 133(1): 212-229. https://doi.org/10.1111/jam.15510
47. Helland MH, Wicklund T, Narvhus JA. 2004. Growth and metabolism of selected strains of probiotic bacteria in milk- and water-based cereal puddings. *Int Dairy J* 14(11): 957-965. https://doi.org/10.1016/j.idairyj.2004.03.008
48. Parhi P, Song KP, Choo WS. 2021. Viability, storage stability and *in vitro* gastrointestinal tolerance of *Lactiplantibacillus plantarum* grown in model sugar systems with inulin and fructooligosaccharide supplementation. *Fermentation* 7: 259. https://doi.org/10.3390/fermentation7040259
49. Barajas-Álvarez P, González-Ávila M, Espinosa-Andrews H. 2023. Recent advances in probiotic encapsulation to improve viability under storage and gastrointestinal conditions and their impact on functional food formulation. *Food Rev Int* 39(2): 992-1013. https://doi.org/10.1080/87559129.2021.1928691
50. Paseephol T, Sherkat F. 2009. Probiotic stability of yoghurts containing jerusalem artichoke inulins during refrigerated storage. *J Func Foods* 1(3): 311-318. https://doi.org/10.1016/j.jff.2009.07.001
51. Bounouala FZ, Roudj S, Karam NE, Recio I, Miralles B. 2017. Casein hydrolysates by *Lactobacillus brevis* and *Lactococcus lactis* proteases: peptide profile discriminates strain-dependent enzyme specificity. *J Agric Food Chem* 65(42): 9324-9332. https://doi.org/10.1021/acs.jafc.7b03203
52. Lucatto JN, da Silva-Buzanello RA, de Mendonça SNTG, Lazarotto TC, Sanchez JL, et al. 2020. Performance of different microbial cultures in potentially probiotic and prebiotic yoghurts from cow and goat milks. *Int J Dairy Technol* 73(1): 144-156. https://doi.org/10.1111/1471-0307.12655
53. Mukhtar H. 2006. Biosynthesis of protease from *Lactobacillus paracasei*: kinetic analysis of fermentation parameters. *Indian J Biochem Biophys* 43(6): 377-381.
54. Alcántara C, Bäuerl C, Revilla-Guarinos A, Pérez-Martínez G, Monedero V, Zúñiga M. 2016. Peptide and amino acid metabolism is controlled by an OmpR-family response regulator in *Lactobacillus casei*. *Mol Microbiol* 100(1): 25-41. https://doi.org/10.1111/mmi.13299

55. García-Cano I, Rocha-Mendoza D, Ortega-Anaya J, Wang K, Kosmerl E, et al. 2019. Lactic acid bacteria isolated from dairy products as potential producers of lipolytic, proteolytic and antibacterial proteins. *Appl Microbiol Biotechnol* 103(13): 5243-5257. https://doi.org/10.1007/s00253-019-09844-6

56. Ertekin B, Guzel-Seydim ZB. 2010. Effect of fat replacers on kefir quality. *J Sci Food Agric* 90(4): 543-548. https://doi.org/10.1002/jsfa.3855

57. GOST 12.1.007-76 Occupational Safety Standards System. Noxious Substances. Classification and General Safety Requirements. [https://online.zakon.kz/Document/?doc_id=1037913&pos=1;-16#pos=1;-16] [Accessed May 20, 2024]