

## Article

# Effect of Fermentation on the Biochemical Parameters Antioxidant Capacity and Dispersed Composition of Plant Beverages Based on Barley and Hemp Seeds

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**Abstract:** Enzymatic processes play a key role in the production of grain-containing food due to their effect on the nutritional properties, rheological characteristics, and contribution to improving the functional and antioxidant properties. Eight samples of beverages based on barley grain and hemp seeds were produced (control beverages and beverages fermented by bifidobacteria and propionic acid bacteria). It was found that lactic acid accumulated during fermentation alongside a gradual shift in the pH level in the acidic direction. A comparative analysis of the DPPH activity revealed the highest values for barley-based beverages, ranging from 71.0 to 100.7%, while for the hemp seed-based beverages, the DPPH activity was 64.1–97.9%. The maximum values of DPPH activity were observed during fermentation with a combination of bifidobacteria and propionic acid bacteria concentrates. The highest concentration of polyphenolic compounds and flavonoids was found in barley-based beverages fermented with *Propionibacterium freudenreichii* (1.26 mg GAE/g and 0.11 mg EQ/g) and a combination of *Propionibacterium freudenreichii* and *Bifidobacterium longum* (1.24 mg GAE/g and 0.14 mg EQ/g). Studies have shown an increase in the nutrient content for fermented beverages compared to the control samples. The barley-based beverages exhibited the largest average dynamic particle diameter, and all beverage samples showed a more uniform particle size distribution after microbial fermentation.

**Keywords:** barley-based beverages; hemp seed-based beverages; bifidobacteria; propionic acid bacteria; DPPH activity; polyphenolic compounds; flavonoids



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## 1. Introduction

Grain raw materials have countless benefits for human health due to their rich content of bioactive compounds, macronutrients, micronutrients, and phytochemicals. Nutritionally, they are an important source of unsaturated fatty acids, dietary fibers, vitamins, and minor nutrients (such as polyphenols, flavonoids, carotenoids, tocopherols, and phytosterols). Cereals and oilseeds are characterized by a well-balanced mineral ratio and have considerable antioxidant potential, anti-inflammatory and anti-allergic activity [1–3]. The bioactive compounds of whole grains have significant beneficial effects on the gut microbial composition and physiological functions. Due to these positive healthy effects, functional grain-based foods have become very popular on all continents [4].

Enzymatic processes play a key role in the production of grain-containing products due to their effect on organoleptic characteristics and the improvement in bioavailability and bioaccessibility as well as nutritional and functional properties. Depending on the process type and microbial species, microbial enzymatic activity can increase the levels of certain bioactive molecules with physiological functions [5,6]. Fermentation can positively modify food composition and be an effective tool to prevent microbial contamination and increase the food shelf life [7–9]. It has been shown that the biotechnological food processing methods can effectively reduce the levels of anti-nutritional factors such as

lectins, phytic acid, proteinase inhibitors, oxalic and tannic acids as well as decrease the amount of toxic components and may be the best alternative to minimizing the adverse effects of these compounds in diets [10–12].

The increased consumption of fermented grain products has been associated with protection against a number of diseases such as obesity, cardiovascular disease, type 2 diabetes, and cancer [1]. Controlled fermentation involves the use of specific starter cultures such as lactic acid bacteria, yeasts, fungi, *Bacillus* species, and other microorganisms. These species have been isolated, identified, and adapted to improve the reliability and reproducibility of fermentation [13,14]. Selecting appropriate starter cultures for specific grain-based matrices is an industrial approach to regulate, accelerate, and standardize fermentation. The starters are capable of generating metabolites (volatile and non-volatile) that provide particular flavor attributes to fermented grain products [7].

The authors have previously established the expediency of using the metabolic profiling of fermented products to register metabolite modifications during the fermentation process and achieving the targeted sensory quality and nutritional value of the final product. The effectiveness of the metabolomics approach in detecting the optimal combinations of *L. plantarum* strains to achieve the desired functional properties, flavor, and antioxidant profiles characteristic of grain fermented systems has been proven. The study took into consideration parameters such as the combination of volatile compounds, flavonoids, polyphenols, and antioxidant activity [15].

Microorganisms require nutrients and favorable environmental conditions for their growth and metabolic activity. Fermentation contributes to the enrichment of the grain substrate with protein, the consumption of carbohydrates, and enhances the bioavailability of nutrients [16].

Grain-based beverages have a huge potential to act as potential vehicles for functional compounds such as antioxidants, dietary fiber, minerals, prebiotics, and vitamins; they are an excellent choice for a healthy diet [17–19]. Functional beverages are one of the most developed segments and consumers appreciate their nutritional characteristics. Non-dairy milk is a rich source of highly valuable proteins, unsaturated fatty acids, vitamin B, and isoflavones [20]. However, plant-based milk substitutes also have various adverse health effects including low protein content, low bioavailability of minerals and vitamins, and anti-nutrients; these obstacles can be overcome through fermentation [18,19]. The common stages of the production of milk substitutes include wet grinding, filtration, addition of ingredients, sterilization, homogenization, aseptic packaging, and cold storage. Technological additives are used to improve the stability, taste, and preservation [21–23].

