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Effect of sourdough at different concentrations on quality and shelf life of bread

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ABSTRACT

The objective of this work was to study the effect of sourdough obtained with selected exopolysaccharide (EPS)-producing lactic acid bacteria (LAB) strains on the quality of bread and its shelf life. Two sourdough concentrations were used in order to ascertain the best bread composition. Fresh bread quality was studied by means of microbiological, physical, chemical and mechanical analysis, whereas physical, thermal and mechanical properties were investigated to study the product shelf life. The results showed that dough prepared with 30 g/100 g of sourdough had a negative impact on bread quality properties in the absence of EPS-producing LAB strains, whereas the opposite was observed in the presence of EPS-producing strains: bread samples at 30 g/100 g of sourdough showed higher volume, higher moisture content and better mechanical properties during storage than samples at 20 g/100 g of sourdough. Moreover, 30 g/100 g of sourdough showed a protective effect on bread staling, thus confirming the effect of sourdough concentration and the positive role of EPS on functional properties.

1. Introduction

Baked products are perishable foods that undergo severe physical, physiochemical, sensory and microbial changes during storage (Robertson, 1993). The time-dependent loss in quality of flavour and texture is generally described as bread staling. Crumb firmness significantly increases, crispness of the bread crust decreases, and the bread loaf loses its fragrance, assuming a stale flavour. These complex physical and chemical phenomena are a consequence of a retrogradation of the starch granules gelatinized during baking, an interchange of moisture between the starch and protein constituents of bread, an increase in interaction between the protein fraction and starch, a redistribution of water in bread and a removal of aromatic molecules (Parker & Ring, 2001; Piazza & Masi, 1995; Schiraldi & Fessas, 2001).

The use of sourdough has a long tradition and still plays an important role in bread-making. Sourdough is obtained by spontaneous fermentation of a mixture of flour, water and salt; recent years have seen the use of specific cultures and control of the fermentation process. Its use in baking and its ability to improve the quality and extend the shelf life of bread has been widely described (Arendt, Ryan, & Dal Bello, 2007; Gocmen, Gurbuz, Kumral, Dagdelen, & Sahin, 2007; Katina, Heinio, Autio, & Poutanen, 2006; Martinez-Anaya, 2003). Lactic acid bacteria (LAB) produce a number of metabolites which have been shown to have a positive effect on the texture and staling of bread, e.g. organic acids, exopolysaccharides (EPS) and/or enzymes. EPS can improve the viscoelastic properties of dough, increase loaf volume, reduce crumb hardness and prolong shelf life (Poutanen, Flander, & Katina, 2009; Tieking & Gänzle, 2005). Moreover, the transformation of amino acids or peptides to aroma compounds contributes substantially to food flavour. In particular, the conversion of glutamate by LAB enables the targeted optimization of food flavour (Gänzle, 2009; Plessas et al., 2011). The *in situ* production of EPS has the advantage of avoiding the use of bread improvers such as expensive hydrocolloids (Arendt et al., 2007; Palomba et al., 2012; Pepe, Ventorino, Cavella, Fagnano, & Brugno, 2013; Tieking, Korakli, Ehrmann, Gänzle, & Vogel, 2003). However, *in situ* production of exopolysaccharides during sourdough fermentation is challenged by simultaneous acidification due to metabolic activities of the bacteria, which may significantly diminish the positive technological impact of EPS (Katina et al., 2009). The formation of alternative products from sucrose like organic acids are of special importance for application of *in situ* produced EPS. In particular, lactate and acetate have previously been identified to significantly affect dough rheology, bread volume and crumb hardness, and may counterbalance the positive effect of EPS (Kaditzky & Vogel, 2008).

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Lacaze, Wick, and Cappelle (2007) have developed a new process to obtain a dextran-rich sourdough in using a specific LAB strain (*Leuconostoc mesenteroides* LMGP-16878) able to produce a sufficient amount of high molecular weigh (HMW) dextran, ensuring a significant impact on bread volume. The sourdough obtained allows improvements in freshness, crumb structure, mouth feel and softness of all kinds of baked goods from wheat-rich dough products to rye sourdough breads. Katina et al. (2009) showed the potential of *Weissella confusa* to produce significant amounts of polymeric dextran and isomaltooligosaccharides in wheat sourdough without strong acidification. Dextran-enriched *W. confusa* sourdoughs showed increased viscosity and improved bread quality. Di Cagno et al. (2006) reported that as shown by carbohydrate consumption, the synthesis of EPS was found from sucrose only. Moreover, compared with a EPS-negative strain (*Lactobacillus sanfranciscensis* SF17), sourdough started with EPS-positive strains (*Weissella cibaria* WC4, *Lactobacillus plantarum* PL9), fermented at 30 °C for 24 h, increased its viscosity, and the resulting bread had higher specific volume and lower firmness. The performance of *L. sanfranciscensis* TMW 1.392 and its levansucrase deletion mutant in wheat dough and their impact on bread quality was studied by Kaditzky, Seitter, Hertel, and Vogel (2008). The authors reported that *in situ* production of EPS was not sufficient to achieve the same positive effects of EPS, as they partially overlapped with effects resulting from enhanced acidification. LAB strains and/or fermentation conditions must be found to maximize *in situ* EPS production while at the same time optimizing acid production to a certain quotient which allows acceptable volume, crumb structure and flavour of breads. Thus, when EPS-producing strains are screened for dough applications, their metabolite pattern, the pH at the end of fermentation and fermentation quotient (the molar ratio of lactate/acetate) should be considered.

In a previous work (Palomba et al., 2012) we showed that sourdough obtained with selected EPS-producing LAB strains after 15 h of fermentation at 30 °C with 5% sucrose resulted in improved viscoelastic properties. Thus, the aim of this work was to study the effect of the sourdough obtained with selected EPS-producing LAB strains on bread quality during shelf life. Microbiological and chemical analysis of sourdough and physical, thermal and mechanical properties of bread were investigated in order to study the evolution of product quality during time. Two sourdough concentrations were studied in order to find the best bread dough composition.

2. Materials and methods

2.1. Sourdough fermentation

Sourdough fermentation was obtained by using two different starters selected on the basis of exopolysaccharide production (Palomba et al., 2012): EPS-positive (EPS+) composed by *Leuconostoc lactis* 95A and *Lactobacillus curvatus* 69B2, and EPS-negative (EPS−) consisting of *L. lactis* 68A and *L. curvatus* 68A2. These LAB strains were previously isolated from sweet baked products (Palomba, Blaiotta, Ventorino, Saccone, & Pepe, 2011) and selected through quantitative analysis on solid media containing sucrose (Palomba et al., 2012). The sourdoughs were prepared by mixing 500 g of wheat flour, 280 g of tap water, 25 g of sucrose and a cell suspension to achieve viable counts of about $5.0 \cdot 10^7$ cfu/g and an incubation period of 15 h at 30 °C.

