

# Development of Iranian rice-bran sourdough breads: physicochemical, microbiological and sensorial characterisation during the storage period

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## RESEARCH ARTICLE

### Abstract

Rice bran (RB) as a functional by-product was used to develop healthy-nutrition breads. Moreover, the sourdoughs fermented by three lactic acid bacteria (LAB) including *Lactobacillus plantarum*, *Lactobacillus delbrueckii* and *Leuconostoc mesenteroides* were applied to reduce negative effects of RB on the quality characteristics of leavened bread. Physicochemical, microbiological and sensorial features of the breads by adding the different RB-sourdoughs (10%) were compared to control bread during storage time. Results showed that *L. plantarum* and *L. delbrueckii* had a similar behaviour in reducing the pH and titratable acidity in the breads and sourdoughs. A firmer crumb was observed due to low moisture content in the control compared with the breads prepared from three LAB at the end of storage time ( $P < 0.05$ ). The loaf volume of breads obtained from sourdoughs fermented by *L. mesenteroides* was significantly higher than the control bread. Microbiological assays revealed that growth rate of *Aspergillus niger* and *Penicillium italicum* can be significantly retarded by the application of each of three type of RB-sourdoughs. Although overall acceptability of the control bread was comparable to the sourdough breads until fifth day of storage, it was significantly lower than other breads on the seventh day.

**Keywords:** rice bran, sourdough, bread quality, fermentation, sensory evaluation

## 1. Introduction

Rice bran (RB) is defined as the outer brown coat of the rice kernel which is obtained by milling rice. Unfortunately, despite the many nutritional benefits of RB, it is still an underutilised co-product which mostly goes to animal feed. Recently attention has been paid to nutritive value of this functional ingredient as many researchers are trying to incorporate it in the different food formulations (De Delahaye *et al.*, 2005). A wide variety of compounds in the RB are responsible for these healthy-functional benefits. For example, RB is a good source of dietary fibres as its total content in this fraction ranged from 20 to 51% (Saunders, 1990). These fibres include hemicellulose, arabinogalactan, arabinoxylan, xyloglycan, proteoglycan, arabinofuranoside and raffinose (Kahlon, 2008). RB also contains approximately 12-15% protein which is superior to other cereals. It is rich in essential amino acids such as

lysine and has a high protein efficiency ratio as well as it is a good source of hypoallergenic protein (Wang *et al.*, 1999). Also, RB has a wide range of minerals, vitamins and antioxidants (more than 100 different types) such as, tocoferol, tocotrienol, ferulic acid, oryzanol, phytosterol, squalene, polyphenols and lipoic acid (Sairam *et al.*, 2011). Besides the nutritional and health effects of RB, it can have beneficial effects on the technological properties of various foods. For example, the water binding capacity of RB dietary fibres is more than wheat bran and its emulsifying capacity is significantly greater than the commercial fibre sources as FIBREX (Abdul-Hamid and Luan, 2000). Moreover, the protein of this food ingredient exhibited favourable high foaming ability which can improve air incorporation and leavening in the baked product (Carroll, 1990). The hygroscopic properties of RB can slow staling of baked products.

Several researchers have investigated the effect of adding bran on the bread properties. They concluded that bran addition to the dough has a significant negative effect on the bread characteristics such as decrease in overall acceptance, an increase in crumb firmness, a darker crumb colour, reduction in specific volume and increase in density of crumb texture (Gan *et al.*, 1992; Lai *et al.*, 1989a).

Different solutions have been reported to reduce the negative effects of bran and/or to improve the quality of breads formulated with bran. These include pre-treatments of the bran (soaking) or heat treatment (Nelles *et al.*, 1998), the addition of exogenous enzymes (hemicellulases and pentosanases) (Haseborg and Himmelstein, 1988), the application of different surfactants and reduction of the particle size of bran (Lai *et al.*, 1989b). It is also reported that fermentation processes incorporating bran in sourdough can be an effective method for removing the negative effects of bran (Katina *et al.*, 2006).

Due to the sourdough process, RB becomes a nutritional food ingredient that contains many beneficial compounds with useful biological functions (Kim and Han, 2012). The RB-sourdough can be also improved the taste, flavour and texture of produced breads (Park *et al.*, 2008). In fact, these beneficial effects of RB-sourdough are related to metabolites produced by lactic acid bacteria (LAB) and bakery yeast in the fermentation process. To the best of our knowledge, no special work was performed on the development of healthy-functional bread based on the dough fortified RB-sourdough fermented by LAB. Thus, the aim of this study was to produce RB-sourdough bread and investigate its physical, chemical, microbiological and sensory features for finding the role of RB-sourdough in the improvement of bread quality during storage time.

