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Probiotic Wheat Drinks: Study of Secondary Metabolites and Bioactive Compounds

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Abstract

Background and Objective: Nowadays, there are a wide variety of probiotic beverages made from animal-derived ingredients that contain beneficial microorganisms for human health. In contrast, probiotic beverages made from plant-based sources are much less common, despite their organic acids, which are biologically active substances. The aim of the study was to quantitatively assess the concentration of secondary metabolites of yeasts and lactic acid bacteria in a fermented grain drinks, as well as sensory characteristics of the drinks.

Material and Methods: Probiotic beverage samples were produced wheat as their primary grain ingredient. Fermentation process involved use of various lactic acid bacteria strains, including *Lactobacillus delbrueckii*, *Lactobacillus brevis*, *Lactobacillus buchneri*, *Lactobacillus plantarum* and *Lactobacillus fermentum* as well as various strains of *Saccharomyces cerevisiae* yeasts. Additionally, commercially manufactured soft drinks made from grain-based ingredients were used as the basis for the comparison. Contents and concentrations of organic acids were analyzed using high-performance liquid chromatography, following the guidelines by the government standard. This technique involved separation of specific organic acids on a solid support using reversed-phase mechanism.

Results and Conclusion: The probiotic wheat drink contained 1300 $mg.dm^{-3}$ lactic acid, suggesting the presence of lactic acid fermentation. Detection of citric and succinic acids of respectively 80 and 152 $mg.dm^{-3}$ indicated heteroenzymatic nature of lactic acid fermentation. Therefore, development of aromas described as clove, fruity and banana-like was expected, generally considered favorable in the context of probiotic wheat drinks. Data make it possible to predict creation of the flavor profiles of fermented drinks from vegetable raw materials using complex combinations of lactic acid bacteria and yeasts.

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1. Introduction

In the food industry, numerous beverages derived from animal sources contain beneficial microorganisms such as traditional yogurts, kefirs and sour creams. In contrast, probiotic beverages sourced from plant materials are significantly less prevalent. Cereals possess substantial potentials as a foundation for probiotic beverages. An example from eastern Europe is the customary drink of kvass, often produced using ryes and rye malts. Production of probiotic beverages from various other cereal types, fruits and vegetables is interested as well [1]. Cereals offer certain advantages more than those fruits and vegetables do due to their high yield, extended shelf lif, consistent compositions and ease of processing. Additionally, the cereal-based beverage industry, including producer of beers, can readily generate probiotic beverages from cereal materials. These beverages confer health advantages by promoting favorable microflora within the human digestive system and leveraging positive effects of metabolic byproducts produced by microorganisms [2]. Important byproducts include organic acids, which are qualified as biologically active substances [3]. These natural safe compounds offer generalized fortifying effects on the body and helps in stabilizing a broad array of vital substances critical to human well-being [4].

Organic acids such as lactic, acetic, malic, wine, citric and formic acids are highly prevalent in a variety of foods, predominantly those of plant origin. These acids actively participate in oxidation reactions, engendering beneficial compounds that facilitate elimination of accumulated toxins and salts, hydration of organs, sensation of satiety and quenching of thirst. Each organic acid contributes to specific roles within organic processes [5]. Lactic acid holds significance within chemical reactions in animals and humans, playing critical roles in metabolic processes, muscle functions, nervous system operations and brain activities [6]. A study by Chasovshchikov and Pomozova [7] comparatively assessed organic acid compositions in kvass wort, kvass as a probiotic beverage and kvass-based drinks. Study highlighted the broad spectrum of organic acids originating from the fermentation of cereal-derived wort in fermented kvass. Non-fermented kvass drinks were dominated by malic acid, which originated from the kvass wort concentrate. In contrast, fermented kvass was primarily characterized by succinic acid. This differentiation in succinic and malic acid concentrations could effectively differentiate between the fermented kvass and its other variations. Thus, these findings suggested potentials for precise quantification of individual organic acids resulting from combined fermentation by lactic acid bacteria (LAB) and yeasts. It is worthnoty that the authors of this study used a range of 16.8- 24.9 cm^3 of 1-N sodium hydroxide solution per 100 cm^3 of drink without discriminating due to the compositions.

One promising avenue in the fermentation kvass production involves use of probiotic microorganisms in combined starter cultures. This approach extends a variety of available drinks while enhancing their quality and functional attributes. Probiotic microorganisms, which include non-pathogenic and non-toxigenic living microorganisms representative of the protective factions within the normal human intestinal microbiota, positively affect the human body. They achieve this effect by preserving normal composition and biological activity of the digestive tract microflora, primarily composed of genera such as *Bifidobacterium*, *Lactobacillus*, *Lactococcus* and *Propioni-bacterium* species [8]. A study by Ponomareva and Borisova [9] included synthesis of primary and secondary substances by LAB in production of acidic ales. It also included roles of these metabolites in shaping tastes and aromas of such beverages. It is noteworthy that the content of lactic acid during grain fermentation can vary significantly ranging 318-486 $mg.dm^{-3}$ with acetic acid tanging 0.1-220 $mg.dm^{-3}$ due to the specific LAB strains. Hence, it is important to investigate presence of other organic acids during plant material fermentation, including citric, succinic and malic acids because of their roles in tastes.

Sensory attributaes, including tastes and aromas, play critical roles in fermentation products, arising from various compounds in small quantities [10]. Significantly, LAB and yeasts include fermentation by-product specificity linked to the strains [11-13]. Potentials for cereals as foundations for the probiotic beverages are substantial. Wheat with a global spread, especially in the European Union (EU) and Russia, is a prime candidate. Therefore, this article analyzed organic acid and secondary metabolite compositions from a combined alcoholic and lactic acid fermentation when wheat served as the raw material for the production of probiotic drinks. The study tried to establish connections between particular secondary metabolites generated during the combined fermentation and sensory profiles of the wheat-based beverages. Physicochemical and organoleptic characteristics of the beverages were studied as well, their probiotic natures were established and changes in the product during storage were investigated.

