



Biomodification of a plant base from cereal flour to produce an alternative fermented drink

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ABSTRACT

Background: Cereals are the most promising raw material for producing functional fermented drinks. Buckwheat possesses a unique chemical composition, high nutritional value, and significant physiological activity. However, cereals lack easily digestible sugars and nitrogen compounds for lactic acid bacteria.

Objective: The study focused on determining whether enzymatic hydrolysis of buckwheat flour can provide a cereal base with soluble sugars and free amino nitrogen to support the rapid growth of lactic acid bacteria and improve the sensory characteristics of fermented products.

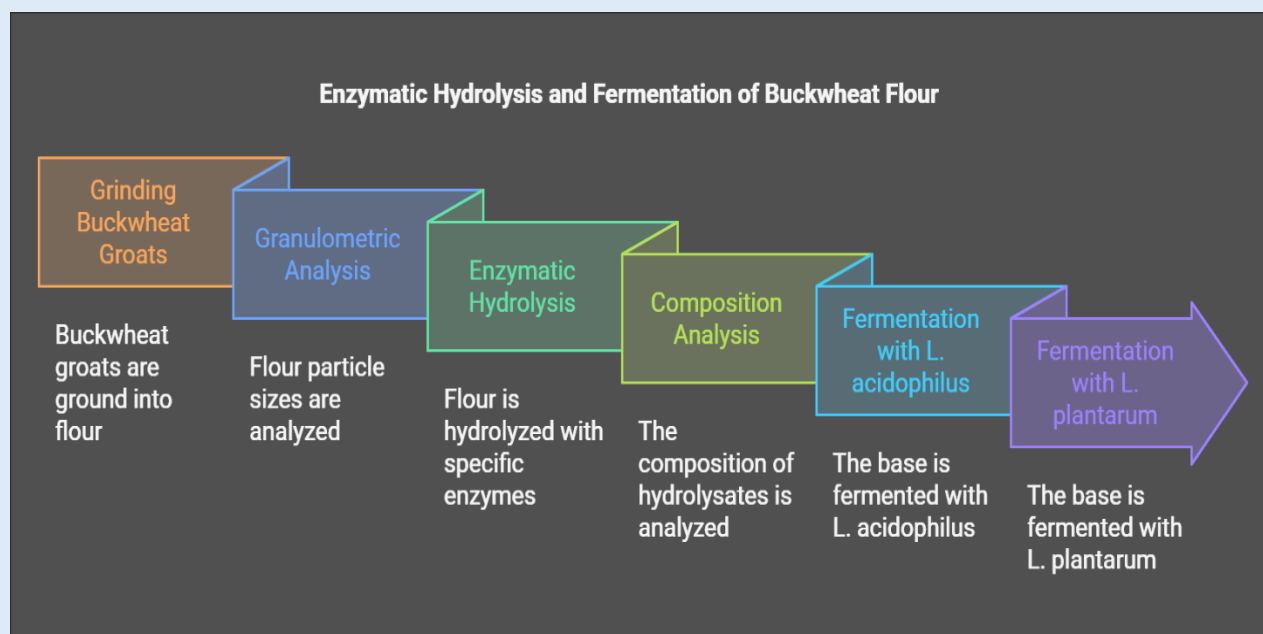
Methods: The grain base was obtained from buckwheat groats. The groats were ground into flour. The granulometric analysis of the flour was conducted using a laser analyzer. Flour samples with different particle sizes were hydrolyzed with α -amylase, protease, and glucoamylase. The hydrolysis temperature was selected based on the temperature at

which the viscosity of the flour-water mixture reached its maximum. The hydrolysates' solids, sugars, and free amino nitrogen were analyzed using refractometry, HPLC, and spectrophotometry. The buckwheat bases were fermented with *L. acidophilus* and *L. plantarum*. The number of lactic acid microorganisms in the fermented bases was determined by the plate count method on MRS agar. Sensory properties (taste, aroma, and texture) were evaluated using a developed 5-point scale.

Results: The study found that hydrolysis of buckwheat flour using α -amylase, protease, and glucoamylase produced a cereal base with 19.15 ± 0.05 g/L glucose, 29.54 ± 0.06 g/L maltose, and 104.72 ± 0.15 mg/100 g of free amino nitrogen. The biomodification resulted in changes to the cereal base composition, favorable for developing lactic acid bacteria cultures. During fermentation of the biomodified base with *L. acidophilus*, a pH level of 4.5 ± 0.1 , a cell count of 10^8 CFU/g, and high taste and aroma scores were achieved within 5 hours. For *L. plantarum*, the changes ensured a pH of 4.5 ± 0.1 and a cell count of 10^7 CFU/g after 7 hours, but lactic fermentation's characteristic taste and aroma were not pronounced.

Conclusion: The buckwheat flour was processed by grinding groats and using enzymatic hydrolysis with α -amylase, protease, and glucoamylase, resulting in a cereal base with a promising composition for the following fermentation. Additionally, the approach offers a model for processing other cereals, contributing to developing alternative plant-based fermented beverages with enhanced functional and sensory properties.

Keywords: buckwheat flour, cereal-based fermented beverage, dairy alternatives, enzymatic hydrolysis, lactic acid bacteria, fermentation.



Graphical Abstract: Biomodification of a plant base from cereal flour to produce an alternative fermented drink

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INTRODUCTION

Alternative fermented plant-based products are attracting increasing attention worldwide [1-3]. Dairy product alternatives have already been developed and are widely available. They are derived from various plant-based raw materials, such as soy, rice, almonds, coconut, and oats [3]. Functional products derived from fermented cereals can improve gut health and boost immunity [4-8]. New sources of cereals are being searched for to expand the functional properties and taste of alternative products. Buckwheat has a unique chemical composition, high nutritional value, and physiological activity [9-12].

Lactic acid bacteria (including probiotics) are incredibly demanding in the presence of easily digestible sugars and nitrogen compounds in the environment [13-14]. The plant base of a fermented alternative drink should have functional properties and be a favorable environment for starter cultures. Glucose or maltose in the substrate is essential for the development of lactic acid bacteria during fermented cereal product production [15-19]. Sufficiently digestible nutrients in the cereal and buckwheat bases minimize fermentation duration and enhance bacterial metabolic activity [19]. Therefore, to obtain a fermented alternative drink from buckwheat flour with good sensory properties, it is necessary to preliminarily modify the plant base so that its chemical composition provides activity and a high growth rate of starter cultures. The required composition of the buckwheat base can be achieved by starch and protein hydrolysis of flour [20]. Research data on the development of lactic acid bacteria in a plant base from buckwheat flour is not enough [21-22].

The purpose of this study was to determine the main parameters of buckwheat flour hydrolysis (the degree of destruction of flour particles, the type and dose of enzymes, the temperature, and duration of the process) to obtain a base of an alternative fermented drink with soluble sugars and free amino nitrogen, which

ensured rapid growth of lactic acid bacteria and favorable sensory properties of the product.