Probiotic drinks can be produced by fermenting a plant base with specific cultures [24,25]. When analyzing the physico-chemical characteristics of grain-based drinks inoculated with *Lactobacillus acidophilus*, *Lactobacillus plantarum*, and *Lactobacillus reuteri*, the titrated acidity, soluble solid content, free amine nitrogen, and acetaldehyde concentration increased; this proved the prebiotic properties of grain raw materials [26]. Studies have shown that probiotic cultures of *L. fermentum KKL1* have a significant effect on the preparation of rice beverages and improve their antioxidant activity and functional characteristics [27]. By selecting the proper processing and fermentation conditions, functional beverages with appropriate nutritional and sensory properties can be produced [28].

As one a cereal, barley possesses high concentrations of vitamins, essential minerals, dietary fiber,  $\beta$ -glucan, arabinoxylans, and cellulose, in addition to relatively high levels of protein, unsaturated fatty acids, and phenolic compounds [29]. Barley and malt are also potential substrates for probiotic microorganisms as they contain easily digestible nutrients, can improve lactobacilli hardiness to the aggressive environment of the gastrointestinal tract, and can support the growth of single and mixed strains of probiotic microorganisms [30,31]. Hemp seeds contain a great deal of physiologically valuable components; they are a priority raw material in the production of functional foods. Industrial hemp seeds are recommended as a source of essential components such as high bioavailability protein

with a balanced amino acid composition, omega-3 fatty acids, dietary fiber, vitamins, and minerals [32,33].

The purpose of the study was to investigate the effect of technological factors on the biochemical parameters, antioxidant activity, and dispersed composition of fermented grain-based beverages.

## 2. Materials and Methods

### 2.1. Raw Materials and Ingredients

For our research, we used Nurgush barley grains and Nadezhda technical hemp seeds harvested in 2021. These varieties of cultures are the most widespread for cultivation and processing in our region.

The nutritional composition of Nargush barley is 10.30 g protein, 2.41 g fat, 59.7 carbohydrates, 14.5 g fiber, and Nadezhda hemp seeds—21.90 g protein, 30.2 g fat, 19.8 g carbohydrates, and 21 g fiber.

Bacterial preparations produced by Propionics LLC (Ulan-Ude) containing probiotic starter cultures were used for the microbiological fermentation of grain beverages:

- Propionix, a concentrated microbial mass of strain *Propionibacterium freudenreichii* subsp. *shermanii* KM 186 with activity  $10^{10}$ – $10^{11}$  CFU/cm<sup>3</sup>;
- Bacterial liquid concentrate of *Bifidobacterium longum* B 379M with activity  $10^{11}$ – $10^{12}$  CFU/cm<sup>3</sup>.

### 2.2. Manufacturing of Fermented Grain-Based Beverages

The production of plant-based beverages involved the following stages and parameters. First, impurities were removed from the grain. The grain was washed with running water, then soaked at  $25.0 \pm 3.0$  °C for 8–12 h in order to swell the proteins and polysaccharides, loosen shells, and release intermolecular bonds. The grains were subjected to wet milling with a hydromodule of 1:8 on a micromill to obtain particles with the smallest size. The resulting milled grain was mixed with a mechanical stirrer for 60–90 min to obtain a homogeneous polydispersity system. The emulsion was then filtered through a series of screens with decreasing mesh sizes; the mesh of the final filter was no greater than 100 microns. The beverages were pasteurized at 60–75 °C for 5 min to increase the stability of the food system, inactivating the microorganisms and enzymes. The beverages were cooled to a temperature of 38 °C and the thermophilic strains of the microorganisms were introduced. The beverages were fermented at 38–40 °C for 8–10 h until a weak clot was formed and a pH level below 4.7 was achieved. The beverages were then cooled to  $4 \pm 2$  °C and stored for 72 h (Figure 1).

A total of eight experimental batches of fermented beverages were produced including unfermented plant-based drinks; fermented drinks with bifidobacteria; fermented drinks with propionic acid bacteria; and fermented drinks with a combination of bifido- and propionic acid bacteria (Table 1). Five replicates were made for each beverage sample for the rheological and biochemical studies.

### 2.3. Methods of Analyses

#### 2.3.1. Analyses of Viscosity and Biochemical Composition

The intensity of metabolic processes in the obtained beverages during fermentation were determined: active acidity (pH level), dynamic viscosity, level of antioxidant activity, and content of polyphenolic components and flavonoids. The biochemical composition was also determined: the content of dry matter, protein, fat, and carbohydrates.

The active acidity of the samples was determined by immersing the electrode of a multiparameter stationary pH meter (edge HI 2002-02 by Hanna Instruments, Romania) in the beverage emulsion for 2 min. The pH level was studied every 2 h during fermentation. Lactic acid accumulation was determined by titrimetric analysis of 0.1 N NaOH using phenolphthalein (0.1% wt./vol. in 95% ethanol) as an indicator and subsequent conversion to lactic acid concentration (g/100 mL).

