Chapter

# The Possibility of Obtaining Buckwheat Beverages Fermented with Lactic Acid Bacteria and Bifidobacteria

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## Abstract

In this study, we aimed to examine the effect of four different industrial starter cultures containing lactic acid bacteria and bifidobacteria on the selected characteristics of beverages prepared from buckwheat and stored at 4°C for 28 days. We estimated the pH of the beverages during fermentation and storage under refrigerated conditions. We also determined the number of lactic acid bacteria and bifidobacteria and performed a chromatographic analysis of the carbohydrates. According to the results, the tested starter cultures effectively fermented the buckwheat beverage. The viable cell count of the starter microflora was sufficient to demonstrate the health-promoting properties of buckwheat. The pH of beverages was stable during the refrigerated storage. However, the carbohydrate content of the stored beverages changed, which indicates a constant biochemical activity of the microflora.

**Keywords:** buckwheat, health, lactic acid bacteria, lactic acid fermentation, bifidobacteria, probiotics

## 1. Introduction

In recent years, the eating habits of people have changed dramatically due to various reasons. One such reason is consumer awareness of the impact of food on human health. Products that have a natural composition, that are unprocessed, and are nongenetically modified are preferred the most by the consumers. Another important factor, which determines people's eating habits, is food allergies and intolerances, which eliminate the possibility of consumption of a particular food product. Food allergies and metabolic disorders have led to an increased demand for allergen-free food products that meet the daily requirements for protein and other nutrients. For example, in the case of gluten intolerance, it is impossible to eat food products containing gluten. For such individuals, an alternative food product is, among others, buckwheat, which, as a gluten-free pseudocereal, can be used as groats, flour in baking bread or cakes.

Buckwheat has a rich composition and high nutritional value and can be an ideal base for products that are enriched with lactic acid bacteria (LAB), including probiotics. They are defined as a functional food because when they are administered in adequate amounts, they confer specific health benefits to the consumer. Consuming functional foods helps to reduce the risk of developing diseases of affluence, such as diabetes, obesity, or cancer. Buckwheat beverage enriched with LAB and bifidobacterial is one such functional food. Its unique taste and nutritional value might be utilized to develop a new product dedicated to people with disorders of the digestive system, as well as for people who want to stay healthy.

Fermented plant-based products represent a better way to substitute dairy products that cannot be consumed by people with food allergies or intolerance. The plant-based products gain a pro-health value after the process of fermentation and at the same time, they require minimal processing. Furthermore, the probiotic LAB have a positive effect on human health by regulating the functions of the intestinal microbiota. They keep the digestive system healthy and increase immunity. They have anticarcinogenic and antiallergenic effects [1]. Food intolerances are not related to the immune system; they are caused by sensitivity to certain food ingredients, e.g. gluten [2]. At present, approximately 20% of the population is affected due to food intolerance [3]. So far, the detailed mechanism of food intolerance is not known, but it may be related to the neuroendocrine system of the digestive system [4]. In the case of treatment available for gluten intolerance, elimination of gluten from the diet is recommended. Any amount of gluten might be harmful to individuals who are gluten-intolerant. According to the literature, more than 50–100 mg of gluten per day can prove to be harmful to such individuals [5].

## 2. Plant-based beverages as an alternative for dairy-based probiotic beverages

Buckwheat is a dicotyledonous plant and is referred to as a pseudocereal. It is classified as a secondary plant. It has a tap root system, which is 1 meter long, and has a straight stem, 60–90 cm high, and brown in color; it bears pink or white flowers. Different products are made from different parts of the plant. The grains are used to produce buckwheat flour and buckwheat, while straw, after threshing the seeds, is added to various types of fodder. During the flowering season, buckwheat provides nectar to the bees [6, 7]. Buckwheat kernels contain gluten-free protein and have well-balanced amino acid profile. The flour is a rich source of minerals such as copper, zinc, manganese, potassium, magnesium, phosphorus, and potassium. It is also rich in polyphenols such as rutin, orientin, vitexin, quercetin, isovitoxin, and isoorientin. Among the aforementioned polyphenols, rutin shows the strongest anti-inflammatory, anticancer, and protective effect. In terms of flavonoid content, tartar buckwheat seeds contain approximately 40 mg/g, whereas common buckwheat seeds contain 10 mg/g [8–10]. A previous study [11] reported that sucrose is the predominant sugar in buckwheat, whereas xylose, glucose, arabinose, and melibiose are present in much smaller quantities. According to a previous study [12], sucrose accumulates in large quantities when the dry matter content is increased. It mainly occurs in the central part of the ovule and the seed coat. Buckwheat also contains *R*-tocopherol, which shows vitamin E activity [13]. In addition, the ethanolic extract of buckwheat contains four catechins: epicatechin, catechin 7-O-β-D-glucopyranoside, (−)-epicatechin 3-O-p-hydroxybenzoate, and (−)-epicatechin-3-O-(3,4-di-O) gallate-methyl [13]. It should be noted that the content of individual components in the plant may change depending on the environmental factors such as temperature, UV radiation, and damage caused by pests. Genetic factors are also of great importance, and the influence of the height of cultivation to sea level has been recently demonstrated [14].

