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The effect of different compositions of starter cultures developed from phytic acid-degrading lactic acid bacteria on the sensory quality of bread

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Abstract

Sourdough is an initial product used in cereal fermentation, usually prepared by adding lactic acid bacteria (LAB) strains to a mixture of wheat and water. Phytic acid present in wheat flours negatively affects the bioavailability of bread. LAB is found to have phytase activity. The present work thus aimed to determine the effect of starter culture combinations developed from phytic acid-degrading LAB (PAD-LAB) strains isolated from sourdough samples on bread sensory quality. De Man, Rogosa, and Sharpe (MRS) agar, MRS Broth, M17 agar, M17 broth, sodium phytate, and mass spectrometry were used to isolate and identify PAD-LAB from sourdough samples. The extracellular phytase activity of PAD-LAB strains was determined by spectrophotometry. Sourdough samples were prepared using different strains having high phytase activity, and breads produced from these samples were evaluated using sensory attributes. Extracellular phytase activities of 30 strains were determined by the spectrophotometric method, showing the highest value at 1109.7 U/mL, and lowest at 386.9 U/mL, with a mean of 685.1 U/mL. Sourdough samples were prepared using five different strains with high phytase activity as starting cultures; and of the four sourdough samples selected, the count of LAB increased (7.80 - 9.87 log CFU/g), pH decreased (3.53 - 4.57), and TTA (total titratable acidity) values increased (10.17 - 14.29 mL) during the fermentation period. In the sensory evaluation, bread produced with starter culture combination CS30 was preferred. In conclusion, it was determined that the bread produced with PAD-LAB strains has an advantage in terms of sensory quality as compared to the control samples.

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Introduction

Sourdough is the starting product used in cereal fermentation, usually prepared by adding lactic acid bacteria (LAB) strains to the mixture of wheat and water to improve its sensory, nutritional, functional, and technological properties. It is possible to develop starter cultures that will standardise sourdough bread production by determining the cultures that play a role during fermentation. For this reason, starter cultures used in sourdough production should be chosen very carefully (Gobbetti *et al.,* 2016; Plessas *et al.,* 2020).

Phytic acid, formed during the ripening of cereal grains, accumulates rapidly with other storage substances such as starch and oil. In cereals, approximately 1 - 2% of grain weight is phytic acid, which can sometimes increase up to 3 - 6% (Poudel *et al.,* 2019). Whole-grain bread is a good source of biologically active compounds. However, phytate functions as a highly negatively charged ion over a wide pH range, thus creating a chelate with positively charged divalent and trivalent ions such as Ca^{2+}, Mg^{2+}, $Fe^{2+/3+}$, Zn^{2+}, and Mn^{2+}. This reduces the absorption of metals in the small intestine, and negatively affects its bioavailability (Lopez *et al.,* 2000; Megat Rusydi and Azrina, 2012; Mohite *et al.,* 2013; Gupta *et al.,* 2015). Many studies have demonstrated that a diet based on foods high in phytate can cause iron deficiency anaemia (the most common nutrient deficiency globally) and deficiencies in mineral absorption (WHO, 2017; Grases *et al.,* 2017). Some studies have demonstrated that LAB possess phytase activity. It is therefore possible to reduce phytic acid and phytate with LAB's phytase (phytic acid-degrading; PAD) activities, especially in bread made of whole wheat flour. Phytic acid and phytate can be reduced through enzymatic (phytase) degradation by adding PAD-LAB to foods with high phytate content. PAD-LAB strains can be selected from LAB strains used in sourdough fermentation (Anastasio *et al.,* 2010; Cizeikiene *et al.,* 2015; Nuobariene *et al.,* 2015).

LAB play an important role in sourdough

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fermentation to improve bread quality and nutritional value (Lim *et al.,* 2018). Using sourdough in bread-making enhances bread volume, structure (crust properties and inner colour), and sensory quality (taste and aroma). In particular, one of the most important goals of bread production with sourdough is to improve the taste and aroma (Papadimitriou *et al.,* 2019). Acidification and enzymatic processes that occur during bacterial development contribute to bread's flavour and texture properties. Acids formed in bread prepared with sourdoughs degrade phytic acid, thereby increasing the mineral bioavailability (Karaman *et al.,* 2018). The present work thus aimed to determine the effect of starter culture combinations with PAD-LAB strains isolated from sourdough samples on the sensory quality of bread.

