

Article

Upcycling Wheat-Rye Bread and Chokeberry Waste into Sustainable Fermented Beverages with Potential Probiotic Properties

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Abstract

Increasing food consumption and waste generation are today's most difficult economic and environmental challenges. In line with the sustainable production and consumption concept, wasted food, as a source of valuable resources, can be reused to produce new products of higher value than the raw materials from which they were made. This concept was used in this work to design products that are a plant-based alternative to fermented milk drinks, which arouse great interest among consumers due to their health-promoting properties. This study aimed to design potential probiotic beverages from food industry waste, including wheat-rye bread and chokeberry pomace, using lactic acid fermentation with different strains of lactic acid bacteria (LAB) and to evaluate selected quality features of the obtained beverages. In the first stage of the research, the group of LAB strains was tested for their efficiency in bakery waste fermentation, and then the potential probiotic properties of chosen LAB strains (*Lactiplantibacillus plantarum* A7, *Lacticaseibacillus paracasei* INV001, *Lacticaseibacillus rhamnosus* INV002, *Lentilactobacillus buchneri* P7, *Loigolactobacillus coryniformis* INV014) were characterized according to FAO/WHO requirements. For the prepared beverages, microbiological quality, antioxidant properties, and the content of polyphenolic compounds were determined. It was found that bakery and chokeberry waste may constitute a good base for obtaining fermented beverages with some beneficial properties, including a high number of potentially probiotic bacteria, reaching 10⁸ CFU/mL, and antioxidant properties, which positively verified their functional properties. The research confirms the high potential of lactic acid fermentation in managing food waste to create innovative, sustainable food products with probiotic properties.

Keywords: waste management; circular economy; plant-based alternatives; sustainable food production; innovative beverages



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1. Introduction

The expected population growth and increasing demand for food contribute to the growing problem of food waste and waste generation. Food waste is one of the most difficult economic and environmental problems, posing a huge challenge and requiring

sustainable solutions in production and consumption [1,2]. It is estimated that one-third of the food produced is wasted each year, and the global annual waste of edible food is 1.3 billion tones [3–5]. Food is wasted throughout the whole food supply chain, from raw materials, through production, to distribution, and by the final consumer. In developed countries, food waste most often occurs after food reaches the consumer, while in developing countries, it occurs both during agricultural production and food production [6–9]. This indicates a strong need for proper food waste management, which would support achieving sustainable development goals and could reduce hunger worldwide [1,10,11]. Effective actions should focus especially on the improvement of two areas: technologies and techniques for managing food waste more efficiently and cost-effectively, in connection with the principles of the circular economy. Currently, available technologies are mostly focused on the use of waste, e.g., to obtain fertilizer, concentrating mainly on minimizing the amount of food waste [12,13]. However, it is worth noting that these activities can also aim at new possibilities of waste valorization, which is profitable, although less popular [1,14]. Effective food waste management can also reduce the impact of food waste on global warming, considering that industry is responsible for approximately 22% of total global greenhouse gas emissions and 30% of total energy consumption [15]. Food waste is estimated to have a global carbon footprint of approximately 4.4 Gt CO₂ equivalent per year, representing 8% of total anthropogenic greenhouse gas emissions [16].

Wasted food is also a valuable resource that can be reused in line with the concept of a circular economy, which involves minimizing waste through reusing, recycling, or implementing more efficient and innovative technologies [13,14]. Therefore, one of the strategies is to create upcycled foods. The term was coined for food produced using ingredients derived from food that would otherwise become food waste. This includes the use of inferior quality, damaged food, by-products, and waste from production processes, which are not suitable for sale as a stand-alone product but still have nutritional value, so their non-use contributes to food waste [17–19].

Designing fermented products allows the use of by-products or waste from the food industry, thus being one of the proposals for their management, especially since the market for plant-based alternatives, including fermented plant-based food, has been growing exponentially in recent years. It is even considered the dominant dietary trend today [20,21]. A significant impact on the growth of this segment was noted during the COVID-19 pandemic, as consumers' interest in the products' health-promoting properties, especially immune system support, increased. Europe has the largest market of fermented foods, which is divided into different product groups, including dairy alternatives, meat alternatives, sauces, and seasonings.

Fermented plant-based products, such as beverages, are of particular interest for consumers, including conventional (e.g., bakery) and emerging products such as meat and dairy alternatives, with the largest market share held by fermented bakery products [21,22]. It is worth noting that innovative plant-based fermented products are considered more sustainable than traditional dairy beverages because they have a smaller carbon footprint and use fewer natural resources [23,24]. Moreover, the transformation of bioactive compounds occurring during the fermentation process can improve the organoleptic and nutritional quality of the product [21]. Therefore, it is emphasized that fermented plant-based beverages show improved digestibility and increased bioavailability of nutrients, which is due to, among others, the promotion of the synthesis of selected vitamins, the degradation of anti-nutritional compounds, the improvement of protein digestibility [24] and the accumulation of biologically active compounds [25]. The advantages of these products make fermented plant-based beverages an alternative to traditional milk beverages [26], being

a good choice for people struggling with milk allergy or lactose intolerance, but also for those who pay attention to a balanced diet or follow plant-based diets [24].

Wheat-rye bread waste and chokeberry pomace with the appropriately selected lactic acid bacteria were used to design the fermented beverages in the presented work. Bread is a product that is commonly consumed all over the world, and at the same time, it is a commonly wasted food, especially in developed countries, which is a serious problem [27]. However, bread is rich in carbohydrates, contains proteins, little fat, and trace amounts of phosphorus, therefore, bakery waste could be valorized into value-added products [28]. In turn, chokeberry fruits contain very high amounts of polyphenolic compounds, making it one of the most potent antioxidant fruits of all [29]. Interestingly, in addition to these fruits, processed products and derived waste are also rich in polyphenols [30]. The literature reports that both chokeberry fruits and their extracts exhibit anti-diabetic, antimutagenic, and anti-cancer effects, as well as protect the cardiovascular system and liver [29]. In vitro studies also indicate high antioxidant properties of chokeberry, both in processed and unprocessed form, as chokeberry ingredients are considered to be the most active ABTS radical scavengers, which confirms this activity [30,31].

The combination of these two types of products allows the use of nutrients in bread and enriches the drink with valuable polyphenolic compounds from chokeberry waste. Therefore, this study aimed to design potentially probiotic beverages from wheat-rye bread waste and chokeberry pomace using lactic acid fermentation with appropriately selected strains of lactic acid bacteria (LAB) and evaluate selected quality features and safety of the obtained beverages.

2. Materials and Methods

2.1. Chemicals, Materials, and Microorganisms

2.1.1. Chemicals

Microbiological media used for the studies were obtained from BioMaxima (Poland) and included De Man–Rogosa–Sharpe (MRS) Agar and Broth, Plate Count Agar (PCA), TBX Agar, VRBG Agar, Sabouraud dextrose with chloramphenicol Agar, Trypticasein Soy Agar (TSA), Trypticasein Soy Broth (TSB), Nutrient Broth (NB), Brain Heart Infusion Agar and Broth (BHI), Yeast Extract Peptone Dextrose Agar and Broth (YPD), and Mueller–Hinton Broth. Chemical reagents used to determine potential probiotic and antioxidant properties of LAB strains such as glucose (Chempur, Piekary Śląskie, Poland), Phosphate Buffered Saline (PBS) (pH 7.4) (BioShop, Burlington, ON, Canada), hydrochloric acid, sodium chloride and toluene (Chempur, Poland), bile extract (Sigma, Darmstadt, Germany), Folin–Ciocalteu reagent (Sigma, Germany), sodium carbonate (Chempur, Poland), gallic acid (Sigma, Germany), methanol (98%) (Stanlab, Poland), 2,2'-azino-bis[3-ethylbenzothiazoline-6-sulfonic acid] (ABTS) diammonium salt (Sigma, Germany), Trolox (6-hydroxy-2,5,5,8-tetramethylchromane-2-carboxylic acid) (Sigma, Germany), potassium persulfate (Chempur, Poland), used in the research were of analytical grade. Antibiotics used to determine the sensitivity of LAB strains, including ampicillin and tetracycline (Sigma, Germany), erythromycin (Acros Organics, Geel, Belgium), streptomycin (Fisher Bioreagents, Pittsburgh, PA, USA), kanamycin, and chloramphenicol (BioShop, Canada), were used to prepare solutions immediately before the test. The following reagents were used for genetic identification: DNA Genomic Mini AX Bacteria+ Spin, PCR Mix Plus Green, and DNA marker 3 (A&A Biotechnology, Gdańsk, Poland), PCR reaction primers 1492R and S-D-Bact-008 (27F) (Future Synthesis, Poznań, Poland), and Biotechnology Grade agarose (BioShop, Canada).

2.1.2. Plant-Based Materials

The ground stale wheat-rye bread and chokeberry pomace were the base for preparing the beverages. The wheat-rye bread, for the duration of this study, was stored sealed in a dry, shaded place at room temperature (± 20 °C). Chokeberry pomace was kept frozen at -18 °C. Before preparing the beverage, pomace was dried at 60 °C for 2 h and ground using an analytical mill (IKA® A11, Sigma-Aldrich, Steinheim, Germany).

