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Yeast whey-enriched bread: Nutritional profile and potential functional relevance

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ABSTRACT

Background: Microorganisms are an innovative biotechnological option for creating new yeast whey-enriched bread. Products obtained with the help of microorganisms can have a beneficial effect on human health and be successfully used in the food industry, as well as in the nutraceutical and pharmaceutical fields. The biotechnological potential of yeast and lactic acid bacteria is confirmed by their wide application in the fermentation of food products. At the same time, autochthonous strains in different matrices often demonstrate higher functional and technological indicators, which justify the microbiological perspective of their potential use in obtaining yeast whey-enriched bread. In this regard, it is of interest to study the potential of autochthonous cultures of yeast and lactic acid bacteria of ttkhmor - bread sourdough used in Armenia since ancient times.

Objective: The study aims to isolate indigenous yeasts from ttkhmor and explore their potential for developing prototype of enriched bread for health.

Novelty: This work advances prototype of enriched bread development by leveraging a two-stage co-cultivation of indigenous yeast and lactic acid bacteria in whey, yielding high yeast biomass and improving shelf life and spoilage

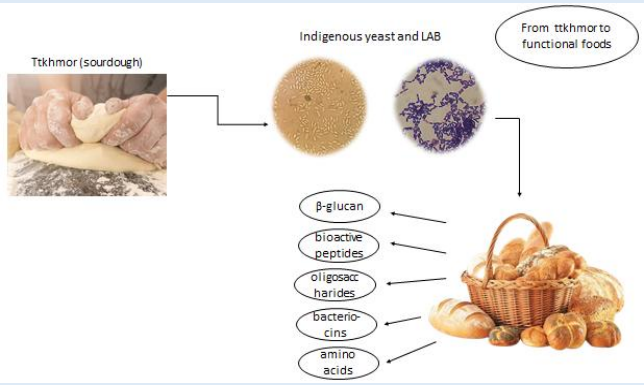
resistance versus commercial yeast controls—while maintaining comparable physicochemical quality. The approach links sustainable whey valorization with measurable technological and nutritional benefits.

Methods: Samples of ttkhmor and handicraft production dough were collected from rural areas of Armenia. Yeast α-glucosidase activity was determined in Yeletsky gasometric device. The nutritional value of bakery products was researched by analyzing the amino acid composition of yeast strain proteins. The quality indicators of bread were studied by physicochemical parameters. Sensory assessment of bread quality was carried out by closed tasting of the samples under study.

Results: More than 150 ttkhmor samples were collected from different geographical zones of Armenia. A total of 498 yeast isolates and 338 LAB isolates were obtained from the ttkhmor samples. Seven representative strains selected from the yeasts with high fermentation activity were subjected to species identification by sequencing. The yeast strain *Saccharomyces cerevisiae* Sevan-60 (deposited in Microbial Depository Center under the number MDC-9792) isolated from the ttkhmor of Tsaghkunk village, Sevan region (mountain zone) was selected based on its antimicrobial activity against *Bacillus subtilis* and biochemical characteristics. The biological value of the yeast biomass of strain Sevan-60 is characterized by a high content of essential and aromatic amino acids. The parameters used for strain evaluation were related to enzymatic activity and utilization of lactic acid as the sole carbon source.

Conclusion: Taken together, the above studies indicate the potential of using ttkhmor microorganisms in creating yeast whey-enriched bread. A method has been developed for two-stage co-cultivation of lactic acid bacteria and the indigenous *S. cerevisiae* Sevan-60 strain to obtain fermented whey with a high yeast biomass yield of 47 g/l for use in baking. Bread quality assessment showed that bread baked using fermented whey, with comparable physicochemical parameters (volume yield and porosity), was superior to the control bread prepared with commercial industrial yeast in terms of spoilage resistance and longer shelf life. At the same time, the experimental yeast whey-enriched bread combines all the useful components of a functional product characteristic of the combined use of yeast, LAB, and whey, in particular, high functional indicators of yeast protein as well as whey protein.

Keywords: Ttkhmor, yeast, LAB, whey proteins, baking, prototype of enriched bread, human health.



Graphical Abstract: Yeast and LAB as a source of bioactive compounds.