Materials and methods

Collection of sourdough samples

From May 2020 to July 2020, 12 sourdough samples (eight from Marmara, and four from Aegean) were collected from traditional bakeries from different regions in Turkey, and later brought to the laboratory under sterile condition and suitable temperature (4°C).

Chemicals and reagents

De Man, Rogosa, and Sharpe (MRS) agar (Merck 1.10660, Darmstadt, Germany), MRS broth (Merck 1.10661), M17 agar (1.15108 Merck), and M17 broth (Merck 1.15029) were prepared and used for isolation, purification, identification, and storage of LAB strains from sourdough samples. Physiological saline solution (PSS) (8.5 g of NaCl dissolved in water, autoclaved 15 min at 121°C, and cooled to room temperature) was used for dilutions, and 20% glycerol (Merck 10494) was used to store the cultures. MRS/M17 broth (52.2 g/L) (pH 6.2) was prepared by adding a 0.1% sodium phytate (Sigma Aldrich 68388, Saint Louis, USA) and 0.2% glucose, sterilised at 121°C for 15 min at 1.2 atm pressure to isolate the PAD-LAB strains. Reference strain (*Escherichia coli* ATCC® 25922™), 1 µL CHCA (alpha-cyano-4-hydroxycinnamic acid) matrix solution, and VITEK® MS (bioMerieux, Marcy I'Etoile, France) device were used to identify the PAD-LAB strains. Sodium acetate (Sigma Aldrich W302406), acetic acid (Sigma Aldrich W200603) buffer (100 mM), 500 µL of 10% (w/v) trichloroacetic acid solution (TCA) (Sigma Aldrich T3399), and spectrophotometer were used to determine the extracellular phytase activities of the isolated and identified PAD-LAB strains. NaOH (Sigma Aldrich 221465) was used for TTA (total titratable acidity) analysis of sourdough and bread samples. All chemicals and reagents were prepared according to the instructions by Songré-Ouattara *et al.* (2008), Raghavendra and Halami (2009), Dubois *et al.* (2012), and International Organization for Standardization (ISO, 2014).

Isolation of LAB strains from sourdough samples

Physiological saline (90 mL) was added to 10 g of sourdough samples under aseptic condition, and homogenised. Dilutions (10^{-2} and 10^{-3}) were prepared also using physiological saline, and 1 mL was transferred on MRS or M17 agar, and incubated for 24 - 48 h at 37°C (NÜVE EN-500, Ankara, Turkey). Following incubation, the morphology and purity of single LAB colonies were examined under light microscopy. Matte and cream-colored colonies presumed to be LAB were transferred to fresh Petri dishes by streaking method. The pure cultures were stained, and Gram-positive cocci and bacilli were detected under light microscopy. A catalase test was further applied, and catalase-negative strains were selected (ISO, 2013; 2014; Harrigan and McCance, 2014; Muryany *et al.,* 2017).

Characterisation of LAB strains using mass spectrometry

The PAD-LAB strains were characterised using VITEK® MS (bioMerieux, Marcy I'Etoile, France) following the manufacturer's instructions. A reference strain of *E. coli* ATCC® 25922™ served as positive control (Dubois *et al.,* 2012).

Detection of PAD-LAB strains

MRS/M17 broths with sodium phytate were inoculated with 200 µL of active culture, and incubated at 37°C for 24 h to identify the PAD-LAB strains. Following incubation, 100 µL of culture was taken from the broths, and inoculated on MRS/M17 agar with sodium phytate and incubated at 37°C for 24, 48, and 96 h (NÜVE EN-500, Ankara, Turkey). Subsequently, the colonies forming transparent zone were washed to see if they were caused by phytase or acid production. Colonies with transparent zones after washing were considered PAD-LAB, and the diameters of the zones were measured in millimetres (Bae *et al.,* 1999; Songré-Ouattara *et al.,* 2008).

Determination of extracellular phytase activity of PAD-LAB strains

Extracellular phytase activities of PAD-LAB strains were determined by quantifying the inorganic

phosphate released from sodium phytate. One-unit phytase activity (U) is defined as the amount of enzyme that produces one nmol inorganic phosphorus per min at 50°C (Haros *et al.,* 2005). Phytase enzyme activity was calculated by incubating the sourdough mix prepared with cell suspension (250 µL) and 2 mM substrate in 100 mM sodium acetate-acetic acid buffer (250 µL) for 15 min at 50°C (NÜVE EN-500, Ankara, Turkey). The reaction was stopped by adding 10% (w/v) trichloroacetic acid solution (TCA) (500 µL). A blank tube was prepared by adding 10% TCA solution before adding the substrate. Finally, the inorganic phosphate was calculated at 700 nm using the iron sulphate-ammonium molybdate method using a UV-VIS spectrophotometer (Shimadzu UV-1280, Kyoto, Japan) (Raghavendra and Halami, 2009).