2.2. Microorganisms, pH and TTA of dough and sourdough

Differential microbial counts of LAB strains were determined on modified Chalmers agar plates (Pepe, Villani, & Coppola, 2001). The pH and acid equivalent were determined by standard methods (AACC, 1975) and total titratable acidity (TTA) was expressed as 0.1 mol equi/L NaOH/10 g of dough.

2.3. Preparation and storage condition of bread

Bread samples were prepared using two different amounts of sourdough: 30 g/100 g of dough (formulation P1), 20 g/100 g of dough (formulation P2). The recipes for the sourdough breads are given in Table 1. Breads were prepared by mixing flour, sugar, salt, yeast (baker yeast, Paneangeli, Italy), sourdough and water. The amount of water to be added was determined so as to have always dough with a consistency of 500 Brabender units (BU). All the ingredient were mixed for 20 min with a spiral mixer (Mod. F100 OEM, Bozzolo (MN), Italy). The dough was divided into 300 g loaves and moulded manually. The loaves were proofed in pans (60 min at 35 °C, RH 70%) and baked in an electric oven (Mod. Modulo, Moretti Forni S.p.a., Pesaro, Italy) at 180 °C for 50 min. After cooling, loaves were characterized and then packed in polymeric bags (PET + COEX EVOH/PE) and stored at 4 °C for 1, 5, 8, 11, 15 days.

2.4. Fresh bread characterization

Bread samples after cooling and before packaging were identified as “fresh bread”. Characterization of fresh bread were performed my means of physical and mechanical analysis.

2.4.1. Physical measurement

Fresh bread volume was determined by applying the rapeseed displacement method. For each sample six measurements were carried out. Colour of crust fresh bread samples was measured with a tristimulus colorimeter (Minolta Chroma Meter model CR 300, Milan, Italy) with a circular measurement area ($D = 8$ mm). The colorimeter was calibrated using a white standard plate ($L = 100$).

Table 1
Sample recipes.

Samples	Recipes									
	Flour (g)	Water (g)	Salt (g)	Yeast (g)	Sugar (g)	Sourdough EPS+ (g)	Sourdough EPS− (g)	Total amount of flour (g)	Total amount of water (g)	Total dough weight
P1+	1000	456	37	20	21	694		1431	697	2228
P1−	1000	380	33	20	18		686	1426	619	2137
P2+	1000	414	34	17	19	406		1252	555	1890
P2−	1000	465	38	18	22		407	1253	607	1950

P1+ = 30 g/100 g of sourdough EPS-positive; P1− = 30 g/100 g of sourdough EPS-negative; P2+ = 20 g/100 g of sourdough EPS-positive; P2− = 20 g/100 g of sourdough EPS-negative.
Total amount of flour in the recipe is calculated as the sum of added flour and flour contained in the sourdough. Total amount of water is calculated as the sum of added water and water contained in the sourdough.

Chromatic coordinates (L^*, a^*, b^*) were reported as the average of six measurements on each sample.

2.4.2. Mechanical analysis

All samples were submitted to a compression test by means of an Instron Universal Testing Machine (Instron Ltd., mod. 4467, High Wycombe, GB), equipped with a 1 kN load cell. Cylindrical samples (diameter 17 mm, height 17 mm) were placed between parallel plates and compressed to a final deformation of 80%, at a crosshead speed of 10 mm/min. For each sample seven measurements were made. True stress–Hencky strain relationships were derived from load–displacement curves and the mechanical behaviour of the bread during storage time was studied by means of a semi-empirical mathematical model (Masi, Sepe, & Cavella, 1997):

$$\sigma_{(\varepsilon)} = \frac{K_1}{K_2}\left[-1+(1+\varepsilon)^{K_2}\right]+K_3\varepsilon^{K_4} \quad (1)$$

Where $\sigma_{(\varepsilon)}$ is the true stress (kPa), k_1 correspond to the elasticity modulus (kPa), k_1/k_2 ratio is a measure of the yield stress, ε is the Hencky strain (%), k_3 and k_4 parameters allow the estimation of the Poisson modulus (ν) by eq. (2):

$$k_3 = \left[\frac{k_1}{3}(1-2\upsilon)\right]^{\frac{1}{a}} \quad (2)$$

where $a = 1/k_4$ is a measure of the resistance offered by the crumb structure to compaction.

2.5. Shelf life of bread

To study the evolution of the sourdough bread quality during storage, the moisture content, differential scanning calorimetry (DSC) and mechanical properties were measured. Mechanical properties were performed as reported in Section 2.4.2.

2.5.1. Moisture content measurement

The moisture content of samples was determined by the gravimetric method. Bread samples were oven-dried at 100 °C and accurately weighed at regular time intervals until constant weight was reached. Three measurements were performed for each sample. The moisture content was expressed as grams of water over grams of total weight (g/100 g).

2.5.2. Differential scanning calorimetry (DSC)

Calorimetric analysis was carried out by means of a DSC calorimeter (Q200, TA Instruments, Milan, Italy). A sample of approximately 10 mg was taken from bread 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 10 °C/min. Melting heat of retrogradated starch (ΔH), initial transition temperature (T_o), peak transition temperature (T_{pk}) and final transition temperature (T_f) were calculated. Average values of three measurements were calculated for each sample.

2.6. Statistical analysis

All experimental results are reported as mean value ± standard deviation. Experimental results of the characterization of the fresh samples were submitted to ANOVA and *t*-test to determine statistically significant difference ($p \leq 0.05$) among samples. A full factorial design was used to study the effect of time, sourdough concentration and strain type on the quality indices of bread samples. There were six levels of storage time (0, 1, 5, 8, 11, 15), two levels of sourdough concentration (20 g/100 g, 30 g/100 g) and two levels of strain type (EPS+, EPS−). Three replicates were performed for each sample for a total of 72 samples. ANOVA analysis was performed on the data to evaluate the effect of time (A), sourdough concentration (B), strain type (C) and the interaction effect (A × B; B × C; A × C; A × B × C) on the quality attributes. Duncan's test was performed to find out the source of the significant differences within samples. Significance of differences was defined at $p \leq 0.05$. All statistical analyses were performed using the SPSS software (SPSS Inc. 15.0, Chicago, 2002).