INTRODUCTION

The importance of functional foods is growing due to the demonstration of their positive impact on health. An important stage in the development of functional foods is the justification of the selection of raw materials and ingredients based on their biological activity [1-4]. Functional foods, due to their bioactive compounds, have antioxidant properties and demonstrate biological activity in the prevention of several serious diseases [5-6].

For thousands of years, bread has remained one of the main food sources in the human diet, and the production of sourdough bread was among the earliest biotechnological processes [7-8]. Bread can be considered a functional food due to its ability to serve as a carrier of various bioactive compounds, thereby improving its nutritional value and potential health benefits [9-11]. A review of studies on functional bakery products shows that bread is the most frequently analyzed product. The production of functional bread has become a trend [7]. Sourdough bread is one of the most widely consumed products worldwide [12-14]. Due to the lactic acid bacteria present in the sourdough microflora and metabolites partially produced by yeast, sourdough bread has a low glycemic index, high content of minerals and antioxidants [15-17]. However, bread is particularly prone to microbial attacks. Due to the potential health risks associated with the use of chemical preservatives to prevent microbial spoilage of bread, there is growing interest in biological methods in the baking industry [18-20]. Among methods of bread biopreservation, microbial fermentation using strains of lactic acid bacteria and yeast is currently of considerable interest due to their antimicrobial activity and ability to extend shelf life [21-26]. Compared to commercial baker's yeast, *Saccharomyces cerevisiae* sourdough yeast contains more amino acids, vitamins, and minerals, and also has an anti-inflammatory effect. Yeast protein biomass is a bioavailable product, which also contains trace elements (zinc, magnesium, iron) and B vitamins (riboflavin, biotin, folic acid) [27-28]. Yeast protein contains all the essential amino acids that are lacking in plant proteins. Essential amino acids, including lysine, histidine, methionine, valine, leucine, and isoleucine, play a critical role in various metabolic processes, and their deficiency can lead to multiple health risks [29]. Thus, yeast can significantly increase the nutritional value and functional properties of bread, making it a more attractive food product [30-31]. Regarding the use of *S. cerevisiae* in the production of fermented products, it is worth noting its broad functional potential for health. Thus, *S. cerevisiae* yeast has a beneficial effect on the intestinal microflora, which has a positive impact on various symptoms of gastrointestinal discomfort [32]. The probiotic activity of this yeast culture for the treatment of multiple types of diarrheas is determined by its antimicrobial, antitoxin, and immunomodulatory effects [33]. In addition, the β-glucan in the cell wall of S. cerevisiae yeast has potential prebiotic properties [34]. Mannoproteins contained in the cell wall of *S. cerevisiae* yeast also have high biological activity and antimicrobial properties [35]. Overall, *S. cerevisiae* yeast is considered an important model organism for modulating aging and validating bioactive compounds for health promotion in the functional food industry [36].

It is known that the use of whey in bakery is effective not only for improving the technological characteristics of bakery products (volume, porosity) but also provides sensory and nutritional benefits (increased calcium content, improved moisture retention in the finished product, which enhances the perception of freshness by consumers, increased microbiological safety) [37-38]. Whey proteins account for 20% of all milk proteins and have great potential as a basis for functional foods [39-

42]. The content of whey proteins in functional foods could help maintain blood pressure and reduce cardiovascular disease risk factors [43].

The study aimed to isolate, characterize, and select technologically valuable strains of S. cerevisiae from traditional sourdough ttkhmor for cultivation on whey, with the use of fermented yeast starter in bread baking to obtain a prototype of enriched bread.

METHODS

Selection of Yeast Strains: Fermentation and enzymatic capacities of active yeast strains were determined according to the methods described in the guides [44, 45]. The power for CO_2 formation was studied in Dunbar tubes. The dough-raising capacity (DRC) of yeast and osmosensitivity were determined using the pop-up ball method with standard and increased salt concentrations.

α-glucosidase and β-fructofuranosidase activities of yeast strains and the fermentation capacity of dough semi-finished products were determined in an Eletsky gasometric device.

Analysis of the quality indicators of dough and bread: The moisture content of the studied semi-finished products was determined using the Chizhova device. Titratable acidity was expressed in Neumann degrees. Physicochemical indicators of bread quality (volume yield, crumb porosity structure, crust character) were studied 16 hours after baking. Organoleptic assessment of bread quality and staling time was conducted using the closed-tasting method [45].