For this purpose, the authors used *Leuconostoc mesenteroides*, *Lactobacillus delbrueckii* and *Lactobacillus plantarum* for fermentation of the RB-sourdough. *L. plantarum* has a wide application in cereal products. It has the potential to improve the quality of bread also it provides good resistance to mould formation (Moore *et al.*, 2008; Todorov *et al.*, 1999).

*L. mesenteroides* has a very good ability to produce exopolysaccharides that result in improved quality and nutritional value of bread (Tieking and Gänzle, 2005). Moreover it can extend the shelf life of bread by retarding the growth of moulds (Zotta *et al.*, 2008). Significant antimicrobial activity of *L. delbrueckii* reported by Şimşek *et al.* (2006) also research conducted by (Plessas *et al.*, 2008) showed that use of *L. delbrueckii* can improve the flavour of sourdough breads. So using these three bacteria can produce breads with desirable properties.

## 2. Materials and methods

### Materials

Wheat flour ( $0.59 \pm 0.02\%$  ash,  $13.9 \pm 0.07\%$  moisture,  $11.79 \pm 0.03\%$  protein) and RB ( $89.80 \pm 0.12\%$  dry matter,  $16.36 \pm 0.04\%$  protein,  $8.12 \pm 0.06\%$  ash and  $19.20 \pm 0.07\%$  fat) were prepared from Nanavarar Co. (Tehran, Iran).

### Microorganism and cultivation media

*Leu.* 1591 (*L. mesenteroides* ssp. *mesenteroides*, strain PTCC1591), *Lb.* 15996 (*L. delbrueckii* ssp. *lactobacillus delbrueckii*, strain DSM 15996) and *Lb.* 20179 (*L. plantarum* ssp. *plantarum*, strain ATCC 20179) were obtained from Bioprocess Engineering Laboratory (BPEL), University of Tehran, Iran. These LAB were inoculated into De Man, Rogosa and Sharpe (MRS) broth (Merck Chemical Co., Darmstadt, Germany). Incubation was performed for 24 h at 28, 37 and 37 °C, respectively for *Leu.* 1591, *Lb.* 15996 and *Lb.* 20179.

In order to analyse anti-mould static potential of the produced breads, *Aspergillus niger* and *Penicillium italicum* were provided from the Persian-type culture collection in Iran and potato dextrose agar (PDA) was supplied by Merck Chemical Co.

### Preparation of sourdough

For the preparation of the sourdough to produce the final breads, RB and water were mixed to obtain the dough yield (DY) which is defined as ratio of dough to flour mass. The percentage of DY obtained in this stage was 300. The resultant mixture was pasteurised for 20 min at 70 °C in a boiling water bath (BWB 8; Tuttnauer LTD, Jerusalem, Israel) and then stored at room temperature. In the next step, the inoculation by the three LAB strains in an initial cell density of  $10^7$  cfu/ml was carried out to obtain the fermented RB-dough.

In order to achieve the above constant cell density, for every 100 ml of sourdough, 15 ml of the cultivated MRS broth was centrifuged at 4,000 rpm for 10 min, the biomass washed twice (with distilled/sterile water) and incorporated into the mixture of water and RB under sterile conditions. Finally, incubation was done at 37 °C for *Lb.* 15996 and *Lb.* 20179 and at 28 °C for *Leu.* 1591 for 16 h.

### Baking procedure

For the production of bread, 10% (w/w on flour basis) of RB-sourdough was added to the formulation and mixed using a mixer (Escher M120 Premium; Escher Mixers,

Lago di Vico, Italy) for 10 min at 25 °C. Then, the dough was divided into 100 pieces with a constant weight of 50g (about 8 kg dough per batch was prepared and 100 breads were prepared from each batch). The pieces of dough were transferred to baking trays and then placed in a proofer (Enkomak, Ankara, Turkey) at 35 °C for 90 min with an 85% RH. The samples were baked at 230 °C for 18 min. The bread formulation used is summarised in Table 1. The quality attributes of the breads were evaluated during storage time at 1, 3, 5 and 7 day. All of the practical experiments were carried out at Nanavaran Co.

