EFFECT OF SELECTED LACTIC ACID BACTERIA ON BUCKWHEAT BEVERAGE FERMENTATION

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Abstract
The buckwheat (*Fagopyrum esculentum*) has been an essential food ingredient for centuries. Fermentation of buckwheat using lactic acid bacteria (LAB) provides an opportunity to improve its nutritional value and bioavailability of nutrients and develop new functional beverages. This study investigated the effect of selected strains of LAB on the fermentation of buckwheat beverage base, analyzing pH changes, LAB growth patterns and sugar profile changes prior to and at the end of fermentation. Buckwheat samples were prepared at 8% and 10% (w v^{-1}) aqueous suspensions, with further sample inoculation with *Lactobacillus plantarum subsp. plantarum, Lactobacillus acidophilus, Lacticaseibacillus paracasei subsp. paracasei, Lacticaseibacillus rhamnosus* strains. The fermentation was carried out at 37 °C for a maximum of 8 hours, analyzing samples after 2, 3, 4 and 8 hours of fermentation. The initial pH of the buckwheat suspension was 7.57 (8% buckwheat base) and 7.44 (10% buckwheat base) depending on the concentration. During the first 4 hours of fermentation, the pH decreased rapidly in all samples. At the end of fermentation, the pH varied from 4.34 to 4.17 in all samples. The growth of LAB during fermentation was monitored using the plate count method. Faster growth of the studied LAB strains was observed in buckwheat (10%) substrate samples with added *L.plantarum* and *L.paracasei* from 8.59 to 8.75 $\log_{10}$CFU mL^{-1}. With the mentioned strains, the sucrose content in buckwheat samples decreased during fermentation, i.e. by 57%. The results show that lactic acid bacteria induced fermentation help to produce nutrient-rich buckwheat-based beverages.
Keywords: buckwheat, lactic acid bacteria, fermentation, sugars.

Introduction

Fermented foods and beverages are estimated to make up 1/3 of human diet (Mota de Carvalho et al., 2018). The consumption of fermented products in the daily diet can provide 1.0 % of the gut's commensal microbiota, with 10^{10} to 10^{11} bacteria being ingested (Tsafrakidou et al., 2020). Fermentation significantly increases the nutritional value of buckwheat (*Fragopyrum esculentum*), increasing the bioavailability of nutrients, reducing anti-nutritional factors, and adding beneficial compounds synthesized via fermentation.

The chemical composition of buckwheat consists of 63% carbohydrate, including 9.9% fiber, 11.7% protein, 2.4% fat, 11% water and 2% minerals, as well as balanced amino acid composition, abundant vitamin P and rutin, which reduce cholesterol content in the blood and makes buckwheat an almost perfect food (Mahata, 2018). The sucrose content in buckwheat grains is generally lower than in leaves and reaches of up to 48.13 g kg^{-1} total solids (Nešović et al., 2021). However, the amount of sucrose can vary between different buckwheat varieties under the same growing conditions, and the ripening time of the grains also depends on the genetic characteristics of the varieties (Nešović et al., 2021). The protein composition and technological properties of buckwheat grains indicate that buckwheat is an excellent raw material for gluten-free and vegan-friendly food product production. Buckwheat's energy value, protein and carbohydrate content are equivalent to wheat, while the fiber content is 40% higher and the fat content is 60% higher than wheat. 100 g of buckwheat contains 110 mg of calcium, 4 mg of iron, and magnesium, phosphorus and manganese are significantly higher than wheat (Pirzadah & Malik, 2020). Buckwheat is characterized with low glycemic index. The complex carbohydrates found in buckwheat are absorbed slowly into the bloodstream, providing longer satiety and supporting energy (Mahata, 2018).

New functional plant-based foods with probiotics are still being developed. Plant-based foods help probiotic bacteria reach the intestinal tract targeted due to the presence of indigestible fiber, such as cellulose (Küçükgöz & Trząskowska, 2022). Fermented plant-based products are becoming increasingly popular dairy analogous due to the absence of lactose or allergic proteins, lower saturated fats, also environmental and economic aspects should be taken into consideration (Hidalgo-Fuentes et al., 2024).

This study investigated the effect of *Lactobacillus plantarum subsp. plantarum, Lactobacillus acidophilus, Lacticaseibacillus paracasei subsp. paracasei, and Lacticaseibacillus rhamnosus* on the fermentation of buckwheat and fermentation outcomes.