The resistance of experimental and control bread samples baked with commercial yeast to "potato" disease was determined by the degree of infection. The duration of the determination was up to 120 hours.

The amino acid composition of yeast proteins was determined using an automatic amino acid analyzer, AAA-339, manufactured by "Microtechn". Hydrolysis was carried out with 6N HCl at 110 °C for 24 hours [44].

Whey parameters: Unfiltered cottage cheese whey with a dry matter content of 6.4-6.5% and titratable acidity of 62-65°T was used for the experiment.

Molecular genetic analysis of yeast: The yeast genomic DNA was extracted and purified from the investigated strains for 18S rRNA PCR amplification. For the 18S rRNA gene amplification, the following primers FD1 (5'-ACCTGGTTGATCCTGCCAG-3') and RD1 (5'-TACAAAGGGCAGGGACAGG-3') were used. PCR amplification of the 18S rRNA gene was conducted under the following conditions: Initial denaturation: 95°C for 2 min; Cycling: 30 cycles of denaturation at 95°C for 30 sec, annealing at 59°C for 30 sec, extension at 72°C for 2 min; Final extension: 72°C for 5 min. DNA electrophoresis was conducted using a 0.8% agarose gel (Agarose I™, VWR® tablets) in 40 mM Tris-Acetate-EDTA buffer, pH 8.0, with the gel run at 100 volts for 35 minutes. DNA bands were visualized using "Millipore" GelRed® nucleic acid stain. NEB's TriDye™ 1 kb Plus DNA ladder was employed as a reference for agarose gel sizing [46]. Sequencing was carried out at Macrogen Company (Korea). For comparative analysis of nucleotide sequences, the BLAST program was used.

Statistical analysis: All studies of yeast enzymatic activity were performed in 5 replicates. The obtained data were statistically analyzed using the mean square deviation method. Statistical significance level was considered at p-value <0.05.

RESULTS AND DISCUSSION

Biological properties of strains DRC and enzymatic activity: Considering the large number of isolated yeast

cultures, the initial criterion for selecting strains for the study was their gas-forming ability. During the analysis, it was noted that, according to gas formation, the studied strains are divided into weak, medium and strong gas formers: weak, with the release of carbon dioxide up to 3 ml, making up 30% of the studied strains; medium - 4-5 ml, the same 30% and strong - with the release of 6 ml or more CO_2 in 24 hours, making up 40% of the studied strains. Further studies of technologically important DRC indicators, zymase, and maltase activity showed that among the most powerful gas-forming strains, releasing 8-9 ml CO2, the Sevan 60 strain demonstrated a 1.85-fold higher DRC value than the commercial strain. However, the α-glucosidase and β-fructofuranosidase activity indices are a more objective assessment of the enzymatic activity of baker's yeast strains [47]. It is known from the literature that yeast strains differ mainly in maltase activity, the index of which is more important in bread baking [48]. The best indices for maltase activity up to 30 minutes were shown by the strains Sevan 60, Goris 201, Sisian 310, and Goris 323. The Sevan 60 strain showed a more than 21% improvement in enzymatic activity, correlating with the maltase activity characteristic, compared to the factory reference industrial strain.

Biochemical Characteristic: The biotechnological potential of yeast is confirmed by the rich practice of its use in the fermentation of food products [14, 49]. In the fermentation process, yeast plays a key role in improving nutritional properties [50]. In addition to its main role in bread production (dough fermentation), *S. cerevisiae* yeast enriches bread with almost all essential amino acids that determine the functional value of baked bread [28]. The body cannot synthesize essential amino acids and must obtain them from food. However, plant foods, unlike animal protein products (meat, fish, eggs, etc.), do not contain sufficient quantities of essential amino acids to meet human needs [51]. A study of the amino acid composition of yeast biomass protein showed that the highest content of essential acids is found in the proteins of the Sevan 60 and Goris 323 strains. The sum of essential amino acids in the Goris 323 and Sevan 60 strains is 38.5 and 45.2%, and the sum of aromatic amino acids (phenylalanine + tyrosine) is 5.1 and 5.3%, respectively. Yeast protein of the Sevan 60 strain contains: 7.8% lysine, which plays a vital role in muscle growth, as well as in the regulation of hormones, antibodies and enzymes; 5.6% isoleucine and 8.7% leucine, which help regulate blood sugar and produce hormones; 2.8% histidine, which is responsible for the formation of blood cells and is metabolized in the body into histamine, which is critical for immunity; 1.6% methionine, which helps remove heavy metals such as lead and mercury from the body; 7.9% valine.