## Analytical methods

### Total titratable acidity and pH

The pH values of samples including breads, doughs and sourdoughs were measured using a glass pH electrode (MP230; MettlerToldo, Zurich, Switzerland) by preparation of an aliquot of 10 g of sample blended with 90 ml of distilled water. The values of titratable acidity (TTA) were expressed as the amount (ml) of 0.1 N NaOH needed to achieve a final pH of 8.5 (Katina *et al.*, 2006).

### Moisture content

In order to determine the moisture content of the crumb and crust of the breads, an homogenised sample from these portions was used. Then 3–5 g of each sample was weighed and dried using a hot-air oven at 130 °C for 2 h (ISIRI, 2010). The samples after cooling in the desiccator were weighed using an analytical balance with an accuracy of  $10^{-2}$  and then calculated by the weight loss as a percentage of the initial weight of the sample.

### Bread volume

Bread volume was measured using the rapeseed displacement method (American Association Cereal Chemistry 10-05; AACC, 2001). Four loaves were used for each evaluation.

### Texture evaluation

Puncture tests and texture profile analysis were carried out to evaluate the crust and crumb texture using an Instron Universal Testing Machine (Testometric machine, M350-10CT; Testometric Co. Ltd., Rochdale, UK) equipped with a 50 kg compression load cell and integrator. The crust hardness was determined by means of a stainless steel probe with 3 mm diameter and test velocity was 1 mm/s. Maximum peak force (N) was measured from the penetration curve and expressed as crust hardness. For the analysis of the texture profile, cube-shaped pieces of the crumb (25×25×25 mm) were separated from the centre of each bread and then compressed using an aluminium probe (40×40 cm) with speed of 1 mm/s to 50% of their original height.

### Anti-fungistatic potential determination

*A. niger* and *P. italicum* were selected to represent bread spoilage fungi and cultivated on PDA at 25 °C for 7 days. Then, the spores were harvested and counted by hemocytometer lam to achieve a final concentration of  $1 \times 10^5$  spore/ml (Kam *et al.*, 2007). Identical volumes (300 µl) of this suspension were sprayed on each bread slice. Slices of breads without spore addition as a reference sample were prepared. All the slices were placed in a polyethylene bag, stored in the incubator at 28 °C and observed daily to verify the presence of mould growth.

### Sensory evaluation

Sensory analysis of the breads was carried out during the storage time by 30 panellists. The panellists were asked to indicate on a questionnaire whether they found any difference between samples, and if so, which sample they most preferred. This evaluation was based on the apparent colour (crumb and crust), firmness/softening degree, chewiness, crumb porosity, resilience and overall acceptability of the coded samples. An hedonic 5-point structured scale, in which 5 corresponded to most liked and 1 to most disliked was used. Each sample was presented twice, and the samples were presented in random order.

**Table 1. Ingredients used in baking breads (means  $\pm$  standard deviation).**

| Bread type      | Ingredients (g) |             |            |                 |            |            |                     |            |               |            |
|-----------------|-----------------|-------------|------------|-----------------|------------|------------|---------------------|------------|---------------|------------|
|                 | Flour           | Gluten      | Salt       | Water           | Sugar      | Yeast      | Rice bran sourdough | Soy flour  | Vegetable oil | Improver   |
| Control bread   | 5,000 $\pm$ 100 | 100 $\pm$ 5 | 50 $\pm$ 0 | 2,500 $\pm$ 100 | 75 $\pm$ 5 | 75 $\pm$ 5 | 0 $\pm$ 0           | 50 $\pm$ 5 | 50 $\pm$ 5    | 50 $\pm$ 5 |
| Sourdough bread | 5,000 $\pm$ 100 | 100 $\pm$ 5 | 50 $\pm$ 0 | 2,700 $\pm$ 100 | 75 $\pm$ 5 | 75 $\pm$ 5 | 500 $\pm$ 10        | 50 $\pm$ 5 | 50 $\pm$ 5    | 50 $\pm$ 5 |

Statistical analysis

Each experiment was carried out in triplicate using prepared samples and the values reported are means of these repetitions. The obtained data were subjected to analysis of variance (ANOVA) using SPSS 13 software (SPSS Inc., Chicago, IL, USA). The means were compared using the Duncan's multiple ranges test at a significant level of  $P<0.05$ . Drawing the graphs and fitting models were performed by Microsoft Office Excel 2010 (Microsoft Inc., Redmond, WA, USA).

