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Preventing of bread mould spoilage and reducing the use of calcium propionate in bread by using antifungal lactic acid bacteria

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ABSTRACT

In this study, 8 lactic acid bacteria with antifungal activity and suitable for sourdough production were used in sourdough bread production to determine the effect of bread on prolonging shelf life and to compare with calcium propionate used as a chemical preservative in the food industry/bakery. For this, *Weissella cibaria* 908, *Leuconostoc pseudomesenteroides* 2619, *Fructilactobacillus sanfranciscensis* 2709, *Levilactobacillus brevis* 2216Y, and *L. plantarum* subsp. *plantarum Y201* isolates were selected for bread production by paying attention to changes in total acidity and pH values, blistering volumes, organic acid production profiles in bread dough. Total 9 different types of bread were produced. Among them at the end of the study, it was observed that the shelf life of bread containing selected antifungal lactic acid bacteria mixed culture (1:1:1:1:1) and 0.15% calcium propionate was prolonged compared to commercially available breads where 0.3% calcium propionate is allowed. Thus, the use of calcium propionate can be reduced by half. It was observed that there was no significant difference (P>0.05) between the commercially sold and the produced sourdough bread in terms of general admissibility and it was concluded that it can be used in sourdough bread production.

1. Introduction

Food product, depending on the contents and storage conditions, has low quality and certain shelf life due to microbial, enzymatic, chemical or physical changes. Changes in microbial origin can cause taste, odor, color, and textural disorders. Also, toxin formation or the development of pathogen microorganisms threaten consumer health. In many countries, bread, which is the main foodstuff, is generally easily perishable like other foods. Two main factors that shorten the shelf life of bread are staling and microbiological deterioration (Mohsen, Aly, Attia, & Osman, 2016). The main species responsible for the microbial degradation of bakery products are: *Aspergillus*, *Fusarium* and *Penicillium*, which are members of the genus. The presence of mould carries the risk of potential mycotoxin production that can cause public health problems as well as major economic losses (Legan, 1993). Therefore, propionic acid and its salts, packaging modified atmosphere, radiation, pasteurization of packed bread and other biological control methods can be utilized for preventing or minimizing mould growth on bread (Dal Bello et al., 2007; Legan, 1993). In recent years, the interest in biological (biopreservation) methods to control microbiological deterioration instead of natural food preservatives has increased because of increasing negative consideration against the use of chemicals in food products (Chen et al., 2021; Nodem Sohanang et al., 2021).

Biopreservation methods can be defined as methods of prolonging the shelf life of food and making the product safer via some microorganisms that are naturally found in food or added as protective culture and/or using their antimicrobial products. Microorganisms and/or their metabolites used as protective agents are required to inhibit pathogens and/or prolong the shelf life while causing little undesirable changes in the sensory properties of food products (Devlieghere, Vermeiren, & Debevere, 2004). Sourdough bread, which draws attention to the production of antimicrobial metabolites and including lactic acid bacteria, is a very suitable food for the use of the biopreservation method. Sourdough fermentation provides an increase in the nutritional value and shelf life of bread while concurrently improving its organoleptic properties.

The interaction of yeasts lactic acid bacteria is of great importance in sourdough production. In sourdough microbiota, there are *Saccharomyces cerevisiae*, which is most effective and dominant in bread swelling, and some other yeasts and lactic acid bacteria that belong to the genus *Lactobacillus* (Mohsen et al. 2016). The products produced as a result of the metabolic activities of these microorganisms and the resulting

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changes greatly affect the rheology, flavor, nutrients and functional properties of sourdough-based baked products (De Vuyst & Vancanneyt 2007; Mohsen et al. 2016). For these reasons, it is important to use lactic acid bacteria, which are found in natural microbiota with antifungal activity in sourdough production, to gain the organoleptic properties expected from lactic acid bacteria and to extend the shelf life of the bread.

In this study, lactic acid bacteria with Antifungal activity and suitable industrial properties were isolated from sourdough bread and used in sourdough bread production; to increase the shelf life of breads, to reduce the amount of calcium propionate chemical additive which is used as a preservative in bakery, and to produce bread of standard quality.

