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ORIGINAL ARTICLE

The effect of flours of different immature cereal grains on sourdough and sourdough bread: Microbiological, rheological, textural and sugar profiles

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Abstract

In this study, the utilization of LAB (*Lactiplantibacillus plantarum*, *Levilactobacillus brevis*, *Furfurilactobacillus rossiae*, *Weissella cibaria*) cultures with selected flours of different immature cereal grains (wheat, barley, oat and rye) on sourdough and sourdough bread was investigated. In total, 16 different sourdough breads were produced and microbiological, pH, TTA and rheological properties of sourdoughs at 0, 8 and 24 h were determined. At the beginning of the fermentation, while sourdoughs showed elasticity, they showed viscoelastic properties after 24 h of fermentation. The sugar profile of cereal grains at the stage of milk formation and the sugar groups presented in EPSs isolated from sourdoughs were revealed by HPLC analysis. The sugar groups in sourdoughs were glucose, xylose and arabinose, while the sugars in the immature cereal grains were glucose, fructose and mannose. The highest EPS production level (1882.69 ± 8.16) was observed in immature barley flour and *Lpb. plantarum* containing sourdough and all sourdoughs were found to contain glucan type EPSs. It was observed that the amount of EPS produced by the related species differed significantly under different grain conditions. Besides, it was observed that the sourdough breads produced were harder than the control bread.

Novelty Impact Statement: Wheat, barley, oat and rye immature grains and four distinct LAB strains were used during sourdough fermentation. Immature grain type and LAB strain used were determinants for in situ exopolysaccharide (EPS) production. Viscoelastic properties of sourdough and textural properties of sourdough bread were affected by grains and LAB strain utilized.

1 | INTRODUCTION

Sourdough is particularly defined as a mixture of cereal flours such as wheat and rye with water and is important for the development of bread (Sakandar et al., 2018). Yeast and lactic acid bacteria (LAB) have a positive interaction in sourdough bread. Therefore, it is a traditional product with high nutraceutical factors (antioxidants, vitamins, minerals) and long shelf life due to their biochemical reactions. Generally, the taste and aroma of sourdough bread are formed by the fermentation of LAB and yeast. Dough properties, bread texture, taste and sensory properties are provided by microbiological activity. As a result, the staling of the bread could be delayed and microbial spoilage could be prevented (Aplevicz et al., 2014; Clément et al., 2018; Yu et al., 2018). The microbiota of sourdough has also important roles in the production of specific metabolites such as organic acids, antimicrobial agents, exopolysaccharides (EPS) and various specific enzymes that might have a positive effect on the texture and staling delay of bread (Alkay et al., 2020).

Another important parameter for LABs in sourdough fermentation process can be the usage of immature flours with high dietary fiber and fructooligosaccharides (FOS) content (Babaoglu et al., 2020). Studies have shown that cereal grains harvested at the milk stage of FOS, which is among dietary sources, have 10 times more FOS content than mature wheat grains (Pepe et al., 2013). It has been emphasized in recent studies that immature wheat, which contains FOS, protein and antioxidant components, is a prebiotic that improves the textural properties and nutritional value of the food product (Casiraghi et al., 2013; Pepe et al., 2013). In addition, it has been stated that FOS stimulates EPS production by LABs. EPSs are considered biochemicals or hydrocolloids that are a good alternative to additives (Göktepe & Akın, 2020). Sourdough LABs are an important source of EPS production and have a significant impact on many beneficial technological properties such as dough's viscoelasticity, dough rheology, bread volume, firmness, breadstick and shelf life (Poutanen et al., 2009; Tieking & Gänzle, 2005; Torrieri et al., 2014). In recent studies, it has been emphasized that EPS produced by LABs might increase the technological properties of dough and bread, and the use of bread additives such as expensive hydrocolloids can be avoided by using EPS-producing LABs (Gezginc & Kara, 2019; Palomba et al., 2012; Pepe et al., 2013; Tieking et al., 2003). Demand for sourdough products has increased in the food industry in recent years. Studies on the effect of immature flours (wheat, barley, oats, rye) on the quality of sourdough bread and the fermentation activity of LAB cultures have been limited.

The main aim of this study was to determine the technological quality on sourdough bread of selected LAB cultures with immature wheat, barley, rye and oat flours. For this purpose, four immature cereal grains (wheat, barley, rye, oats) were collected from Bayburt city of Turkey. The sourdoughs were made by mixing four selected LAB strains (*Lactiplantibacillus plantarum*, *Levilactobacillus brevis*, *Furfurilactobacillus rossiae*, *Weissella cibaria*) with immature flours and wheat flour. Microbiological analysis, pH values, TTA values and viscoelastic properties of sourdoughs were recorded during fermentation process. At the same time, EPS production amounts and sugar profiles in dough were determined. Then, sourdough bread was produced and its textural properties were characterized.

2 | MATERIALS AND METHODS

2.1 | Material

In this study, immature wheat flour (BU) (*Triticum aestivum*), immature rye flour (CU) (*Secale cereale*), immature oat flour (YU) (*Avena sativa*) and immature barley flour (AU) (*Hordeum vulgare*) were used. Immature cereals were collected from Bayburt city in Turkey. Codes of immature cereal flours and LAB used in sourdough are given in Table 1.