3. Results and discussion

Total titratable acidity and pH

Kinetic changes of pH and TTA values for the RB-sourdoughs are shown in Figure 1. The ability of *Leu.* 1591 to reduce the pH is poor since the TTA value in sourdough was less than two other bacteria. In fact, *Lb.* 20179 and *Lb.* 15996 as homo-fermentative bacteria, can produce more acid compared with *Leu.* 1591 that is a hetero-fermentative bacterium.

The pH and TTA values of dough and breads are summarised in Table 2. Generally, pH values of the breads were higher than their dough. It may possibly be due to the removal of organic acids by the heating process (Hansen and Schieberle, 2005). As expected, the pH values of control breads were significantly more than the breads produced from different sourdoughs due to the acid production by applied LAB. However, a similar behaviour in pH and TTA values of the breads and sourdoughs obtained from fermentation process by *Lb.* 20179 and *Lb.* 15996 was observed (Table 2).

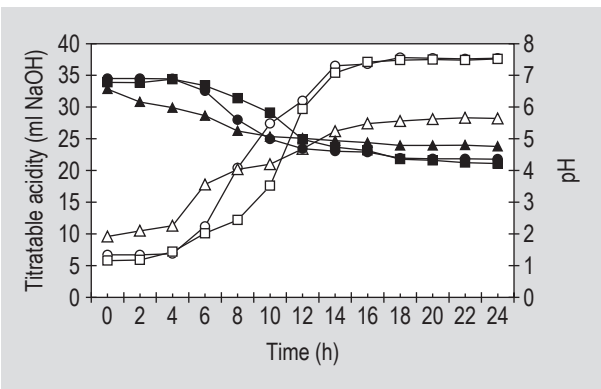


Figure 1. Kinetic behaviour of pH (black symbol) and total titratable acidity (white symbol) values of the sourdoughs obtained from *Leuconostoc mesenteroides* ssp. *mesenteroides*, strain PTCC1591 (*Leu.* 1591; triangle), *Lactobacillus plantarum* ssp. *plantarum*, strain ATCC 20179 (*Lb.* 20179; circle) and *Lactobacillus delbrueckii* ssp. *lactobacillus delbrueckii*, strain DSM 15996 (*Lb.* 15996; square) fermentation.

Table 2. pH and total titratable acidity (TTA) values of the doughs and breads produced in this study.<sup>1</sup>

| Rice bran-bread type <sup>2</sup> | Used dough              |                        | Produced bread         |                        |
|-----------------------------------|-------------------------|------------------------|------------------------|------------------------|
|                                   | pH                      | TTA                    | pH                     | TTA                    |
| <i>Leu.</i> 1591                  | 6.48±0.00 <sup>b</sup>  | 4.10±0.33 <sup>b</sup> | 6.54±0.08 <sup>b</sup> | 3.00±0.22 <sup>b</sup> |
| <i>Lb.</i> 20179                  | 6.34±0.12 <sup>bc</sup> | 4.70±0.25 <sup>a</sup> | 6.48±0.12 <sup>a</sup> | 4.10±0.11 <sup>b</sup> |
| <i>Lb.</i> 15996                  | 6.25±0.08 <sup>c</sup>  | 5.20±0.41 <sup>a</sup> | 6.42±0.12 <sup>a</sup> | 4.40±0.32 <sup>b</sup> |
| Control                           | 6.89±0.11 <sup>a</sup>  | 1.50±0.12 <sup>c</sup> | 6.92±0.03 <sup>c</sup> | 1.60±0.13 <sup>a</sup> |

<sup>1</sup> Values (means ± standard deviation) with the same letter in a same column are not significantly different ( $P<0.05$ ).

<sup>2</sup> *Leu.* 1591 = *Leuconostoc mesenteroides* ssp. *mesenteroides*, strain PTCC1591; *Lb.* 20179 = *Lactobacillus plantarum* ssp. *plantarum*, strain ATCC 20179; *Lb.* 15996 = *Lactobacillus delbrueckii* ssp. *lactobacillus delbrueckii*, strain DSM 15996.