2. Materials and Methods

2.1 Raw materials

Light wheat malt produced by BESTMALZ, Heidelberg, Germany, was selected as a base of probiotic drink. The choice of light wheat malt was associated with the fact that this raw material was appropriate from a legal standpoint for use in beverages of the "kvass" type. Additionally, there were significant potentials for developing wheat-based beverages, as the overall harvested crop quantities were increasing. Since the organoleptic characteristics of the drink directly depended on the selected cultures of the microorganisms, LAB with homofermentative and heteroenzymatic fermentation were used in the study. Thus, LAB of the following strains of *Lactobacillus* (*L.*) *delbrueckii* WLP 677 (White Labs, CA, USA), *L. brevis* WLP 672 (White Labs, CA, USA), *L. buchneri* 5335 (Wyeast Laboratories, Ore, USA), *L. plantarum* WLP 693 (White Labs, CA, USA) and *L. fermentum* ME-3 (Wyeast Laboratories, Ore, USA) were used to ferment the beverage. Table 1 shows the characteristics of these LAB.

Moreover, the following strains of *Saccharomyces* (*S.*)*cerevisiae* were used, including *S. cerevisiae* TUM 175 and *S. cerevisiae* W68 (Bavarian State Brewery Weihenstephan, Bavaria, Germany), *S. cerevisiae* WLP 300 Hefeweizen Ale (White Labs, CA, USA) and *S. cerevisiae* Safale WB-06 (Fermentis by Lesaffre, O-de-France, France). Table 2 presents characteristics of these strains.

Characteristics of LAB and *S. cerevisiae* yeast were provided by the manufacturer in the certificates for each strain of the microorganisms. During the study, only pure yeast cultures were used. Industrial probiotic drinks made from grain raw materials ["Ochakovsky" kvass (Ochakovo, Moscow, Russian Federation) and "Russian Pattern" kvass (Trekhsosensky, Ulyanovsk region, Russian Federation)] were used in the study as well.

2.2 Production of wheat-based probiotic drink

A probiotic drink made from wheat raw materials was prepared based on the following scheme (Figure 1).

Table 1. Characteristics of the lactic acid bacteria

LAB	Fermentation type	Fermentation temperature °C	Optimal pH	Acid production intensity
L. delbrueckii	Homofermentative. Do not synthesize CO_2 when fermenting sugars	32-50	5.0-6.0	High
L. brevis	Heterofermentative. They synthesize CO_2 during the fermentation of sugars. Diacetyl is not synthesized	34-37	4.0-6.0	Middle
L. buchneri	Heterofermentative. Synthesize CO_2 when fermenting sugars	37	5.5- 6.2	Middle
L. plantarum	Homofermentative /heterofermentative. Synthesize glycerin, diacetyl, butanediol-2,3, succinic acid, acetaldehyde	30	5.0-6.0	High
L. fermentum	Heterofermentative. They synthesize CO_2 during the fermentation of sugars. Can synthesize acetaldehyde depending on the composition of the medium	37-40	5.0-6.0	Middle

L.= *Lactobacillus*

Table 2. Characteristics of *Saccharomyces cerevisiae* strains

Yeast strains	Organoleptic (taste and aroma)	Optimum fermentation temperature°C	Flocculation
S. cerevisiae TUM 175	Pronounced phenolic and spicy tones, as well as floral aromas.	18-22	High
S. cerevisiae W 68	Light spicy notes of clove are combined with fruity aromas (isoamyl acetate and ethyl acetate). Banana flavor is palpable, but not dominant.	18-23	High
S. cerevisiae WLP 300	Promotes the appearance of banana and clove notes.	20-22	Low
S. cerevisiae Safale WB-06	The strain provides the essential and phenolic notes characteristic of a typical wheat beer. Pronounced carnation.	18-24	Low

S. =Saccharomyces

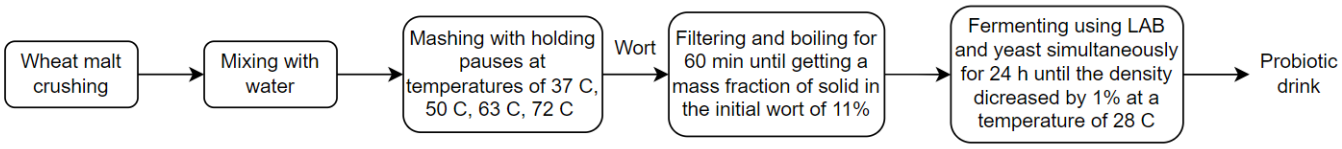

Figure 1. Production of the wheat-based probiotic drink

This process was regulated by standard technological instructions and complied with the norms of the state standard of GOST 31494-2012 [14]. The temperature paused the hydrolysis process of non-starch polysaccharides, proteins and starch.

Thus, 20 samples of probiotic drinks with various combinations of LAB and yeasts were prepared for the experiment based on the selected recipe and technology. Combinations of LAB and yeast are presented in Table 3.