MATERIAL AND METHODS

Parboiled buckwheat groats were obtained from buckwheat species *Fagopyrum esculentum* Moench. The total protein content of buckwheat groats was 20.7 ± 0.6 %, total fat content 6.1 ± 0.3 %, moisture content 11.6 ± 0.2 %, ash 3.6 ± 0.1 %, and total carbohydrates 57.8 ± 0.5 %, including 42.4 ± 1.0 % of starch.

The total protein content in groats was determined by the Kjeldahl method. The fat content was determined by the Soxhlet method. The ash content was determined by incinerating the samples. The moisture content was measured by drying at 130 °C.

Probiotic cultures, *Lactobacillus acidophilus* BZ-AV and *Lactiplantibacillus plantarum* 207-8, from ITMO University's microbial culture collection, were used to ferment the buckwheat bases.

The following enzymes were used for flour hydrolysis: α -amylase Amilolux-ATS (Sibbiopharm Ltd, Berdsk, Russian Federation) with an activity of 2000 U/cm³, protease Distizym Protacid (Erbslöh, Geisenheim, Germany) with an activity of 350 U/cm³ and glucoamylase Distizym AG-alpha (Erbslöh, Geisenheim, Germany) with an activity of 6500 U/cm³.

Buckwheat flour preparation: To obtain buckwheat flour, buckwheat groats were ground on a rotary vortex mill of fine grinding PBM 600C (Novosfera Ltd, Novocherkassk, Russian Federation) with a capacity of 40 – 400 kg/h and 22.7 kW power. The mill made it possible to produce flour with different particle sizes to study the effect of the degree of particle destruction on the rate of soluble solids accumulation in the hydrolysate.

The particle size distribution of buckwheat flour was analyzed using the Microsizer 201C (VA Install Ltd, Saint Petersburg, Russian Federation), which measures

particles ranging from 0.2–600 μm . The particle size at 10 % (Dv10), 50 % (Dv50), and 90 % (Dv90) of the volume distribution were calculated using the Microsizer 201C software.

Hydrolysis temperature of starch in buckwheat flour: A mixture of buckwheat flour and water in a ratio of 1:3 at 30 °C was prepared in a 1.5 L beaker and covered with a lid with a built-in stirrer. Then, α -amylase was added to the mixture at 0.5 U/g of starch. The obtained mixture was placed in a LOIP LB-212 water bath (LOIP Ltd, St. Petersburg, Russian Federation) at 30 °C, and the viscosity of the mixture was measured on a Fungilab S.A. Visco Basic Plus viscometer (Fungilab SA, Barcelona, Spain) with an R3 spindle at 20 rpm. The mixture was heated to 90 °C at a 1 °C/min heating rate. The viscosity was measured at 40, 50, 60, 70, 80 and 90 °C temperatures. The temperature at which the mixture's viscosity reached its maximum corresponded to the hydrolysis temperature of buckwheat flour.

Hydrolysis of buckwheat flour: The enzymes were introduced as follows: α -amylase in doses of 0.25, 0.4, and 0.6 U/g of starch, protease in the dose of 0.2 U/g of flour, and glucoamylase in doses of 2, 4, and 6 U/g starch. These doses were selected based on the manufacturer's recommendations. The α -amylase without or with the protease was added at the beginning of the hydrolysis at the starch gelatinization temperature equal to 40 °C. Glucoamylase was added after the primary hydrolysis at 60 °C and kept for 30 min.

Total soluble solids (TSS): Total soluble solids (in °Bx) during hydrolysis were determined with the fully automatic desktop refractometer Index Instruments PTR 46 (Index Instruments Ltd., Huntingdon, England) at 20 °C. The procedure was repeated until the TSS value (in °Bx) reached a plateau.

The content of sugars in the hydrolysates: The determination of glucose and maltose content was based on the AOAC 977.20 method [23]. High-Performance Liquid Chromatography (HPLC) on a Shimadzu Prominence LC-20 chromatograph (Shimadzu Europa GmbH, Duisburg, Germany) with a RID-20A refractive index detector was carried out. Chromatography was performed on the Rezex RPM Monosaccharide Pb²⁺ column at 85°C. The mobile phase was deionized water at a 600 $\mu\text{l}/\text{min}$ flow rate.

Free amino nitrogen (FAN): Free amino nitrogen in the obtained hydrolysates was determined by colorimetric method with ninhydrin on a KFK-3-01 spectrophotometer (JSC ZOMZ Ltd, Sergiev Posad, Russian Federation) at a wavelength of 570 nm.

Acid production rate of starter cultures: Active cultures of *L. acidophilus* BZ-AV and *L. plantarum* 207-8 in 5 % were added to the sterilized buckwheat bases. The mixtures were incubated at 37 \pm 2 °C with *L. acidophilus* BZ-AV and at 30 \pm 2 °C for *L. plantarum* 207-8. Incubation temperatures were chosen according to the culture's specifications. pH was measured on an Expert-001 pH meter (Ekoniks-Expert Ltd, Moscow, Russian Federation) every nine hours.

The specific growth rate of starter culture: The formula calculated the specific growth rate of the bacterial cultures in the buckwheat bases:

$$\mu = \frac{\ln X - \ln X_0}{\tau - \tau_0},$$

where X_0 was the number of CFU/ml at τ_0 ; X - the number of CFU/ml at τ , τ_0 was 3 hours after the cultures were added to the sterile cereal base. τ corresponded to when the pH reached 4.5 \pm 0.1 or the lowest possible value. The beginning of the log phase of culture growth was the initial time. The number of lactic acid

microorganisms (in CFU/ml) was determined using the pour plate technique on MRS agar medium (NPC Biocompass LLC, Uglich, Russia). The plates were incubated anaerobically in an aerostat (model AE-01, NIKI MLT LLC, St. Petersburg, Russia) at 37 ± 2 °C for *L. acidophilus* BZ-AV and at 30 ± 2 °C for *L. plantarum* 207-8 for 72 ± 3 hours.

Sensory evaluation of the fermented bases: The sensory properties of the fermented buckwheat bases were determined according to the developed 5-point hedonic scale (1 = very poor, unacceptable, 3 = satisfactory, 5 = excellent). The evaluation was carried out simultaneously by 15 panelists. 30 ml of each sample of fermented buckwheat base at 4 ± 2 °C was presented to each panelist

in numbered cups. The following organoleptic properties were determined: taste, aroma, and texture [24-25].

Statistical analysis: The Bonferroni (Dunn) t-test was used to determine significant differences at $P < 0.05$. The experiments were conducted with three repetitions ($n = 3$). Statistical analysis was performed using GraphPad Prism 9 software (GraphPad Software, San Diego, USA).

RESULT AND DISCUSSION

Particle size distribution of buckwheat flour: The size of flour particles significantly impacted the availability of starch for enzymes and affected the rate of hydrolysis [26-28]. The particle size distribution in buckwheat flour A and B samples is shown in Fig. 1.