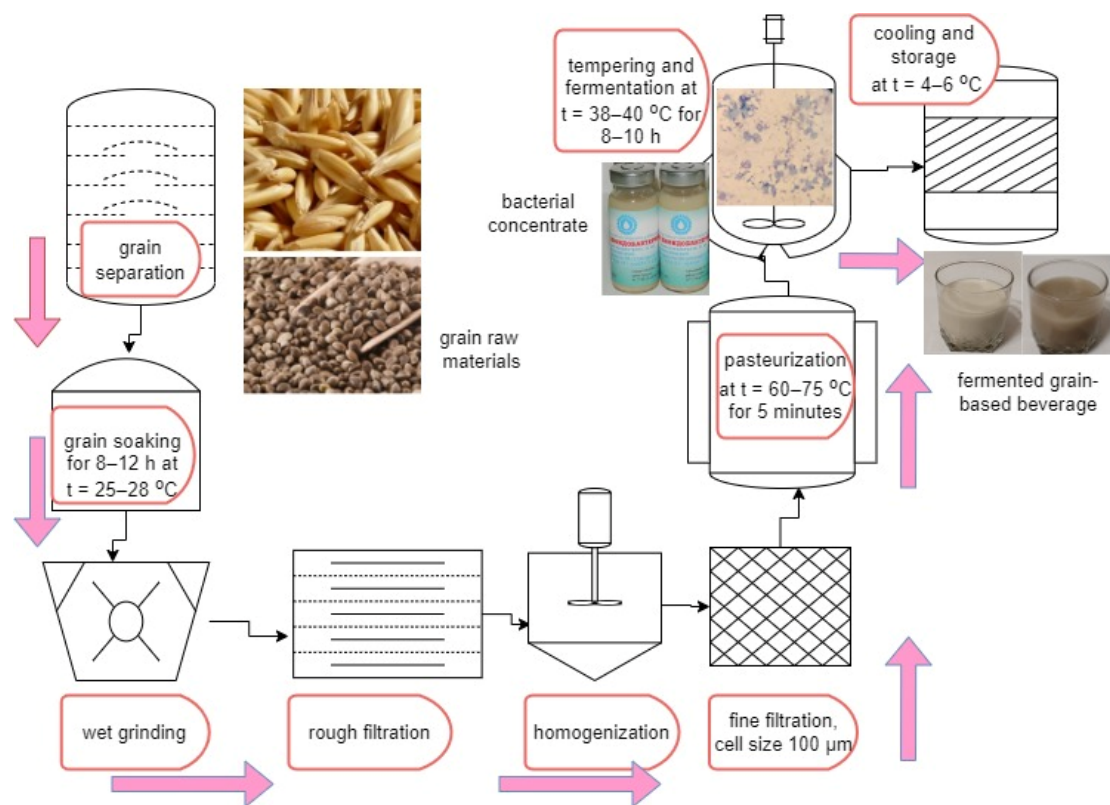


Figure 1. The production stages of the fermented grain-based beverages.

Table 1. The component composition of the beverage samples.

Designation of Samples	Plant Milk from Barley Grain, %	Plant Milk from Hemp Seeds, %	<i>Bifidobacterium longum</i> Concentrate, %	<i>Propionibacterium freudenreichii</i> Concentrate, %
Unfermented barley milk	100	–	–	–
Barley milk, fermented by <i>Bif. longum</i>	98	–	2	–
Barley milk, fermented by <i>Pr. freudenreichii</i>	98	–	–	2
Barley milk, fermented by <i>Bif. longum</i> and <i>Pr. freudenreichii</i>	98	–	1	1
Unfermented hemp milk	–	100	–	–
Hemp milk, fermented by <i>Bif. longum</i>	–	98	2	–
Hemp milk, fermented by <i>Pr. freudenreichii</i>	–	98	–	2
Hemp milk, fermented by <i>Bif. longum</i> and <i>Pr. freudenreichii</i>	–	98	1	1

The dynamic viscosity of the samples was determined using an AND SV vibro viscometer. The measurement was carried out for 60.0 s at 22.0 ± 2.0 °C.

The content of dry matter was measured using a digital refractometer Abbemat 500 (Anton Paar, Graz, Austria). The total nitrogen content was assayed by the Kjeldahl method with nitrogen converted to equivalent protein content using a factor of 6.25 (Methods 992.15 and 992.23); total fat was determined via the Soxhlet method (Methods 920.39 C and 960.39).

### 2.3.2. Investigation of Polyphenol Content and Antioxidant Activity Extraction of Phenolic

The beverage samples were extracted with 5-fold 80% (*v/v*) ethanol in a water bath at 40 °C for 3 h. The extracted solution was centrifuged at 8000 g for 15 min, and the supernatant was evaporated to dryness using a rotary evaporator at 50 °C [34]. The phenolic extracts were then redissolved in 80% (*v/v*) ethanol for further analysis (phenolic samples).

#### Antioxidant Activity Analysis

##### DPPH Radical (DPPH) Scavenging Activity Measurement

The DPPH radical scavenging activity was determined according to the method of Xiao, Rui, et al. [34]. Specifically, 2 mL of phenolic sample was added to 2 mL of DPPH solution (0.4 mmol/L), and the mixture was allowed to stand in the dark for 30 min. Then, the absorbance was recorded at 515 nm. The DPPH radical scavenging activity (%) =  $[1 - \text{absorbance of sample} / \text{absorbance of control}] \times 100$ .

##### Detection of Total Phenolic Content (TPC)

The TPC was determined using the Folin–Ciocalteu colorimetric method [35]. Briefly, 0.2 mL of the phenolic sample was added to 2.3 mL of distilled water and oxidized with 2 mL of 0.5 mol/L Folin–Ciocalteu reagent for 4 min. The reaction was neutralized by adding 2 mL of 75 g/L saturated sodium carbonate. After 2 h of incubation in the dark, the absorbance at 760 nm was recorded by Jenway spectrophotometer (6405 UV/Vis, UK). The total phenolic content was expressed in gallic acid equivalent (GAE) (i.e., mg GAE/g dry weight (DW)) [34].