Preparation of sourdoughs with combinations of PAD-LAB starter cultures

Five different PAD-LAB strains with high phytase activity were incubated in 5 mL of MRS/M17 broth for 24 h at 37°C, and the cell pellets were washed by centrifugation at 4°C. Thirty-one different combinations were created to determine the synergistic effect of starter culture combinations. The cell pellet, 150 g of whole wheat flour, 2 g of table salt, and 350 mL of drinking water were mixed for 5 min using a mixer to prepare sourdough. The mixture was then fermented at 35°C for 24 h, and kneaded for 1 min by adding 50 g of whole wheat flour and 25 mL of drinking water to the dough mixture every 24 h. The fermentation continued for 3 d at 35°C (Paramithiotis *et al.,* 2005).

LAB enumeration, and physicochemical analysis of sourdough samples

Serial dilutions (10^{-1} to 10^{-7}) of the homogenised sample (10 mL) were prepared. Each dilution (100 µL) from 10^{-4} to 10^{-7} was spread on MRS/M17 agar, and incubated at 37°C for 24 h, and the viable bacterial were counted from Petri dishes containing 30 - 300 colonies. LAB of sourdough samples were enumerated at 24, 48, and 72 h (Tharmaraj and Shah, 2003; Songré-Ouattara *et al.,* 2008). Distilled water (90 mL) was added to 10 g of sourdough samples, and homogenised in a mixer. The pH was measured during the fermentation at 0, 24, 48, and 72 h, and simultaneously titrated to pH 8.5 with 0.1 N NaOH, and TTA (total titratable acidity) was calculated (Paramithiotis *et al.,* 2006).

Preparation of sourdough bread

Whole wheat flour (400 g), table salt (5 g), and drinking water (240 mL) were kneaded using a mixer for 2 min. The sourdough samples prepared with starter culture and spontaneous fermentation (control) was added to 20% of the bread dough weight, and mixed for 4 min. The doughs were fermented at 35°C and 90% relative humidity for 3 h, and refrigerated overnight (12 h) at 4°C. At the end of the period, it was cooked in traditional stone-based ovens for 30 ± 5 min (Menteş *et al.,* 2007; García-Mantrana *et al.,* 2016).

Determination of specific volume, pH, and TTA values of sourdough bread samples

The baked sourdough bread samples were kept at room temperature for 2 h, then weight (g) and volume (mL) were calculated based on the displacement principle, and recorded. The specific volume (mL/g) was calculated by dividing the bread volume by its weight (Ding *et al.,* 2019). pH and TTA values of sourdough bread samples were determined by the same method applied to sourdough samples (Paramithiotis *et al.,* 2006).

Sensory analysis of bread produced with sourdough prepared with PAD-LAB starter cultures

The purpose of sensory quality analysis is to measure and analyse human responses to external stimuli perceived by the sense of sight, smell, taste, touch, and hearing. Thirteen panellists evaluated the sensory quality parameters of whole wheat bread produced by starter cultures and control (spontaneous fermentation). The panellists were asked about crust colour, interior colour, smell, taste, pore structure, and whole wheat bread samples' general preferences. In terms of sensory quality, aromatic odour, and taste, a slightly sour taste, and yellowish or light bread colour were considered as desired properties. Weak aroma and odour, neutral or very sour taste, and dark bread colour were considered undesired properties. Scores were given as 1 (very bad), 2 (bad), 3 (medium), 4 (good), and 5 (very good) (ISO, 1994; Paramithiotis *et al.,* 2005; Chambers, 2019).

Statistical analysis

SSPS 22 package (SPSS Inc., Chicago, Illinois, USA) program was used for statistical analysis and data evaluation. Means and standard deviations were calculated for all variables. A significant difference was accepted at $p < 0.05$ using one-way analysis of variance (ANOVA). The significance of the differences between means was determined using the Multiple Comparison (*post hoc*) Duncan's test. All measurements were

performed in triplicate, and repeated twice.