2. Materials and methods

2.1. Materials

In this study, eight antifungal potential LAB isolates (Weissella cibaria 908, Lactiplantibacillus plantarum subsp. plantarum 2114, Leuconostoc pseudomesenteroides 2619, L. plantarum subsp. plantarum 2702, Fructilactobacillus sanfranciscensis 2709, Levilactobacillus brevis 2216Y, L. pentosus Y118 and L. plantarum subsp. plantarum Y201eight LAB) previously isolated from Turkish spontaneous sourdough and selected for their antifungal activity against Aspergillus favus, Aspergillus niger, and Penicillium expansum (Arsoy, Gul, & Con, 2022; Yurttas, 2018) were studied. Aspergillus niger ATCC 16888, Aspergillus flavus MAM200682 and Penicillium expansum were used as indicators to prove the effect of antifungal activity on mould growth in bread.

2.2. Bread production

The method given by Gerez, Torino, Rollán, & Font de Valdez (2009) was used in the production of bread dough using antifungal LAB isolates. Antifungal LAB isolates were grown in MRS broth (Merck, Darmstadt, Germany) at 32 °C under anaerobic conditions for 16 hours to be used as a starter in bread production. Then, the cultures were harvested by centrifugation at 4500 rpm for 10 min, washed twice with sterile saline water and resuspended to obtain cultures contain 9 log CFU/mL of cells. Flour (250 g), sucrose (5 g), skimmed milk (12.5 g), and tap water (750 mL) were mixed by a dough blender to form dough and then microbial cultures were added to dough to obtain approximately 7 log CFU/g. Semi-liquid fermented dough to be used in the preparation of bread dough was obtained by fermenting at 32ºC for 16 h.

Considering the method given by Gerez, Torino, Obregozo, & De Valdez (2010), dough containing commercial wheat flour (1000 g), NaCl (15 g), sucrose (20 g), skim milk (50 g), margarine (10 g), water (550 mL) and *Saccharomyces cerevisiae* (7 log CFU/g) (Pakmaya Yeast Co., İzmir, Turkey) for the production of control bread has been prepared. Calcium propionate (0.3%, w/w) was added to the dough to be used as antifungal control. In the preparation of bread dough to be produced with antifungal LAB isolates as starter, semi-liquid fermented dough was added instead of only 20%, 40% and 60% of the water to be used in the dough prepared with the same composition.

Bread doughs prepared with the compositions described above were kneaded in a mixer (KitchenAid) for an average of 7 min and fermented at 27°C for 1 h. Afterwards, the doughs were divided into 160 g pieces and left to ferment for 10 min by airing to be folded by hand. After shaping, dough samples were placed in pans and left for final fermentation at 35°C. Final fermentation was continued until the dough height reached 7 cm prove height (2.5-3 hours). The fermented doughs were baked in a 200 °C oven (Özköseoğlu, PFS9-E, Turkey) for 20 min and then cooled to room temperature (Koca & Anil, 2007). Determination of the characteristics of the breads was carried out after cooling (90 minutes) to room temperature.

2.3. Determination of physicochemical properties of sourdough and bread

The pH values of the doughs were measured by immersing the glass electrode of the pH meter (Hanna instruments, HI2002-02 Edge) directly into the dough. For the determination of pH values of bread samples, bread sample (5 g) was mixed with 25 mL of distilled water and the mixture was homogenized by using ultraturrax (IKA-Werke, T25 Basic, Germany). Then, the volume was filled up to 50 mL with distilled water, and then the pH value of the bread was measured with a pH meter.

In order to determine the total titratable acidity of the dough and bread samples, 25 mL of distilled water was added to 5 g of dough or bread sample and the mixture was homogenized by mixing with ultraturrax. It was then filled up to 50 mL with distilled water and titrated with 0.1 NaOH until the pH value as 8.1 with a pH meter. The amount of 0.1 N NaOH consumed in the titration was recorded and the calculation was made in terms of equivalent lactic acid.

The swelling power of the dough was evaluated by subtracting the initial volume (cm^3) from the final volume (cm^3) of the 20 g dough fermented in a measuring cylinder at 30 ºC for 2 h (Gerez et al., 2010).

The total fermentation time of the dough samples were determined by adding the waiting times in all fermentation periods from kneading to placing in the oven.

Bread volume was determined based on the principle of rapeseed replacement using the method given by Greene & Bovell-Benjamin (2004).

