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ORIGINAL PAPER

The effects of wheat sourdough on glutenin patterns, dough rheology and bread properties

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Abstract Sourdough was prepared with cellular suspension containing 10^9 cfu of each strain mL^{-1} and incubated at 28 °C for 24 h and at 37 °C for 4 h. Two different sourdough levels (20 and 40%) were used in bread dough preparation. The bread doughs were proofed at 30 °C and 85% relative humidity for 60/120/180 min. When glutenin changes that occurred in samples 17, 18, 19, and 20 (40% SD 28) are compared with those that appeared in controls, it is obvious that, the relative intensities of some of the protein bands slightly decreased and a few fainter protein bands appeared (which did not exist in controls). A few fainter protein bands corresponding to the MM $\approx$ 25 kDa (high-mobility region) and the MM $\approx$ 66 kDa (low-mobility region) were appeared in the same samples. In the samples prepared with 20% sourdoughs incubated at 28 or 37 °C, the bands were still evident after 180 min of proof. This can be explained that glutenin fractions were not hydrolysed in these applications due to the delay in pH drop. The use of 40% sourdough incubated at 28 °C for 24 h resulted in sticky doughs and breads with lower volume, harder texture, unsatisfactory crumb grain and unpleasant flavour than the rest of the samples. The use of sourdoughs incubated at 37 °C for 4 h caused positive effect on loaf volumes, specific loaf volumes and crumb structure.

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Introduction

Sourdough fermentation has a well-established role in improving the flavour and structure of bread. Sourdough fermentation can modify healthiness of cereals in a number of ways: it can improve the texture and the palatability of the whole grain, fibre-rich or gluten-free products, stabilise or increase the levels of various bioactive compounds, retard starch bioavalibility (low glyceamic index products) and improve mineral bioavailability [1].

The use of sourdough in wheat bread production clearly improves the dough properties, bread texture and flavour, delays the staling process, prevents bread from mould and bacterial spoilage [2, 3].

Sourdough microflora generally contain a complex mixture of yeasts (mainly *Saccharomyces cerevisiae*) and hetero- and homofermentative lactic acid bacteria (LAB). LAB play a key role during fermentation. LAB cause rapid acidification of the raw material through the production of organic acids, mainly lactic acid. Also, their production of acetic acid, ethanol, aroma compounds, bacteriocins, exopolysaccharides, and several enzymes is of importance. In this way, they enhance self life and microbial safety, improve texture and contribute to the pleasant sensory profile of the end product [4]. LAB exhibit proteolytic activity [5, 6]. The proteolytic systems of LAB release amino acids and small peptides, which can promote growth and metabolic activities of other microorganisms and also enhance flavour development and rheological parameters [7, 8].

According to Spicher et al. [9], *Lactobacillus sanfranciscensis* is a better microorganism for sourdough bread

baking. The proteolytic system of *L. sanfranciscensis* was characterized and includes proteinase, dipeptidase, and aminopeptidase activities [5]. However, a screening of several strains of *L. sanfranciscensis* for proteolytic activity toward gluten indicated that they only weakly hydrolyse wheat proteins [10]. Other important sourdough bacterial strains include *Lactobacillus brevis* var. *lindneri*, *Lactobacillus plantarum* and *Lactobacillus fermentum* [11]. In addition to this, Gerez et al. [12] also concluded that pediococci strains isolated from sourdough were proteolytic active on gluten.

Cereal flour, LAB, and yeasts contain proteinases and peptidases [13, 14] that can contribute to the proteolytic events [15] in different ways. Thiele et al. [16] concluded that proteolysis during sourdough fermentation and the rheological consequences of gluten degradation are mainly related to the pH-mediated activation of cereal enzymes; the indigenous proteases of flour, in fact, are able to degrade cereal prolamins under acid conditions. The degradation of gluten proteins influences the rheology of wheat sourdoughs and, consequently, the texture of bread.

When flour is mixed with water to form a dough, the protein matrices in the individual cells are brought together to form a continuous network [17]. This confers the viscoelasticity that is necessary for producing high-quality bread with a light porous crumb structure of a well-leavened loaf [18]. In the making of leavened bread, the viscoelastic properties of the gluten network allow the entrapment of carbon dioxide released during fermentation, leading to a light porous crumb structure, although its mechanism is still not completely understood. The gluten fraction is highly cohesive and has a combination of two physical properties; it is elastic but also exhibits extensibility (or viscous flow) [19].

Wheat gluten proteins are classically divided into two groups, the gliadins and glutenins, based on their extractability (gliadins) or unextractability (glutenin) in aqueous alcohols. This property is largely determined by the ability of the component proteins to form inter-chain disulphide bonds, with the glutenins consisting entirely of disulphide-stabilised polymers. Reduction of these inter-chain bonds allows the separation of the glutenin subunits into low-molecular-weight (LMW) and high-molecular-weight (HMW) groups [20]. Disulphide bonds are, therefore, widely considered to be essential for glutenin viscoelasticity [21]. The LMW glutenin subunits (LMWgs) have molecular weights of 36,000–44,000 and are closely related to gliadins. The HMW glutenin subunits (HMWgs) have molecular weights of 95,000–136,000 [22]. One group of proteins appears to be of greatest importance in determining elasticity. This is the HMWgs [19]. Glutenins have greater importance in explaining the variation that occur in dough properties and loaf volume [23, 24] but HMWgs have a strong influence on bread-making quality [25–27].

