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Physicochemical, textural and microbiological properties of optimised wheat bread formulations as affected by differently fermented sourdough

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RESEARCH ARTICLE

Abstract

The aim of the study was to evaluate the physicochemical properties, microbiological and textural features of optimised wheat bread formulations consisting of sourdough (A) prepared with two different fermentation methods [spontaneous fermentation (F1) versus starter of *lactic acid bacteria* (LAB) added fermentation (F2)], instant active dry yeast (B) and wheat bran (C) during their shelf life. The optimised levels for F1 fermentation type 11.45% for sourdough, 1.10% for dry yeast and 1.58% for wheat bran; and for F2 fermentation type 6.99% for sourdough, 1.02% for dry yeast and 38.84% for wheat bran were determined according to results. The acidic content of the sourdough improved the crust thickness, volume and colorimetric properties of the bread, significantly (P<0.05). The effects were much more pronounced in optimised bread $(OB)_{F2}$. The retrogradation phenomenon during the shelf life was evaluated with the result of rate of staling (RS) and loss of springiness (LS) values which determined by using texture profile analysis parameters, and diferential scanning calorimetry (DSC) thermograms obtained during the shelf life. RS (7.14 for CB, 4.55 for OB_{F1}, and 2.90 for OB_{F2}), and LS (62.1 for CB, 51.6 for OB_{F1}, and 39.7 for OB_{F2}) decreased significantly (P<0.05) by addition of sourdough. Therefore, CB had the most hardness texture at the end of the shelf life. All bread samples exhibited moisture loss during their shelf life especially in the first three days but demonstrated different tendencies. OB_{F2} sample had the highest moisture content in contrast to CB. Although no endothermic area could be determined on DSC thermograms on day 0, the initial tendency of the bread samples, especially CB and OB_{F1} was clearly seen. On day 5 thermograms, an increase in endothermic peak areas due to starch retrogradation was observed (413.792 mJ for OB_{F1}, 510.107 mJ for OB_{F2} and 768.962 mJ for CB). The results showed that sourdough improved the staling properties of bread. We found that the textural properties, the loaf and staling qualities of sourdough breads (OB_{F1} and OB_{F2}) were higher than that of CB. Furthermore, the F2 fermentation method had a much more pronounced effect in terms of textural properties examined.

Keywords: texture profile analysis, sourdough, bread, response surface methodology, staling

1. Introduction

Baked products undergo physical, chemical, microbial and sensory alterations during storage. The time-dependent loss in appearance, texture and aroma is generally described as bread staling. Depending on consuming time, the crispness of bread crust decreases, crumb firmness significantly increases and bread gains a stale flavor. Staling can be evaluated by means of examination of the revealed physical, chemical or microbiologic events (Torrieri *et al.,* 2014).

In bread-making, the usage of sourdough has an ancient tradition and still plays an important role in bakery products. The sourdough is especially used to improve volume, flavour, shelf life and enhance the nutritional value of the bread. While the traditional sourdough is obtained by spontaneous fermentation of a mixture of various cereal flours, salt and water; the use of specific starter cultures, such as lactic acid bacteria (LAB) to control the fermentation process during baking and its ability to improve quality and extend shelf life of bread has widely

been described in various studies in recent years (Arendt *et al.,* 2007; Gocmen *et al.,* 2007; Katina *et al.,* 2006; Torrieri *et al.,* 2014).

The metabolites produced by the influence of the mutual interactions of LAB and yeast in the sourdough fermentation may affect the texture and staling of bread. The metabolites, such as exopolysaccharides (EPS), organic acids and/or enzymes, have been known to have positive effects on bread quality. For instance, EPS can increase loaf volume, reduce crumb hardness, improve the elasticity of the dough and prolong shelf life; they may also act as bread improvers similar to various hydrocolloids (Arendt *et al.,* 2007; Palomba *et al.,* 2012; Poutanen *et al.,* 2009). The organic acids which one of the important metabolites give acidic flavor as a primarily result and affect the enzyme activity in the dough, in addition to various other effects on bread quality (Gobbetti *et al.,* 2014).

The traditional sourdough process performed under uncontrolled fermentation conditions is based on the back-sloping process requires long-term high labor. Therefore, industrial utilisation of wheat sourdough has not gained wide acceptance in many countries. Recently, the controlled sourdough processes have been developed by utilising yeast and LAB with special technological properties. This kind of a fermentation process offers the option of using selected yeast or LAB as a starter culture that can be appropriate for bakery product with desired properties.

In our previous studies, we explained that optimised sourdoughs obtained from two different fermentations (F1 and F2) resulted in improved bioavailability and various bioactive properties (Hayta and Hendek Ertop, 2017) and affected microtextural features (Hayta and Hendek Ertop, 2018) of the bread. In this study by using the response surface methodology (RSM) with the same responses (bread value (BV) and bread desirability (BD) and factors (level of bran, sourdough, and yeast), the effect of the optimised sourdoughs on physicochemical, textural and microbiological properties of bread was examined during shelf life.