Table 3. Combinations of the lactic acid bacteria and yeasts

Sample	Combination of microorganisms	Sample	Combination of microorganisms
1	*L. delbrueckii* WLP 677,S. cerevisiae TUM 175	11	*L. delbrueckii* WLP 677, S. cerevisiae WLP 300
2	*L. brevis* WLP 672,S. cerevisiae TUM 175	12	*L. brevis* WLP 672, S. cerevisiae WLP 300
3	*L. buchneri* 5335, S. cerevisiae TUM 175	13	*L. buchneri 5335, S. cerevisiae* WLP 300
4	*L. plantarum* WLP 693, S. cerevisiae TUM 175	14	*L. plantarum* WLP 693, S. cerevisiae WLP 300
5	*L. fermentum* ME-3, S. cerevisiae TUM 175	15	*L. fermentum* ME-3, S. cerevisiae WLP 300
6	*L. delbrueckii* WLP 677, S. cerevisiae W 68	16	*L. delbrueckii* WLP 677,S. cerevisiae Safale WB-06
7	*L. brevis* WLP 672, S. cerevisiae W 68	17	*L. brevis* WLP 672, S. cerevisiae Safale WB-06
8	*L. buchneri* 5335, S. cerevisiae W 68	18	*L. buchneri* 5335, S. cerevisiae Safale WB-06
9	*L. plantarum* WLP 693, S. cerevisiae W 68	19	*L. plantarum* WLP 693, S. cerevisiae Safale WB-06
10	*L. fermentum ME-3, S. cerevisiae W 68*	20	*L. fermentum* ME-3,S. cerevisiae Safale WB-06

L.= Lactobacillus,, S. =Saccharomyces

The following mixing algorithm was used to create combinations of microorganisms. For each of the four yeast strain variants, five variants of LAB strains were added. Thus, it was possible to explore all 20 resulting combinations of yeast and LAB. Mixing of dry commercial yeast and LAB preparations was carried out in a 1:1 ratio and introduced into a solution. The mixture included 1 g per 1000 cm^3.

2.3 Analysis

Content of ethyl alcohol was assessed through distillation based on the state standard of GOST 6687.7-88 [15]. Titratable acidity of the beverage was assessed by titration with NaOH in presence of phenolphthalein indicator based on GOST 6687.4-86 [16]. Organoleptic characteristics of the beverage were assessed based on GOST 6687.5-86 [17]. Additionally, the major standardized indicators (appearance, transparency, color, aroma and taste) were investigated using sensory profiles with the specified descriptors. A tasting panel consisting of nine individuals were participated in the study and the final results were reported as the arithmetic mean for each descriptor. The maximum possible score for each sample was 25. Composition and mass concentration of organic acids in the final beverage samples were assessed using high performance liquid chromatography (HPLC) based on GOST 32771-2014 [18]. The method was based on the assessment of individual organic acids by their separation on a solid support using reversed-phase mechanism. A phosphate buffer with pH 2.4 was used as eluent. The flow rate of the eluent was 1 cm^3 min^{-1}. The elution mode was isocratic. Mass fraction of the major substance in the control samples of the organic acids was not less than 99.5%.

Identification and quantitative calculation of the peaks acids were carried out at individual maximums of their light absorption in ultraviolet region of the spectrum by comparing them with the retention time in calibration solutions. To assess concentration of the secondary metabolites that were not linked to organic acids (e.g., aldehydes, esters and higher alcohols), gas chromatography method was used with a flame ionization detector based on GOST 33408-2015 [19]. Concentration of the major substance in the control samples of compounds was not less than 99.5%. In this study, instrumental analysis was used as the most representative method. The number of viable cells was assessed based on the analysis of the colony number after inoculation of the liquid product in nutrient agar [20]. All experiments were carried out in triplicate and results were expressed as mean ±SD (standard deviation). Furthermore, MS Excel was used for the analysis.

3. Result and Discussion

The standardized indicators for kvass based on GOST 31494-2012 [14] were the content of ethyl alcohol and titratable acidity. Thus, the quantity of ethanol in kvass should not exceed 1.2% vol. and the titratable acidity should be assessed in the range of 1.5-7 cm^3 of 1 $mol.dm^{-3}$ NaOH solution per 100 cm^3 of the beverage (acid unit, AU). Assessments of these indicators on the samples were carried out and the following results were reported (Table 4).

Thus, it was established that four samples contained the optimal physicochemical characteristics, including Sample 1, *L. delbrueckii* WLP 677 and *S. cerevisiae* TUM 175; Sample 4, *L. plantarum* WLP 693 and *S. cerevisiae* TUM 175; Sample 11, *L. delbrueckii* WLP 677 and *S. cerevisiae* WLP 300; and Sample 14, *L. plantarum* WLP 693 and *S. cerevisiae* WLP 300. Although ethanol content and titratable acidity values of the 16 samples did not include ranges recommended by the standard [14], the authors believed that they could still be used in the food industry following individual adjustments to fermentation conditions. Organoleptic analysis was carried out for the selected four samples (Figure 2). Then, these samples were investigated for the probiotic characteristics.

As a result of this study and previous studies [21], it was detected that from the sensory perception, combination of *L. plantarum* WLP 693 and *S. cerevisiae* TUM 175 included the best results. It was further used as a sample for the detailed investigation of secondary metabolites. Thereby, qualitative and quantitative compositions of the organic acids were assessed for the probiotic wheat drink. The chromatogram is shown in Figure 3 and a quantitative calculation of the major peaks of the organic acids is present in Table 5.