##### Determination of Total Flavonoid Content (TFC)

The contents were spectrophotometrically measured based on the formation of a flavonoid–aluminum complex [35]. Briefly, 1.00 mL of the sample was mixed with 0.10 mL of 5.0% NaNO<sub>2</sub> for 6.0 min. Then, 0.10 mL of 10.00% AlCl<sub>3</sub>·6H<sub>2</sub>O solution was added to the mixture for another 5 min. After adding 1.0 mL of 1.0 mol/L NaOH, the reaction solution was mixed well and allowed to stand for 15 min. The absorbance was measured at 510 nm. Quercetin was used as a standard in order to establish the calibration curve. The TFCs were calculated and expressed in quercetin equivalent (i.e., mg EQ/g dry weight DW).

### 2.3.3. Determination of Dispersed Composition

The study of the dispersed composition and the analysis of the particle size in the samples were carried out by the method of laser dynamic light scattering on a laser diffraction analyzer Microtrac S3500. Program: Microtrac FLEX 10.6.1.

## 2.4. Statistical Analyses

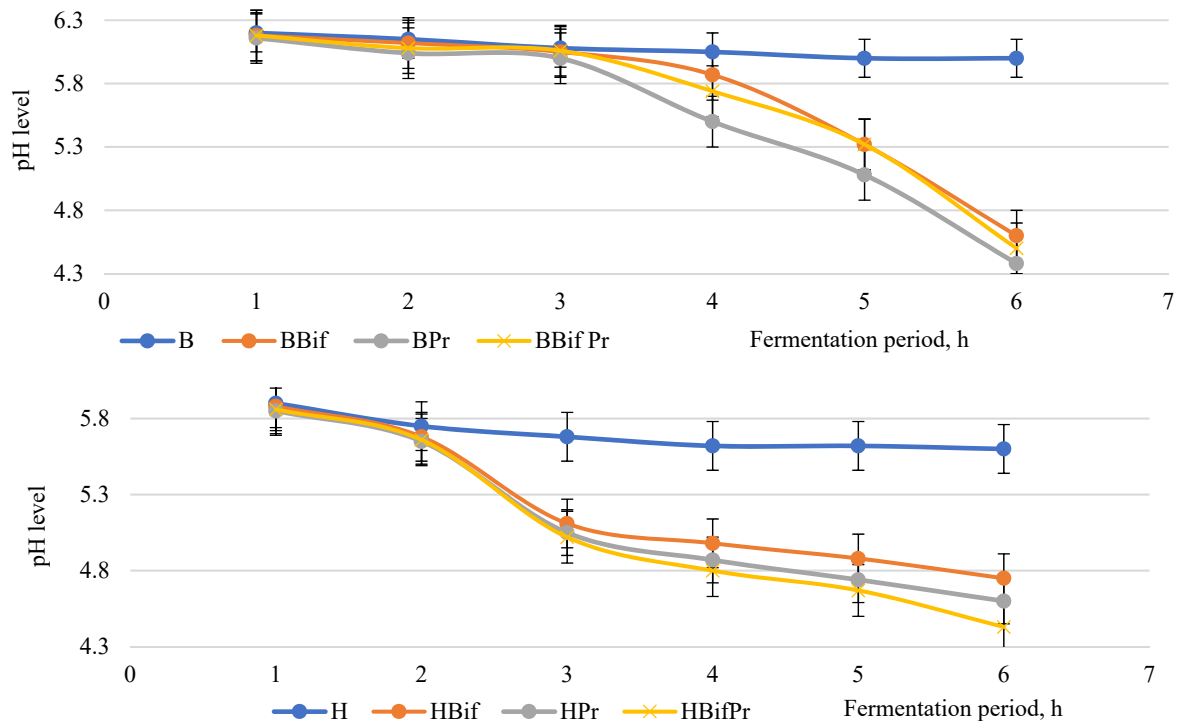
The analyses were performed in five replicates. Results were expressed as the mean values of the five replicates ± the standard deviation. Probability values of  $p \leq 0.05$  were taken to indicate statistical significance. The data were analyzed via one-way ANOVA analysis of variance using the free web-based software offered by Assaad et al. [36].

## 3. Results and Discussion

### 3.1. Analysis of Viscosity and Biochemical Parameters of Fermented Beverages

The accumulation of acidity during the fermentation of the biological system affects the inhibition of pathogenic microflora, impacts shelf life, and helps form the flavor profile characteristic of fermented grain-based beverages. During the fermentation period, a gradual shift in the pH level toward acidity was established in the samples. In the barley-based beverages, the pH value decreased more pronouncedly after 6 h of fermentation as the organic acids accumulated; after 6–10 h, the pH level decreased from approximately 5.70 to 4.38–4.6. In the hemp seed-based beverages, the pH value decreased more strongly over the first 4 h (from 5.86 to 5.02–5.11), reducing more slowly throughout the remainder

of the fermentation period. It was found that propionic acid bacteria most intensively accumulated acidity, both when used on their own and in combination with bifidobacteria (Figure 2).