2.1.3. Microorganisms

Thirteen strains of lactic acid bacteria (LAB) were used in the first stage of study to prepare the fermented beverages: *Lentilactobacillus buchneri* P7, *Levilactobacillus brevis* INV009, *Loigolactobacillus coryniformis* INV014, *Limosilactobacillus fermentum* INV003, *Lactiplantibacillus plantarum* A7, *Lacticaseibacillus paracasei* INV001, *Lacticaseibacillus rhamnosus* INV002, *Leuconostoc mesenteroides* INV011, *Pediococcus pentosaceus* INV010, *Lactobacillus helveticus* INV008, *L. sakei* JS032, *Lacticaseibacillus zeae*, and *Streptococcus thermophilus* INV017. Antimicrobial properties of selected LAB strains were assessed against ten indicator microorganisms, Gram-positive bacteria: *Micrococcus luteus* ATCC 4698, *Bacillus subtilis* DSM 4451, *Staphylococcus aureus* ATCC 33868, *Enterococcus faecalis* ATCC 19433, and *Listeria monocytogenes* ATCC 19111; Gram-negative bacteria: *Serratia marcescens* PCM 549, *Escherichia coli* ATCC 25922, *Pseudomonas paraeruginosa* ATCC 9027, *Salmonella* Enteritidis ATCC 13076, and yeast, *Rhodotorula mucilaginosa* DKK 040. The culture conditions of each microorganism are shown in Table 1. All strains used originated from the collection of the Department of Natural Science and Quality Assurance, Poznań University of Economic and Business, Poland. The LAB strains were identified using the MALDI-TOF MS method. When testing the probiotic potential of the LAB strains, they were re-identified. Before each use, LAB strains were cultured in MRS Broth for 24 h at 37 °C. The strains were stored long-term in cryoprobes in MRS Broth, mixed 1:1 with 80% glycerol at -23 °C.

Table 1. List of indicator microorganisms with culture conditions.

Microorganism	Medium	Incubation Temperature
Gram-Positive Bacteria		
<i>Bacillus subtilis</i> DSM 4451	PCA	37 °C
<i>Enterococcus faecalis</i> ATCC 19433	BHI	37 °C
<i>Listeria monocytogenes</i> ATCC 19111	BHI	37 °C
<i>Micrococcus luteus</i> ATCC 4698	TSA	30 °C
<i>Staphylococcus aureus</i> ATCC 33868	PCA	37 °C
Gram-Negative Bacteria		
<i>Escherichia coli</i> ATCC 25922	TSA	37 °C
<i>Pseudomonas paraeruginosa</i> ATCC 9027	PCA	37 °C
<i>Salmonella</i> Enteritidis ATCC 13076	BHI	37 °C
<i>Serratia marcescens</i> PCM 549	TSA	30 °C
Yeasts		
<i>Rhodotorula mucilaginosa</i> DKK 040	YPD	30 °C

2.2. Bread-Waste Beverage Fermentation with LAB

Bread-waste beverages without (BWBs) and with chokeberry pomace (BWB + CPs) were prepared according to the procedure described by Juś et al. [32]. First, 10 g of ground stale wheat-rye bread was soaked in 200 mL of boiling water in a sterile Simax and left to soften for 24 h at room temperature. Next, glucose (5 g) and varying amounts of dried, ground chokeberry pomace (5–10 g) were added (under laminar chamber), depending on the stage of the experiment (Figure 1). Finally, the mixtures were inoculated with 1 mL of

LAB cultures (density 10^{10} CFU/mL) per 100 mL and incubated at 30 °C for 24 h. After fermentation, total LAB counts were estimated by the standard plate count method by culturing microorganisms on MRS Agar at 30 °C for 24–48 h. This study was conducted in three parallel repetitions.

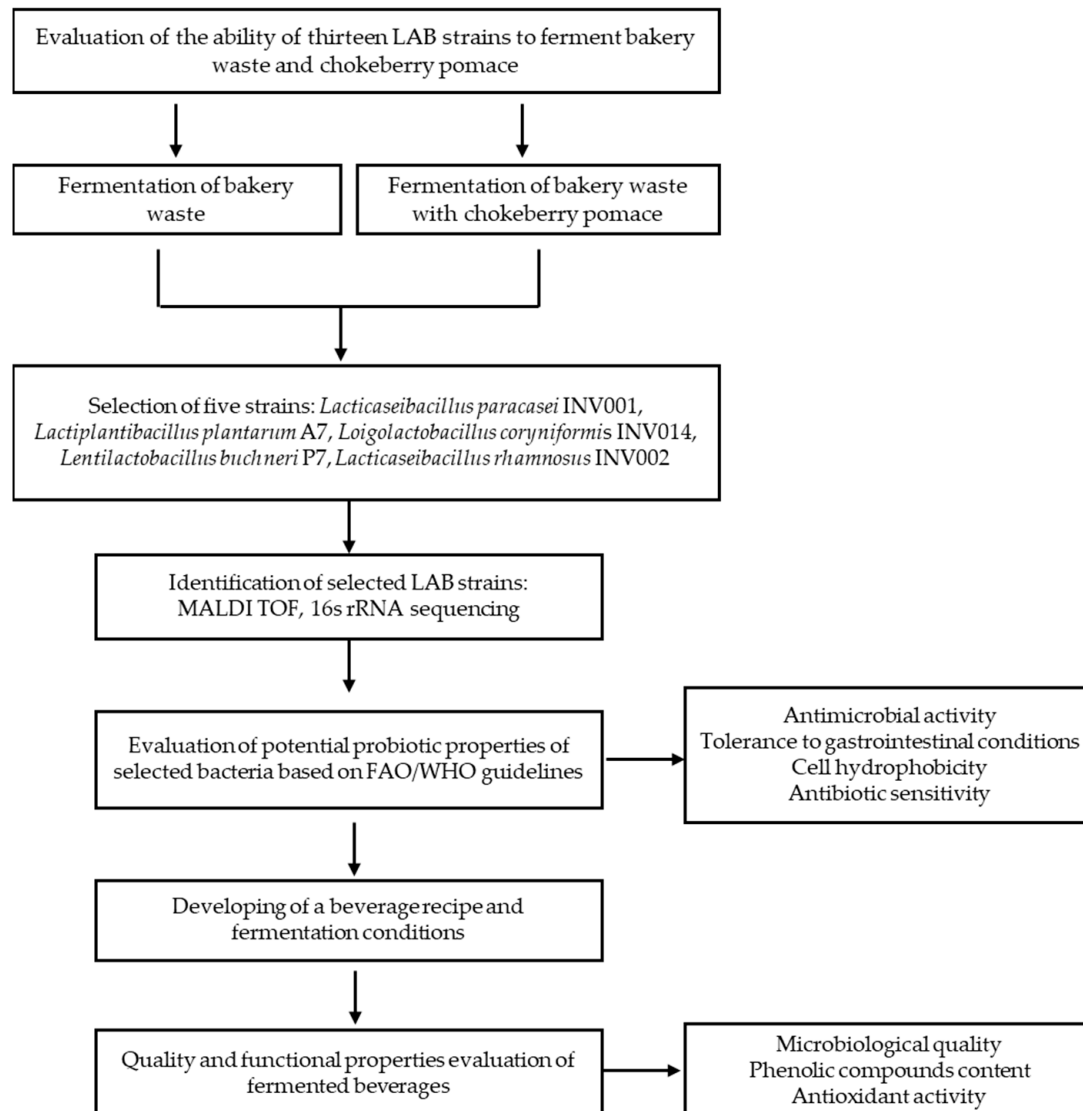


Figure 1. The scheme of fermented beverages development.

2.3. Characterization of the Probiotic Potential of Selected LAB Strains

2.3.1. Identification of Selected LAB Strains

Identification of selected LAB strains was performed by the matrix-assisted laser desorption-ionization (MALDI) time-of-flight mass spectrometry (TOF/MS) method using ribosomal protein profile analysis [33] and 16S rRNA gene sequencing [34]. To identify isolates by the MALDI-TOF MS method, single colonies with homogeneous growth, prepared by a series of reduction plating were sent for identification to the Life Sciences Park in Krakow (Poland) at the Jagiellonian Center of Innovation.

Genetic identification was performed in the following stages: genomic DNA isolation, polymerase chain reaction (PCR), agarose gel electrophoresis, sequencing of PCR reaction products, and their analysis [34]. Isolation of genomic DNA from 24-h cultures of the test strains was performed according to the procedure included with the Genomic Mini AX Bacteria+ Spin kit (A&A Biotechnology).