2.4. Determination of organic acid

To determine the organic acid content of the dough and bread, 10 g of dough sample was homogenized with 60 mL of pure water, completed to 100 mL with pure water, and centrifuged for 5 minutes at 4500 rpm. After taking 20 mL of the supernatant part, adding 5 mL of Carrez I solution (potassium II hexaferrocyanate; 0.085 mol/L) and mixing then 5 mL of Carrez II (zinc sulfate, 0.25 mol/L) solution was added and waited for a while adjusting to pH 8 (±0.5) with 0.5 mol/L NaOH. The volume was completed to 50 mL with distilled water, passed through filters with a pore size of 0.22 µm at twice and 10 µL of filtrate was injected into HPLC (UHPLC, Agilent 1290 Infınıty II) (Robert et al., 2006). Solvent A (100 mM KH_2PO_4, pH 2, 65%) and solvent B (water containing 1% acetonitrile; 35%) were used as mobile phase. The analysis was carried out with a photodiode array (PDA) detector at 210 nm wavelength, using ACE 5, C18 column (250x4.6 mm, batch no:V11-6053), at 25 ºC constant temperature and 25 min at 0.85 mL/min flow rate. The organic acid contents and quantities of the samples were determined by making use of the calibration curve based on the exit time and areas of the peaks. The fermentation rate of bread is also from the amount of lactate and acetate, using the formula of Fermentation Rate (FQ) = lactate / acetate (Gerez et al., 2009).

2.5. Microbiological analysis

The LAB and yeast-mould counts of the dough samples were determined using the pouring method. LAB count was determined using 200 µg/mL MRS agar (Merck, Darmstadt, Germany) with cycloheximide, and yeast-mould count was determined using DRBC agar (Merck, Darmstadt, Germany). Petri dishes were incubated for 48 hours at 32°C for LAB count and 72 hours at 30°C for yeast-mould count. The results obtained by counting all the developed colonies were expressed as log CFU/g (Gerez et al., 2010; Gerez et al., 2009).

2.6. Determination of bread moulding

Half of the breads baked and cooled from dough prepared with and without the addition of antifungal LAB isolate and calcium propionate were sliced 7 mm thick and the other half was unsliced. One mL of conidial mould suspension containing 10^4 cfu/mL cells was spread on the surfaces of slices and whole breads per 100 g of bread. Then it was packed in polyethylene packages and kept at 25°C. Daily observations were made for significant mould growth in packaged slices and whole breads (Gerez et al., 2009).

2.7. Sensory properties of bread

Sensory evaluation of bread samples was carried out by 13 trained panelists. Breads were prepared as two separate tables with parallels, coded with three-digit random numbers and presented to the panelists. Panelists were asked to evaluate breads in terms of crust color, crust structure, inner color, pore structure, texture, odor, taste and general acceptance. A standard ten-point hedonic scale from 1 (very bad) to 10 (very good) was used to measure acceptance-preference of product. The panelists were also asked to mark the wrong situations they saw in the breads on the form.

2.8. Statistical Analysis

The statistical analysis of the data obtained was made with a single-factor ANOVA and Tukey Multiple Comparison Test using the Windows-based SPSS 20.0 statistical package program in the 95% confidence interval (P<0.05).

3. Results and Discussion

3.1. Effect of antifungal lab isolates on semi-liquid dough and bread dough properties

Based on the acid production capabilities and the bulking volumes of doughs samples, 8 different antifungal LAB isolated from sourdoughs were selected for the production of sourdough bread. Fermentation time of semi-liquid dough and the rate of semi-liquid dough to be used were determined. It has been determined that there are significant differences between the antifungal LAB isolates related with acid production capacities and pH reduction in semi-liquid dough (Figure 1a). The semi-liquid dough produced using LAB isolates showed a pH of around pH 6.0 and close to each other at the beginning of the fermentation process. After 12 and 16 h fermentation, pH values decreased and significantly different (P<0.05) among the samples. At the end of 16 h fermentation, it decreased to 3.5-5.5 pH value depending on starter LAB type. Similarly, the total acidity (around 0.07%) values that were close to each other at the beginning of fermentation, differed at the end of fermentation (16 hours) and increased to 0.10-0.38% (Figure 1b). Based on these results, it was decided that LAB isolates had a significant effect on the pH value and organic acid accumulation after the 8th h of fermentation, this decline continued at the 16th h and it was appropriate to perform 16 h fermentation for all semi-liquid dough to be prepared.