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) is used routinely in the study of wheat proteins. The technique is used to determine HMWgs (a class of proteins linked to bread quality) or LMWgs composition, to look for changes occurring in proteins during dough mixing and baking or during storage of food products [28, 29].

The aim of the present study was to investigate the influence of sourdough on dough rheology, bread properties and the electrophoretic patterns of glutenins.

Materials and methods

Materials

Commercial bread-making wheat flour (Type 550) containing 12% protein (dry basis), 0.55% ash (dry basis), 14% moisture, 27.2% wet gluten was obtained from the Toru Flour Milling Co. Ltd. (Bandırma, Turkey). It was obtained from a mixture of wheat cultivars. Commercial compressed bakers' yeast (1.5%, w/w, flour basis) and salt (1.5%, w/w, flour basis) were used to prepare bread doughs. *L. plantarum* (DSMZ 20174) and *L. sanfranciscensis* (DSMZ 20663) were purchased from DSMZ-Deutsche SammLung von Mikroorganismen und Zellkulturen Gmbh, Germany.

Methods

Flour analysis

Moisture, ash, protein ($N \times 5.7$) and wet gluten contents were determined using International Association for Cereal Science and Technology (ICC) Standard Methods No. 110/1, 104/1, 105/2, 106/2, respectively [30–33]. Farinograms were performed using a farinograph (Brabender OHG, Duisburg, Germany) with a 300 g mixing bowl according to the ICC Standard Method No. 115/1 [34]. Extensograms were also carried out according to ICC Standard Method No. 114/1 [35]. Sourdough was premixed with the flour at the beginning of the mixing period prior to the addition of water in farinograph and extensograph analysis as described by Crowley et al. [36]. Each result is the average of three measurements.

Inoculum preparation

The strains were routinely propagated for 24 h at 37 °C in modified MRS broth (Oxoid) with the addition of fresh yeast extract (5% v/v) and 28 mmol L^{-1} maltose at a final pH of 5.6. When used for sourdough fermentation, lactic acid bacterial cells were incubated until the late exponential phase of growth was reached (ca. 12 h). Twelve-hour-old LAB cells were harvested by centrifugation at 9000 $\times$ *g* for 10 min

Table 1 Formulations of bread doughs prepared with sourdough incubated at 37 °C for 4 h

	Control	20% Sourdough	40% Sourdough
Flour (g)	1500	1200	900
Sourdough (g)	0	600	1200
Added water (mL)	900	640	300
Salt (g)	22.5	22.5	22.5
Baker's yeast (g)	22.5	22.5	22.5
Total water in recipe (mL)	900	940	900

at 4 °C, washed twice with 20 mmol L^{-1} sterile phosphate buffer (pH 7.0), and resuspended in the same buffer at a concentration of ca. 10^9 cfu mL^{-1} [37].

Sourdough preparation

One kilogram of wheat flour, 700 mL of tap water and 300 mL of cellular suspension containing 10^9 cfu of each LAB strain mL^{-1} were used to produce 2 kg of dough (dough yield 200) with a continuous high-speed mixer (60 × *g*; dough mixing time, 5 min). The resultant dough was divided into two pieces and poured into large beakers, covered and then one piece of these doughs was placed in an incubator at 28 °C for 24 h and another piece was also placed in an incubator at 37 °C for 4 h.

Bread-making procedure

Bread doughs were prepared as described in Tables 1 and 2. Sourdoughs were used at different levels (20 and 40%) in bread doughs [36]. Doughs based on 5 kg flour quantity were mixed in high-speed mixer. Final dough temperatures were in the range of 25–27 °C. The dough was rested in bulk for 20 min, scaled into 350 g portions, moulded, placed in tins of size (98 mm × 280 mm × 80 mm) and placed in the proofer that was set to 30 °C and 85% relative humidity for 60/120/180 min. Baking was carried out at 230 °C for 30 min. The oven was pre-steamed before (0.3 L water) and upon loading (0.7 L water) via the injection of water. The loaves were depanned and allowed to cool for 120 min at room temperature. All individual loaves were sealed in polyethylene bags and stored at 25 °C. Bread dough containing baker's yeast alone in the same amount was included in the test series as a control. Dough samples were taken at 0, 60, 120 and 180 min and their pH values were immediately measured by Metrohm 654 pH meter (Herisau, Switzerland). Sample numbers codes are shown in Table 3.