Polymerase chain reaction (PCR) was performed to amplify a 16S rRNA gene sequence fragment. The primers used were forward S-D-Bact-0008-c-S-20 (27F) (AGAGTTTGATCCTGGCTCAG) and reverse 1492R (TACGGYTACCTTGTTACGACTT) [35]. The primers were diluted in ultrapure water according to the manufacturer's recommendations (10 µL of primer in 190 µL of ultrapure water). Amplification was performed using PCR Mix Plus Green (2005-100Z, A&A Biotechnology). Samples were prepared in sterile 25 µL Eppendorf tubes with the composition: PCR Mix Plus Green 12.5 µL, 1492R primer 1 µL, S-D-Bact-0008 (27F) primer 1 µL, DNA template 1 µL, ultrapure water 9.5 µL. The PCR reaction was carried out immediately in a Biometra thermocycler according to the scheme: initial denaturation (95 °C, 180 s), primer binding (45 °C, 30 s), elongation (78 °C, 120 s), 30 cycles: denaturation (94 °C, 30 s), primer binding (45 °C, 30 s), elongation (72 °C, 120 s), completed with denaturation (95 °C, 30 s), primer binding (45 °C, 30 s), elongation (72 °C, 420 s). Next, electrophoresis was performed using a 1% agarose gel with Midori Green Advance dye (MG04, NIPPON Genetics Europe) and 1xTBE buffer for 45 min at 5 V/cm. PCR products were compared with a DNA marker (3035-500, A&A Biotechnology) with a range of 100–3000 base pairs. Observation under UV light, using an Omega Lum G gel analyzer (Aplegen), made it possible to observe the efficiency of the polymerase chain reaction performed. PCR products were sent to Genomed SA in Warsaw (Poland) for sequencing. The GeneDoc 2.7 program provided by the National Resource for Biomedical Supercomputing (NRBSC) was used to analyze the obtained sequences. Next, the sequences obtained were compared with the BLAST® database of genetic nucleotide sequences belonging to the National Center for Biotechnology Information (NCBI).

2.3.2. Antimicrobial Activity

The antimicrobial activity of five selected LAB strains (Figure 1) was determined by the well diffusion method against ten indicator microorganisms (Table 1). From 24-h cultures of indicator microorganisms maintained under optimal conditions (Table 1), suspensions were prepared in physiological saline with an optical density of 0.5 on the McFarland scale, and inoculation was performed using the flood method in three parallel replicates. After the medium had solidified, 10 mm diameter wells were cut out with a sterile cork borer. Next, 100 µL of a 24-h LAB culture with an optical density of 10 on the McFarland scale was introduced into each well. The plates were incubated for 24 h at 30 °C or 37 °C, depending on the requirements of the indicator microorganisms. After incubation, the diameter of the growth inhibition zone, including the wells' diameter, was measured.

2.3.3. Susceptibility to Antibiotics

The sensitivity of LAB strains to six antibiotics: ampicillin, tetracycline, erythromycin, streptomycin, kanamycin, and chloramphenicol in the concentration range of 0.125–256 µg/mL, depending on the substance, was determined using the microplate method. The antibiotics and their used concentrations were selected following EFSA guidelines [36]. First, two-fold dilutions of antibiotic solutions were made in a liquid medium consisting of a Mueller–Hinton and MRS Broths (9:1) mixture in 96-well U-bottom microplates. Then, bacterial suspensions were prepared from 24-h cultures in physiological saline with an optical density of 0.5 on the McFarland scale and diluted 1:100 in the medium. Next, 100 µL of the prepared bacterial suspension was added to the wells of a microplate with the prepared antibiotic dilutions. The microplates were incubated for 24 h at 37 °C, after which the optical density was measured at 600 nm wavelength using a BioTek Epoch 2 (BioTek Instruments Limited, Winooski, VT, USA) microplate spectrophotometer. The negative control was a medium with antibiotics, without the addition of bacterial suspension. The positive control was a medium without the addition of antibiotics. The minimum

inhibitory concentration (MIC) value was determined based on the measurements taken and compared with the cut-off values defined by the EFSA [36].

2.3.4. Acidic pH and Bile Salts Tolerance

The low pH and bile salt tolerance were performed using sterile 96-well U-bottom microplates. The LAB suspensions were prepared by centrifuging 2 mL of a 24-h culture for 5 min at 10,000 rpm and washing twice with PBS solution (phosphate buffered saline), centrifuging the biomass each time. Finally, 2 mL of the culture was reconstituted by adding MRS Broth. The preparation of the low pH environment consisted of adjusting the medium pH to 2.0 and 3.0 by adding HCl to MRS broth. Similarly, solutions were prepared to determine LAB tolerance for bile salts. Bile salt extract was added to MRS Broth at final concentrations of 1%, 0.5%, and 0.25%, and the solutions were filtered through a syringe filter with a 0.22 µm pore size. Each well of the microplate was filled with 180 µL of the appropriate pH medium or 180 µL of MRS medium with bile salts and 20 µL of the prepared tested strain cell suspension. The first absorbance measurement at 600 nm was taken immediately after preparation, followed by incubation for 24 h at 37 °C, with absorbance measurements taken every hour using a BioTek Epoch 2 (BioTek Instruments Limited, Winooski, VT, USA) microplate spectrophotometer. The negative control was MRS Broth without added bacterial culture. The positive control was a bacterial culture suspended in MRS Broth at pH 6.5 [37].

2.3.5. The Cell Surface Hydrophobicity Test

The cell hydrophobicity was assessed according to the method described by Taheri et al. [38] and Prasath et al. [39] with some modifications. Tested strains were prepared from a 24-h culture by centrifugation for 5 min at 7500 rpm in a centrifuge 5804R (Eppendorf AG, Hamburg, Germany), and washed twice with PBS solution [39], each time centrifuged under the same conditions. The biomass was resuspended in saline until an optical density of 0.5 was achieved with an absorbance measurement of 600 nm. To sterile Falcon-type tubes, 3 mL of bacterial suspension and 1 mL of toluene were added, then shaken vigorously for 90 s and allowed to stand for 15 min for phase separation. The absorbance of the aqueous phase was measured at 600 nm using Genesys 10S UV-VIS spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). The final result is a calculation of the percentage decrease in optical density of suspensions of the tested strains caused by cell adhesion to toluene [38].

2.4. Evaluation of the Quality and Functional Properties of Fermented Beverages

2.4.1. Microbiological Quality and Safety of Fermented Beverages

The microbiological quality and safety of beverages were assessed by the standard plate count method after 0, 24, and 48 h of fermentation. The tests were performed for total bacterial count (PCA, 30 °C), LAB count (MRS Agar, 37 °C), fungi count (yeasts and molds) (Sabouraud dextrose with chloramphenicol Agar, 30 °C), Enterobacteriaceae count (VRBG Agar, 37 °C) and the presence of *Escherichia coli* (TBX Agar, 37 °C). The plates were incubated for 24–48 h. The assessment of pH value for the beverages was performed with Thermo Scientific Orion Star A111 pH meter (Waltham, MA, USA). Each sample was analyzed three times at room temperature (22.0 ± 2.0 °C).

2.4.2. Determination of Total Phenolic Content (TPC) of Fermented Beverages

Total phenolic content was determined using the Folin–Ciocalteu method on 48-well microtiter plates described by Włodarska et al. [40]. Samples were centrifuged in a Minispin centrifuge (Eppendorf) for 5 min at 10,000 rpm. Samples were used for the tests without dilution and diluted 1:1 with demineralized water. The reference sample was

demineralized water. The Folin–Ciocalteu reagent was added to the wells with the sample, left for 3 min, next a clear aqueous solution of sodium carbonate 20% was added. Subsequently, demineralized water was introduced into each well and left in a dark place for 2 h. The measurements were performed at 765 nm using a BioTek Epoch 2 microtiter spectrophotometer. Total phenolic content was expressed as mg gallic acid per 100 mL of beverage, calculated from the gallic acid standard curve equation.

2.4.3. Determination of Antioxidant Activity (TEAC) of Fermented Beverages

The antioxidant activity of the tested beverages was determined using the TEAC test based on the ABTS reaction and absorbance decrease. The samples were centrifuged in a MiniSpin centrifuge (Eppendorf) for 5 min at 10,000 rpm, and the measurement was performed in a Genesys 10S UV–VIS spectrophotometer (Thermo Fisher Scientific) [41]. The samples of the tested beverages were diluted to a concentration of 10 mg/mL. TEAC values were expressed in mM Trolox equivalent per liter of beverage. TEAC values were calculated as the equation of the sample dilution ratio and the corresponding Trolox standard curve.

2.5. Statistical Analysis

The results of the studies are presented as the arithmetic mean ($\pm$ standard deviation) from three–six parallel replicates. The selected results were subjected to one-way analysis of variance (ANOVA) using Tukey’s test with a significance level of $p < 0.05$. For the TPC and TEAC results, correlation analysis was performed using Pearson’s and Spearman’s correlation coefficients. Microsoft Excel® (Microsoft 365 MSO) and IBM SPSS Statistics 29 (PS IMAGO PRO 10.0) programs were used for statistical analyses.