2. Materials and methods

Materials

The wheat flour (59.2% water absorption, 14.3% moisture, 11.3% protein, 0.63% ash) and wheat bran samples were obtained by a wheat flour producer (Cesur Milling Company, Trabzon, Turkey). The instant active dry yeast (Dr. Oetker) and salt were supplied by a local supermarket. The chemicals used were acquired from Sigma (Darmstadt, Germany) and Merck (Darmstadt, Germany).

Experimental design and optimisation

The levels of yeast and sourdough, the bran content of sourdough, were considered as three independent factors. The two main responses that were examined were bread value (BV) and bread desirability (BD) as described by Hayta and Hendek Ertop (2017). The response surface methodology (RSM)-central composite rotatable design (CCRD) desirability function was used to measure the effect of the three factors on the responses (Design Expert 7.0.0, Stat-Ease Inc., Minneapolis, MN, USA). The experimental levels of factors were: 0, 0.41, 1.00, 1.59, 2.00% for instant active dry yeast; 5, 9.05, 15.00, 20.95, 25.00% for sourdough; and: 0, 8.11, 20.00, 31.89, 40.00% for wheat bran. These levels obtained with our previous trials and offered by literature. The three factors and five replicates at the center point led to a set of 20 experiments. The bread samples were produced according to the experimental design. The experimental design was applied to both fermentation methods (F1 and F2) described in our previous studies (Hayta and Hendek Ertop, 2017, 2018).

The BD values were estimated by using a 'five-point hedonic scale' sensory evaluation test. ('5' as 'I like it very much' and '1' as 'I didn't like it at all' respectively) (Hayta and Hendek Ertop, 2017, 2018; Olapade and Adetuyi, 2007). BV is a parameter which enables the evaluation of texture, crumb properties and volume of bread samples together. BV was calculated using the equation below as described by Pelshenke *et al.* (1964):

$$BV = \left[\frac{\text{pore factor} \times \text{volume factor}}{100}\right] \pm \text{crumb value} \qquad (1)$$

The 'Dalmann scale' method recognised by Pelshenke *et al.* (1964) was slightly modified and Image Pro Plus 6.0 (Media Cybernetics Inc., Rockville, MD, USA) software was used to determine the pore factor as decribed in our previous studies (Hayta and Hendek Ertop, 2017, 2018). Images of both faces of two central slices (20 mm thickness) were scanned (600 dpi) with a flatbed scanner (Model Scanjet 8200, HP, Cupertino, CA, USA). The images converted to grayscale were calibrated by applying appropriate filters to measure pore size and their distribution with Image-Pro Plus 6.0 software. The number and the area of pores were characterised by enumerating the pores by the software. For classification of the pores, the five pre-selected dimensional classes based on pores area (class1 = 0.05-0.49 mm^2; class 2 = 0.50-0.99 mm^2; class 3 = 1.00-4.99 mm^2; class 4 = 5.00-49.99 mm^2; class 5=>50 mm^2) was used (Bianchia *et al.*, 2008). The pore numbers of each class which were calculated using the software were multiplied with their own coefficient (for class 1:1.0, class 2: 0.8, class 3:0.6, class 4:0.4, class 5:0.2) (Hayta and Hendek Ertop, 2017), and pore factors were calculated. Since Class 1 contained pores with the smallest area, the highest coefficient was given to Class 1, similar to Dallman Scale.

Preparation of sourdoughs

The dough yield (DY) value, which represented the proportion between water and flour, was 200 for the sourdoughs (Chavan and Chavan, 2011).

Sourdough prepared by spontaneous fermentation (F1)*:* The 'back-sloping method' was used. The wheat flour (200 g) and water (200 g) were mixed and fermented spontaneously until the dough reached a pH value below 4.5.

Sourdough prepared by starter (F2): LAB (*Lactobacillus delbrueckii, Lactobacillus brevis* and *Lactobacillus plantarum*) were activated on MRS broth culture to obtain a cellular suspension of 10^7cfu/ml. The bacterial suspensions were washed two times and each LAB culture, which was 10^7cells/ml, was added at a ratio of 1% (Wu *et al.*, 2012).

Preparation of bread samples

The straight dough method described by Keswet *et al.* (2003) were used as slightly modified. The production method followed the main steps of premixing, kneading, fermentation for 40 min, shaping, proofing for 50 min and finally baking at 185 °C for 25 min. For preparing the dough, the flour (300 g), water (59%), sourdough, dry yeast and salt (1.5% based on dry matter) were added to the kneading bowl of the mixer (KitchenAid KSM150PSER, Antwerp, Belgium) and mixed for 15 min.

Physicochemical properties

To measure pH, the sample was homogenised with distilled water (1:9, w/v) by an ultra turrax (T25, IKA, Königswinter, Germany). The pH value was measured. The mixture was titrated with 0.1 N NaOH. The total titratable acidity (TTA) was calculated (Rizzello *et al.*, 2016). The volume was determined by applying the rapeseed displacement method. The breads were weighed, and specific volumes of the breads were calculated (Artan *et al.*, 2010). The colour profile of the bread crust and bread crumb were determined on five different points using a colorimeter (CR400; Konica-Minolta, Tokyo, Japan) as L^*, a^* and b^*. Mean values were then calculated (Torrieri *et al.*, 2014). The crust thicknesses (mm) of the samples were measured at five different points using a digital caliper.