3. Results and discussion

3.1. Cell enumeration, pH and TTA of dough and sourdough

As reported in Table 2 sourdough samples fermented by both EPS+ and EPS− starter cultures as well as dough samples obtained with 20 g/100 g and 30 g/100 g of sourdough showed similar microbial content and pH values. In particular, LAB content in EPS+ and EPS− sourdough increased by about 1 log cycle after 15 h of incubation at 30 °C, reaching cumulative counts of about 10^8 CFU g^{-1}. After leavening, bread dough achieved viable counts of about 5×10^8 CFU/g (Table 2) and 5×10^7 CFU/g (data not shown) of LAB and yeast, respectively. As expected, differences were detected between dough and sourdough samples in which the prolonged fermentation time led to an increase in acidimetric characteristics (pH = 4.00–4.02; TTA = 7.4–6.4). On comparing values from the analysis of dough samples obtained with EPS+ and EPS− starter cultures, the TTA was greater in sourdough started with of EPS+ LAB when 30 g/100 g of sourdough was used, reaching values of 6.50 ± 0.70 (sourdough 20 g/100 g) and 6.70 ± 0.58 (sourdough 30 g/100 g) ml of 0.1 mol equi/L NaOH/10 g (Table 2). Higher acidity could negatively affect bread quality since it can cause off-flavour and reduce loaf volume as well as diminish crumb softness suitable for optimal wheat bread preparation (Kadizky et al., 2008). The presence of EPS could promote additional metabolic activity, increasing the production of lactate, acetate and ethanol (Korakli, Pavlovic, Ganzle & Vogel, 2003; Pepe et al., 2004) and also contributing to improve flavour, texture and shelf-life of bread. After 15 h of fermentation at 30 °C with the starter EPS+ in wheat sourdough production of EPS reached the final concentration of about 2.3 ± 0.04 g kg^{-1}, whereas the EPS− LAB were unable to produce EPS (Palomba et al., 2012).

Table 2
Microbial content and acidification properties (pH and TTA) of sourdough and dough obtained with EPS+ and EPS− starter after 15 h of incubation at 30 °C. The sourdoughs were prepared with 5 g/100 g of sucrose.

Experimental conditions	Starter EPS+[a]	Starter EPS−[a]
Sourdough		
LAB (Log CFU/g)	8.62 ± 0.29	8.40 ± 0.29
pH	4.00 ± 0.11	4.02 ± 0.28
TTA	7.40 ± 0.95	6.40 ± 0,42
Dough (20 g/100 g)		
LAB (Log CFU/g)	7.63 ± 0.38	7.63 ± 0.06
pH	4.95 ± 0.01	5.03 ± 0.05
TTA	6.50 ± 0.70	4.75 ± 0.35
Dough (30 g/100 g)		
LAB (Log CFU/g)	7.75 ± 0.20	7.50 ± 0.38
pH	4.73 ± 0.05	4.75 ± 0.09
ATT	6.70 ± 0.58	5.83 ± 0.30

Microbial count, pH and TTA values detected in all experimental conditions immediately after dough mixing, were in the range of 7.56 ± 0.20–7.70 ± 0.23 log CFU ml^{-1}, 5.87 ± 0.02–5.89 ± 0.05, 0.70 ± 0.02–0.75 ± 0.01 0.1 mol equi/L NaOH/10 g, respectively.

[a] Data listed in the table refer to the analysis of sourdough and dough samples at the end of the fermentation period. Values represent the average of three replication ($n = 3$).

3.2. Fresh bread characterization

3.2.1. Physical characteristics

Bread volume changes from a maximum of 990 cm^3 for sample P2− to a minimum of 666 cm^3 for sample P1− (Table 3). ANOVA analysis showed that sourdough concentration ($p < 0.0001$), strain type ($p < 0.0001$) and their interaction ($p < 0.0001$) have a significant effect on fresh bread volume. In particular, for samples obtained with EPS− LAB strains, samples obtained with 20 g/100 g of sourdough (P2−) had a higher volume than samples obtained with 30 g/100 g of sourdough (P1−). These results are in agreement with Katina et al. (2009) who observed a roughly 10% reduction in bread volume in the presence of 40% of sourdough compared to the unsoured control. Normally the addition level of sourdoughs in wheat baking is 7.5–10% (Lorenz & Brummer, 2003) since intense acidification can decrease bread volume (Barber, Ortola, Barber, & Fernandez, 1992). By contrast, for EPS+ LAB strains, no significant differences were observed between samples obtained with 20 g/100 g or 30 g/100 g of sourdough. The results obtained for samples EPS+ may be explained by the production of exopolysaccharides that can counterbalance the negative effect of the acidification. The effect of strain type was significant for both bread recipes ($p < 0.05$). For samples P1 the bread volume was higher in presence of the starter EPS+; they also showed a different shape, with a smaller cross section and a greater height than the EPS− samples. This was probably due properties of EPS considered bio-thickeners or hydrocolloids that stabilize the rheological properties of dough and improve texture and flavour and ensure an extended shelf-life (Tieking & Gänzle, 2005). In addition, the crust of EPS− samples was broken both longitudinally and transversally. For samples P2 the bread volume obtained with strain EPS− was moderately higher than that of samples EPS+; as regards shape, the samples were quite similar and the crust was smooth for both.

It may be supposed that when EPS+ strains are used, a higher percentage of sourdough can be added to the bread without an adverse effect on bread volume. Kaditzky et al. (2008) reported that when sourdough was added to bread at 10%, no effect of the levan producer strain (*L. sanfranciscensis* TMW 1.392) on bread volume was observed, whereas at the same concentration, a dextran-producing strain (*L. mesenteroides* LMGP-16878) caused a volume increase up to 12% (Lacaze et al., 2007). Di Cagno et al. (2006) showed that the breads containing sourdough at 20% fermented for 24 h with EPS+ strains showed higher volume when compared to the EPS− strains. Katina et al. (2009) also reported that EPS+ *W. confusa* sourdough bread at 40% showed higher volume than EPS− *W. confusa* sourdough bread, whereas for *L. mesenteroides* no differences were observed in terms of volume between the two strains due to a different acidification level. As reported by Gocmen et al. (2007) the improving effects of wheat sourdough on bread making performance is closely dependent on the sourdough incubation temperature and time, inoculum level and proof time.

The colour of the bread crust in terms of chromatic coordinates is reported in Table 3. Samples obtained with EPS+ strains had a lighter crust than EPS− ones. ANOVA results highlighted a significant effect of sourdough concentration on L^* ($p < 0.0001$) and a^* ($p < 0.0001$), of strain type on L^* ($p < 0.0001$) and b^* ($p < 0.0001$), and of their interaction only on a^* ($p < 0.0001$). Bread obtained with 20 g/100 g of sourdough (P2) showed the highest value of L^*. Only at 30 g/100 g (P1) of sourdough, the L^* of bread obtained with EPS+ strains was higher than the control obtained with EPS− strains, i.e. the crust of samples at 30 g/100 g of sourdough is darker than that of samples at 20 g/100 g, and the same was observed at 30 g/100 g of sourdough for EPS− samples vis-à-vis EPS+. Moreover, parameter a^* (redness) was higher for samples P1 (30 g/100 g) both for EPS+ and for EPS−, whereas the effect of EPS was significant only for bread P2 (20 g/100 g). For parameter b^*, the effect of bread composition was significant only for EPS− strains, with bread P2 having a lower b^* value, i.e. the samples are less yellow (Komlenick, Ugarcic-Hardi, Jukic; Planinic, Bucic-Kojik & Strelec, 2010; Rizzello, Nionelli, Coda, Di Cagno & Gobetti, 2010). EPS+ bread always showed a higher b^* value than EPS− samples.