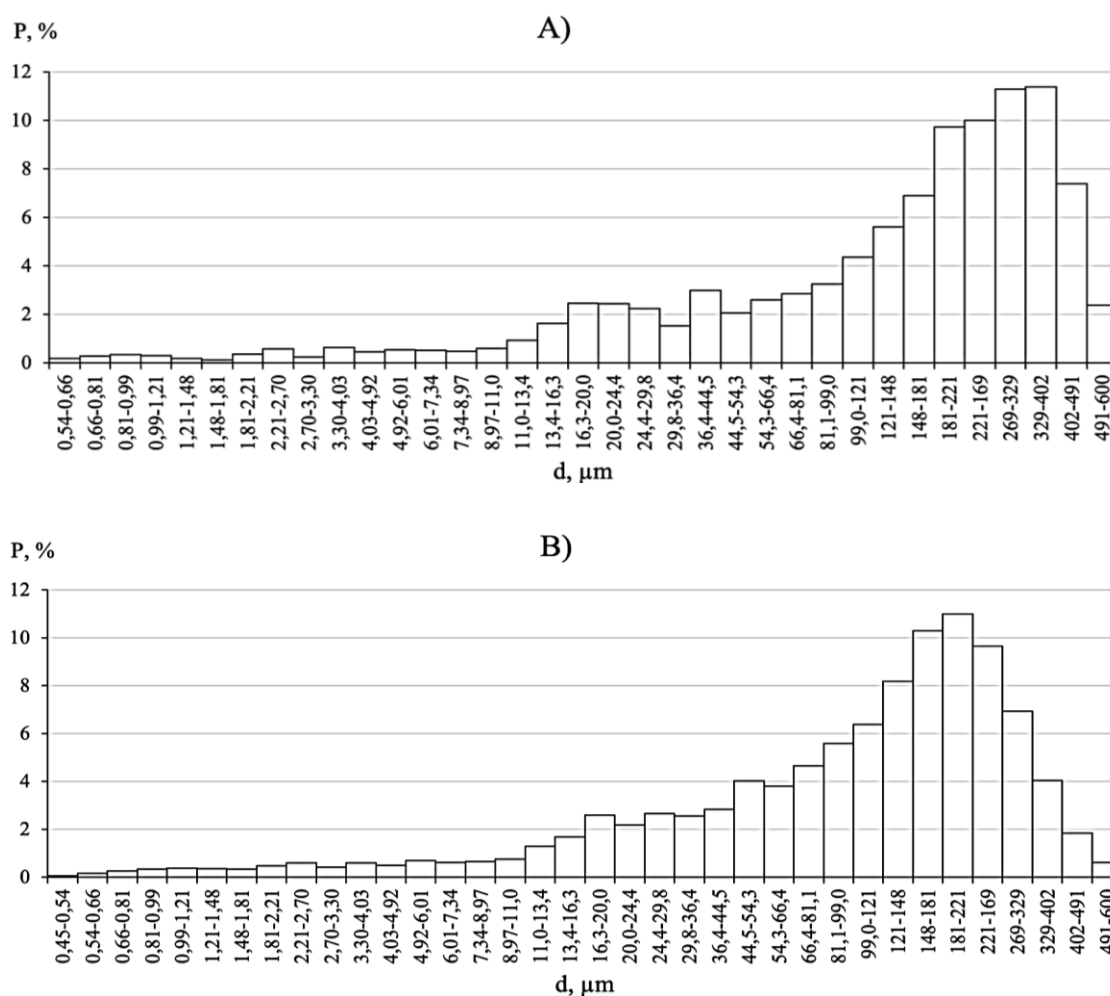


Figure 1. The particle size distribution in the flour samples

A) – sample A; B) – sample B

In sample A, 10 % of the particles were less than 18.6 μm , 50 % were less than 190 μm , and 90 % were less than 401 μm . In sample B, particle sizes at 10 %, 50 %, and 90 % were 16.1 μm , 129 μm , and 299 μm , respectively.

Hydrolysis temperature: The hydrolysis temperature of the flour corresponded to the temperature at which the mixture's viscosity reached its maximum and began to decrease. Starch dissolution occurred within the flour at this temperature, making it more available to amylolytic enzymes [29-33]. The results obtained are shown in Fig. 2.

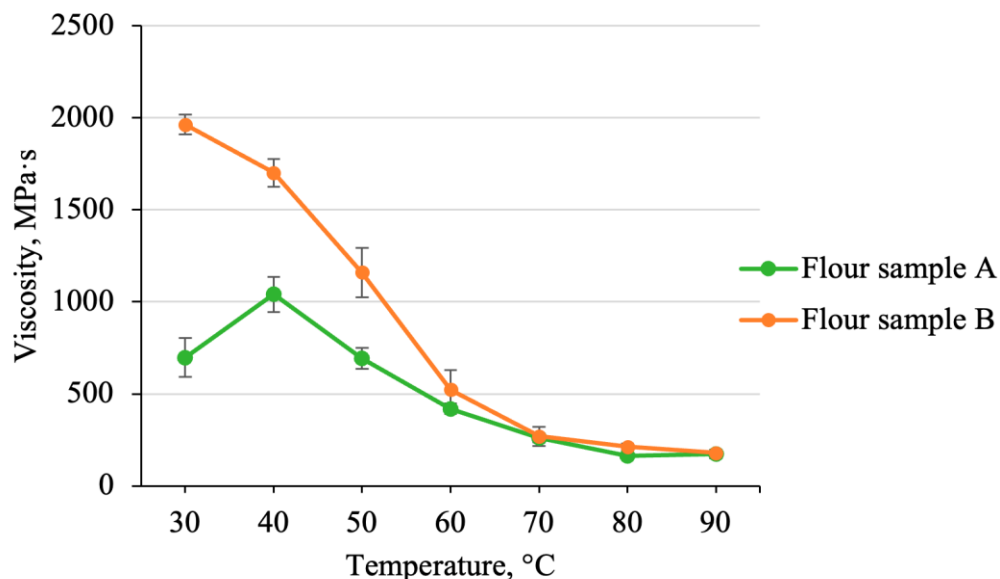


Figure 2. Change in the viscosity of a mixture of buckwheat flour and water at different temperatures.

The mixture with flour sample A reached its maximum viscosity at 40 °C. The mixture's viscosity with flour sample B gradually decreased and did not reach an intermediate maximum while the temperature increased. The absence of a viscosity peak in flour sample B can be explained by structural changes in starch granules caused by the grinding process. During fine grinding, starch granules were disrupted and separated from surrounding molecules, and their crystalline structure was also altered. Additionally, the increased surface area due to a more significant number of smaller particles enhanced water absorption, leading to a smooth decrease in viscosity without a peak [26, 34-35].

Studies have shown that the temperature of starch gelatinization in flour composition, and hence the temperature of buckwheat flour hydrolysis, depends on

the degree of particle destruction. For flour sample A, the gelatinization temperature was 40 °C. For sample B, any hydrolysis temperature could be chosen from 30 to 90 °C. The hydrolysis temperature of 40 °C was optimal for flour sample B since the mixture viscosity allowed its agitation at this temperature, and the impact on flour components and energy costs were minimal compared to the higher temperatures.