Antimicrobial Activity: One of the global food safety issues is the prevention of food spoilage. In baking, chemical preservatives, in particular calcium propionate, are widely used to prevent microbial spoilage of bread [21]. At the same time, the use of various chemical preservatives in dough-making to suppress the growth of pathogens that cause bread spoilage is not recommended [26]. To solve this problem, the baking industry has sought processing methods that make bread safe and extend its shelf life, including replacing harmful chemical preservatives. Yeast is known to have antibacterial activity, which manifests itself when co-cultivated with other microorganisms [25]. Studies of

these antibiotic properties, including those related to B. subtilis, have shown that yeast cell metabolites effectively suppress the growth of bacteria that cause spoilage in bakery products. Moreover, a correlation has been established between the antimicrobial properties of yeast strains in relation to test microbes and their increased enzymatic activity [44]. Studies of the antagonistic properties of Sevan 60, Goris 201, Sisian 310, and Goris 323 strains with high maltase activity in relation to *B. subtilis* have shown that yeast cultures form significant zones of growth inhibition of test objects up to 18 mm in size. It should be noted that the strains Sevan 60, Sisian 310, and Goris 323, unlike the strain Goris 201, formed stable zones of inhibition of the growth of the test object regardless of the fermentable sugar of the medium (glucose, sucrose, or maltose). The use of these strains in the bakery industry will eliminate the use of various chemical preservatives, obtain more natural, healthy bread, and extend its shelf life.

Phylogenetic sequencing: The genetic profiles of autochthonous yeast strains isolated from ttkhmor were analyzed by Macrogen (Korea). The results of the comparative analysis confirmed the phylogenetic relationship of the technologically valuable strain Sevan 60 with *S. cerevisiae*.

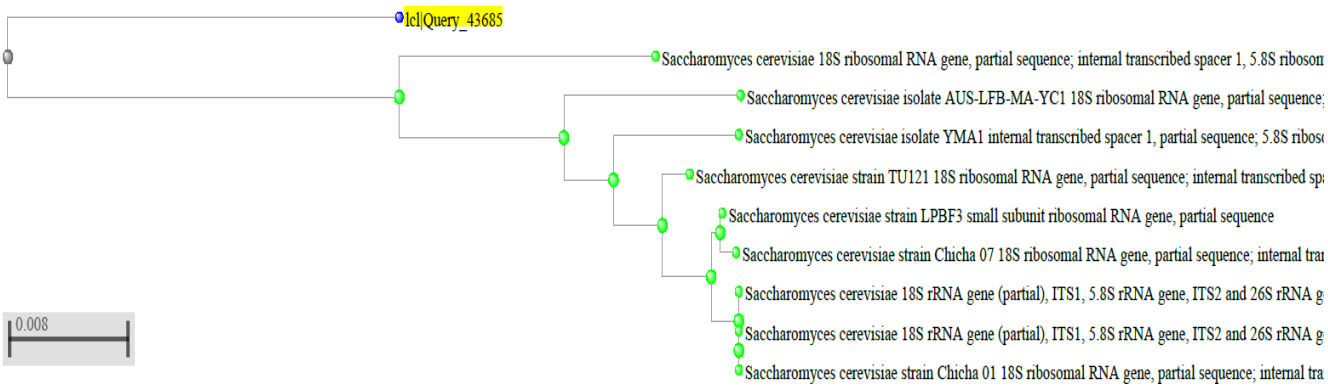


Figure 1. Phylogenetic tree of strain *S. cerevisiae* Sevan 60. Constructed using the neighbor joining method.