Results and discussion

Characterisation of LAB strains using mass spectrometry

A total of 12 sourdough samples from the traditional bakery were examined microbiologically, and 62 LAB strains were isolated. Of these, eight LAB species were characterised; *Enterococcus faecium* (*n* = 3), *Lactobacillus casei* (*n* = 5), *L. fermentum* (*n* = 4), *L. lactis* (*n* = 6), *L. brevis* (*n* = 8), *L. pentosus* (*n* = 7), *L. plantarum* (*n* = 4), and *Pediococcus pentosaceus* (*n* = 9). Bartkiene *et al.* (2020) evaluated the antimicrobial and antifungal properties of LAB isolated from spontaneous sourdough, similar to this study, and identified similar LAB species. LAB flora in sourdough depends on the environmental conditions prevailing throughout the production, from dough preparation to fermentation. It is also possible that the microorganisms in the environment and the flour can survive in some sourdough. Various researchers have also suggested that the variety of LAB in sourdough varies with geographic location (Liu *et al.,* 2018; Sadeghi *et al.,* 2019; Maidana *et al.,* 2020).

In a study conducted to evaluate the diversity of LAB in Ya'an (Sichuan, China) sourdoughs, it was reported that the isolated LAB strains included *L. plantarum, L. pantheris, L. lactis, L. raffinolactis, Leuconostoc citreum, Leu. pseudomesenteroides, Leu. Mesenteroides*, and *Weissella viridescens* (Liu *et al.,* 2018). Another study investigating LAB's biological diversity isolated *E. faecium, , E. mundtii, L. lactis, L. rhamnosus* and *W. cibaria* from spontaneously fermented chia sourdough in Argentina (Maidana *et al.,* 2020). LAB isolated from traditional Chinese sourdough was identified as *L. plantarum* (Li *et al.,* 2019), while that from rice bran sourdough was *L. brevis* (Sadeghi *et al.,* 2019).

Detection of PAD-LAB strains and determination of extracellular phytase activities

All isolated strains (*n* = 46) were screened for phytase activity, and 30 revealed phytase activity in phytate medium, and thus identified as PAD-LAB strains. Extracellular phytase activities of PAD-LAB strains were determined by measuring the amount of inorganic phosphate released from sodium phytate by spectrophotometry. The isolates showing the highest activity were identified, ranging from 386.9 to 1,109.7 U/mL, with an average of 685.1 U/mL (Figure 1).

The level of phytase activity of LAB depends on the number and type of strains used to ferment the dough (Hashemi *et al.,* 2019). Various researchers isolated different PAD-LAB strains from sourdough. Nuobariene *et al.* (2015) identified PAD-LAB from Lithuanian sourdoughs, and found that the phytase-active isolates belonged to *L. panis, L. reuteri, L. fermentum*, and *P. pentosaceus*. Hashemi *et al.* (2019) also measured the phytase activities of three LAB strains (*L. casei, L. fermentum*, and *L. plantarum*) during fermentation of the dough, and found that the three lactobacilli strains significantly reduced phytate concentration during the 24 h fermentation.

In the study investigating the possible use of *L. brevis* in Barbari bread to enhance the nutritional bioavailability and decrease phytate, bread doughs were prepared in seven different combinations, with and without *L. brevis*. As a result, *L. brevis* IBRC-M10790 showed a better yield in phytate reduction than other combinations alone (approximately 96.5% reduction in phytate content). Thus, it could be used effectively to increase the nutritional properties of Barbari bread (Hadaegh *et al.,* 2019).

In another study, LAB and yeast strains with phytate-degrading ability were isolated from traditional Iranian sourdough, and identified molecularly. The isolated yeasts were *Kluyveromyces marxianus, K. lactis, and K. aestuarii*, while *E. faecium, P. pentosaceus*, and *Leu. citreum* were the isolated LAB (Fekri *et al.,* 2020).

Preparation of sourdoughs with combinations of PAD-LAB starter cultures

The five LAB strains demonstrating the high phytase activity were *P. pentosaceus* EM11 (1,109.7 U/mL), *L. pentosus* EM46 (957.9 U/mL), *L. fermentum* EM14 (932.7 U/mL), *L. brevis* EM21 (870.8 U/mL), and *L. lactis* EM63 (764.4 U/mL). Thirty-one different combinations were created using these five strains, and sourdough samples were prepared (Figure 1 and Table 1).