Materials and Methods

The research was carried out at the Food Institute and Valmiera Technical School. The grain specimens under examination were harvested in 2024. The unprocessed green buckwheat variety 'AIVA' was purchased from the local producer (Kaņepītes, Latvia). Buckwheat beverage base was prepared with different grain concentrations - 8 % buckwheat (w v^{-1}) (coded as A), and 10% (w v^{-1}) (coded as B). The buckwheat was mixed with tap water, and boiled for 20 minutes. The insoluble buckwheat particles were separated through a sieve (size diameter 2 mm). The clarified beverage base was sterilized at 121 °C for 15 minutes in the sterilizer (Systec D-65, Systec GmbH, Germany), after sterilization the samples were cooled down to fermentation temperature 37 °C.

Single strain DVS starter cultures (Mediterrania Biotechnologie, Italy) - LBP 10 U *(Lactobacillus*

plantarum subsp. plantarum), LA 10 U *(Lactobacillus acidophilus)*, LCP 10 U *(Lacticaseibacillus paracasei subsp. paracasei)*, LCR 10 U *(Lacticaseibacillus rhamnosus)* were used for the study. 0.02 U of starter cultures were used for 1000 ml of the substrate inoculation. The fermentation process was provided up to reaching pH 4.3-4.5 at 37 ºC temperature using BD 23 serial incubator (Binder, Germany).

The pH of the substrate was analyzed initially and further every 2-3 hours during the fermentation, and the colony forming units of lactic acid bacteria were determined initially and after 4 and 8 hours of fermentation.

Microbiological analyses

Lactic acid bacteria were determined using MRS medium (Scharlau, Spain). Incubation of plates was provided in anaerobic conditions (37 °C, 72 hours) at an aerostat using anaerobic sacket BSM (Merck KGaA Darmstadt, Germany). MRS agar was prepared within LVS CEN ISO/TS 11133 – 1:2009. Pepton water was used for the preparation of corresponding dilutions. Microbiological analyses were performed in a laminar flow cabinet Biological Safety Cabinet SHANDONG 11237 BBC 86 (BIO BASE, China). The horizontal method of microorganism counting was used according to the LVS EN ISO 4833-1:2014 (ISO, 2014). After incubation, the colony forming units (CFU) of probiotic bacteria were determined using the automatic colony counter SCAN500 (INTERSCIENCE, France), and software version 8.6.12.0 v3.4 (INTERSCIENCE, France). The results were expressed in $\log_{10}$CFU mL^{-1}.

pH

The pH was measured using Milwaukee MW102-FOOD digital pH meter (Milwaukee Electronics Kft., Hungary). The electrode was calibrated with pH 4.00 and pH 7.00 buffer solutions. To ensure accuracy, each sample was tested in three replications.

Sugar analyses

Sugar analyses were conducted using high-performance liquid chromatography (HPLC). Buckwheat suspension samples were centrifuged for 10 min at 15000 rpm in Microtube PrO-Research Centrifuge (UK). 10 µL of the sample was injected into HPLC system Shimadzu. The equipment utilized included a Shimadzu LC-20 Prominence system chromatograph (Shimadzu Corporation, Japan) equipped with a photodiode M20A detector, LC-20A solvent delivery system, CBM-20A system controller, and LC solution data system software (Shimadzu Corporation, Japan). The chromatographic separation was carried out, using a deionised water as mobile phase at a flow-rate of 1.0 mL min^{-1} maintaining the column temperature at 40 °C. Peak areas were utilized for quantitative analysis. The calibration curves were prepared for raffinose or sucrose (Merck, USA) solution in water. Sugar (sucrose and raffinose) analyses were compared with the control sample - buckwheat substrate (B) without fermentation.

Statistical analysis

The Microsoft Excel 2013 software, designed for Windows 11, version 10.0.6.1000 operating systems, was employed for data processing. The pH, sugar, LAB CFU analyses were prepared in three replications, the mean value and standard deviation were calculated. A one-way analysis of variance (ANOVA) and Tukey's test were employed to assess the data. The resulting p-value indicated whether a statistically significant difference existed among the samples, with the significance level defined as $p < 0.05$.

Results and Discussion

The results of pH analyses are shown in Table 1. *L. acidophilus, L. paracasei* and *L. rhamnosus* metabolize carbohydrates, producing lactic acid and other metabolites, lowering the pH from 7.57 to 4.17 in the samples during 8 hours of fermentation. In samples with 8 and 10% of buckwheat grains, the pH decreases more rapidly within 4 or 8 hours. Zhou et al., 2022 indicated that a significant drop in pH was achieved during 8-12 hours of fermentation, while other researchers (Cardinali et al., 2021) found pH lower than 4.2 during 24 hours. It depends on the amount of dry matter in the buckwheat base, the fermentation temperature and the amount of starter added. For instance, Cardinali et al. (2021) reported initial pH values of 6.95 to 7.42 for different buckwheat bases. This difference could be due to variations in buckwheat species, solids and substrate preparation methods.

