The impact of dairy starter cultures on selected qualitative properties of functional fermented beverage prepared from germinated white kidney beans

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Summary

The fact that most probiotic beverages are milk-based naturally evokes the researchers to develop other than dairy products based on plant materials. Thus, the samples of beverages were produced from germinated seeds of white kidney bean "Piękny Jaś Karłowy" using two yoghurt starter cultures. The content of stachyose and raffinose in prepared products after the fermentation process was lower by about 31 % and 17 %, respectively, than that before the fermentation (2.73 mg·kg^{-1} and 0.43 mg·kg^{-1}, respectively), but we did not find a significant reduction in verbascose. In each sample, the levels of riboflavin, niacin and pyridoxine were significantly lower (by approximately 88 %, 45 % and 76 %, respectively) after fermentation than before it. We did not observe any significant changes in the contents of thiamine and cyanocobalamin. A change in the water-holding capacity was noticed during the cold storage for 28 days, values being within the range of 49–57 %. The population of starter microorganisms met the criteria of the "minimum therapeutic" level immediately after the fermentation and during the 28-days storage period at 6 °C. The samples of beverages with a natural taste had low acceptability ratings, but the use of fruit flavours increased overall the acceptability by approximately 15 %.

Keywords

bean-based beverages; raffinose-series oligosaccharides; organoleptic properties; lactic acid bacteria; fermentation

Fermented beverages, such as yoghurts, are usually produced from milk of animals, namely, cows', goats' or ewes' milk. These products are characterized by attractive sensory properties with acidity accepted by consumers, as well as by increased nutritional and functional values due to the presence of live cells of lactic acid bacteria (LAB) and bifidobacteria. Current trends in the research of new sources of proteins as well as the increasing spread of vegan diet inspires the research community to search for vegetable sources for food production, for example, bean seeds. LAB and bifidobacteria are widely used for fermentation of safe functional beverage products with several additional health benefits for the consumers who aim to follow a health-promoting diet. Modern technologies offer non-dairy-fermented foods obtained from various plant materials [1–3]. In Poland, the varieties of beans, such as white beans, especially White Kidney Bean "Piękny Jaś Karłowy" (*Phaseolus vulgaris*), are most popular.

An important component of legume seeds are oligosaccharides, including stachyose, raffinose and verbascose. They are called raffinose-series oligosaccharides (RSO), raffinose family oligosaccharides, galactosyl-saccharose oligosaccharides

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or α-galactosides [4, 5]. The action of oligosaccharides on the human body is considered positive, because their presence in the diet stimulates the growth of bifidobacteria in the colon, which makes them natural biologically active food ingredients with a variety of health-promoting effects. Despite its ubiquity in the diet, RSO have some limitations for some food products, such as unpleasant beany flavour and its content of raffinose and stachyose, which produce flatulence. A characteristic α-galactosidic bond between saccharose and galactose is very important as humans do not possess the α-galactosidase enzyme that is necessary for hydrolysing the bond typical for these oligosaccharides, so they cannot be digested when consumed [6]. Intact oligosaccharides reach the colon, where they are fermented by microorganisms that contain α-galactosidase. Studies showed that various processing methods, such as soaking, germination, autoclaving, fermentation, repeated boiling or enzyme treatment, reduce the content of indigestible oligosaccharides [4, 7–9].

The aim of this study was to use two yoghurt starter cultures for fermentation of germinated beans to prepare functional fermented bean beverages characterized by attractive qualitative features, including microbiological, chemical and nutritional characteristics, and by acceptability for consumers.

MATERIALS AND METHODS

Materials

Bean seeds of white kidney bean "Piękny Jaś Karłowy" (*Phaseolus vulgaris*) of Polish origin (year of harvest 2017) were purchased in the local market. Two commercially available lyophilized industrial yoghurt starter cultures, namely, Yo-Mix 205 LYO (DuPont Danisco, France; consists of *Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Lactobacillus acidophilus*, *Bifidobacterium lactis* with saccharose and maltodextrins as carriers) and FD-DVS ABY-3 Probio-Tec (Chr. Hansen, Hørsholm, Denmark; consists of *S. thermophilus*, *Lb. delbrueckii* subsp. *bulgaricus* and documented probiotic strains *Lb. acidophilus* La-5 and *Bifidobacterium animalis* subsp. *lactis* BB-12) were used in the study. Yo-Mix 205 LYO is a thermophilic yoghurt-type starter culture that is used to produce a mild yoghurt of medium thickness. ABY-3 is a yoghurt culture used to obtain products with high viscosity, clear aroma, mild flavour and low post-acidification. It is suitable for cup set, stirred and drinking yoghurts. Fruit flavours for yoghurt production (strawberry, peach-mango, apricot, forest fruits, banana–kiwi and red orange) were obtained from local representatives of food additive producers Bakoma-Bis (Kozienice, Poland) and Wild Poland (Mrągowo, Poland).