3. Results

3.1. Screening of LAB Strains Based on the Ability of Bread and Chokeberry Fermentation

In the first stage of the experiment, the ability of 13 LAB strains to ferment bread waste, both without and with the addition of dried chokeberry pomace, was assessed to select strains showing the highest process efficiency (Figure 2). The efficiency of the fermentation process was determined based on the total number of lactic acid bacteria and the value of the increase in the LAB number after fermentation compared to 0 h. Most LAB strains reached cell counts above 7 log CFU/mL after 24 h of fermentation. Lower counts were obtained in beverages fermented by *L. helveticus* INV008 and *L. zeae* (below 7 and 6 log CFU/mL, respectively). The addition of dried chokeberry pomace did not negatively affect the number of LAB in BWB, as indicated by the comparable number of LAB population in beverages without and with the addition of chokeberry. The key criterion for selecting strains was the difference in LAB numbers between 0 and 24 h of incubation. The highest difference was observed in beverages inoculated with *L. plantarum* A7 and *L. paracasei* INV001, where the difference in the number of LAB between 0 and 24 h of fermentation was above 3 log CFU/mL in both variants (without and with the addition of chokeberry pomace). High LAB growth rates were also noted in beverages inoculated with *L. rhamnosus* INV002, *L. coryniformis* INV014, and *L. buchneri* P7, which were above 2.8 log CFU/mL and above 2.5 log CFU/mL for the BWB and BWB + CP variants, respectively. Based on the above analysis, strains: *L. paracasei* INV001, *L. plantarum* A7, *L. coryniformis* INV014, *L. buchneri* P7 and *L. rhamnosus* INV002 were selected as the most effective.

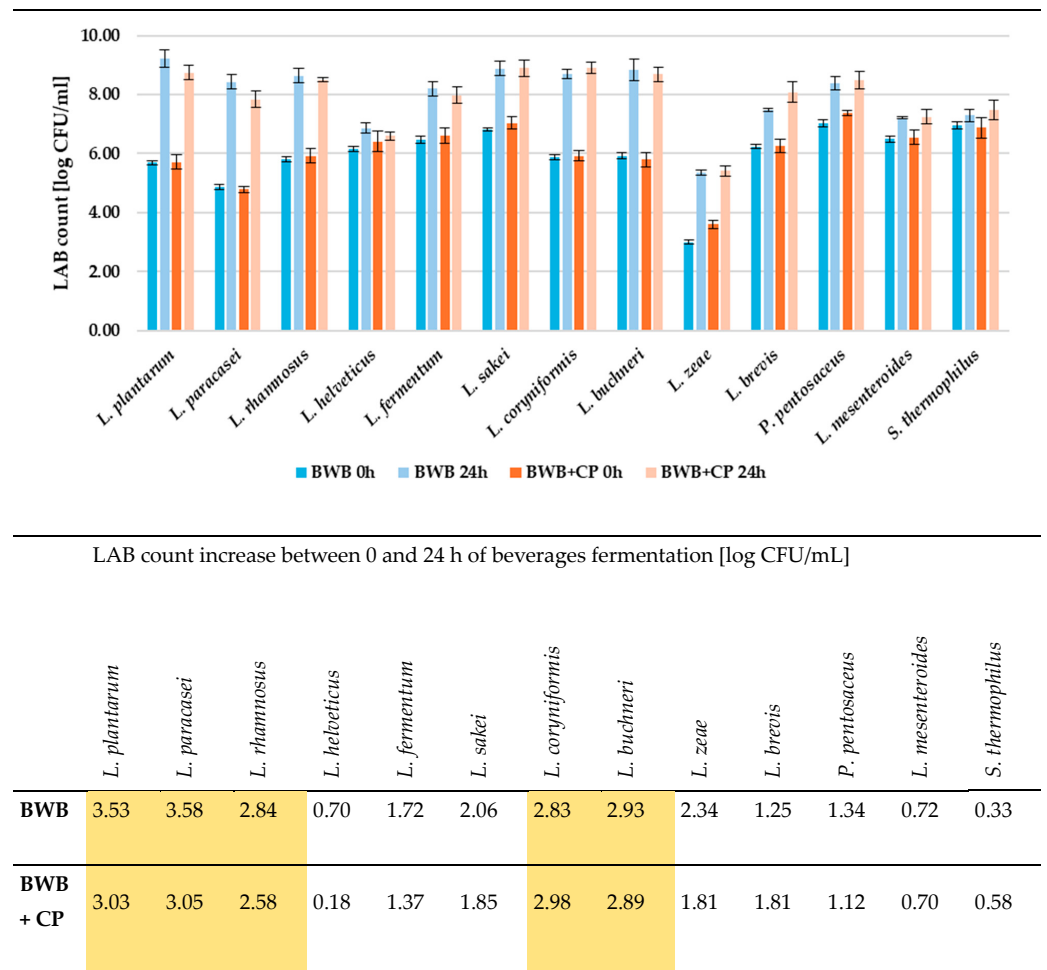


Figure 2. Total count and increase in LAB number in BWBs after 24 h fermentation. BWB—bread-waste beverages without additives; BWB + CP—bread-waste beverages with addition of chokeberry pomace. Yellow highlighting—results obtained for strains selected for the next stage.

3.2. Characterization of the Probiotic Potential of the Selected Strains

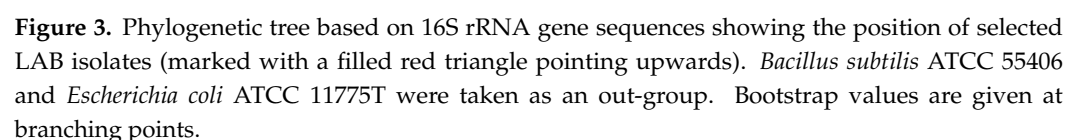
The conducted screening tests allowed the selection of five LAB strains (*L. paracasei* INV001, *L. plantarum* A7, *L. coryniformis* INV014, *L. buchneri* P7, *L. rhamnosus* INV002), which met the assumptions of the project concerning the development of a fermented beverage based on waste and by-products of the food industry. The added value of the designed beverages was the usage of appropriately selected strains. Therefore, in addition to their fermentation abilities, their potential probiotic properties were determined. Functional features, including antimicrobial properties, antibiotic sensitivity, resistance to digestive tract conditions, and hydrophobicity of cells, were assessed according to FAO/WHO criteria [42].

3.2.1. Identification of LAB

As mentioned earlier, the LAB strains used in the presented studies were identified using the MALDI-TOF MS method, while for five selected strains, species affiliation was also verified by genetic identification. A fragment of the 16S rRNA gene sequence was sequenced, which also enabled phylogenetic classification. The identification results, together with the percentage of identity after comparing the obtained sequence with the BLAST[®] genetic database, are presented in Table 2. The value of the identification index was interpreted according to the report of the ordered analysis: ≥ 2.00 —identification with high certainty, 1.70–1.99—identification with low certainty, and 0.00–1.69—no identifica-

Table 2. Results of the identification of five LAB strains.

The genetic identification confirmed the results of identification using MALDI-TOF MS. Based on the obtained 16S rRNA sequences, molecular phylogeny analysis was performed, and the phylogenetic tree was constructed using the neighbor-joining method (no. of bootstrap replications = 2000) (Figure 3) [43–46]. Following the phylogenetic analysis, LAB strains INV001 and INV002 were placed in the cluster making up the *Lacticaseibacillus* genus, subgroups *L. paracasei* and *L. rhamnosus*, respectively. The strain P7 was placed in the *Lentilactobacillus* cluster, *L. buchneri* subgroup. Next, A7 was allocated in the cluster of *Lentilactobacillus*, *L. plantarum* subgroup. Finally, the INV014 strain was placed in the *Loigolactobacillus* cluster, subgroup *L. coryniformis*. The conducted phylogenetic analysis also confirmed the correctness of the isolated LAB strains identification (Figure 3).



3.2.2. Antimicrobial Properties of Selected LAB Strains

The antimicrobial activity of LAB strains (Figure 4) was determined against ten indicator microorganisms selected on the EFSA's requirements for assessing probiotic potential and potential commercialization [47]. Figure 4 shows the results of the assessment.

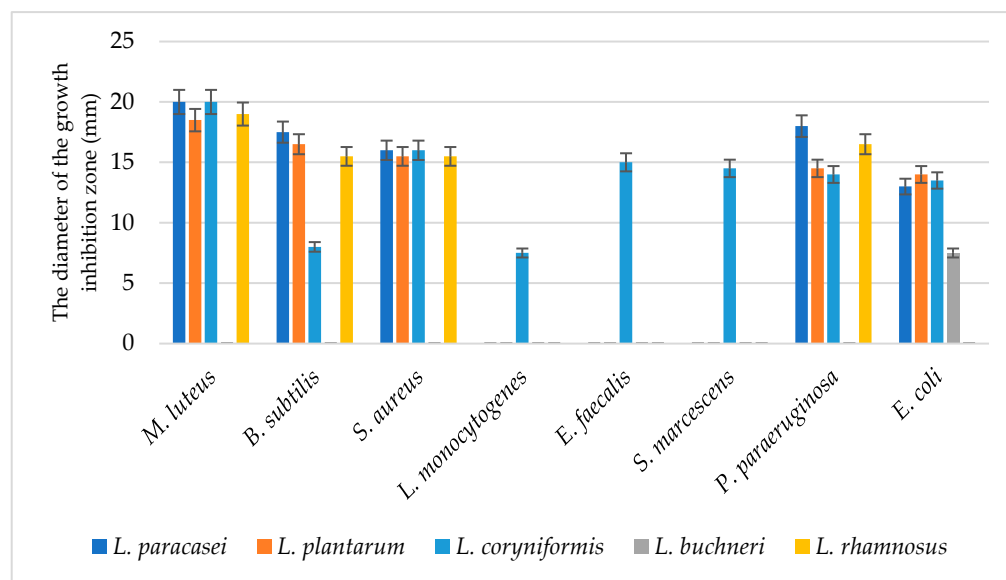


Figure 4. The antimicrobial properties of LAB strains.