**Figure 2.** The pH level during the fermentation of grain-based beverages. The error bars represent the standard deviation of measurements ( $n = 5$ ). Designation of samples: B (H) unfermented barley (hemp) milk; BBif (HBif) barley (hemp) milk, fermented by *Bifidobacterium longum*; BPr (HPr) barley (hemp) milk, fermented by *Propionibacterium freudenreichii*; BBifPr (HBifPr) barley (hemp) milk, fermented by a combination of *Bifidobacterium longum* and *Propionibacterium freudenreichii*.

The noticeable decrease in pH during fermentation might be explained by the decomposition of the grain starch and the generation of a large number of sugars accessible for bacteria. This decline in pH can serve to protect the beverage against spoilage-associated bacteria and pathogens.

We observed significant accumulation of lactic acid in the beverages after fermentation—the acid content was 4–6 times higher compared to the unfermented samples. Moreover, propionic bacteria and a consortium of bifidobacteria and propionic bacteria produced organic acids most intensively during metabolism. The levels of lactic acid detected in the fermented beverages were correlated to the values reported in maize porridges supplemented with malted barley and inoculated with lactobacilli strains [37].

The barley-based beverages had a higher dynamic viscosity (2.29–3.36 mPa·s). These results were to be expected, since the barley shells contain insoluble structural polysaccharides (cellulose, hemicellulose); in addition, the grain endosperm contains a significant amount of mucus-forming substances (pentosans and soluble  $\beta$ -glucan) that easily swell in water, forming low viscosity gels. Moreover, the fermentation process had a greater effect on the viscosity increase in the barley-based beverages. When the samples were fermented with *Bifidobacterium longum*, the viscosity increased by 29.70% compared to the unfermented drink. With a combined starter (*Bifidobacterium longum* and *Propionibacterium freudenreichii*), the viscosity increased by 46.72% after fermentation. Non-starch barley polysaccharides absorb up to 800.0% of water at room temperature, forming viscous solutions.  $\beta$ -glucan does not crystallize, leading to high water solubility and increased hydrolyzability by enzymes (Table 2) [38].

**Table 2.** The viscosity and biochemical composition of the grain-based beverages.

Indicators	Designation of Samples			
	B	BBif	BPr	BBifPr
Dynamic viscosity, mPa·s	2.29 ± 0.005 <sup>a</sup>	2.97 ± 0.001 <sup>b</sup>	2.79 ± 0.004 <sup>b</sup>	3.36 ± 0.005 <sup>c</sup>
Lactic acid content, g/100 mL	0.18 ± 0.005 <sup>a</sup>	0.81 ± 0.007 <sup>b</sup>	0.99 ± 0.006 <sup>bc</sup>	1.17 ± 0.008 <sup>c</sup>
Dry matter content, %	3.86 ± 0.054 <sup>a</sup>	4.42 ± 0.067 <sup>ac</sup>	4.96 ± 0.063 <sup>b</sup>	4.68 ± 0.072 <sup>c</sup>
		Including:		
Protein content, %	1.38 ± 0.021 <sup>a</sup>	1.67 ± 0.023 <sup>b</sup>	1.77 ± 0.034 <sup>b</sup>	1.68 ± 0.030 <sup>b</sup>
Fat content, %	0.38 ± 0.003 <sup>a</sup>	0.35 ± 0.003 <sup>a</sup>	0.35 ± 0.004 <sup>a</sup>	0.40 ± 0.004 <sup>a</sup>
Carbohydrate content, %	1.89 ± 0.025 <sup>a</sup>	2.16 ± 0.044 <sup>ab</sup>	2.56 ± 0.030 <sup>bc</sup>	2.38 ± 0.032 <sup>c</sup>

Indicators	Designation of Samples			
	H	HBif	HPr	HBifPr
Dynamic viscosity, mPa·s	0.85 ± 0.002 <sup>a</sup>	1.01 ± 0.003 <sup>b</sup>	0.99 ± 0.005 <sup>b</sup>	1.21 ± 0.002 <sup>c</sup>
Lactic acid content, g/100 mL	0.17 ± 0.005 <sup>a</sup>	0.75 ± 0.006 <sup>b</sup>	1.08 ± 0.006 <sup>bc</sup>	0.99 ± 0.007 <sup>c</sup>
Dry matter content, %	4.67 ± 0.048 <sup>a</sup>	6.31 ± 0.059 <sup>b</sup>	5.77 ± 0.050 <sup>c</sup>	6.55 ± 0.067 <sup>b</sup>
		Including:		
Protein content, %	1.67 ± 0.040 <sup>a</sup>	2.25 ± 0.056 <sup>b</sup>	2.06 ± 0.045 <sup>b</sup>	2.34 ± 0.068 <sup>b</sup>
Fat content, %	1.35 ± 0.020 <sup>a</sup>	1.77 ± 0.034 <sup>b</sup>	1.56 ± 0.030 <sup>a</sup>	1.82 ± 0.041 <sup>b</sup>
Carbohydrate content, %	1.42 ± 0.024 <sup>a</sup>	1.86 ± 0.042 <sup>bc</sup>	1.68 ± 0.025 <sup>b</sup>	1.81 ± 0.030 <sup>c</sup>

The values are means (M) ± standard deviation of five replicates (s). Different letters in the same row (M<sup>a, b, c</sup>) refer to a significant difference at ( $p \leq 0.05$ ). Designation of samples: B (H) unfermented barley (hemp) milk, BBif (HBif) barley (hemp) milk, fermented by *Bifidobacterium longum*; BPr (HPr) barley (hemp) milk, fermented by *Propionibacterium freudenreichii*; BBifPr (HBifPr) barley (hemp) milk, fermented by *Bifidobacterium longum* and *Propionibacterium freudenreichii*.