For measuring moisture and moisture loss (ML), 3 hours after baking, the crust and crumb of a slice was taken from the bread (Poinot *et al.*, 2008), were homogenised, and 5 g was weighed out. Moisture was measured by oven drying at 105 °C to constant weight and then calculated. ML during the shelf life was also determined between the 2nd and 8th day.

Organic acid content

The conditions recommended by Kritsunankula *et al.* (2009) were modified to be used for HPLC (Thermo Fisher Scientific Inc., Waltham, MA, USA) separation of the organic acids. 50 µl standard/sample solutions were injected into an isocratic mobile phase of 1% of acetonitrile in 99% of 0.05 M KH_2PO_4 buffer (pH 2.5), which was flowed at a rate of 0.7 ml/min. The standard lactic acid peak was obtained at 3.4 min, and the acetic acid peak at 4.1 min. The injected zone was passed through the C18 column (5 µm particle size, 150 mm length, 4.6 mm i.d.) and the UV detector (210 nm detection wave length) respectively. All experiments were performed at column temperature of about 45±1 °C and room temperature of about 25±1 °C. The chromatograms, peak areas and retention times were evaluated. Calibration graph was constructed by plotting peak areas obtained versus concentrations of the organic acid.

Texture profile analysis

Crumb texture was determined on day 0, 3, 5 and 8 of storage. The three bread slices (20 mm thickness) taken from the centre of each loaf were used to evaluate physical crumb texture. TPA was performed using a texture analyser (TA-XT.Plus, Stable Micro Systems, Godalming, UK) equipped with a 50-mm aluminium cylindrical probe. Instrument settings were: the test speed 5 mm/s; the applied force 0.98 N for compress the middle of the bread crumb to 50% of its original height. The waiting time between the compression cycles was 5 s. The values calculated by the TPA software were chosen to describe the crumb textural parameters of the bread samples: hardness (peak force of the first compression cycle), springiness and chewiness (area of the second compression cycle divided by the area of the first compression cycle multiplied by springiness). The measurements were performed on day 0, 3, 5 and 8. Rate of staling (RS) was calculated using the following equation (Hager *et al.*, 2012):

$$RS = \frac{\text{crumb hardness day 5-crumb hardness day 0}}{\text{crumb hardness day 0}} \quad (2)$$

Moreover, loss of springiness was calculated by means of springiness values on day 0 and 5 using the following equation:

$$LS = \left[\frac{\text{crumb springiness day 5-crumb springiness day 0}}{\text{crumb springiness day 0}}\right] \times 1000 \quad (3)$$

Differential scanning calorimetry

Analysis of bread samples was carried out using a diferential scanning calorimetry (DSC) calorimeter (Perkin Elmer, Waltham, MA, USA) on day 0 and 5. A sample of approximately 10 mg was taken from each bread samples

and tightly packed into an aluminium pan. The pan was closed with a lid and weighed. All samples were heated from 2 to 100 °C at a rate of 10 °C/min. The method used by Katina (2005) and Torrieri *et al.* (2014) was performed for evaluating endothermic enthalpy (ΔH), peak area (mj) and peak temperature (°C) in the obtained thermograms.

Enumeration of mould growth during shelf life

To determine mould growth during 8 days, the method used by Dal Bello *et al.* (2007) was slightly modified. The loaf of bread was sliced at 20 mm thickness and stored at the room temperatures in open fridge bags. 10 g samples were taken from the bread loaf and prepared dilutions. Appropriate dilutions were placed on YGC agar and incubated at 27 °C for 48 hours. The colonies were then counted (Hendek Ertop and Coşkun, 2018).

Statistical analysis

The obtained results by the optimisation were validated experimentally. Variance of analyses (ANOVA) and one sample *t*-test (SPSS 17.0.1) were used for the comparison ($P<0.05$) of the results (Katina *et al.*, 2006).

3. Results and discussion

Optimisation

The result of BV and BD values were subsequently analysed by the software as the responses. The 'lack of fit' and 'sequential model sum of squares' tests were performed (Table 1) for the responses (BD and BV). 'Lack of fit' was determined as insignificant ($P<0.05$) and 'quadratic function' was approved appropriate function ($P<0.05$) in terms of both responses. The effects of factors on BD and BV were evaluated (Table 1 and 2). While the influences of instant active dry yeast and sourdough usage on BD were statistically significant ($P<0.05$) for fermentation type F1, the influence of bran usage on BV was statistically significant ($P<0.05$). The effects of sourdough and bran usage on BD and BV were determined as statistically significant ($P<0.05$) for F2. The model was also determined as statistically significant ($P<0.05$) for both responses and both fermentations. Statistical parameters, ANOVA results and the first solutions offered by software were given in Table 1 as explained in Hayta and Hendek Ertop (2017).

Final equations were coded with the following factors.

