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Effect of Fermentation on Microbiological, Physicochemical and Physical Characteristics of Sourdough and Impact of its Use on Bread Quality

Alba CASADO[1], *Alberto ÁLVAREZ*[1], *Leticia GONZÁLEZ*[1], *Domingo FERNÁNDEZ*[2], *José L. MARCOS*[3] *and María E. TORNADIJO*[1]*

[1]*Department of Food Hygiene and Technology,* [2]*Institute of Food Science and Technology, ICTAL and* [3]*Department of Applied Chemistry and Physics, University of León, León, Spain*

**Corresponding author: metorr@unileon.es*

Abstract

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The chemical, microbiological, and texture characteristics and rheological properties of sourdoughs were studied in the course of fermentations at 25 and 35°C over a period of 72 hours. After 6 h of fermentation, pH decreased rapidly in the sourdough at 35°C. However, after 72 h the pH values were slightly lower in the sourdough at 25°C. To obtain sourdough with an optimum pH, 11 h were required at 25°C and 9 h at 35°C. Acidity increased more slowly in sourdough at 25°C. However, values were similar in both types of dough after 24 h, but higher in dough at 25°C after 72 hours. Counts of lactic acid bacteria were slightly higher in sourdough at 35°C after 6 h, but after 24 h the sourdough at 25°C showed higher counts. Sourdough evinced elastic behaviour, with a larger elastic modulus in dough at 25°C. Both the phase angle and the relationship between elastic and viscous modulus indicated that dough was an elastic solid that lost elasticity over time. The best mechanical characteristics were noted in dough fermented at 25°C during 24 hours. Bread made with sourdough was rated more highly.

Keywords: dough fermentation; microbial counts; rheology; sensory quality; texture

Sourdough is defined as a dough made up of a mixture of wheat or rye flour, drinking water and possibly salt, subjected to natural fermentation in order to increase the microbiota native to the flour itself (Onno 1996; Hammes & Gänzle 1998). It provides active microbiota depending on the type of sourdough and its adaptation to the medium, together with organic (lactic and acetic) acids, which contribute to the taste and aroma of bread (Arendt *et al.* 2007). These metabolites give the product greater stability. Moreover, the incorporation of sourdough also affects the texture and rheology of dough and of bread (Clarke *et al.* 2002; Arendt *et al.* 2007). Hence, the inclusion of sourdough directs the fermentation of dough and has a positive impact on the texture, smell and taste of the baked product. In addition, it increases the storage life of products and may make them more digestible. Bread obtained from sourdough must have a maximum pH of 4.3 and an acetic acid content of at least 900 ppm (Onno 1996). The use of sourdough is widespread in countries like France, Germany, and Italy. In other countries like Spain, the bread industry has replaced bread-making procedures using sourdough with techniques employing compressed yeast and flour improvers. This leads to the production of bread with a more homogeneous appearance and composition, but not of better quality.

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Sourdough ecosystems are characterised by stable associations of yeasts and lactic acid bacteria, particularly lactobacilli (Huys *et al.* 2013; De Vuyst *et al.* 2014). These microbial associations reflect the metabolic capacities of the species involved and point to the trophic relationships that are of importance in these ecosystems. Among the main parameters influencing sourdough fermentation, mention must be made of the hydration of the dough and the fermentation temperature. Each kind of flour has its own capacity to absorb water, affecting the fermentation process. The temperature has an impact on the dynamics of the microbial population and the metabolic activity of the microorganisms. Homofermentative and facultative heterofermentative lactobacilli like *Lactobacillus fermentum* and *L. plantarum* predominate when the fermentation temperature is above 30°C. In contrast, heterofermentative lactobacilli like *L. sanfranciscensis* predominate when it is lower than 30°C (De Vuyst *et al.* 2014).

The aim of this study was to investigate changes in physicochemical, microbiological, and texture parameters, together with the rheological variations in sourdough fermented at 25 and 35°C, and to discover the optimum length of fermentation. In addition, the effect of sourdough on the texture and the sensory properties of bread was also studied.

MATERIAL AND METHODS

Sourdough production. Four batches of sourdough (SD) were made from a consignment of dough provided by an industrial bakery in the city of Astorga in the Province of Leon in Spain. This had a pH of 5.37 and a titratable acidity of 0.38% of lactic acid. To the quantity of flour used, 25% of its weight in the dough provided, 50% in water and 2% in salt were added. The batches were incubated at two different temperatures, 25°C (SD25) and 35°C (SD35) over a fermentation period of 72 hours.

Bread making. Two types of bread were made in duplicate, a total of four batches, two batches being bread with sourdough (sourdough bread – SDB) and the other two bread without sourdough (plain bread – PB). The bread-making process was performed using a Moulinex bread machine which allowed working conditions to be kept constant. The time spent making the dough (kneading and fermentation) was 2 h and 11 min, of which approximately 1 h and 25 min were spent on fermentation. Baking was completed in 58 minutes. The bread was allowed to cool for 1 h before tests were performed.