Hydrolysis of buckwheat flour with α -amylase: To achieve a high content of soluble sugars in the buckwheat bases with the shortest flour hydrolysis time, it was necessary to find the optimal dose of the α -amylase. The best dose of α -amylase was determined by the rate of increase in the content of TSS in the mixture. The obtained results are presented in Table 1.

Table 1. Change of total soluble solids in the samples of buckwheat flour during hydrolysis with α -amylase

Enzyme doze, U/g starch	TSS, °Bx										
	Time, h										
	0	30	60	90	120	150	180	210	240	270	300
Flour sample A											
0.25	12.3± 0.15 ^a	14.0± 0.12 ^a	14.8± 0.06 ^a	15.5± 0.15 ^a	16.0± 0.15 ^a	16.4± 0.06 ^a	16.7± 0.12 ^a	17.1± 0.10 ^a	17.5± 0.06 ^a	17.7± 0.10 ^a	18.0± 0.06 ^a
0.4	12.5± 0.10 ^a	14.3± 0.17 ^{ab}	15.2± 0.31 ^{ab}	16.4± 0.15 ^b	17.2± 0.10 ^b	17.7± 0.31 ^b	18± 0.26 ^b	18.2± 0.21 ^b	18.5± 0.12 ^b	18.8± 0.10 ^b	18.8± 0.06 ^b
0.6	12.5± 0.25 ^a	14.6± 0.10 ^b	15.5± 0.12 ^b	16.6± 0.06 ^b	17.4± 0.10 ^b	17.8± 0.06 ^b	18.1± 0.15 ^b	18.5± 0.12 ^b	18.6± 0.12 ^b	18.8± 0.06 ^b	18.8± 0.06 ^b
Flour sample B											
0.25	12.6± 0.15 ^a	13.3± 0.21 ^a	14.5± 0.15 ^a	15.4± 0.21 ^a	16.4± 0.15 ^a	17.3± 0.10 ^a	17.9± 0.10 ^a	18.0± 0.10 ^a	18.0± 0.06 ^a	18.1± 0.06 ^a	18.1± 0.06 ^a
0.4	12.8± 0.15 ^a	14.9± 0.10 ^b	16.2± 0.25 ^b	17.1± 0.25 ^b	17.7± 0.32 ^b	18.1± 0.12 ^b	18.4± 0.10 ^b	18.6± 0.06 ^b	18.8± 0.10 ^b	18.9± 0.10 ^b	19.0± 0.06 ^b
0.6	12.8± 0.06 ^a	15.1± 0.42 ^c	16.5± 0.35 ^b	17.3± 0.15 ^b	17.8± 0.21 ^b	18.2± 0.15 ^b	18.6± 0.12 ^b	18.7± 0.10 ^b	18.8± 0.12 ^b	18.9± 0.06 ^b	19.0± 0.06 ^b

^{a-c}The results are presented as mean values \pm SD (n=3). Identical letters in the column indicate no significant differences between the tested samples at $p < 0.05$.

Table 1 shows that with both flour samples and all doses of α -amylase, the prominent TSS content increase is observed after 270 min. The fastest TSS content growth, about 18 °Bx in all flour samples, was observed with the doses of α -amylase 0.4 and 0.6 U/g starch. The hydrolysis time to 18 °Bx TSS was 30 minutes shorter with flour B.

Thus, the optimal dose of the α -amylase for the hydrolysis of both buckwheat flour samples was 0.4 U/g starch. The hydrolysis time decreased with an increase in

the degree of flour destruction, which was 180 min for flour A and 150 min for flour B.

Hydrolysis of buckwheat flour with α -amylase and protease: The use of proteases in the hydrolysis of flour can provide enough available nitrogenous compounds in the cereal base [36-37]. The hydrolysis with protease was carried out under the same conditions described for the α -amylase. The change in FAN measured the effectiveness of the protease. The data is presented in Table 2.

Table 2. Change in free amino nitrogen content in buckwheat flour samples

Enzyme		FAN (mg/100g)
α -amylase (U/g starch)	protease (U/g flour)	
Flour sample A		
0.4	-	66.64±0.19 ^a
0.4	0.2	92.18±0.14 ^b
Flour sample B		
0.4	-	73.44±0.23 ^a
0.4	0.2	102.31±0.25 ^b

^{a-c} The results are presented as mean values \pm SD (n=3). Identical letters in the column indicate no significant differences between the tested samples at $p < 0.05$.

The introduction of the protease led to an increase in FAN content in both samples by about 1.5 times. The content of FAN in flour B was 11 % higher. Thus, adding

the protease in the dose of 0.2 U/g flour was shown to be expedient in providing nitrogen sources to the lactic acid bacteria.

Influence of glucoamylase on the chemical composition of buckwheat bases: For the breakdown of maltose to glucose, it was necessary to introduce a glucoamylase

during flour hydrolysis. The study results on the effect of the glucoamylase dose on maltose and glucose content in the buckwheat base are presented in Table 3.

Table 3. The content of maltose and glucose in buckwheat bases

Enzyme		Content	
α -amylase (U/g starch)	glucoamylase (U/g starch)	maltose, g/L	glucose, g/L
Flour sample A			
0.4	-	13.47±0.04 ^a	1.66±0.04 ^a
0.4	2	18.25±0.06 ^b	10.85±0.04 ^b
0.4	4	20.28±0.03 ^c	15.24±0.06 ^c
0.4	6	20.34±0.07 ^c	15.39±0.04 ^c
Flour sample B			
0.4	-	21.16±0.04 ^a	2.46±0.05 ^a
0.4	2	28.84±0.06 ^b	17.65±0.09 ^b
0.4	4	28.89±0.08 ^b	17.75±0.05 ^b
0.4	6	28.87±0.08 ^b	17.89±0.05 ^b

^{a-c} The results are presented as mean values ± SD (n=3). Identical letters in the column indicate no significant differences between the tested samples at $p < 0.05$.

During the hydrolysis of flour A, the most significant increase in glucose content was observed with the dose of 4 U/g starch of glucoamylase. The increase in the dose of glucoamylase to 6 U/g starch increased glucose content by less than 1 %. During the hydrolysis of flour B, the most significant increase ($P < 0.05$) in glucose content was observed with 2 U/g starch of glucoamylase. An

insignificant ($P > 0.05$) increase in the content of maltose in the bases with glucoamylase could probably be explained by the hydrolysis of the residual starch by α -amylase. Thus, for the hydrolysis of flour A, the best dose of glucoamylase was 4 U/g starch and 2 U/g starch for flour B. The summarized data of the soluble solids in the bases are presented in Table 4.

Table 4. Content of glucose, maltose, and FAN in buckwheat bases.