Preparation of fermented bean-based beverages

Bean-based beverages were prepared by following the method that we had developed previously in our laboratory. First, the beans were cleaned by removing stones and any other contaminants. One hundred grams of healthy and unbroken bean seeds were subjected to the process of germination in the kitchen germinator at 25 °C for 72 h, every 24 h the seeds being moistened with tap water. Then, the seeds were weighed and transferred to a bigger container filled with water. The total weight of bean seeds and water was 1 000 g. They were stirred with a kitchen blender for 20 min. The homogenate was boiled and stirred continuously to gelatinize the starch contained in the beans. Then, the beverage was filtered through a 0.1 mm sieve. Consequently, the beverage was collected in a clean container and poured into a 170 ml jar, sterilized at 121 °C for 20 min and allowed to cool down to 45 °C.

The substrate was inoculated with starter cultures in accordance with the manufacturers' instructions, following their reconstitution in sterile water. The mixture was stirred well and retained at 43 °C for 4 h to carry out the fermentation process. Then it was placed in the refrigerator at 6 °C to stop the fermentation process.

The unflavoured beverages obtained were subjected to physicochemical and microbiological analysis, whereas flavoured beverages were subjected to organoleptic evaluation. Fruit additives were added in accordance with the recommendations of their producers at a dose of 20 g per 100 g. The addition process was carried out under aseptic conditions. After the addition of flavours, fermented bean-based beverages were stored in the refrigerator at 6 °C until their analysis.

Chemical analysis

The dry matter, protein, fat, RSO and cyanocobalamin were determined in all samples. The dry matter was determined using oven-drying method at 102 °C to a constant weight [10]. Protein was determined using the Kjeldahl method, crude protein content being calculated as $N \times 6.25$ [10].

The RSO concentration was determined using high-performance liquid chromatography (HPLC). The preparation of samples for the determination of the oligosaccharides content included the following steps: homogenization of

8 g of each sample in 32 g of methanol (HPLC-grade; Sigma-Aldrich, St. Louis, Missouri, USA) using an automatic shaker and an ultrasonic bath (for 30 min), sample centrifugation (16000×*g*, 4 °C, 30 min) and concentration of the samples, reducing the liquid volume 8-fold in a water bath at 75 °C. The chromatographic analysis was performed on a set of devices: DeltaChrom Pump Injector (S6020 Needle Injection Valve, Sykam, Fürstenfeldbruck, Germany), DeltaChrom Temperature Control Unit (Sykam), refractive index detector (S3580 RI Detector, Sykam), pre-column Guard Column Sugar-D (10 mm × 4.6 mm, 5 μm; Cosmosil, Nacalai Tesque, Kyoto, Japan), and column Sugar-D (250 mm × 4.6 mm, 5 μm; Cosmosil). The HPLC separation parameters were as follows: flow 1 ml·min^{-1}, oven temperature 30 °C, range of detector 10000 mV, and sample rate 2 Hz. The mobile phase was a mixture of acetonitrile (HPLC-grade, Sigma-Aldrich) and deionized water in a ratio of 60 : 40. All analyses were performed in triplicate. Relevant external standards of raffinose, stachyose and verbascose (Sigma-Aldrich) were determined through the analysis of samples. The concentration of oligosaccharides was calculated on the basis of the standard curve and was expressed as milligrams of sugar per litre of samples.