Crust browning is due to caramellization and Maillard reactions, belonging to the non-enzymatic or non-oxidative browning category (Fennema, 1993). Crust colour depends both on the physico-chemical characteristics of the raw dough (i.e. water content, pH, reducing sugars and amino acid content) and on the operating conditions applied during baking (i.e. temperature, air speed, relative humidity, heat transfer conditions) (Purlis & Salvadori, 2007; Zanoni, Peri, & Bruno, 1995). Such non-enzymatic browning reactions transform sugars and amino acids as the crust develops. Crusty sourdough fermented bread has more flavour than non-crusty bread and shows an increase in the concentration of different compounds.

The quality characteristics of bread are strongly dependent on manufacturing conditions as well as specific microbial proteolytic (Pepe et al., 2004) and acidification activities (Pepe et al., 2004) that can directly and indirectly affect both sugar and amino acid composition acting in the Maillard and Strecker reactions. Moreover, as reported by Lindenmeier and Hofmann (2004), the decrease in the pH value induced by microbial acid formation during sourdough fermentation is the clue for producing high amounts of compounds such as pronyl-L-lysine with antioxidant activity in baking products. Thus we can speculate that the production of dextran by EPS+ LAB strains during sourdough fermentation had a significant effect on crust colour that was more evident with the increase in sourdough concentration added to the dough.

3.2.2. Mechanical properties

The mechanical properties of bread are discussed in terms of model parameters (Table 3). ANOVA results showed a significant effect of composition and strain type on k_1, that is related to the bread hardness; it assumes the lowest value for samples P1+ (30 g/100 g) and P2− (20 g/100 g). Our experimental results demonstrate that EPS positive sourdough has a beneficial effect when added at 30 g/100 g (softer bread) while it has a detrimental effect at 20 g/100 g (harder bread). The opposite holds for EPS negative sourdough. The elastic modulus of the crumb has been shown to depend on the size, shape and distribution of the crumb alveoli, which are related to the behaviour of the gluten network during the expansion of CO_2 in leavening and baking (Fessas & Schiraldi, 1998).

All the other model parameters showed slight differences among the samples, but the effect of the recipe and sourdough were the same as for k_1; these results are in agreement with those

Table 3
Volume, colour and mechanical properties (k_1: elasticity modulus; k_1/k_2: measure of the yield stress; ν: Poisson modulus; a: measure of the resistance offered by the crumb structure to compaction) of fresh bread.

Sample (see Table 1)	Volume (ml)	Colour			Mechanical properties			
		L	a	b	k_1 (kPa)	k_1/k_2	ν	a
P1+	934aA	59.7aB	7.3aA	29.7aA	6bB	0.2aA	0.15aB	0.25bA
P1−	666bB	58.2bB	7.5aA	26.2bB	9aA	2.5aA	0.19aA	0.37aA
P2+	917bA	63.8aA	5.8aB	29.7aA	11aA	5aA	0.24aA	0.29aA
P2−	990aA	63.1aA	5.2bB	27.3bA	7bB	0.5aA	0.13bA	0.27aB

Data are reported as average of three replication ($n = 3$); Statistics in small letters compare samples EPS+ and EPS− in each sample and parameter; Statistics in capital letters compare samples at different concentrations of sourdough (P1–P2) for each EPS and parameter. Different letters indicate statistically significant differences.

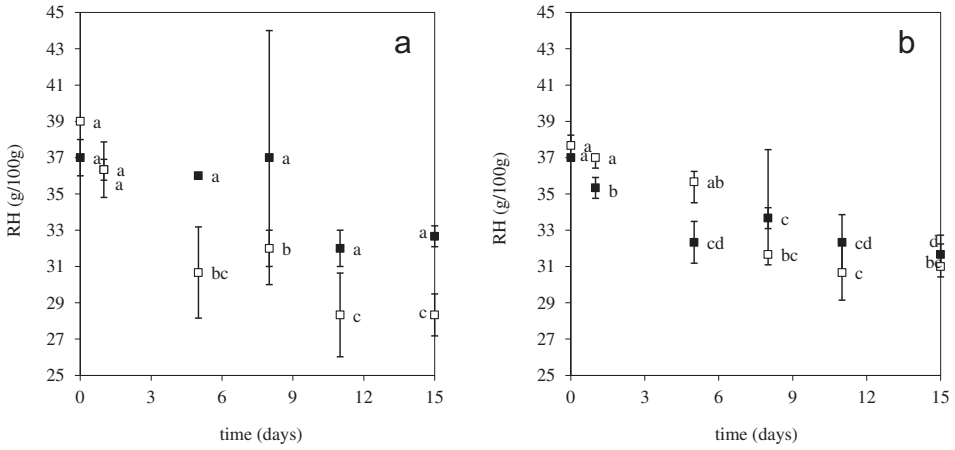

Fig. 1. Relative humidity (RH%) of bread crumbs during storage time at 4 °C. a) 30 g/100 g of sourdough; b) 20 g/100 g sourdough. ■ EPS+; □ EPS−. Data are reported as average of three replications ($n = 3$). Statistics in small letters compare the storage times in each sample. Different letters indicate statistically significant differences.

concerning volume. A reduction in bread hardness in the presence of sourdough obtained with EPS-producing strains was also reported for milk bread (Lacaze et al., 2007) and wheat bread (Arendt et al., 2007; Di Cagno et al., 2006; Katina et al., 2009).

3.3. Bread shelf life

3.3.1. Moisture content

The moisture content of bread samples during storage time is reported in Fig. 1. For sample P1 (30 g/100 g), ANOVA results highlighted a significant effect of storage time only for EPS− samples, whereas the effect of time was not significant for EPS+ samples that assume an average value during storage of 35% (Fig. 1a). Strain type has a significant effect on moisture content at all times during storage and EPS+ samples always showed a higher moisture content vis-à-vis EPS− samples.

For P2+ and P2− samples (20 g/100 g), the moisture trend during storage was the same (Fig. 1b). ANOVA analysis showed that storage time had a significant effect on moisture content whereas the strain type had no effect.

These results suggest that only with 30 g/100 g of sourdough the quantity of EPS produced is enough to reduce moisture redistribution inside the bread and hence reduce the reduction in moisture content of the crumb over time. For bread obtained with 20 g/100 g of sourdough (P2), it is not possible to observe a protective effect of strain type, perhaps due to the lower quantity of EPS.

3.3.2. Differential scanning calorimetry (DSC)

Experimental data obtained by thermal analysis are reported in Table 4. ANOVA analysis showed that storage time had a significant effect on melting enthalpy of recrystallized amylopectin and that, for both samples, strain type had no significant effect on the change of enthalpy during storage ($p > 0.05$). Similar results have been reported by Andreu, Collar, and Martinez-Anaya (1999) and Kaditzky et al. (2008) who studied the thermo properties of dough formulated with enzymes and starters. As reported by other authors (Corsetti et al., 1998, 2000; Katina et al., 2006; Ribotta, Cuffini, Leon, & Anon, 2004), with storage time 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. Only for samples EPS+ did the bread recipe have a significant effect ($p < 0.05$) on the kinetics of starch retrogradation. Thus, 30 g/100 g of sourdough seems to have a protective effect on bread staling.