Moisture content

The changes in moisture content in the crust and crumb of produced breads are shown in Figure 2A and 2B. As considered in this figure, the moisture content in crumb decreased during storage period, while amount in the crust increased. This phenomenon was probably due to different levels of vapour pressure and water content in the crust and crumb which can lead to the water migration from crumb to crust (He and Hoseney, 1990). High moisture content in the crust makes it soft and leathery and this is a sign of the staling process. The results showed that there was no difference among moisture content of the crumb between the three treatments with LAB. However, the moisture content of the control bread on fifth and seventh day after the production was significantly lower than the breads obtained from different sourdoughs. The moisture content of the crust of the control bread was sharply increased. Thus it can be concluded that the moisture in the control bread faster transferred to the crust, while with the prepared breads with various RB-sourdoughs, moisture loss was slower and therefore it can be expected that these breads keep their freshness for a longer time period. This phenomenon is because of the RB hygroscopicity and its water-binding capacity that helps to retain moisture in the crumb (Carroll, 1990). Moreover, the water distribution among the various regions of bread (gluten or crystalline and amorphous starch) has a significant effect on the staling and structural changes of the bread during baking, such as starch gelatinisation, protein denaturation and the formation of colour and flavour and is dependent on the water presence (Katina *et al.*, 2006; Pomeranz, 1991). Thus, water-binding capacity in RB-sourdoughs has a significant effect on quality properties of bread.



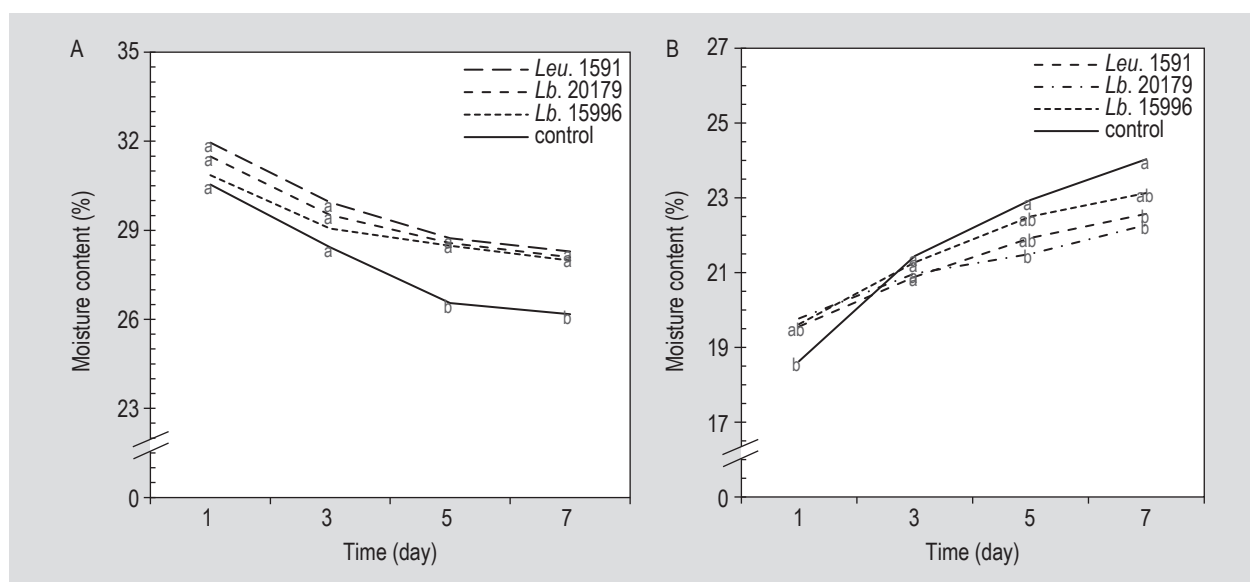


Figure 2. The moisture changes (A) crumb and (B) crust of the produced breads during storage time. Values marked by the same letter on the same day of storage are not significantly different ( $P < 0.05$ ). *Leu. 1591* = *Leuconostoc mesenteroides* ssp. *mesenteroides*, strain PTCC1591; *Lb. 20179* = *Lactobacillus plantarum* ssp. *plantarum*, strain ATCC 20179; *Lb. 15996* = *Lactobacillus delbrueckii* ssp. *lactobacillus delbrueckii*, strain DSM 15996.

On the other hand, the use of sourdough in the bread formula led to a significant increase in the pore size of the crumb texture in comparison with the control bread (data not shown). Water transfer from the crumb to crust can be disrupted due to the spongy texture and air spaces. Therefore, water migration from crumb to crust in breads obtained from RB-sourdoughs was slower than the control bread.

