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The effect of exopolysaccharide producing *Lactobacillus plantarum* strain addition on sourdough and wheat bread quality

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RESEARCH ARTICLE

Abstract

The effect of addition of exopolysaccharide (EPS) producing *Lactobacillus plantarum* strains on sourdough and wheat bread quality was determined in this study. The exopolysaccharide production of 40 different *L. plantarum* isolated from sourdough has been examined. Three different levels (high, mid and low) of EPS producer isolates were selected as additive to sourdough. Two types of flour with different quality properties were used for bread making and commercial yeast and selected EPS producer strains. Ten different dough and bread samples were prepared under controlled conditions. Chemical, physical, microbiological, textural, colour and sensory analyses were performed in dough and bread samples. The pH values of bread dough decreased during the fermentation period and at the end of fermentation the titratable acidity (TTA) value was found to be in the range of 0.29-0.50%. Statistical analysis showed that pH value, TTA value, moisture content (37.95-42.0%), firmness (8.00-13.23 N), bread volume (370-433 ml), specific volume (2.74-3.28 ml/g), bread crumb L (72.61-74.92), and b (14.87-16.39) colour values, bread crust L (42.39-58.29), a (5.64-9.04) and b (13.04-19.64) colour values, organic acid (lactic, acetic and propionic acid) contents of bread crumb and crust differed significantly ($P<0.05$) according to different treatments. In the sensory evaluation, sourdough and EPS forming strains added breads were preferred by panellists. The best sensory evaluation scores have been obtained with sourdough bread containing a high-EPS producing strain (BioLp57) and flour type A. Consequently, the use of EPS producing *L. plantarum* and sourdough has contributed positive effects on bread quality criteria, except for bread volume.

Keywords: lactic acid bacteria, exopolysaccharide, sourdough, *L. plantarum,* bread

1. Introduction

Bread production using sourdough has been an important application for a long time. Traditional sourdough is achieved by spontaneous fermentation of a mixture of yeast, homo- and hetero fermentative lactic acid bacteria (LAB), flour, water and salt (Torrieri *et al.*, 2014). In recent years, demand for sourdough based bakery products has been increased (Corsetti, 2013). Using sourdough in bread making improves technological properties of the dough, enhances the nutritional properties of the breads, prevents microbial deterioration, and slows down the staling rate (Arendt *et al.*, 2007; Chavan and Chavan, 2011). The predominant microorganisms isolated from traditional sourdough sustained by continuous propagation are yeasts and LAB. Sourdough contains natural LAB strains, which have rich metabolic activities, making sourdough important for bakery industry. Several lactobacilli (*Lactobacillus. plantarum*, *Lactobacillus fermentum* and *Lactobacillus sanfranciscensis*) and yeast (*Saccharomyces cerevisiae* and *Saccharomyces exigus*) species were isolated from sourdough (Mariotti *et al.*, 2014; Torrieri *et al.*, 2014).

LAB produces metabolites, such as organic acids, antimicrobial substances, exopolysaccharides (EPS) and various specific enzymes, which have a positive impact on the texture and stale of bread. Current studies show that sourdough LAB are an important source of production of EPS and have an effect on many useful technological properties, such as viscoelasticity of dough, dough rheology, bread

volume, firmness, bread staling and shelf life (Poutanen *et al.*, 2009; Tieking and Gänzle, 2005; Torrieri *et al.*, 2014). The EPS produced by the LAB increases the technological properties of the dough and bread and with employment of EPS producing LAB the use of bread additives, such as expensive hydrocolloids can be avoided (Palomba *et al.*, 2012; Pepe *et al.*, 2013; Tieking *et al.*, 2003). The formation of EPS *in situ* from sucrose has been reported to promote the production of additional metabolites, such as mannitol, glucose, and acetate, which contribute to the quality of the end-product. Oligo- and homopolysaccharides produced from carbohydrates by LAB have received increasing attention owing to their potential industrial applications, such as texturizing agents and prebiotics (Naessens *et al.*, 2005). Two different application methods, either addition of pure EPS substance or the use of EPS producing LAB strains are used during the fermentation of dough and bread making stages. The production of EPS during the sourdough fermentation has favourable technological effects and EPS producer isolates improve the sensory properties of food products. In addition, it was demonstrated that EPS provide additional nutritional properties like prebiotic attributes (Katina *et al.*, 2009). In particular, to improve the quality of bakery products with distinct nutritional properties, the identification of new raw materials, appropriate technologies, and specific microbial strains are necessary (Pepe *et al.*, 2013).

In recent years, some studies have been performed defining the Turkish sourdough microbial strains and their use in bread production, showing their importance for bread technology (Dertli *et al.*, 2016; Gül *et al.*, 2005; Şimşek *et al.*, 2006). In this study, *L. plantarum* strains were isolated from sourdough samples collected from different regions of Turkey and their EPS productions were determined. In addition, two different wheat flours, three selected EPS producing strains, sourdough used for bread making and the effects on bread quality were examined.

2. Materials and methods

Materials

Forty LAB strains were obtained from Kahramanmaraş Sütçü Imam University's Food Engineering Culture Collection. LAB strains were identified by using species specific PCR amplification and three isolates were selected and used as bread quality developer. Two different types of wheat flour (A and B) obtained from Güzelun Factory (Konya, Turkey) were analysed and used for bread making. A flour had better bread making quality as compared to B flour. Sourdough (from an artisanal bread bakery) and commercial yeast were bought from traditional bakeries and Pakmaya Company (Kartepe, Turkey), respectively.