The B group vitamins was determined simultaneously according to the extraction method developed by Costa et al. [11]. A combination of acid and enzymatic hydrolysis was used in the preparation of samples to determine the concentration of B-group vitamins. The sample preparation included the following steps: treatment of 25 g of the sample for 3 h at 37 °C with a set of enzymes containing pancreatin, lipase and amylase (Kreon 10000; BGP Products, Warsaw, Poland), then treatment for 1 h at 37 °C by 45 ml of 0.1 mol·l^{-1} H_2SO_4 (Avantor Performance Materials, Gliwice, Poland), ultrasonication for 1 h at 25 °C, addition of 50 mmol·l^{-1} phosphate buffer, freezing at –18 °C for 1 h, filtration through qualitative medium flow filter paper (Grade 1, 11 μm, Whatman, Maidstone, United Kingdom) and filtration through a syringe membrane filter of 0.4 μm pore size (Merck Millipore, Burlington, Massachusetts, USA). The samples were kept in amber vials, away from visible light at all stages of extraction. Chromatographic analysis was performed on a set consisting of a DeltaChrom Pump Injector (S6020 Needle Injection Valve, Sykam), DeltaChrom Temperature Control Unit (Sykam), S3580 UV Detector (Sykam), and column 5C18-MS-II (150 mm × 4.6 mm, 5 μm; Cosmosil). The mobile phase consisted of a solution of 50 mmol·l^{-1} K_2HPO_4 (Avantor Performance Materials) and methanol (HPLC-grade, Sigma-Aldrich) in a ratio of 80 : 20 (to determine cyanocobalamin and riboflavin) or 99 : 1 (to determine thiamine, niacin and pyridoxine). The mobile phase flow was 1 ml·min^{-1}, oven temperature was set to 35 °C, and the detection wavelength of the UV detector was 220 nm. All analyses were performed in triplicate. With samples analysed, relevant external standards of B vitamins (Sigma-Aldrich) were determined at different concentrations of the component.

pH determination

The pH values of samples was measured directly using the pH-meter CP-505 (Elmetron, Zabrze, Poland). The analysis was performed in triplicate. The pH-meter was calibrated against pH 4.0 and pH 7.0 buffer solutions. The result was read with an accuracy of 0.01 pH unit.

Water-holding capacity

The water-holding capacity (*WHC*) of samples was determined when 40 g of sample was centrifuged at 16128 ×*g*, at 4 °C for 20 min. The resulting liquid was removed and weighed. The examination was performed in triplicate. *WHC* was defined by Eq. 1 and expressed as percentage:

$$WHC = \frac{m_1}{m_2} \times 100 \quad (1)$$

where m_1 is the mass of precipitate after centrifugation in grams, and m_2 is the mass of sample in grams.

Microbiological examination

Total viable counts of yoghurt bacteria were determined using plate technique [12]. De Man, Rogosa and Sharpe (MRS) agar (Merck, Darmstadt, Germany) was used to determine the counts of *Lactobacillus* sp., M17 agar (Merck) was used to determine the counts of *Streptococcus* sp., and MRS with clindamycin-ciprofloxacin (MRS-CC) agar was used to determine the counts of *Lb. acidophilus* [13]. Bifidus selective medium (BSM) agar (Sigma-Aldrich) with selective BSM supplement (Sigma-Aldrich) was used to determine the counts of *Bifidobacterium* sp. The plates inoculated with diluted samples were incubated at 37 °C for 72 h under aerobic (for streptococci) or anaerobic (for lactobacilli and bifidobacteria) conditions. After incubation, the grown colonies were counted and calculated as colony forming units in 1 g of the original beverage sample, and then expressed as decadic logarithm. The examination was performed in triplicate.

Sensory evaluation

Sensory analysis was carried out in accordance with ISO 8586:2012 [14]. The prepared samples were presented to a 17-member panel of potential consumers. The panel comprised 11 female and 6 male university students. All sensory evaluations of the samples were done at the same time by each evaluator. The samples were presented under natural lights in 170 ml jars with number codes. The samples were assessed for appearance, consistency, taste, smell and overall acceptability using a standard 9-point hedonic scale varying from 1 = dislike extremely to 9 = like extremely, according to the description of scale described by ZIARNO et al. [3]. The evaluation form also provided space for evaluators' remarks. The sensory evaluation of samples was conducted on the 1st day of storage at 6 °C. The informed consent was obtained from all subjects involved in the sensory evaluation.