### Loaf volume measurement

Loaf volume is one of the important qualitative properties in bakery industries. One of the main problems with the application of bran in bakery products is that it often reduces loaf volume. The use of sourdough can have a positive or negative effect on loaf volume. This effect depends on the acidification rate and enzymatic activity that modifies the properties of gluten network. In this experiment, the loaf volume of bread obtained from sourdoughs fermented by *Leu. 1591* was significantly higher than the control bread (Figure 3). It seems that the lower acidity of these breads improves the protein network and therefore increases  $\text{CO}_2$  retention in the dough. Gobbetti *et al.* (1994) investigated the interaction LAB and yeasts in sourdough and reported that hetero-fermentative LAB can increase yeast activity. *Leu. 1591* as a hetero-fermentative bacterium can enhance the production of  $\text{CO}_2$  by the yeast for leavening.

### Texture analysis

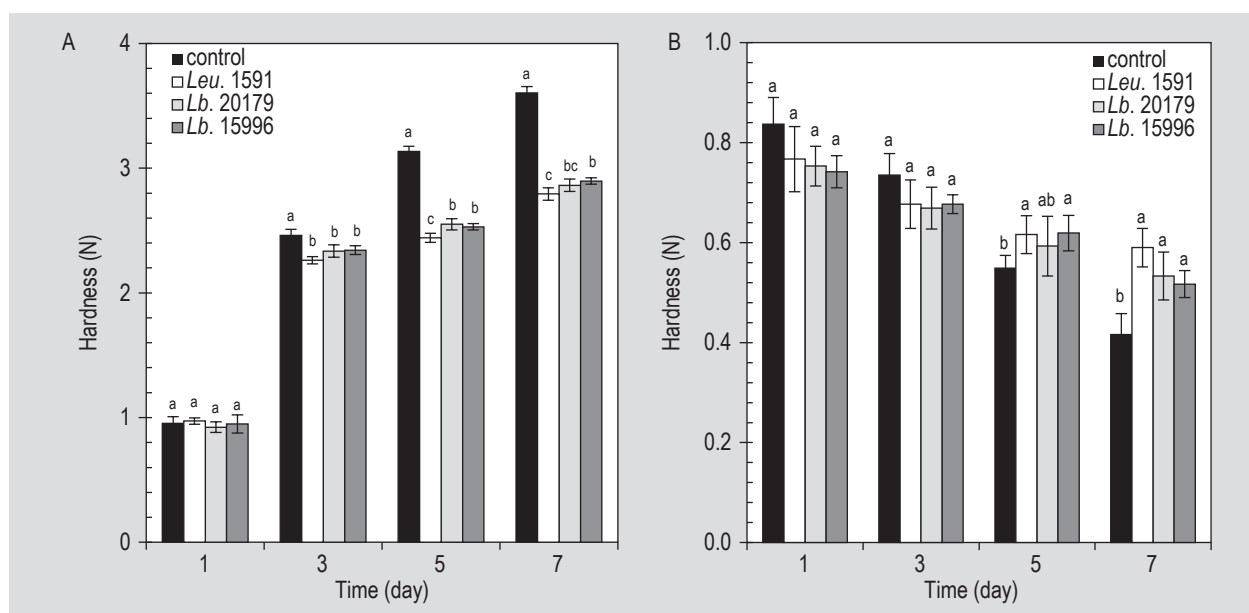
The quality changes in bakery products during the storage period decrease consumer satisfaction. The crumb hardness



Figure 3. Comparison of amounts of the loaf volume of breads produced with different sourdoughs and control bread. Columns marked by the same letter are not significantly different ( $P < 0.05$ ). *Lb. 15996* = *Lactobacillus delbrueckii* ssp. *lactobacillus delbrueckii*, strain DSM 15996; *Leu. 1591* = *Leuconostoc mesenteroides* ssp. *mesenteroides*, strain PTCC1591; *Lb. 20179* = *Lactobacillus plantarum* ssp. *plantarum*, strain ATCC 20179.

as one of the main changes largely decreases consumer perception of freshness (Ahlborn *et al.*, 2005). In this study, the hardness of crumb and crust were measured during 7 days storage to examine the effect of RB-sourdough addition on the textural characteristics.