Genotypic identification of EPS producing Lactobacillus plantarum *strains*

LAB strains were activated in De Man, Rogosa and Sharpe (MRS) broth. Activated bacteria were grown in MRS agar medium for 24 h at 37 °C. All isolates were tested for Gram reaction and catalase activity. A single colony from the developing strains was dissolved in 10 µl of pure sterile water and 1 µl of the solution was taken and used for PCR as template DNA. For each bacterial culture, 4 µl of PCR buffer (mix), 1 µl of forward (F) Primer GCCGCCTAAGGTGGGACAGAT, 1 µl of reverse primer (R) TTACCTAACGGTAAATGCGA, 23 µl of sterile distilled water, 1 µl of dNTP and 1 µl of DNA were added to the PCR mixture. The PCR cycle started with a 5 min pre-denaturation step at 94 °C followed by 30 cycles with 1 min of denaturation at 95 °C, 1 min of annealing at 55 °C and 1 min extension at 72 °C with and final extension step for 10 min at 72 °C. All PCR assays were performed on an Eppendorf Mastercycler Gradient. Electrophoresis of PCR products for LABs was performed in gel containing 1% agarose (Sisto *et al.*, 2009; Walter *et al.*, 2000).

Microbial growth condition

LABs were isolated from dough samples and serial decimal dilutions were prepared and left to incubate at 37 °C for 24 h in MRS broth. Developing strains were transferred to MRS agar (Merck, Germany) by spread plate method and incubated at 37 °C for 24 h. For yeast count, 0.1 ml of prepared dilutions were spread on Potato Dextrose Agar plates and incubated at 25 °C for 3-5 days. At the end of incubation, yeast colonies were counted (Zeinab *et al.*, 2008).

Determination of EPS production quantities

Valerie and Rawson (1999)'s method was used to determine EPS production quantities of *L. plantarum* strains. Total EPS production quantities were determined spectrophotometrically by the phenol sulfuric acid method using glucose as standard (Dubois *et al.*, 1956).

Flour analysis

Flour analyses (moisture, ash, protein, dry and wet gluten contents) were performed according to International Association for Cereal Science and Technology (ICC) Standard Methods No. 110/1, 104/1, 105/2 and 106/2, respectively (ICC, 1976, 1984, 1990, 1994). Extensograms were also carried out according to ICC Standard Method No. 114/1. Farinograph tests were performed using a farinograph (Brabender OHG, Duisburg, Germany) with a 300 g mixing bowl according to the ICC Standard Method No. 115/1. For flour and bread quality determination, gluten index, Zeleny sedimentation test and falling number test

were carried out according to American Association of Cereal Chemists International (AACCI, 2000) methods. Each result was given as the average of three independent measurements.

Bread making procedure

Two different types of flour (A and B), salt, water, sourdough, baker's yeast were used for bread making. The sourdough was purchased from an artisanal bakery and three different EPS producing *L. plantarum* strains (BioLp57, BipLp47 and BioLp39) were added to the sourdough. *L. plantarum* added sourdough was prepared by adding a suspension of strains (7 log cfu/ml; biomass was collected by centrifugation at 5,000 rpm for 5 min at 4 °C, the supernatant was discarded, the residue washed twice in NaCl solution (0.85%, w/v) and resuspended in water for use as starter culture in sourdough) and kneading for 3 min.

For each type of flour (A, B), five different bread samples were prepared. 100 g of flour, water (according to the farinograph absorption value of flour), 2% non-iodized salt mix was used for all doughs of bread samples. Control samples were prepared with addition of 3% baker's yeast to mix (Control A, Control B). Other samples were obtained with addition of 30% sourdough to mix instead of baker's yeast to contain; sourdough (A1 and B1), BioLp57 strain added sourdough (A2 and B2), BipLp47 strain added sourdough (A3 and B3), BioLp39 strain added sourdough (A4 and B4). Bread samples were prepared according to AACCI Method 10-10B (AACC International, 2000) modified by Ozturk *et al.* (2009). 160 g dough was cut after kneading and left fermentation for 4 h at 22-24 °C. Samples were baked at 220 °C for 25 min after the fermentation (Table 1).

Table 1. Different treatment combinations used in bread making.

Treatment	Combination
Control A	Commercial yeast with A flour
A1	Sourdough bread with A flour
A2	*L. plantarum* strain BioLp57 with A flour and sourdough bread
A3	*L. plantarum* strain BioLp47 with A flour and sourdough bread
A4	*L. plantarum* strain BioLp39 with A flour and sourdough bread
Control B	Commercial yeast with B flour
B1	Sourdough bread with B flour
B2	*L. plantarum* strain BioLp57 with B flour and sourdough bread
B3	*L. plantarum* strain BioLp47 with B flour and sourdough bread
B4	*L. plantarum* strain BioLp39 with B flour and sourdough bread

Chemical analyses of dough and bread samples

The determination of pH was done using a pH meter. TTA was analysed using 0.1 N NaOH to reach final pH 8.5 and calculated as % lactic acid (Elgün *et al.*, 1998; Paramithiotis *et al.*, 2006). Organic acids (lactic acid, acetic acid, propionic acid) were assayed by high-performance liquid chromatography (HPLC) using 0.5% metaphosphoric acid as a mobile phase. The method of organic acid determination has been modified according to the Gezginc *et al.* (2015).

Physical analyses of bread samples

The ash content of bread samples was determined according to AACCI Method 08-01.01 (AACC International, 2000). The weight and volume of the breads were measured after baking and cooling the loaves at room temperature for 2 h. The volume of bread was determined by rapeseed displacement method and the specific volume was obtained by dividing volume by weight. After 24 h bread crust and crumb colour (L, a, b) values were determined in triplicate with Hunter LabScan Colorimeter (HunterLab MiniScan XE Plus) (Elgün *et al.*, 1998). Colour determination was followed by texture analysis (Stable Microsysytems, TA-XT plus, Godalming, Surrey, England). Firmness of bread was determined according to the standard method AACCI Method 74.09 (AACC International, 2000) from the midpoint of overlapping two slices of bread with a thickness of 1.25 cm.

Sensory analysis

Bread were sliced about 12 h after they were made (thickness 15 mm) and presented to panellists. A panel of 7 non-specialists was used to evaluate the sensory characteristics of the bread samples. Evaluation of the breads was done using seven hedonic scales for crumb colour, crust colour, pore structure, chewiness, taste, flavour and general taste (Hematian *et al.*, 2010). The linear scales ranged from 1 (very bad) to 7 (very good).