- For fermentation type F1:

$$BV = +154.65 - 1.61 \times A - 4.25 \times B - 16.87 \times C - 4.78 \times A \times B - 8.75 \times A \times C - 0.59 \times B \times C - 12.92 \times A^2 + 11.79 \times B^2 + 3.49 \times C^2$$

$$BD = +3.24 - 0.48 \times A - 0.47 \times B - 0.06 \times C - 0.31 \times A \times B - 0.19 \times A \times C - 0.00 \times B \times C - 0.56 \times A^2 + 0.15 \times B^2 + 0.42 \times C^2$$

- For fermentation type F2:

$$BV = +154.65 - 1.61 \times A - 4.25 \times B - 16.87 \times C - 4.78 \times A \times B - 8.75 \times A \times C - 0.59 \times B \times C - 12.92 \times A^2 + 11.79 \times B^2 + 3.49 \times C^2$$

$$BD = +3.24 - 0.48 \times A - 0.47 \times B - 0.06 \times C - 0.31 \times A \times B - 0.19 \times A \times C - 0.00 \times B \times C - 0.56 \times A^2 + 0.15 \times B^2 + 0.42 \times C^2$$

The 'numerical optimisation' was performed in the study. The responses (BD and BV) were evaluated together according to the desirability function which was based on

Table 1. Statistical parameters of optimisation; *P*-values for model selection and lack of fit tests: model and independent variable factors.[1]

		Fermentation type (*P*-values[2])			
		F1		F2	
		BV	BD	BV	BD
Model selection and lack of fit test	Quadratic	0.0041	0.0066	0.0030	0.0040
	Lack of fit	0.1990	0.1178	0.1092	0.1304
Model and independent variable factors	Model	0.0062	0.0121	0.0082	0.0005
	A	0.6824	0.0152	0.4068	0.2089
	B	0.2907	0.0169	0.0481	<0.0001
	C	0.0013	0.7131	0.0338	0.0364

[1] BV = bread value; BD = bread desirability; F1 = spontaneous fermentation; F2 = starter LAB added fermentation; A = dry yeast; B = sourdough; C = bran.
[2] Values are statistically significant ($P<0.05$).

Table 2. Statistical parameters of optimisation; *P*-values for model selection and lack of fit tests: ANOVA results of quadratic function.[1]

Response	Fermentation type			
	F1		F2	
	BV	BD	BV	BD
R^2	0.835	0.808	0.825	0.906
Intercept	154.65	3.24	143.45	2.74
A, %	-1.61	-0.48	-3.39	-0.14
B, %	-4.25	-0.47	-8.81	-0.73
C, %	-16.87	-0.06	-9.63	-0.25
AB	-4.78	-0.31	-4.15	0.47
AC	-8.75	-0.19	-3.66	-0.03
BC	-0.59	0.00	-12.49	-0.16
A^2	-12.92	-0.56	-10.58	-0.30
B^2	11.79	0.15	7.98	0.23
C^2	3.49	0.42	14.22	0.32

[1] BV = bread value; BD = bread desirability; F1 = spontaneous fermentation; F2 = starter LAB added fermentation; A = dry yeast; B = sourdough; C = bran.

the evaluation of multiple quality characteristics. The first solution from the 30 solutions offered by the software that have desirability value of 1 was selected and applied to the study (Table 3).

Experimental validation of optimisation results

The bread samples were prepared by the use of optimised levels (Table 3) in a form of three replicates. The BV and BD values of optimised samples were determined for both fermentation type (F1 and F2), and the mean values were calculated. It was also evaluated whether there was a statistically significant ($P<0.05$) difference between estimated values from the model and the mean of the bread samples by applying the one sample *t*-test. The results of the one sample *t*-test for each response are BV: 190.22±7.29 ($P=0.229$); BD: 4.57±0.06 ($P=0.130$) for F1 and BV: 202.26±5.12 ($P=0.071$); BD: 4.67±0.12 ($P=0.338$). Between the results obtained from the validation test were found the statistically insignificant differences ($P>0.05$). The results indicated that the model obtained with optimisation was experimentally successful.

Determination of differences between optimised breads (OB_{F1} and OB_{F2}) and control bread (CB)

The bread samples (OB_{F1} and OB_{F2}) prepared/validated according to the optimised model were compared with CB in terms of several quality properties.

Physicochemical properties of bread samples

Initially, the acetic acid and lactic acid contents of the sourdough samples prepared with two different fermentations were determined as 0.206 mg/g acetic acid and 1.491 mg/g lactic acid for F1; 0.447 mg/g acetic acid and 1.104 mg/g lactic acid for F2. The results show that lactic acid levels for both fermentation type were higher than that of acetic acid. However, the level of acetic acid in F2 sourdough was higher than that F1 sourdough. Then the organic acid composition of the bread samples prepared with F1 and F2 sourdough were determined and the similar acidic composition was also found in the bread samples (Table 4). The level of acetic acid in OB_{F2} (0.577 mg/g) bread was higher than that of OB_{F1} (0.237 mg/g) bread sample. The acidic composition of the sourdough bread samples was seriously strong than of the CB sample. Moreover, the sourdough bread samples (OB_{F1} and OB_{F2}) exhibited seriously acidic composition than of the CB sample. The difference between pH, TTA values and the amounts of organic acids of the bread samples were found statistically significant ($P<0.05$).