As shown in Figure 4A, crumb hardness increases was significantly with storage time. On the first day of measurement, there was no significant difference in



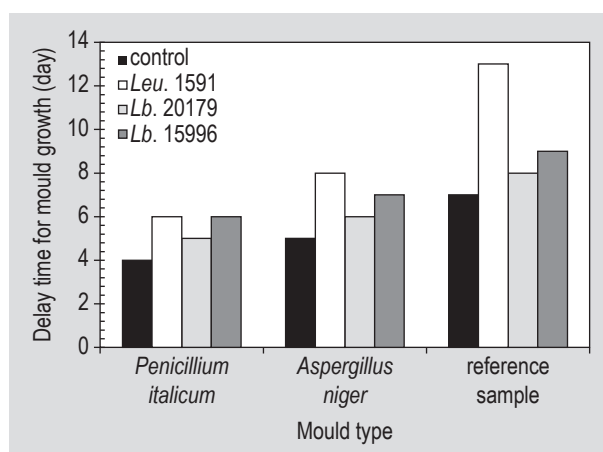
**Figure 4.** Hardness changes of the (A) crumb and (B) crust of breads produced with different sourdoughs and control bread during storage time. Columns marked by the same letter on the same day of storage are not significantly different ( $P < 0.05$ ). *Leu. 1591* = *Leuconostoc mesenteroides* ssp. *mesenteroides*, strain PTCC1591; *Lb. 20179* = *Lactobacillus plantarum* ssp. *plantarum*, strain ATCC 20179; *Lb. 15996* = *Lactobacillus delbrueckii* ssp. *lactobacillus delbrueckii*, strain DSM 15996.

hardness values between the samples, but after the third day, the hardness of the control bread was significantly higher than the other samples. It can be concluded that RB-sourdoughs can lead to a delay in bread crumb hardening. Many researchers have observed that the softness values were increased by the adding sourdough. An increase of dough acidity due to the sourdough application and bacterial activity could increase the positive charge on the dough and the proteins solubility. Increased proteolysis and modification of the gluten network help bread softening. Corsetti *et al.* (1998) and Katina (2006) reported that the protein network in the bread structure can be improved using fermented sourdoughs and thus increasing gas retention, loaf volume and softness of the produced bread. On the other hand, water re-distribution in bread is affected by acidification process and helps the freshness of the bread (Corsetti *et al.*, 2000). Moreover, the high water-binding capacity of RB can lead to moisture retention in the bread for a longer time and delays bread hardening. The breads prepared with sourdough fermented by *Leu. 1591* on the fifth and seventh day after baking were significantly softer than the other breads. This fact could be due to the mild acidity in these breads along with the production of exopolysaccharides. From a technological point of view, exopolysaccharides produced by LAB induced important effects on the quality properties such as dough water absorption, dough rheology and machinability, loaf volume and bread staling (Tieking and Gänzle, 2005). Crust hardness gradually reduced as a result of moisture transfer from the crumb to crust, this is responsible for

the formation of a soft and leathery crust (Figure 4B). Our results showed that there was no significant difference in the crust hardness on the first and third day after bread baking. However, due to the fast water uptake in the crust of the control bread on the fifth and seventh day after baking, they were significantly softer than the breads produced from different sourdoughs. On the other hand increased proteolysis in the sourdough breads can affect the protein structure as it opens the gluten network that leading to improved crispness retention in the crust of produced breads (Primo-Martín *et al.*, 2006).

### Anti-fungi static potential

The anti-fungi static potential in the different breads is shown in Figure 5. As depicted in Figure 6, the resistance of control bread to mould growth in all cases was lower than the breads prepared from the fermented sourdoughs. Thus, a positive effect in retarding the growth of moulds due to the sourdough addition was observed. This fact is related to some of the bacterial metabolites with strong antimicrobial activities such as organic acids,  $\text{CO}_2$ , ethanol, hydrogen peroxide, diacetyl, fatty acids, phenyllactic acid and reuteran (Chavan and Chavan, 2011). When no mould was inoculated (reference sample), maximum delay for the mould growth (13 day) were found for the breads prepared with sourdough fermented by *Leu. 1591*. The highest anti-mould activity against *A. niger* growth was observed with this treatment. Maximum resistance to *P. italicum* was observed in breads obtained from RB-sourdough fermented



**Figure 5. Resistance to mould formation in the different breads.** *Leu. 1591* = *Leuconostoc mesenteroides* ssp. *mesenteroides*, strain PTCC1591; *Lb. 20179* = *Lactobacillus plantarum* ssp. *plantarum*, strain ATCC 20179; *Lb. 15996* = *Lactobacillus delbrueckii* ssp. *lactobacillus delbrueckii*, strain DSM 15996.