LAB enumeration and physicochemical analysis of sourdoughs

During the fermentation of sourdough samples, LAB count, pH, and TTA values were examined. Four sourdough samples (CS6, CS14, CS25, and CS30) that gave the best results in whole wheat bread production were selected (Table 1). The four selected sourdough samples' pH, ranging from 5.28 - 6.21 after kneading, decreased after fermentation (3.53 - 4.57). In addition, an increase in LAB number (after fermentation, 7.80 - 9.87 log

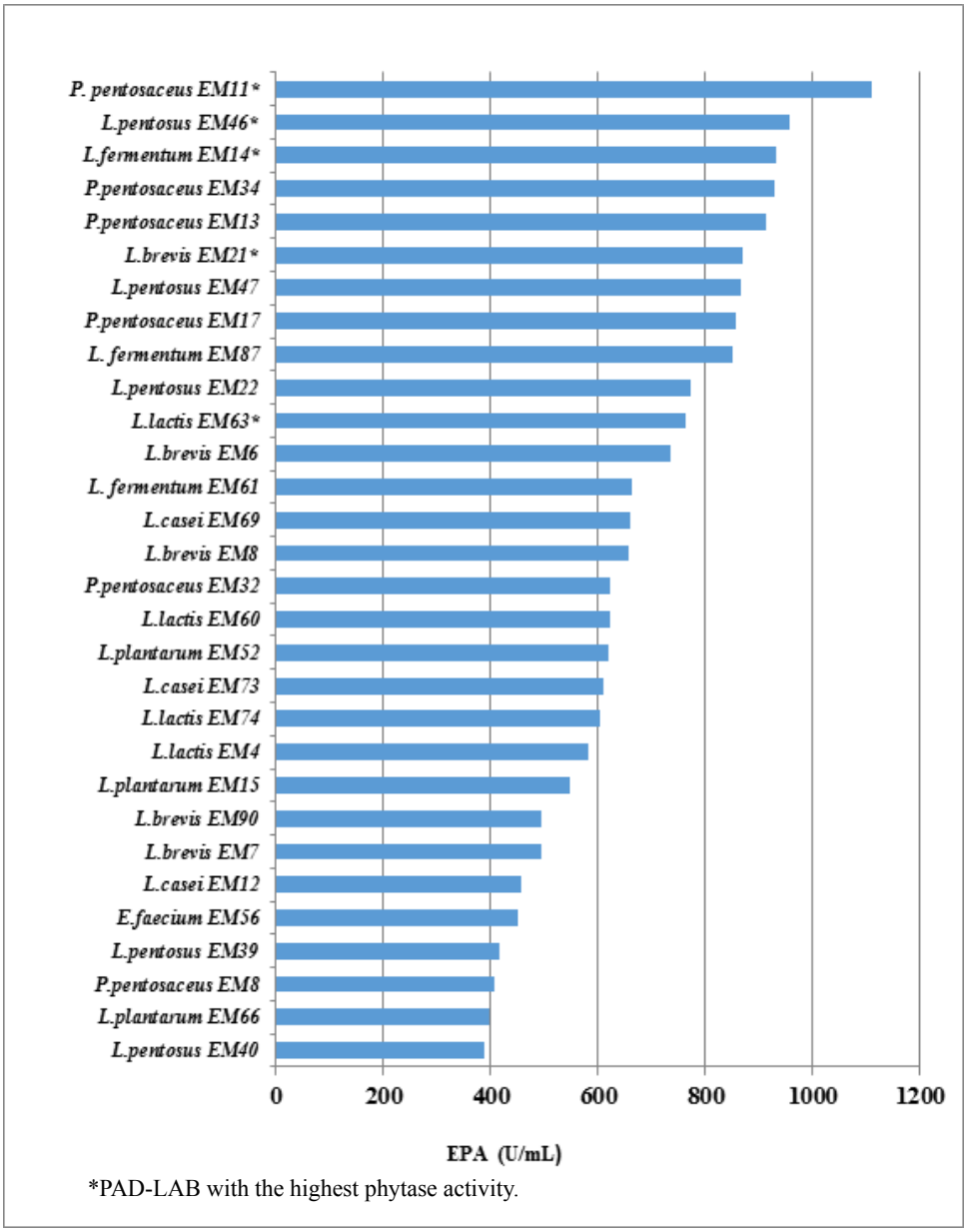

Figure 1. Extracellular phytase activity of PAD-LAB strains.

CFU/g) and values of TTA (after fermentation, 10.17 - 14.29 mL) of these samples was also observed (Table 1).