The viscosity of the hemp-based fermented beverages was measured at 0.85–1.21 mPa·s. We found that the combined starters of bifidobacteria and propionic acid bacteria most strongly increased the dynamic viscosity of the beverages. The observed patterns were associated with the synergistic action of microorganisms toward the hydrolysis of structural polysaccharides and the synthesis of exopolysaccharides in the food system. In analogous studies, researchers have demonstrated that rice extracts fermented with probiotic cultures were similar to yoghurts and traditional lactic acid drinks in their rheological properties (viscosity and flow characteristics) [39].

Studies have shown an increase in the content of nutrient constituents during the fermentation of plant materials by probiotic microorganisms [40,41]. In our experiment, we observed an increase in the dry matter content in fermented beverages; for barley-based beverages, the dry matter value increased by 14.5–28.5% ( $p \leq 0.05$ ) and for hemp-based beverages by 23.6–40.3% ( $p \leq 0.05$ ) (compared to the control sample). The highest levels of proteins and lipids were observed in the hemp seed-based fermented beverages, meaning that these beverages can be considered as sources of macronutrients (Table 2).

Numerous authors reporting an increase in protein content during the fermentation of grain-deriving food have ascribed this to the activities of proteolytic enzymes produced by the fermenting microorganisms and protein synthesis during fermentation [40]. The mechanism of protein increase was also ascribed to an increase in the microbial mass, resulting in an extensive hydrolysis of the protein molecules to simple peptides [41]. Some studies have reported that the increase in protein content during fermentation was attributed to the release of protein from plant tissues by the enzymatic breakdown of cellulose, with the simultaneous degradation of tannins and phytic acid by the action of microbial enzymes [42].

### 3.2. Antioxidant Activity and Polyphenol Content in Fermented Beverages

The activity of probiotic microorganisms in the food system is associated with a number of enzymatic processes, leading to effective proteolysis, thereby increasing the concentration of potentially bioactive peptides as well as the release of active forms of

polyphenolic compounds and increased antioxidant activity. A comparative analysis of the DPPH activity, which is related to the ability of biomolecules to neutralize free radicals, revealed the highest values for barley-based beverages, ranging from 71.0 to 100.7%, while for the hemp seed-based beverages, the DPPH activity was 64.1–97.9%. There was a significant increase in the antioxidant capacity of beverages fermented with probiotic microorganisms. Thus, an increase of 25.0–29.2% ( $p \leq 0.05$ ) in DPPH activity was observed when the samples were fermented with propionic acid bacteria, while the maximum values of antioxidant activity were observed in the beverages fermented with a combination of bacterial concentrates, in which DPPH increased by 41.8–52.8% ( $p \leq 0.05$ ) compared to the non-fermented beverages (Table 3).

**Table 3.** The antioxidant activity and polyphenol content in the fermented grain-based beverages.

Designation of Samples	DPPH Activity, %	Content of Polyphenols, mg GAE/g	Content of Flavonoids, mg EQ/g
Unfermented barley milk	71.03 ± 2.45 <sup>a</sup>	1.17 ± 0.04 <sup>ab</sup>	0.105 ± 0.012 <sup>d</sup>
Barley milk, fermented by <i>Bif. longum</i>	85.54 ± 4.25 <sup>b</sup>	1.23 ± 0.05 <sup>bc</sup>	0.105 ± 0.013 <sup>d</sup>
Barley milk, fermented by <i>Pr. freudenreichii</i>	88.70 ± 3.54 <sup>bc</sup>	1.27 ± 0.04 <sup>c</sup>	0.110 ± 0.014 <sup>d</sup>
Barley milk, fermented by <i>Bif. longum</i> and <i>Pr. freudenreichii</i>	100.72 ± 5.36 <sup>c</sup>	1.24 ± 0.05 <sup>bc</sup>	0.140 ± 0.015 <sup>f</sup>
Unfermented hemp milk	64.12 ± 3.22 <sup>a</sup>	1.13 ± 0.03 <sup>a</sup>	0.029 ± 0.009 <sup>a</sup>
Hemp milk, fermented by <i>Bif. longum</i>	84.35 ± 5.53 <sup>b</sup>	1.17 ± 0.03 <sup>ab</sup>	0.044 ± 0.010 <sup>b</sup>
Hemp milk, fermented by <i>Pr. freudenreichii</i>	82.79 ± 4.85 <sup>b</sup>	1.20 ± 0.04 <sup>b</sup>	0.078 ± 0.011 <sup>c</sup>
Hemp milk, fermented by <i>Bif. Longum</i> and <i>Pr. freudenreichii</i>	97.95 ± 6.62 <sup>c</sup>	1.17 ± 0.04 <sup>ab</sup>	0.109 ± 0.013 <sup>d</sup>

The values are means (M) ± standard deviation of five replicates (s). Different letters in the same column (M<sup>a, b, c</sup>) refer to a significant difference at ( $p \leq 0.05$ ).