The word "probiotic" was borrowed from Greek, wherein "probios" means "for life." Probiotics are mainly bacterial strains from the genera *Lactobacillus* and *Bifidobacterium*. However, before a strain is considered a probiotic, clinical trials must be conducted to prove its health-promoting properties [15, 16]. There should be mutual benefit between the human body and the probiotic bacteria (on the basis of symbiosis). The intestines are one of the most important organs in maintaining the body's normal immunity. About 70% of the entire population of immune cells is associated with intestinal mucosa [1]. Literature shows that probiotics regulate the functioning of the bacterial microflora in the intestines through certain mechanisms [17]; for example, they compete with the pathogenic bacteria for the same receptors and nutrients; they produce lactic acid and acetic acid, which lower the pH of the environment and inhibit the colonization of pathogenic microorganisms; they produce mucus; and finally, they synthesize B vitamins.

Growing consumer needs have increased the demand for functional food, which means that the food industry introduces more and more interesting and a variety of products. Currently, Europe, Japan, and the United States are the largest markets for functional products [18]. Functional foods must contain one or more compounds that trigger specific changes in the body. In particular, they should help to reduce the risk of civilization diseases, which are the greatest threat to society; for example, cancer, diabetes, heart disease, osteoporosis, neurodegenerative diseases, and hypertension [19]. It is noteworthy that functional foods help to optimize the physiological functions of the body so that it is possible to initiate repair processes and maintain health. It cannot be treated as a drug in specific disease entities, but only as a support in therapy [20]. Compounds that can be used in functional foods are polyphenols, sterols, carotenoids, probiotics, and prebiotics [21].

There are different categories of functional foods. The simplest ones are unprocessed conventional foods, for example, tomatoes, kale, raspberries, and broccoli. These foods contain a high content of ellagic acid and lycopene. is the next category is modified foods—this category contains foods that are modified by enrichment with specific ingredients. For example, orange juice with added calcium to support bone health, bread supplemented with folic acid, which is especially dedicated to pregnant women, and margarine enriched with plant stanols. The third category of functional food is a medical food, which is used in specific disease cases and can only be administered under the supervision of a doctor. These foods include supplements for phenylketonuria and diabetes and kidney and liver disease. The latter type is special-purpose food, which includes infant formulas, gluten-free foods, lactose-free foods, and foods used in a slimming diet. Therefore, it may be one of the food products that provides the necessary nutrients. In the case of some categories of food, for example gluten-free food, some components of the material are removed to avoid the aggravation of the disease [22].

Fermented foods are grouped as functional foods. Since the beginning of human civilization, fermented foods formed the basis of food, and although people were not aware of it back then, they had a positive effect on their health [23]. Fermented foods can be obtained by the spontaneous or controlled growth of microorganisms and the enzymatic conversion of their main components. Currently, fermented foods can be produced very fast, which allows for the production of thousands of various products [24]. The fermentation process of some food products gives them new health properties and features that were not present in the starting material. Furthermore, recent clinical trials have shown an existing relationship between the consumption of fermented milk products and maintaining a healthy body weight [25]. Other studies have shown that regular consumption of fermented foods such as yogurt reduces the risk of heart disease and type 2 diabetes [26, 27].

## 3. Buckwheat beverages fermented with industrial probiotic cultures

In this study, we aimed to investigate the effect of four different bacterial cultures containing LAB and bifidobacteria on the selected features of buckwheat beverage. With regard to this, we performed fermentation of the selected cultures with buckwheat beverages and evaluated the parameters.