To produce the bread, the following ingredients were used: high-protein (11%) flour (100 parts) together with 50% of the weight of flour in water, 2% in salt, 2% in yeast in the batches without sourdough and 1% in those with sourdough, 1% in sugar and 5% in sunflower-seed oil. The batches made with sourdough included it in the proportion of 20% of the weight of flour used.

Sampling. Samples were taken in duplicate of each dough just after it was made, and then after 6, 24, 48, and 72 h of fermentation at the two different temperatures used (25 and 35°C), in total twenty samples of dough. Samples of bread were also taken from each batch made, both with and without sourdough. Physical, chemical, microbiological, texture, and rheological analyses were undertaken, together with a sensory analysis of the bread samples.

Physicochemical analysis in sourdough. From each sample 10 g were taken, to which 90 ml of water-acetone mix (5 : 95) were added, and this was then homogenised for approximately 1 minute. The pH value was determined with a previously calibrated pH-meter. The same mixture was assessed with a calibrated solution of NaOH (0.1 N). A note was made of what volume of NaOH was needed to reach a pH of 8.5, phenolphthalein being used as an acidity indicator. The results for titratable acidity (T.A.) were expressed as percentage of lactic acid.

In order to discover the optimum period of fermentation the pH of the dough fermented at 25°C and at 35°C was measured over the time necessary to reach pH values in the range of 4.2 to 4.3. In addition, a check was made on the effects that refrigeration for 18 h at 4°C had on the physical and chemical characteristics of sourdough after the optimum pH value was attained.

The humidity content of the dough subjected to different fermentation temperatures (25 and 35°C) was determined. An approximately 15 g amount was weighed from each sample into a previously dried nickel capsule, then it was homogenised with a rod and placed in a kiln at 102 ± 1°C for 4 h (AACC, method 44-15.02). The percentage of humidity was calculated from the difference in weight of the sample before and after drying, relative to the weight of the sample when fresh.

Microbial changes in sourdough. A Stomacher homogenizer was used to blend 25 g of dough with 225 ml of 0.1% sterile peptone water for 2 minutes.

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Thereafter decimal dilutions were prepared by mixing 10 ml of this solution with 90 ml of 0.1% sterile peptone water. These were then sown in duplicate on different culture media so as to determine any presence of the following microbial groups: total aerobic mesophilic microorganisms on standard plate count agar (PCA) (APHA 1960), with plates being incubated at 30°C for 48 h; *Enterobacteriaceae*, on violet red bile glucose agar (VRBGA), with incubation at 37°C for 24 h; general counts of lactic acid bacteria (LAB), on de Man-Rogosa-Sharpe (MRS) agar, after incubation at 30°C for 3 days, and of lactobacilli in particular, on Rogosa agar, incubating at 30°C for 5 days; and moulds and yeasts, on oxytetracycline glucose yeast extract agar (OGYEA), after incubation at 25°C for 5 days. Counts were expressed as the logarithm of colony-forming units per gram (log CFU/g).

Texture of sourdough, dough, and bread. Texture parameters (hardness, adhesiveness, cohesiveness, springiness, gumminess, resilience, and chewiness) were determined with a model TA-XT2i texturometer (Stable Micro Systems, UK). This analysis involved a double compression test known as Texture Profile Analysis (TPA), which allowed values to be obtained for the various texture parameters. A cylindrical probe 75 mm in diameter was used at a temperature of 20°C. The pre-trial velocity was set at 10 mm/s, the trial speed at 0.5 mm/s and the post-trial speed at 10 mm/s, a double cycle test to 60% compression level, a force of 0.98 N and a time of thirty-five seconds. The results were processed with the Texture Expert software package (Stable Micro Systems, UK).

For these analyses samples of dough were taken after zero hours (just kneaded) and after 6 h of fermentation. After 24 h the dough became too viscous to be analysed with the texturometer. The weight of the samples was 3.2 g, and they were given a spherical shape. Samples of bread made with and without sourdough were also taken, exclusively from the crumb portion, which was cut into cubes with the side length of 25 mm.

Rheological properties of sourdough. Samples of sourdough were analysed at zero hours (just kneaded) and after 6, 24, 48, and 72 h of fermentation at 25 and 35°C. These samples were subjected to dynamic oscillatory measurements using an AR2000ex Rheometer (TA instruments, USA) with plate-plate geometry. An upper steel plate 40 mm in diameter was used, and sanded so as to avoid slippage. The base was a Peltier plate thermostatically controlled to be at a temperature of 20°C. Both the probe and the distance between the plates were calibrated prior to the analysis by means of a 'zero gap'. The force applied was standardised at 1 N for all the samples. The sample was adjusted to a height of 2 mm on the plate. A mechanical spectrum was applied, with frequency sweeps between 0.1 and 20 Hz. All the samples were analysed in duplicate with the equipment built-in software, Rheology Advantage Data Analysis version 5.7 (TA Instruments, USA), being used for the integration of results.

Sensory analysis of bread. Analysis was performed by a panel of 26 testers who carried out an acceptability test that compared bread made with and without sourdough. They evaluated various sensory parameters referring to the colour, aroma and taste of the bread. Intensity of each attribute was scored on a scale from 1 (slight) to 3 (strong).