Barley and hemp are rich in biologically active compounds such as phytosterols and polyphenols. In particular, polyphenols can be divided into several classes, namely flavonoids, phenolic acids, lignans, stilbenes, tannins, and diterpenes. These compounds possess different molecular weight and chemical structure and are widespread in plants in their free and bound forms [43]. Phenolic compounds are known for their antioxidant activity; they play a key role in the treatment and prevention of a number of diseases (such as cardiovascular and neurodegenerative diseases) and cancer [44–46]. In our experiment, the highest concentration of polyphenolic compounds and flavonoids was found in barley-based beverages fermented with *Propionibacterium freudenreichii* (1.27 mg GAE/g and 0.11 mg EQ/g) and a combination of *Propionibacterium freudenreichii* and *Bifidobacterium longum* (1.24 mg GAE/g and 0.14 mg EQ/g, respectively). Moreover, the total concentration of polyphenolic compounds in the samples increased by 5.3–8.6% ( $p \leq 0.05$ ) when they were fermented with propionic bacteria compared to the control (Table 3).

The effect of microbial fermentation on the content of polyphenols and flavonoids in fermented grain-based food has been reported in numerous studies [47,48]. Starter cultures increase the solubility and extractability of polyphenolic compounds; the effectiveness of these reactions depends on the strain and the specific enzymatic activity of bacteria. The fermentative processes that occur during the ripening of beverages contribute to the release of polyphenols from glycosylated protein complexes. This is especially valuable because the phenolic compounds must be in a soluble form in order to enter the bloodstream and manifest their beneficial properties. The increased content of polyphenolic compounds



can be associated with an increase in acidity as well as the activity of microbial enzymatic systems. On the another hand, microbial metabolism could modify the bioactive substances in grain, leading to the synthesis of new substances such as phenolic compounds.

Reduced pH value promotes the activation of cellulose degrading enzymes to accelerate the release of intra-cell compounds [34,44]. Studies have been conducted on probiotic plant-based beverages including a mixture of flour from barley, millet, and moth beans (using germinated and ungerminated seeds). Fermentation with a probiotic culture of *L. acidophilus* improved the overall acceptability, functional properties, and polyphenol content during fermentation [49]. Researchers have found that protein extracts from brewers' grain waste fermented by *Rhizopus oligosporus* demonstrated high antioxidant activity and excellent functional properties [50].

Microbial fermentation by *Lactobacillales* can ensure the health-promoting properties of buckwheat and quinoa and increased phenolic acid and tyrosol content in non-wheat grains [51]. The fermentation of whole-grain oats with a combination of *Lactobacillus plantarum* and *Rhizopus oryzae* has been proven to significantly increase the total content of phenols and DPPH activity [52].

### 3.3. Dispersed Composition of the Beverage Food System

Native biopolymers in a biological system interact specifically, whereas in processed food systems, they are mostly denatured and interact nonspecifically. Most food components have limited miscibility at the molecular level and form multicomponent, heterophase, nonequilibrium disperse systems. Dispersive composition analysis is applicable to the study of interrelationships and nonspecific interactions between individual structures. Mechanical stirring, changes in temperature and/or pH, enzymatic hydrolysis, and certain other treatment methods significantly change the size and homogeneity of individual particles of the disperse system.

While analyzing the disperse composition of the food system of beverages, we determined the average hydrodynamic diameter from the numerical particle size distribution and investigated the particle distribution profiles. We found that the disperse composition of the beverages differed significantly, depending on the type of grains being used (Figure 3).

In the barley-based beverages, the average dynamic diameter ranged from 21.37 to 30.97  $\mu\text{m}$ ; the smallest particle diameter was found in the samples fermented with a combination of bifidobacteria and propionic acid bacteria (10.37  $\mu\text{m}$ ). In addition, a more uniform particle size distribution was found in the beverages after microbial fermentation. The larger particle diameter of barley-based drinks was primarily due to the hydration properties of soluble dietary fibers that swelled in water, generated intermolecular bonds, and formed larger agglomerates. These features provide the beverage food system with a higher dynamic stability and prevent the separation of phases. The water hydration facilitates electrostatic interactions, hydrogen bonding, and noncovalent complexing (ionic surfactant or dispersion interaction) between oligosaccharides, oil, fiber, sugars, and protein, resulting in larger particle sizes [53]. When studying the disperse composition of rice flour after treatment, researchers have found that the particle distribution profiles illustrated noticeably increased particle sizes in samples that were subjected to enzymatic processing. This indicates a chemical interaction between the initially catabolized complex classes of substances [54].

Hemp seeds include soluble globular proteins and a high concentration of lipids. During fermentation, the hydrolysis of protein and carbohydrate macromolecules occurs and the emulsion stabilizes due to the surface-active properties of the peptides and fatty acids. The average hydrodynamic particle diameter of hemp seed beverages after fermentation was reduced by 51.7–85.1% ( $p \leq 0.05$ ) compared to the unfermented samples (Figure 3). According to the principles of emulsification (Stokes' law), smaller particle sizes ensure the stability of oil–water and water–oil emulsions [53].