The influence of yeast strain Sevan 60 on the intensification of the dough-making process and the quality of baked bread: Liquid yeast produced in bakery establishments is commonly utilized to either supplement or replace commercial yeast. It consists of *S. cerevisiae* and *Lactobacillus delbrueckii* and undergoes fermentation in a pre-saccharified flour brew. A distinctive feature of liquid yeast is its beneficial effect on biochemical processes in the dough due to the shift in the pH of the dough to a more acidic range.

The study of the effect of yeast *S. cerevisiae* Seva 60 with high fermentative ability and enzymatic potential on the process of preparing liquid yeast, dough and quality of bread showed that the volume of CO_2 released at the fermentation period of the experimental dough was 18% higher, and the proofing time of dough pieces was reduced by 10 minutes, as compared to the controls. Experimental samples of bread were distinguished by the best state of elasticity and porosity of the crumb. With the same weight as the control sample, the volume yield of the experimental samples was 26% higher, and the porosity was 11% (Table 1).

Table 1. Physical-chemical assessment of liquid yeast, pre-dough, dough and bread (weight 0.7 kg), prepared using test and production strains (n=5; P<0.05).

Indicators	**Strains of yeast *S. cerevisiae***	
	Berlinskaya14 (industrial)	**Sevan 60**
	Liquid yeast	
Fermentation activity ml CO_2/20g	15.00±2.64	17.50±2.00
Number of yeast cells, mln/g	125.00±2.23	140.00±1.73
Moisture content, %	88.00±1.26	88.00±1.34
Titratable acidity, °N	7.00±1.09	9.00±1.14
DRC, min	20.00±2.64	10.00±2,23
	Pre-dough	
Fermentation activity ml CO_2/20g	14.70±1.70	18.50±1.73
Number of yeast cells, mln/g	110.00±1,37	125.00±1,58
Moisture content, %	45.00±1.04	45.00±1.14
Titratable acidity, °N	5.50±1.34	5.50±1.73
DRC, min	25.00±1.73	13.00±2.00
Fermentation duration, min	210.00±1.34	150.00±1.34
	Dough	
Fermentation activity ml CO_2/20g	14.20±2.82	16.80±2.44
Number of yeast cells, mln/g	115.00±1,58	135.00±2.00
Moisture content, %	44.30±1.14	44.00±1.09
Titratable acidity, °N	4.00±1.26	4.20±1.34
DRC, min	17.00±2.23	8.00±1.58
Fermentation duration, min	80.00±1.26	60.00±1.14
Dough proofing duration, min	50±1.14	40±1.09
	Bread (loaf)	
Weight, g	700.00±5.00	700.00±5.00
Moisture content, %	43.60±1.26	43.30±1.34
Titratable acidity, °N	3.00±1.55	3.20±1.61
Volume yield, ml/100 g	422.00±2.64	532.00±2.00
Increase of volume yield to control, %	-	+26
Porosity, %	68.00±2.23	76.00±1.73
Increase of porosity to control, %	-	11
	Organoleptic evaluation	
Appearance	Matte crust with cracks	Gloss crust, no cracks
Crumb	Porosity is unevenly developed	Evenly developed porosity
Taste, aroma	Without a distinct aroma	With a pleasant, distinct aroma

The best physical and chemical parameters of intensification of the dough preparation process by the Sevan 60 strain compared to the production strain served as a prerequisite for studying the possibility of reducing the consumption of yeast for kneading dough. The consumption of liquid yeast decreased by 22.3 and 44.5% of the norm provided for by the recipe. The control dough was prepared with the addition of 45% liquid production

yeast to the flour mass. The study of the dynamics of gas formation in the experimental test (Fig. 2) has shown that the greater the initial content of yeast in the dough, the faster the gas formation rate curves reach their maximum.