Statistical analysis

Tests were carried out in triplicate, and average values were reported. The standard deviation was calculated. Data obtained from this study were subjected to variance analysis in the SPSS program (BM Corporation, Armonk, NY, USA), and the average of the major sources of variation found were compared with the Duncan Multiple Comparison Test. Experimental design was carried out according to randomised design. Results of $P<0.05$ and $P<0.01$ were considered significant.

3. Results and discussion

Molecular identification of *Lactobacillus plantarum* isolates

All LAB isolates that were catalase-negative, Gram positive and rod shaped (determined by phenotypic methods) were selected for further analysis. Single growing colonies were picked and identified as *L. plantarum* if they showed a visible 318 bp long PCR amplicon using species specific primers (Kunduhoglu *et al.*, 2012; Tabasco *et al.*, 2007).

Determination of EPS production quantities

A total of 40 *L. plantarum* were cultivated on medium containing excessive amounts of glucose for EPS production. The EPS production quantities of strains varied between 75.973 mg/l and 411.575 mg/l (Figure 1). From these, a low (BioLb39), mid (BioLb47) and high (BioLb57) EPS producer isolate was selected and used as an additive for bread making. Adesulu-Dahunsi *et al.* (2018) reported that EPS production by *L. plantarum* was approximately 1.36-2.18 g/l. Depending mainly on starter and strains used, the EPS production may change.

Quality analysis of flour and dough samples

Two different wheat flours (A and B) were used in this study. Moisture content, protein content, ash content, wet gluten content, dry gluten content, gluten index value, Zeleny sedimentation value, falling number, pH, farinograph degree of softening, development time and stability values of flour A were 11.8%, 13.1% (dry matter basis, dmb), 0.79%, 28.5%, 10.3%, 98.8%, 34 ml, 333s, 6.1, 48 BU, 2.14 min and 12.10 min, respectively, while those were found as 12.8%, 9.7% (dmb), 0.55%, 20.5%, 6.9%, 95.8%, 23 ml, 278s, 5.7, 54 BU, 1.42 min and 8.53 min, respectively, for flour B. Higher protein content, wet gluten content, dry gluten content, gluten index value, Zeleny sedimentation value, farinograph development time and stability, lower farinograph degree of softening indicated that flour A had better quality characteristics for bread making compared with flour B. The extensograph data are also useful in studying changes of flour strength, an important parameter correlated with bread quality. Extensibility, dough energy, maximum resistance to extensibility values of flour A were 186/179/163 mm, 118/126/115 cm^2 and 470/522/534 EU for 45, 90, 120 min, respectively, while those were found as 118/95/87 mm, 75/74/55 cm^2 and 471/645/520 for 45, 90, 120 min, respectively, for flour B.

Enumeration of lactic acid bacteria and yeast in dough samples

Two different type of flours (A, B) were used for bread making and three different EPS producing *L. plantarum* strains (BioLp39, BipLp47 and BioLp57) were added to sourdough for texture developing. Control group dough contained only flour and commercial yeast. Different treatment combinations used in bread making were shown in Table 1.

The results of the microbiological analysis of experimental samples showed that LAB was dominant during dough fermentation (Table 2). Statistically significant differences ($P<0.01$) were observed for LAB and yeasts in dough samples. The total number of LAB ranged from 1.8×10^7 to 10.6×10^7 log cfu/g and reached a maximum level at the end of fermentation. When flour type and added starter effect on microbial number were analysed, flour A and moderate level EPS producing strain (BioLp47) had the highest (10.6×10^7 log cfu/g) microbial mass. The lowest

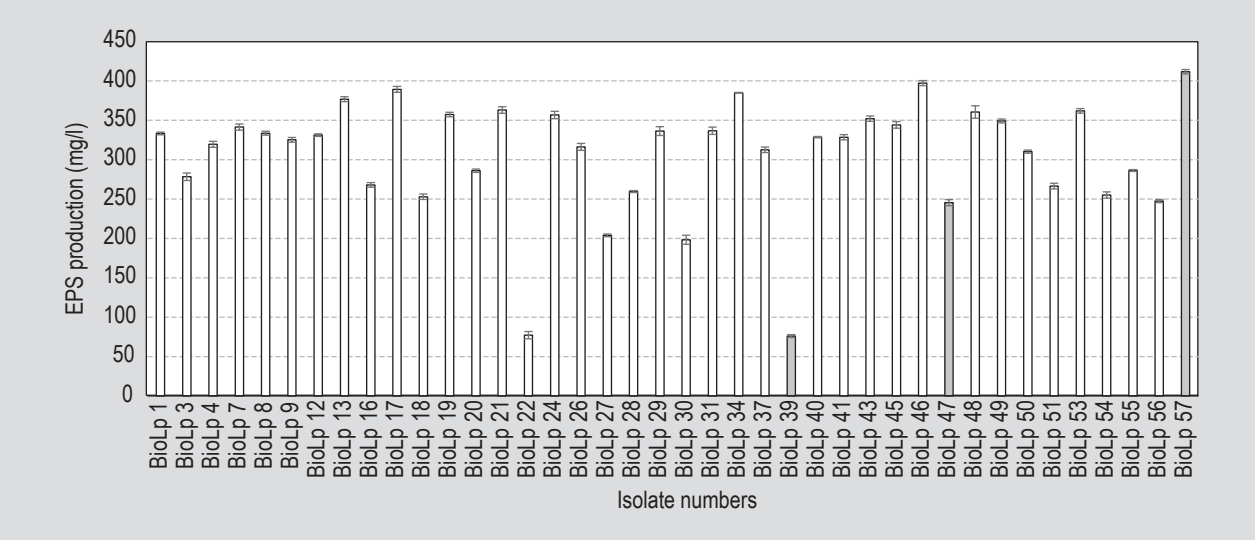

Figure 1. Exopolysaccharide production (mg/l) of isolates. Grey bars indicate the selected strains used in further experiments.