Table 2 Formulations of bread doughs prepared with sourdough incubated at 28 °C for 24 h

	Control	20% Sourdough	40% Sourdough
Flour (g)	1500	1200	900
Sourdough (g)	0	600	1200
Added water (mL)	900	600	225
Salt (g)	22.5	22.5	22.5
Yeast (g)	22.5	22.5	22.5
Total water in recipe (mL)	900	900	825

Bread evaluation

Three loaves were used for each analysis. Two hours after baking, the loaf volume was measured using the rapeseed displacement method, and after 6 h, the loaf weight was also recorded. Specific loaf volume (mL g^{-1}) was calculated. The internal properties of the bread samples were determined using the method of Pelshenke et al. [38]. A panel of 10 non-specialists was used to evaluate the sensory characteristics of the sourdough breads. They were asked to evaluate the overall acceptance of each loaf concerning general properties. Then, they were asked to evaluate separately the crust for colour, odour, taste, chewiness, the crumb additionally for porosity. The ranking scale ranged from 0 (unacceptable) to 5 (ideal) [39].

Protein extraction of dough samples

The bread dough samples were taken at 0, 60, 120 and 180 min of final dough fermentation and immediately freeze-dried. Wheat proteins and their fractions were extracted sequentially from freeze-dried dough samples using the following solvents in three steps [40]: (1) 1 mol L^{-1} NaCl and 50 mmol L^{-1} Tris–HCl buffer, pH 8.0 (*albumins and globulins*); (2) 55% 1-propanol in H_2O (*alcohol solubles*); (3) sodium dodecyl sulphate sample buffer (SDS-SB) with 5% mercaptoethanol (ME) as reducing agent (*glutenins*).

Hundred milligrams of each dough sample was weighed and put into a 2 mL eppendorf tube. In each phase of the extraction, 1 mL of extraction solvent was used and each centrifugation was done at 11.000 × *g* for 10 min. In the first extraction step, the organic acid produced by LAB was removed from the salt-soluble proteins by extracting the samples at room temperature with 1 mol L^{-1} NaCl and 50 mmol L^{-1} Tris–HCl buffer (pH 8.0) for 60 min. After centrifugation and two washes (with deionized water), the supernatant (*albumins and globulins*) was removed. In the second step, the precipitate was mixed with 1 mL of 55% 1-propanol, and the suspension was incubated for 30 min at 50 °C. The supernatant obtained after centrifugation of this suspension contained the alcohol-soluble proteins (*alcohol solubles*). After two washes with 55% 1-propanol, the precipitate was extracted with SDS-SB with 5% ME at 50 °C for 60 min (step 3). After centrifugation, the supernatant was obtained. This fraction contained *glutenins*. SDS-SB was

Table 3 Sample numbers and codes

Sample numbers	Sample codes	Percentage of sourdough (%)	Sourdough incubation parameters (°C/h)	Proof time of bread doughs (min)
1	Control	0	Control	0
2	Control	0	Control	60
3	Control	0	Control	120
4	Control	0	Control	180
5	20% SD 37	20	37/4	0
6	20% SD 37	20	37/4	60
7	20% SD 37	20	37/4	120
8	20% SD 37	20	37/4	180
9	40% SD 37	40	37/4	0
10	40% SD 37	40	37/4	60
11	40% SD 37	40	37/4	120
12	40% SD 37	40	37/4	180
13	20% SD 28	20	28/24	0
14	20% SD 28	20	28/24	60
15	20% SD 28	20	28/24	120
16	20% SD 28	20	28/24	180
17	40% SD 28	40	28/24	0
18	40% SD 28	40	28/24	60
19	40% SD 28	40	28/24	120
20	40% SD 28	40	28/24	180

prepared by mixing 10 mL of 0.5 mol L^{-1} Tris–HCl (pH 6.8), 8 mL of glycerol, and 16 mL of 10% SDS, with a trace of bromophenol blue [40].

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

Glutenin fractions were analysed by sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis (PAGE) using a cooled vertical slab gel system (C.B.S. Scientific Company Inc., CA, USA). PAGE, in the presence of SDS, was carried out by using a modified method of Laemmli [41]. Glutenin extracts were heated at 95 °C for 5 min before run [14]. Electrophoresis was performed using 12.5% acrylamide separating gel and 3% acrylamide stacking gel containing 0.1% SDS. Buffers and reagents were prepared as described by Shi and Jackowski [42]. Samples (8 μL) and molecular weight standard (Sigma Marker Wide Range, Sigma) (10 μL) were applied to each gel. Gels were run at a 25 mA constant current for 5 h at 20 °C using Series 90 Mid Range Power Supplies (Thermo EC, USA). After electrophoresis, the gels were stained overnight with Coomassie Brilliant Blue-G 250 according to Ng and Bushuk [43]. SDS-PAGE gels were analysed and molecular weights and band intensities were measured by Ingenius Syngene Bio Imaging System (Synoptics Group, Cambridge, UK).

Statistical analysis

The standard deviation was calculated by analysis of variance (ANOVA) using Minitab Statistical Package [44]. Furthermore, Duncan's multiple range test was used to determine the differences between variances by using MSTAT Statistical Package [45].