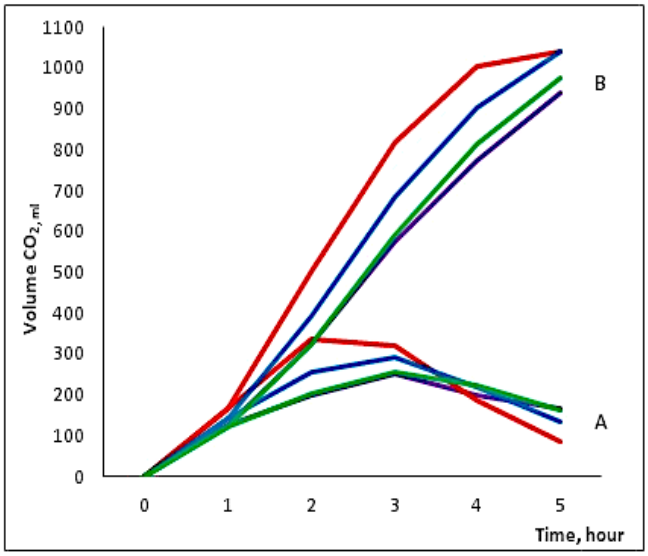


Figure. 2. The rate of formation CO_2 in the dough at different dosages of yeast strain of Sevan 60

strain Sevan 60 (dosage of liquid yeast is 45% to the mass of flour)

strain Sevan 60 (dosage of liquid yeast is 35% to the mass of flour)

strain Sevan 60 (dosage of liquid yeast is 25% to the mass of flour)

control liquid production yeast (dosage of yeast is 45% to the mass of flour)

A - Dynamics of CO_2 formation.

B - The amount of CO_2 formed in 5 hours.

The maximum gas formation rate at the introduction of the usual dosage of yeast (45% of liquid yeast of the strain *S. cerevisiae* Sevan 60 to the mass of flour) was observed 2 hours later after the start of fermentation and amounted to 334±2.44 ml/h. With a decrease in the dosage of yeast by 22.3 and 44.5%, the maximum gas formation in the experimental test occurred 3 hours later and amounted to 291.0 ±1.73 ml/h and 256.0 ±2.23ml/h, accordingly (Table 2).

Table 2. Gas formation parameters at dough fermentation (n= 5; P<0.05).

Fermentation duration, h	Control, dosage of yeast 45%		Strain Sevan 60 with dosage of yeast, %					
			45%		35%		25%	
	CO_2, ml	Gas formation rate, ml/h	CO_2, ml	Gas formation rate, ml/h	CO_2, ml	Gas formation rate, ml/h	CO_2, ml	Gas formation rate, ml/h
1	127	127.00±1.73	166	166.00±2.23	140	140.00±2.44	121	121.00±2.64
2	323	196.00±1.37	500	334.00±2.44	393	253.00±2.44	323	202.00±2.44
3	573	250.00±1.34	818	318.00±1.73	684	291.00±1.73	589	256.00±2.23
4	773	200.00±1.37	1002	184.00±2.23	904	220.00±2.00	813	224.00±1.73
5	937	164.00±1.30	1088	86.00±2.00	1038	134.00±2.23	966	163.00±2.00

At all selected dosages of yeast, the total amount of CO_2 released in the experimental samples exceeded the control bread by 4.1-16.1%. At the same time, in terms of physicochemical parameters, the prototype bread samples in all variants with reduced dosages of yeast had a volume yield on 13.4-15.6%, and the porosity was higher by 8.2-9.6% (Fig. 2).

The technology of co-cultivation and symbiosis of lactic acid cultures and *S. cerevisiae* strains in obtaining liquid yeast is fundamentally applicable for use on other substrates, including for the purpose of utilizing cottage cheese whey. Based on a set of microbiological studies, a two-stage way for culturing a consortium of microorganisms on whey has been developed. Since pure genetic lines of *S. cerevisiae* yeast cultures do not assimilate lactose as a carbon source, curd whey is pre-fermented with a LAB culture (for 48 hours until acidity reaches 172-180°T) in order to transform lactose into lactic acid, which is assimilated by saccharomycetes. After this, *S. cerevisiae* Sevan 60 yeast grown on malt wort is added to the curd whey in an amount of 5% of the whey volume and kept for 48 hours at a temperature of 32°C with aeration and shaking at 200 rpm until acidity reaches 85-90°T with a yeast biomass yield of 31 g/l and 47 g/l in variants using the LAB *L.casei* MDC-9644 and *L.lactis* MDC-10881 strains, respectively. The baking qualities of the mother starter were assessed by means of control baking of bread on thick pre- dough. The starter was added in an amount of 45% of the flour weight. Bread quality assessment showed that bread baked using fermented whey, with comparable volume yield (532.0±1.67 and 529.0±1.84 ml/100 g) and porosity (79% in both cases), outperformed the control bread prepared with commercial yeast in organoleptic qualities and demonstrated a significantly longer shelf life (Table 3). After baking, the experimental and control bread samples were thermostatted to test the antimicrobial activity of the yeast strain Sevan 60. Incubation of the bread samples at 37°C showed that while the control bread samples showed signs of spoilage after 48 hours, the bread baked with fermented whey remained defect-free even after 120 hours, despite a small difference in acidity of 0.5 °N.