TABLE 1 Codes of immature flours and LAB used in sourdough

BU-ED1	Immature wheat flour—*Furfurilactobacillus rossiae*
CU-ED1	Immature rye flour—*Furfurilactobacillus rossiae*
YU-ED1	Immature oat flour—*Furfurilactobacillus rossiae*
AU-ED1	Immature barley flour-*Furfurilactobacillus rossiae*
BU-ED10	Immature wheat flour—*Lactiplantibacillus plantarum*
CU-ED10	Immature rye flour—*Lactiplantibacillus plantarum*
YU-ED10	Immature oat flour—*Lactiplantibacillus plantarum*
AU-ED10	Immature barley flour—*Lactiplantibacillus plantarum*
BU-E25	Immature wheat flour—*Levilactobacillus brevis*
CU-E25	Immature rye flour—*Levilactobacillus brevis*
YU-E25	Immature oat flour—*Levilactobacillus brevis*
AU-E25	Immature barley flour—*Levilactobacillus brevis*
BU-N9	Immature wheat flour—*Weissella cibaria*
CU-N9	Immature rye flour—*Weissella cibaria*
YU-N9	Immature oat flour—*Weissella cibaria*
AU-N9	Immature barley flour—*Weissella cibaria*

2.2 | Bacterial strains and culture conditions

Furfurilactobacillus rossiae ED1, *Lactiplantibacillus plantarum* ED10, *Levilactobacillus brevis* E25, *Weissella cibaria* N9 (Dertli et al., 2016) cultures were used as sourdough isolates and LAB strains were grown in MRS medium (de Man, Rogosa and Sharpe) at 37°C for 24–48 h. Then, the supernatant was removed with the centrifuge (4500 *g* 15 min 4°C). The remaining pellets were dissolved in sterile water and used to produce sourdough for inoculation.

2.3 | Preparation of sourdough samples

The sourdoughs were prepared by mixing water (26 ml), wheat flour (40 g) and immature cereal flour (2 g each separately [wheat, barley, oat and rye flour]), and the lactobacilli strains were inoculated (10^7 CFU/ml) into wheat flour doughs. Dough yield of sourdough was calculated as approximately 165. Acid control doughs (acidified to pH 4.8 by acetic acid addition) without bacterial inoculum were prepared and incubated under the same conditions.

2.4 | Determination of pH, total titratable acidity and enumeration of LAB

Ten grams of sourdough sample were transferred into 90 ml of sterile physiological solution (0.8%) and homogenized for 2 min in a Stomacher (Interscience, Bag mixer). After decimal dilutions, 20 µl of

these suspensions were taken and was planted on MRS agar by spot method. LABs were counted after incubation for 48–72 h at 30°C under anaerobic conditions and the results were expressed as log CFU/g. The pH values of 0, 8 and 24 h of sourdough samples were determined by a pH-meter (WTW Inolab 7110). Total titratable acidity (TTA) was measured on 2 g of dough samples, which were homogenized with 18 ml of distilled water and expressed as the amount (mL) of 0.1 N NaOH to achieve a pH of 8.5 and results are given in % lactic acid.

2.5 | Determination of EPS production levels and isolation of EPS from sourdough

Isolation of EPS from sourdough was performed according to the method described by Van Geel-Schutten et al. (1999). Before drying the pellets, the phenol-sulfuric test was performed according to DuBois et al. (1956). Briefly, 200 µl of sample was placed in the spectro cuvette and then 600 µl of 98% sulfuric acid was added, 120 µl of 5% phenol was added and it was waited for 5 min for color development. Then, the OD490nm in the relevant cuvettes was measured (UV-1800-240 V, Shimadzu) and the EPS amount of the samples was determined using the glucose curve.

2.6 | Determination of sugar in sourdough by HPLC analysis

The monosaccharide composition was determined according to the method specified by İspirli et al. (2019). For HPLC conditions, a CARBOsep CHO682 Pb Column and RID-10 A refractive index detector were used. The mobile phase was H_2O and flow rate was 0.7 ml/min and column temperature was 25°C.

2.7 | HPLC analysis of sugars in cereal grains

HPLC analysis was performed to reveal sugar profiles in cereal grains in milk formation stage. 100 mg of sample was weighed and 4 ml of 80% ethanol was added. It was then hydrolyzed in a water bath (at 80–90°C) for 20 min followed by centrifugation at 7500 *g*, 4°C for 10 min. From the supernatant 400 µl sample was taken and ethanol was removed with a rotary evaporator. The remaining solid component was dissolved in 75 µl of water and the same amount of acetonitrile was added and finally passed through a filter (0.45 µm) by means of an injector to remove impurities (Shimbata et al., 2011). The samples thus prepared were injected into a high-pressure liquid chromatography (HPLC-RID, Shimadzu) system with a refractive index detector. Injection volume was determined as 20 µl and CARBOsep CHO-682 Pb column was used as column. The column temperature was kept constant at 85°C and deionized water was used as the mobile phase.

2.8 | Determination of dynamic rheological analysis of sourdough

Amplitude sweep test was applied in strain range of 0.1–100 Pa, 25°C and 10 rad/s and determination of linear viscoelastic region. And then frequency sweep test was applied in a strain of 0,5 % 25°C with a frequency range of 10–100 rad/s. Calculated storage (G') and loss modulus (G'') values. R^2 determination coefficient was calculated by power law model.

$$G' = K'(\omega)^{n'}$$

$$G'' = K''(\omega)^{n''}$$

2.9 | Bread production

In this study, 16 different sourdough were prepared. The sourdough used in the preparation of the bread dough was used after being kept in the air-conditioning cabinet for 24 h with the method mentioned before. For sourdough bread, 50 g wheat flour, 14 g sourdough, 0.95 g salt and 27.5 ml water were mixed. It was left for 60 min for main fermentation in a cabinet containing 25°C, 86% humidity. After aeration, it was left to intermediate fermentation under the same conditions. After the dough was shaped, it was left for final fermentation for 120 min in a cabinet containing 75% humidity at 25°C and the cooking process has been carried out. Both bread made from acidified control dough and 1% and 3% commercial yeast bread were used as control bread. Breads were baked at 210°C for 30 min. It was then left to cool for texture analysis for at least 1 h.

2.10 | Determination of textural properties of sourdough breads

The textural properties of sourdough breads were performed according to a modified method described by Rizzello et al. (2010). Briefly, texture profile analysis (TPA) was performed with a TA. HD Plus Texture Analyzer, using a 35-mm flat-end aluminum compression disc (probe P/35). The selected settings were as follows: test speed 50 mm/min, 25% deformation of the sample.

2.11 | Statistical analysis

One way analysis of variance (ANOVA) was performed on the results using Minitab version 17.3.1 (Minitab, Inc.) and JMP version 9. Significant differences between the samples were determined as $p < 0.05$. The difference between the two parallels was given as mean ± standard deviation.