Table 4. Contents of ethanol and titratable acidity in the beverages

Sample	Ethanol, % vol.	Acidity, AU	Sample	Ethanol, % vol.	Acidity, AU
1	0.8±0.05	5.5±0.23	11	0.7±0.05	5.9±0.30
2	1.3±0.06	5.5±0.23	12	0.9±0.04	7.1±0.35
3	1.4±0.07	5.6±0.28	13	0.8±0.04	7.2±0.35
4	0.9±0.05	5.8±0.29	14	0.7±0.04	6.3±0.32
5	1.3±0.06	5.8±0.29	15	0.9±0.04	7.2±0.35
6	1.0±0.05	7.1±0.35	16	1.7±0.08	3.5±0.12
7	0.7±0.03	7,5±0.36	17	1.4±0.07	3.6±0.18
8	0.8±0.04	7.5±0.36	18	1.5±0.06	3.5±0.12
9	0.9±0.04	7.2±0.35	19	1.5±0.06	3.7±0.19
10	1.0±0.05	7.2±0.35	20	1.4±0.06	3.9±0.20

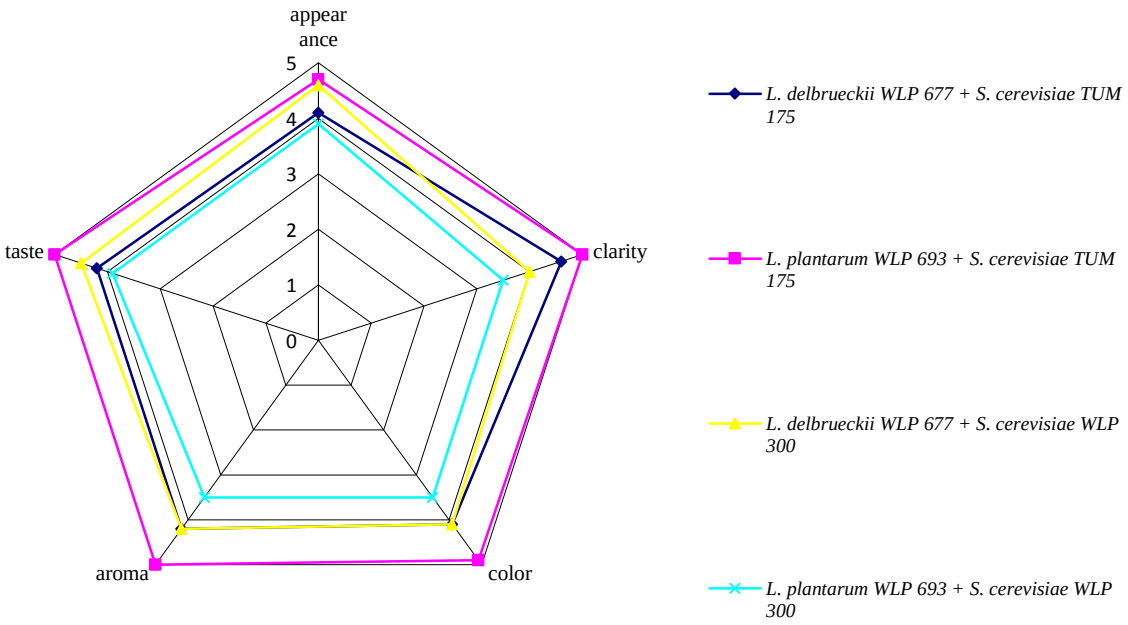

Figure 2. Organoleptic profiles of the samples

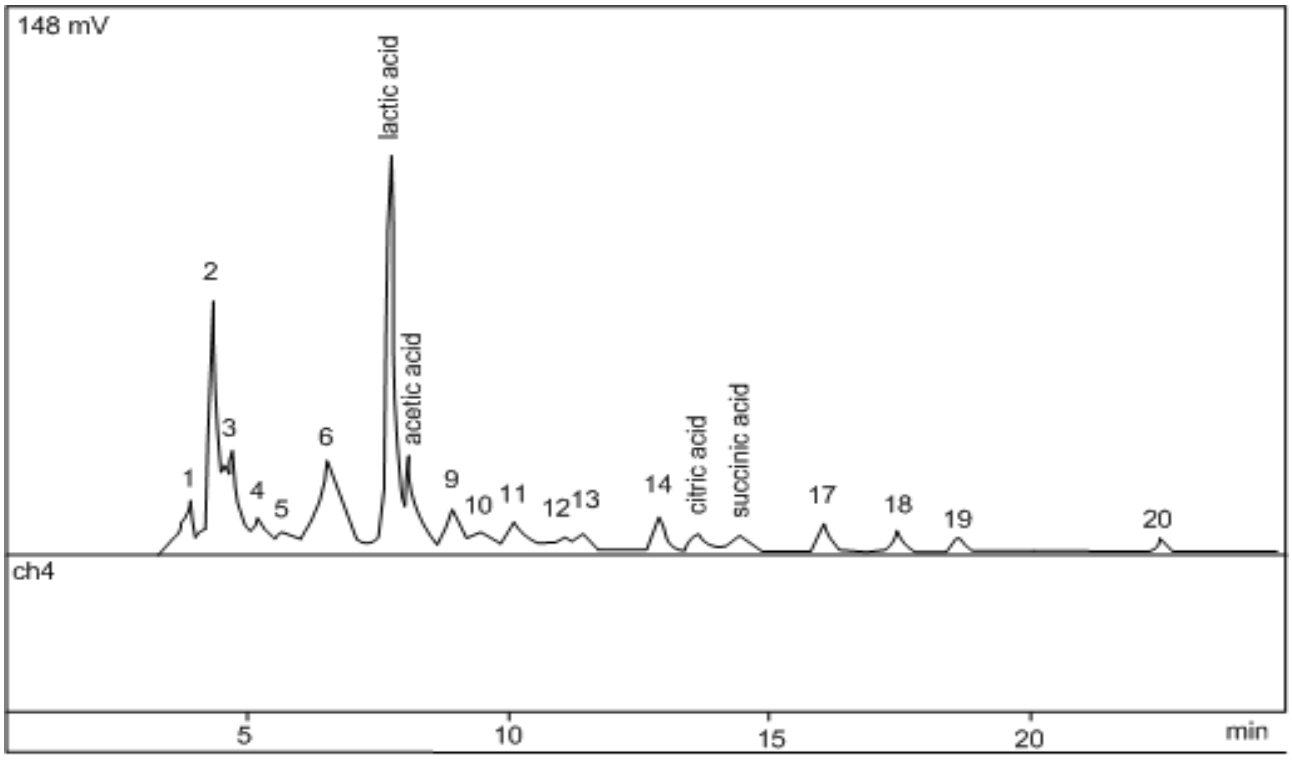

Figure 3. Chromatogram of the contents of organic acids in the probiotic wheat drink

As reference samples for the content of organic acids, "Russian Pattern" rye kvass with alcohol fermentation and "Ochakovsky" rye kvass with alcohol and lactic fermentations were investigated. The nomenclature of organic acids in kvass samples was represent majorly by lactic, acetic, succinic, malic and citric acids. Results of comparing the probiotic drink with two other kvass samples are present in Table 6.

