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RHEOLOGICAL, TEXTURAL AND PHYSICOCHEMICAL PROPERTIES OF BUCKWHEAT SOURDOUGH BREAD PREPARED WITH DIFFERENT LACTIC ACID BACTERIA STRAINS

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ABSTRACT

The aim of this study is to make buckwheat sourdough with three different lactic acid bacteria (LAB) strains and to determine its quality on wheat bread. After 24 hours fermentation, microbiological count, pH and TTA values and viscoelastic properties of buckwheat sourdoughs were examined. While the *Limosilactobacillus fermentum* 29GT-19 strain providing the highest value (10^9 CFU / g) in microbiological count, it was not observed in the chemically acidified control buckwheat dough. pH values changed from 3.8 to 4.6. The elastic modulus (*G´*) of all sourdoughs was found to be higher than the viscous modulus (*G´´*) and a positive correlation was found between rheology graph with increase acidity. The prepared buckwheat doughs were then added to wheat flour as 10% and 20% (w / w). At the same time, commercial yeast bread was made as control bread. In the chemical composition of the breads, *Levilactobacillus brevis* KCO-48 (dough with 20% buckwheat) sourdough bread had the highest ash content (1.26%) and moisture content. But mostly, the ash content of the breads was low, and the amount of moisture was higher than commercial yeast bread. The breads were generally found to have high crumb hardness (from 11.48 N to 31.34 N). As the concentration of buckwheat sourdough increased, the hardness of the bread increased. The lowest hardness (5.21 N) was determined in commercial yeast wheat bread. The results of this study have had a positive effect on the quality of wheat bread of buckwheat sourdough.

Keywords: LAB, buckwheat flour, sourdough, sourdough bread, rheology

INTRODUCTION

In the past decade, the demand for gluten-free products such as bread has increased **(Gallagher *et. al*., 2004)**. Particularly, buckwheat's both adaptation to negative environments and high basic nutrient content are considered as alternative products for the production of functional foods **(Moroni *et. al*., 2012)**. Thereby, in various parts of the world to make various food products are used common buckwheat (*Fagopyrum esculentum Moench*) and Tartar buckwheat (*Fagopyrum tataricum (L.) Gaertn*.) **(Bonafaccia *et. al*., 2003)**. Cereals such as buckwheat (*Fagopyrum esculentum* Moench), amaranth and quinoa are included in the pseudocereal. Buckwheat is preferred due to its nutritional properties. Because it increases the processing and marketing opportunities of the food industry **(Rayas-Duarte *et. al*., 1998; Qian and Kuhn, 1999)** and constitutes an important part of the antioxidant activity source in functional foods **(Holasova *et. al*., 2002)**. Due to its antioxidant and anti-inflammatory properties, it appears with the presence of routine and quercetin flavonoids in buckwheat grain and its products **(Lukšič *et. al*., 2016)**. Flavonoids and / or polyphenols are found in significant amounts in the hull of buckwheat **(Watanabe *et. al*., 1997)**. Since carbohydrates contained in buckwheat are digested more slowly than other carbohydrates, it has also been associated with diabetes prevention **(Mariotti *et. al*., 2008)**, cholesterol-lowering and prebiotic activities **(Alvarez-Jubete *et. al*., 2009; Li *et. al*., 2010; Préstamo *et. al*., 2003)**. In addition to buckwheat and sourdough bread is known to have positive effects on health **(Różyło *et. al*., 2015)**. The application of sourdough technology to improve the quality of gluten-free bread has been of interest for the past few years **(Moroni *et. al*., 2009).** Sourdough is a mixture formed by mixing flour and water and fermented with yeast and lactic acid bacteria. Often it is responsible for the acidification of dough, the synthesis of aroma components, exopolysaccharide (EPS), enzymes and antifungal components **(Hammes *et. al*., 2005; Poutanen *et. al*., 2009; Moroni *et. al*., 2011).** In recent studies, it has been said that fermentation of gluten-free substances, gluten-free dough and bread can be used for nutritional, rheological, textural and shelf-life properties **(Moroni *et. al*., 2011)**. Bread prepared from buckwheat has been found to have higher oleic acid, magnesium and dietary components compared to wheat bread (**Alvarez-Jubete *et. al*., 2010; Moroni *et. al*., 2012**). However, the use of buckwheat in wheat bread reduces the digestibility of wheat and white tannins. Thereby, some technological problems has occurred. It is also difficult due to the content of coffee that gives bitter taste to buckwheat products **(Li and Zhang, 2001)**. For this reason, the way to increase the flavor, digestibility and baking performance of buckwheat flour is based on the production of buckwheat sourdough **(Hammes *et. al*., 2005)**. However, until now, studies with buckwheat sourdough have been limited.
The purpose of this study, it is the determination of the quality of the bread by making the buckwheat sourdough with the *Levilactobacillus brevis* KCO-48, *Limosilactobacillus. fermentum* 29GT-19, *Companilactobacillus paralimentarius* 06B-2 strains isolated from sourdough, determining the rheological properties of these doughs and then adding them to wheat bread in 10% and 20%.