3 | RESULTS AND DISCUSSION

3.1 | LAB counts, pH and TTA levels of sourdoughs

LAB counts, pH and TTA levels of sourdoughs were monitored during 0th, 8th and 24th hour of fermentation. *Levl. brevis* E-25 and *W. cibaria* N9 numbers between 0–8 and 8–24 h of fermentation in different sourdough samples prepared with the addition of different immature flours (wheat, rye, oat, barley) showed similar growth profiles. At the end of the 24th hour, *Lpb. plantarum* ED-10 strain showed the best growth, and the growth level was about 2 log units higher than other strains and this rate was close to *Levl. brevis* E-25 growth level in this period (Table 2). LAB numbers increased in all sourdough samples at the end of fermentation for 24 h. The results were similar to previous sourdough studies. Several previous studies have examined the LAB counts in sourdough prepared with starters. For instance, *Furl. rossiae* numbers were observed to be at 9.36 log CFU/g (Rizzello et al., 2013) and 9.8 log CFU/g levels (Garofalo et al., 2012). In addition, our results are interesting in terms of showing the effect of cereal grains on LAB species. For example, the flour of immature wheat did not show important stimulating properties in the development of three other species except *Lpb. plantarum* ED-10. Although there were some proportional differences in sugar monomers in these grains, no significant differences were observed. Therefore, these results can be thought to be based on the biochemical and genetic differences of bacteria (such as the order and speed of metabolizing sugar monomers).

LAB strains show a good growth capacity because they provide a good adaptation to the ecosystem of sourdough and have the ability to be used as a starter culture for bakery products (Palomba et al., 2011). Also, sourdough LABs are responsible for the production of metabolites such as lactic acid, acetic acid and ethanol. These metabolites affect the characteristics of food products such as flavor, texture, taste and shelf life (Gänzle, 2014; Gänzle & Ripari, 2016). Our results showed that although the sugar profiles of different immature cereal grains were highly similar, they had different effects on LAB species. One of the most important features of sourdough fermentation is a decrease in pH proportional to the growth of lactic and acetic acid producing LABs (De Vuyst et al., 2009). Due to the decrease of pH during fermentation, enzymatic activity changes and increment in protease activity might create weakness in gluten network and cause partial starch degradation. Thus, the use of sourdough provides a softer dough formation (Belz, 2016).

When looking at Table 2, it can be observed that sourdough prepared with *Lpb. plantarum* ED10 strain had the lowest pH value. In addition, *W. cibaria*-N9 during this period grown well in sourdough with immature barley flour. However, it did not have a significant effect on pH. The reason for this may be a low amount of acid production by this strain. Between 8 and 24 h, the pH change in each sourdough sample was close to each other and a decrease of approximately 1.5 units in pH was observed. This result can be important as it might suggest that the differences in the amount of acids produced as well as the number of bacteria can be extremely important in the pH decrease. The pH and acidity level of the dough is an important indicator of the fermentation activities of LAB and yeast (Đukić et al., 2014).

TA values of sourdoughs were ranged between 0.27% and 0.54% at 0 h, while these values were ranged between 1.01% and 1.52% at 24 h of fermentation (Table 2). As can be seen from the results, the tendency of acidity change coincides with pH and a more significant relationship was found between the change in bacteria number and acidity. Increased acidity leads to protein degradation and control of the activity of proteolytic enzymes. An increase in acidity is necessary for good fermentation, control of enzyme activity, elasticity and prolongation of shelf life (Pepe et al., 2013).

3.2 | Evaluation EPS production in distinct sourdoughs

EPS extraction was performed from sourdough samples and EPS production quantities were determined by phenol-sulfuric acid test. During fermentation of sourdough, EPS production can take place at different levels. In the results, the highest production amount of EPS was obtained from sourdough with immature wheat flour and immature barley flour, and the lowest was sourdough with immature rye flour. At the same time, the highest EPS producing strain was *Lpb. plantarum* ED10 (1882.692 ± 8.16 μg/g), while the lowest EPS producing strain was *Levl. brevis* E25 (929.23 ± 31.54 μg/g). A direct relationship could not be established between EPS production results and the use of immature cereals. This situation may be due to the metabolism of LAB species and the fact that these components are not preferred enough due to the high sugar content in the environment might also affect these findings (Table 3).

3.3 | Determination of sugars in both immature flours and sourdough by HPLC

Another important feature of sourdough environment can be its unique characteristics with presence of distinct sugars. EPSs can be also recognized as sugar sources for distinct LABs, and oligosaccharides can be also formed during EPS production (Patel et al., 2012). EPS production has technological importance in the production of fermented foods. These technological advantages improve the rheology and texture of fermented food formulations (Dilna et al., 2015; Lee et al., 2011). In additional studies, it was emphasized that EPS provides prebiotic properties (Katina et al., 2009). LABs produce carbohydrates such as oligo- and homopolysaccharides. These carbohydrates are used as texturizing agents and prebiotics. Their interest is growing due to their potential industrial applications (Naessens et al., 2005). The interest in sourdough LABs is that EPSs such as glucan and fructan are capable of triggering major structural changes (Galle & Arendt, 2014).

HPLC process was applied to detect sugar groups in sourdoughs in our study. Glucose, fructose, galactose, arabinose, mannose and