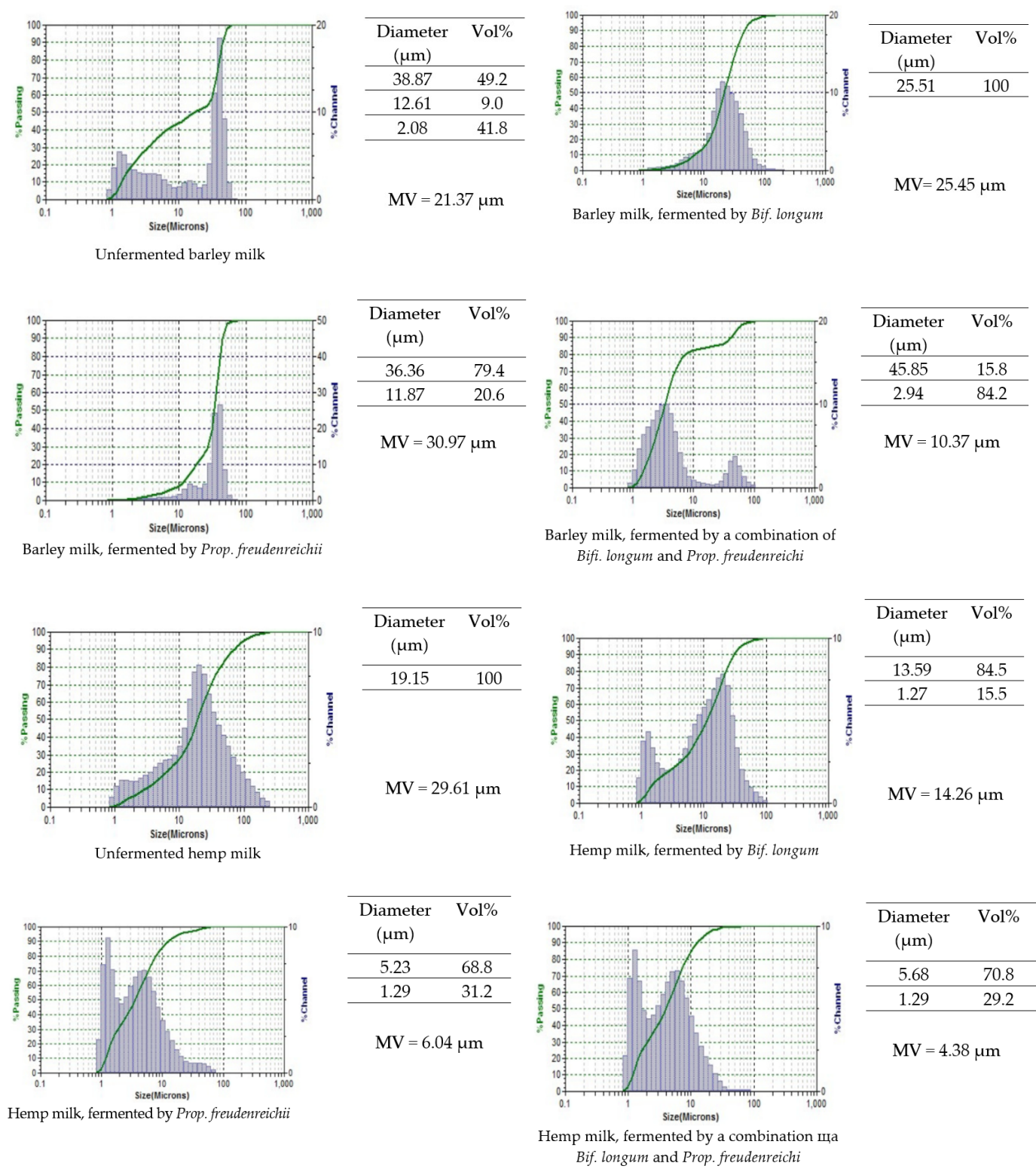


Figure 3. The dispersed composition of the grain-based beverage food system.

Metabolic by-products of probiotic microorganisms also affect the hydrodynamic stability and particle size of food systems. Researchers have shown that ultra-high molecular weight exopolysaccharides contribute to the stability and viscosity of soybean beverages. Thus, soybean beverages fermented with starter cultures do not require the inclusion of complex and expensive stabilizers, resulting in lower production costs [46].

#### 4. Conclusions

Enzymatic processes play a key role in the production of grain-containing food due to their effect on the nutritional properties, rheological characteristics of the finished product, and their contribution to improving the functional and antioxidant proprieties. During the microbial fermentation of plant beverages, a number of physico-chemical and colloidal processes were observed. We found that lactic acid accumulated during fermentation alongside a gradual shift in the pH level in the acidic direction. These biochemical processes contributed to the release and accumulation of polyphenolic compounds and, as a consequence, an increase in the antioxidant activity of fermented grain-based beverages. The maximum values of DPPH activity were observed during fermentation with a combination of bifidobacteria and propionic acid bacteria.

Microbial fermentation significantly affected the rheological properties and dispersion composition of the beverages. Fermentation with bifidobacteria and propionic acid bacteria positively affected the viscosity of grain-based beverages. The barley-based beverages exhibited the largest average dynamic particle diameter, and all beverage samples showed a more uniform particle size distribution after microbial fermentation. The average particle diameter of hemp beverages after fermentation was significantly reduced. This ensured the hydrodynamic stability of the disperse system of the beverage.

In future studies, it is planned to expand research for more varieties of grain crops. To take full advantage of the benefits of fermentation in the production of functional products including those with antioxidant properties, it is necessary to carefully select the grain substrate and industrial strains of microorganisms, taking into account the principles of metabolomics. In addition, in order to comprehensively improve the food quality and nutritional value, it is necessary to optimize fermentation process parameters based on mathematical modeling methods.

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