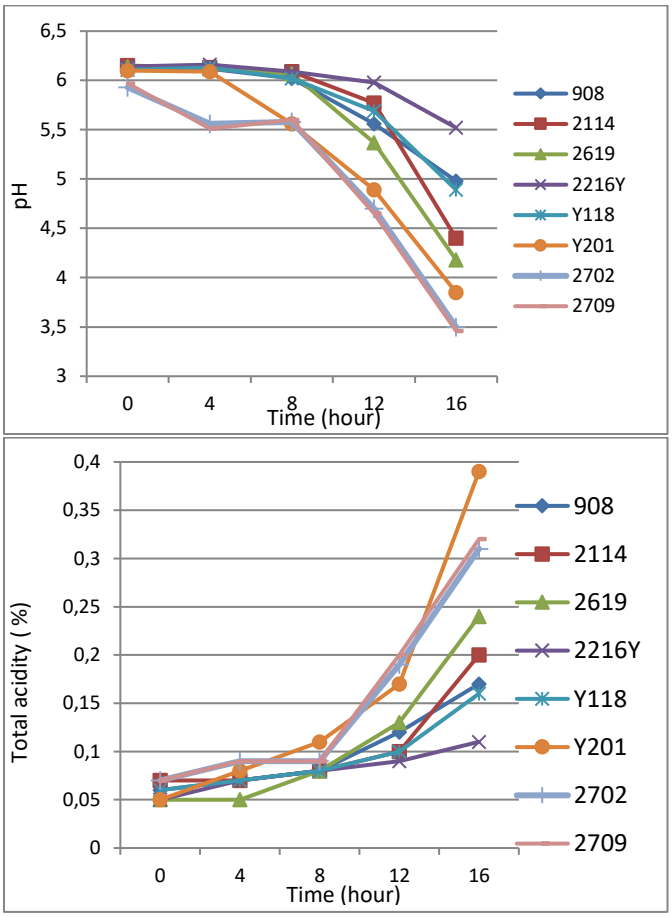


Figure 1. Change of pH and total acidity values of liquid dough produced using LAB isolates during fermentation (*W. cibaria* 908, *L. plantarum* subsp. *plantarum* 2114, *Leu. pseudomesenteroides* 2619, *L. plantarum* subsp. *plantarum* 2702, *L. sanfranciscensis* 2709, *L. brevis* 2216Y, *L. pentosus* Y118, *L. plantarum* subsp. *plantarum* Y201)

To determine the rate of using semi-liquid dough obtained by applying fermentation for 16 h in bread making and to select the appropriate isolates, the dough samples were prepared by adding semi-liquid dough samples in the ratio of 20 to 60% and their acid production and swelling strengths were determined.

The pH values of bread dough samples which were prepared by using fermented semi-liquid dough samples, initially varying 5.43-6.65 pH decreased to 4.49-6.05 pH after 4 h fermentation. Semi-liquid dough addition rates (20% 40% and 60%) were found to be effective on the pH values reached after 4 h of fermentation in the dough samples (P<0.05). Both the fermented semi-liquid dough addition rates and the LAB isolate types that were added as a starter were effective on the swelling power of bread dough (P<0.05). In general, the swelling power was low in the dough in which the total acidity value and pH was high low, respectively.

Considering the effect of antifungal LAB isolates on total acidity, pH and swelling volume in bread dough. It has been decided that it is suitable to use in the production of bread by adding 60% of semi-liquid dough containing *W. cibaria* 908, *Leu. pseudomesenteroides* 2619, *L. sanfranciscensis* 2709, *L. plantarum* subsp. *plantarum* Y201, and *L. brevis* 2216Y isolates.

3.2. Effect of antifungal LAB isolates selected as starter on important properties in bread production

In this study, total 9 different breads was produced. Five of these with semi-liquid fermented dough containing single culture, the others; LAB Mixed Culture 1 (5 LAB isolates combined in 1:1:1:1:1 ratio) bread dough with semi-liquid fermented dough, LAB Mixed Culture 2 (5 LAB isolates combined in 1:1:1:1:1 ratio + 0.15% CaP) bread dough with semi-liquid fermented dough, Control 1 only yeast (*S. cerevisiae*) without semi-liquid fermented dough and Control 2 bread dough containing 0.3% CaP and Yeast (*S. cerevisiae*).