Results and discussion

Effect of sourdough on electrophoretic patterns of glutenins

The electrophoretic patterns of glutenin fractions are given in Fig. 1. When glutenin changes that occurred in samples 17, 18, 19, and 20 (40% SD 28) are compared with those that appeared in controls, it is obvious that, the relative intensities of some of the protein bands slightly decreased and a few fainter protein bands appeared (which did not exist in controls). In these samples, the intensity of bands with MM (molecular mass) of 104 kDa, decreased compared with controls from 3150 to 2849, 4188 to 2545, 4882 to 2814, 5438 to 3343, respectively. Furthermore, the intensity of bands at 95 kDa also slightly decreased the rate of 9.9, 1.8 and 24.2% in samples 18, 19 and 20 (40% SD 28) compared with controls (data not shown). A few fainter protein bands corresponding to the MM $\approx$ 25 kDa (high-mobility region) and the MM $\approx$ 66 kDa (low-mobility region) appeared in the same samples (Fig. 1, black arrow). The arrows of 66 and 25 kDa point out potential hydrolysis products. The pH values of these samples were 4.20, 4.17, 4.11 and 4.05, respectively (Fig. 2).

Since the literature about the effects of sourdough proteases on electrophoretic patterns of glutenins is scarce, it was decided to compare the findings of the present study with

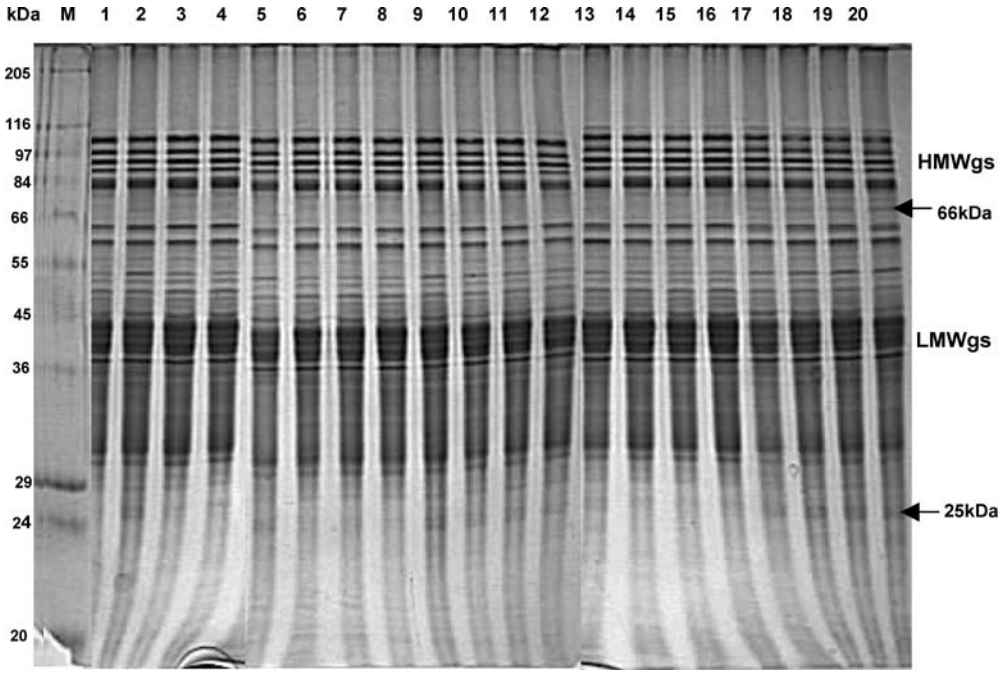

Fig. 1 SDS-PAGE of glutenin fractions in the bread doughs. M, Sigma Marker Wide Range, molecular weight marker; sample codes are given in Table 3

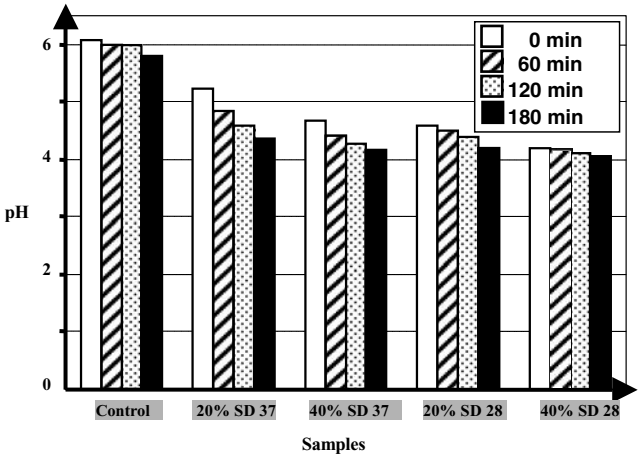

Fig. 2 pH changes of bread loaves in the final fermentation

that of Sivri et al. [46], who investigated the changes in electrophoretic patterns caused by another protease source (*Eurygaster* spp.). These researchers reported that proteolytic enzymes of wheat bug (*Eurygaster* spp.) caused significant decreases in the relative intensity of most of the glutenin bands in the electrophoretic patterns of bug-damaged wheats samples after incubation due to proteolytic activity. They observed that some HMWgs bands disappeared completely in the glutenin patterns of bug-damaged wheats after incubation. The changes in LMWgs were less than those of HMWgs. However, in the present study, the changes in electrophoretic patterns were not substantial because of the lower protease activity in the sourdough applications.