In this respect several authors have reported beneficial effects of biological acidification on bread staling (Corsetti et al., 1998, 2000) due to the metabolite products of fermentation (Kaditzky et al., 2008; Pepe et al., 2004) and in particular due to proteolytic activity of LAB (Thiele, Gänzle, & Vogel, 2003; Thiele, Grassl, & Gänzle, 2004). Moreover, the differences in moisture content between samples during storage may also justify these results. In general, high bread water content has been reported to increase shelf life (He & Hoseney, 1990) and delay starch retrogradation (Andreu

Table 4
Thermal properties of bread during storage: melting heat of retrograded starch (ΔH), initial transition temperature (T_0), peak transition temperature (T_p), final transition temperature (T_m).

Sample (Table 1)	Time (days)	ΔH (J/g)	T_0 (°C)	T_p (°C)	T_m (°C)	ΔH (J/g)	T_0 (°C)	T_p (°C)	T_m (°C)
		EPS+				EPS−			
P1	1	0.6 ± 0.3aA	53 ± 5aA	67 ± 2aA	78 ± 4aA	0.8 ± 0.3a	53 ± 3a	66 ± 4a	81 ± 8a
	5	0.7 ± 0.6aA	51 ± 2aB	65 ± 3aA	81 ± 8abA	1.5 ± 0.4bA	50 ± 2bA	65 ± 1aA	81 ± 3aA
	8	1.4 ± 0.7bA	53 ± 3aB	67 ± 2aB	82 ± 5abA	1.3 ± 0.4bA	54 ± 3aA	69 ± 5bA	84 ± 6aA
	11	1.8 ± 0.6bA	52 ± 3aB	65 ± 2aB	84 ± 5bA	1.4 ± 0.3bA	51 ± 1aA	64 ± 1aA	79 ± 2aA
	15	2.7 ± 0.4cB	51 ± 1aB	66 ± 2aB	89 ± 3c	1.5 ± 0.9bA	53 ± 3abA	65 ± 3aA	80 ± 5aA
P2	1	1.0 ± 0.1aB	52 ± 3aA	69 ± 3aA	89 ± 6aB	n.d	n.d.	n.d.	n.d.
	5	1.9 ± 0.6bB	49 ± 2aA	64 ± 3bA	86 ± 7abB	1.9 ± 0.4aA	52 ± 2aB	67 ± 2aB	86 ± 2abB
	8	1.9 ± 0.4bB	49 ± 3aA	64 ± 3bA	83 ± 4bA	1.7 ± 0.6aA	53 ± 3aA	68 ± 5aA	87 ± 2aA
	11	2.1 ± 0.4bA	49 ± 1aA	63 ± 1bA	82 ± 3bA	2.4 ± 0.5bB	50 ± 3aA	65 ± 2aA	84 ± 3cB
	15	2.1± 0.9bA	49 ± 1aA	64 ± 3bA	84 ± 2bA	1.6 ± 0.2aA	52 ± 1aA	67 ± 1aA	84 ± 1bcB

P1: 30 g/100 g of sourdough; P2: 20 g/100 g of sourdough.
Data are reported as average of three replication ($n = 3$). Statistics in small letters compare the storage time in each sample and parameter; Statistics in capital letters compare the different samples for each storage time and parameter. Different letters indicate statistically significant differences.

et al., 1999). Indeed, sample P1 (30 g/100 g) showed a constant moisture content whereas for samples P2 (20 g/100 g) a decrease in moisture content was observed after the first days of storage. As reported by Piazza and Masi (1995), moisture redistribution throughout the loaf during storage has an effect on bread freshness. In agreement with them, Katina et al. (2006) showed that redistribution of water can have a role in the staling process. Nonetheless, after 15 days of storage samples P1 (30 g/100 g) reached a higher value of enthalpy than samples P2 (20 g/100 g). The same results were reported by Corsetti et al. (1998) who reported a very high final increment of enthalpy in sourdough breads produced by homo- (*Lactobacillus farciminis* A80) and heterofermentative (*Lactobacillus fructivorans* DD10) strains. Moreover, according to Czuchajowska and Pomeranz (1989), these results, together with the slight change in moisture during storage for samples P1+, indicated that the retrogradation of amylopectin may not be avoided by reducing moisture loss.

For both samples, storage time did not affect the initial temperature (T_0), whereas termination temperature (T_f) increased with storage time. The effect of storage time on peak temperature (T_p) was significant only for sample P2 (Table 4).

As reported previously (Czuchajowska & Pomeranz, 1989), bread staling is very complex and concomitant causes may have either augmenting or opposing effects. Our results highlighted that the specific activity of EPS-producing LAB strains and sourdough concentration, which involves properties other than acidification, appeared to be predominant. The production of organic acids (Barber et al., 1992), bacterial hydrolysis of starch and proteolysis of gluten subunits (Pepe, Villani, Oliviero, Greco, & Coppola, 2003) are activities involved in bread staling which may explain the different effects of LAB starters.

3.3.3. Mechanical analysis

Fig. 2 shows the stress–strain curves of bread samples at time zero and samples after 15 days of storage. Analysis of the individual curves shows a linear elasticity at low stress followed by a plateau of roughly constant stress, leading into a final regime of steeply rising stress. Each regime is associated with a mechanism of deformation. Linear elasticity is controlled by cell wall bending and, if there are closed cells, by cell face stretching. In compressive loading when a critical stress is reached the cells begin to collapse (by elastic buckling in elastomeric foam, by the formation of plastic hinges in a foam which yields, by brittle fracture in brittle foam), to which the plateau is associated. When the cells have almost completely collapsed, opposing cell walls touch and further deformation compresses the cell wall material itself, which corresponds to the densification region (Gibson & Ashby, 1997).

Mechanical behaviour of bread samples at time zero differs only at high deformation: the P1+ sample has a more pronounced densification region. More substantial differences among samples are evident during storage. EPS+ sourdough has a higher preservative effect on bread structure if added at 30 g/100 g, whereas EPS− sourdough has the same effect if added at 20 g/100 g. In all cases, increasing the storage time of the samples increases the Young's modulus, raises the plateau stress and reduces the strain at which densification starts.

In order to better describe the behaviour of the bread and the effect of fermentation strains, recipe and storage time on bread mechanical properties, a semi-empirical model able to correlate the instrumental response at large deformations was used (Eqs. (1) and (2)). In Fig. 2, broken and continuous lines represent the model fitting of the experimental data. As may be seen, the model well described bread behaviour under low and high deformation.