Statistical analysis

All results were reported as arithmetic average and standard deviation calculated from three replicates. Analysis of variance (ANOVA) was used to determine differences between the mean scores. Differences between the mean obtained by ANOVA were ascertained using Tukey's comparison test. Significance was accepted at $p < 0.05$.

RESULTS AND DISCUSSION

When developing a recipe of fermented bean-based beverage, we did not try to make the basic chemical composition similar to that of cows' milk. Our intention was to develop and study the composition of a specific product, which is fermented and accepted in this form by consumers. According to our examination, the basic chemical composition of fermented bean-based beverage included dry matter of 98 ± 3 g·kg^{-1}, protein content of 23 ± 1 g·kg^{-1} and fat 2 ± 1 g·kg^{-1}.

Oligosaccharides content

The content of oligosaccharides in the bean-based beverage samples obtained from the germinated beans (Tab. 1) was significantly lower than the average content of these sugars in the beans [15]. The differences found could be explained not only by diluting the bean mass with water (to obtain a beverage) but also, and perhaps most importantly, by germinating the bean seeds. As mentioned in the introduction, various techniques have been tested and used to reduce the level of oligosaccharides from the raffinose family. Such techniques include soaking, germinating and cooking. MBITHI et al. [16] stated that, in a bean-seed germinated for 96 h, a reduction in the starch content was found. In addition, the content of reducing sugars increased 50-fold (from 1.9 g·kg^{-1} to 94.6 g·kg^{-1} of dry matter), but non-reducing sugars increased approximately twice (from 18.7 g·kg^{-1} to 41.6 g·kg^{-1} of dry matter). A similar tendency was observed in the case of germination of other grains and seeds [6, 17]. The presence of these oligosaccharides is the cause of excessive gas formation and digestive discomfort in most mammals.

Research by many scientists demonstrated the ability of LAB and bifidobacteria to ferment the oligosaccharides available in plant matrices [18–21]. In our study, application of the fermentation process to the bean matrix resulted in further reduction of the content of oligosaccharides (Tab. 1). In the case of stachyose and raffinose, the reduction was statistically significant, whereas in the case of verbascose, the change was not significant. There were no significant differences ($p < 0.05$) between the used starters in terms of the reduction level of the oligosaccharides. Our starter cultures for fermentation were multispecies, containing bacteria of the genus *Bifidobacterium* in addition to LAB. Literature data confirm that LAB strains show high activity of enzymes such as α- and β-galactosidase [22–24]. Very often, the enzymatic activity correlates with the catabolism of α-galactosidase. This is the characteristic of the strains from the species *Lactococcus lactis* subsp. *lactis* biovar *diacetylactis*, *Lb. plantarum* and *Lb. casei* subsp. *casei*, while β-galactosidase activity is usually high in strains of the species *Lb. plantarum* and *Leuconostoc mesenteroides*. Strains from the species *Lc. lactis* subsp. *lactis* biovar *diacetylactis*, *Lb. plantarum* and *Lb. casei* subsp. *casei* have moderate to high galactosidase activity. The ability of these strains to hydrolyse oligosaccharides from the raffinose family has also been demonstrated [21]. WANG et al. [20] suggested that such changes favourably improve the digestibility of products in which these oligosaccharides are present, so it is a good way to obtain a functional bean-based beverage. BORDIGNON et al. [25] reported that some strains of *Bifidobacterium* rather prefer galacto-oligosaccharides than saccharose during fermentation of soymilk. SCALABRINI et al. [19] found that *Bifidobacterium* strains metabolized raffinose in soymilk, as opposed to yoghurt cultures, which did not reduce raffinose and stachyose during growth in soymilk. These findings led to a conclusion that these strains have a high α-galactosidase activity. Overall, the saccharose content in soymilk could be significantly reduced even by *S. thermophilus* St1342, *B. lactis* B94 and

Tab. 1. Content of oligosaccharides in beverages before and 1 day after the fermentation.

Oligosaccharide	Plant beverages before fermentation	Plant beverages fermented by Yo-Mix 205 LYO	Plant beverages fermented by FD-DVS ABY-3 Probio-Tec
Stachyose [mg·kg^{-1}]	2.73 ± 1.43^{a}	0.88 ± 0.10^{b}	0.80 ± 0.09^{b}
Raffinose [mg·kg^{-1}]	0.43 ± 0.13^{a}	0.08 ± 0.01^{b}	0.07 ± 0.01^{b}
Verbascose [mg·kg^{-1}]	0.15 ± 0.05^{a}	0.06 ± 0.04^{a}	0.10 ± 0.02t^{a}

Values are expressed as mean ± standard deviation (n = 3). Values within a row with the same letters in superscript are not significantly different at the p-value of 0.05.