Enzyme		Content		
glucoamylase (U/g starch)	protease (U/g flour)	FAN (mg/100g)	glucose (g/L)	maltose (g/L)
Flour sample A				
-	-	66.64±0.19 ^a	1.66±0.04 ^a	13.47±0.04 ^a
4	-	68.17±0.24 ^a	15.24±0.06 ^b	20.28±0.03 ^b
-	0.2	92.18±0.14 ^b	1.79±0.05 ^a	14.04±0.04 ^a
4	0.2	95.21±0.20 ^c	17.74±0.07 ^c	21.37±0.06 ^b
Flour sample B				
-	-	73.44±0.23 ^a	2.46±0.05 ^a	21.16±0.04 ^a
2	-	74.51±0.23 ^a	17.65±0.09 ^b	28.84±0.06 ^b
-	0.2	102.31±0.25 ^b	2.70±0.05 ^a	21.33±0.05 ^a
2	0.2	104.72±0.15 ^c	19.15±0.05 ^c	29.54±0.06 ^b

Note: all samples are obtained with 0.4 U/g starch of α -amylase

^{a-c} The results are presented as mean values ± SD (n=3). Identical letters in the column indicate no significant differences between the tested samples at $p < 0.05$.

After hydrolysis using identical enzyme combinations, the glucose, maltose, and FAN content was higher in the base made from flour B, which had greater particle destruction. Additionally, the hydrolysis time for flour B was 30 minutes shorter (see Table 1). Thus, flour with a finer grind is preferable for preparing the cereal base of a fermented drink. Subsequent experiments were conducted exclusively with hydrolysates obtained from flour B samples. Samples were fermented with the following combinations and doses of enzymes:

- sample 1 – α -amylase (0.4 U/g starch),

- sample 2 – α -amylase (0.4 U/g starch) and glucoamylase (2 U/g starch),
- sample 3 – α -amylase (0.4 U/g starch) and protease (0.2 U/g flour),
- sample 4 – α -amylase (0.4 U/g starch), protease (0.2 U/g flour), and glucoamylase (2 U/g starch).

Acid production rate of probiotic starter cultures: The high acid production activity of these bacteria shortened fermentation time and reduced the risk of microbial contamination [38-43]. The pH change during the fermentation of buckweat bases with cultures *L. acidophilus* BZ-AV and *L. plantarum* 207-8 is shown in Fig. 3.

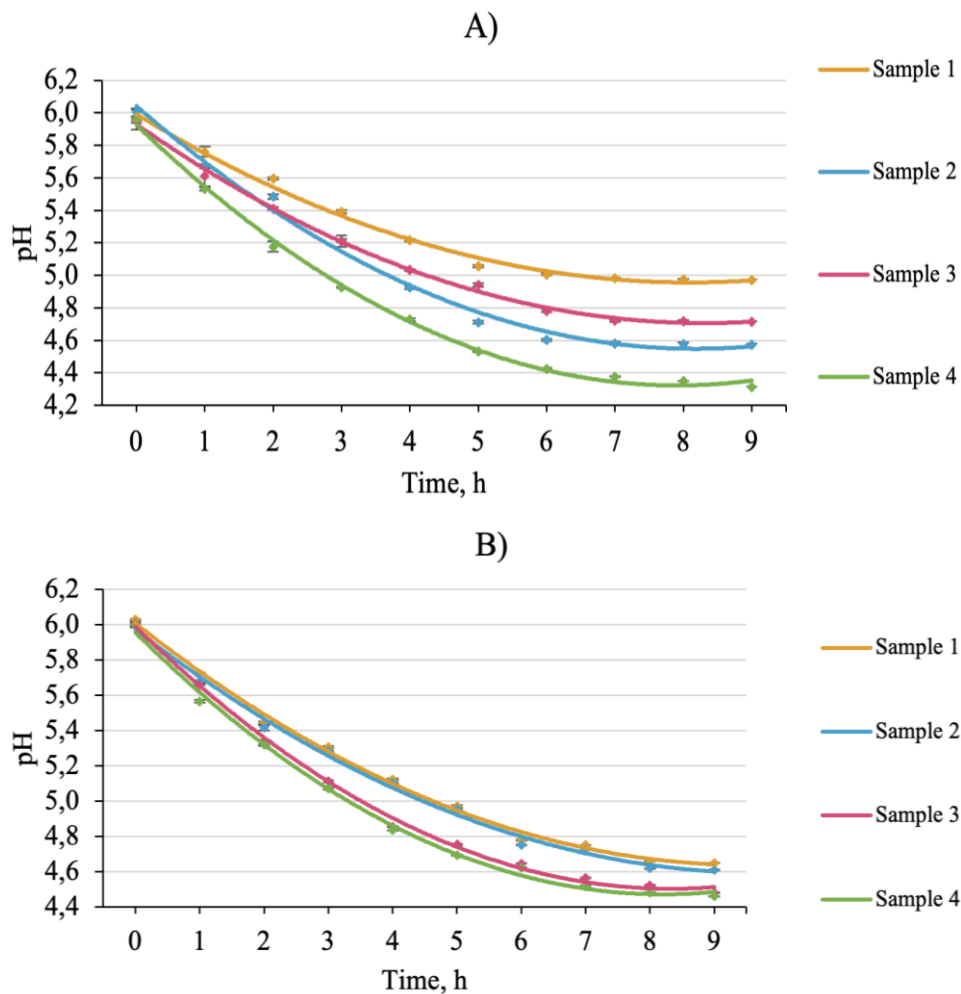


Figure 3. pH changes of buckweat bases during the fermentation with A) – *L. acidophilus* BZ-AV; B) – *L. plantarum* 207-8

The highest acid production rate of *L. plantarum* was observed during the fermentation of samples 4 and 3. The pH value in samples 3 and 4 reached 4.5 ± 0.1 after 7 hours. The pH in sample 2 decreased to 4.6 after 9 hours of fermentation and only 4.7 in sample 1. Both cultures exhibited the shortest time reaching the pH level of 4.5 ± 0.1 when developed in sample 4. *L. plantarum* 207-8 also showed a high acid production rate in sample 3, probably due to the utilization of both glucose and

maltose. Adding three enzymes during the treatment of the buckwheat base made it possible to reduce the fermentation time of the buckwheat base by *L. acidophilus* BZ-AV to five hours.

The growth rate of starter cultures: The specific growth rate of *L. acidophilus* BZ-AV and *L. plantarum* 207-8 during their growth in the buckwheat bases is presented in Table 5.

Table 5. Specific growth rate of probiotic cultures in buckwheat bases

Buckwheat base	Specific growth rate, μ , h^{-1}	
	<i>L. acidophilus</i> BZ-AV	<i>L. plantarum</i> 207-8
Sample 1	0.60 ± 0.02^a	0.59 ± 0.02^a
Sample 2	0.71 ± 0.03^c	0.62 ± 0.03^a
Sample 3	0.67 ± 0.03^b	0.75 ± 0.04^b
Sample 4	0.91 ± 0.04^d	0.78 ± 0.04^b

^{a-d} The results are presented as mean values \pm SD (n=3). Identical letters in the column indicate no significant differences between the tested samples at $p < 0.05$.

The specific growth rate of *L. acidophilus* BZ-AV in sample 4 was significantly higher than in the other samples. The specific growth rate of *L. plantarum* 207-8 was almost the same in samples 4 and 3 and samples 1 and 2. This culture showed the highest growth rate in samples 4 and 3, which indicated the ability of *L. plantarum* to utilize maltose.