In samples 6, 7 and 8 (20% SD 37) and 14, 15 and 16 (20% SD 28), the bands were still evident after 180 min of proof. This can be explained by the fact that glutenin fractions were not hydrolysed in these applications due to the delay in pH drop. Similar results were obtained by Di Cagno et al. [6], who determined that glutenins were not hydrolysed by sourdough LAB. Hydrolysis of glutenins is mainly dependent on the pH, and the wheat proteinases that degrade gluten proteins have their optimum activity at pH values of ≤ 4.0. In accordance with previous observations that all HMWgs were digested in doughs fermented at pH $<$ 4.0 [16]. By using the fluorescence-labelled wheat proteins, it was shown that proteolytic breakdown of proteins was enhanced at low pH. The effect of acidification and endogenous wheat proteinases, which have an optimum pH at 3.0–4.0, must be considered important for proteolysis in the dough, especially for long-term sourdough fermentation [47]. In the present study, dough samples 6, 7 and 8 (20% SD 37) and 14, 15 and 16 (20% SD 28) had the final pH values ranging from 4.20 to 4.85 (Fig. 2), depending on low sourdough level; for this reason, the glutenin fractions were very slowly hydrolysed. This is in keeping with the report by Gobbetti et al. [47].

Effect of sourdough on dough rheology

The results of Brabender farinograph analysis are given in Table 4. The interactive effects between different proof times and different sourdough levels effected the dough machinability (development time, stability and softening degree) and functionality (resistance to extension and extensibility). All sourdough applications resulted in softer doughs with longer development times and lower water

Table 4 Brabender extensograph and farinograph parameters of doughs

Sample codes[a]	Extensograph properties			Farinograph properties			
	Resistance (BU[b])	Extensibility (mm)	Energy (cm^3)	Water absorption (%)	Development time (min)	Stability (min)	Softening degree (BU[a])
1	754	115	128	62.5	1.9	2.5	98
5	776	124	150	59.5	2.6	2.2	105
9	697	129	136	58.5	2.4	2.0	113
13	510	127	99	60.4	3.2	2.1	234
17	198	121	33	58.3	2.2	1.7	319

Data are the average of three replicates independently analysed.

[a]These samples had proof time of 0 h.

[b]BU, Brabender units.

absorptions (Table 4). Generally, softening degrees of the doughs prepared with sourdoughs incubated at 28 °C for 24 h were determined to be higher than that of others. Especially, 40% sourdough incubated at 28 °C for 24 h led to a drastic decrease in the dough stability and formation of a sticky dough. Similar findings were also determined by Collar Esteve et al. [48], who reported that intermediate sourdough level (17.5%) resulted in softer doughs with the longest development times when LAB with yeast were used. They determined that increasing the level of sourdough led to a decrease in the dough stability due to souring action and to an increase in softening degree in the samples with LAB.

The proteolytic enzymes present in the sourdough system degrade various cereal proteins [49, 50] and contribute to cereal proteases [5, 51, 52]. The proteolytic degradation of gluten proteins also alters the formation of the gluten network [53], resulting in a weak and sticky dough. Even minor changes in the gluten structure may cause considerable changes in dough properties [54]. A number of considerations have been put forward, including the direct impact of pH on dough structure, the effect of acid on cereal enzymes, and indeed the effect of the microorganisms alone. A secondary effect of acidification may include changes in the activity of cereal enzymes associated with changes in the pH of the environment [55].

The results of extensograph analysis are shown in Table 4. Generally, sourdough applications led to lower resistance to extension. As the sourdough level increased, the resistance to extension decreased. The lowest resistance was obtained in the sample started with 40% sourdough incubated at 28 °C for 24 h. The highest extensibility was determined in the sample started with 40% sourdough incubated at 37 °C for 4 h. Similar findings were obtained from a previous study of Di Cagno et al. [6].

In the present study, energy values of the samples started with sourdoughs incubated at higher degree for shorter time (37 °C/4 h) were higher than that of control. In contrast, the energy values of the samples started with sourdoughs incubated at lower degree for longer time (28 °C/24 h) were lower than that of control. The lowest energy value was obtained in the sample started with 40% sourdough incubated at 28 °C for 24 h. These results showed that the degradation of gluten, as a result of the proteolytic activity of starter culture, affected the viscoelastic properties of the dough with a loss of resistance to extension. In agreement with Pepe et al. [56], a decreased dough consistency was observed.

Similar results were also obtained by Collar Esteve et al. [48]. They determined that as the amount of added sourdough increased, the maximum resistance to extension and energy decreased in the samples started with LAB.

Low pH is not a direct factor causing the changes, and it is shown that opposite effects are observed in dough rheology by just adding acids to the dough. Tsen [57] and Tanaka et al. [58] determined that the addition of acid, in the presence of salt, resulted in doughs with increased resistance and decreased extensibility.