Fermented plant beverages are very popular in Asia and Africa, for example, boza, togwa, mahewu, makgeolli, or hardalie. The most popular plant-based fermented beverage in Poland and throughout Eastern Europe is kvass. It is a product of milk-alcohol fermentation of wholemeal bread with the addition of yeast, water, and a small amount of sugar. The microorganisms present in kvass are *Lactobacillus casei*, *Leuconostoc mesenteroides*, and *Saccharomyces cerevisiae* [28].

In recent years, many studies have reported the properties of plant-based fermented beverages. The most important feature of this type of product is the ability of LAB to carry out effective fermentation, and the pH value of the resulting product is an important parameter, which indicates the effectiveness of the fermentation process. In this study, this parameter was checked both during the fermentation process and after its completion (28 days).

Kowalska [29] used four yogurt starter cultures to ferment the buckwheat beverage: YO-MIX 207, YO-MIX 205, ABY-3, and VEGE 033. The microbial composition of the starters was as follows:

a. ABY-3 (Chr. Hansen, Denmark)—Streptococcus thermophilus, Lactobacillus delbrueckii subsp. bulgaricus, Lactobacillus acidophilus La-5, Bifidobacterium animalis subsp. lactis BB-12,

b. YO-MIX 207 (DuPont Danisco, Denmark)—*S. thermophilus*, L. delbrueckii subsp. bulgaricus, L. acidophilus, Bifidobacterium lactis,

c. YO-MIX 205 LYO (DuPont Danisco, Denmark)—*S. thermophilus*, L. delbrueckii subsp. bulgaricus, L. acidophilus, B. lactis,

d. VEGE 033 LYO (DuPont Danisco, Denmark)—*S. thermophilus*, L. delbrueckii subsp. bulgaricus, L. acidophilus NCFM, B. lactis HN019.

Buckwheat beverage was prepared with 200 g of boiled buckwheat and blended with 3000 mL of drinking water. Prior to the process of sterilization, the beverage was strained through a fine sieve to get rid of the groats. The strained beverage was sterilized at 121°C for 15 min [30]. Based on the recipe of the buckwheat beverage, which was obtained by mixing the buckwheat in water in the proportion 1:15, the nutritional value of 100 g of buckwheat beverage was calculated [29]:

- Fat—0.16 g (including 0.04 g of saturated acids)
- Carbohydrates—4.69 g (including 0.16 g of sugars)
- Proteins—0.75 g.

The average water content of buckwheat beverage was 87.9% [30].

Kowalska [29] reported that the initial pH of buckwheat (beverage before fermentation at 37°C for 5 h) was on an average 6.550 for the samples intended for fermentation with YO-MIX 207, YO-MIX 205, and ABY-3 cultures, and 6.400 for the samples intended for fermentation with VEGE 033 culture (**Table 1**). The most effective fermentation process was observed in the case of beverage fermented with YO-MIX 207 culture, followed by the beverage fermented with YO-MIX 205. Within 1–2 h of fermentation, both beverages reached an average pH value of 4.8, which was statistically significantly from that of before fermentation (**Table 1**). ABY3 and VEGE 033 cultures were less efficient in terms of acidification, in which case, the pH value did not increase until 3–4 h of the fermentation process. After fermentation for 5 h, all of the beverages reached a pH of 4.5–4.9, which means that all the bacteria carried out the fermentation process efficiently [29]. A previous study [31] also reported similar results for soybean beverage fermented with *S. thermophilus*. However, a previous study [32] conducted on barley malt fermented with *Lactobacillus plantarum* (NCIMB 8826) and *L. acidophilus* (NCIMB 8821) recorded a pH value of approximately 4.0. This difference in pH value might be because of the specificity of plant matrices, as well as the use of various bacterial cultures for fermentation (**Table 2**).

Kowalska [29] also measured the pH of buckwheat beverage during 28 days of refrigerated storage. During refrigerated storage, the most stable pH value was recorded for buckwheat beverage fermented with VEGE 033, ABY-3, and YO-MIX 205 cultures. However, the beverage fermented with YO-MIX 207 culture showed variation in pH value during refrigerated storage.