Thus, *L. acidophilus* BZ-AV, compared to *L. plantarum* 207-8, showed the highest specific growth rate in all samples of buckwheat bases. Sample 4 provided the highest growth rate for both cultures. The

addition of three enzymes during the hydrolysis allowed the preparation of a composition of the cereal base that was favorable for the growth of probiotic cultures *L. acidophilus* BZ-AV and *L. plantarum* 207-8. It provided the minimum time to reach the pH of 4.5 ± 0.1 and a high microbial count in the fermented buckwheat bases.

Sensory properties of fermented buckwheat bases: The sensory evaluation results in the samples fermented with *L. acidophilus* BZ-AB and *L. plantarum* 207-8 are presented in Fig. 4.

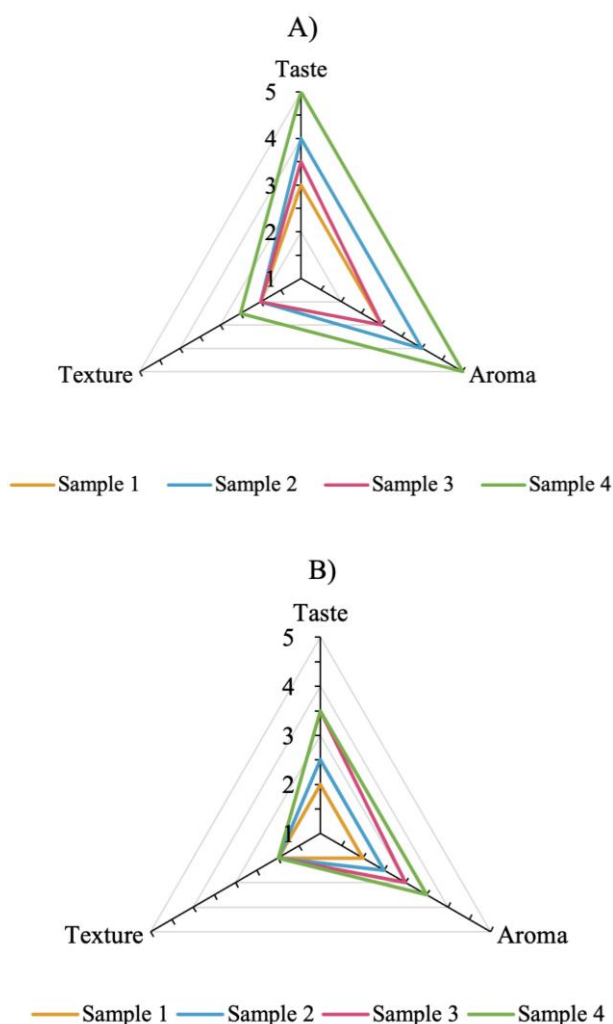


Figure 4. Sensory properties of buckwheat bases fermented with A) – *L. acidophilus* BZ-AV; B) – *L. plantarum* 207-8

All samples of buckwheat bases fermented with *L. acidophilus* BZ-AV had significantly higher taste and aroma scores than those fermented with *L. plantarum* 207-8. Regardless of the culture, fermented sample 4 received the highest scores for taste and aroma, and fermented sample 1 received the lowest scores. All samples had heterogeneous layered textures with flakes and sediment and received unsatisfactory scores of this characteristic.

Samples fermented with *L. acidophilus* BZ-AV had the following sensory properties. Sample 4 had an intense taste and aroma of lactic acid fermentation with a noticeable flavor of buckwheat groats. Sample 2 had a mild taste and aroma of lactic acid fermentation. Samples

1 and 3 received the lowest scores because of an unexpressed taste and aroma of lactic acid fermentation and the presence of off flavors. Some authors suggested a change in the metabolism of *L. acidophilus* in a substrate with a high content of glucose and low molecular weight nitrogenous substances. This content could shift the metabolism towards forming volatile and fatty acids, alcohols, aromatic compounds, and lactic acid [44-45].

The taste and aroma of all samples of buckwheat bases fermented with *L. plantarum* 207-8 had low scores. The scores were lowered for samples 3 and 4 because of a mild taste and aroma of lactic acid fermentation and a predominance of buckwheat taste and smell. Samples 1

and 2 had an unexpressed aroma of lactic acid fermentation, a predominance of buckwheat taste, and aroma and off-flavors. The results could probably be explained by the possible formation of some flavoring compounds of *L. plantarum* in substrates containing hexoses [45-48].

Thus, the biomodification of the plant base from buckwheat groats by flour hydrolysis at a ratio of flour to water of 1:3 with α -amylase, glucoamylase, and protease made it possible to obtain the best taste and aroma of an alternative fermented drink. The homogeneous and firm texture of the drink could be achieved by buckwheat base homogenization. It was likely that *L. plantarum* should be combined with other microorganisms to produce a fermented alternative drink from buckwheat flour.

CONCLUSION

The obtained results showed that the fine grinding of parboiled buckwheat groats and the subsequent hydrolysis with α -amylase, protease, and glucoamylase were favorable during the processing of a cereal base for a fermented drink from buckwheat flour—fine grinding of buckwheat groats allowed to reduce the temperature and time parameters of flour hydrolysis. Managing the dose of α -amylase, protease, and glucoamylase allowed us to obtain the carbohydrate and nitrogen composition of the grain base, which was required to stimulate acid production activity and increase the growth rate of lactic acid bacteria. In addition, these dosages shifted bacterial metabolism towards forming compounds that improved the taste and aroma of alternative fermented drinks and provided high microbial counts in the product. The findings on processing buckwheat bases for fermented alternative drinks could inform studies on other cereal raw materials. In the future, further research was planned on the homogenization of flour bases to ensure the homogeneous viscous texture of the fermented alternative drink and to compose a microbial co-culture to provide high functional properties.

Abbreviations: FAN: free amino nitrogen, TSS: total soluble solids.

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REFERENCES

1. McClements DJ, Newman E, McClements IF: Plant-based milks: a review of the science underpinning their design, fabrication, and performance. *Comprehensive Reviews in Food Science and Food Safety* 2019, 18(6):2047–2067. DOI: <https://doi.org/10.1111/1541-4337.12505>.
2. Silva ARA, Silva MMN, Ribeiro BD: Health issues and technological aspects of plant-based alternative milk. *Food Research International* 2020, 131:108972. DOI: <https://doi.org/10.1016/j.foodres.2019.108972>.
3. Sridhar K, Bouhallab S, Croguennec T, Renard D, Lechevalier V: Recent trends in the design of healthier plant-based alternatives: the nutritional profile, gastrointestinal digestion, and consumer perception. *Critical Reviews in Food Science and Nutrition* 2022, 1–16. DOI: <https://doi.org/10.1080/10408398.2022.2081666>.
4. Tachie CYE, Onuh JO, Aryee ANA: Nutritional and potential health benefits of fermented food proteins. *Journal of the Science of Food and Agriculture* 2024, 104:1223–1233. DOI: <https://doi.org/10.1002/jsfa.13001>.
5. Mukherjee A, Breselge S, Dimidi E, Marco ML, Cotter PD: Fermented foods and gastrointestinal health: underlying mechanisms. *Nature Reviews Gastroenterology & Hepatology*, 21(4), 248-266. DOI: <https://doi.org/10.1038/s41575-023-00869-x>.
6. Martirosyan D.M., Ekblad M. Functional Foods Classification System: Exemplifying through Analysis of Bioactive Compounds. *Functional Food Science* 2022; 2(4): 94-123. DOI: <https://www.doi.org/ffs.v2i4.919>
7. Martirosyan D.M., Lampert T., Lee M. A comprehensive review on the role of food bioactive compounds in functional food science. *Functional Food Science* 2022; 3(2): 64-79. DOI: <https://www.doi.org/10.31989/ffs.v2i3.906>