Table 5 Bread properties

Sample codes	Weight (g)	Loaf volume (mL)	Specific loaf volume (mL g^{-1})
2	303.67 ± 0.58[a] bc	1348.3 ± 2.9 j	4.44 ± 0.02 h
3	295.00 ± 1.73 d	1520.0 ± 0.0 g	5.14 ± 0.029 f
4	292.00 ± 0.00 de	1940.0 ± 5.0 a	6.64 ± 0.015 a
6	305.00 ± 3.61 ab	1421.7 ± 7.6 h	4.66 ± 0.082 g
7	303.00 ± 3.61 bc	1555.0 ± 35.0 f	5.13 ± 0.167 f
8	290.33 ± 0.58 ef	1831.7 ± 2.9 b	6.31 ± 0.02 b
10	305.00 ± 1.00 ab	1360.0 ± 5.0ı j	4.46 ± 0.00 h
11	302.00 ± 4.00 bc	1700.0 ± 10.0 d	5.63 ± 0.067 d
12	288.00 ± 1.73 f	1860.0 ± 20.0 b	6.43 ± 0.085 b
14	305.67 ± 0.58 ab	1380.0 ± 5.0 i	4.51 ± 0.025 h
15	303.67 ± 0.58 bc	1600.0 ± 5.0 e	5.27 ± 0.021 e
16	294.33 ± 0.58 d	1748.3 ± 2.9 c	5.94 ± 0.010 c
18	309.00 ± 1.00 a	1180.0 ± 10.0 k	3.82 ± 0.036 i
19	308.67 ± 0.58 a	1096.7 ± 5.8 m	3.55 ± 0.012 j
20	301.00 ± 2.65 c	1123.3 ± 5.8 l	3.73 ± 0.049 i

Different letters within each column are significantly different ($p \leq 0.001$).

[a]Values are averages and standard deviations of three experiments.

Table 6 Sensory evaluation of the crust, the crumb and the overall acceptance of bread samples

Sample code	Crust evaluation				Crumb evaluation					Overall evaluation
	Colour	Odour	Taste	Chewiness	Colour	Odour	Taste	Chewiness	Porosity	
2	3.92 ± 0.085[a] ab	2.77 ± 0.098 ef	3.72 ± 0.052 a	3.80 ± 0.046 a	3.28 ± 0.046 cd	3.61 ± 0.092 ab	3.19 ± 0.052 b	3.42 ± 0.085 cde	2.28 ± 0.046 i	3.11 ± 0.092 de
3	3.94 ± 0.098 ab	3.61 ± 0.092 ab	3.80 ± 0.046 a	3.86 ± 0.052 a	3.78 ± 0.046 a	3.55 ± 0.092 bc	3.22 ± 0.098 b	4.22 ± 0.052 a	3.39 ± 0.098 cd	3.44 ± 0.098 c
4	3.72 ± 0.191 bc	3.61 ± 0.092 ab	3.47 ± 0.046 a	3.42 ± 0.085 b	3.83 ± 0.000 a	3.55 ± 0.092 bc	3.61 ± 0.092 a	3.83 ± 0.085 b	3.61 ± 0.092 bc	4.28 ± 0.132 b
6	2.83 ± 0.085 f	2.55 ± 0.092 fg	2.08 ± 0.144 f	3.28 ± 0.132 bc	3.50 ± 0.080 b	2.78 ± 0.132 g	2.46 ± 0.183 de	2.69 ± 0.172 g	2.75 ± 0.150 h	2.05 ± 0.092 g
7	3.36 ± 0.178 d	2.78 ± 0.176 ef	2.53 ± 0.291 de	2.99 ± 0.165 ef	3.83 ± 0.170 a	3.11 ± 0.171 f	2.80 ± 0.132 c	3.30 ± 0.132 def	4.01 ± 0.185 a	3.08 ± 0.144 de
8	4.00 ± 0.080 a	3.11 ± 0.171 cd	2.64 ± 0.127 de	2.99 ± 0.165 ef	3.83 ± 0.085 a	3.30 ± 0.132 de	2.50 ± 0.165 cde	3.64 ± 0.206 bc	3.69 ± 0.052 ab	4.83 ± 0.085 a
10	2.83 ± 0.085 f	3.00 ± 0.080 de	2.47 ± 0.207 e	2.83 ± 0.085 f	3.16 ± 0.085 de	3.19 ± 0.052 ef	2.69 ± 0.464 cd	2.64 ± 0.127 g	2.86 ± 0.052 gh	2.95 ± 0.046 e
11	3.61 ± 0.092 c	3.58 ± 0.144 b	3.03 ± 0.122 b	3.14 ± 0.127 cde	3.83 ± 0.085 a	3.63 ± 0.046 ab	3.19 ± 0.052 b	3.11 ± 0.092 f	3.11 ± 0.092 ef	3.45 ± 0.046 c
12	3.86 ± 0.052 ab	3.89 ± 0.098 a	3.03 ± 0.122 b	3.05 ± 0.092 de	3.86 ± 0.052 a	3.83 ± 0.085 a	3.33 ± 0.085 ab	3.69 ± 0.052 b	3.05 ± 0.092 fg	4.95 ± 0.046 a
14	3.30 ± 0.132 de	3.11 ± 0.092 cd	2.94 ± 0.098 bc	3.25 ± 0.085 bcd	3.44 ± 0.098 bc	3.30 ± 0.046 de	3.36 ± 0.052 ab	3.22 ± 0.052 ef	3.27 ± 0.098 de	3.42 ± 0.085 c
15	3.11 ± 0.092 e	3.28 ± 0.132 c	2.75 ± 0.150 bcd	3.22 ± 0.098 bcd	3.27 ± 0.098 cd	3.61 ± 0.092 b	3.33 ± 0.000 ab	3.44 ± 0.098 cd	3.11 ± 0.092 ef	3.19 ± 0.052 d
16	3.11 ± 0.092 e	3.05 ± 0.092 cd	2.69 ± 0.128 cde	3.22 ± 0.098 bcd	2.99 ± 0.165 e	3.39 ± 0.098 cd	2.77 ± 0.098 c	3.27 ± 0.098 def	2.66 ± 0.165 h	4.14 ± 0.127 b
18	2.63 ± 0.046 f	2.39 ± 0.098 g	2.05 ± 0.092 f	2.14 ± 0.127 g	2.97 ± 0.128 e	3.22 ± 0.098 def	2.27 ± 0.098 e	2.30 ± 0.046 h	2.30 ± 0.046 i	2.64 ± 0.127 f
19	1.50 ± 0.165 g	1.61 ± 0.092 h	1.77 ± 0.098 g	2.05 ± 0.092 g	1.75 ± 0.085 g	2.36 ± 0.052 h	1.72 ± 0.098 f	2.08 ± 0.080 i	1.36 ± 0.052 j	1.61 ± 0.092 h
20	1.39 ± 0.098 g	1.44 ± 0.098 h	1.72 ± 0.098 g	2.05 ± 0.092 g	2.11 ± 0.092 f	1.69 ± 0.052 i	1.28 ± 0.046 g	1.72 ± 0.098 j	1.19 ± 0.052 j	1.30 ± 0.176 i