CB obtained by yeast fermentation had the lowest TTA value (2.65%). However, the TTA value was 4.60% for OB_{F1} and 5.34% for OB_{F2}. It was determined that the acidification resulted in a decrease of pH values of the sourdough bread samples clearly. The LAB synthesises lactic acid, acetic acid, ethanol and CO_2 by the heterofermentation of hexoses, and lactic acid by the homofermentation of

Table 3. Optimisation results for F1 and F2 of the model selection and lack of fit tests.

Fermentation type	The level of usage (g)			BV	BD	Desirability
	A	B	C			
F1	1.10	11.45	1.58	197.42	4.65	1.000
F2	1.02	6.99	38.84	212.76	4.75	1.000

[1] BV = Bread value; BD = Bread desirability; F1 = Spontaneous fermentation; F2 = Starter LAB added fermentation; A = dry yeast; B = sourdough; C = bran.

Table 4. Physicochemical properties of bread samples.[1,2]

Bread sample	pH	TTA (%)	Moisture (%)	Crust thickness (mm)	Volume (ml)	Specific volume (ml/g)	Organic acid content (mg/g)	
							Lactic acid	Acetic acid
OB_{F1}	5.29^{b}	4.60±0.012^{b}	38.20±0.14^{b}	4.27±0.06^{a}	2,691±15^{b}	4.029^{b}	0.835±0.08^{a}	0.237±0.05^{b}
OB_{F2}	5.04^{c}	5.34±0.016^{a}	39.68±0.21^{a}	4.29±0.02^{a}	2,732±18^{a}	4.081^{a}	0.610±0.05^{b}	0.577±0.03^{a}
CB	6.15^{a}	2.65±0.067^{c}	38.11±0.10^{b}	3.46±0.13^{b}	2,610±10^{c}	3.915^{c}	0.294±0.05^{c}	0.098±0.00^{c}

Bread sample	Crust			Crumb		
	*L**	*a**	*b**	*L**	*a**	*b**
OB_{F1}	69.31±1.29^{b}	6.00±0.68^{a}	24.68±0.50^{b}	71.23±0.83^{a}	0.49±0.05^{a}	14.27±0.25^{c}
OB_{F2}	66.70±2.56^{c}	5.95±0.78^{a}	25.50±0.42^{a}	67.71±1.54^{b}	0.63±0.04^{a}	15.60±0.74^{a}
CB	71.31±1.76^{a}	4.31±0.90^{b}	22.22±0.57^{c}	71.01±1.39^{a}	-0.26±0.07^{b}	14.75±0.45^{b}

[1] OB_{F1} = optimised bread produced with F1 fermentation; OB_{F2} = optimised bread produced with F2 fermentation; CB = control bread; TTA = total titratable acidity.
[2] Different superscript letters in the same column mean the values are significantly different (P<0.05).

hexoses (Olapade and Adetuyi, 2007). The LAB which were used for F2 fermentation in the present study *L. delbrueckii* was homofermentative, while *L. plantarum* and *L. brevis* were heterofermentative. Therefore, the acetic acid content of sourdough and bread obtained with F2 fermentation was higher than that of F1 sourdough and bread. The fermentation method used in the bread production and its associated microbiota and its metabolites clearly influenced the organic acid content, pH and TTA levels.

During fermentation, a decrease in pH of the dough may promote an increase in protease activity. In addition, LAB also have their own enzyme activity, and increased enzyme activity raises the free amino acid content by hydrolysing proteins (Hansen and Schieberle, 2005), thus it may lead to a high TTA value. A study by Hansen *et al.* (1989), which used two homofermentative LAB (*L. plantarum* and *L. delbrueckii*) and three heterofermentative LAB (*Lactobacillus fermentum, Lactobacillus sanfrancisco,* and *L. brevis*) determined that TTA was increased by heterofermentative LAB.

The crust thickness of CB was lower than those of OB_{F1} and OB_{F2} that contained sourdough. Additionally, the moisture content values of the bread samples and the differences between them were found to be statistically significant (P<0.05). The last stage of the baking is the release of the remaining moisture and formation of the crust. The thick crust formation in the sourdough breads prevented and limited the release of moisture. On the other hand, although OB_{F1} and OB_{F2} had nearly the same crust thickness, their moisture was found different. This is due to the fact that, the acidification may have related to the improved moisture retention. It was indicated in a study conducted by Corsetti *et al.* (2000) that the biological acidification due to fermentation may aid in maintaining bread freshness because it influences moisture redistribution throughout the loaf during storage. Furthermore, the formation of dextrins and exopolysaccharides during fermentation may have enhanced the shelf life by decreasing starch recrystallisation.