TABLE 2 LAB count (log CFU/g), pH and TTA values in sourdough

Sourdough	Fermentation time (h)	LAB (log CFU/g)	pH	TTA (% lactic acid)
Control	0	–	4.83 ± 0.00^{J}	0.71 ± 0.00^{A}
	8	–	4.72 ± 0.01^{F}	0.69 ± 0.01^{B}
	24	–	4.52 ± 0.01^{A}	0.90 ± 0.00^{K}
AU-ED1	0	8.40 ± 0.03^{BCDE}	5.22 ± 0.01^{F}	0.54 ± 0.01^{B}
	8	8.62 ± 0.03^{FG}	4.72 ± 0.01^{F}	0.60 ± 0.00^{FG}
	24	9.19 ± 0.03^{DEF}	3.90 ± 0.01^{BC}	1.08 ± 0.01^{HI}
AU-E25	0	7.22 ± 0.00^{F}	5.45 ± 0.00^{B}	0.36 ± 0.00^{E}
	8	8.79 ± 0.06^{DEF}	4.33 ± 0.04^{K}	0.67 ± 0.01^{B}
	24	9.43 ± 0.15^{BCD}	3.73 ± 0.04^{E}	1.14 ± 0.00^{G}
AU-ED10	0	8.52 ± 0.00^{BCD}	4.57 ± 0.00^{N}	0.44 ± 0.00^{D}
	8	8.93 ± 0.10^{CDE}	4.18 ± 0.01^{L}	0.63 ± 0.01^{CD}
	24	9.67 ± 0.21^{BC}	3.39 ± 0.01^{F}	1.52 ± 0.01^{A}
AU-N9	0	8.07 ± 0.02^{DE}	5.03 ± 0.00^{I}	0.44 ± 0.00^{D}
	8	9.28 ± 0.14^{B}	4.97 ± 0.00^{B}	0.54 ± 0.01^{H}
	24	9.67 ± 0.21^{BC}	3.87 ± 0.00^{BC}	1.01 ± 0.00^{J}
BU-ED1	0	8.40 ± 0.01^{BCDE}	5.15 ± 0.00^{G}	0.43 ± 0.00^{D}
	8	8.64 ± 0.06^{EFG}	4.91 ± 0.01^{BC}	0.58 ± 0.01^{G}
	24	8.99 ± 0.09^{EFG}	3.93 ± 0.00^{BC}	1.15 ± 0.01^{G}
BU-E25	0	7.22 ± 0.00^{F}	5.54 ± 0.00^{A}	0.27 ± 0.00^{G}
	8	8.96 ± 0.00^{CD}	4.48 ± 0.01^{HI}	0.62 ± 0.01^{CDE}
	24	9.42 ± 0.13^{BCD}	3.86 ± 0.03^{CD}	1.15 ± 0.01^{G}
BU-ED10	0	8.23 ± 0.00^{CDE}	4.74 ± 0.01^{K}	0.34 ± 0.00^{F}
	8	8.83 ± 0.01^{DEF}	4.55 ± 0.07^{GH}	0.46 ± 0.00^{K}
	24	9.43 ± 0.15^{BCD}	3.39 ± 0.01^{F}	1.31 ± 0.01^{D}
BU-N9	0	9.21 ± 0.03^{A}	5.22 ± 0.01^{F}	0.35 ± 0.01^{E}
	8	9.60 ± 0.12^{A}	4.58 ± 0.01^{HI}	0.53 ± 0.01^{HI}
	24	9.69 ± 0.01^{BC}	3.94 ± 0.01^{B}	1.07 ± 0.01^{I}
CU-ED1	0	8.00 ± 0.00^{E}	5.12 ± 0.00^{H}	0.44 ± 0.00^{D}
	8	8.69 ± 0.00^{DEFG}	4.78 ± 0.00^{DEF}	0.61 ± 0.01^{DEF}
	24	8.78 ± 0.05^{G}	3.90 ± 0.01^{BC}	1.18 ± 0.01^{F}
CU-E25	0	7.37 ± 0.21^{F}	5.25 ± 0.00^{E}	0.44 ± 0.00^{D}
	8	8.96 ± 0.00^{CD}	4.39 ± 0.00^{JK}	0.64 ± 0.00^{C}
	24	9.22 ± 0.00^{DEF}	3.78 ± 0.01^{DE}	1.24 ± 0.01^{E}
CU-ED10	0	8.16 ± 0.14^{CDE}	4.66 ± 0.00^{L}	0.54 ± 0.01^{B}
	8	8.83 ± 0.01^{DEF}	4.11 ± 0.00^{L}	0.60 ± 0.00^{FG}
	24	10.12 ± 0.00^{A}	3.39 ± 0.01^{F}	1.37 ± 0.01^{C}
CU-N9	0	8.70 ± 0.01^{B}	5.21 ± 0.00^{F}	0.35 ± 0.01^{E}
	8	9.22 ± 0.00^{BC}	5.12 ± 0.01^{A}	0.44 ± 0.00^{K}
	24	9.67 ± 0.21^{BC}	3.87 ± 0.01^{BC}	1.01 ± 0.00^{J}
YU-ED1	0	7.45 ± 0.33^{F}	5.03 ± 0.00^{I}	0.46 ± 0.00^{C}
	8	8.69 ± 0.00^{DEFG}	4.85 ± 0.01^{CD}	0.67 ± 0.01^{B}
	24	8.78 ± 0.05^{G}	3.89 ± 0.01^{BC}	1.10 ± 0.01^{H}
YU-E25	0	7.22 ± 0.00^{F}	5.39 ± 0.00^{C}	0.43 ± 0.00^{D}
	8	9.22 ± 0.00^{BC}	4.37 ± 0.00^{K}	0.74 ± 0.00^{A}
	24	9.31 ± 0.00^{CDE}	3.78 ± 0.01^{DE}	1.10 ± 0.01^{H}

(Continues)

TABLE 2 (Continued)

Sourdough	Fermentation time (h)	LAB (log CFU/g)	pH	TTA (% lactic acid)
YU-ED10	0	8.08 ± 0.12^{DE}	4.63 ± 0.01^{M}	0.53 ± 0.01^{B}
	8	8.41 ± 0.09^{G}	4.45 ± 0.01^{IJ}	0.50 ± 0.01^{J}
	24	9.60 ± 0.06^{BCD}	3.38 ± 0.01^{F}	1.43 ± 0.01^{B}
YU-N9	0	8.60 ± 0.08^{BC}	5.33 ± 0.01^{D}	0.45 ± 0.01^{C}
	8	8.96 ± 0.00^{CD}	4.81 ± 0.01^{DE}	0.51 ± 0.01^{IJ}
	24	9.60 ± 0.06^{BCD}	3.86 ± 0.03^{C}	1.07 ± 0.01^{I}

Note: Different letters in the same column are statistically different ($p < 0.05$).

Abbreviations: AU, Barley Flour; BU, Wheat Flour; ÇU, Rye Flour; E25, *Levilactobacillus brevis*; ED1, *Furfurilactobacillus rossiae*; ED10, *Lactiplantibacillus plantarum*; N9, *Weissella cibaria*; YU, Oat Flour.