As the results indicate, the tested LAB strains inhibited the growth of indicator microorganisms to a varying extent, and each of them was characterized by a different spectrum of activity. No inhibition of the growth of *S. Enteritidis*, and the yeast *R. mucilaginosa* was observed; therefore, they were not included in the graph. Among the tested LAB strains, the *L. coryniformis* INV014 strain showed the widest spectrum of activity, inhibiting the growth of eight indicator microorganisms (*M. luteus*, *B. subtilis*, *S. aureus*, *L. monocytogenes*, *E. faecalis*, *S. marcescens*, *P. paraeruginosa*, and *E. coli*) to a strong or moderate degree. Three strains: *L. paracasei* INV001, *L. rhamnosus* INV002, and *L. plantarum* A7 inhibited the growth of four–five indicator microorganisms, and the strain *L. buchneri* P7 showed activity only against *E. coli*. Comparing the sensitivity of the indicator microorganisms used, no trend was observed, while the most strongly inhibited microorganism by most LAB strains was *M. luteus*. Based on such results, it can be concluded that mixtures of different LAB strains may have a beneficial effect on their ability to inhibit the growth of pathogenic microorganisms and thus expand the scope of their action.

3.2.3. Antibiotic Sensitivity of Selected LAB Strains

The results of the sensitivity assessment of the tested LAB strains to selected antibiotics are presented in Table 3. The numbers in brackets indicate the breakpoints established by the EFSA [47], and the symbol “n.r.” (not required) indicates that this value was not established by the EFSA for a given microorganism. The microorganism was considered resistant when the obtained value exceeded the breakpoint.

All strains tested showed sensitivity to ampicillin, kanamycin, erythromycin, tetracycline, and chloramphenicol; however, the MIC values differed between strains. The highest sensitivity to kanamycin was demonstrated by strain *L. coryniformis* INV014, while the strain *L. paracasei* INV001 showed the highest susceptibility to erythromycin, tetracycline, and chloramphenicol among all tested strains. Sensitivity to streptomycin was variable. Two strains, *L. paracasei* INV001 and *L. rhamnosus* INV002, showed resistance, while the MIC

value for the strains *L. buchneri* P7 and *L. coryniformis* INV014 was at the cut-off. Similarly, MIC value for the *L. plantarum* A7 was at the cut-off in case of ampicillin sensitivity.

Table 3. Sensitivity of selected LAB strains to antibiotics (expressed as MIC—minimal inhibitory concentration).

Strain	MIC [$\mu\text{g/mL}$]					
	Ampicillin	Kanamycin	Streptomycin	Erythromycin	Tetracycline	Chloramphenicol
<i>L. paracasei</i> INV001	1 (4)	32 (64)	128 (64)	<0.0625 (1)	0.5 (4)	1 (4)
<i>L. plantarum</i> A7	2 (2)	32 (64)	128 (n.r.)	0.25 (1)	4 (32)	2 (8)
<i>L. coryniformis</i> INV014	2 (4)	8 (64)	64 (64)	0.5 (1)	2 (8)	4 (4)
<i>L. buchneri</i> P7	1 (2)	16 (64)	64 (64)	0.25 (1)	2 (128)	4 (4)
<i>L. rhamnosus</i> INV002	1 (4)	32 (64)	64 (32)	0.125 (1)	1 (8)	4 (4)

EFSA breakpoints (mg/L) for each LAB isolate are presented in the brackets, and isolates with the MIC higher than the EFSA breakpoint value are considered as resistant strains.

3.2.4. Tolerance to Gastrointestinal Conditions

The survival of potentially probiotic bacteria under low pH conditions and the presence of bile salts in in vitro tests is crucial because it directly influences the determination of the dose of the microorganism to be administered to the experimental host in in vivo tests. Hydrochloric acid can disrupt the activity of macromolecules, and a reduced pH can inhibit the metabolism and growth capacity of *Lactobacillaceae* [48]. Adding hydrochloric acid to the MRS Broth allowed obtaining pH values corresponding to the conditions in the human stomach: pH 2.0 (without food) and pH 3.0 (after a heavy meal) [49]. The results of the determination of low pH tolerance and bile salt tolerance by the tested LAB strains are shown in Table 4. Low pH tolerance was expressed as a percentage of microbial growth compared to the control sample (pH 6.5). Strain *L. paracasei* INV001 showed the best tolerance at both values of reduced pH, while the percentage of tolerance did not change (87%). High tolerance was also observed for strain *L. buchneri* P7, showing higher survival at pH 2.0 (87%) than at pH 3.0 (78%). Strains *L. rhamnosus* INV002 and *L. coryniformis* INV014 showed similar tolerance at both pH values. In contrast, the lowest tolerance was observed for strain *L. plantarum* A7, reaching values of 62% at pH 2.0 and 71% at pH 3.0. Figures S1–S10 in the Supplementary Materials show the growth of the tested strains in low pH conditions during 24 h of incubation in more detail. Comparison of cultures on a medium with reduced pH and control medium showed that differences in the growth of strains appeared already within the first 3 h. All strains showed moderate to strong tolerance to low pH values, which emphasizes the usefulness of the tested strains in terms of probiotic potential.

Table 4. Results of survival assessment in the gastrointestinal tract.

Strain	Low pH Tolerance [%]		Bile Salts Tolerance [%]			Hydrophobicity [%]
	pH 2.0	pH 3.0	0.25% Bile Salts	0.5% Bile Salts	1% Bile Salts	
<i>L. paracasei</i> INV001	87	87	53	41	74	98.6
<i>L. plantarum</i> A7	62	71	56	42	74	98.6
<i>L. coryniformis</i> INV014	72	80	23	30	54	83.6
<i>L. buchneri</i> P7	87	78	44	50	62	97.5
<i>L. rhamnosus</i> INV002	81	85	38	36	69	98.3

The results of the bile salt tolerance determination for the tested LAB strains are shown in Table 4. Tolerance was determined as the percentage of microbial growth compared to the control sample (no bile salts present). Three concentrations of bile salts were used in this study: 0.25%, 0.5% and 1%. In the control sample, all strains showed similar optical density, indicating comparable growth under optimal conditions. However, with increasing bile salt concentration, a decrease in optical density was observed in all strains. The highest tolerance at the highest salt concentration (1%) was shown by strains *L. paracasei* INV001 and *L. plantarum* A7, reaching the same value of 74% survival compared to the control sample. Strain *L. buchneri* P7 showed moderate tolerance (62% at 1% bile salts), while strain *L. coryniformis* INV014 showed the lowest bile salt tolerance. Based on the results, it was observed that all tested LAB strains showed greater tolerance to 1% bile salt concentration in the environment, compared to lower concentrations. This trend may be related to the ability of lactic acid bacteria, potentially probiotics, to hydrolyze bile salts using the enzyme bile salt hydrolase (BSH) [50]. Strains with higher bile salt tolerance may have greater industrial utility in developing potential probiotic products.

3.2.5. Cell Hydrophobicity

An important parameter for assessing the probiotic potential of bacteria is cell hydrophobicity. High cell hydrophobicity increases the potential ability to adhere to intestinal epithelial cells and survive in the intestines themselves, which increases the chance for better colonization of the intestinal epithelium. In addition, greater adhesion to carbohydrates increases the ability of cells to self-aggregate, which prolongs their residence time in the intestines [50]. The test results are presented in Table 4, and based on them, the microorganisms were divided into three groups depending on the result: highly hydrophobic (>50%), moderately hydrophobic (20–50%), and hydrophilic (<20%) [51]. All tested LAB strains can be described as highly hydrophobic. The lowest hydrophobicity was observed for the *L. coryniformis* INV014 strain (83.6%), while the remaining strains obtained a result above 95%. The same percentage of hydrophobicity was achieved by *L. paracasei* INV001 and *L. plantarum* A7 strains (98.6%).