MATERIAL AND METHODS

Material

Buckwheat flour was provided by Prof. Dr. Kağan Kökten (Bingol University Agricultural Faculty Field Crops Department). Buckwheat was developed in Turkey/Bingol conditions.

Method

Providing bacterial strains and environmental conditions

A 3 different lactic acid bacteria strains were used for the preparation of buckwheat sourdough. These strains are *Liml. fermentum* 29GT-19, *C. paralimentarius* 06B-2, *Levl. brevis* KCO-48. Bacterial strains were taken from 20% glycerol at -80 °C and left for 24 h incubation at 37 °C in 20 mL De Man, Rogosa and Sharpe (MRS) broth (Merck, Germany).

Preparation of buckwheat sourdough

Bacterial strains developing in MRS broth were centrifuged at 5000 x rpm for 10 minutes and the supernatant was removed. The remaining pellet was washed with sterile water and then re-suspended in sterile water. These suspensions were inoculated to dough (10^7 CFU / g dough) prepared from buckwheat flour. For the dough, 50 g of buckwheat flour and 87.5 g of sterile water were used. After 12 h at 35 °C, it was regenerated for back-slopping (10% w / w) and left fermentation at 35 °C for 24 h. Acetic acid was used for control buckwheat sourdough.

Determination of microbiological, pH and titratability acidity (TTA) of buckwheat sourdough

After 24 h fermentation, 10 g of buckwheat sourdough was taken for microbiological analysis of the dough and it was homogenized in the stomacher in 90 mL serum saline (0.85 % FTS). Serial dilutions were then prepared and selected dilutions were sown on the MRS agar (Merck, Germany) by spot method (20 µL each spot) for microbiological counting. It was then incubated at 37 °C in aerobic conditions. On the other hand, for pH and TTA, 5 g buckwheat sourdough were taken and dissolved in 45 mL sterile water. Following, pH values were determined by pH meter and titrated to pH 8.5 with 0.1 N NaOH. The amount of NaOH spent was given in % lactic acid.

Determination of Rheological Properties of Buckwheat sourdoughs

An aircontrolled rheometer (Anton Paar, MCR-302) with Rheoplus module was used for rheological analysis of buckwheat sourdough. Parallel plate configuration (diameter 25 mm, 2 mm gap) was adjusted. In order to determine the viscoelastic properties of sour doughs, Frequency scanning tests were prepared at room temperature (21-23 °C) with controlled strain (0.05%) in the range of 1–10 Hz and was calculated as G' elastic or storage module as G'' viscose or loss modulus **(İspirli *et. al.*, 2020)**.

Preparation of buckwheat sourdough breads

Control bread was made from wheat flour. For this, 100 g of wheat flour, 62.4 g of water, 2 g of salt and 2 g of commercial yeast were used. For buckwheat sourdough breads; 2 different doughs (10% and 20% buckwheat sourdough) and 2 different breads were prepared. Buckwheat sourdough and acidified control dough were the same as the amount of flour and water in bread prepared with control wheat flour. The dough fermentation for bread making was performed according to the method determined by **Alkay *et al.*, (2020)**.

Determination of textural properties of breads

In order to determine the textural properties of the breads, the breads were left to cool after cooking. Texture Analyzer TA Plus device was used for measurements. The cooled breads were cut into slices to be 2.5 cm. The analysis was carried out with a 35 mm cylinder probe, an approach rate of 55 mm / min, a compression ratio of 25 % and a maximum load of 50 N. The results were recorded in parallel **(Alkay *et al.*, 2020; Alkay *et. al.*, 2022)**.