**Table 1** shows the pH value of buckwheat beverage before and after fermentation with cultures tested by Kowalska [29]. Similar results were obtained by Ziarno et al. [33]. They reported the change in pH value of bean beverage (initial pH of 6.58) after fermentation, which was 4.47 and 4.45 when fermented with YO-MIX 205 and ABY-3 cultures, respectively. At 6°C, the pH value of beverages fermented with YO-MIX 205 and ABY-3 cultures respectively decreased to 4.40 and 4.39 on day 7, 4.34 and 4.29 on day 21, and 4.33 and 4.27 on day 28 [33]. This shows that LAB continued the process of fermentation during the entire storage period, which was not observed in the research conducted by Kowalska [29].

Bacterial cell count is a very important parameter in determining the quality of the product and its health properties [34]. Manufacturers frequently check this parameter in fermented beverages. The minimum acceptable number of live LAB cells that should be present in fermented beverages is 7 log(CFU/mL) and at least 6 log(CFU/mL) for strains with probiotic properties, including probiotics of the genus *Bifidobacterium* [35].

Kowalska [29] found that the changes in the number of live LAB and bifidobacteria in beverages fermented with the YO-MIX 205 and YO-MIX 207 cultures were

| Fermentation time [h] | Buckwheat beverages fermented by: | | | |
|---|---|---|---|---|
| | YO-MIX 207 | YO-MIX 205 | ABY-3 | VEGE 033 |
| 0 | 6.550 ± 0.212$^{a}$ | 6.550 ± 0.212$^{a}$ | 6.550 ± 0.212$^{a}$ | 6.400 ± 0.000$^{a}$ |
| 1 | 5.185 ± 0.481$^{b}$ | 5.020 ± 0.389$^{b}$ | 5.610 ± 0.721$^{ab}$ | 5.770 ± 0.000$^{ab}$ |
| 2 | 4.840 ± 0.226$^{b}$ | 4.805 ± 0.163$^{b}$ | 5.085 ± 0.262$^{b}$ | 5.170 ± 0.000$^{b}$ |
| 3 | 4.730 ± 0.127$^{b}$ | 4.675 ± 0.163$^{b}$ | 4.860 ± 0.085$^{b}$ | 4.910 ± 0.000$^{b}$ |
| 4 | 4.640 ± 0.057$^{b}$ | 4.600 ± 0.212$^{b}$ | 4.825 ± 0.106$^{b}$ | 4.880 ± 0.000$^{b}$ |
| 5 | 4.590 ± 0.127$^{b}$ | 4.595 ± 0.276$^{b}$ | 4.790 ± 0.156$^{b}$ | 4.950 ± 0.000$^{b}$ |

*Note: $^{a,b}$—values in columns with the same letter do not differ statistically significantly for* α = 0.05.

**Table 1.**
*pH values of buckwheat beverage during the fermentation process (mean ± standard deviation) (based on [29]).*

| Storage time [day] | Buckwheat beverage fermented by: | | | |
|---|---|---|---|---|
| | YO-MIX 207 | YO-MIX 207 | YO-MIX 207 | YO-MIX 207 |
| 0 | 4.590 ± 0.127[b] | 4.595 ± 0.276[a] | 4.790 ± 0.156[a] | 4.950 ± 0.000[a] |
| 7 | 4.750 ± 0.071[ab] | 4.750 ± 0.071[a] | 4.850 ± 0.071[a] | 4.900 ± 0.000[a] |
| 21 | 4.875 ± 0.035[ab] | 4.850 ± 0.071[a] | 4.850 ± 0.071[a] | 5.000 ± 0.000[a] |
| 28 | 4.920 ± 0.028[a] | 4.935 ± 0.049[a] | 4.925 ± 0.035[a] | 5.000 ± 0.000[a] |

*Note: [a,b]—values in columns with the same letter do not differ statistically significantly for α = 0.05.*

**Table 2.**
*pH values of buckwheat beverage fermented with different starter cultures (mean values and standard deviations) (based on [29]).*

very similar. Interestingly, after fermentation, there was a slight reduction in the number of viable bacterial cells compared to the state before fermentation. In addition, during the refrigerated storage of the fermented beverage, there were fluctuations in the number of LAB cells, both lactobacilli and lactic streptococci, as well as bifidobacteria. The number of viable cells of lactobacilli, lactic streptococci, and bifidobacteria on day 28 was over 7 log(CFU/mL), which indicated the potential health-promoting properties of the tested beverages fermented with the YO-MIX 207 and YO-MIX 205 cultures.