3.3. Effect of starter antifungal LAB isolates on bread dough properties

To determine the effect of antifungal LAB isolates on bread dough properties, pH, total acidity, swelling volume and microbial contents were determined by taking samples from the liquid dough, dough (before main fermentation) and final dough (after last fermentation) samples. The data obtained are given in Table 1. Selected 5 LAB isolates, which were used either individually or mixed cultures, reduced the pH values of the doughs as 0.39 to 0.98 and increased the total acidity values as 0.10-0.41% compared to the control samples. Similarly, Gerez et al. (2010) and Robert et al. (2006) reported that pH values reached after fermentation are in these ranges. The pH values indicated that the target decrease can be achieved with these LAB isolates. There is a statistical difference between the decreases in the pH value of different LAB isolates in the dough samples ($P<0.05$). While there was no statistically significant difference between the LAB numbers of dough containing different LAB isolates, this value was determined as low as expected (4.68 ± 0.13 and 4.44 ± 0.09 CFU/g) in the control groups and it was found to be significantly different from those containing LAB isolate. This number, which is determined as 8.41 log CFU/g after kneading in bread dough prepared by adding semi-liquid doughs prepared using LAB isolates, was determined as 8.71 log CFU/g after 4 h fermentation. The average number of 7.62 log CFU/g yeast determined at the beginning of the dough prepared for the production of sourdough bread did not change much after 4 h of fermentation (7.59 log CFU/g). It is thought that a small increase in the number of LAB in the initial and final fermentation steps can be attributed to the adaptation of the LAB's to the medium due to the addition of LAB as semi-liquid sourdough and a small decrease in the number of yeast can be associated with the inability to fully adapt to the environment due to the use of yeast directly in bread dough or the counting method. Similar studies that are in line with these results are also included in the literature (Lefebvre, Gabriel, Vayssier, & Fontagné-Faucher, 2002; Robert et al., 2006).

The swelling volumes of Control 1 and Control 2 bread doughs were 50-51 cm^3, while the swelling volumes of dough prepared with *W. cibaria* 908 and Mixed Culture 2 were significantly lower with a value of 44 cm^3 and below. The swelling volumes of the dough containing other LAB isolates (between 48-52 cm^3) were determined to be very close to the control dough. This indicate that the selected LAB isolates are sufficient for this feature, which is important in the bakery.

It has been determined that LAB selected for sourdough production have an important effect on organic acid content, which is important in terms of shelf life, aroma, taste and texture of their breads, and that there are significant differences between the control and dough samples containing LAB isolates. While the lactic and acetic acid levels in the dough samples containing LAB isolates individually ranged between 27.46-62.58 mM and 2.81-27.01 mM, respectively; those that contain mixed cultures were determined between 71.91-105.10 mM and 23.47-37.08 mM, respectively, and there was a statistically significant difference ($P<0.05$) between single and mixed cultures. It has been determined that lactic and acetic acid are dominant in sourdough and the highest amount of these acids in the dough that contains the mixed culture (Table 2). Likewise, Mohsen et al. (2016) reported that the use of LAB together with yeast leads to increasing the level of lactic and acetic acid more than other organic acids because acetic and lactic acid are essential organic acids in fermentation of sourdough. Propionic acid was determined only in Mixed Culture 2 and Control 2 breads, in which calcium propionate was added. The amount detected was also proportional to the added calcium propionate. Lactate/acetate (FQ) value, which was reported to be important in terms of sourdough bread quality and the optimum value between 2-2.7 by Hammes & Ganzle (1998), was found very close to these values in mixed cultures. Gerez et al. (2010) reported that FQ values are determined to be the best between 1.3 and 2.8 in the doughs prepared with *Lactobacillus brevis* CRL 772 and CRL 796, *L. plantarum* CRL 778, *L. reuteri* CRL 1100 strains.