3.3. Development of Fermented Beverage Based on Wheat Bakery Waste and Chokeberry Pomace

3.3.1. Development of a Basic Beverage Recipe

The strains tested for their probiotic potential were used to develop a recipe for upcycled fermented beverages based on wheat bakery waste and chokeberry pomace. First, the maximum amount of chokeberry pomace added that would not inhibit the growth of starter cultures was determined (Figure 5). The main idea was to add as much additive as possible to the beverage while maximizing the use of the fruit waste without affecting the number of bacteria. During the fermentation dried chokeberries in the amount of 5 g, 7.5 g and 10 g were added to 200 ml of the beverage base, and their effect was observed for 48 h, determining the number of bacteria every 24 h. Due to the method of preparation of the beverages, including the use of sterile bottles and boiling water for soaking the bread, the inoculation base was characterized by high microbiological purity (Tables S1 and S2 in the Supplementary Material). Studies have shown that regardless of the additive introduced, intensive bacterial growth was observed in all samples. However, the population size varied, depending mainly on the LAB strain. In beverages inoculated with *L. paracasei* INV001, *L. plantarum* A7, and *L. rhamnosus* INV002, the cell number reached a level comparable to samples without chokeberry, regardless of the amount of the additive. After 48 h, the bacterial count was above 9 log CFU/mL in beverages inoculated with *L. paracasei* INV001 and above 8 log CFU/mL in samples inoculated with *L. plantarum* A7 and *L. rhamnosus* INV002. In most cases, no significant differences were observed between

the individual beverage variants. In turn, greater variation in results was observed in beverages inoculated with *L. coryniformis* INV014 and *L. buchneri* P7. While the addition of chokeberry in the range of 5–7.5 g did not significantly affect the growth of these strains, 10 g of dried chokeberry significantly slowed down the growth of both strains. The difference observed between the number of bacteria in the control beverage with *L. coryniformis* INV014 and the variant with the addition of 10 g of chokeberry exceeded one logarithmic unit. For *L. buchneri* P7, significant differences were also observed between the variants, although the difference between the cell number in the control beverage with *L. buchneri* P7 and the variant with 10 g added was approximately 0.25 log units.

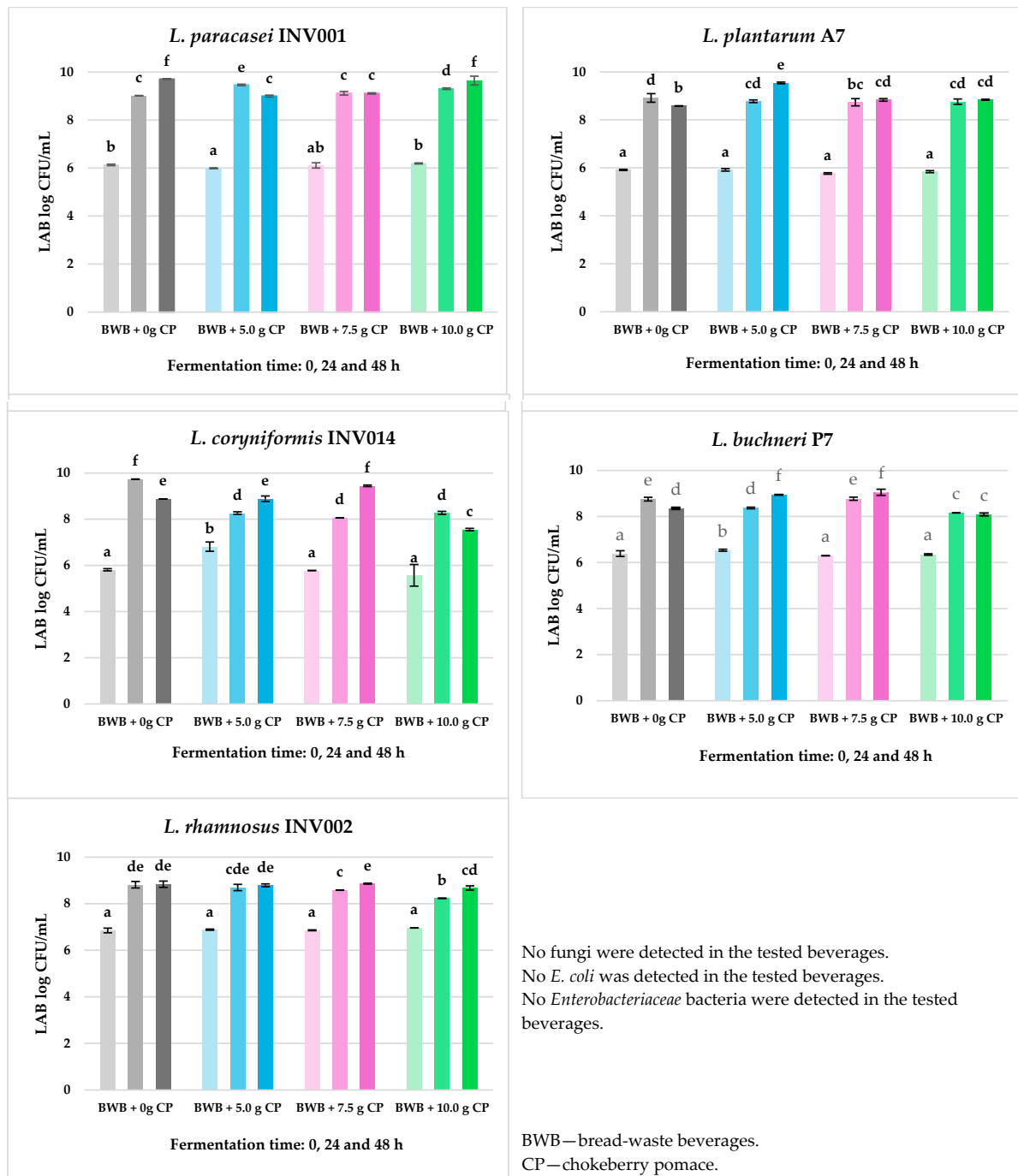


Figure 5. Microbiological quality of BWBs with the addition of different amounts of dried chokeberry pomace. Averages with different lowercase letters (a–f) for each LAB strain are significantly different at $p < 0.05$ based on one-way analysis of variance (ANOVA) using Tukey's test.

3.3.2. Determination of Total Phenolic Compounds (TPC) and Antioxidant Activity (TEAC) of Fermented Beverages

The final version of fermented beverages was a combination of bioferments prepared with five LAB strains and with 5% addition of chokeberry pomace (10 g/200 mL). The functionality of prepared beverages was determined by assessing the content of polyphenolic compounds and antioxidant properties and by monitoring the number of LAB and pH value (Table 5). The effect of fermentation time and strain on the lactic acid bacteria population was assessed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test ($p < 0.05$). The analysis showed significant differences between groups.

At 0 h (pre-fermentation samples), the mean values ranged from 5.05 log CFU/mL (INV014) to 6.94 log CFU/mL (P7). Significant differences were observed between beverages inoculated with *L. coryniformis* INV014 and beverages inoculated with *L. paracasei* INV001, *L. buchneri* P7, and *L. plantarum* A7, with the beverage inoculated with *L. coryniformis* INV014 showing the lowest value ($p < 0.05$). After 24 h of fermentation, all samples showed a significant increase in LAB counts compared to their 0-h counterparts ($p < 0.05$). The means ranged from 5.88 log CFU/mL (INV014) to 9.07 log CFU/mL (INV002). Beverages inoculated with *L. paracasei* INV001, *L. rhamnosus* INV002, *L. buchneri* P7, and *L. plantarum* A7 formed a homogeneous group with no significant differences between them ($p < 0.05$). Whereas, for *L. coryniformis* INV014 beverage consistently the lowest LAB values were observed both before and after fermentation. This suggests strain-specific effects on the process outcome. The pH values of the prepared beverages after 24 h of fermentation were 2.54–2.75, significantly lower than the initial values, indicating that the fermentation process was proceeding correctly. It is worth mentioning that the relatively low pH values at 0 h of fermentation (in the range of 3.31–3.45) result from the strong acidification of the beverage base after the addition of chokeberry pomace (Table S2 in Supplementary Material).

Table 5. Results of determination of LAB count, total phenolic compounds (TPC), and antioxidant activity (TEAC) of fermented beverages.

Strain in The Beverage	Fermentation Time							
	0 h	24 h	0 h	24 h	0 h	24 h	0 h	24 h
	LAB Count (log CFU/mL)		pH Value		TPC (mg GAE/L)		Antioxidant Activity (μM Trolox/mL)	
<i>L. paracasei</i> INV001	6.274 ^{bc} ± 0.098	8.785 ^e ± 0.255	3.31 ^C ± 0.04	2.54 ^A ± 0.01	369.24 ^G ± 3.68	350.93 ^F ± 3.22	0.446 ^h ± 0.069	0.383 ^g ± 0.021
<i>L. plantarum</i> A7	6.942 ^d ± 0.159	8.826 ^e ± 0.386	3.39 ^{CD} ± 0.07	2.75 ^B ± 0.04	345.40 ^F ± 5.01	317.30 ^E ± 3.54	0.414 ^{gh} ± 0.018	0.370 ^{fg} ± 0.015
<i>L. coryniformis</i> INV014	5.047 ^a ± 0.108	5.878 ^b ± 0.116	3.45 ^D ± 0.03	2.72 ^B ± 0.02	462.47 ^I ± 9.05	507.17 ^J ± 16.54	0.448 ^h ± 0.024	0.537 ⁱ ± 0.024
<i>L. buchneri</i> P7	6.431 ^{bcd} ± 0.090	8.653 ^e ± 0.126	3.38 ^{CD} ± 0.07	2.60 ^A ± 0.05	424.52 ^H ± 16.60	423.24 ^H ± 9.57	0.463 ^h ± 0.027	0.378 ^g ± 0.039
<i>L. rhamnosus</i> INV002	6.714 ^{cd} ± 0.076	9.066 ^e ± 0.263	3.36 ^{CD} ± 0.02	2.58 ^A ± 0.01	301.55 ^E ± 7.00	356.46 ^{FG} ± 8.63	0.315 ^f ± 0.003	0.368 ^{fg} ± 0.012

Different letters indicate statistically significant differences ($p < 0.05$) based on one-way analysis of variance (ANOVA) using Tukey's test. Letter ranges refer to each analysis separately: "a–e" for LAB count, "A–D" for pH value, "E–J" for TPC, and "f–i" for TEAC. The beverages were prepared according to the recipe described in point 2.2 with 5% (10 g) addition of chokeberry pomace.