Statistical analysis. A single-factor analysis of variance (ANOVA) was used and Fisher's least significant difference (LSD) test was applied to check whether there were differences in the physical and chemical characteristics of dough. This covered fermentation at both temperatures used (25 and 35°C) and also the treatments applied to the types of dough with regard to the same fermentation time.

RESULTS AND DISCUSSION

Physicochemical characteristics of sourdough. The values of pH and titratable acidity of the sourdough samples fermented at two different temperatures are shown in Table 1. As may be seen from this table, pH values of dough fermented both at 25°C (SD25) and at 35°C (SD35) dropped over 72 h of fermentation period. The initial pH for the dough was 5.98. Acidification rate of the sourdough was faster at 35°C than at 25°C. This fact became evident by the pH values obtained in the first 6 hours. However, from this moment, the pH values were lower in samples held at 25°C, showing that a cooler fermentation temperature allows the acidifying activity of microorganisms to be prolonged over a greater time. It should be recalled that the growth and metabolism of lactic acid bacteria cease when dough reaches a pH between 3.6 and 3.8 (Thiele *et al.* 2004). Values of titratable acidity (TA) (Table 1) underwent an increase in both types of dough, and after 6 h they were higher in dough fermented at 35°C. Nevertheless, after 24 h of fermentation there were no significant differences ($P < 0.05$) between

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Table 1. Changes in the pH and titratable acidity (T.A.) expressed as percentage of sourdough lactic acid during 72 h of fermentation at 25 and 35°C

Sourdough sample		Fermentation time (h)				
		0	6	24	48	72
SD25	pH	5.98 ± 0.01[a,f]	5.13 ± 0.01[b,f]	3.9 ± 0.04[c,f]	3.84 ± 0.02[c,f]	3.89 ± 0.01[c,f]
	T.A.	0.13 ± 0.001[a,f]	0.33 ± 0.01[b,f]	0.87 ± 0.01[c,f]	0.85 ± 0.01[c,f]	1.08 ± 0.01[d,f]
SD35	pH	5.98 ± 0.01[a,f]	4.81 ± 0.01[b,g]	3.99 ± 0.02[c,f]	3.95 ± 0.04[c,g]	3.98 ± 0.01[c,f]
	T.A.	0.13 ± 0.001[a,f]	0.52 ± 0.01[b,g]	0.85 ± 0.01[c,f]	1.16 ± 0.01[d,g]	0.92 ± 0.01[e,g]

SD25 – sourdough fermented at 25°C; SD25 – sourdough fermented at 35°C; [a–e]different superscripts indicate significant differences ($P < 0.001$) during fermentation for the samples at each fermentation temperature and in relation to each characteristic; [f–g]different superscripts indicate significant differences ($P < 0.05$) between fermentation temperatures in relation to the same fermentation time and for each parameter

the two sorts of sourdough. The TA of dough batches held at 25°C remained more or less constant, whilst in dough kept at 35°C an increase in acidity was observed until forty-eight hours had gone by, where after it dropped until 72 h had passed. The highest values of TA were noted in samples fermented at 35°C after 48 h, while samples fermented at 25°C reached their highest acidity after 72 hours.

The fermentation temperature for dough is a major factor in the dynamics of the microbial community and the kinetics of metabolites. It affects the fermentation quotient, which refers to the relationship between the molar concentrations of lactic acid relative to acetic acid (Banu *et al.* 2011; Vogelmann & Hertel 2011). Homofermentative and facultative heterofermentative lactic acid bacteria (LAB) cause rapid acidification at high temperatures over short periods of time, principally producing lactic acid. Heterofermentative LAB are prevalent in sourdough fermented at low temperatures (< 30°C) and over longer times. They produce a mix of lactic acid, acetic acid, and ethanol in varying proportions (De Vuyst *et al.* 2014).

The optimum value of the sourdough pH should lie between 4.2 and 4.5 (De Vuyst & Neysens 2005), and hence the fermentation time required at 25 and 35°C will be somewhere between six and 24 hours. Checking the changes in pH until it reached a value of around 4.3 allowed the optimum fermentation period for each of the two temperatures to be discovered. It was observed that samples fermented at 35°C reached a pH of 4.28 after nine and a half hours, whilst samples kept at 25°C took 11 h to attain a pH of 4.35. It was also possible to see that an increase in the volume of sourdough held at 35°C was greater than that occurring in dough at 25°C. This may be attributed primarily to a higher level of fermentation activity, producing more gas, which in turn lowers the dough density. From twenty-four hours onwards the increase in the volume was stabilised.

The fermented doughs were refrigerated at 4°C for 18 h in order to determine whether refrigeration had any noticeable effect on pH. The dough fermented at 25°C had a pH value of 4.18, while the dough processed at 35°C showed a value of 4.11 after refrigeration. This indicates that refrigerated storage for some hours is possible without affecting these parameters and hence other physical and chemical characteristics of dough. The refrigeration temperature used inhibited the growth and activity of lactic acid bacteria, and may be used as a method for preserving sourdough over short periods of time.