3.4. Effect of starter antifungal LAB isolates on bread properties

The fermented dough samples are baked at 200 °C in an oven for 20 min and then cooled to room temperature. In the produced breads, lowest acidity and highest pH values (0.30% and 0.32% acidity; 5.73 and 5.56 pH, respectively) in Control 1 and Control 2 samples were detected, which did not use the lactic starter as in dough ($P>0.05$). pH and acidity values were determined between 4.47-4.69 and 0.59-0.51% in bread produced with sourdough containing lactic starter cultures ($P>0.05$). The highest pH value was determined as 5.22 pH and total acidity 0.38% in the bread produced using *W. cibaria* 908. An increase in pH was observed by the heat treatment of the doughs. In a study conducted by Robert et al. (2006), LAB strains were added and left in the incubation for 15-20 h and the pH value drops below 4.0. When they make bread, it is stated that the pH rises above 4.0 and there is also a decrease in the total acidity. It has been stated by Rizzello, Cassone, Coda, & Gobbetti (2011) that when sourdough is added to wheat bread with a pH of 5.0, pH drops to around 4.0 and reaches higher titration acidity than others. These data are compatible with the results of the study.

The use of sourdough in bread making did not significantly change the physical properties of bread, such as weight and volume, compared to control bread. The specific volumes ranged 3.46-3.93 cm^3/g of the breads were determined and there was no statistically significant difference between them ($P>0.05$). It is an important finding in terms of the bakery. Robert et al. (2006) also stated that the addition of sourdough does not affect the physical properties of bread, such as volume and weight.

As in the bread samples, lactic and acetic acid were determined in all of the products produced using a lactic starter culture, while Control 1 and Control 2 samples were not detectable at the level of lactic and acetic acid. Propionic acid was only detected in bread with calcium propionate (Table 3). The highest amount of lactic acid was obtained in the bread made with Mixed Culture 2 (80.79 mM) and this value was followed by *L. sanfranciscensis* 2709, *L. brevis* 2216Y, Mixed Culture 1 isolates, which were not statistically significant ($P>0.05$). To obtain the highest amount of lactic acid in Mixed

Table 1. pH, total acidity, microbial content and swelling volume values of bread dough produced with antifungal LAB isolates, produced with the liquid dough after kneading and before baking.

Starter culture	Dough type	pH	Total acidity (%)	LAB number (log cfu/g)	Yeast-mould number (log cfu/g)	Swelling volume (cm^3)
Control 1	Dough (BF)*	5.21±0.06^{a}*	0.23±0.03^{a}	4.15±0.14^{a}	7.61	51
(S. cerevisae)	Dough (AF)*	5.15±0.06^{D}	0.40±0.04^{A}	4.69±0.13^{A}	7.63	
Control 2	Dough (BF)	5.31±0.07^{a}	0.23±0.04^{a}	4.12±0.19^{a}	7.62	50
(*S. cerevisae* + 0.3% CaP)	Dough (AF)	5.07±0.07^{D}	0.42±0.01^{AB}	4.44±0.09^{A}	7.54	
	Liquid dough	4.24	0.28	8.63	3.13	
***W. cibaria* 908**	Dough (BF)	5.27±0.21^{a}	0.28±0.01^{ab}	7.43±0.67^{b}	7.42	<44
	Dough (AF)	4.68±0.09^{C}	0.50±0.03^{ABC}	8.45±0.01^{BC}	7.77	
	Liquid dough	3.18	0.64	8.81	1.00	
***L. pseudomesenteroides* 2619**	Dough (BF)	4.97±0.15^{a}	0.36±0.00^{ab}	8.13±0.53^{b}	7.30	49
	Dough (AF)	4.09±0.01^{A}	0.63±0.01^{CDE}	8.17±0.44^{BC}	7.61	
	Liquid dough	3.25	0.65	9.00	2.43	
***L. sanfranciscensis* 2709**	Dough (BF)	5.14±0.23^{a}	0.31±0.02^{ab}	8.47±0.11^{b}	7.52	48
	Dough (AF)	4.18±0.06^{AB}	0.67±0.09^{DEF}	8.69±0.30^{BC}	7.43	
	Liquid dough	3.25	0.63	8.72	2.33	
***L. plantarum* subsp. *plantarum* Y201**	Dough (BF)	5.00±0.12^{a}	0.48±0.16^{b}	7.87±0.16^{b}	7.95	52
	Dough (AF)	4.41±0.13^{BC}	0.57±0.02^{BCD}	7.94±0.11^{B}	7.49	
	Liquid dough	3.34	0.58	9.77	<1.00	
***L. brevis* 2216Y**	Dough (BF)	4.88±0.21^{a}	0.42±0.007^{ab}	8.59±0.11^{b}	7.81	51
	Dough (AF)	4.14±0.01^{AB}	0.73±0.04^{EF}	8.95±0.07^{C}	7.86	
	Liquid dough	3.32	0.59	9.20	<1.00	
Mixed Culture 1	Dough (BF)	5.14±0.28^{a}	0.39±0.007^{ab}	8.62±0.54^{b}	7.56	48
(All LAB Isolates)	Dough (AF)	4.15±0.10^{AB}	0.76±0.04^{EF}	8.94±0.03^{C}	7.89	
	Liquid dough	3.42	0.55	9.29	<1.00	
Mixed Culture 2	Dough (BF)	5.20±0.05^{a}	0.40±0.021^{ab}	8.45±0.18^{b}	7.60	44
(All LAB Isolates + 0.15% CaP	Dough (AF)	4.39±0.01^{BC}	0.81±0.04^{F}	8.83±0.17^{C}	7.56	