The data in Table 5 indicate that the initial content of polyphenolic compounds in the beverages as well as antioxidant activity results differ after inoculation with the selected strains. Both tested values varied significantly depending on the microbial strain used and

the fermentation time. Statistical analysis revealed significant differences between strains at both time points, as indicated by differing letter annotations.

The highest content of phenolic compounds at 0 h of fermentation was observed in the *L. coryniformis* INV014 and *L. buchneri* P7 beverages, with a value of 462.47 mg GAE/L and 424.52 mg GAE/L, respectively. It is worth noting that the highest TPC value in the beverage inoculated with strain *L. coryniformis* INV014 significantly exceeded all other beverages. Beverages with strains *L. paracasei* INV001, *L. plantarum* A7, and *L. buchneri* P7 showed intermediate but significantly different values. The least amount of phenolic compounds, before fermentation, was shown in the *L. rhamnosus* INV002 beverage.

Fermentation led to an increase or decrease in the content of phenolic compounds in the beverages tested. The greatest increase in the total content of phenolic compounds was observed for *L. rhamnosus* INV002 beverage (18.21% reaching 356.46 mg GAE/L), which was a value significantly higher than all other beverages. A similar trend was observed in the *L. coryniformis* INV014 beverage, where the process led to a 9.67% increase in TPC, reaching a value of 507.17 mg GAE/L. It is worth noting that this was the beverage with the highest total content of phenolic compounds. In the other beverages, the fermentation process led to a decrease in the concentration of phenolic compounds. The largest decrease in TPC (8.14%) was observed in the beverage inoculated with *L. plantarum* A7, reaching a value of 317.30 mg GAE/L, which was also the lowest result obtained after the fermentation process.

The antioxidant activity results presented in Table 5 indicate that after the fermentation process, similar trends of decrease or increase in antioxidant activity were observed as in the case of the TPC; however, the dynamics of these changes were different. The highest antioxidant activity before the fermentation process was demonstrated for the beverage inoculated with *L. buchneri* P7, with a value of 0.463 μM Trolox/mL, and the lowest for the beverage inoculated with *L. rhamnosus* INV002, with a value of 0.315 μM Trolox/mL. At 0 h, beverages inoculated with strains *L. paracasei* INV001, *L. buchneri* P7, and *L. coryniformis* INV014 exhibited comparably high antioxidant activity. For the beverage inoculated with strain *L. plantarum* A7 no significant differences were observed comparing with the highest-performing beverages or *L. rhamnosus* INV002 beverage.

After 24 h of fermentation, a change in antioxidant activity was observed in all samples. *L. coryniformis* INV014 beverage showed the highest increase in antioxidant activity (19.87%), reaching a value of 0.537 μM Trolox/mL, significantly exceeding all other strains. In the beverage inoculated with *L. rhamnosus* INV002, the increase was slightly lower (14.13%), reaching a value of 0.368 μM Trolox/mL. In turn, in the other samples, a decrease in antioxidant activity was observed. The greatest decrease was observed in the beverage inoculated with *L. buchneri* P7 (18.36%), where the result of antioxidant activity was 0.463 μM Trolox/mL after 24 h of fermentation. It is worth noting that the value of antioxidant activity did not differ significantly in the beverages inoculated with strains *L. paracasei* INV001 and *L. buchneri* P7 as well as inoculated with strains *L. rhamnosus* INV002 and *L. plantarum* A7.

A strong positive correlation was observed between the total phenolic compounds (TPC) and antioxidant activity (TEAC). When all samples were analyzed across, the relationship was highly significant ($p < 0.01$) (Table S3). These findings indicate that a higher phenolic content is consistently associated with greater antioxidant capacity.

4. Discussion

The agricultural and food industries generate significant amounts of waste, which, due to their diverse nutritional and biologically active components, can be transformed into products with high utility value [52]. The growing interest in reusing food waste in recent years has led to proposals for innovative food products, pharmaceuticals, and

biomaterials [53]. At the same time, consumers are increasingly interested in healthy eating and are looking for innovative functional food products that provide significant nutritional value and health benefits [54]. In response to consumer expectations and the challenges facing the food production sector, the trend of upcycling is becoming increasingly common in waste management and waste prevention. The beverages designed in this paper are examples of upcycling, as they utilize plant-based waste and, on the other hand, utilize potentially probiotic strains of lactic acid bacteria and the fermentation process, offering consumers a product of higher quality than the raw material used. It is also worth emphasizing that the beverages designed are in line with current trends in the development of dairy-free fermented products, meeting the needs of individuals suffering from allergies or intolerances to milk components. Plant-based fermented beverages are also gaining popularity among vegans, as health-conscious consumers seek plant-based, sustainable, and gut-friendly alternatives to traditional beverages while avoiding animal-derived ingredients [55]. It is worth noting that the global vegan food market grew from USD 14.44 billion in 2020 to USD 15.77 billion in 2021 and is expected to continue growing for the next few years [56]. This trend is particularly noticeable among young consumers, who demand alternative, healthier eating practices than previous generations [57]. The number of people adopting veganism worldwide is steadily increasing, putting veganism at the forefront of demand for healthy eating [58].

It is worth noting that bakery waste are rarely used as a raw material for fermentation [32,59], although bread waste is a growing problem in many developed countries. Our previous research has shown that bread waste can be a base for obtaining fermented beverages characterized by a high number of lactic acid bacteria and antibacterial properties against microorganisms such as *E. coli*, *P. fluorescens*, and *S. saprophyticus* [32]. Sigüenza-Andrés et al. [59] developed eight probiotic plant-based beverages from discarded bread flour using a monoculture of *L. rhamnosus* or a coculture of lactic acid bacteria and *Bifidobacterium* strains. Depending on the variant and process used, the resulting products varied in terms of the amount of organic acids and individual volatile compounds responsible for the aroma of fermented beverages. The beverage produced using mixed culture gained consumer recognition in a sensory test. In turn, Nguyen et al. [60] investigated the potential for biovalorization of surplus bread on the market to develop probiotic fermented beverages using the probiotics *L. rhamnosus* GG (LGG) and *S. cerevisiae* CNCM I-3856, both individually and in combination. They noted that proper formulation and dry matter content were important, as a 5% initial dry matter content resulted in better probiotic growth and higher cell counts compared to higher concentrations of bread, which resulted in excessive stickiness and poor texture.

The fermentation of fruit pomace or juice is reported much more often [61,62]. The combination of bread and fruit waste in the beverages designed in this work made it possible, on the one hand, to provide nutrients such as proteins, carbohydrates, vitamins, and minerals from bread and also to enrich the product with polyphenolic compounds originated from fruit waste. According to literature data, the use of chokeberry waste in fermented beverages also concerns the fermentation of juice rather than pomace, and studies on black chokeberry juice fermented by lactic acid bacteria are still scarce. Some authors, such as Zamfir et al. [63], similarly to this work, used a combination of cereal raw material with the addition of a raw material rich in polyphenolic compounds, in this case red beetroot or carrot. The additive affected the biological activity of the resulting beverages.