The organoleptic profile of wheat light kvass is naturally formed due to the major by-products of the metabolism of LAB, complexity of the composition and quantity of which is assessed by the culture of microorganisms. Using gas

chromatographic method [19], it was detected that kvass included a multicomponent composition. Acetaldehyde, ethyl acetate, ethyl lactate, ethyl formate, isobutanol, isopropanol, isoamylol, benzaldehyde, diacetyl, acetone, 1-propanol and methanol were detected in the samples. For these compounds, the corresponding taste and aromatic associations were assessed. Mass concentration of the volatile components for three drinks samples are shown in Table 7. Roles of the yeast and LAB in flavor formation is unequal. Yeast primarily contribute to the accumulation of ethyl formate, ethyl acetate and methanol while LAB produce isopropanol, 1-propanol, isobutanol and isoamylol. Some substances are chemical derivatives resulting from the interaction of metabolites such as ethyl lactate. Certain compounds are synthesized by the yeast and LAB, including acetaldehyde, diacetyl and benzaldehyde [22,23].

The four selected samples of wheat-based probiotic drinks through combined alcoholic and lactic acid fermentations were investigated for colony-forming units (CFUs) after 24 h of co-fermentation at 28 °C. Results are present in Table 8.

In this study, it was reported that the final drinks could be addressed as probiotics because they contained $4*10^7$ to $2*10^8$ cells per 1 cm^3 of the product. These indicators met the standards for probiotic products. Industrial interest represents preservation of samples during storage. Based on the regulations [14], the maximum allowable ethanol content in beverages should not exceed 1.2% vol. This value was adopted as a threshold. Storage of the samples was carried out at 4 ºC ±0.5. Results are present in Figure 4.

Table 5. Concentrations of organic acids in the probiotic wheat drink

№ (fig. 3)	Time	Height	Area	Concentration	Title
	min	mV	mV*sec	$mg.dm^{-3}$	
7	7.718	89.934	1039.8254	1316.0342	Lactic acid
8	8.056	21.7244	330.6512	501.8564	Acetic acid
15	13.604	4.6031	126.2642	78.9896	Citric acid
16	14.373	4.0183	109.1082	158.8652	Succinic acid
Sum	40.001	119.5392	1605.8490	2055.7454	

Table 6. Composition of the major organic acids in kvass

Name of acid	Mass concentration of organic acid $mg.dm^{-3}$		
	probiotic drink	"Ochakovsky" kvass	"Russian Pattern" kvass
Lactic	1317.72±181.58	2716.76±374.37	336.19±88.25
Citric	80.14±24.09	110.30±33.16	1113.45±150.76
Succinic	152.71±38.96	190.16±48.51	202.53±51.66
Malic	-	667.61±88.39	594.86±78.76
Acetic	496.50±62.11	466.41±61.71	479.86±63.48

L.= Lactobacillus,, S. =Saccharomyces

Table 7. Mass concentrations of the volatile components

Component Name	Associative terms	Mass concentration of components $mg.dm^{-3}$		
		probiotic drink	"Ochakovsky" kvass	"Russian Pattern" kvass
Acetaldehyde	green apples	31.95±4.79	13.08±1.96	11.03±1.65
Acetone	sharp, solvent	0.18±0.03	1.52±0.23	0.58±0.09
Ethyl formate	forest raspberries	0.37±0.06	0.63±0.09	1.20±0.18
Ethyl acetate	pear, at high concentrations similar to solvent	0.80±0.12	1.16±0.17	0.60±0.09
Methanol	low odor of alcohol	< 0.01	< 0.01	< 0.01
Diacetyl	buttery, dairy, sweet milk, rancid	4.18±0.63	-	0.32±0.05
Isopropanol	smell of alcohol	0.12±0.02	0.85±0.13	0.06±0.01
1-Propanol	orange, banana, apricot; at high concentrations - burning, smell of alcohol	6.15±0.92	5.18±0.78	3.29±0.49
Isobutanol	smell of alcohol	18.14±2.72	15.48±2.32	8.42±1.26
Isoamylol	smell of alcohol, wine	18.59±2.79	36.24±5.44	29.03±4.35
Ethyl lactate	fruity, coconut flavor	0.34±0.05	1.39±0.21	0.27±0.04
Benzaldehyde	almond, smell and taste of marzipan and almond oil	2.63±0.39	1.30±0.20	1.57±0.24
Sum		83.45	78.83	56.37

Table 8. The microbial colony forming units in probiotic wheat drinks

№	Combination of microorganisms	CFU.cm^{-3}	№	Combination of microorganisms	CFU.cm^{-3}
1	*L. delbrueckii* WLP 677 *S. cerevisiae* TUM 175	4.0*10^{7}	11	*L. delbrueckii* WLP 677 *S. cerevisiae* WLP 300	4.0*10^{7}
4	*L. plantarum* WLP 693 *S. cerevisiae* TUM 175	2.0*10^{8}	14	*L. plantarum* WLP 693 *S. cerevisiae* WLP 300	1.0*10^{8}