microbial mass was observed when flour B was used. During fermentation, the number of yeasts ranged between 1.0×10^6 and 27.6×10^6 log cfu/g, with the highest number of yeasts measured at the end of the fermentation. The maximum number of yeast reached 27.6×10^6 log cfu/g in flour A dough, while addition of LAB isolates decreased the yeast level (2.2×10^6 log cfu/g) in dough. Statistically significant differences ($P<0.01$) were observed for LAB and yeasts in dough samples. Similar studies on LAB and yeast content of sourdough were performed by Gül *et al.* (2005) and Lattanzi *et al.* (2013). These studies found that LAB and yeast contents were similar (6.3-9.2 and 4.9-7.6 log cfu/g, respectively).

pH changes during dough fermentation

A decrease in pH during fermentation was found when EPS producing *L. plantarum* were added to dough (Figure 2). pH values after kneading, at mid-phase of fermentation and at the end of fermentation ranged between 5.61-5.84, 5.35-5.52 and 4.87-5.22, respectively. The highest pH value was determined when only yeast (Control A) was used as starter (pH 5.22) and the lowest pH value was observed when the highest EPS producing strain was used with yeast (treatment A2). It was found that pH changes of the doughs depended on the added EPS producing strain, however, differences between the doughs were statistically insignificant ($P>0.05$). When *L. plantarum* was used as a starter, Clarke *et al.* (2002) measured pH value of 4.1 for dough, while Gül *et al.* (2005) found a pH ranging between 3.60-5.55.

Determination of organic acid accumulation in dough

Increasing levels of organic acid (lactic acid, acetic acid and propionic acid) content were measured during fermentation (Table 3). The highest lactic, acetic and propionic acid contents were found when the highest EPS producing strain was added to flour A (treatment A2). When the three LAB strains were compared during fermentation, the EPS production potential increased with the content of organic acid accumulation in dough. Lactic acid formation content in different doughs was statistically significant ($P<0.01$), but not between the three different fermentation stages ($P>0.05$). The level of acetic acid and propionic acid formation in the doughs was statistically significant ($P<0.05$) between doughs and periods. Lefebvre *et al.* (2002) determined lactic acid and acetic acid production in dough as 0.5 g/l and 0.1 g/l, respectively. Robert *et al.* (2006) reported that wheat sourdoughs produced with a freeze-dried starter contained 0.32-0.55 g/100 g lactic acid and 0.03-0.08 g/100 g acetic acid. Mainly depending on starter and fermentation stage, flour type may change organic acid production.

Physicochemical characteristic of dough and bread

TTA (%), moisture content (%), firmness (N), ash content (%), weight (g), volume (ml) and specific volume (ml/g) of bread samples are shown in Table 4. The highest acidity value (0.50%) was found for treatment A2 containing the highest EPS-producing strain, while the lowest acidity value was measured in Control B dough (0.29%). When the acidity levels of the starter added samples were examined, it was seen that BioLp 57 produced a statistically higher percentage acidity than the lower EPS-producing strains ($P<0.01$). The titration acidity of the bread ranged between

Table 2. Enumeration of lactic acid bacteria and yeast in dough samples.[1,2]

Dough samples	Lactic acid bacteria log cfu/g			Yeast log cfu/g		
	After kneading	Mid-fermentation	End of fermentation	After kneading	Mid-fermentation	End of fermentation
Control A	–	–	–	$15.5\times10^6\pm1.48^{a}$	$12.3\times10^6\pm5.20^{b}$	$27.6\times10^6\pm4.81^{a}$
A1	$1.80\times10^7\pm0.28^{d}$	$2.00\times10^7\pm1.86^{f}$	$3.60\times10^7\pm3.96^{d}$	$1.1\times10^6\pm0.18^{e}$	$1.7\times10^6\pm1.57^{g}$	$2.2\times10^6\pm0.48^{f}$
A2	$7.94\times10^7\pm1.06^{a}$	$8.02\times10^7\pm4.37^{a}$	$10.40\times10^7\pm0.71^{a}$	$2.4\times10^6\pm1.09^{c}$	$3.85\times10^6\pm0.21^{c}$	$4.2\times10^6\pm1.13^{c}$
A3	$7.03\times10^7\pm0.64^{b}$	$7.50\times10^7\pm2.12^{b}$	$10.60\times10^7\pm2.55^{a}$	$1.0\times10^6\pm0.08^{f}$	$3.0\times10^6\pm1.41^{e}$	$3.5\times10^6\pm2.12^{d}$
A4	$6.42\times10^7\pm0.57^{bc}$	$7.11\times10^7\pm2.76^{bc}$	$7.55\times10^7\pm2.19^{c}$	$1.5\times10^6\pm0.07^{d}$	$3.0\times10^6\pm2.90^{e}$	$3.1\times10^6\pm2.02^{de}$
Control B	–	–	–	$10.7\times10^6\pm1.03^{b}$	$14.2\times10^6\pm0.29^{a}$	$18.3\times10^6\pm2.44^{b}$
B1	$1.90\times10^7\pm0.85^{d}$	$2.60\times10^7\pm0.14^{e}$	$3.30\times10^7\pm1.17^{d}$	$1.2\times10^6\pm0.35^{de}$	$1.6\times10^6\pm0.11^{g}$	$2.2\times10^6\pm1.17^{f}$
B2	$7.22\times10^7\pm0.97^{ab}$	$7.83\times10^7\pm0.79^{ab}$	$9.25\times10^7\pm0.11^{bc}$	$2.6\times10^6\pm1.24^{c}$	$3.3\times10^6\pm2.89^{d}$	$3.3\times10^6\pm1.03^{de}$
B3	$7.03\times10^7\pm1.70^{b}$	$7.60\times10^7\pm1.80^{b}$	$9.86\times10^7\pm0.53^{b}$	$1.3\times10^6\pm0.99^{d}$	$2.5\times10^6\pm2.61^{f}$	$3.6\times10^6\pm1.98^{d}$
B4	$5.70\times10^7\pm2.29^{c}$	$6.31\times10^7\pm1.06^{d}$	$7.20\times10^7\pm0.03^{cd}$	$1.2\times10^6\pm1.13^{de}$	$1.5\times10^6\pm0.04^{gh}$	$2.4\times10^6\pm0.21^{f}$

[1] Different letters within each column are significant at $P<0.01$.