The difference between L^* values in bread crust were statistically significant (P<0.05). While CB had the highest (71.31) lightness value, the level was lower for sourdough breads OB_{F1} and OB_{F2}. The a^* and b^* values for OB_{F1} and OB_{F2} were higher and this statistically significant ($P<0.05$) than that of CB. Colour and appearance are the most important factors influence consumer preferences of bakery products (Purlis, 2010). The formation of the yellow-gold-brown colours often attributed to a non-enzymatic chemical, specifically the caramelisation and Maillard reactions simultaneously taking place during the baking (Purlis and Salvadori, 2009). The reactions lead to colour changes at different levels in crumb and crust of bakery products (Artan *et al.*, 2010). The microflora produces simple sugars as metabolites during sourdough fermentation. The simple sugars produced at the end of sourdough fermentation may have promoted to chemical browning reactions and may have resulted in the increase of the a^* value of crust in particular. Because Amadori rearrangement required H^+, a decrease in pH value during fermentation probably positively effected the browning reactions. The duration of fermentation has been reported to influence colour of the bakery product through the

formation of various compounds as precursors of brown pigments (Martinez Anaya, 1996).

The difference between the volume and specific volume values of breads was statistically significant *(P*<0.05). ANOVA analysis showed that the fermentation methods have a significant (*P*<0.05) effect on bread volume. OB_{F2} had the highest volume value followed by OB_{F1} and CB respectively. The improving effect of wheat sourdough in bread making performance has been linked to fermentation process and interaction of microbiota. It was indicated by the previous studies that the addition of sourdough increases the specific volume and volume of bread (Dal Bello *et al.*, 2007; Farahmand *et al.*, 2015; Wu *et al.*, 2012). The gluten structure in acidic dough containing sourdough has been known to have a better gas holding capacity (Gobbetti *et al.*, 1995). It is caused by the solubility of pentosans during the sourdough process (Corsetti *et al.,* 2000) and by the increase in endogenous enzyme activities due to lower pH (Clarke *et al.*, 2003). Moreover, the effect was also attributed to the water retention capacity improved promoted by fermentation of dough (Gobbetti *et al.*, 1995).

Textural properties

To describe the texture of the optimised breads (OB_{F1} and OB_{F2}) and control bread (CB), crumb hardness, chewiness and springiness were shown in Table 5. According to the TPA results, the greatest change in textural characteristics of the bread samples occurred in the first three days. The hardness value of all breads increased during the shelf life. While the CB sample had the softest crumb, the optimise breads (OB_{F1} and $OB_{F2)}$ had harder texture on day 0. Due to its higher fibre content, crumb hardness of OB_{F2} was about two times higher than that of CB. However, CB reached the highest degree of hardness compared to the other samples on the 8th day.

The RS parameter was calculated using the hardness values of the breads; while the highest RS value (7.14) was found in CB, the values were found to be lower in the sourdough bread samples OB_{F1} (4.55) and OB_{F2} (2.90). The addition of sourdough slowed the rate of staling during shelf life.

The 'crumb springiness' was described by Hager *et al.* (2012), as a value exhibiting the recovery of the sample after compression, so it is important in separating soft, soggy bread from soft but resilient bread. CB and OB_{F1} had the highest crumb springiness values (0.998 and 0.989 respectively), while OB_{F2} showed the lowest crumb springiness value (0.981). The springiness values of the bread samples decreased during their shelf life. A remarkable decrease in the springiness values of the breads occurred between day zero and the 3rd day. The LS values calculated by using mean springiness parameters on day 0 and 5 were found to be the highest for CB compared to OB_{F1} and OB_{F2}.

Chewiness gives an indication of the energy required to masticate a solid food. The chewiness values increased during the shelf life. CB had the lowest chewiness (406.4±32.3) on day 0, which is significantly lower than that of OB_{F1} (597.4±90.0) and OB_{F2} (706.2±114.2) samples. However, CB had the highest value (2540.3±518.7) on the

Table 5. Properties of bread samples during shelf life.[1,2]

Bread sample	Days	Textural parameters					Moisture	ML	Mould growth (log (cfu)/g)
		Hardness	Springiness	Chewiness	RS	LS			
OB_{F1}	0	721.8±109.9	0.989±0.001	597.4±90.0	4.55^{b}	51.6^{b}	38.20±0.14^{a}		0
	3	2,075.2±201.7	0.948±0.034	1,126.8±189.9			35.81±0.22^{b}	6.26^{a}	1.30
	5	4,003.7±381.2	0.938±0.010	1,743.7±167.0			34.89±0.06^{c}	2.57^{b}	4.83
	8	5,200.9±581.6	0.928±0.028	2,425.7±329.8			34.56±0.37^{c}	0.95^{c}	5.67
OB_{F2}	0	883.3±173.3	0.981±0.014	706.2±114.2	2.90^{c}	39.7^{c}	39.68±0.21^{a}		0
	3	3,154.2±357.5	0.953±0.010	1,721.9±313.3			36.39±0.28^{b}	8.30^{a}	1.00
	5	3,443.6±565.7	0.942±0.012	1,742.5±404.8			36.45±0.15^{b}	0.11^{b}	4.63
	8	5,287.8±521.4	0.929±0.018	2,494.8±518.2			36.09±0.42^{b}	0.99^{b}	5.51
CB	0	484.7±41.1	0.998±0.002	406.4±32.3	7.14^{a}	62.1^{a}	38.11±0.10^{a}		0
	3	2,352.6±260.7	0.948±0.013	1,351.2±161.1			35.04±0.10^{b}	8.05^{a}	1.30
	5	3,943.6±702.8	0.936±0.020	1,879.8±414.8			34.46±0.26^{c}	1.66^{b}	2.30
	8	5,461.5±521.4	0.935±0.012	2,540.3±518.7			34.38±0.19^{c}	0.23^{b}	5.08

[1] Different superscript letters in the same column mean the values are significantly different (*P*<0.05).