The main component increasing the value of the products are selected lactic acid strains, which were chosen based on their fermentation abilities and then extensively tested to determine their probiotic properties. Interest in probiotics is constantly growing, as evidenced by the rapidly growing probiotic market. According to a report published by

Grand View Research, it is expected to grow at a compound annual growth rate (CAGR) of 14.0% from 2023 to 2030 (GVR) [64]. They are used, among other things, as dietary supplements and food ingredients, encompassing an increasingly wide range of both dairy and non-dairy products including traditional and newly developed probiotic products. Studies have shown that the choice of strain is very important not only due to its probiotic properties, but also due to the changes it introduces in the food matrix. Authors often describe changes in the content of biologically active compounds such as polyphenols and biological properties, including antioxidant activity. In this work, both an increase and a decrease in total polyphenol content and antioxidant activity were observed. Moreover, both strain and fermentation time markedly influence the polyphenolic content and antioxidant capacity of the beverage. In particular, the beverage from the *L. coryniformis* INV014 strain showed consistently higher polyphenolic content and antioxidant activity, making it a promising candidate for applications where such properties are desired. However, in some beverages, a decrease in both the amount of polyphenols and antioxidant activity was noted. Literature data generally emphasize that the fermentation process causes an increase in the content of polyphenolic compounds. Zamfir et al. [63] prepared beverages with three selected strains (*L. plantarum* BR9, *L. plantarum* P35, and *La. acidophilus* IBB801) in different combinations and observed an increase in the content of polyphenolic compounds and antioxidant properties during fermentation in all beverages, reaching significantly higher values after 24 h compared to the unfermented substrates. Wang et al. [65] investigated the influence of lactic acid bacteria on the phenolic profile, antioxidant activities, and volatiles of black chokeberry juice fermented with *L. plantarum*, *La. acidophilus*, and *L. rhamnosus*. In all fermented samples, an increase in the total phenolic and total flavonoid contents were observed with *La. acidophilus* demonstrated the highest contents of these compounds. Also, an increase in the reducing power capacity was noted. Similarly to our work, some authors also observed a decrease in the phenolic content. Szutowaska et al. [66] described fermented green kale juice, emphasizing that the product's properties depended on the starter cultures used. Significant differences were observed between the prepared fermented juice variants in the content of phenolic compounds, vitamins, carotenoids, and antibacterial activity. Juice inoculated with the *L. plantarum* strain showed the lowest loss of total phenolic compounds, while *L. sakei* reduced their content the most. The authors also noted a decrease in carotenoid content after juice fermentation in all cultures, with the greatest losses observed in juices inoculated with *L. plantarum* and the MIX B culture (*L. plantarum* and *L. sakei*). Harbaum et al. [67] also observed a decrease in the content of flavonoids and some hydroxycinnamic acids during the fermentation process of two pak choi cultivars and two Chinese mustard cultivars. The authors suggested that this may be due to degradation or structural changes. Markkinen et al. [68], tested the impact of *L. plantarum* fermentation on acids, sugars, and phenolic compounds, among others, in black chokeberry juices prepared with and without pectinolytic enzyme treatment. The authors determined that *L. plantarum* fermentation led to the reduction in flavanols' content by 9–14%, as well as hydroxycinnamic acids by 20–24% in the *Aronia mitschurinii* 'Viking' juices. On the other hand, anthocyanins level remained unaffected after the malolactic fermentation. The authors suggested that hydrolysis of both chlorogenic acid and neochlorogenic acid leads to the formation of quinic acid and caffeic acid. Furthermore, *L. plantarum* fermentation of bog bilberry juice performed by Wei et al. [69] also led to the decrease in anthocyanins, phenolic acids, flavanols and flavanol values. These studies are in accordance with the results presented in this manuscript as the TPC value for the *L. plantarum* fermented beverages decreased by 9%, for the *L. paracasei* beverages by 5% but increased for the rest of the tested microorganisms. The impact on the bioactive compounds amount is undeniably related to the microbial strain used for the fermentation. Literature

data report that β -glucosidase released by *L. plantarum* during the fermentation process may cleave anthocyanins into anthocyanidins and sugar moieties [70]. Lactic acid fermentation results in changes in the initial bioactive compounds' variety and amount due to the different mechanisms. The composition of fruit juices subjected to lactic acid fermentation is related to phenolic acid reductases playing crucial role in quality modulation via reductive modification [71]. Ricci et al. [72] proved that during *L. casei*, *L. paracasei*, *L. plantarum* and *L. rhamnosus* cherry juice fermentation, these enzymes catalyzed the biotransformation of phenolics. Metabolization of caffeic acid to dihydrocaffeic acid and converting *p*-coumaric acid into 4-ethylphenol were observed. Interestingly *L. plantarum* 285 completely degraded caffeic acid and totally metabolized *p*-coumaric and protocatechuic acids. Furthermore, all of the tested LAB strains produced phenyllactic acid and *p*-hydroxyphenyllactic acid. Lactic acid fermentation metabolites result in changes in the final products' color, aromatic characteristics as well as improve its stability through redox equilibrium regulation [71,72]. Nevertheless, non-phenolic antioxidants as for example ascorbic acid or copper, occurring in the product, also may have contribution to the ABTS radical-scavenging capacity [73,74].

In addition to functional properties, consumer safety is undoubtedly an important criterion for microorganisms used in food production. The bacterial species selected in this study are included on the QPS list [75], but the safety of strains used in food products is increasingly being emphasized, and antibiotic susceptibility is a significant trait among the tested traits. Intrinsic antibiotic resistance can be considered a beneficial trait of probiotic bacteria when used during antibiotic therapy. However, attention is drawn to acquired resistance, which is associated with the risk of this trait spreading within the gastrointestinal microbiome and the environment. Lactic acid bacteria used in the food industry may possess intrinsic resistance to antibiotics but should not possess potentially mobile antibiotic resistance genes [76]. Literature data indicate that LAB exhibit intrinsic resistance to aminoglycosides (gentamicin, kanamycin, neomycin, and streptomycin), vancomycin, ciprofloxacin, and trimethoprim, and are generally susceptible to β -lactams (penicillin and ampicillin) as well as protein synthesis inhibitors (tetracycline, erythromycin, chloramphenicol, and linezolid) [77–79]. However, studies indicate that acquired resistance to tetracycline, erythromycin, clindamycin, and chloramphenicol is also observed in lactic acid bacteria isolated from fermented foods [80,81], and transfer of resistance from lactobacilli to other organisms is possible [82,83]. In studies of bacterial susceptibility to antibiotics, both phenotypic and genomic assessments are recommended. However, phenotypes are not always reflected in the genomic program of the tested LAB. Sometimes, the presence of a resistance gene for a given antibiotic does not coincide with the phenotype, because other mechanisms, such as efflux or cell surface impermeability, may be involved in resistance [84,85], and sometimes this may result from disturbances in the expression of resistance genes [79]. Current quality control standards for probiotics do not require the inclusion of acquired or transferred antibiotic resistance determinants in their assessment. However, some authors point out the need to consider regulatory changes in the future [85,86].

5. Conclusions

Appropriate food waste management, as well as the implementation of circular economy elements into manufacturing processes, are essential components of sustainable production and consumption. This study involved research aimed at developing a prototype of an innovative, fermented beverage based on food industry waste (wheat-rye bread waste and chokeberry pomace) with potential probiotic properties. It can therefore be concluded that the adopted concept aligns with current sustainable production trends (circular economy, natural processing processes), environmental (reduction and

reuse of waste), and social (consumption of products with health-promoting potential). Five selected strains of lactic acid bacteria (LAB) were used to develop the final beverage prototypes. These strains demonstrated the greatest potential for fermenting bread waste and maintained their height growth rate in the presence of chokeberry pomace. All LAB strains were characterized according to FAO/WHO guidelines, confirming their probiotic potential. Nevertheless, no *in vivo* studies were conducted; therefore, probiotic status of selected LAB strains remains to be confirmed. The addition of chokeberry pomace enriched the beverages with polyphenolic compounds, thus contributing to an increase in their antioxidant activity. However, it should be emphasized that, depending on the strain used, the TPC content and antioxidant properties changed during the 24-h fermentation period. These observations confirm the strain-dependent nature of selected LAB properties and the need for appropriate selection of bacterial cultures for fermentation to achieve products with the desired quality characteristics.

In summary, carefully selected LAB strains should be considered as an interesting element of sustainable food industry waste processing, aimed at obtaining functional, sustainable products. The conclusions from this study can therefore provide a valuable source of information for developing sustainable product strategies, thus incorporating them into the overall food waste management system.

For future investigation plans, despite the conducted tests confirming the probiotic potential of selected LAB strains, their probiotic status needs to be confirmed by *in vivo* studies. Furthermore, the studies represent a proof of concept of innovative plant-based fermented beverages at the laboratory scale. Consumer acceptance, including sensory evaluation and toxicological validation, of the products has to be assessed before the mass-scale production and consumption stages.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/su17188502/s1>, Table S1: Microbiological quality and pH value of the bread waste soaked in boiling water after 24 h, Table S2: Microbiological quality and pH value of the bread-waste beverages with chokeberry pomace without LAB inoculation, Figure S1: Low pH tolerance of strain INV001, Figure S2. Low pH tolerance of strain INV002, Figure S3. Low pH tolerance of strain P7, Figure S4. Low pH tolerance of strain A7, Figure S5. Low pH tolerance of strain INV014, Figure S6. Bile salts tolerance of strain INV001, Figure S7. Bile salts tolerance of strain INV002, Figure S8. Bile salts tolerance of strain P7, Figure S9. Bile salts tolerance of strain A7, Figure S10. Bile salts tolerance of strain INV014, and Table S3. Pearson's and Spearman's correlations between TPC and TEAC (all samples, 0 h + 24 h).

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Abbreviations

The following abbreviations are used in this manuscript:

BWB	Bread-waste beverages
LAB	Lactic acid bacteria
CP	Chokeberry pomace
WHO	World Health Organization
FAO	Food and Agriculture Organization
TPC	Total phenolic compounds
MALDI-TOF MS	Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry
EFSA	European Food Safety Authority
GAE	Gallic acid equivalent
CFU	Colony forming unit

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