The smallest variation in the population of lactobacilli, lactic streptococci, and bifidobacteria was recorded for beverages fermented with ABY-3 culture (**Table 3**) [29]. Contrary to buckwheat beverages fermented with the YO-MIX 207 (**Table 4**) and YO-MIX 205 (**Table 5**), there were no such significant changes in the number of bacterial cells. After fermentation, the number of bifidobacterial cells decreased the most. After 7 days of refrigerated storage (4°C), there was a slight change in the number of lactobacilli, lactic streptococci, and bifidobacteria. After 28 days of storage, the average bacterial cell count was 8.0 log(CFU/mL) for lactobacilli, 7.8 log(CFU/mL) for lactic streptococci, and 8.0 log(CFU/mL) for bifidobacteria. The number of viable cells of lactobacilli, lactic streptococci, and bifidobacteria on day 28 was over 7 log(CFU/mL), which indicated the potentially health-promoting properties of the tested buckwheat beverages fermented with the ABY-3 culture [29].

According to Kowalska [29], in the case of buckwheat beverage fermented with VEGE 033 (**Table 6**), the greatest proportion in the population of bacterial cells prior to fermentation were lactic streptococci [29]. In the beverages fermented with the VEGE 033 culture, the lower number of bifidobacterial cells was found (during the entire period of cooling storage) compared to the buckwheat beverage fermented with ABY-3 culture. On the 7th day of storage of the samples of buckwheat beverages fermented with the VEGE 033 culture, the number of streptococcal cells was on average 8.2 log(CFU/mL). The number of viable lactobacilli, lactic streptococci, and bifidobacteria cells in the beverage fermented with VEGE 033 culture on day 28 was over 7 log(CFU/mL) [29].

A previous study conducted on rice beverage reported low counts of bacterial cells [36]. Prior to fermentation, the number of bacterial cells in rice beverage was lower than that observed for buckwheat beverage in the research conducted by Kowalska [29] - the population of LAB was 5.0 log(CFU/mL). However, after 16-hour fermentation process, the bacterial population increased to 8.1 log(CFU/mL) and remained at this level until the end of the fermentation process [29]. However, the previous study [37] reported that after fermentation of corn or rice-based beverages, the microbial cell population was at the level of 7–8 log(CFU/mL). This number indicates that the product has probiotic properties [38].

| Determination time | Number of lactobacilli [log(CFU/mL)] | Number of bifidobacteria [log(CFU/mL)] | Number of lactic streptococci [log(CFU/mL)] |
|---|---|---|---|
| Before fermentation | 8.2 ± 0.2[a] | 8.2 ± 0.4[a] | 8.2 ± 0.4[a] |
| After fermentation | 7.9 ± 0.3[a] | 7.7 ± 0.1[b] | 8.0 ± 0.3[a] |
| 7 day of storage | 8.1 ± 0.3[a] | 8.2 ± 0.2[ab] | 8.0 ± 0.2[a] |
| 28 days of storage | 8.0 ± 0.2[a] | 8.0 ± 0.2[ab] | 7.8 ± 0.1[a] |

*Note: [a,b]—values in columns with the same letter do not differ statistically significantly for α = 0.05.*

**Table 3.**
*The population of live cells of lactic acid bacteria and bifidobacteria in buckwheat beverage fermented with ABY-3 culture and stored for 28 days under refrigerated condition (mean ± standard deviation) (based on [29]).*

| Determination time | Number of lactobacilli [log(CFU/mL)] | Number of bifidobacteria [log(CFU/mL)] | Number of lactic streptococci [log(CFU/mL)] |
|---|---|---|---|
| Before fermentation | 8.7 ± 0.3[a] | 8.5 ± 0.2[a] | 8.4 ± 1.1[a] |
| After fermentation | 7.8 ± 0.2[b] | 7.2 ± 0.1[c] | 7.9 ± 0.3[a] |
| 7 day of storage | 8.0 ± 0.3[b] | 8.1 ± 0.3[ab] | 8.0 ± 0.4[a] |
| 28 days of storage | 7.8 ± 0.0[b] | 7.7 ± 0.1[b] | 7.7 ± 0.3[a] |

*Note: [a–c]—values in columns with the same letter do not differ statistically significantly for α = 0.05.*