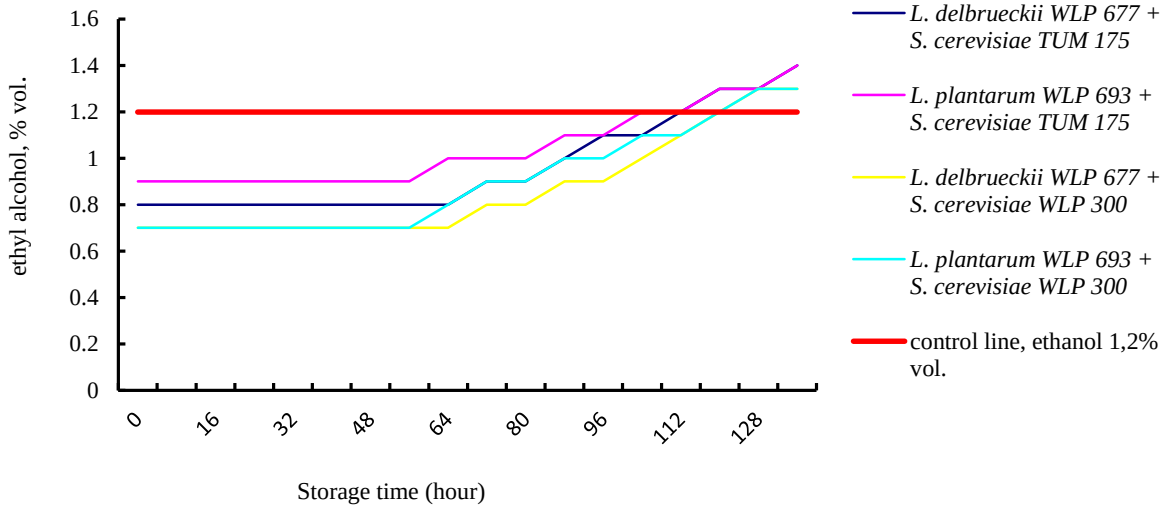

Figure 4. Dynamics of the ethanol accumulation during sample storage

Results of this study revealed that the storage time of the products without deviations from the normal values ranged from 104 h for the combination of *L. plantarum* WLP 693 and *S. cerevisiae* TUM 175 to 120 h for the combination of *L. delbrueckii* WLP 677 and *S. cerevisiae* WLP 300. Sensory characteristics of the beverages were unchanged during this time. This outcome allowed considering of the beverage as a short shelf-life product, needing mandatory refrigeration storage at 4 ºC ±0.5. The CFU content was calculated at the point of establishing the maximum permissible ethanol content. Results are present in Table 9.

When storing products for 4-5 d at 4 ºC ±0.5, content of the probiotic microorganisms did not decrease but rather slightly increased. This factor was addressed as positive.

3.1 Basic indicators and organoleptics

Study on the 20 samples revealed that four of them met the standards well that were set for this type of products [14], while the 16 samples either showed increased acidity (more than 7.5 AU) or excessive alcohol content (more than 1.2% vol.). This was attributed to the fact that under various combinations of yeast and LAB, certain strains dominated others. Sensory characteristics of the selected samples were positively accepted. The favorable combination of color, appearance, transparency, aroma and taste was achieved through a successful symbiosis of *L. plantarum* WLP 693 and *S. cerevisiae* TUM 175 strains. Their secondary metabolites complemented each other in the sensory profile of the beverage. This sample was detailed analyzed to assess qualitative and quantitative contents of the secondary metabolites. The three other samples that met the baseline standards were assessed in terms of storage stability to better understand dynamics of this process.

3.2 Organic acids

The lactic acid content, registering at 1317.72 mg.dm^{-3} in the wheat probiotic and 2716.76 mg.dm^{-3} in "Ochakovsky" rye kvass, provided good evidence of lactic acid fermentation. In contrast, the relatively diminished lactic acid content in "Russian Pattern" rye kvass suggested absence of lactic acid fermentation within the specific sample. Presence of citric acid as 80.14 mg.dm^{-3} and succinic acid as 152 mg.dm^{-3} indicated the heterogeneous enzymatic nature of lactic acid fermentation. Similar traits were identified in "Ochakovsky" rye kvass sample. In contrast, significant citric acid concentration of 1113.45 mg.dm^{-3} in "Russian Pattern" rye kvass revealed incorporation of this component. Regarding its roles as an acidity regulator, citric acid is limited for use in the food industry. Verification was detected in presence of acetic acid at 496.5 mg.dm^{-3}, verifying a heterozygous enzymatic lactic fermentation process. As established, acetic acid stands as a verified metabolite resulting from the hydrolysis of pentosans, which are constituents of the kvass ingredients [24] (Figure 5).

Table 9. The microbial colony forming units in probiotic wheat drinks after storage

№	Combination of microorganisms	CFU.cm^{-3}	№	Combination of microorganisms	CFU.cm^{-3}
1	*L. delbrueckii* WLP 677 *S. cerevisiae* TUM 175	4.5*10^{7}	11	*L. delbrueckii* WLP 677 *S. cerevisiae* WLP 300	4.4*10^{7}
4	*L. plantarum* WLP 693 *S. cerevisiae* TUM 175	2.3*10^{8}	14	*L. plantarum* WLP 693 *S. cerevisiae* WLP 300	1.1*10^{8}