Lb. casei Lc279 after 48 h [26]. In turn, GRANITO et al. argued that besides the type of bacteria used for fermentation, the time of carrying out the fermentation process is also of great importance [27].

B-group vitamins content

Germination and lactic acid fermentation processes can change the B-group vitamins content, but the direction of these changes is dependent on the cultures and processing parameters [28–30]. In our experiments, we examined the level of B-group vitamins before and after the fermentation of the bean beverage matrix obtained on the basis of germinated beans (Tab. 2). Our goal was not to show the effect of fermentation or germination on the final product, but to check the nutritional value of the drink obtained in terms of the level of discussed vitamins. We observed that the obtained bean-based beverages differed slightly in the level of some B-group vitamins, namely, riboflavin, niacin and pyridoxine. The level of these three vitamins was statistically significantly lower in the fermented samples than in beverages before fermentation. We did not observe significant changes in the content of other vitamins. Our results are in line with those obtained by CHAMPAGNE et al. [31], though studies conducted by other investigators suggest that many LAB and bifidobacteria are capable of the biosynthesis of vitamin B [30, 32]. Some researchers reported that the germination process of legume seeds influenced the level of vitamins from B group. EL-ADAWY [33] showed a significant increase in riboflavin content (from 1.733 g·kg^{-1} to 2.013 g·kg^{-1} dry matter) and slightly lower increase in pyridoxine content (from 4.663 g·kg^{-1} to 4.830 g·kg^{-1} dry matter) after 3 days of germination of chickpea seeds. At the same time, the content of thiamine significantly decreased (from 4.533 g·kg^{-1} to 2.833 g·kg^{-1} of dry matter) and the content of niacin significantly decreased (from 16.027 g·kg^{-1} to 15.186 g·kg^{-1} of dry matter).

Acidity

Fermentation of a bean-based beverage resulted in a reduction of the pH value from the initial level (an average of pH 6.58) to final pH 4.47 and pH 4.45 for samples fermented by the Yo-Mix 205 LYO 289 culture and the FD-DVS ABY-3 Probio-Tec culture, respectively (Tab. 3). For each of the tested starter cultures, we observed a significant reduction in the pH value resulting from the course of the lactic acid fermentation process. During further refrigerated storage of the fermented bean beverage, pH decreased to an average of pH 4.33 and pH 4.27 for samples fermented by the Yo-Mix 205 LYO culture and the FD-DVS ABY-3 Probio-Tec culture, respectively. Changes caused by fermentation and storage were statistically significant, but we did not find statistically significant differences between the cultures tested.

The acidity level of the fermented bean beverage is important from the point of view of

Tab. 2. Contents of B-group vitamins in beverages before and 1 day after the fermentation.

Vitamin	Plant beverages before fermentation	Plant beverages fermented by Yo-Mix 205 LYO	Plant beverages fermented by FD-DVS ABY-3 Probio-Tec
Thiamine [mg·kg^{-1}]	0.69 ± 0.04^{a}	0.60 ± 0.04^{a}	0.65 ± 0.06^{a}
Riboflavin [mg·kg^{-1}]	0.20 ± 0.06^{a}	0.13 ± 0.06^{b}	0.22 ± 0.04^{a}
Niacin [mg·kg^{-1}]	2.34 ± 0.10^{a}	0.68 ± 0.02^{b}	1.43 ± 0.28^{c}
Pyridoxine [mg·kg^{-1}]	0.55 ± 0.04^{a}	0.32 ± 0.07^{b}	0.52 ± 0.02^{a}
Cyanocobalamin [ng·kg^{-1}]	1.38 ± 0.11^{a}	1.67 ± 0.34^{a}	1.35 ± 0.71^{a}

Values are expressed as mean ± standard deviation (n = 3). Values within a row with the same letter in superscript are not significantly different at the p-value of 0.05.

Tab. 3. pH values of beverages before, 1 day after the fermentation process and during refrigerated storage.