[2] For control, no lactic acid bacteria was added and determined.

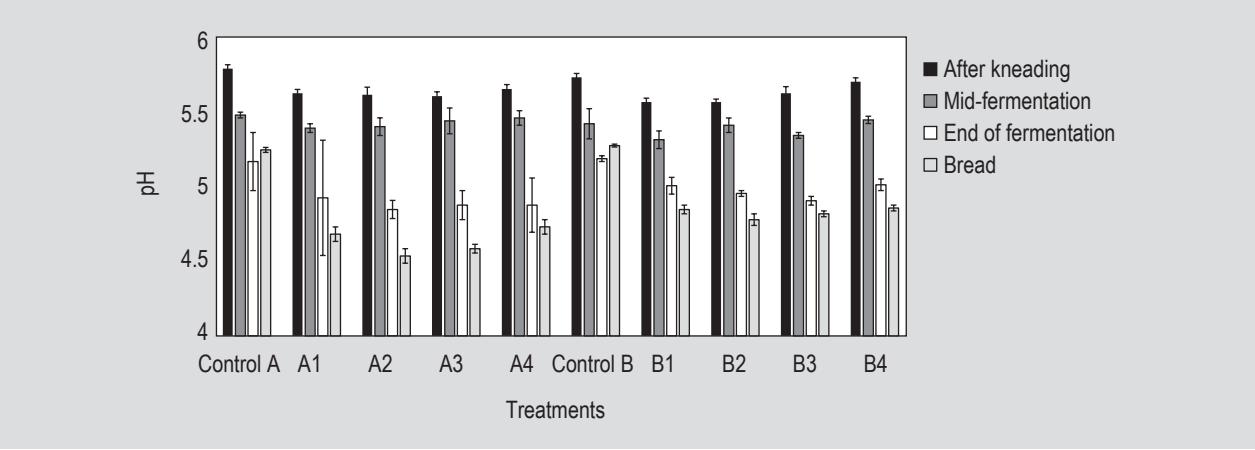

Figure 2. pH values of dough during fermentation and bread. Treatments are described in Table 1.

0.20 to 0.40% in this study. Küçükçuban (2012) determined that TTA values of sourdough changed between 0.12% and -0.60% with different fermentation time. With respect to some studies, differences in acidity could result from fermentation stage and temperature.

Statistically significant (P<0.01) differences were found between moisture content and firmness parameters of bread samples. Bread B4 contained the highest moisture level (42.0%) and bread A3 the lowest (37.95%). The moisture contents of the control breads were 38.73 (Control A) and 39.00 (Control B), while the firmness ranged from 8.00 to 13.23 N (Table 4). The moisture content of bread produced with flour A was lower and firmness was softer than bread produced with flour B. Therdthai and Jitrakbumrung (2014) reported that the moisture content of sourdough bread was 43.60%. Crowley *et al.* (2002) determined the moisture content in breads containing different amounts of added sourdough and found it ranged between 44.4-46.3%. Gül *et al.* (2005) found the moisture contents of the sourdough breads with mixed cultures between 37.00-39.90%. Bread firmness was reported by Sandra *et al.* (2012) ranging between 2.4 and 10.6 N, and by Di Cagno *et al.* (2006) between 11.9-27.2 N after 24 h. In a similar study, Rinaldi *et al.* (2015) found that the firmness of sourdough breads produced by durum wheat flour and soft wheat flour ranged between 1.1-8.7 N after 1, 3 and 5 days storage. These differences in firmness of bread are thought to be due to conditions, such as formulation, storage conditions and firmness value measuring time.

The ash contents ranged between 1.92 and 2.04% with no significant differences (P>0.05), as shown in Table 4. Sit and Saikia (2014) reported the amount of ash ranged between 1.4 and 1.6%, but our results showed higher ash values. Edeghor *et al.* (2016) determined that the amount of ash produced by the yeast starter was 1.12% and the amount of ash produced with yeast and lactic starter was 3.40. The differences in ash content are thought to be due to the chemical properties of the used flour and the differences in formulation.

After determination of weight and volume of the breads, the specific volume was calculated by dividing bread volume by weight. The weight, volume, specific volume values of the breads are shown in Table 4. Bread weight variations between samples were not statistically significant (P>0.05), while bread volume and specific volumes between samples were statistically significant (P<0.01). The lowest bread weight was obtained from Control A bread (132.33 g) while the highest bread weight was for B2 bread (135.58 g). Volume and specific volume values of breads were between 370-433 ml and 2.74-3.28 ml/g, respectively. While the control groups had the highest volume and specific volume values, only sourdough added B1 bread showed the lowest value. Gül *et al.* (2005) reported that specific volume values of sourdough breads produced by mixed cultures of *L. plantarum* ranged between 1.70-2.16 ml/g; values found by Crowley *et al.* (2002) ranged between 3.40-3.18 ml/g. Torrieri *et al.* (2014) found in their study that volume of sourdough (30% of EPS + strain) added bread was higher than the volume of EPS-sourdough bread. According to similar studies, these differences in specific volume may be due to the proportion of sourdough used, duration and temperature of dough fermentation, differences in bacterial strains used as starter cultures, and differences in amount of EPS production.

Determination of bread crumb and crust colour values

The colour values (L: brightness, a: red-green and b: yellow-blue) were determined at crumb and crust of the bread samples (Table 5). Colour measurements were done 12 h after baking of the breads.