Chemical analysis of breads

Chemical composition of buckwheat sourdough breads and control breads were examined according to standard methods. The standard methods used are AACC 44-15A for moisture content, AACC 0801 for ash determination and proteins AACC 46-12 (multiplying the nitrogen content by factor 5.7) **(AOAC, 2002).**

Statistical Analysis

One-way analysis of variance (ANOVA) was used to determine the difference between strains depending on the measured characteristics. Minitab version 17.3.1 (Minitab, Inc., State College PA, USA) and JMP version 9 was used for this purpose.

RESULTS

Microbiological, pH and TTA results of buckwheat sourdough

In our study, buckwheat sourdough prepared by using 3 different lactic acid bacteria was left for fermentation for 24 hours and microbiological count, pH and TTA value were measured and summarized in **Table 1**. The LAB count ranged from 10^7 to 10^9 CFU / g and buckwheat sourdough containing *Liml. fermentum* strain reached the highest ($p<0.05$) LAB count. In addition, the LAB count was not determined on chemically acidified buckwheat dough. The pH values were close to each other and the lowest ($p<0.05$) pH value was observed in *Levl. brevis* KCO-48 buckwheat sourdough. TTA values were calculated as % lactic acid.
As the fermentation developed, the pH change occurred and thus positively correlated with the increase in titration acidity. TTA also provided information on the metabolic activity and growth of lactobacilli during fermentation **(Bender *et. al.*, 2018).**

Table 1 Microbiological counts, pH and TTA values of buckwheat sourdough at the end of 24 hours.

Buckwheat sourdoughs	Microbiological cultivation (log CFU /g)	pH	TTA (% lactic acid)
***Liml. fermentum* 29GT-19 buckwheat sourdough**	9.30±0.14 ^{A}	4.65±0.03 ^{A}	1.08±0.07 ^{D}
***C. paralimentarius* 06B-2 buckwheat sourdough**	7.64±0.22 ^{B}	4.41±0.03 ^{B}	1.12±0.21 ^{C}
***Levl. brevis* KCO-48 buckwheat sourdough**	8.84±0.20 ^{A}	4.09±0.01 ^{C}	1.74±0.07 ^{B}
Chemically acidified control buckwheat sourdough	-	3.80±0.01 ^{D}	4.76±0.14 ^{A}

Within the column, different superscript uppercase letters show differences between the strains.

Viscoelastic properties of buckwheat sourdough

Viscoelastic properties of our prepared buckwheat sourdoughs were examined at the end of 24 hours. It is shown in **Figure 1** that the elastic modulus (G') of all buckwheat sourdough is higher than its viscous module (G'') (data not provided).

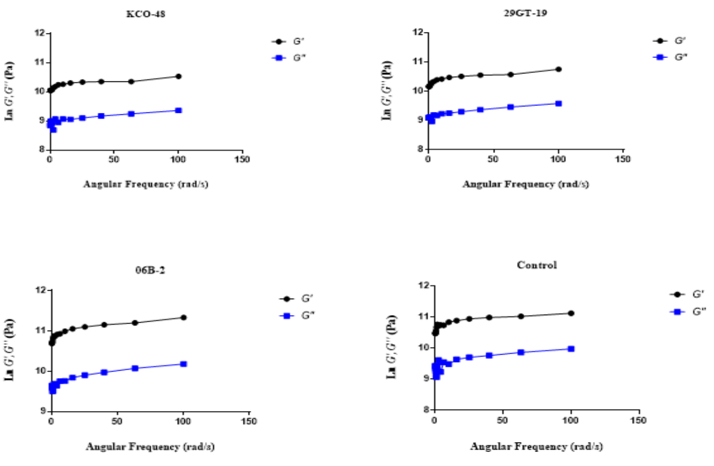

Figure 1 Viscoelastic behavior of buckwheat sourdough

All sourdoughs were observed to exhibit a solid, elastic-like behavior. While the highest ($p<0.05$) value of the elastic module is observed *C. paralimentarius* 06B-2 in buckwheat sourdough, the lowest ($p<0.05$) value *Liml. fermentum* 29GT-19 buckwheat sourdough reached.

Chemical properties of buckwheat sourdough breads

Ash, moisture and protein values were evaluated to determine the chemical properties of buckwheat sourdough breads. As shown in Table 2, 10% and 20% use of buckwheat sour dough in breads did not make any difference in values, and mostly values were found to be close to each other. Bread made from obligatory hetero-fermentative *Levl. brevis* KCO-48 strain had the highest ash content (1.26). It was determined that the ash content of the bread varied between 0.62% and 1.26%. The moisture content of all breads was found to be higher than commercial yeast control bread and their moisture composition ranged from 41.0% to 50.2%.