BF: Before Fermentation, AF: After Fermentation, LAB: Lactic Acid Bacteria, CaP: Calcium Propionate; *: Values indicated by different lowercase letters (a-b) and different capital letters (A-F) in the same column are different from each other (P<0.05). Values are expressed as the mean of two experiments which were twice analysed.

Table 2. Organic acid content (nM) of bread dough

Starter culture	Lactic acid	Acetic acid	Propionic acid	Lactate/acetate ratio
W. cibaria 908	27.46±1.01^{a*}	27.01±0.11^{e}	-	1.02
L. pseudomesenteroides 2619	62.34±1.79^{cd}	3.68±0.30^{ab}	-	16.96
L. sanfranciscensis 2709	55.38±5.46^{c}	6.22±1.63^{bc}	-	9.35
L. plantarum subsp. *plantarum* Y201	41.31±1.95^{b}	2.81±0.31^{a}	-	14.75
L. brevis 2216Y	62.58±0.18^{cd}	7.09±0.38^{c}	-	8.84
Mıxed Culture 1 (All Lab Isolates)	105.10±0.23^{e}	37.08±0.18^{f}	-	2.83
Mıxed Culture 2 (All Lab Isolates + 0.15% CaP)	71.91±2.81^{d}	23.47±0.23^{d}	16.16	3.06
Control 1 (*S. cerevisae*)	-	-	-	-
Control 2 (*S. cerevisae* + 0.3% CaP)	-	-	28.25	-

mM: Mili Molar, CaP: Calcium Propionate; *: Values indicated by different lowercase letters (a-f) in the same column are different from each other (P <0.05). Values are expressed as the mean of two experiments which were twice analysed.

Culture 2 bread, in which starter LAB's are added one to one; it indicates that the combination of lactic cultures causes positive interactions. This positive relationship was observed in the study by Plessas et al. (2011), but it was not observed in the study by Gerez et al. (2009). The bread produced with *Weissella cibaria* 908 strain has the lowest lactic acid (33.77 mM) and is statistically different from all other breads (P<0.05). The highest acetic acid level was determined in bread made from *W. cibaria* 908 strain (44.24 mM) and statistically differentiated from others (P<0.05). The amount of propionic acid determined in breads made with Control 2 and Mixed Culture 2 added with calcium propionate is proportional to the added calcium propionate. Lactate/acetate (FQ) value is close to the desired level in breads prepared with *L. plantarum* subsp. *plantarum* Y201 and Mixed Culture 2. Different from our study, Rizzello et al. (2011) stated that they effectively define formic acid and phenyllactic acid (24.7 mM and 0.4 mM) on mould growth in breads. Similarly, Russo et al. (2017), Dal Bello et al. (2007) and Lavermicocca, Valerio, & Visconti (2003) stated that effectively define phenyllactic acid, phenyllactic acid and lactic acid, phenyllactic acid, lactic acid and two cyclicdipeptides on mould growth in breads respectively.