The dough's rich nutritional content as an energy source for LAB supported its proliferation and growth. As a result of LAB's continued metabolic activity during fermentation, the organic acids and increased lactate concentration led to decreased pH (Hashemi *et al.,* 2019). The ripe sourdough's pH varies depending on the type of starter culture and processing, and usually lies between 3.5 - 4.5 (Fekri *et al.,* 2020).

Hadaegh *et al.* (2019) reported that adding *L. brevis* as PAD-LAB to the dough to increase the nutritional properties of bread reduced the pH significantly. They revealed that sourdoughs containing bacteria had a lower pH (4.55) than sourdoughs without bacteria (5.39). Fekri *et al.* (2020) reported that while the LAB-free control group's pH was 5.73, the pH of sourdoughs containing *E. faecium, P. pentosaceus*, and *Leu. citreum* were 3.78, 3.41, and 4.27, respectively. Similarly, in the present work, the control group's pH was 4.57, while the average pH of the four selected sourdough samples was 3.67. Thus, the sourdough samples prepared using starter culture combinations had a 20% lower pH value than the sourdough samples prepared by spontaneous fermentation. In contrast, the LAB number and TTA value in the experimental group were 22.8 and 22.6% higher than the control sample, respectively (Table 1).

Specific volume, pH, and TTA values of sourdough breads produced with sourdough containing PAD-LAB starter cultures

The pH, TTA values, and specific volume of the sourdough bread samples were determined. As seen in Table 2, the control sample's pH was higher

Table 1. Microbiological and physicochemical results of sourdough samples prepared with PAD-LAB combinations.

Combination code	LAB (log CFU/g)			pH				TTA (mL)		
	24 h	48 h	72 h	0 h	24 h	48 h	72 h	0 h	24 h	48 h	72 h
C*	6.75	6.98	7.80	6.21	5.82	5.38	4.57	2.15	6.35	9.23	10.31
CS1	7.16	8.09	8.72	5.93	5.16	4.84	3.95	2.43	4.76	10.68	11.10
CS2	6.82	7.93	8.60	6.30	5.59	5.17	4.70	1.97	4.56	9.44	12.67
CS3	7.34	7.81	8.63	5.41	4.84	4.48	3.86	1.75	8.61	9.74	13.16
CS4	6.56	7.54	6.96	6.26	5.77	5.32	4.81	1.67	4.03	8.60	11.81
CS5	8.60	8.98	9.16	6.12	5.69	4.86	4.62	2.17	5.26	10.20	9.88
CS6**	7.19	9.17	9.57	6.12	4.80	3.65	3.68	2.32	6.96	12.38	10.17
CS7	7.47	8.33	8.71	6.27	5.82	5.15	4.80	2.35	4.32	10.83	12.39
CS8	6.44	7.25	8.32	6.48	5.92	5.72	4.98	2.18	5.96	11.68	10.65
CS9	7.70	8.67	9.04	5.46	4.97	4.75	4.35	1.88	5.41	12.79	9.48
CS10	7.25	8.47	9.23	5.38	4.64	4.28	4.12	1.93	5.66	9.45	11.50
CS11	6.35	7.02	7.36	6.49	5.94	5.13	4.78	2.32	4.30	11.78	9.95
CS12	7.56	8.42	8.86	6.01	5.51	4.84	4.66	2.14	5.17	9.61	11.40
CS13	6.49	7.80	8.51	5.83	5.13	4.80	4.18	1.98	4.30	12.80	10.70
CS14**	8.31	9.37	9.18	5.48	4.36	3.74	3.79	1.81	6.48	11.39	12.60
CS15	7.68	8.03	8.69	5.64	4.91	4.55	4.27	2.31	5.16	10.94	9.66
CS16	6.97	7.19	8.28	6.19	5.89	4.95	4.13	2.44	5.42	9.51	10.37
CS17	7.60	8.46	8.54	6.37	5.42	4.75	4.26	2.24	4.15	9.36	10.45
CS18	7.04	7.81	8.99	5.92	5.23	4.87	4.12	1.74	5.67	9.55	12.42
CS19	5.62	6.96	8.60	5.78	4.93	4.66	4.26	2.42	5.76	9.21	12.60