Storage time [d]	Plant beverages fermented by Yo-Mix 205 LYO	Plant beverages fermented by FD-DVS ABY-3 Probio-Tec
Before fermentation	6.58 ±0.10^{a}	
0	4.47 ± 0.12^{b}	4.45 ± 0.09^{b}
1	4.43 ± 0.11^{b}	4.43 ± 0.05^{b}
7	4.40 ± 0.17^{b}	4.39 ± 0.05^{bc}
14	4.37 ± 0.12^{c}	4.34 ± 0.03^{c}
21	4.34 ± 0.10^{c}	4.29 ± 0.06^{cd}
28	4.33 ± 0.11^{c}	4.27 ± 0.08^{d}

Values are expressed as mean ± standard deviation (n = 3). Values with the same letters in superscript are not significantly different at the p-value of 0.05.

Tab. 4. Water-holding capacity values of beverages during refrigerated storage.

Storage time [d]	Plant beverages fermented by Yo-Mix 205 LYO	Plant beverages fermented by FD-DVS ABY-3 Probio-Tec
0	49.8 ± 1.0^{a}	54.9 ± 0.9^{a}
1	51.4 ± 2.6^{a}	56.7 ± 2.6^{a}
7	49.2 ± 5.7^{a}	54.2 ± 6.1^{a}
14	50.2 ± 5.7^{a}	55.3 ± 6.0^{a}
21	51.8 ± 5.3^{a}	57.2 ± 5.6^{a}
28	52.3 ± 6.4^{a}	57.8 ± 6.9^{a}

Values are expressed as mean ± standard deviation (n = 3). Values with the same letters in superscript are not significantly different at the p-value of 0.05.

the oligosaccharides hydrolysis discussed previously. Mital et al. [34] showed that α-galactosidase is active in the *Lactobacillus* genus in the range of pH 4.5–8.0. The results of acidity measurements of the fermented-bean beverage samples in our study suggested that, at the end of the fermentation process, the activity of α-galactosidase was so small that the oligosaccharides were not degraded. It can also be assumed that, during the refrigerated storage, the distribution of oligosaccharides in question was significantly or completely inhibited.

Water-holding capacity

In frames of our study, we were able to obtain functional bean-based beverage fermented with the use of commercial starter cultures that are used industrially for the production of yoghurt. The issue of using starter cultures for producing fermented beverages from ordinary bean seeds has not been discussed so far in the scientific literature in such a wide range as in our studies. Thus, we attempted to determine the quality of fermented beverages, i.e. *WHC* of beverages during the 28-days period of refrigerated storage (Tab. 4). Statistical analysis of the collected results showed that there was no statistically significant difference in the *WHC* value during 28-day storage of the fermented bean beverage samples, although the values seemed to slowly increase. Also, there were no differences between the samples fermented with individual cultures. It is difficult to interpret the obtained data, because there are no similar results in the scientific literature. Additionally, interpreting our results referring to *WHC* changes of yoghurts obtained on the basis of milk or soya does not seem a good idea. However, fermented drinks with a relatively low water-holding capacity are still considered as low-quality products.

The comparison of fermented bean beverages obtained in these studies with milk yoghurts or fermented soya-based beverages is difficult, if not impossible. The consistency of milk products is the result of protein aggregation (by low pH value) and also disulphide bonding (between κ-casein and denatured whey proteins) [35]. Meanwhile, in the fermented bean-based beverage samples, no protein aggregation phenomena are observed, suggesting that bean carbohydrates contribute to the water-holding capacity, mainly starch gelled with thermal peel preceding fermentation.

Population of starter microflora

In our experiments, the number of live cells of lactobacilli and streptococci in both fermented plant-based beverages was at a similar level (Tab. 5). One of the directions of scientific research on fermented bean-based beverages is to obtain a product containing, at the right level, live cells of LAB, possibly probiotic strains, similar to the levels in fermented milk products [36]. The high levels of LAB during the entire shelf life of fermented plant-based beverages are taken as a parameter of product quality and of its health-promoting properties. The minimum level of live cells in fermented products should be at least 7 log CFU·ml^{-1} or 7 log CFU·g^{-1} for starter culture bacteria, or at least 6 log CFU·ml^{-1} or 7 log CFU·g^{-1} for additional microorganisms (i.e. probiotic strains) until the end of the shelf life of the product [37]. This criterion pertaining to additional microorganisms is called the "minimum therapeutic" level. In our fermented bean-based beverage sample, the levels of starter culture bacteria and additional microorganisms were at the "minimum therapeutic" level both immediately after fermentation and also during the entire shelf life.