TABLE 3 EPS production in sourdough

Sourdough code	EPS production (μg/g)
BU-ED1	1808.5 ± 174.06^{AB}
CU-ED1	1289.61 ± 16.86^{G}
YU-ED1	1545.77 ± 5.98^{DE}
AU-ED1	1627.31 ± 0.54^{BCD}
BU-ED10	1853.08 ± 10.88^{A}
CU-ED10	1503.85 ± 25.02^{DEF}
YU-ED10	1613.08 ± 1.09^{BCD}
AU-ED10	1882.69 ± 8.16^{A}
BU-E25	1429.23 ± 18.49^{DEFG}
CU-E25	1330 ± 27.196^{FG}
YU-E25	929.23 ± 31.54^{H}
AU-E25	1350 ± 65.27^{EFG}
BU-N9	1590 ± 52.22^{CD}
CU-N9	1054.23 ± 9.25^{H}
YU-N9	1532.31 ± 42.45^{DEF}
AU-N9	1761.54 ± 25.02^{ABC}

Note: Different letters in the same column are statistically different ($p < 0.05$).

Abbreviations: AU, İmmature barley flour; BU, İmmature wheat Flour; CU, İmmature Rye Flour; E25, *Levilactobacillus brevis*; ED1, *Furfurilactobacillus rossiae*; ED10, *Lactiplantibacillus plantarum*; N9, *Weissella cibaria*; YU, İmmature Oat Flour.

xylose were used as standards. In all of the sourdoughs made with immature flours and selected LABs, sugars found were glucose, xylose and arabinose (Figure 1).

In our study, the presence of xylose and arabinose in sourdough samples might suggest the presence of water-soluble arabinoxylan which is normally present in a small proportion of flour. The increase in solubility of arabinoxylans might be positively affected by the fermentation of LAB species in sourdough, and this has positive effects on the quality of the produced bread (Neumann et al., 2006). One reason for this might be the fact that pH dropped by sourdough process resulted in the formation of optimum pH environment for xylinases in sourdough environment (Rasmussen et al., 2001). As a result, the formation of arabinoxylans in soluble form and consumption together with bread is very important because of their potential prebiotic effects (Neyrinck et al., 2012).

Another important point was the uncovering of sugars in immature cereal flours. The amounts of monosaccharides found in flours obtained from all grains in milk maturation stage are shown in Figure 2. In the results, it was determined that there were glucose, fructose and mannose as sugar monomers. It was observed that fructose was the highest in immature wheat flour, while glucose and mannose were low. These three sugar monomers were also found in immature oat flour and immature barley flour. However, while the glucose-fructose ratio in immature rye flour was similar to that of immature wheat flour, mannose was observed in trace amounts in this flour. As a result, all four contained different amounts of usable sugar, and it is thought that their promoting effects on LAB species may be different. However, although certain changes in bacterial numbers were observed, this effect was not achieved at the desired level.

3.4 | Dynamic rheological analysis

Parameters such as rheological properties, acidification and flavor development are important in fermentation processes. It is emphasized that rheological properties are affected depending on the type of microorganisms, metabolic activities and changes in pH. In this context, viscoelastic properties of sourdough samples at 0th, 8th and 24th hours were measured under 0.5% strain in frequency screening test and frequency dependent change of G' and G'' values and results were given in Table 4. The data obtained as a result of the analysis in the study were adapted according to the power-law model. The samples showed viscoelastic properties as can be seen in the results. Elastic properties were found to be higher since it was initially $K' > K''$. As the fermentation progressed, the elastic property decreased, and the viscous property became dominant for $K'' > K'$ (Table 4). In addition, the R^2 determination coefficient was found to be 0.99 in all of the sourdough samples. The relationship between elastic (G') and viscous modulus (G'') values in the produced sourdoughs was more stable as expected. While the elastic modulus was

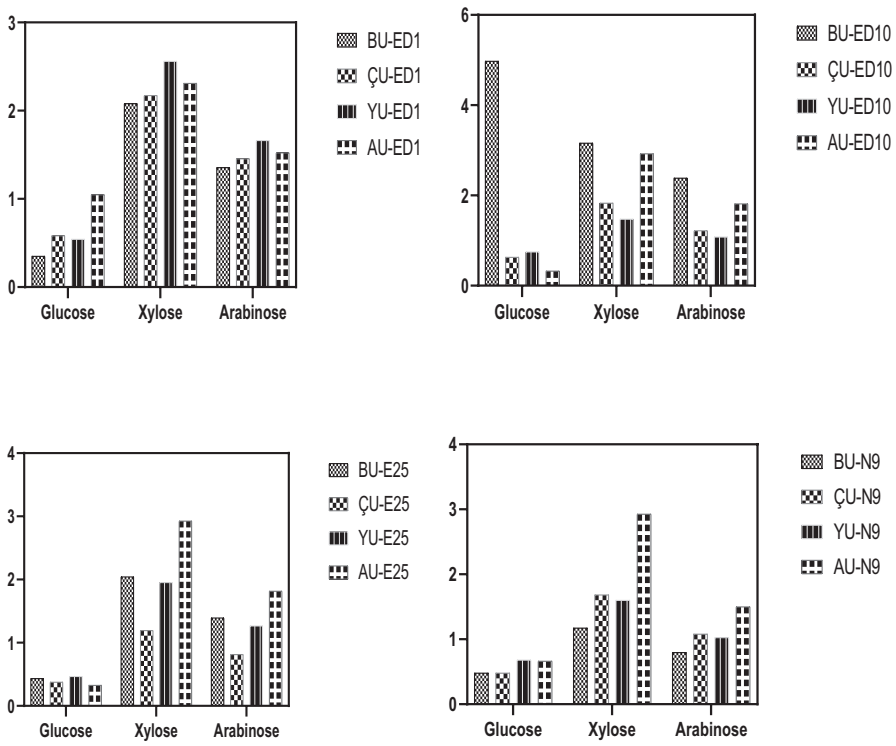

FIGURE 1 Sugar production amounts of EPS obtained from sourdough samples. AU, İmmature barley flour; BU, İmmature wheat flour; CU, İmmature Rye flour; E25, *Levilactobacillus brevis*; ED1, *Furfurilactobacillus rossiae*; ED10, *Lactiplantibacillus plantarum*; N9, *Weissella cibaria*; YU, İmmature oat flour.