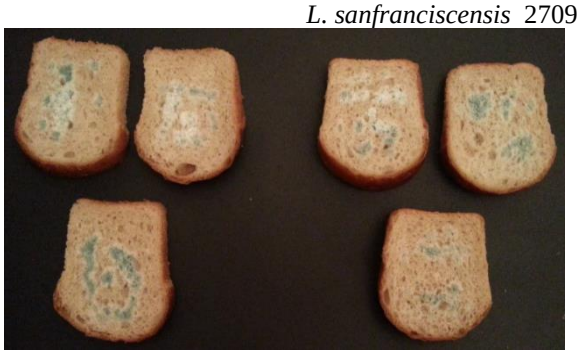
The effect of LAB isolates on the moulding of bread was evaluated by making daily observations. The results obtained are given in Table 4. Due to the fact that the shelf life cannot be extended with the use of single lactic starter culture, it is aimed to reduce the amount of CaP, which is legally allowed to be

Table 3. Organic acid amounts (mM) and fermentation rates of breads produced using LAB isolates (Lactate/acetate)

Starter culture	Lactic acid	Acetic acid	Propionic acid	Lactate/acetate ratio
W. cibaria 908	33.77±2.94^{a*}	44.24±0.89^{c}	-	0.76
L. pseudomesenteroides 2619	67.47±0.32^{c}	10.73±3.42^{a}	-	6.62
L. sanfranciscensis 2709	76.90±2.56^{cd}	16.31±4.08^{ab}	-	4.89
L. plantarum subsp. *plantarum* Y201	56.46±1.40^{b}	18.03±0.87^{ab}	-	3.14
L. brevis 2216Y	76.22±4.12^{cd}	9.20±0.66^{a}	-	8.32
Mıxed Culture 1 (All Lab Isolates)	71.68±0.20^{cd}	12.11±0.31^{a}	-	5.92
Mıxed Culture 2 (All Lab Isolates + 0.15% CaP)	80.79±2.80^{d}	21.39±2.14^{b}	15.83	3.79
Control 1 *(S.cerevisae)*	-	-	-	-
Control 2 (*S.cerevisae* + 0.3% CaP)	-	-	31.59	-

mM: Mili Molar, CaP: Calcium Propionate; *: Values indicated by different lowercase letters (a-d) in the same column are different from each other (P <0.05). Values are expressed as the mean of two experiments which were twice analysed.

L. sanfranciscensis 2709 / A. *niger* (5th day)



L. plantarum subsp. *plantarum* Y201 / *P. expansum* (3rd day)

L. pseudomesenteroides 2619 / A. *flavus* (3rd day)

Figure 2. Situations of breads produced with different LAB cultures and grafted with different indicator mould types on the 3rd day of the sliced breads and on the 5th day of the non-sliced breads

used in the bread by 0.3%. For this purpose, bread production was carried out using CaP in combination with mixed lactic starter culture. It has been shown that in bread where 0.15% CaP is used in combination with a mixed culture, a longer shelf life can be achieved than Control 2 breads with 0.3% CaP. Sample photos are given in Figure 2, which was shown the moulding status of unsliced and sliced breads produced with different LAB cultures and grafted with different indicator mould types.

In the analysis carried out to determine the sensory quality of the breads, the breads are similar in terms of crust color, crust structure, inner color, pore structure, texture, smell, taste and general acceptability properties and there is no difference between them (p>0.05); It has been determined that they have acceptable values by the consumer. These results were compatible the results of the study conducted by Mohsen et al. (2016), Gerez et al. (2010), Ryan, Dal Bello, & Arendt (2008) and Gerez et al. (2009).

4. Conclusions

This Bread which is an indispensable part of meals can easily be spoiled if it is stored under unfavorable conditions. It becomes inconsumable when especially with the development of mould and poses a great risk for health with possible toxin production. Chemical preservatives such as calcium propionate are used to prevent this problem and to extend the shelf life of bread. To reduce the use of chemical preservatives in bread, it is a rational way to use antifungal LAB isolated from sourdough as a natural preservative in bread production. Based on this strategy; 8 lactic acid bacterial isolates were determined, which have been identified as having antifungal activity isolated from sourdoughs, their antifungal properties were tested against *A. flavus, A. niger* and *P. expansum* moulds that cause deterioration in bread and *S. cerevisiae* yeast used as a starter in bread production. Following this, some properties and shelf life of the bread dough and bread produced by using these semi-dough and semi-liquid doughs were determined.

At the end of these studies, *W. cibaria* 908, *L.*

pseudomesenteroides 2619, L. *sanfranciscensis* 2709, *L. plantarum* subsp. *plantarum* Y201 and *L. brevis* 2216Y isolates were selected for bread production by paying attention to changes in total acidity and pH values in bread dough, blistering volumes, organic acid production profiles and the isolates to be selected from different types. As a result of this study, the use of mixed culture (1:1:1:1:1) caused a 50% reduction in usage of calcium propionate to extend the shelf life.

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