*C: Control (spontaneous fermentation); CS1: EM46; CS2: EM14; CS3: EM21; CS4: EM63; CS5: EM11; CS6: EM46 + EM14; CS7: EM46 + EM21; CS8: EM46 + EM63; CS9: EM46 + EM11; CS10: EM14 + EM21; CS11: EM14 + EM63; CS12: EM14 + EM11; CS13: EM21 + EM63; CS14: EM21 + EM11; CS15: EM63 + EM11; CS16: EM46 + EM14 + EM21; CS17: EM14 + EM21 + EM63; CS18: EM21 + EM63 + EM11; CS19: EM63 + EM46 + EM11; CS20: EM63 + EM46 + EM21; CS21: EM11+ EM46 + EM21; CS22: EM11 + EM14 + EM21; CS23: EM11 + EM63 + EM14; CS24: EM46 + EM63 + EM14; CS25: EM46 + EM14 + EM11; CS26: EM46 + EM14 + EM21 + EM63; CS27: EM14 + EM21 + EM63 + EM11; CS28: EM46 + EM21 + EM63 + EM11; CS29: EM46 + EM14 + EM63 + EM11; CS30: EM46 + EM14 + EM21 + EM11; and CS31: EM46 + EM14 + EM21 + EM63 + EM11. **Culture combinations selected as starter culture in the production of sourdough whole wheat breads.

than sourdough yeast samples, while the TTA value and specific bread volumes were lower. The control samples, pH, TTA, and specific bread volumes were 5.84, 2.78 mL, and 1.97 mL/g, respectively. However, the sourdough samples' pH value varied between 5.01 and 5.36, while the TTA values ranged from 3.79 to 3.98 mL, and specific bread volumes ranged from 2.10 to 2.87 mL/g. Whole wheat bread produced with sourdough prepared with CS30 PAD-LAB starter culture combination (B2 coded bread sample) had the lowest pH value (5.01), while it had the highest TTA value (3.98 mL) and specific bread volume (2.87 mL/g).

Various studies showed results similar to our findings that sourdough improves the physicochemical properties of bread dough. Savkina *et al.* (2019) developed a new microbial starter culture composition from dough samples using different sourdough amounts. They reported that four of the five samples had a higher specific volume (11.5 -

Table 2. Physicochemical results of bread samples

	pH	TTA (mL)	Specific volume of bread (mL/g)
CB	5.84 ± 0.21	2.78 ± 0.60	1.97 ± 0.03
B1	5.36 ± 0.18	3.82 ± 0.28	2.10 ± 0.07
B2	5.01 ± 0.09	3.98 ± 0.35	2.87 ± 0.02
B3	5.23 ± 0.12	3.85 ± 0.42	2.31 ± 0.03
B4	5.17 ± 0.13	3.79 ± 0.24	2.56 ± 0.06

CB = Control (spontaneous fermentation); B1 = CS25 (EM46 + EM14 + EM11); B2 = (CS30: EM46 + EM14 + EM21 + EM11); B3 = (CS14: EM21 + EM11); B4 = (CS6 - EM46 + EM14).

15.4%) than the control group. In contrast, only one sample was 7.7% lower than the control group since the large quantity of sourdough may have inhibited yeast. Xu *et al.* (2019) determined that sourdough gave a higher bread volume than chemically acidified dough. In another study, it was reported that the volume of all bread samples increased during fermentation, and the increase in sourdough bread was slightly but significantly higher than the control group (Rizzello *et al.,* 2019).

Sourdough contains lactic and acetic acids produced by LAB, which increase the acidity of the dough. Depending on the level of lactic acidification, sourdough fermentation causes a specific smell and sour taste and enhances bread volume (Savkina et al., 2019).

Sensory analysis of sourdough breads produced with sourdough containing PAD-LAB starter cultures

The panellist evaluations of the crust colour, interior colour, smell, taste, pore structure and general preference of the bread samples are presented in Table 3. For breads produced from sourdough samples using starter culture combinations, the crust colour scores were between 3.23 to 4.38, the interior colour scores were between 3.08 to 4.08, the smell scores were between 2.85 to 4.38, the taste scores were between 2.62 to 4.31, and the pore structure scores were between 2.77 to 4.23. The general preference scores were between 3.08 to 4.31, while the average scores were between 2.94 to 4.28 (Figure 2). Bread prepared from spontaneous fermentation had lower scores. Savkina *et al.* (2019) compared the crust colour, pore structure, taste, and smell characteristics of the bread obtained from different doughs. They reported that the bread produced with sourdough had a higher score in all parameters. Meng *et al.* (2020) also reported that sourdough improved the sensory qualities of fermented rice cakes. In contrast, Rizzello *et al.* (2019) showed that the crust colour and pore structure evaluations of bread made with sourdough were high, but there was no significant difference in the overall taste.