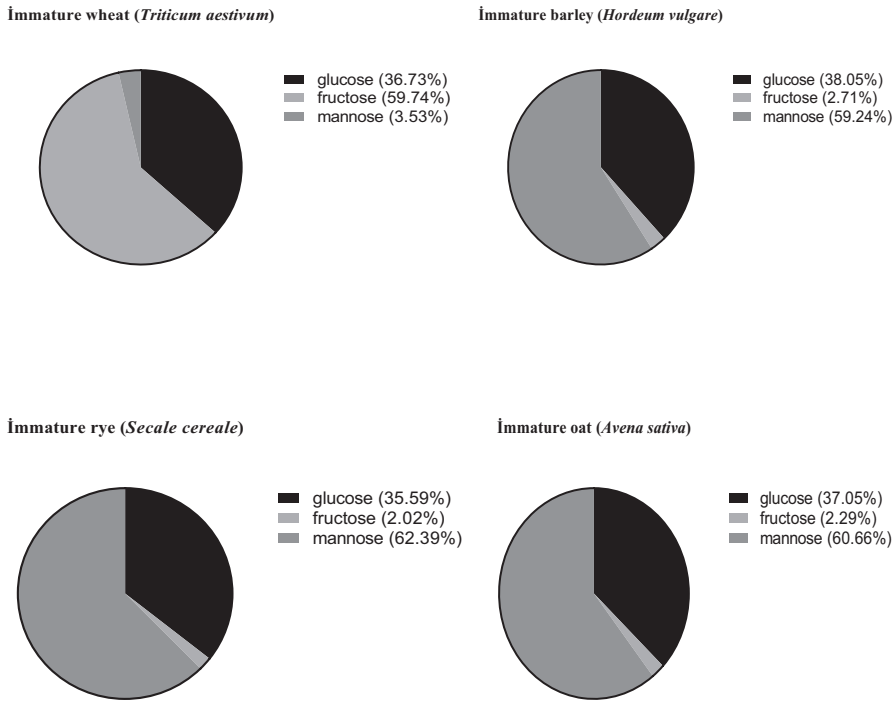

FIGURE 2 The amount of sugars in the cereal grains at the stage of milk formation.

high at the beginning, it was very close to each other at the end of the 24th hour. Similar qualities were also recorded in the control dough (Table 4). Although there were various differences in EPS production in these sourdoughs, a direct relationship could not be revealed between the measurement of elastic and viscous modulus values and EPS production levels.

Clarke et al. (2004) noted that there was a significant decrease in the elasticity and hardness of sourdough during 24-h fermentation. These show that cereal proteases with optimum acidity play an important role in the rheological changes that occur during sourdough fermentation. The use of sourdough has changed the functioning of the dough and provided a softer dough (Bleukx & Delcour, 2000; Thiele et al., 2002). The results we obtained are important in that they show the change in elastic and viscous modules due to fermentation and especially with the effect of pH decrease, but it was not possible