**Table 4.**
*The population of live cells of lactic acid bacteria and bifidobacteria in buckwheat beverage fermented with YO-MIX 207 culture and stored for 28 days under refrigerated condition (mean ± standard deviation) (based on [29]).*

| Determination time | Number of lactobacilli [log(CFU/mL)] | Number of bifidobacteria [log(CFU/mL)] | Number of lactic streptococci [log(CFU/mL)] |
|---|---|---|---|
| Before fermentation | 7.9 ± 0.8[a] | 7.8 ± 0.7[a] | 8.4 ± 0.8[a] |
| After fermentation | 7.6 ± 0.2[a] | 7.0 ± 0.2[a] | 7.8 ± 0.5[a] |
| 7 day of storage | 7.9 ± 0.6[a] | 7.6 ± 0.7[a] | 8.1 ± 0.5[a] |
| 28 days of storage | 7.6 ± 0.1[a] | 7.5 ± 0.3[a] | 7.8 ± 0.2[a] |

*Note: [a]—values in columns with the same letter do not differ statistically significantly for α = 0.05.*

**Table 5.**
*The population of live cells of lactic acid bacteria and bifidobacteria in buckwheat beverage fermented with YO-MIX 205 culture and stored for 28 days under refrigerated conditions (mean ± standard deviation) (based on [29]).*

However, another group of researchers [39] used different strains of LAB for fermentation of soy milk, including *L. delbrueckii* subsp. bulgaricus and *L. acidophilus*, which were also used in this study. The cell population of all cultures was 8 log(CFU/mL), which is similar to the results of this study with buckwheat. In each bacterial culture, *L. delbrueckii* subsp. bulgaricus and *L. acidophilus* were present, but their strain was different.

Kowalska [29] found that in all plant-based products, there were similarities in the population of LAB, despite the diversity of the *Lactobacillus* strains used. The

| Determination time | Number of lactobacilli [log(CFU/mL)] | Number of bifidobacteria [log(CFU/mL)] | Number of lactic streptococci [log(CFU/mL)] |
|---|---|---|---|
| Before fermentation | $8.7 \pm 0.0^{a}$ | $7.1 \pm 0.1^{b}$ | $9.0 \pm 0.1^{a}$ |
| After fermentation | $7.1 \pm 0.1^{d}$ | $7.3 \pm 0.0^{b}$ | $7.9 \pm 0.0^{c}$ |
| 7 day of storage | $8.1 \pm 0.1^{b}$ | $8.2 \pm 0.2^{a}$ | $8.2 \pm 0.1^{b}$ |
| 28 days of storage | $7.7 \pm 0.0^{c}$ | $7.4 \pm 0.1^{b}$ | $7.5 \pm 0.0^{d}$ |

*Note: $^{a–d}$—values in columns with the same letter do not differ statistically significantly for α = 0.05.*

**Table 6.**
*The population of live cells of lactic acid bacteria and bifidobacteria in buckwheat beverage fermented with VEGE 033 culture and stored for 28 days under refrigerated condition (mean ± standard deviation) (based on [29]).*

good growth of LAB in plant-based beverages can be explained by the high amounts of mono and disaccharides in the plant media.

A previous study performed fermentation of bean beverages with ABY-3 culture [33]. Prior to fermentation, the number of viable lactobacilli was 7.7 log(CFU/mL), which gradually decreased during the cold storage. On days 7 and 28 of storage, the population of lactobacilli was 7.5 log(CFU/mL) and 6.9 log(CFU/mL), respectively. According to a previous study [30], the observed lower bacterial cells after the cold storage period may result from antimicrobial compounds produced by bacteria, e.g. hydrogen peroxide, bacteriocins, or organic acids. In contrast, in the research conducted by Kowalska [29], the number of viable lactobacilli in the buckwheat beverage fermented with ABY-3 culture was slightly higher. Prior to fermentation, on days 7 and 28 of storage, the number of viable lactobacilli was 8.2 log(CFU/mL) and 8.0 log(CFU/mL), respectively. The better growth on buckwheat substrate might be due to higher sugar content and availability in plant media.