8. Zhou J. V., Martirosyan D. Functional Foods for Cholesterol Management: A Comparison Between the United States and Japan. *Functional Food Science* 2024; 4(6): 228-250. DOI: <https://www.doi.org/10.31989/ffs.v4i6.13>
9. Jin J, Ohanenye IC, Udenigwe CC: Buckwheat proteins: functionality, safety, bioactivity, and prospects as alternative plant-based proteins in the food industry. *Critical Reviews in Food Science and Nutrition* 2022, 62(7):1752–1764. DOI: <https://doi.org/10.1080/10408398.2020.1847027>.
10. Mir NA, Riar CS, Singh S: Nutritional constituents of pseudo cereals and their potential use in food systems: a review. *Trends in Food Science & Technology* 2018, 75:170–180. DOI: <https://doi.org/10.1016/j.tifs.2018.03.016>.
11. Škrabanja V, Kreft I: Nutritional value of buckwheat proteins and starch. In: *Molecular Breeding and Nutritional Aspects of Buckwheat*. Cambridge: Academic Press; 2016:169–176. DOI: <https://doi.org/10.1016/B978-0-12-803692-1.00013-4>.
12. Sofi SA, Ahmed N, Farooq A, Rafiq S, Zargar SM, Kamran F, Dar TA, Mir SA, Dar BN, Mousavi Khaneghah A: Nutritional and bioactive characteristics of buckwheat, and its potential for developing gluten-free products: an updated overview. *Food Science & Nutrition* 2023, 11(5):2256–2276. DOI: <https://doi.org/10.1002/fsn3.3166>.
13. Abdul-Abbas SJ, Al Alnabi DIB, Al-Hatim RR, Al-Younis ZK, Al-Shawi SG, Bokov DO, Abdelbasset WK: Effects of mixed strains of *Rhizopus oryzae* and *Lactobacillus* on corn meal fermentation. *Food Science and Technology* 2022, 42: e73621. DOI: <https://doi.org/10.1590/fst.73621>.
14. Ganguly S, Sabikhi L, Singh AK: Effect of probiotic fermentation on physico-chemical and nutritional parameters of milk-cereal based composite substrate. *Journal of Food Science and Technology* 2022, 59(8):3073–3085. DOI: <https://doi.org/10.1007/s13197-021-05350-8>.
15. Yang X, Hong J, Wang L, Cai C, Mo H, Wang J, Fang X, Liao Z: Effect of lactic acid bacteria fermentation on plant-based products. *Fermentation* 2024, 10:48. DOI: <https://doi.org/10.3390/fermentation10010048>.
16. Horlacher N, Oey I, Agyei D: Learning from Tradition: Health-Promoting Potential of Traditional Lactic Acid Fermentation to Drive Innovation in Fermented Plant-Based Dairy Alternatives. *Fermentation* 2023, 9:452. DOI: <https://doi.org/10.3390/fermentation9050452>
17. Gunkova PI, Buchilina AS, Maksimiuk NN, Bazarnova YG, Girel KS: Carbohydrate Fermentation Test of Lactic Acid Starter Cultures. *IOP Conference Series: Earth and Environmental Science* 2021, 852:012035. DOI: <https://doi.org/10.1088/1755-1315/852/1/012035>
18. Khushboo, Karnwal A, Malik T: Characterization and selection of probiotic lactic acid bacteria from different dietary sources for the development of functional foods. *Frontiers in Microbiology* 2023, 14. DOI: <https://doi.org/10.3389/fmicb.2023.1170725>
19. Molina GES, Shetty R, Xiao H, Wätjen AP, Tovar M, Bang-Berthelsen CH: Development of a novel lactic acid bacteria starter culture approach: From insect microbiome to plant-based fermentations. *LWT* 2022, 167:113797. DOI: <https://doi.org/10.1016/j.lwt.2022.113797>
20. Wang P, Ma T: Production of Bioactive Peptides from Tartary Buckwheat by Solid-State Fermentation with *Lactiplantibacillus plantarum* ATCC 14917. *Foods* 2024, 13:3204. DOI: <https://doi.org/10.3390/foods13193204>
21. Yang X, Hong J, Wang L, Cai C, Mo H, Wang J, Fang X, Liao Z: Effect of Lactic Acid Bacteria Fermentation on Plant-Based Products. *Fermentation* 2024, 10:48. DOI: <https://doi.org/10.3390/fermentation10010048>
22. Chen M, Xia H, Zuo X, Tang D, Zhou H, Huang Z, Guo A, Lv J: Screening and characterization of lactic acid bacteria and fermentation of gamma-aminobutyric acid-enriched bamboo shoots. *Frontiers in Microbiology* 2024, 15:1333538. DOI: <https://doi.org/10.3389/fmicb.2024.1333538>
23. Cunnif P (Ed.): *Official Methods of Analysis of AOAC International*. 16th ed. AOAC Method 977.20, vol. II, 1995, p. 25 (Chapter 44).
24. Anjushree M., Satish A., Sunil L., Shivakumara CS. Fortified noni (*Morinda citrifolia* L.) cookies: formulation, properties, antioxidant activity, sensory traits. *Bioactive Compounds in Health and Disease* 2023; 6(7): 145-154. DOI: <https://www.doi.org/10.31989/bchd.v6i7.1150>
25. Thatsanasuwan N., Duangjai A., Suttirak P., Phanthurat N. Proximate composition and sensory attributes of gluten-free pasta made from jackfruit seeds. *Functional Foods in Health and Disease* 2023; 13(1):11-21. DOI: <https://www.doi.org/10.31989/ffhd.v13i1.1039>
26. Angelidis G, Protonotariou S, Mandala I, Rosell CM: Jet milling effect on wheat flour characteristics and starch hydrolysis. *Journal of Food Science and Technology* 2016, 53:784–791. DOI: <https://doi.org/10.1007/s13197-015-1990-1>
27. de la Hera E, Gomez M, Rosell CM: Particle size distribution of rice flour affecting the starch enzymatic hydrolysis and hydration properties. *Carbohydrate Polymers* 2013, 98:421–427. DOI: <https://doi.org/10.1016/j.carbpol.2013.06.002>