TABLE 4 Viscoelastic values of sourdoughs

Fermentation time (h)	Strains	*K′* FLOUR TYPE				*K″* FLOUR TYPE				*n′* FLOUR TYPE				*n″* FLOUR TYPE			
		AU	BU	ÇU	YU	AU	BU	ÇU	YU	AU	BU	ÇU	YU	AU	BU	ÇU	YU
0th	Control*	$7140.9^{a,A,x}$	$7140.9^{a,A,x}$	$7140.9^{a,A,x}$	$7140.9^{a,A,x}$	$2434.5^{a,A,x}$	$2434.5^{b,A,x}$	$2434.5^{b,A,x}$	$2434.5^{ab,A,x}$	0.25	0.25	0.25	0.25	0.33	0.33	0.33	0.33
	ED1	$5411.3^{b,B,x}$	$5530.4^{b,A,x}$	$4688.8^{b,B,x}$	$3689.7^{d,C,x}$	$2257.9^{a,B,x}$	$2530.1^{b,B,x}$	$3075.9^{a,A,x}$	$2352.5^{abc,B,x}$	0.22	0.23	0.29	0.32	0.3	0.28	0.33	0.30
	E25	$5171.9^{b,B,x}$	$7353.1^{a,A,x}$	$7199.6^{a,A,x}$	$5227.2^{bc,B,x}$	$2361.2^{a,C,x}$	$3133.2^{a,A,x}$	$2767.2^{ab,B,x}$	$2250.9^{bc,C,x}$	0.21	0.24	0.21	0.23	0.32	0.32	0.3	0.28
	ED10	$5171.9^{b,B,x}$	$6699,4^{a,A,x}$	$5341,3^{b,B,x}$	$5262^{c,B,x}$	$2004.8^{b,C,x}$	$2454.6^{b,B,x}$	$2664.5^{b,A,x}$	$2085.8^{c,C,x}$	0.19	0.22	0.24	0.27	0.31	0.31	0.28	0.29
	N9	$3112.7^{c,B,x}$	$2942.4^{c,B,x}$	$2742.9^{c,B,x}$	$6127.2^{b,A,x}$	$1464.9^{c,BC,x}$	$1614.7^{c,B,x}$	$1402.9^{c,C,x}$	$2625.8^{a,A,x}$	0.22	0.27	0.35	0.20	0.30	0.29	0.32	0.35
8th	Control*	$1643.2^{c,A,y}$	$1643.2^{bc,A,y}$	$1643.2^{c,A,y}$	$1643.2^{c,A,y}$	$837.12^{cd,A,y}$	$837.1^{b,A,y}$	$837.1^{c,A,y}$	$837.1^{d,A,y}$	0.27	0.27	0.27	0.27	0.37	0.37	0.37	0.37
	ED1	$2640.9^{a,B,y}$	$1758.3b^{c,C,y}$	$1566.9^{c,C,y}$	$3103.0^{a,A,y}$	$1557.2^{a,A,y}$	$900.9^{b,C,y}$	$797.2^{c,D,y}$	$1335.2^{a,B,y}$	0.31	0.28	0.19	0.35	0.32	0.36	0.31	0.32
	E25	$1672.9^{c,C,y}$	$3293.1^{a,A,y}$	$3385.7^{a,A,y}$	$2173.8^{b,B,y}$	$1050.4^{b,B,y}$	$1514.4^{a,A,y}$	$1478.9^{a,A,y}$	$1181.5^{b,B,y}$	0.37	0.24	0.21	0.24	0.36	0.33	0.32	0.31
	ED10	$2164.1^{b,A,y}$	$2122.9^{b,A,y}$	$2409.2^{b,A,y}$	$1139.3^{d,B,y}$	$994.9^{bc,AB,y}$	$854.4^{b,B,y}$	$1141.7^{b,A,y}$	$797.3^{d,B,y}$	0.25	0.24	0.23	0.35	0.33	0.34	0.32	0.32
	N9	$1264.2^{d,B,y}$	$1442.5^{c,B,y}$	$1305.5^{c,B,y}$	$2061.1^{b,A,y}$	$710.8^{d,AB,y}$	$544.8^{c,B,y}$	$784.5^{c,AB,y}$	$996.4^{c,A,y}$	0.31	0.29	0.27	0.23	0.34	0.35	0.32	0.32
24th	Control*	$442.8^{b,A,z}$	$442.8^{b,A,z}$	$442.8^{b,A,z}$	$442.8^{a,A,z}$	$344.3^{a,A,z}$	$344.3^{a,A,z}$	$344.3^{a,A,z}$	$344.3^{a,A,z}$	0.38	0.38	0.38	0.38	0.46	0.46	0.46	0.46
	ED1	$644.4^{a,A,z}$	$488.9^{a,AB,z}$	$446.1^{a,AB,z}$	$480.1^{a,B,z}$	$165.7^{c,C,z,}$	$334.2^{a,A,z,}$	$249.8^{b,B,z}$	$334.2^{a,A,z}$	0.30	0.27	0.31	0.25	0.53	0.46	0.47	0.39
	E25	$54.9^{c,D,z}$	$188.3^{e,B,z}$	$381.9^{b,A,z}$	$80.7^{d,C,z}$	$243.9^{b,A,z}$	$233.9^{b,A,z}$	$173.2^{d,B,z}$	$159.2^{c,B,z}$	0.78	0.48	0.40	0.62	0.42	0.44	0.46	0.44
	ED10	$322.1^{b,AB,z}$	$368.3^{c,A,z}$	$307.9^{d,B,z}$	$242.7^{c,C,z}$	$206,9^{bc,AB,z}$	$217,8^{b,A,z}$	$202,0^{cd,B,z}$	$160,8^{c,C,z}$	0.44	0.29	0.36	0.31	0.47	0.46	0.5	0.46
	N9	$267.0^{b,B,z}$	$276.5^{d,B,z}$	$435.2^{b,A,z}$	$398.4^{b,A,z}$	$212.0^{b,B,z}$	$160.6^{c,C,y}$	$218.6^{c,B,z}$	$258.2^{b,A,z}$	0.39	0.29	0.28	0.28	0.45	0.46	0.46	0.39
R^2		0.999	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99

Note: $^{a-d}$Within each column, different superscript lowercase letters show differences between the strains within each flour type ($p < 0.05$).

Note: $^{A-D}$Within each row, different superscript uppercase letters show differences between the flour types within each strain ($p < 0.05$).

Note: $^{X-Z}$Within each row, different superscript uppercase letters show differences between the incubation times with respect to same strain type and flour type ($p < 0.05$).

*Control samples were not treated with starter culture.

TABLE 5 Texture analysis results of sourdough with immature flour additives

Strains	Hardness				Springiness				Cohesiveness				Gumminess				Chewiness				Resilience			
	AU	BU	CU	YU	AU	BU	CU	YU	AU	BU	CU	YU	AU	BU	CU	YU	AU	BU	CU	YU	AU	BU	CU	YU
Control	20.57^{aA1}	20.57^{aA1}	20.57^{aA1}	20.57^{aA1}	0.86^{aD4}	0.86^{aB4}	0.86^{aE4}	0.86^{aB4}	0.87^{aB4}	0.87^{aA4}	0.87^{aBC4}	0.87^{aAB4}	17.93^{aA2}	17.93^{aA2}	17.93^{aA2}	17.93^{aA2}	15.39^{aA3}	15.39^{aA3}	15.39^{aA3}	15.39^{aB3}	0.57^{aA4}	0.57^{aB4}	0.57^{aB4}	0.57^{aAB4}
ED1	8.10^{aC1}	6.44^{bcC1}	5.45^{cC1}	6.66^{bC1}	0.91^{aC3}	0.89^{aB4}	0.92^{aD3}	0.92^{aB4}	0.83^{aC3}	0.81^{aB4}	0.82^{aD3}	0.80^{aD5}	6.74^{aC2}	5.23^{bC2}	4.48^{bC2}	5.36^{bC2}	6.14^{aC2}	4.67^{bC3}	4.12^{bD2}	4.90^{bC3}	0.57^{aA3}	0.48^{aE5}	0.51^{aC3}	0.47^{aD6}
ED10	7.80^{aC1}	6.89^{aC1}	6.64^{aC1}	5.61^{aC1}	0.92^{aC3}	0.95^{aA3}	0.95^{aCD2}	0.94^{aB2}	0.84^{aC3}	0.87^{aB3}	0.83^{aD2}	0.83^{aC2}	6.49^{aC2}	5.89^{aC2}	5.51^{aC1}	4.68^{aC1}	5.96^{aC2}	5.59^{aB2}	5.24^{aD1}	4.41^{aC1}	0.49^{bC3}	0.56^{aBC3}	0.48^{bD2}	0.49^{bCD2}
E25	13.81^{bcB1}	17.68^{aB1}	11.99^{cB1}	16.20^{abB2}	0.86^{bD4}	0.95^{abA4}	0.98^{aBC3}	1.75^{aA3}	0.89^{bA4}	0.90^{aA4}	0.88^{bA3}	0.89^{abA3}	12.27^{bcB3}	15.86^{aB2}	10.61^{cB2}	14.46^{abB2}	10.51^{bB2}	14.98^{abA3}	10.37^{bB2}	23.98^{aA1}	0.60^{cA4}	0.61^{aA4}	0.60^{bcA3}	0.61^{abA3}
N9	5.25^{aD1}	6.16^{aC1}	5.78^{aC2}	6.14^{aC1}	0.93^{bBC2}	0.97^{bA3}	1.66^{aA4}	0.96^{bB4}	0.86^{aB2}	0.85^{aB3}	0.86^{aC5}	0.86^{aB4}	5.24^{aC2}	4.96^{aC3}	5.30^{aC2}	4.17^{bD1}	5.08^{bBC2}	8.26^{aC1}	5.08^{bC3}	0.53^{abCD4}	0.54^{aC6}	0.52^{aBC4}		
% 3 yeast	2.46^{aE1}	2.46^{aD1}	2.46^{aD1}	2.46^{aD1}	0.97^{aAB3}	0.97^{aA3}	0.97^{aBC3}	0.97^{aB3}	0.87^{aAB3}	0.87^{aAB3}	0.87^{aAB3}	0.87^{aAB3}	2.15^{aE2}	2.15^{aD2}	2.15^{aD2}	2.15^{aD2}	2.08^{aE2}	2.08^{aD2}	2.08^{aE2}	2.08^{aC2}	0.52^{aBC4}	0.52^{aD4}	0.52^{aC4}	0.52^{aC4}
% 1 yeast	2.63^{aE1}	2.63^{aD1}	2.63^{aD1}	2.63^{aD1}	0.98^{aA3}	0.98^{aA3}	0.98^{aA3}	0.98^{aB3}	0.87^{aAB3}	0.87^{aB3}	0.87^{aBC3}	0.87^{aB3}	2.29^{aE2}	2.29^{aD2}	2.29^{aD2}	2.29^{aD2}	2.26^{aE2}	2.26^{aD2}	2.26^{aE2}	2.26^{aC2}	0.53^{aB4}	0.53^{aCD4}	0.53^{aC4}	0.53^{aBC4}