Kowalska [29] verified the content of carbohydrates using high-performance liquid chromatography. The results showed the presence of 7 carbohydrates: xylose, melibiose, fructose, arabinose, glucose, sucrose, and maltose. The initial (before fermentation) content of carbohydrate in the fermented buckwheat beverage was 4.598 g in 100 g of the product. The chromatographic analysis includes only a few selected carbohydrates, whereas the calculated value of carbohydrate content takes into account all such compounds, including starch. Therefore, it can be concluded that as a result of the cooking and sterilization of buckwheat beverage in an aqueous solution, some complex carbohydrates or polysaccharides might be released, which were determined by chromatography [29].

Immediately after the end of fermentation of buckwheat beverages, the highest total carbohydrate content was found in the beverage fermented with the ABY-3 culture (**Table 7**), whereas the lowest was found in the beverage fermented with the YO-MIX 207 culture (**Table 8**). It should be noted that both the ABY-3 culture and the YO-MIX 207 culture had a rich microbiological composition, which not only included LAB but also included bifidobacteria of different strains [29].

In the case of beverage fermented with YO-MIX 205 culture (**Table 9**), we obtained statistically significant differences in terms of carbohydrate content before and after fermentation and during cold storage.

Contrary to the buckwheat beverages fermented with the YO-MIX 205 and YO-MIX 207 cultures, the beverage fermented with VEGE 033 culture (**Table 10**) contained a low amount of xylose after fermentation. In this case, the xylose content decreased slightly. As in the beverages fermented with the

| Carbohydrates [g/100 g beverage] | Before fermentation | After fermentation | After 7 days of storage | After 28 days of storage |
|---|---|---|---|---|
| Xylose | 0.000[e] | 0.129[e] | 0.065[f] | 0.193[b] |
| Fructose | 0.096[d] | 0.322[b] | 0.132[e] | 0.000[e] |
| Arabinose | 0.000[e] | 0.241[d] | 0.294[c] | 0.000[e] |
| Glucose | 2.958[a] | 0.280[c] | 0.251[d] | 0.152[c] |
| Melibiose | 0.000[e] | 0.000[f] | 0.318[b] | 0.153[c] |
| Sucrose | 1.544[b] | 2.300[a] | 1.591[a] | 0.698[a] |
| Maltose | 0.218[c] | 0.000[f] | 0.000[g] | 0.000[e] |
| All | 4.598 | 3.273 | 2.650 | 1.196 |

*Note: [a–e]—values in columns with the same letter do not differ statistically significantly for α = 0.05.*

**Table 7.**
*Content of carbohydrates in buckwheat beverages fermented with ABY-3 culture (based on [29]).*

| Carbohydrates [g/100 g beverage] | Before fermentation | After fermentation | After 7 days of storage | After 28 days of storage |
|---|---|---|---|---|
| Xylose | 0.000[f] | 0.000[f] | 0.000[e] | 0.143[c] |
| Fructose | 0.096[d] | 0.115[c] | 0.076[d] | 0.164[b] |
| Arabinose | 0.000[e] | 0.069[d] | 0.086[c] | 0.000[g] |
| Glucose | 2.958[a] | 0.204[b] | 0.186[b] | 0.102[d] |
| Melibiose | 0.000[e] | 0.000[e] | 0.000[f] | 0.087[e] |
| Sucrose | 1.544[b] | 1.436[a] | 1.388[a] | 0.751[a] |
| Maltose | 0.218[c] | 0.000[f] | 0.000[f] | 0.000[f] |
| All | 4.598 | 1.824 | 1.736 | 1.247 |

*Note: [a–g]—values in columns with the same letter do not differ statistically significantly for α = 0.05.*

**Table 8.**
*Content of carbohydrates in buckwheat beverages fermented with YO-MIX 207 culture (based on [29]).*

YO-MIX 205 and YO-MIX 207 cultures, the content of sucrose, glucose, and maltose also decreased, and the content of arabinose increased. The chromatographic analysis also did not detect the presence of melibiose. Statistical analysis showed significant differences in the results of carbohydrate content during cold storage of the samples.

Our results show differences in the fermentation abilities of the tested starter cultures, resulting from different biochemical activities (mainly saccharolytic and fermentation) of the strains present in the tested cultures [29].

It can be assumed that the changes in the content of carbohydrates during refrigerated storage were due to the changes taking place in the analyzed samples; for example, the biochemical activity of LAB and bifidobacteria, as well as enzymatic changes [29]. Due to the lack of information, it is difficult to compare the results of this study with that of others.