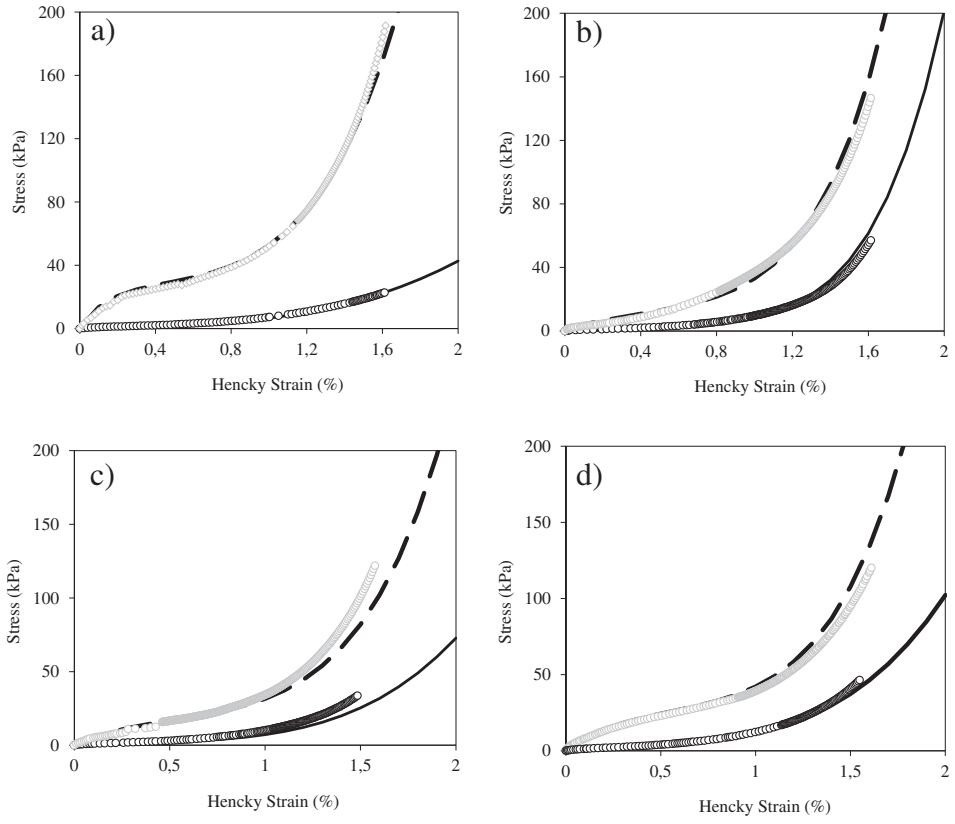

Fig. 2. Stress–Hencky strain curve of bread crumbs at 30% (a: EPS−; b: EPS+) and 20 g/100 g of sourdough (c: EPS−; d: EPS+) at time zero (continuous line) and after 15 days (dotted line) of storage at 4 °C.

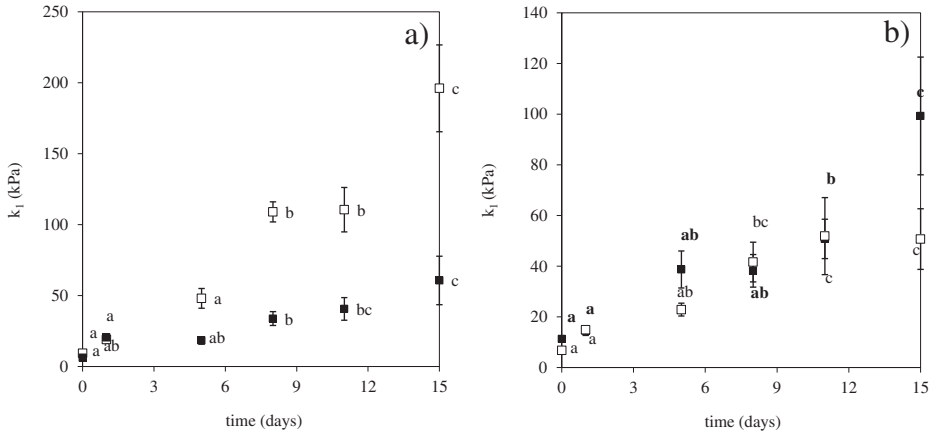

Fig. 3. Model parameter k_1 that correspond to the elasticity modulus (kPa) of bread crumbs during storage time at 4 °C. a) 30 g/100 g of sourdough; b) 20 g/100 g sourdough. ■ EPS+; □ EPS−. Data are reported as average of three replications ($n = 3$). Statistics in small letters compare the storage times in each sample. Different letters indicate statistically significant differences.

Fig. 3 shows the variation of the k_1 parameter of samples at 30 g/100 g (Fig. 3a) and 20 g/100 g (Fig. 3b) as a function of storage time. ANOVA results showed that time had a significant effect on k_1 both for EPS positive ($p < 0.001$) and for EPS negative strains ($p < 0.001$), with the first significant differences at 8 days of storage for both bread types. Moreover, strain types also have a significant effect since after four days of storage the P1+ samples had a lower k_1 than P1− samples.

These results show that at 30 g/100 g of sourdough bread made with EPS+ strains of lactobacilli proves less rigid during storage, hence appearing to slow down bread staling. Di Cagno et al. (2006) reported that EPS from lactobacilli (*W. cibaria* WC4 or *L. plantarum* LP9) reduce crumb firmness, giving a softer crumb than the bread obtained with sourdough started with the EPS− strain. Improved softness of fresh EPS-enriched wheat bread has also been reported by Kaditzky et al. (2008) and Katina et al. (2009).

The increment of the k_1 modulus for EPS− samples can be justified by an extensive hydrolysis of wheat protein, which may also cause bread flattening (Corsetti et al., 2000). The elastic modulus of the crumb has been shown to depend on the size, shape and distribution of the crumb alveoli, which are related to the gluten network during the expansion of CO_2 in leavening and baking (Fessas & Schiraldi, 1998). Fig. 3 shows the changes of k_1 for samples obtained with 20 g/100 g of sourdough. ANOVA results showed that time had a significant effect on k_1 both for P2+ ($p < 0.001$) and for P2− ($p < 0.001$), with first differences after 8 days of storage for samples P2− and after 11 days of storage for samples P2+. Strain type had no effect on k_1 over time, showing that upon reducing the sourdough in bread dough, the actual quantity of EPS is insufficient to have a protective effect on the mechanical properties of bread during storage. Mechanical results are in agreement with moisture content results, highlighting the significant effect of both sourdough concentration, LAB strain type and their interaction.

The Poisson modulus of bread P1 (30 g/100 g) changed from a minimum of 0.15 ± 0.02, at time zero, to a maximum of 0.47 ± 0.02, after 15 days of storage at 4 °C (Fig. 4a). These values fall within the Poisson modulus values (ν) typical of a foam (Gibson & Ashby, 1997). An increase in the Poisson modulus (ν) indicates that the bread is less compressible (Rohm, Jaros, & deHaan, 1997) with increasing storage time. The latter had a significant effect on the Poisson modulus (ν) for both samples ($p < 0.0001$). Furthermore, based on the results of the Duncan test, it is noted that after eight days of storage at 4 °C the modulus no longer varies as a function of time. The strain type also had a significant effect on the Poisson modulus ($p < 0.001$). At all storage times, P1+ showed a lower Poisson modulus than P1−. The above results show that bread made with EPS positive strains is more compressible during storage, confirming the positive effect of EPS on bread staling.