The pH values ranged from 4.20 to 4.60 (Cardinali et al., 2021; Kowalska & Ziarno, 2020) compared to our study where pH changed from 4.17 up to 4.88 at the end of fermentation. The study results on fermented oat and buckwheat beverages showed that pH of around 4.30 - 4.40 was well-accepted by consumers (Salman, 2024). Some studies have found that pH drops below 4.00 can negatively impact sensory properties and probiotic viability. After 4 hours of fermentation in 8% buckwheat substrate samples, the growth of probiotic bacteria ranged from 7.30 to 8.49 $\log_{10}$CFU mL^{-1} (see Table 2). In contrast, a higher LAB growth was found in 10% buckwheat substrate samples, where LAB ranged from 7.67 to 8.75 $\log_{10}$CFU mL^{-1}.

After eight hours of fermentation, the probiotic bacteria CFU in samples ranged from 8.54 to 8.97 $\log_{10}$CFU mL^{-1}, but the rapid growth of LAB was noticed in samples fermented with *L. plantarum* and *L. paracasei*.

Results are expressed as mean value ± standard deviation (n = 2). Significant differences between LAB CFU in fermented buckwheat after 4 and 8 h fermentation in columns are marked with different lower letters. Tukey HSD test $p \leq 0.05$.

L. plantarum and *L. paracasei* are widely used in sourdough bread production due to their ability to adapt different environmental conditions (di Renzo et al., 2018). *L. plantarum* is efficient in utilizing various sugars (Figure 1), contributing to rapid fermentation (Wang & Ma, 2024).

Table 1
The changes of pH during fermentation

Single strain culture	***Buckwheat sample***	***Fermentation time, h***				
		0	***2***	***3***	***4***	***8***
L. plantarum	A	7.57±0.01	6.26±0.01	5.27±0.02	4.90±0.00	4.31±0.01
L. acidophilus			6.35±0.02	5.23±0.01	4.96±0.00	4.26±0.01
L. paracasei			6.00±0.02	5.08±0.01	4.86±0.01	4.17±0.01
L. rhamnosus			7.08±0.01	6.85±0.00	6.34±0.01	4.98±0.01
L. plantarum	B	7.44±0.01	5.48±0.01	4.85±0.01	4.34±0.01*	-
L. acidophilus			5.51±0.00	4.95±0.00	4.55±0.01*	-
L. paracasei			5.46±0.01	4.82±0.01	4.55±0.01*	-
L. rhamnosus			6.66±0.02	5.54±0.01	5.00±0.01	4.72±0.01

*Fermentation process was finished reaching pH 4.30-4.50.

Table 2
Effect of single strain cultures on fermentation patterns

Single strain culture	***Buck-wheat sample***	***log₁₀ CFU mL⁻¹***	***log ₁₀ CFU mL⁻¹***
		4 h	***8 h***
L. plantarum	A	8.32±0.42^{c}	8.97±0.45^{a}
L. acidophilus		7.76±0.39^{b}	8.54±0.43^{a}
L. paracasei		8.49±0.42^{c}	8.95±0.45^{a}
L. rhamnosus		7.30±0.37^{b}	8.56±0.43^{a}
L. plantarum	B	8.75±0.44^{b}	8.97±0.45^{a}
L. acidophilus		7.83±0.39^{ab}	8.54±0.43^{a}
L. paracasei		8.59±0.43^{b}	8.95±0.45^{a}
L. rhamnosus		7.67±0.38^{a}	8.56±0.43^{a}