Different letters within each column are significantly different ($p \leq 0.001$) [a]Values are averages and standard deviations of 30 (10 panellists × 3 experiments) different experiments

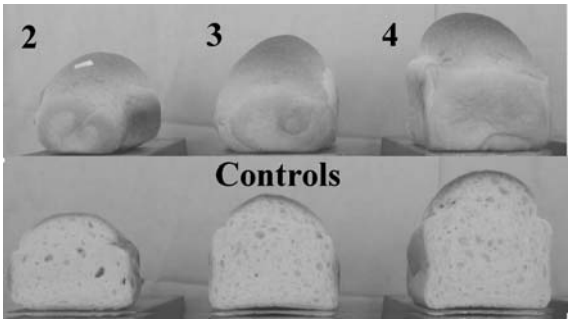

Fig. 3 Crumb structures and external appearances of controls

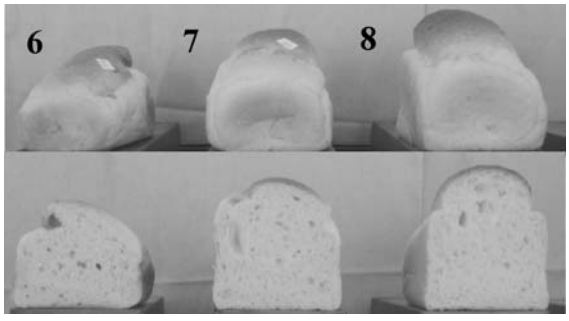

Fig. 4 Crumb structures and external appearances of 20% SD 37

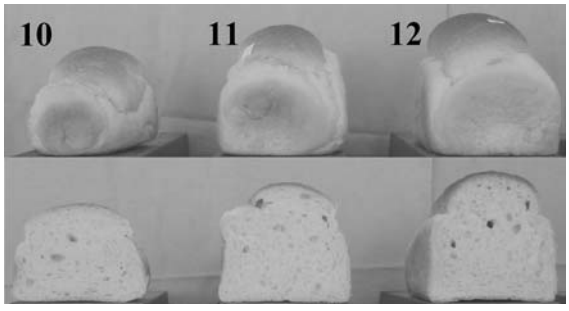

Fig. 5 Crumb structures and external appearances of 40% SD 37

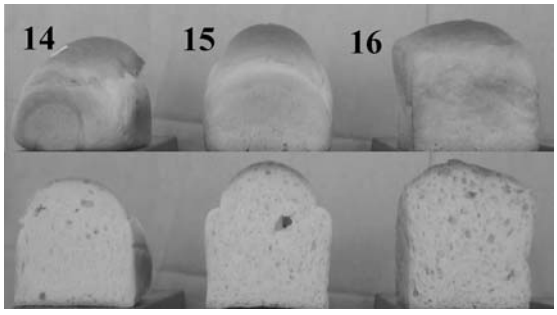

Fig. 6 Crumb structures and external appearances of 20% SD 28

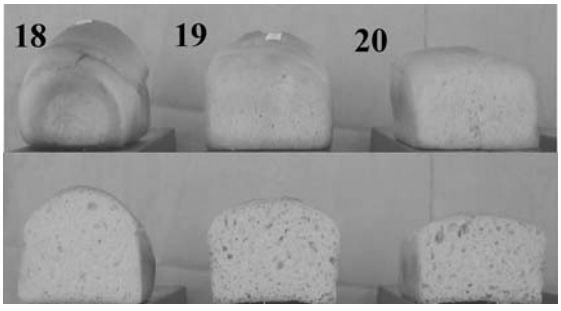

Fig. 7 Crumb structures and external appearances of 40% SD 28

Effect of sourdough on bread properties

The effects of different sourdough levels and different proof times of bread loaves were measured (Tables 5 and 6, and 3–7). Loaf volume data (Table 5) showed a significant difference ($p \leq 0.001$) between controls and other samples as well. Control bread (4) proofed for 180 min had the highest loaf volume (1940 mL) with compact light porous crumb structure (Table 5 and Fig. 3). Breads (8 and 12) prepared from 20 and 40% sourdoughs incubated at 37 °C for 4 h and proofed for 180 min had higher loaf volumes (1831 and 1860 mL, respectively) than other applications (Table 5). On the other hand, the breads (18, 19 and 20) prepared with 40% sourdough incubated at 28 °C for 24 h and proofed for 60, 120 and 180 min had lower loaf volumes (1180, 1096 and 1123 mL, respectively), harder texture, unsatisfactory crumb grain (Table 5 and Fig. 7) and unpleasant flavour than the rest of the samples. It can be explain that doughs of these samples had the lowest resistance, energy and stability and the highest softening degree. For this reason, they became sticky and wet and could not retain CO_2 formed in fermentation.

Significant differences ($p \leq 0.001$) can also be observed in specific loaf volumes (Table 5). The highest values were obtained in samples 4, 8 and 12 as loaf volumes. Generally, the use of sourdoughs incubated at 37 °C for 4 h caused positive effect on loaf volumes, specific loaf volumes and crumb structure, whereas the sourdoughs incubated at 28 °C for 24 h resulted in poor-quality breads exhibiting a low degree of acceptance (Figs. 4–7).

Collar Esteve et al. [48] determined that the bread volume was greater in the samples containing LAB and yeast than that of no-yeast and uninoculated samples. Crumb grain improved as the percent sourdough increased. Crumb structure was, in general, opened and uniform, and similar to that of control breads. The more elastic and softer crumbs corresponded to breads with higher volume and smoother grain. Similar results were obtained in the samples prepared with 20 and 40% sourdoughs incubated at 37 °C for 4 h.