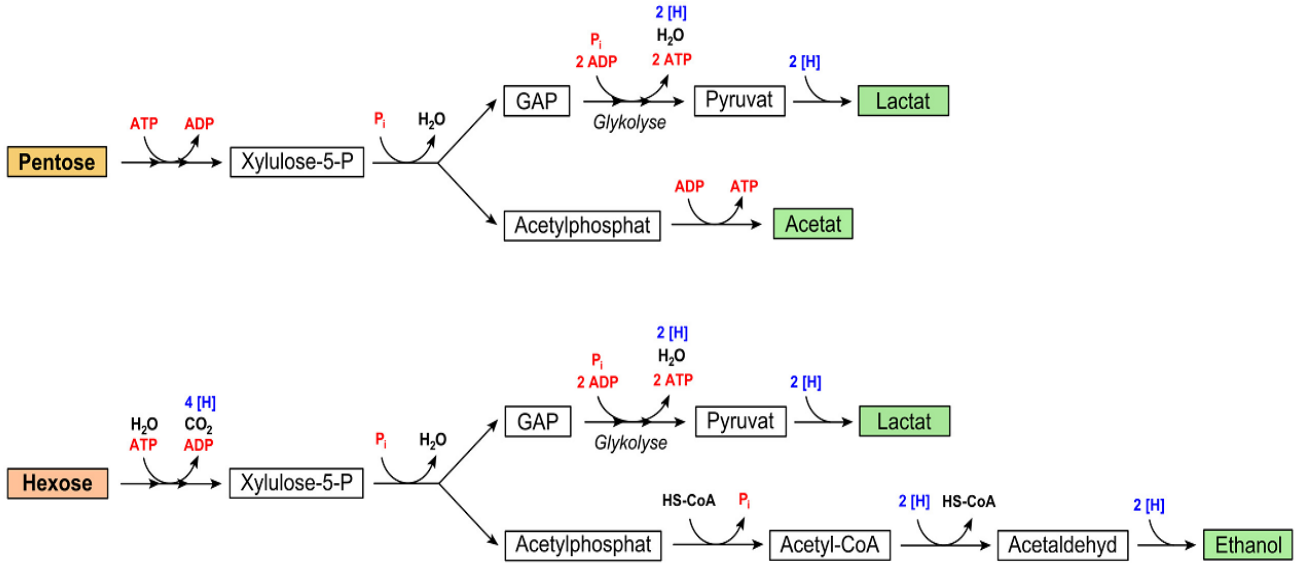

Figure 5. Metabolism pathways during lactic acid fermentation [24]

Increased concentration of acetic acid (466 mg.dm^{-3}) was observed in "Ochakovsky" rye kvass, showing a heterozygous enzymatic route of lactic acid fermentation. In contrast, substantial presence of acetic acid (479.86 mg.dm^{-3}) in the third reference sample, "Russian pattern" kvass, might indicate a concurrent acetic acid fermentation route due to the existence of accompanying microflora. This could be due to a parallel "background" lactic acid fermentation, as revealed by the marginal accumulation of lactic acid in this sample (336.9 mg.dm^{-3}). Absence of malic acid in the probiotic wheat drink demonstrated that the *L. plantarum* WLP 693 strain fermented pentoses, yielding lactic, acetic, succinic and citric acids (presence of malic acid needed absence of the fermentation process as during fermentation, malic acid was transformed into lactic acid). Furthermore, this finding was similar to the finding of Chasovshchikov and Pomozova [7], supporting that malic acid was a fundamental constituent of grain concentrates and present in limited quantities in products generated through fermentation. It is noteworthy that the aggregation of diverse organic acids with their relative proportions has facilitated development of a different profile for the wheat probiotic beverage. Considering that organic acids are biologically active substances, their presence significantly contributes to the overall vitality of the human body.

3.3 Esters, higher alcohols, aldehydes

In a sample of kvass with alcohol and lactic acid fermentations, significantly increased levels of isoamylol (36.24 mg.dm^{-3}), acetone (1.52 mg.dm^{-3}), isopropanol (0.85 mg.dm^{-3}) and isobutanol (15.48 mg.dm^{-3}) were identified. These compounds contributed to the aroma and taste of the beverage. In contrast, kvass with alcohol fermentation alone exhibited lower concentrations of metabolic byproducts, compared to other samples. Based on data in Table 6, microbial metabolism byproducts that contribute to the sensory profile of the probiotic drinks include acetaldehyde, isobutanol, 1-propanol, diacetyl, benzaldehyde and isoamylol. Aroma of the wheat light kvass is characterized by the prevalent fruit notes such as green apples, oranges, bananas, apricots, almonds and forest raspberries with traces of milk aroma and alcohol scent. Of particular interest is the unique pattern of secondary metabolite accumulation within the wheat raw material sample. For example, isoamyl acetate (ether), a part of the group of compounds formed in tandem with 4-vinyl guaiacol during wheat raw material processing, stands out. The threshold for perceiving this compound aroma ranged 1.0-1.6 mg.dm^{-3}, characterized by hints of cloves and bananas. This contributed positively to the creation of a distinctive taste profile. In the probiotic drink sample of this study, a higher concentration of acetaldehyde (31.95 mg.dm^{-3}) was detected, compared to the findings of other researchers (21.57 mg.dm^{-3}) [25]. This difference could be attributed to use of non-typical wheat raw materials and

specific strain characteristics, governing secondary metabolites. Significantly, the acceptable sensory concentration for acetaldehyde in fermented beverages typically is 40 $mg.dm^{-3}$ [26,27]. Since this threshold was not exceeded in the current sample, no buttery or milky aromas were detected in the drink.

3.4 Probiotics

After incubation, it was shown that the selected combinations of microorganisms included various degrees of biomass accumulation (Table 7). Significantly, samples with *L. plantarum* showed the highest count of CFU, reaching approximately $1\text{-}2*10^8$ per 1 cm^3. This difference was attributed to the significant acid-forming activity of these bacteria. Interestingly, this effect occurred independently of the yeast symbiotic strain. An aspect increases in the context of incubation temperature, which was set atypical for LAB at 28 °C. These bacteria conventionally propagate at optimal temperatures of 37-39 °C [28,29]. However, decision to operate at a lower temperature was motivated by the concern of excessive ethyl alcohol accumulation in the final product due to the increased yeast activity. Since probiotic beverages were non-alcoholic, this compromised condition for LAB and yeasts seemed appropriate. Furthermore, the ultimate CFU count was similar to the international standards, governing the microorganism content in food products. Significantly, these were similar to recommendations for balanced nutrition such as those outlined in MR 2.3.1.1915-04 [3].