Table 2 Ash, moisture and protein values of buckwheat sourdough breads

Buckwheat sourdough bread	Ash (%)	Moisture (%)	Protein (%)
Liml. fermentum 29GT-19 buckwheat sourdough bread (10% buckwheat sourdough)	0.80 ^{E}	50.2^{A}	8.7^{CD}
Liml. fermentum 29GT-19 buckwheat sourdough bread (20% buckwheat sourdough	0.82 ^{E}	48.1^{AB}	9.3^{BC}

C. paralimentarius 06B-2 buckwheat sourdough bread (10% buckwheat sourdough)	0.62 F	46AB	8.2DE
C. paralimentarius 06B-2 buckwheat sourdough bread (20% buckwheat sourdough)	0.67 F	43.3AB	8.5D
Levl. brevis KCO-48 buckwheat sourdough bread (10% buckwheat sourdough)	0.92 C	46.9AB	9.5B
Levl. brevis KCO-48 buckwheat sourdough bread (20% buckwheat sourdough)	1.26 A	45.2AB	10.3A
Chemically acidified control bread (10% buckwheat dough)	0.85 DE	42.04AB	8.6D
Chemically acidified control bread (20% buckwheat sourdough)	0.90 CD	41.21AB	8.7CD
Commercial yeast bread	1.08 B	39.5B	7.7E

Within the column, different superscript uppercase letters show differences between the strains

Table 3 Textural properties of buckwheat sourdough

Strain Code	Hardness(N)	Springiness(mm)	Cohesiveness	Gumminess	Chewiness (N x mm)	Resilience
***Liml. fermentum* 29GT-19 buckwheat sourdough bread (10% buckwheat at sourdough)**	11.48±1.46 DE	0.94±0.01 A	0.90±0.01 A	1.40±0.37 B	16.09±2.00 A	0.60±0.03 A
***Liml. fermentum* 29GT-19 buckwheat sourdough bread (20% buckwheat at sourdough**	19.34±0.86 BCD	0.89±0.03 A	0.89±0.01 A	1.39±0.63 B	9.67±1.18 AB	0.63±0.03 A
***C. paralimentarius* 06B-2 buckwheat sourdough bread (10% buckwheat sourdough)**	21.57±1.82 BC	0.92±0.03 A	0.87±0.01 A	1.93±0.16 AB	19.68±0.19 A	0.60±0.01 A
***C. paralimentarius* 06B-2 buckwheat sourdough bread (20% buckwheat sourdough)**	24.12±0.62 BC	0.91±0.01 A	0.87±0.01 A	1.94±1.23 AB	17.84±1.99 A	0.60±0.01 A
***Levl. brevis* KCO-48 buckwheat sourdough bread (10% buckwheat sourdough)**	17.63±2.32 CD	0.94±0.03 A	0.88±0.01 A	1.96±0.09 AB	18.18±0.17 A	0.62±0.01 A
***Levl. brevis* KCO-48 buckwheat sourdough bread (20% buckwheat sourdough)**	21.72±1.32 BC	0.92±0.03 A	0.87±0.01 A	1.57±0.23 AB	14.86±2.72 AB	0.60±0.02 A
Chemically acidi fied control bread (10% buckwheat sourdough)	27.41±4.07 B	0.93±0.01 A	0.89±0.01 A	3.39±0.29 AB	2.93±0.66 C	0.57±0.03 A
Chemically acidi fied control bread (20% buckwheat sourdough)	37.34±3.22A	0.88±0.02 A	0.88±0.01 A	2.45±0.30 AB	2.41±0.65 C	0.63±0.03 A
Commercial yeast bread	5.27±0.75 E	1.18±0.31 A	0.87±0.01 A	6.86±3.71 A	2.41±0.66 C	0.63±0.03 A

Within the column, different superscript uppercase letters show differences between the strains

It is seen that the hardness of the starter buckwheat sourdough bread varies between 11.48 ± 1.46 and 24.12 ± 0.62. Accordingly, it can be said that cohesiveness is similar in all examples, but chewiness is difficult compared to commercial yeast bread. Some images of sourdough bread are given in **Figure 2.**