The ability of fermented plant-based beverages to positively impact human health depends on the raw material (matrix) used in the production. The previous study showed that the LAB starter culture should be carefully selected for the specific type of fermented plant beverage due to the variable survival of lactobacilli and streptococci [38]. In the case of a fermented bean beverages, during 28 days of storage, there was a significant reduction in the population of *S. thermophilus*, *Lb. acidophilus* and *Bifidobacterium* spp., however, these were not below 7 log CFU·ml^{-1} and 6 log CFU·ml^{-1}, respectively (Tab. 5). The good survival may be explained by the presence of oligosaccharides, which may provide a carbon source for live bacterial cells during fermentation. Utilization of oligosaccharides present in beans, especially by bifidobacteria and some lactobacilli, is the result of the enzymatic activity of these microorganisms. The metabolic and enzymatic potential of these LAB is connected with their natural environment and, at the same time, gives the possibility of their widespread use in many branches of the food industry and for fermentation of various substrates in food production [39].

Sensory evaluation

The sensory evaluation of samples was performed after 1-day storage and fermentation at 6 °C. Tested samples of a natural fermented bean beverage had low acceptability ratings (Tab. 6), including unappealing flavour and colour, regardless of the starter culture used in their production. Generally, volatile and non-volatile off-flavour compounds of bean-based products belong to the categories of constituents including aldehydes, alcohols, ketones, acids, sulphur compounds, and others. These compounds are responsible for

Tab. 5. Population of lactic acid bacteria in beverages.

Storage time [d]	*Streptococcus thermophilus* [log CFU·ml^{-1}]	*Lactobacillus* spp. [log CFU·ml^{-1}]	*Lactobacillus acidophilus* [log CFU·ml^{-1}]	*Bifidobacterium* spp. [log CFU·ml^{-1}]
Beverages fermented by Yo-Mix 205 LYO				
0	8.9 ± 0.1^{a}	7.5 ± 0.1^{a}	7.6 ± 0.2^{a}	7.5 ± 0.1^{a}
1	8.6 ± 0.2^{a}	7.4 ± 0.1^{a}	7.5 ± 0.2^{a}	7.4 ± 0.1^{a}
7	7.6 ± 0.3^{ab}	7.4 ± 0.1^{a}	7.0 ± 0.1^{a}	6.7 ± 0.1^{a}
14	7.7 ± 0.0^{b}	7.0 ± 0.1^{a}	6.8 ± 0.2^{a}	6.6 ± 0.2^{ab}
21	7.3 ± 0.1^{b}	6.9 ± 0.1^{a}	6.6 ± 0.3^{ab}	6.4 ± 0.2^{b}
28	7.0 ± 0.0^{b}	6.8 ± 0.1^{a}	6.2 ± 0.2^{b}	6.0 ± 0.1^{b}
Beverages fermented by FD-DVS ABY-3 Probio-Tec				
0	8.8 ± 0.1^{a}	7.7 ± 0.3^{a}	7.5 ± 0.2^{a}	7.5 ± 0.3^{a}
1	8.6 ± 0.2^{a}	7.6 ± 0.3^{a}	7.3 ± 0.3	7.4 ± 0.3^{a}
7	7.9 ± 0.2^{ab}	7.5 ± 0.3^{a}	7.0 ± 0.1^{a}	6.6 ± 0.5^{ab}
14	7.8 ± 0.2^{ab}	7.3 ± 0.4^{a}	6.9 ± 0.1^{a}	6.4 ± 0.2^{ab}
21	7.5 ± 0.1^{b}	7.0 ± 0.0^{a}	6.8 ± 0.1^{ab}	6.2 ± 0.2^{b}
28	7.2 ± 0.1^{b}	6.9 ± 0.1^{a}	6.3 ± 0.4^{ab}	6.0 ± 0.1^{b}

Values are expressed as mean ± standard deviation (n = 3). Values within a column with the same letter in superscript are not significantly different at the p-value of 0.05.