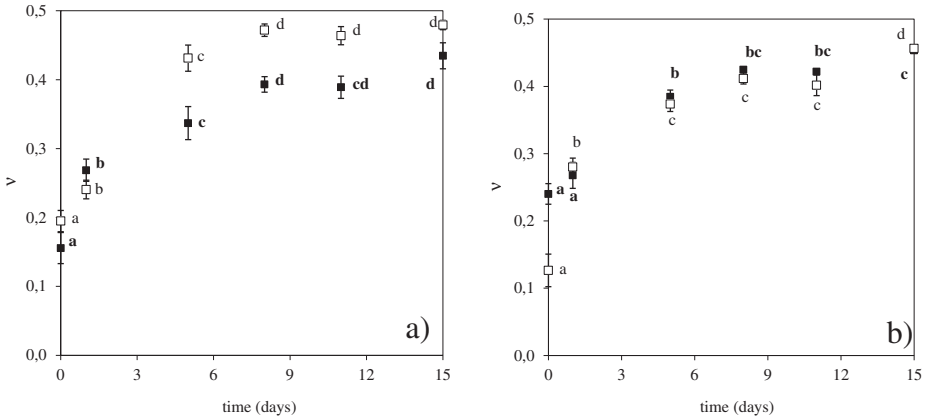

Fig. 4. Poisson module (ν) of bread crumbs during storage time at 4 °C. a) 30 g/100 g of sourdough; b) 20 g/100 g sourdough. ■ EPS+; □ EPS−. Data are reported as average of three replications ($n = 3$). Statistics in small letters compare the storage times in each sample. Different letters indicate statistically significant differences.

The Poisson modulus (ν) of P2 bread (20 g/100 g) changed from a minimum of 0.13 ± 0.02 at time zero to a maximum of 0.45 ± 0.05 after 15 days of storage at 4 °C (Fig. 4b).

Storage time has a significant effect on the Poisson module (ν) both for samples P2+ ($p < 0.0001$) and for P2− ($p < 0.0001$). Furthermore, based on the results of the Duncan test, the first statistically significant differences compared to the fresh sample (time zero) are observed after one day of storage at 4 °C for the EPS− sample, after five days of storage at 4 °C for the EPS+ sample, and after eight storage days the Poisson module remains constant.

For samples P2, ANOVA results pointed out that strain type had no effect on the Poisson module ($p > 0.05$). The compressibility module did not change during storage time, for all the samples investigated, assuming an average of 0.2 ± 0.1 kPa for all samples.

Comparison of the two formulations showed that for EPS− samples, formulation had a significant effect on k_1 ($p < 0.0001$) and the Poisson modulus ($p < 0.0001$), and samples at 20 g/100 g of sourdough showed a lower k_1 value and a lower Poisson modulus with respect to samples at 30 g/100 g of sourdough at any storage time.

4. Conclusion

Results showed that the addition of 30 g/100 g of sourdough, obtained using selected EPS producing-lactic acid bacteria, had a positive effect on bread volume and crumb texture. Shelf life study highlighted that 30 g/100 g of sourdough had a negative impact on bread quality in the absence of the EPS-producing strain, whereas the opposite is observed in the presence of EPS-producing strains: samples at 30 g/100 g of sourdough showed higher moisture content, better mechanical properties during storage than samples at 20 g/100 g of sourdough. Moreover, 30 g/100 g of sourdough showed a protective effect on bread staling, thus confirming the effect of sourdough concentration and the positive role of EPS functional properties.

References

American Association of Cereal Chemists (AACC), Inc.. (1975). *Cereal laboratory methods, method 44-15A*. St. Paul, Minn: American Association of Cereal Chemists, Inc.

Andreu, P., Collar, C., & Martinez-Anaya, M. A. (1999). Thermal properties of doughs formulated with enzymes and starters. *European Food Research and Technology, 209*(34), 289–293.

Arendt, E. K., Ryan, L. A. M., & Dal Bello, F. (2007). Impact of sourdough on the texture of bread. *Food Microbiology, 24*, 165–174.

Barber, B., Ortola, C., Barber, S., & Fernandez, F. (1992). Storage of packaged white bread. III. Effect of sourdough and addition of acids on bread characteristics. *LWT – Food Science and Technology, 194*, 442–449.

Corsetti, A., Gobbetti, F., Balestrieri, F., Paletti, F., Russi, L., & Rossi, J. (1998). Sourdough lactic acid bacteria effects on bread firmness and staling. *Journal of Food Science, 63*(2), 347–351.

Corsetti, A., Gobbetti, M., De Marco, B., Balestrieri, F., Paletti, F., Russi, L., et al. (2000). Combined effect of sourdough lactic acid bacteria and additives on bread firmness and staling. *Journal of Agricultural and Food Chemistry, 48*, 3044–3051.

Czuchajowska, Z., & Pomeranz, Y. (1989). Differential scanning calorimetry, water activity and moisture contents in crumb center and near-crust zones of bread during storage. *Cereal Chemistry, 66*, 305–309.

Di Cagno, R., De Angelis, M., Limitone, A., Minervini, F., Carnevali, P., Corsetti, A., et al. (2006). Glucan and fructan production by sourdough *Weissella cibaria* and *Lactobacillus plantarum*. *Journal of Agricultural and Food Chemistry, 54*, 9873–9881.

Fennema, O. W. (1993). *Quìmica de los alimentos* (2nd ed.). Zaragoza, Spain: Editorial Acribia S.A.

Fessas, D., & Schiraldi, A. (1998). Texture and staling of wheat bread crumb: effect of water extractable proteins and pentosans. *Thermochimica Acta, 323*, 17–26.

Gänzel, M. G. (2009). From gene to function: metabolic traits of starter cultures for improved quality of cereal foods. *International Journal of Food Microbiology, 134*, 29–36.

Gibson, L. J., & Ashby, M. F. (1997). In C. A. Harper (Ed.), *Cellular solids: Structure and properties* (2nd ed.). New York, USA 0975: Mcgraw-Hill.

Gocmen, D., Gurbuz, O., Kumral, A. Y., Dagdelen, A. F., & Sahin, I. (2007). The effects of wheat sourdough on glutenin patterns, dough rheology and bread properties. *European Food Research and Technology, 225*, 821–830.

He, H., & Hoseney, R. C. (1990). Changes in bread firmness and moisture during long-term storage. *Cereal Chemistry, 67*, 603–605.

Kaditzky, S., Seitter, M., Hertel, C., & Vogel, R. F. (2008). Performance of *Lactobacillus sanfranciscensis TMW 1.392* and its levansucrase deletion mutant in wheat dough and comparison of their impact on bread quality. *European Food Research and Technology, 227*, 433–442.

Kaditzky, S., & Vogel, R. F. (2008). Optimization of exopolysaccharide yield in sourdoughs fermented by lactobacilli. *European Food Research and Technology, 228*, 291–299.

Katina, K., Heinio, R. L., Autio, K., & Poutanen, K. (2006). Optimization of sourdough process for improved sensory profile and texture of wheat bread. *LWT – Food Science and Technology, 39*, 1189–1202.