Statistically significant differences (P<0.01) were observed for L and b, but not for a at crumb colour of the breads. The L value of the breads ranged from 72.61 to 74.92, the

Table 3. Organic acid contents of certain periods of dough (g/100 g).[1]

Dough samples[2]	Lactic acid			Acetic acid			Propionic acid		
	After kneading	Mid-fermentation	End of fermentation	After kneading	Mid-fermentation	End of fermentation	After kneading	Mid-fermentation	End of fermentation
Control A	0.190±0.001^{dA}	0.200±0.001^{cdB}	0.209±0.001^{deA}	0.126±0.001^{gB}	0.159±0.001^{eA}	0.159±0.001^{eA}	0.090±0.001^{eB}	0.101±0.001^{cA}	0.104±0.001^{cA}
A1	0.219±0.002^{bA}	0.219±0.001^{cA}	0.220±0.001^{cA}	0.141±0.001^{eB}	0.200±0.001^{bA}	0.200±0.001^{dA}	0.100±0.001^{cB}	0.100±0.001^{cB}	0.105±0.001^{cA}
A2	0.232±0.002^{aB}	0.234±0.001^{aB}	0.244±0.001^{aA}	0.194±0.001^{aB}	0.217±0.001^{aA}	0.217±0.001^{aA}	0.111±0.001^{aB}	0.108±0.001^{aB}	0.118±0.00^{aA}
A3	0.222±0.003^{bB}	0.229±0.001^{abA}	0.231±0.001^{aA}	0.174±0.001^{bC}	0.201±0.001^{bB}	0.212±0.001^{bA}	0.101±0.001^{cB}	0.101±0.001^{cB}	0.113±0.001^{bA}
A4	0.219±0.001^{bB}	0.225±0.001^{abA}	0.228±0.001^{bA}	0.144±0.001^{eC}	0.171±0.000^{dB}	0.216±0.001^{aA}	0.101±0.001^{cA}	0.090±0.001^{dA}	0.102±0.001^{cdA}
Control B	0.189±0.001^{fA}	0.189±0.001^{deA}	0.190±0.001^{gA}	0.120±0.001^{hB}	0.123±0.001^{iB}	0.132±0.001^{gA}	0.093±0.00^{1dB}	0.090±0.001^{dB}	0.099±0.000^{eA}
B1	0.190±0.001^{fB}	0.192±0.001^{deAB}	0.195±0.001^{fA}	0.129±0.001^{fgA}	0.127±0.001^{hA}	0.130±0.001^{gA}	0.100±0.001^{cA}	0.092±0.001^{dB}	0.090±0.001^{fB}
B2	0.213±0.001^{cC}	0.221±0.001^{bB}	0.231±0.001^{bA}	0.168±0.003^{cC}	0.176±0.001^{cB}	0.206±0.000^{cA}	0.103±0.001^{bB}	0.102±0.001^{cB}	0.115±0.001^{bA}
B3	0.209±0.001^{dA}	0.209±0.001^{cA}	0.210±0.001^{dA}	0.151±0.001^{dB}	0.152±0.001^{fB}	0.210±0.000^{bA}	0.105±0.001^{bB}	0.105±0.003^{bB}	0.116±0.001^{abA}
B4	0.199±0.001^{eB}	0.181±0.001^{eC}	0.207±0.001^{eA}	0.130±0.001^{fB}	0.143±0.001^{gA}	0.143±0.000^{fA}	0.100±0.000^{cA}	0.101±0.000^{cA}	0.101±0.001^{deA}

[1] Values are mean ± standard deviation (n=4). Means followed by different lower-case superscript within the same row or uppercase superscript within the same column differ significantly (P<0.05).
[2] Treatment descriptions can be found in Table 1.

Table 4. Physicochemical characteristic of dough and bread samples.[1]

Bread samples[2]	Dough	Bread						
	TTA end of fermentation (%)	TTA (%)	Moisture content (%)	Firmness (N)	Ash (%)	Weight (g)	Volume (ml)	Specific volume (ml/g)
Control A	0.32^{g}±0.01	0.23^{f}±0.01	38.73^{g}±0.02	8.00^{e} ±0.55	2.04±0.01	132.33±0.08	433^{a}±5.77	3.28^{a}±0.04
A1	0.44^{c}±0.01	0.38^{b}±0.01	38.78^{f}±0.01	9.96^{cd}±0.35	1.97±0.02	134.21±0.91	402^{b}±7.64	2.99^{b}±0.07
A2	0.50^{a}±0.01	0.40^{a}±0.02	38.38^{i}±0.01	9.60^{c}±0.10	2.03±0.03	133.88±0.02	408^{b}±2.65	3.05^{b}±0.02
A3	0.48^{b}±0.03	0.38^{b}±0.01	37.95^{j}±0.03	9.87^{cd}±0.06	1.97±0.10	133.22±0.09	404^{b}±2.00	3.03^{b}±0.02
A4	0.46^{b}±0.01	0.37^{bc}±0.01	38.50^{h}±0.01	10.60^{c}±0.10	1.92±0.03	133.34±0.40	400^{b}±2.00	3.00^{b}±0.01
Control B	0.29^{h}±0.01	0.20^{g} ±0.01	39.00^{e}±0.02	13.23^{a}±0.65	1.98±0.08	134.18±0.14	405^{b}±5.00	3.02±0.04
B1	0.41^{f}±0.01	0.35^{de}±0.00	41.12^{c}±0.02	12.73^{ab}±0.49	1.96±0.05	135.23±0.60	370^{d}±3.46	2.74^{c}±0.04
B2	0.44^{cd}±0.00	0.35^{de}±0.01	41.06^{d}±0.01	12.02^{b}±0.23	1.95±0.05	135.58±0.75	380^{c}±5.00	2.80^{c}±0.03
B3	0.42^{de}±0.00	0.34^{e}±0.00	41.90^{b}±0.04	12.10^{b}±0.90	1.96±0.04	134.15±0.90	374^{cd}±5.29	2.79^{c}±0.02
B4	0.42^{ef}±0.02	0.36^{cd}±0.02	42.00^{a}±0.01	12.20^{b}±0.60	1.93±0.03	134.07±0.13	370^{d}±5.00	2.76^{c}±0.03

[1] Values are mean ± standard deviation. Values with different superscript letters within rows differ significantly at P<0.05; TTA = titratable acidity (%).
[2] Treatment descriptions can be found in Table 1.