The initial humidity content of the sourdough was 42%, and this value remained more or less constant during fermentation. Nevertheless, there were slight, but not significant differences between the types of dough in the course of fermentation ($P < 0.001$) and between doughs fermented at different temperatures ($P < 0.05$). The higher the temperature, the less water is fixed by proteins, owing to the weakening of hydrogen bonds. When the ambient temperature is higher than that of the dough, the surface may undergo drying. Fermented sourdough had a hard, dry surface, which may possibly have acted as a protective crust preventing a higher loss of humidity from the rest of the dough. Such a function for this crust seemed more evident in sourdough fermented at 35°C.

Changes in microbial groups during sourdough preparation. The type and quality of cereal flour used to make sourdough influences the stability of the dough produced, since this raw material is a major source of nutrients for the autochthonous microbiota,

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Table 2. Changes in the microbial counts (log CFU/g) obtained in the different culture media from the sourdough fermented at 25°C and 35°C during 72 hours

Microbial group	Fermentation temperature (°C)	Fermentation time (h)				
		0	6	24	48	72
Aerobic mesophilic bacteria	25	7.05	6.88	8.89	8.69	8.16
	35	7.05	6.96	8.67	7.48	7.84
Lactic acid bacteria	25	6.77	6.8	7.89	7.57	7.56
	35	6.77	6.89	7.26	7.06	6.83
Lactobacilli	25	6.55	6.03	7.73	7.44	6.89
	35	6.55	6.22	7.29	7.05	6.83
Moulds and yeasts	25	6.04	5.56	7.08	7.25	7.30
	35	6.61	6.73	7.15	6.96	6.76
Enterobacteria	25	–	2.42	–	–	–
	35	–	1.74	–	–	–

fundamentally made up of species of heterofermentative LAB (Corsetti *et al.* 2003; Vrancken *et al.* 2010). Sourdough constitutes a complex ecosystem characterised by low pH, high concentrations of carbohydrates, low concentrations of O_2 and high LAB counts (≥ 10^8 CFU/g), as compared with the figures (≤ 10^7 CFU/g) found for yeasts (De Vuyst *et al.* 2014).

The changes in the microbial counts of sourdough fermented at 25 and 35°C are shown in Table 2. The counts obtained on PCA for dough that had just come from kneading were 7 log CFU/g. These values rose above 8 log CFU/g after 24 h of fermentation, and were slightly higher in dough fermented at 25°C. This type of dough continued to have these counts more or less constant, or with only a slight drop, until the end of fermentation. In contrast, in dough processed at 35°C a decline of one logarithmic unit could already be seen in the time period between 24 and 48 h of fermentation. These counts reflect the majority population in sourdough, made up of lactic acid bacteria and yeasts. Between 6 and 24 h of fermentation it proved possible to see an increase in the counts on MRS agar and Rogosa agar for dough fermented at both temperatures used. The values reached were between 7 and 8 log CFU/g, this being related to the increase in the population of LAB. From 24 h onwards, the counts on MRS agar and Rogosa agar gradually fell in dough fermented both at 25 and 35°C. The competitive relationships and microbial associations that are established over the process of fermentation lead at one period of the procedure to a stable ecosystem in which lactobacilli are particularly dominant. The drop in pH that occurs as the fermentation progresses causes some species to be gradually replaced by others more tolerant to acids (Weckx *et al.* 2010). In fact, it has proved possible to isolate more than sixty species of lactobacilli from various types of sourdough, including homofermentative, facultative heterofermentative, and obligate heterofermentative ones (Corsetti & Settanni 2007; De Vuyst *et al.* 2014). The counts on OGYEA showed an increase all the way through until 72 h for dough fermented at 25°C, while for dough held at 35°C the maximum counts were reached after 24 h, whereafter there was a slow decline until the full seventy-two hours of fermentation were completed. The higher fermentation temperature for this sourdough possibly had a negative effect on the metabolism of yeasts. Yeasts tend to be sensitive to undissociated acetic acid and to a lesser degree to lactic acid (Gänzle *et al.* 2007). In fact, a higher temperature (35°C) favours bacterial activity and triggers a rise in the fermentation quotient, while the metabolism of yeasts is enhanced when fermentation takes place at 25°C (Onno 1996). Lactobacilli and yeasts establish a stable association in sourdough, thanks to their growth requirements in respect of temperature, pH and organic acids, and as a function of their ability to metabolize carbohydrates (Gänzle *et al.* 2007). The ratio of LAB to yeasts in sourdough made without added yeasts is generally 100 : 1 (Ottogalli *et al.* 1996). Lactobacilli constitute a population well adapted to the physical and chemical conditions in sourdough, competing with the remaining microbial life present until they become the dominant microbiota (Messens & De Vuyst 2002). This permits the propagation of sourdough over long periods if

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Table 3. Values of the Texture Profile Analysis (TPA) of sourdough during fermentation (0 and 6 h) at 25 and 35°C (data are the mean values ± standard deviations of three replicates)