The sensory evaluation of the crust, the crumb and the overall evaluation of the experimental breads are shown in Table 6. Concerning the odour and taste of the crust, significant differences ($p \leq 0.001$) were observed. The same conclusion can be drawn from the sensory evaluation of the crumb. These results were confirmed by the overall evaluation as well. When all of the sensory properties are observed, the addition of 40% sourdoughs incubated at 28 °C for 24 h also showed the lowest sensory values like their farinograph and extensograph values. Unpleasant flavour de-

tected in these samples was related to high inuculum level of sourdough incubated for long time (24 h). Atypical flavour was also reported by Collar Esteve et al. [48], who indicated that unpleasant flavour was generally related to low percentage of sourdough when homofermentative starters were used and high percentage of sourdough addition in heterofermentative samples.

Conclusion

In this study, as samples 17, 18, 19 and 20 (40% SD 28) are compared with the controls, the relative intensities of some of the protein bands slightly decreased and a few fainter protein bands appeared in the samples 18, 19 and 20 (which did not exist in controls). Slight decreases in the relative intensity of some protein bands indicated that the glutenins slowly degraded during sourdough fermentation in some samples, as their pH values were still above the optimum for the proteolytic enzymes. A drastic decrease was observed in the dough stability of these samples. As a result, the lowest loaf volumes and the unsatisfactory sensory values were determined in the same samples.

In samples 6, 7 and 8 (20% SD 37) and 14, 15 and 16 (20% SD 28), the bands were still evident after 180 min of proof. This can be explained by the fact that glutenin fractions were not hydrolysed in these applications due to the delay in pH drop.

The improving effects of wheat sourdough on the breadmaking performance were closely dependent on the sourdough incubation temperature and time, the sourdough inoculum level and the proof time. The use of 40% sourdough incubated at 28 °C for 24 h resulted in sticky doughs and breads with lower volume, harder texture, unsatisfactory crumb grain and unpleasant flavour than the rest of the samples. The results of the present study indicated that the use of 20 and 40% sourdoughs incubated at 37 °C for 4 h can be recommended because these loaves had an open crumb porosity, good crumb elasticity, high specific loaf volumes and satisfactory sensory properties.

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