Tab. 6. Organoleptic characteristics of natural fermented plant beverages after 1-day refrigerated storage..

Feature	Plant beverages fermented by Yo-Mix 205 LYO	Plant beverages fermented by FD-DVS ABY-3 Probio-Tec
Appearance	5.7 ± 0.4^{a}	5.9 ± 0.4^{a}
Smell	5.9 ± 0.3^{a}	6.5 ± 0.5^{a}
Consistency	6.8 ± 0.6^{a}	7.6 ± 0.4^{a}
Taste	6.0 ± 0.5^{a}	6.6 ± 0.4^{a}
Overall acceptability	6.1 ± 0.4^{a}	6.6 ± 0.6^{a}

Values are expressed as mean ± standard deviation (n = 21). Values within a row with the same letter in superscript are not significantly different at the p-value of 0.05.

Tab. 7. Overall acceptability of flavoured fermented plant beverages after 1-day refrigerated storage.

Taste	Plant beverages fermented by Yo-Mix 205 LYO	Plant beverages fermented by FD-DVS ABY-3 Probio-Tec
Peach-mango	7.1 ± 0.5^{a}	7.2 ± 0.4^{a}
Strawberry	6.4 ± 0.6^{ab}	7.4 ± 0.4^{a}
Forest fruits	6.5 ± 0.3^{b}	6.7 ± 0.4^{ab}
Apricot	7.3 ± 0.3^{a}	7.3 ± 0.5^{a}
Red orange	8.1 ± 0.6^{a}	8.3 ± 0.3^{c}
Banana-kiwi	8.3 ± 0.4^{ac}	8.3 ± 0.4^{ac}

Values are expressed as mean ± standard deviation (n = 21). Values with the same letter in superscript are not significantly different at the p-value of 0.05.

grassy-beany and green flavours noted in bean-based products. It is interesting that fermented bean-based beverage had milder aroma compared to the beverage before fermentation. The best-assessed feature was viscosity, which was probably due to the lactic acid fermentation process, which improved this sensory attribute. The use of fruit flavours improved the perception of basic features assessed in our study (Tab. 7). Fruit flavours decreased unpleasant off-flavours of bean-based products. Depending on the flavouring additive used, the obtained samples of fermented bean beverages received better ratings in comparison to natural samples. Our results showed that fermented bean-based beverages with banana–kiwi and red orange flavours had significantly higher overall acceptability than samples with strawberry or forest fruits flavours. A possible explanation of this fact may result from different intensities of these flavours and from various masking effects of these additives on the bean flavours of the samples. A similar effect was observed with other fermented plant beverages [40].

CONCLUSIONS

Germinated or sprouted seeds of various plant species, including legumes, are food products that are becoming popular among consumers. In our study, we showed the possibility to obtain a functional fermented beverage from sprouted white kidney beans using two starter cultures containing *S. thermophilus*, *Lb. delbrueckii* subsp. *bulgaricus*, *Lb. acidophilus* and *Bifidobacterium* spp., industrially used in the production of dairy yoghurts. It is evident from this study that fermented beverage can be produced from germinated beans with a good level of sensory acceptability (with the addition of the flavouring additives, especially) and a good physicochemical and microbiological quality, as well as high levels of live LAB and bifidobacteria. We did not notice any difference in *WHC* during the storage of both tested culture samples for 28 days. The levels of starter culture bacteria and additional microorganisms met the criteria of the “minimum therapeutic” level both immediately after fermentation and during 28-days storage at 6 °C. In this way, the mixed culture of LAB and bifidobacteria can be used to obtain a functional food of vegetable origin. In each sample, the levels of riboflavin, niacin and pyridoxine were significantly lower after fermentation than before fermentation. We did not observe any significant changes in the content of thiamine and cyanocobalamin. Although we did not observean increase in the level of the tested B-group vitamins due to the process of lactic acid fermen-

tation, we observed a significant reduction in the level of RSO causing bloating in humans, which probably will encourage the potential consumers to consume this product. The content of stachyose and raffinose after fermentation was lower than that before the fermentation. We did not find a statistically significant reduction in verbascose. In this study, fruit flavours were incorporated into bean-based fermented beverages to enhance their acceptability. The choice of appropriate fruit flavour would enhance acceptability of the products.

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