[2] OB_{F1} = optimised bread produced with F1 fermentation; OB_{F2} = optimised bread produced with F2 fermentation; CB = control bread; RS = rate of staling; LS = loss of springiness; ML = moisture loss.

8th day. CB was the bread that lost chewiness the most compared to its value on day 0. Regarding overall textural properties, the sourdough breads, especially $OB_{F2,}$ were found to be more favourable compared to CB.

Moisture and moisture loss

The conservation of initial moisture in bread is an important criterion for the sustainability of eating quality. Moreover, ML is expressed as an increase in sensory hardness. The breads differed significantly in their moisture content (Table 5; $P<0.05$). OB_{F2} showed the highest moisture content in contrast to CB. All bread samples had ML during their shelf life but demonstrated different tendencies. This was expected due to the different amounts of sourdough and bran added based on the experimental optimised formulation. The ML parameter indicated a relative loss of moisture of the bread samples compared to the previous day. It was established that the highest ML value for all of the bread samples was obtained on the 3rd day. This situation is similar to TPA results. Although ML continued after the 3rd day, the rate was lower.

Starch retrogradation

Experimental thermograms obtained by DSC are shown in Figure 1. Since retrogradation did not proceed on day 0, no endothermic area could be determined on the thermograms. However, the initial tendency of the bread samples, especially CB, to retrogradation was clearly seen in thermograms on day 0. Thermograms and the data showed that storage time had a significant effect on melting enthalpy of recrystallised amylopectin and that fermentation types had a significant effect on the change of enthalpy of the three samples during their storage. During storage, the increment of the percentage of retrogradated starch leads to an increase in the peak area of the transition observed between 50 and 85 °C (Katina *et al.*, 2006; Ribotta *et al.*, 2004). In this study, it was determined that storage caused an increase in melting enthalpy (ΔH) of recrystallised