Fermentation time/temperature	Hardness (kg·m/s^2)	Adhesiveness (kg·m^2/s^2)	Cohesiveness	Springiness	Gumminess (kg·m/s^2)	Resilience	Chewiness (kg)
0 h	3.657 ± 0.598^a	0.797 ± 0.420^a	0.523 ± 0.046^a	0.743 ± 0.156^a	1.925 ± 0.417^a	0.065 ± 0.006^a	1.462 ± 0.501^a
6 h/25°C	5.128 ± 0.458^a	2.799 ± 1.229^b	0.655 ± 0.020^b	0.929 ± 0.087^b	3.359 ± 0.339^b	0.097 ± 0.011^b	3.139 ± 0.547^a
6 h/35°C	5.649 ± 0.874^a	3.341 ± 0.461^b	0.627 ± 0.014^b	0.971 ± 0.023^b	3.549 ± 0.592^b	0.108 ± 0.013^b	3.391 ± 0.585^a

$^{a–b}$different superscripts indicate significant differences ($P < 0.05$) between the values of the texture parameters of sourdough just mixed (t = 0 h) and after 6 h of fermentation at both temperatures

water and flour are added at the appropriate, perhaps irregular, intervals (Zugić-Petrović *et al.* 2009; Komlenić *et al.* 2010). Moreover, the development of acidifying microbiota was sufficient to control the population of *Enterobacteriaceae*, since this microbial group was not detected beyond twenty-four hours of fermentation in any of the types of dough.

Sourdough texture. Batches of sourdough fermented for 6 h at 25 and 35°C showed higher values of all the texture parameters considered relative to dough that had just been kneaded (0 h). Significant differences were found in all parameters before and after fermentation. The values of the parameters were slightly higher in dough fermented at 35°C than in batches treated at 25°C, although the differences were not significant ($P < 0.05$), as may be seen from Table 3.

With lower pH values, there is a net positive charge and proteins become more soluble. An increase in the solubility of proteins promotes intramolecular electrostatic repulsion among the proteins in gluten and makes protein groups more reactive (Komlenić *et al.* 2010). However, strong intermolecular repulsion forces prevent the formation of new linkages. The fermentation of sourdough causes the dough to have less elasticity and firmness. This could be related with the weakening of the gluten structure caused by the repulsion occurring at a molecular level (Angioloni *et al.* 2006).

Rheological properties of sourdough. Sourdough fermented at 25°C showed an increase in the values of the elastic component G' and viscous component G'' over the first 24 hours. These values then dropped abruptly as the 48 h mark was reached, and thereafter they showed a gradual decline until 72 h had passed. In contrast, dough fermented at 35°C showed a gradual decrease in the elastic modulus G' and viscous modulus G'' from the start through until 72 h had elapsed, although this drop was sharper over the first 6 h (Figure 1).

As may be seen from Figure 1, the elastic modulus G' was always larger than G'', which indicates that dough had an elastic solid behaviour (Moroni *et al.* 2011). This is linked to the protein content and the gluten network obtained, and elasticity is lost over time (Sofou *et al.* 2008). It is to be noted that in sourdough fermented at 25°C the differences between the elastic and viscous modulus were more marked than in dough fermented at 35°C, particularly after the first 24 h of fermentation. The largest elastic modulus seen in sourdough occurred when it was fermented at 25°C (Figure 2). This is related to firmer dough and the formation of a more stable network of gluten, whilst lower values, as observed in dough fermented at 35°C, indicate that the elastic component is reduced, showing less elasticity and simultaneously less firmness in the dough. The protein matrix undergoes degradation as fermentation proceeds and sourdough fermented at 35°C had lessened elasticity and viscosity, becoming more fluid. Furthermore, the drop in pH in sourdough fermented at 35°C may possibly trigger the activation of enzymes (amylases, proteinases, hemicellulases) that hydrolyse gluten components (Gänzle *et al.* 2008; Poutanen *et al.* 2009). Indeed, variations in pH alter the rheological

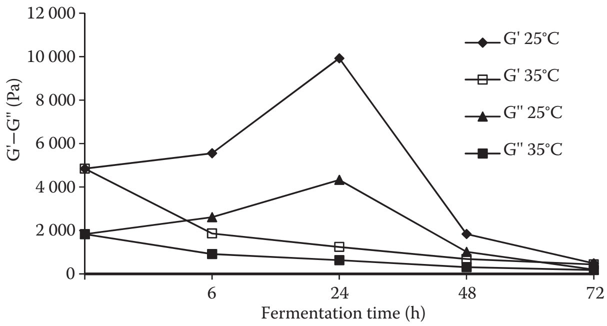

Figure 1. Changes in the elastic modulus (G') and viscous modulus (G'') of sourdough fermented at 25 and 35°C for 72 h at a frequency of 1 Hz