Table 3. Technological mode of dough preparation and physical-chemical assessment of quality indicators of bread prepared using fermented whey and commercial yeast strain (n= 5; P<0.05).

Indicators	Bread sourdough	
	Yeast (commercial)	Yeast whey
	Pre-dough	
Moisture content, %	45.00±1.09	45.00±1.26
Titratable acidity, °N	3.90±1.34	5.50±1.58
Fermentation duration, min	60.00±2.64	120.00±2.82
	Dough	
Moisture content, %	44.20±1.14	44.0±1.14
Titratable acidity, °N	3.50±1.26	4.30±1.34
Fermentation duration, min	40.00±2.00	60.00±2.23
	Dough proofing	
Duration, min	30.00±2.00	35.00±2.00
	Bread (loaf)	
Weight, g	700.00±5.00	700.00±5.00
Moisture content, %	43.80±1.09	43.40±1.14
Titratable acidity, °N	2.80±1.26	3.30±1.34

Indicators	Bread sourdough	
	Yeast (commercial)	Yeast whey
Volume yield, ml/100 g	532.00±1.67	529.00±1.84
Porosity, %	79.00±1.84	79.00±1.48
	Organoleptic evaluation	
Appearance	Matte crust with cracks	Gloss crust, no cracks
Crumb	Porosity is unevenly developed	Evenly developed porosity
Taste, aroma	Unleavened	Distinguished by the characteristic taste and aroma of homemade baking

The presented study results indicate the potential for using ttkhmor microorganisms to create prototype of enriched bread for health through the synthesized bioactive compounds [52]. Bread quality assessment revealed that bread baked using fermented whey, with comparable physicochemical parameters (yield and porosity), outperformed the control bread made with industrial yeast in terms of spoilage resistance and longer shelf life. Furthermore, the experimental bread combines all the beneficial components of a product characteristic of the combined use of yeast, lactic acid bacteria, and whey, unlike similar studies.

CONCLUSIONS

Based on a number of microbiological and technological processes, a method has been developed for two-stage co-cultivation of *L. lactis* MDC-10881 and *S. cerevisiae* Sevan 60 (deposited in Microbial Depository Center under the number MDC-9792) cultures on cottage cheese whey to be used in the bakery industry as a starter for baking an enriched bread for health. Collectively, the above studies indicate that fermented yeast whey has a wide biotechnological potential for use in bakery to improve the quality, sensory, and functional properties of bakery products:

The use of yeast strain S. cerevisiae Sevan 60 in dough fermentation results in bread with a large yield and a characteristic airy texture, which contributes to better digestibility. Yeast strain S. cerevisiae Sevan 60 improves the nutrient content of bread (including essential amino acids), which helps to improve the digestive capacity and immunity of the body. Lactic acid and bacteriocins produced by the L. lactis MDC-10881 strain extend the shelf life of bread by inhibiting the growth of food pathogenic bacteria. In addition, metabolites of lactic acid fermentation have a positive effect on the taste and aroma of fermented bread products. Whey additionally gives bread the character of homemade baking and enriches it with whey protein, which is an immuno-nutrient.

Abbreviations: DRC: dough raising capacity, FD: dilution factor, LAB: lactic acid bacteria, MDC: Microbial Depository Center of the NAS RA.

Competing interests: Authors declare no conflict of interest.

Author Contributions: Author Contributions: Conceptualization - V.B.; software - A.V. and B.H.; validation - V.B., T.S. and V.G.; formal analysis - K.C., S.H., T.D., T.S. and A.V.; data curation - V.B., K.C. and M.K.; writing—original draft preparation - V.B., N.G. and A.Z.; writing—review - V.B. and V.G.; visualization – B.H; funding acquisition – V.B., K.C., A.T., and A.V. All authors have read and agreed to the published version of the manuscript.

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