28. Baskovtceva A., Barakova N., Samodelkin E., Kiprushkina E., Alkhateeb R., Tochilnikov G. Unlocking the Potential of Carrot Pomace: Enzymatic and Impact-Disintegrator-Activator Processing for Elevated Beta-Carotene Concentration in Carrot Powder. *Functional Foods in Health and Disease* 2023; 13(10):505-519.
DOI: <https://www.doi.org/10.31989/ffhd.v13i10.1184>
29. Kim A-N, Rahman MS, Lee K-Y, Choi S-G: Superheated steam pretreatment of rice flours: Gelatinization behavior and functional properties during thermal treatment. *Food Bioscience* 2021, 41:101013.
DOI: <https://doi.org/10.1016/j.fbio.2021.101013>
30. Roy M, Dutta H, Jaganmohan R, Choudhury M, Kumar N, Kumar A: Effect of steam parboiling and hot soaking treatments on milling yield, physical, physicochemical, bioactive, and digestibility properties of buckwheat (*Fagopyrum esculentum* L.). *Journal of Food Science and Technology* 2019, 56:3524–3533.
DOI: <https://doi.org/10.1007/s13197-019-03849-9>
31. Sindhu R, Devi A, Khatkar BS: Physicochemical, thermal and structural properties of heat moisture treated common buckwheat starches. *Journal of Food Science and Technology* 2019, 56:2480–2489.
DOI: <https://doi.org/10.1007/s13197-019-03725-6>
32. Tester RF, Qi X, Karkalas J: Hydrolysis of native starches with amylases. *Animal Feed Science and Technology* 2006, 130:39–54.
DOI: <https://doi.org/10.1016/j.anifeeds.2006.01.016>
33. Wani AA, Singh P, Shah MA, Schweiggert-Weisz U, Gul K, Wani IA: Rice starch diversity: Effects on structural, morphological, thermal, and physicochemical properties—A review. *Comprehensive Reviews in Food Science and Food Safety* 2012, 11:417–436.
DOI: <https://doi.org/10.1111/j.1541-4337.2012.00193.x>
34. Chen Z, Huang Q, Xia Q, Zha B, Sun J, Xu B, Shi YC: Intact endosperm cells in buckwheat flour limit starch gelatinization and digestibility in vitro. *Food Chemistry* 2020, 330:127318.
DOI: <https://doi.org/10.1016/j.foodchem.2020.127318>
35. Lu M, Yang W, Zhang H, Yu Y, Chen F, Hao Y: Study on the Characteristics of Fine Rice Flour by Micro-Crushing and Its Effects on the Quality Improvement of Rice Cakes. *Foods* 2024, 13:3565.
DOI: <https://doi.org/10.3390/foods13223565>
36. Bergsveinson J, Kajala I, Ziola B: Next-generation sequencing approaches for improvement of lactic acid bacteria-fermented plant-based beverages. *AIMS Microbiology* 2017, 3:8–24. DOI: <https://doi.org/10.3934/microbiol.2017.1.8>
37. de Giori GS, Hébert EM: Methods to Determine Proteolytic Activity of Lactic Acid Bacteria. In: *Food Microbiology Protocols*, Humana Press 2001, pp. 197–202.
DOI: <https://doi.org/10.1385/1-59259-029-2:197>
38. Fung WY, Woo YP, Liang MT: Optimization of Growth of *Lactobacillus acidophilus* FTCC 0291 and Evaluation of Growth Characteristics in Soy Whey Medium: A Response Surface Methodology Approach. *Journal of Agricultural and Food Chemistry* 2008, 56:7910–7918.
DOI: <https://doi.org/10.1021/jf801567j>
39. Matejčeková Z, Liptáková D, Valík L: Functional probiotic products based on fermented buckwheat with *Lactobacillus rhamnosus*. *LWT-Food Science and Technology* 2017, 81:35–41. DOI: <https://doi.org/10.1016/j.lwt.2017.03.018>
40. Uğural A, Akyol A: Can pseudocereals modulate microbiota by functioning as probiotics or prebiotics? *Critical Reviews in Food Science and Nutrition* 2020, 12:1–15.
DOI: <https://doi.org/10.1080/10408398.2020.1846493>
41. Huang F, Zhao Y, Hou Y, Yang Y, Yue B, Zhang X: Unraveling the antimicrobial potential of *Lactiplantibacillus plantarum* strains TE0907 and TE1809 sourced from *Bufo gargarizans*: advancing the frontier of probiotic-based therapeutics. *Frontiers in Microbiology* 2024, 15:1347830.
DOI: <https://doi.org/10.3389/fmicb.2024.1347830>
42. Liu Y, Nawazish H, Farid MS, Abdul Qadoos K, Habiba UE, Muzamil M, Tanveer M, Sienkiewicz M, Lichota A, Łopusiewicz Ł: Health-Promoting Effects of *Lactobacillus acidophilus* and Its Technological Applications in Fermented Food Products and Beverages. *Fermentation* 2024, 10:380.
DOI: <https://doi.org/10.3390/fermentation10080380>
43. Grinvald S. A., Barakova N. V., Kiprushkina E. I., Jamaldinova B. A., Ushaeva I. U., Tochilnikov G. V., Sadovoy V. V., Gunkova P. I. The effect of phenolic compounds contained in flour from green buckwheat, flaxseed, grape and dogwood Seeds on the fermentation activity of yeast *S. cerevisiae*. *Functional Foods in Health and Disease* 2024; 14(3): 207-2. DOI: <https://doi.org/10.31989/ffhd.v14i3.1191>
44. Lee PR, Boo CX, Liu SQ: Fermentation of coconut water by probiotic strains *Lactobacillus acidophilus* L10 and *Lactobacillus casei* L26. *Annals of Microbiology* 2013, 63:1441–1450.
DOI: <https://doi.org/10.1007/s13213-013-0607-z>
45. Saha S., Putatunda S., Majumder R., Das A. Changes of volatile flavored compounds during lactic acid fermentation in cereal-based products. *Functional Foods in Health and Disease* 2024; 14(11): 871-882.
DOI: <https://doi.org/10.31989/ffhd.v14i11.1503>

46. Salmerón I, Thomas K, Pandiella SS: Effect of substrate composition and inoculum on the fermentation kinetics and flavor compound profiles of potentially non-dairy probiotic formulations. *LWT - Food Science and Technology* 2014, 55:240–247.
DOI: <https://doi.org/10.1016/j.lwt.2013.07.008>
47. Yeganyan G, Avakyan M, Trachtenberg T, Zhou V, Yeganyan E: Fermented milk product “Narineh” with *Lactobacillus acidophilus* in sequential and classical therapy for *Helicobacter pylori*-associated peptic ulcers. *Bioactive Compounds in Health and Disease* 2024, 7(2):65–78.
DOI: <https://doi.org/10.31989/bchd.v7i2.1314>.
48. Hovhannisyan HG, Danielyan LV, Chichoyan NB, Pashayan MM, Baghdasaryan LG, Melkumyan IE, Barseghyan A: Improvement of functional and sensory properties of fermented dairy drink *Narine* using raw apricot gum. *Functional Foods in Health and Disease* 2024, 14(8):600–614. DOI: <https://doi.org/10.31989/ffhd.v14i8.1398>.