Table 5. Bread crumb and crust colour values.[1]

Bread samples[2]	Crumb colour values			Crust colour values		
	L value	a value	b value	L value	a value	b value
Control A	72.61^{e}±0.71	1.52±0.16	15.46^{b}±0.42	57.67^{a}±1.66	8.98^{a}±0.49	19.64^{a}±0.18
A1	74.36^{ab}±0.43	1.37±0.04	14.87^{c}±0.09	44.94^{d}±163	8.46^{b}±0.47	13.04^{g}±0.81
A2	74.07^{bc}±0.48	1.39±0.04	15.00^{c}±0.17	44.60^{d}±2.24	8.69^{ab}±0.18	13.64^{f}±0.27
A3	74.00^{bcd}±0.66	1.41±0.05	15.08^{c}±0.07	42.56^{e}±0.24	9.04^{a}±0.10	14.08^{f}±0.36
A4	73.97^{bcd}±0.10	1.37±0.02	14.97^{c}±0.02	42.39^{e}±1.09	8.90^{a}±0.06	13.74^{f}±0.32
Control B	73.17^{de}±0.67	1.27±0.07	16.39^{a}±0.34	54.92^{b}±0.08	7.33^{c}±0.01	17.10^{b}±0.06
B1	74.92^{a} ±0.01	1.08±0.03	15.50^{b}±0.27	58.29^{a}±0.51	6.15^{d}±0.03	16.27^{c}±0.01
B2	73.42^{cde}±0.80	1.16±0.07	15.64^{b}±0.09	55.20^{b}±0.14	6.31^{d}±0.03	15.03^{de}±0.03
B3	74.00^{bcd}±0.17	1.23±0.05	15.41^{b}±0.11	52.34^{c}±0.18	6.21^{d}±0.02	14.84^{e}±0.05
B4	73.35^{cde}±0.51	1.14±0.04	15.61^{b}±0.17	54.80^{b}±0.28	5.64^{e}±0.07	15.37^{d}±0.09

[1] Values are mean ± standard deviation. Values having different superscript letters within rows are significantly different at $P<0.01$.
[2] Treatment descriptions can be found in Table 1.

lowest L colour value was found for Control A bread, and the highest for the sourdough added B1 bread. The a value of crumb ranged from 1.08 to 1.52. The lowest value of a was found for B1 bread, while the highest value was measured for the Control A bread. The values of a for control breads (A and B) were 1.52 and 1.27, respectively. The closest value to the control bread for a was reached for sourdough breads added with BioLp47 strain at A3 and B3 treatments. The b values of the breads ranged from 14.87 to 16.39; the lowest b value was found for A1 bread, and the highest for Control B bread.

When measuring bread colour, the breads with added EPS strains yielded the closest results to the control bread. The most yellow crumb colour was determined on Control B bread. The EPS strain-added breads had better crumb L values compared to only yeast-added breads. When crumb colours were evaluated according to the flour used, bread samples made with flour A had a higher a value than those made with flour B and there were not much differences in L values when giving a lower b value.

Rinaldi *et al.* (2015) determined the L and b values of crumbs of sourdough breads produced by soft wheat flour with 30% sourdough additive as 71.6 and 11.5, respectively. Torrieri *et al.* (2014) determined the L, a and b values of breads of EPS-strain and 30% added sourdough bread as 59.7, 7.3 and 29.7, respectively. At the same time, they pointed out that EPS-strain sourdough breads were whiter and more yellow compared to EPS-strain. These differences in bread colour value are thought to be due to chemical and microbiological properties of sourdough, sourdough usage rate, physical and chemical properties of flour, EPS, oven temperature and baking time.

Differences in bread crust colour values (L, a, b) were found to be very significant ($P<0.01$). L values ranged between 42.39 and 58.29 while b values ranged from 13.04 to 19.64 for crust colour. The most effective parameter in the crust colour is the a (redness) value and the crust a value of the breads ranged between 5.64 and 9.04. The closest value to the control flour was determined in sourdough bread made with mid-EPS strain and flour A (A3 bread) and high-EPS strain with B flour (B2 bread).

When bread crust colour was evaluated on flour basis, the bread samples prepared with flour A had higher a and b values and lower L value than those made with flour B. In this context, bread made with flour A was found to have a more desirable crust colour. Therdthai and Jitrakbumrung (2014) determined the L, a and b colour values of 30% sour yeast-added breads as 52.00, 11.63 and 29.82, respectively. These differences in crust colour values are thought to vary depending on the formulation, oven temperature and duration.

Organic acid content of bread crumb and bread crust

The contents of lactic acid, acetic acid and propionic acid in the bread crumb and bread crust varied between treatments and were statistically significant ($P<0.01$) (Table 6). The amount of lactic acid in the bread crumb was 0.200-0.212 g/100 g, that of acetic acid 0.140-0.263 g/100 g and of propionic acid 0.130-0.236 g/100 g. The amount of lactic acid in the bread crust ranged between 0.205-0.217 g/100 g, for acetic acid between 0.150-0.384 g/100 g and for propionic acid between 0.126-0.342 g/100 g. The best bread by organic acid content was sourdough bread A2, made with the highest EPS forming strain and flour A.