A previous study [40] reported contradictory results with respect to sugar content in the cooked buckwheat wort. According to the result of the aforementioned study, glucose was present in the highest quantities. However, in this study, sucrose was found to be the highest after fermentation and after the storage period, which was most likely the result of starch decomposition.

| Carbohydrates [g/100 g beverage] | Before fermentation | After fermentation | After 7 days of storage | After 28 days of storage |
|---|---|---|---|---|
| Xylose | 0.000[e] | 0.000[e] | 0.042[e] | 0.233[c] |
| Fructose | 0.096[d] | 0.099[d] | 0.093[d] | 0.244[b] |
| Arabinose | 0.000[e] | 0.152[c] | 0.873[b] | 0.000[f] |
| Glucose | 2.958[a] | 0.000[e] | 0.000[f] | 0.123[d] |
| Melibiose | 0.000[e] | 0.286[b] | 0.188[c] | 0.075[e] |
| Sucrose | 1.544[b] | 1.514[a] | 1.763[a] | 0.595[a] |
| Maltose | 0.218[c] | 0.000[e] | 0.000[f] | 0.000[f] |
| All | 4.598 | 2.051 | 2.959 | 1.270 |

*Note: [a–f]—values in columns with the same letter do not differ statistically significantly for α = 0.05.*

**Table 9.**
*Content of carbohydrates in buckwheat beverages fermented with YO-MIX 205 culture (based on [29]).*

| Carbohydrates [g/100 g beverage] | Before fermentation | After fermentation | After 7 days of storage | After 28 days of storage |
|---|---|---|---|---|
| Xylose | 0.000[e] | 0.080[e] | 0.625[c] | 0.705[a] |
| Fructose | 0.096[d] | 0.094[d] | 0.000[e] | 0.000[e] |
| Arabinose | 0.000[e] | 0.741[b] | 1.299[b] | 0.264[c] |
| Glucose | 2.958[a] | 0.237[c] | 0.328[d] | 0.106[d] |
| Melibiose | 0.000[e] | 0.000[f] | 0.000[e] | 0.000[e] |
| Sucrose | 1.544[b] | 1.237[a] | 1.598[a] | 0.338[b] |
| Maltose | 0.218[c] | 0.000[f] | 0.000[e] | 0.000[e] |
| All | 4.598 | 2.389 | 3.851 | 1.413 |

*Note: [a–f]—values in columns with the same letter do not differ statistically significantly for α = 0.05.*

**Table 10.**
*Content of carbohydrates in buckwheat beverages fermented by VEGE 033 culture (based on [29]).*

A previous study [41] reported that sucrose was the predominant carbohydrate, whereas xylose, glucose, arabinose, and melibiose were present in much smaller quantities. Another study [42] reported that with an increasing amount of water and lengthening heating time, the content of glucose increases.

According to the literature [43], fermentation of buckwheat beverages with the use of *Propionibacterium freudenreichii* subsp. *shermanii* resulted in a significant increase in the content of fructose, glucose, and galactose. In addition, there was a significant increase in sucrose content.

## 4. Conclusion

The results of this study indicate a high potential of fermented buckwheat beverage as a probiotic product with pro-health properties. The demand for gluten-free cereal beverages is growing among people suffering from celiac disease and food intolerance. Good bacterial survival during the storage period allows achieving a therapeutic effect similar to that caused by consuming fermented milk products, such as kefir, buttermilk, or yoghurt. In addition, an additional advantage of the product is the lack of allergenic milk proteins. More and more people are experiencing side effects after drinking milk and other dairy products such as gas, indigestion, and diarrhea, which are causing them to be excluded from their diet. In such a case, dietary supplements containing probiotic strains are often used to supplement the intestinal microflora and increase the body's immunity. Fermented buckwheat beverages can replace these types of supplements and provide other essential nutrients for the body. The product is dedicated not only to people suffering from disorders of the digestive system but also to healthy people who care about a balanced diet and want to have a healthy lifestyle. In addition to LAB and bifidobacteria, the base of the buckwheat beverage is important, as it is also a medium necessary for the growth of the bacterial population used for fermentation. Our results show that buckwheat can be successfully fermented by LAB and bifidobacteria. Its proven health properties mean that the beverage can be used to prevent civilization diseases such as diabetes, obesity, or cancer.

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## Conflict of interest

Authors have declared that they do not have any conflict of interest in publishing this research.

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