Note: Capital Letters:. Effect of Au, Bu, Yu and Cu on the samples (Vertical Once Way), Little Letters: Effect of AU, BU, YU and CU on the example (Horizontal One Way), Effect of AU, BU, YU and CU in Numbers on Flexibility, Hardness ... (Vertical Once Way).

Abbreviations: AU, Barley Flour; BU, Wheat Flour; ÇU, Rye Flour; E25, *Levilactobacillus brevis*; ED1, *Furfurilactobacillus rossiae*; ED10, *Lactiplantibacillus plantarum*; N9, *Weissella cibaria*; YU, Oat Flour.

to establish a direct relationship between EPS production and these values.

3.5 | Textural properties of sourdough breads

The hardness in bread is generally expressed as a decrease in the softness of the bread interior. In softness, the first is the loss of moisture inside the bread and the second is the retrogradation of starch (Cauvain, 2004). In a study investigating the effect of various sourdough and additives on the hardness and staling of bread, it was emphasized that only sourdough fermentation was effective in delaying retrogradation of starch and this effect was dependent on the degree of acidification and LAB type (Corsetti et al., 2000). Table 5 demonstrates the textural properties of sourdough breads produced in this study. The hardness values of 1% and 3% commercial yeast breads were found between 2.64 N and 2.46 N, respectively, while the hardness values of sourdough breads produced with the use of different starter and immature cereal flours varied between 5.25 N and 20.57 N. The lowest value was sourdough bread containing immature barley flour and *W. cibaria* strain, while the highest value was control bread that was chemically acidified and did not contain sourdough. Chemically acidified bread was close to the firmness value of sourdough breads produced with *Levl. brevis* E25. This result is remarkable in terms of showing the effects of different types on sourdough and bread. The results obtained are important in terms of the emergence of significant differences in sourdough bread produced with different types.

When the stickiness, chewiness and elasticity values were compared statistically, the lowest value was the sourdough bread with *Furl. rossiae* starter, while the highest value was the sourdough bread containing the *Levl. brevis* starter. Mujoo and Ng (2003) observed that bread crumbs made with fructooligosaccharide-rich milk-forming wheat grains were harder and smaller. Our results were also similar. The hardness of sourdough breads may be due to the decrease in moisture content and partial starch retrogradation. Other factors effecting the hardness of sourdough bread can be the high acidity of sourdough which might provide the stimulation of proteolytic and amylolytic activity. As this leads to weakening of the structure of the gluten network, the dough becomes soft and the CO_2 holding capacity is reduced (Bartkiene et al., 2013; Pepe et al., 2013). In addition, acidification causes the bread to have a harder crumb structure (Lynch et al., 2018).

4 | CONCLUSION

According to the results obtained with the use of cereal grains at the stage of milk formation in sourdough, the behavior of *Lpb. plantarum*, *Levl. brevis*, *Furl. rossiae*, *W. cibaria* strains used in the dough environment were relatively different. The pH values and acidity measured in the dough were the most obvious indicators of this phenomenon.

With the addition of these grains, the development of LAB species was affected at different rates. Importantly, rheological properties of sourdough and textural characteristics of sourdough bread were affected at different rates depending on the usage of different immature grains and strain specific conditions were effective for these properties. More studies are definitely required in order to understand the roles of different immature grains in sourdough environments as potential prebiotic as well textural properties effecting agents.

AUTHOR CONTRIBUTIONS

Zühal Alkay: Conceptualization; Investigation; Methodology; Writing—original draft; Writing—review & editing. Mustafa Tahsin Yılmaz: Investigation; Data curation; Writing—original draft. Aslı Muslu Can: Investigation; Writing—original draft; Data curation. Hümeyra İspirli: Investigation; Writing—original draft; Data curation. Enes Dertli: Conceptualization; Investigation; Methodology; Writing—original draft; Writing–review & editing.

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CONFLICT OF INTEREST

The authors have declared no conflict of interest for this article.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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