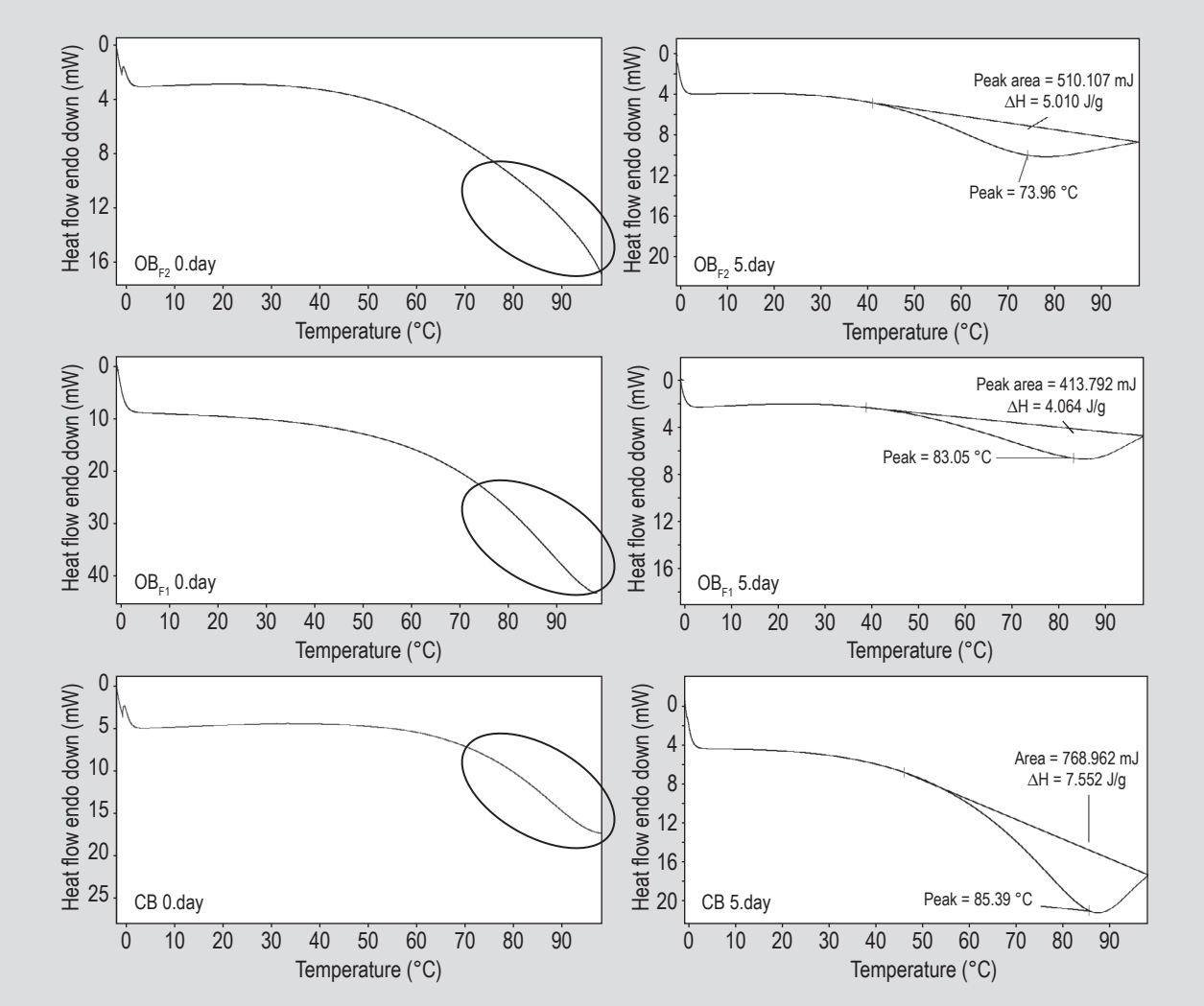

Figure 1. DSC thermogram of bread samples on days 0 and 5.

amylopectin in the breads, which was observed as an increase in endothermic peak areas in DSC thermograms. On the 5th day thermograms, an increase in endothermic peak areas due to starch retrogradation was observed. In addition, it was also observed that, the lowest endothermic peak area was 413.792 mJ for OB_{F1} and 510.107 mJ and 768.962 mJ for CB and OB_{F2} respectively. These results imply that sourdough has a delaying effect on bread staling. In this respect, several studies have reported beneficial effects of biological acidification on bread staling due to the metabolite products of fermentation and particularly, proteolytic activity of LAB (Torrieri *et al.*, 2014). Melting enthalpy of recrystallised amylopectin is affected by the storage process but not by the starter species (Andreu *et al.,* 1999; Kaditzky and Vogel, 2008).

It has been reported that the high bread moisture content increases shelf life (He and Hoseney, 1990) and delays starch retrogradation. In this study, the bread samples with sourdough, especially $OB_{F2,}$ had more moisture content than CB. Therefore, the differences in moisture content between the samples during storage may also justify DSC results.

Mould growth

The quality of bread is lost rapidly during storage not only due to staling but also due to microbial spoilage. Under ambient conditions, mould growth occur on well-packaged wheat bread within 4-6 days (Hager *et al.,* 2012). The examination time for microbial shelf life of the bread samples in this study was 8 days. While any mould growth was not generally observed in the first three days of storage, mould growth was observed from the 3rd day onwards. In OB_{F1} and OB_{F2}, the growth level on the 5th and 8th day was found to be higher than in CB. The microbial shelf life might be extended by slowing the mould growth in breads produced with LAB usage or sourdough fermented spontaneously (Dal Bello *et al.*, 2007). However, in this study, the microbial growth was higher in breads with sourdough (OB_{F1}, OB_{F2}) compared to CB. Depending on the optimisation results, OB_{F1} and OB_{F2} were prepared with different levels of bran, and therefore had different moisture contents. The microbial stability of sourdough breads was mainly compromised because of the high moisture and bran content, especially in OB_{F2}. The study revealed by Plessas *et al.* (2008) indicated that the sourdough usage was the most effective factor against mould growth and 50% usage was an effective rate. In this case, it has been stated that the final pH of bread was 4.3-5.2 and the duration of bread was 8-12 days (Plessas *et al.,* 2008). In this study, the level of sourdough was 11.45% for F1, and 6.99% for F2. It can be said that higher rates of sourdough lead to better results in terms of preventing mould growth.

4. Conclusions

In the study, the effects of sourdough on textural, physicochemical and microbial qualities of bread during its shelf life were evaluated. We found that the textural properties, the loaf and staling qualities of sourdough breads (OB_{F1} and $OB_{F2)}$ were higher than that of CB. Adding heterofermentative and homofermentative co-culture to OB_{F2} changed the organic acid content of the bread. The acidic content of the sourdough improved the crust thickness, volume and colorimetric properties of the bread. The effects were much more pronounced in OB_{F2} prepared with the starter culture. The use of sourdough, especially in OB_{F2} prepared with F2, resulted in a prolonged shelf life in terms of RS, LS and starch retrogradation. When assessed in terms of general textural characteristics, a severe loss in the consuming qualities of breads was observed in the first three days. Similar results were observed in TPA and ML parameters. Furthermore, RS and LS values determined by using TPA parameters, and DSC thermograms were interpreted in terms of staling qualities during the shelf life of the bread samples. The presented results showed that the sourdough improved the staling properties of bread. The spontaneous fermentation method (F1) is presently preferred in the bakery industry. Nonetheless, our results demonstrated that it is appropriate to use lactic starters in bread production. Nowadays, there is a consumer tendency towards bread types that are produced with wheat bran, germ or whole wheat flour due to their nutritional properties. However, the usage these fractions may have an adverse effect on the textural properties and volume of the bread. The result of this study indicated that the usage of the sourdough encouraged the volume despite the bran content. In this respect, the production of bread containing different types of cereals, whole wheat flour, and grain fractions might be improved by the incorporation of sourdough.

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