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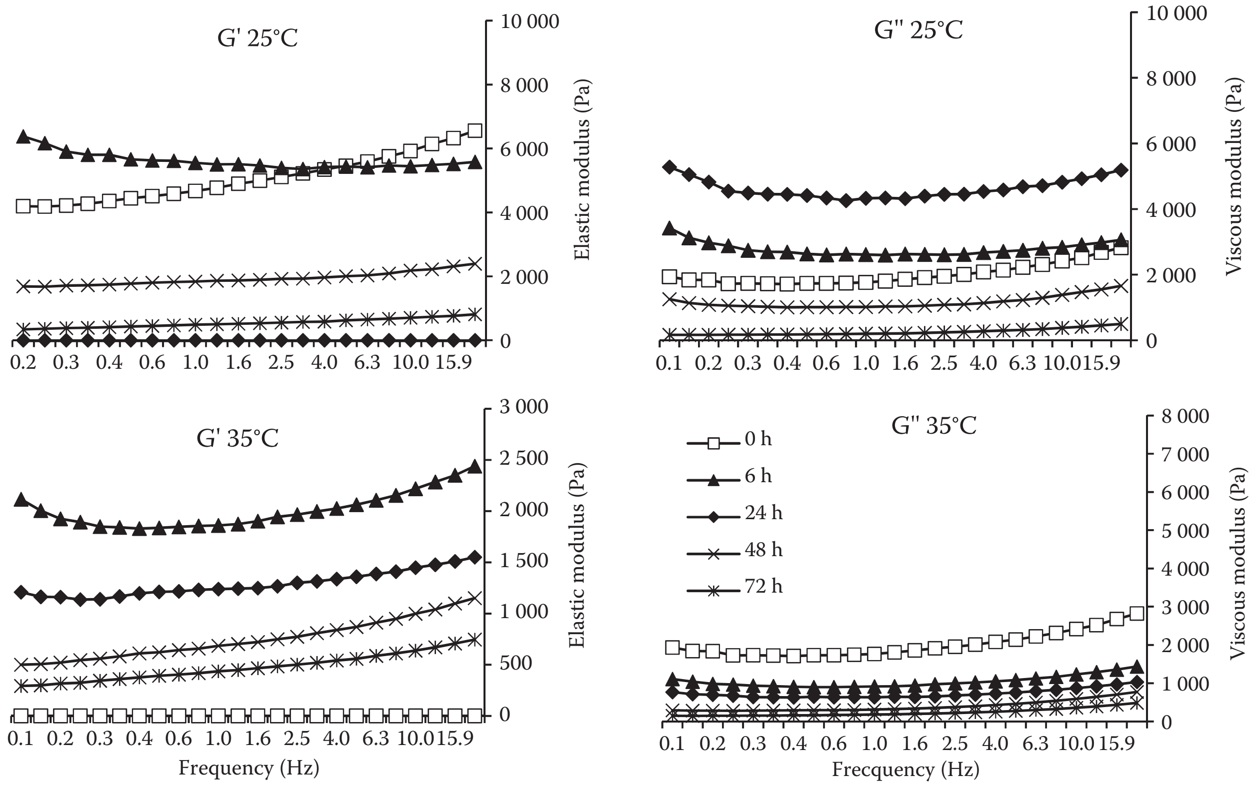

Figure 2. Elastic modulus (G') and viscous modulus (G'') of sourdough fermented at 25 and 35°C for 72 hours

behaviour of dough, and even small physical and chemical changes in the gluten network lead to alterations in rheological characteristics.

Influence of sourdough on bread dough fermentation. Bread dough made without sourdough, when just kneaded, had a pH of 5.86 and titratable acidity of 0.28% of lactic acid. After fermentation it had a pH of 5.77 and TA of 0.36% of lactic acid. In making sourdough bread, the sourdough used had been fermented at 25°C for 11 h and had a pH of 4.35. In this dough the value of pH immediately after kneading was slightly lower (5.63) and TA slightly higher (0.29%). These differences became more marked relative to dough not incorporating sourdough after fermentation was completed, since pH was reduced to 5.05 and TA rose to 0.49% of lactic acid. It should be noted that the fermentation time for this dough in the bread-making process was approximately 1 h, the time set by the equipment used, but this period should be extended, since in making sourdough bread the pH of the dough should be around 4.3. In fact, bread products made with sourdough require longer periods of fermentation.

The increase in TA and decrease in pH observed during the dough fermentation are due to the fermentative activity of the lactic acid bacteria. In general, the drop in pH is smaller than it might be expected from the production of acids, owing to the buffering effect of proteins in wheat. The bread made with sourdough had a more acidic pH (4.98) and TA of 0.4% of lactic acid, whilst the bread made without sourdough showed a pH of 5.86 and TA of 0.28% of lactic acid. The process of acidification promoted by the use of fermented dough is principally used to improve the quality, taste and aroma of wheat bread and to slow down its becoming stale (Brümmer & Lorenz 1991; Arendt *et al.* 2007). In addition, acidification reduces the risk of microbial growth in bread after baking, as values of pH higher than 6.0 are conducive to the growth of *Bacillus mesentericus*, responsible for the change to a stringy consistency known as 'rope in bread'.