Table 6. Organic acid content in bread crumb and bread crust (g/100 g).[1]

Bread samples[2]	Bread crumb			Bread crust		
	Lactic acid	Acetic acid	Propionic acid	Lactic acid	Acetic acid	Propionic acid
Control A	0.202^{e}±0.001	0.172^{d}±0.001	0.161^{g}±0.001	0.213^{bc}±0.001	0.181^{e}±0.001	0.172^{e}±0.002
A1	0.209^{bc}±0.001	0.172^{d}±0.003	0.144^{i}±0.001	0.205^{e}±0.000	0.186^{d}±0.001	0.151^{h}±0.001
A2	0.212^{a}±0.001	0.263^{a} ±0.003	0.236^{a}±0.001	0.217^{a}±0.001	0.348^{a}±0.001	0.342^{a}±0.002
A3	0.211^{ab}±0.001	0.236^{b}±0.001	0.230^{b}±0.001	0.213^{b}±0.003	0.305^{b}±0.001	0.221^{b}±0.001
A4	0.210^{abc}±0.001	0.185^{c}±0.001	0.201^{d}±0.001	0.206^{c}±0.003	0.197^{c}±0.001	0.189^{c}±0.001
Control B	0.200^{e}±0.002	0.149^{e}±0.001	0.148^{h}±0.000	0.212^{bc}±0.001	0.164^{f}±0.001	0.130^{l}±0.000
B1	0.206^{d}±0.001	0.140^{f}±0.001	0.130^{j}±0.003	0.208^{de}±0.001	0.150^{h}±0.001	0.126^{j}±0.000
B2	0.209^{bc}±0.001	0.147^{e}±0.001	0.210^{c}±0.001	0.212^{bc}±0.001	0.159^{g}±0.001	0.157^{g}±0.000
B3	0.208^{bc}±0.001	0.141^{f}±0.001	0.183^{e}±0.000	0.210^{cd}±0.002	0.151^{h}±0.001	0.180^{d}±0.000
B4	0.208^{cd}±0.002	0.140^{f}±0.000	0.164^{f}±0.003	0.206^{e}±0.002	0.150^{h}±0.001	0.165^{f}±0.002

[1] Values are mean ± standard deviation. Values having different superscript letters within rows are significantly different at $P<0.01$.
[2] Treatment descriptions can be found in Table 1.

As in doughs, breads made with flour A have higher organic acid values than those from flour B. Sourdough from flour A bread and sourdough added with EPS had higher organic acid content than control bread and organic acid content generally increased as EPS production increased. Shahid *et al.* (2016) found a lactic acid content between 0.013-0.46 g/100 g, propionic acid content as 0.28 g/100 g, acetic acid content between 0.01-0.17 g/100 g for sourdough breads, and an amount of acetic acid in control bread made with wet yeast as 0.09 g/100 g. Robert *et al.* (2006) determined lactic acid and acetic acid in bread crumb of sourdough breads and found the contents in the range of 0.188-0.408 g/100 g and 0.019-0.181 g/100 g, respectively, and these values are consistent with the results of the present study.

Sensory evaluation

Sourdough and EPS-forming strain added sourdough breads were preferred compared with those produced with commercial yeast in sensory evaluation. The highest sensory score was obtained from the highest EPS forming strain (BioLp 57) added A2 bread (5.86) and breads made with flour A were generally better than those made with flour B. The colour of bread made with flour B was found to be undesirable (Figure 5). The best appearance of crumb and crust structure of breads were obtained for A2 bread (Figure 3 and 4).

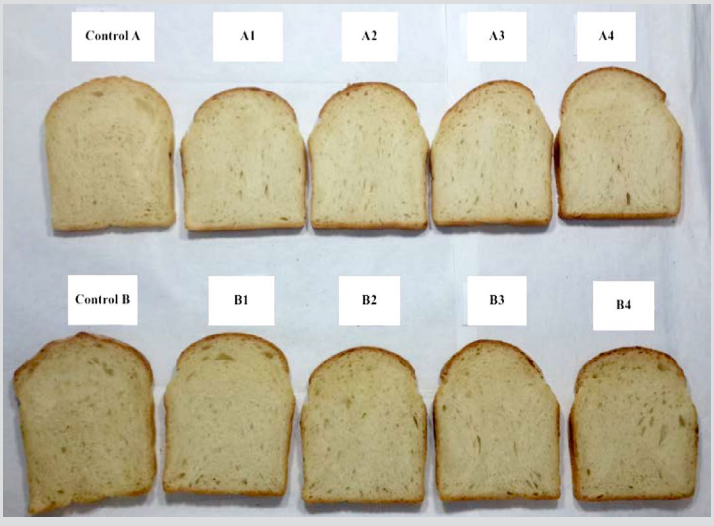

Figure 3. Crumb structures of bread samples.

Figure 4. Crust appearance of bread samples.

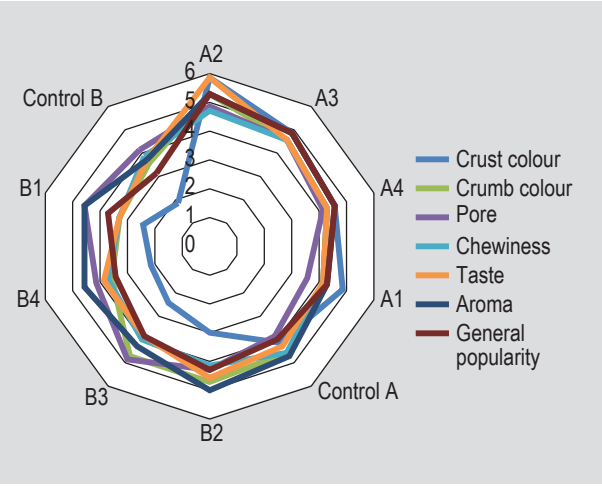

Figure 5. Sensory analysis chart of breads. Treatment descriptions can be found in Table 1.

4. Conclusions

In conclusion, EPS producing *L. plantarum* and sourdough has contributed a positive effect on bread quality criteria, except for bread volume. The best sensory evaluation scores were obtained from sourdough bread containing the highest EPS-producing strain (BioLp 57) with flour type A (bread A2). Therefore, instead of using additives, LAB that produce EPS can be used in bread production, resulting in both a preferred bread and one that can have human health protecting effects. In addition, the use of cultures that synthesise EPS should be emphasised for marketing, as it will also contribute to the economy.

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