Katina, K., Maina, N. H., Juvonen, R., Flander, L., Johansson, L., Virkki, L., et al. (2009). *In situ* production and analysis of *Weissella confusa* dextran in wheat sourdough. *Food Microbiology, 26*, 734–743.

Komlenick, D. K., Ugarcic-Hardi, Z., Jukic, M., Planinic, M., Bucic-Kojik, A., & Strelec, I. (2010). Wheat dough rheology and bread quality effected by *Lactobacillus brevis* preferment, dry sourdough and lactic acid addition. *International Journal of Food Science and Technology, 45*, 1417–1425.

Korakli, M., Pavlovic, M. M., Gänzle, G., & Vogel, R. F. (2003). Exopolysaccharide and kestose production by *Lactobacillus sanfranciscensis* LTH2590. *Applied and Environmental Microbiology, 69*, 2073–2079.

Lacaze, G., Wick, M., & Cappelle, S. (2007). Emerging fermentation technologies: development of novel sourdoughs. *Food Microbiology, 24*, 155–160.

Lindenmeier, M., & Hofmann, T. (2004). Influence of baking conditions and precursor supplementation on the amounts of the antioxidant pronyl-L-lysine in bakery products. *Journal of Agricultural and Food Chemistry, 52*, 350–354.

Lorenz, K., & Brummer, J. M. (2003). Preferments and sourdoughs in German breads. In K. Kulp, & K. Lorenz (Eds.), *Handbook of dough fermentations* (pp. 247–267). New York: Marcel Dekker Inc.

Martinez-Anaya, M. A. (2003). Association and interaction of microorganisms in dough fermentations: effects on dough and bread characteristics. In K. Kulp, & K. Lorenz (Eds.), *Handbook of dough fermentations* (pp. 63–95). New York: Marcel Dekker.

Masi, P., Sepe, M., & Cavella, S. (1997). Mathematical modelling of the compressive stress–strain relationship of foods submitted to large deformations. In R. Jowitt (Ed.), *Engineering & food at ICEF7* (pp. 212–215). Sheffield UK: Sheffield Academic Press. Part 1, section A.

Palomba, S., Blaiotta, G., Ventorino, V., Saccone, A., & Pepe, O. (2011). Microbial characterization of sourdough for sweet baked products in the Campania region (southern Italy) by a polyphasic approach. *Annals of Microbiology, 61*, 307–314.

Palomba, S., Cavella, S., Torrieri, E., Piccolo, A., Mazzei, P., Blaiotta, G., et al. (2012). Wheat sourdough from *Leuconostoc lactis* and *Lactobacillus curvatus* exopolysaccharide-producing starter culture: polyphasic screening, homopolysaccharide composition and viscoelastic behavior. *Applied and Environmental Microbiology, 78*(8), 2737–2747.

Parker, R., & Ring, S. G. (2001). Macromolecular aspect of bread staling. In P. Chinachoti, & Y. Vodovotz (Eds.), *Series in contemporary food scienceBread staling* (pp. 61–71). New York: CRC Press LLC.

Pepe, O., Blaiotta, G., Anastasio, M., Moschetti, G., Ercolini, D., & Villani, F. (2004). Technological and molecular diversity of *Lactobacillus plantarum* strains isolated from naturally fermented sourdoughs. *Systematic and Applied Microbiology, 27*, 443–453.

Pepe, O., Ventorino, V., Cavella, M., Fagnano, M., & Brugno, R. (2013). Prebiotic content of bread prepared with flour from immature wheat grain and selected dextran-producing lactic acid bacteria. *Applied and Environmental Microbiology, 79*, 3779–3785.

Pepe, O., Villani, F., & Coppola, S. (2001). Differential viable count of mixed starter cultures of lactic acid bacteria by using modified Chalmers medium. *Microbiological Research, 155*, 351–354.

Pepe, O., Villani, F., Oliviero, D., Greco, T., & Coppola, S. (2003). Effect of proteolytic starter cultures as leavening agents of pizza dough. *International Journal of Food Microbiology, 84*, 319–326.

Piazza, L., & Masi, P. (1995). Moisture redistribution throughout the bread loaf during staling and its effect on mechanical properties. *Cereal Chemistry, 72*(3), 320–325.

Plessas, S., Alexopoulos, A., Bekatorou, A., Mantzourani, I., Koutinas, A. A., & Bezirtzoglou, E. (2011). Examination of freshness degradation of sourdough bread made with kefir through monitoring the aroma volatile composition during storage. *Food Chemistry, 124*, 627–633.

Poutanen, K., Flander, L., & Katina, K. (2009). Sourdough and cereal fermentation in a nutritional perspective. *Food Microbiology, 26*, 693–699.

Purlis, E., & Salvadori, V. O. (2007). Bread browning kinetics during baking. *Journal of Food Engineering, 80*, 1107–1115.

Ribotta, P. D., Cuffini, S., Leon, A. E., & Anon, M. C. (2004). The staling of bread: an X-ray diffraction study. *European Food Research and Technology, 218*, 219–223.

Rizzello, C. G., Nionelli, L., Coda, R., Di Cagno, R., & Gobbetti, M. (2010). Use of sourdough fermented wheat germ for enhancing the nutritional, texture and sensory characteristics of the white bread. *European Food Research and Technology, 230*, 645–654.

Robertson, G. L. (1993). *Food packaging - Principles and practice*. New York, USA: Marcel Dekker, Inc.

Rohm, H., Jaros, D., & deHaan, M. (1997). A video-based method for determination of average stress-strain relations in uniaxial compression of selected foods. *Journal of Texture Studies, 28*, 245–255.

Schiraldi, A., & Fessas, D. (2001). Mechanism of staling: an overview. In P. Chinachoti, & Y. Vodovotz (Eds.), *Series in contemporary food scienceBread staling*. CRC Press LLC.

Thiele, C., Gänzle, M. G., & Vogel, R. F. (2003). Fluorescence labelling of wheat proteins for determination of gluten hydrolysis and depolymerization during dough processing and sourdough fermentation. *Journal of Agricultural and Food Chemistry, 51*, 2745–2752.

Thiele, C., Grassl, S., & Gänzle, M. G. (2004). Gluten hydrolysis and depolymerization during sourdough fermentation. *Journal of Agricultural and Food Chemistry, 52*, 1307–1314.

Tieking, M., & Gänzle, M. G. (2005). Exopolysaccharides from cereal-associated lactobacilli. *Trends in Food Science and Technology*, 79–84.

Tieking, M., Korakli, M., Ehrmann, M. A., Gänzle, M. G., & Vogel, R. F. (2003). In situ production of exopolysaccharides during sourdough fermentation by cereal and intestinal isolates of lactic acid bacteria. *Applied and Environmental Microbiology, 69*, 945–952.

Zanoni, B., Peri, C., & Bruno, D. (1995). Modelling of browning kinetics of bread crust during baking. *LWT – Food Science and Technology, 28*, 604–609.