From the sensory quality evaluation, the whole-wheat bread produced using sourdough prepared with B2 coded bread sample (CS30:

Table 3. Sensory quality characteristics (1 – 5) of bread from sourdough samples produced using starter culture combinations.

	Crust colour	Interior colour	Smell	Taste	Pore structure	General preference
CB	3.23 ± 0.28^{b}	3.08 ± 0.37^{b}	2.85 ± 0.39^{b}	2.62 ± 0.42^{b}	2.77 ± 0.28^{c}	3.08 ± 0.29^{b}
B1	4.00 ± 0.20^{a}	4.08 ± 0.21^{a}	4.00 ± 0.20^{a}	4.00 ± 0.20^{a}	3.92 ± 0.24^{ab}	3.92 ± 0.24^{a}
B2	4.38 ± 0.14^{a}	4.08 ± 0.18^{a}	4.38 ± 0.18^{a}	4.31 ± 0.21^{a}	4.23 ± 0.20^{a}	4.31 ± 0.21^{a}
B3	4.15 ± 0.22^{a}	3.92 ± 0.21^{a}	4.00 ± 0.23^{a}	3.77 ± 0.17^{a}	4.08 ± 0.18^{ab}	4.23 ± 0.12^{a}
B4	4.15 ± 0.15^{a}	3.77 ± 0.20^{a}	3.69 ± 0.24^{a}	3.85 ± 0.22^{a}	3.46 ± 0.27^{bc}	3.92 ± 0.18^{a}

Means in the same row with different lowercase superscripts differ significantly ($p < 0.05$).

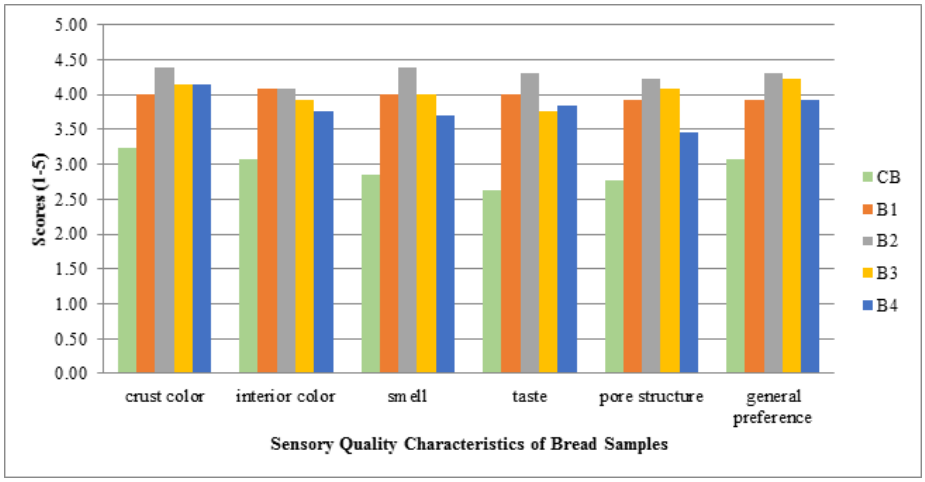

Figure 2. Crust color, interior color, smell, taste, pore structure, and general preference scores according to the sensory analysis made from sourdough samples produced using starter culture combinations.

L. pentosus EM46, *L. fermentum* EM14, *L. brevis* EM21, and *P. pentosaceus* EM11; PAD-LAB starter culture combination) was preferred. The B2 coded bread sample showed the highest score with an average of 4.31 ± 0.21 scores in all parameters (Table 3).

Conclusions

In the present work, phytase-active strains of LAB isolated from sourdough were screened. Strains with the highest phytase activity (PAD-LAB) were determined, and used in the starter culture combinations to produce whole-wheat bread. Overall, breads produced with PAD-LAB strains as the starter culture had significantly better physicochemical and sensory quality as compared to control (spontaneous fermentation). In addition, considering the results of phytic acid degradation and bread quality sensory analysis, it is recommended to use *L. pentosus, L. fermentum, L. brevis*, and *P. pentosaceus* strains together as a starter culture. PAD-LAB strains with high extracellular phytase activity in bread production as a starter culture could increase the sensory quality properties and contribute to their bioavailability. In countries where bread consumption is high, starter cultures with enhanced functional properties in the bakery can positively affect public health. In future studies, advanced biotechnological studies on starter cultures with accepted sensory quality characteristics may be recommended.

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