The counts recorded on the different culture media were very similar for dough with or without sourdough, both before and after fermentation. This was perhaps because the fermentation time for the batches of dough used to make bread was short. The counts obtained on PCA and MRS agar were around 8 log CFU/g. On OGYEA, the counts recorded were slightly higher in dough produced without sourdough, with values around 8 log CFU/g that barely increased after the dough was fermented. In contrast, for dough made with sourdough the counts were

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marginally lower, both before and after fermentation. On VRBGA the counts noted were of the order of 3 log CFU/g in dough fermented without sourdough and 2 log CFU/g when sourdough was incorporated. If the fermentation time were to be increased, it is to be expected that there would be an increase in microbial counts on MRS and Rogosa agar and a decrease in pH until optimum values in the range of 4 to 4.5 were reached.

Influence of sourdough on dough and bread texture. In the bread-making process, dough incorporating sourdough had higher values of adhesiveness, cohesiveness, springiness, gumminess, and chewiness immediately after kneading relative to dough made without sourdough, but on the other hand it had lower figures for hardness and resilience (Table 4). As fermentation progressed, the values of all the parameters rose. Hardness, gumminess, and resilience were lower in dough made with sourdough than in that made without it, while adhesiveness, cohesiveness, springiness and chewiness were higher. In fact, Clarke *et al.* (2002) indicated that dough obtained through biological fermentation was softer. The values of the texture parameters of bread after the dough was baked were in general higher, except for adhesiveness, which was not detected, and cohesiveness, which decreased. Hardness, gumminess, resilience, and chewiness were greater in bread made with sourdough, whilst cohesiveness and springiness were higher in bread not incorporating sourdough (Table 4). The use of a smaller amount of compressed yeast in making the bread that used sourdough resulted in a loaf of smaller volume as its outcome. Its volume and the air present in bread interstices affect the hardness of a loaf and hence its gumminess (hardness × cohesiveness) and chewiness (gumminess × springiness). Thus, the larger the volume of the bread loaf, the lower will be its hardness (Ronda *et al.* 2015).

Influence of sourdough on sensorial properties of bread. Figure 3 shows the various sensory parameters determined for bread made with and without sourdough. The testing panel noted differences between the types of bread, bread made with sourdough being seen as more acceptable by fifteen votes to eleven. The greatest differences between bread made with sourdough (SDB) and bread made without (PB) were detected in respect of acid taste, doughy taste and aroma, which were more intense in SDB. In contrast, no major differences were noted with regard to colour, except a slightly more golden tone of bread made with sourdough.

Table 4. Values of the Texture Profile Analysis (TPA) of dough and bread obtained during the making process (data are the mean values ± standard deviations of three replicates)

	Sourdough addition		Hardness (kg·m/s^2)	Adhesiveness (kg·m^2/s^2)	Cohesiveness	Springiness	Gumminess (kg·m/s^2)	Resilience	Chewiness (kg)
Dough	Yes	just mixed	3.429 ± 0.430^{a}	1.661 ± 1.345^{ac}	0.587 ± 0.091^{a}	0.651 ± 0.228^{a}	2.034 ± 0.544^{a}	0.070 ± 0.015^{a}	1.412 ± 0.924^{ac}
	No		3.778 ± 0.377^{ab}	0.295 ± 0.176^{b}	0.462 ± 0.040^{b}	0.389 ± 0.038^{b}	1.754 ± 0.314^{a}	0.077 ± 0.006^{ab}	0.689 ± 0.189^{a}
	Yes	fermented	4.387 ± 0.533^{b}	2.400 ± 0.594^{c}	0.697 ± 0.016^{c}	0.917 ± 0.044^{c}	3.056 ± 0.352^{b}	0.088 ± 0.009^{b}	2.806 ± 0.363^{b}
	No		5.013 ± 0.170^{c}	0.622 ± 0.466^{ab}	0.624 ± 0.033^{ac}	0.637 ± 0.061^{a}	3.131 ± 0.255^{b}	0.120 ± 0.009^{c}	2.002 ± 0.307^{bc}
Bread	Yes		12.650 ± 3.212^{d}		0.397 ± 0.033^{d}	0.928 ± 0.034^{d}	5.054 ± 1.455^{d}	0.183 ± 0.030^{d}	4.683 ± 1.335^{d}
	No		7.239 ± 3.611^{e}		0.424 ± 0.037^{d}	0.978 ± 0.038^{e}	2.888 ± 0.833^{e}	0.174 ± 0.026^{d}	3.479 ± 1.360^{d}

$^{a-c}$different superscripts indicate significant differences (P < 0.05) between the values of the texture parameters of doughs with and without sourdough addition, just mixed and after fermentation, during bread making; $^{d-e}$different superscripts indicate significant differences (P < 0.05) between the values of the texture parameters of bread with and without sourdough