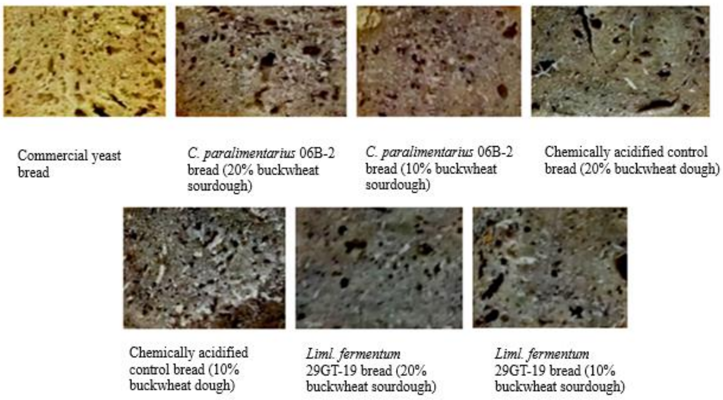

Figure 2 General view of breads made with the addition of buckwheat sourdough

DISCUSSION

In this study, 3 different obligatory hetero-fermentative LAB strains which were previously isolated from wheat sourdough were used in making buckwheat sourdoughs, and sourdoughs were added to wheat bread in certain proportions (10% and 20%) and its effect on functional bread was examined. It is known that pH affects the structure and stability of the dough. Therefore, the pH of sourdough was checked after 24 hours of fermentation. It was observed that there was no significant difference between pH and TTA values and it showed a positive correlation and the values match each other. **Bender *et al.*, (2018)** made both buckwheat sourdough and millet sourdough with 7 different LAB strains and looked at pH values. pH values changed from 3.76 to 4.89. *Lb. fermentum* LMG 6902 and *Lb. paralimentarius* LMG 19152 strains had a pH of 4.20 and 3.92, respectively. Another study was to look at the properties of buckwheat sourdough crackers by two different LAB strains by **Selimović *et al.*, (2014)**. *Lactobacillus plantarum* (Lyoflora B2) and *Lactobacillus brevis* (Lyoflora B4) starters were used. The pH of the sourdough after 24 h fermentation of *Lb. brevis* strain was 3.9. There was an increase in total acidity due to the accumulation of organic acids (lactic, acetic acid) as products of bacterial metabolism during lactic acid fermentation. **Zhou *et al.*, (2022)** reported in their study that the pH value of Tartary buckwheat sourdoughs was 4.51 after 24 h of fermentation. Compared with study results, *Liml. fermentum* 29GT-19 and *Levl. brevis* KCO-48 strains had approximately the same pH. However, *C. paralimentarius* 06B-2 strain exhibited different pH. Generally, it is known that the change in pH and TTA is based on differences in metabolism and acidification rates of LAB strains, the type of acids produced and the buffer capacity of flours. The acidic conditions of the dough have the ability to affect the main constituent components, such as starch and arabinoxylan, which change the quality of gluten-free breads **(Bender *et al.*, 2018)**. Hydrolysis of buckwheat storage proteins is affected by the release of smaller polypeptides during fermentation **(Radović *et al.*, 1996)**. It is said that this may be related to activation of endogenous proteases or proteolytic activity by fermenting LABs **(Gänzle *et al.*, 2007; Moroni *et. al.*, 2012)**. It has been suggested that the hydrolysis of wheat gluten and gliadins during the addition of buckwheat sourdough is caused by the activation of endogenous proteases at acidic pH (**Moroni *et. al.*, 2012)**. Another feature investigated after 24-hour fermentation was the determination of the rheological viscoelastic properties of buckwheat sourdough (data not shown). While the modulus of storage (G') shows the elastic property of a material, the loss module (G'') represents its viscous property (**Olojede *et. al.*, 2020)**. Fermentation significantly affects the rheological properties of the dough. Rheological properties also depend on the type of microorganism, metabolic activity and pH value **(Wehrle and Arendt, 1998).**

Textural properties of buckwheat sourdough breads

Buckwheat sourdough was added to wheat flour at 10% and 20%, and sourdough bread was made and compared with two control breads. One of the control breads was chemically acidified buckwheat bread and the other was made with commercial yeast and the textural properties of the breads were determined. In **Table 3**, it is determined that the hardness of acidified control bread (having 20% buckwheat dough) is highest, while the least hardness is commercially yeast bread (2%).