3.5 Storage

It was established that during refrigerated storage at 4 ºC ±0.5 for 4-5 d, physicochemical, organoleptic and probiotic characteristics were stable. After this time, ethyl alcohol accumulated in quantities exceeding 1.2% vol., which were associated with the yeast activity. For the probiotic beverage, the highlighted time and temperature were acceptable.

5 Conclusion

Findings of the current studies have revealed that probiotic drinks derived from wheat raw materials include various ranges of biologically active organic acids. Specific composition of these acids is directly affected by the choice of grain raw materials and microorganism strains. Example of this phenomenon includes significant accumulation of lactic acid up to 1317 $mg.dm^{-3}$ with the absence of malic acid in the probiotic drink sample. This is resulted from use of a combination of LAB and yeast, as the interaction between these microorganisms and raw materials prevents significant malic acid accumulation. Accumulation of secondary metabolites from combined lactic acid and alcoholic fermentation contributes to different sensory profiles of the probiotic drinks. This attribute is important for the consumers when making their selections. By pairing microorganism strains correctly, it is possible to achieve various taste and aromatic profiles. The probiotic nature and associated health benefits are substantiated by the presence of living LAB cells within the product, ranging from $4*10^7$ to $2*10^8$ cells per 1 cm^3. This presence underscores potentially positive effects of the drink on gut health. Use of wheat raw materials serves to expand the repertoires of ingredients available for crafting traditional Eastern European beverages such as kvass. In contrast to relying on familiar rye raw materials alone, this modification broadens possibilities for creating unique variations of this culturally significant drink.

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7. Conflict of Interest

The authors report no conflict of interest.

8. Authors Contributions

Conceptualization, R.O. and M.A.; methodology, M.A. and A.G.; software, R.O.; data curation, P.B. and I.S.; writing-original draft preparation, M.A. and A.G.; writing—review and editing, P.B. and I.S.

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نوشیدنی‌های زیست‌یار بر پایه گندم: مطالعه متابولیت‌های ثانویه و ترکیبات زیست‌فعال

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واژگان کلیدی
- ترکیبات فعال بیواوژیکی
- باکتری‌های لاکتیک اسید (LAB)
- مخمرها

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چکیده

سابقه و هدف: امروزه، طیف گسترده‌ای از نوشیدنی‌های زیست‌یار تهیه شده از مواد با منشا حیوانی و حاوی میکروارگانیسم‌های مفید برای سلامت انسان وجود دارد. در مقابل، نوشیدنی‌های زیست‌یار با منشا گیاهی، علی‌رغم وجود اسیدهای آلی فعال از نظر زیستی، بسیار کمتر رایج هستند. هدف از این مطالعه ارزیابی کمی غلظت متابولیت‌های ثانویه مخمرها و باکتری‌های لاکتیک اسید در نوشیدنی‌های دانه تخمیر شده و نیز ویژگی‌های حسی نوشیدنی‌ها بود.

مواد و روش ها: نمونه‌های نوشیدنی زیست‌یار برپایه گندم‌ها، به‌عنوان ماده اولیه غلات، تولید شدند. فرآیند تخمیر شامل استفاده از سویه‌های مختلف باکتری اسید لاکتیک، مانند *لاکتوباسیلوس دلبروکی*، *لاکتوباسیلوس برویس*، *لاکتوباسیلوس بوکنری*، *لاکتوباسیلوس پلانتاروم* و *لاکتوباسیلوس فرمنتوم* و همچنین گونه‌های مختلف مخمرهای *ساکارومایسس سرویزیه* بود. علاوه بر این، نوشابه‌های غیرالکلی تولید شده تجاری ساخته شده از مواد تشکیل‌دهنده غلات به عنوان مبنای مقایسه مورد استفاده قرار گرفتند. محتویات و غلظت اسیدهای آلی با استفاده از کروماتوگرافی مایع با کارایی بالا، با پیروی از دستورالعمل‌های استاندارد دولتی مورد تجزیه و تحلیل قرار گرفت. این تکنیک شامل جداسازی اسیدهای آلی خاص بر روی یک تکیه‌گاه جامد با استفاده از مکانیسم فاز معکوس بود.

یافته‌ها و نتیجه‌گیری: لاکتیک اسید به‌میزان $mg.dm^{-3}$ ۱۳۰۰ در نوشیدنی زیست‌یار برپایه گندم، دلالت بر تخمیر لاکتیک اسید دارد. شناسایی سیتریک و سوکسنیک اسیدها به‌ترتیب به‌میزان ۸۰ و ۱۵۲ $mg.dm^{-3}$ ۱۳۰۰ نشان دهنده ماهیت هتروآنزیمی بودن ماهیت تخمیر لاکتیک اسید است. بنابراین، توسعه ترکیبات معطر مطلوب مانند میخک، میوه‌ای و شبیه موز برای نوشیدنی زیست‌یار برپایه گندم انتظار می‌رود. براساس داده‌ها، می‌توان پروفایل ترکیبات معطر نوشیدنی‌های تخمیری حاصل از مواد خام گیاهی با استفاده از مخلوط باکتری‌های لاکتیک اسید و مخمرها را پیش‌بینی کرد.

تعارض منافع: نویسندگان اعلام می‌کنند که هیچ نوع تعارض منافعی مرتبط با انتشار این مقاله ندارند.