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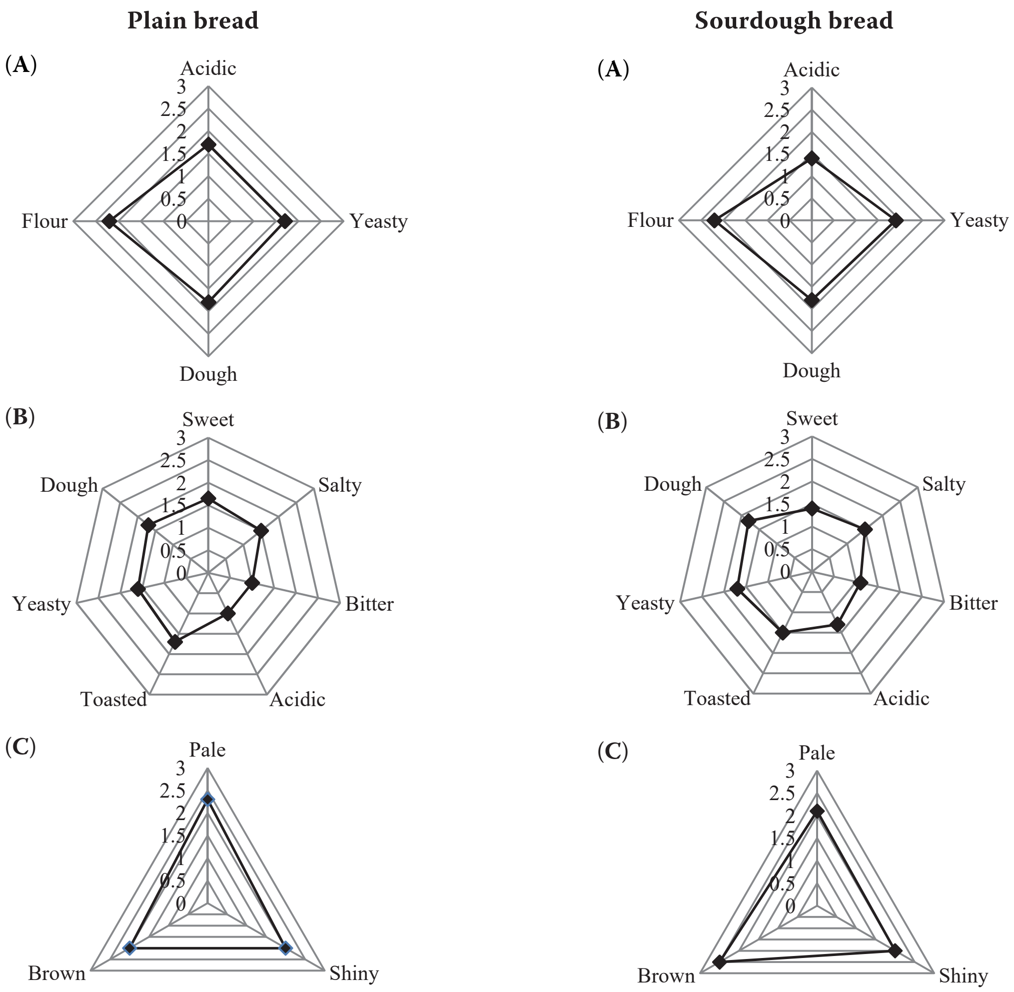

Figure 3. Sensory scores: (**A**) aroma, (**B**) taste, and (**C**) colour of plant bread and bread made without sourdough (intensity of each attribute was scored on a scale from 1 to 3)

The addition of sourdough favours the production of organic acids and improves the aroma of bread. Moreover, the diversity of microbes that it provides, their metabolic activity and the acidity which develops affect rheological properties and thus improve bread's sensory characteristics and texture (Brümmer & Lorenz 1991; Arendt *et al.* 2007). The proteolytic activity of the LAB present in the sourdough produces an increase in the concentration of amino acids, which can act as precursors for aromas (Gänzle *et al.* 2007). The relationship between lactic acid and acetic acid is a major factor affecting the aroma profile and final structure of the product. The acetic acid produced by the heterofermentative LAB is responsible for a shortening and hardening of gluten, whilst lactic acid may result in a more elastic structure of gluten (Komlenić *et al.* 2012). Furthermore, the higher content of amino acids and simpler sugars in the product made with natural yeast, as opposed to bread made with industrial yeast, determines the aroma of a loaf. The alcohol formed, the drop in pH, and the metabolites arising from secondary fermentations participate directly or as precursors in the development of bread taste, aroma, and colour (Onno 1996).

CONCLUSIONS

At the start of fermentation, sourdough fermented at 25°C had higher pH values than those observed in batches of dough fermented at 35°C and a slower increase in acidity. However, after 24 h the development of acidity was slightly greater in dough fermented at the lower temperature. In parallel, the counts of LAB were marginally higher in sourdough fermented at 35°C at the beginning of fermentation, but after twenty-four hours had elapsed, batches of dough fermented at 25°C had the higher counts,

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which remained more or less constant until the end of fermentation. The optimum pH value of sourdough, which lies between 4.2 and 4.3, was reached after around 9 h when fermentation was at 35°C and around 11 h when it was at 25°C. This is related to the increase that temperature produces in the fermentation quotient.

The data arising from analyses of the texture profile and rheology showed that the ideal fermentation time for sourdough at 25°C was below 24 hours. Refrigeration temperature may be used as a method for preserving sourdough over short periods of time. The data also revealed that the mechanical properties of bread made with sourdough were better than those of bread made without it.

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