Buckwheat sourdough fermented by 2 LAB (*C. paralimentarius* 06B-2 and *Levl. brevis* KCO-48) starter was found to have a higher elastic modulus (Table 2). It is said that buckwheat proteins are not completely dissolved at pH 4 in gluten-free dough. It has been suggested that this is due to complex carbohydrates. In addition, it was emphasized that the hardness is caused by the pentosan in buckwheat (**Moore *et. al*., 2008)**. In addition, the presence of organic acids in the dough has been shown in studies that result in increased resistance to viscosity and as a result cause hardness and elasticity **(Moroni *et. al*., 2012)**.

Another feature for gluten-free sourdough bread is the texture of the breads. There are differences between gluten-free bread acidified by LAB and chemically acidified bread. These differences are based on the amount of acid produced and the amount of bacteria used as a starter. Due to the high crumb hardness of buckwheat sourdough breads, it is considered that their availability should be lower for gluten-free bread. For this, buckwheat flour might be used at lower concentrations. The *Liml. fermentum* 29GT-19 strain we used in the study was found to have less crumb hardness compared to the other 2 LAB strains and the bread hardness increased as the buckwheat sourdough concentration increased. It is thought that sourdough should be made after blending with wheat flour due to its poor cooking properties. Because mixing with wheat flour will have a positive effect on bread quality. It can be said that the change in texture parameters results from the chemical composition of buckwheat flour **(Dvořáková *et. al*., 2012)**. **Corsetti *et al*., (1998)** emphasized that a number of physicochemical changes were affected during the storage of bread. They reported that these changes occurred by acidification of sourdough LABs, microbial hydrolysis of starch and proteolysis. Gluten-free breads often contain large amounts of starch. Substances such as dextrin, polypeptides and fatty acids that interfere with starch retrogradation are reduced during sourdough fermentation. This allows fermentation to take place in a shorter time. However, it has been reported that these substances are less degraded in chemically acidified dough and thus make a difference (**Moore *et. al*., 2008)**.

Considering the chemical and microbiological results of sourdough, *Liml. fermentum* 29GT-19 strain reached the most microbiological count. Microbial stability and carbohydrate metabolism can be thought to be effective in the acidification rate of buckwheat sourdough **(Bender *et al*., 2018)**. LABs perform special metabolic activities by synthesizing lactic acid fermentation, proteolysis, exopolysaccharides and compounds with antimicrobial activity. This realizes the texture, flavor, taste, volume and nutritional quality of gluten-free bread properties **(Moroni *et al*., 2009)**. Several studies show that different gluten-free flours such as amaranth, buckwheat, teff or quinoa are used in making sourdough **(Sterr *et al*., 2009; RŘhmkorf *et al*., 2012; Wolter *et al*., 2014; Rizzello *et al*., 2016)**. **Moroni *et al*., (2011)** made spontaneous sourdough using buckwheat and teff flour. One of the analyzes was the microbiological count. In their results, the LAB count was more than 10^9 CFU/ g and was similar to our results. These flours provide nutrients that help the growth of microorganisms.

In addition, it has been stated that the addition of sourdough and gluten-free flour has an effect on the bread quality **(Campo *et al*., 2016; Rinaldi *et al*., 2017)**. Moisture content of foods is generally an indicator of food quality. The amount of moisture affects the sensory, physical and microbial properties of bread. Gluten-free breads are generally considered to have high moisture content. **Diowksz and Sadowka (2021)** used 15% 30% 50% of buckwheat sourdough, guar gum and transglutaminase enzyme in their study. It was stated that the moisture content of the sourdough breads in their study was higher than the control bread (commercial yeast). This value was approximately 46%. Another study was done by **Yeşil and Levent (2022).** In their study, they used fermented buckwheat, quinoa and amaranth flours on bread quality. In the results, it was stated that the moisture content of the breads made from these fermented flours was higher than the control. **Temnikova *et al*., (2021)** reported that the moisture content in sourdough breads containing 10% of buckwheat flour was 45.5 %. These results are similar to the moisture values in our study. In addition, it was observed that the ash content of buckwheat sourdough breads made with the starter cultures selected in this study was low compared to the control bread (commercial yeast).

CONCLUSION

In summary, the effect of different amounts of buckwheat flour on sour dough and bread was evaluated. As a result, it was observed that different LAB types and different amounts of buckwheat flour caused textural differences on sourdough bread. In general, it can be said that buckwheat bread can be difficult to consume in terms of both color and texture, but its consumption is increased when it is made with sour dough. It may be thought that buckwheat flour should be optimized as our study is important for future studies.

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