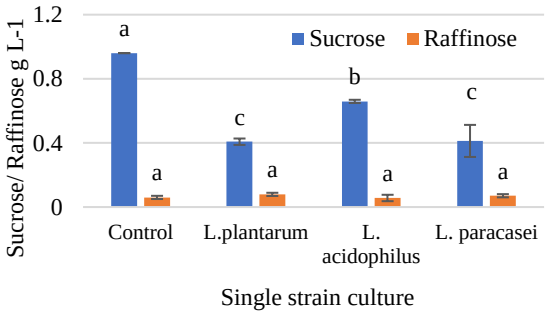
In fermented buckwheat beverage production, *L. plantarum* reached up to 8-9 $\log_{10}$CFU mL^{-1} after 6 hours of fermentation (Matejčeková et al., 2017), but in oat drinks fermented with *L. plantarum*, 8 $\log_{10}$CFU ml^{-1} were reached after 24 hours of fermentation (Gokavi et al., 2005). Likewise, *L. acidophilus* LACA 4 in the peanut-soy milk beverage production reached above 8 $\log_{10}$CFU mL^{-1} after 24 hours of fermentation (Valero-Cases et al., 2020). The lactic acid bacteria growth during fermentation influences the composition of the substrate, selected fermentation patterns.

In general, LAB strains typically reach populations of 7-10 $\log_{10}$CFU mL^{-1} in plant-based beverages during fermentation, with slight variations depending on the specific substrate and fermentation conditions (Salman, 2024). The growth patterns are similar across different plant-based media, including buckwheat, oats, and various fruits and vegetables.

After four hours of fermentation, the sucrose content in the samples decreased rapidly by 31–57%, but after eight hours the reduction was only 27%. Sucrose presents in significant amounts in buckwheat, particularly in the leaves, but it is also found in the grains. The sucrose and raffinose content in buckwheat substrate samples were analysed before and after fermentation. In the control sample the sucrose content was 0.958 g L^{-1} and the raffinose content - 0.056 g L^{-1}. During the first four hours, the sucrose content in buckwheat samples significantly decreased (Figure 1).

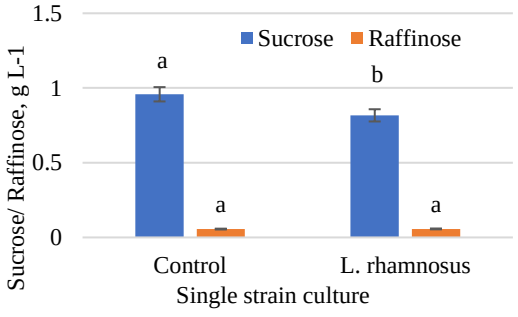
Also, changes were observed in the raffinose concentration due to pH and the LAB strain chosen. Some LAB strains can metabolize raffinose, others may not be efficient, such as *L.plantarum* and *L.paracasei* (Sanyal et al., 2023).

Figure 1
The changes of sucrose and raffinose content in buckwheat substrate samples (B) after 4 hours of fermentation



Results are expressed as mean value ± standard deviation (n = 4). Significant differences between sucrose and raffinose in fermented buckwheat substrate samples after 4 hours are marked with different lower letters (Tukey HSD test p≤0.05).

Figure 2
The changes of sucrose and raffinose content in buckwheat substrate samples (B) after 8 hours of fermentation



Results are expressed as mean value ± standard deviation (n = 4). Significant differences between sucrose and raffinose in fermented buckwheat substrate samples after 8 hours are marked with different lower letters (Tukey HSD test $p \leq 0.05$).

The acidity level after 4 hours of fermentation was pH=4.35-4.55, and after 8 hours of fermentation pH=4.72-4.75. The difference between both groups is not statistically significant $p \leq 0.05$. A greater reduction of sucrose was observed in samples with *L. plantarum* and *L. paracasei*. In contrast, after 8 hours of fermentation in samples with *L. rhamnosus* sucrose concentration was decreased only 27% (Figure 2). The ability of *L. rhamnosus* to metabolize sucrose might be limited compared to other sugars (Bertsch et al., 2020). *L. rhamnosus* GG efficiently metabolize glucose and fructose but has limited ability to metabolize lactose and sucrose (Corcoran et al., 2005; Bertsch et al., 2020), which could be an explanation of our results, as well as longer fermentation process due to availability of sugars for energy balance and multiplication.

Conclusions

In this study, single strain cultures were used to evaluate the buckwheat base fermentation ability with *L. plantarum*, *L. paracasei*, *L. acidophilus* and *L. rhamnosus* strains.

1. The study found that fermentation causes significant changes in pH and LAB growth during fermentation. *L. plantarum* and *L. paracasei* showed the highest potential, indicating their potential for the development of buckwheat beverages.
2. The rapid decrease in pH indicates a fastened fermentation, which influenced also the dose of added starter.
3. The changes of sucrose and raffinose were analyzed during buckwheat substrate fermentation. A reduction of sucrose from 27 to 57% was established by *L. plantarum, L. acidophilus, L. paracasei and L. rhamnosus.*

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