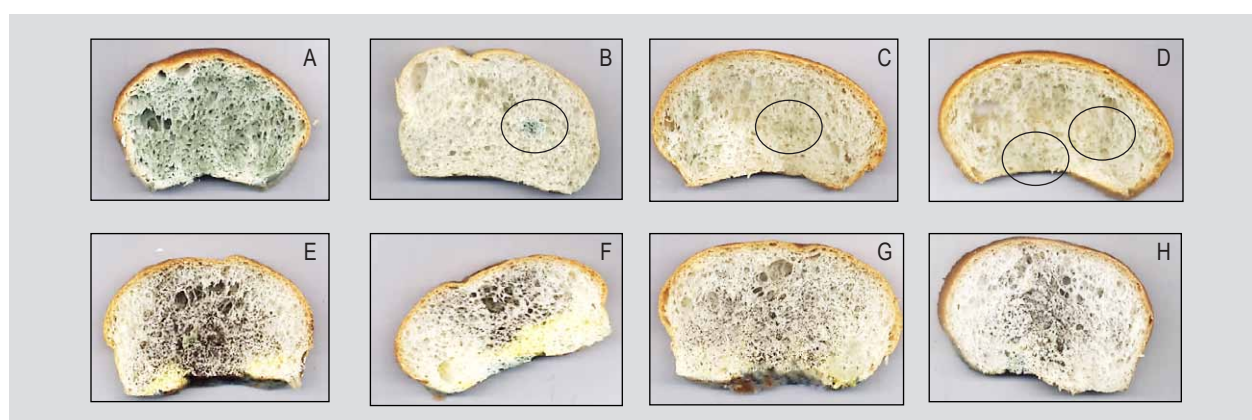
by *Lb. 15996* and *Leu. 1591* (6 ds). Also, the mould resistance of breads produced by sourdoughs of *Lb. 20179* was more than the that of the control bread. According to research conducted by Dalié *et al.* (2010), *L. plantarum* at the end of the logarithmic phase has its highest inhibition on mould growth. However, the presented data demonstrate that the ability of *Lb. 15996* and *Leu. 1591* to prevent the growth of moulds was more than *Lb. 20179* (*L. plantarum*). These results are in agreement with the results reported by Zotta *et al.* (2008) who studied the antifungal activity of *L. mesenteroides* and *Weissella cibaria* and *L. plantarum* isolated from cornetto (a traditional bread produced in Italy). They concluded that anti-mould activities of *L. mesenteroides* and *W. cibaria* were more than *L. plantarum*. This study showed that *L. delbrueckii* also had a good anti-mould activity.

## Sensory evaluation

Evaluation of consumer preference of an essential product such as bread is one of the most important methods for determining its sensory quality. Based on the scores that were given by the panellists, there was no significant different in the colour of crust and crumb. The scores for resilience, chewiness and softness were reduced significantly over time because the breads became stale. Based on this evaluation, a minimum reduction in softness was observed in *Leu. 1591* (from  $4.80 \pm 0.41$  on the first day to  $4.13 \pm 0.64$  on the seventh day) and a maximum reduction was related to the control bread (from  $4.67 \pm 0.49$  on the first day to  $3.40 \pm 0.51$  on the seventh day). The taste acceptance rate is reduced over time due to removal of the volatile compounds from bread structure. Higher proteolytic activity in the sourdough breads led to the breakdown of amino acids that acts as flavour precursors. Furthermore, the formation of acetic and lactic acids by LAB has an important effect on flavour. Nevertheless, it seems that the acidic taste of sourdough breads was not so satisfactory because the control breads had higher scores in comparison with sourdough bread on the same day. And the obtained results from sensory evaluation are summarised in the Table 3.

## 4. Conclusions

The present study has demonstrated that the addition of RB-sourdough to the bread formula had notable effects on the quality characteristics during storage period. It was concluded that the present of RB due to its hygroscopic properties helps to retain moisture in the bread. Therefore, the bread containing RB-sourdough maintains its softness for a longer time. Moreover, the negative effect of adding bran on the bread volume was solved with fermentation by LAB. These bacteria and their acid production had an important role in the formation of taste and texture of the



**Figure 6. Mould formation in breads of control (A, E) and prepared with sourdoughs fermented by *Lactobacillus plantarum* ssp. *plantarum*, strain ATCC 20179 (*Lb. 20179*) (B, F), *Lactobacillus delbrueckii* ssp. *lactobacillus delbrueckii*, strain DSM 15996 (*Lb. 15996*) (C, G) and *Leuconostoc mesenteroides* ssp. *mesenteroides*, strain PTCC1591 (*Leu. 1591*) (D, H) infected by *Penicillium italicum* (A-D) and *Aspergillus niger* (E-H). Image acquisition was performed on the tenth day after production.**

**Table 3. Evaluation of sensory attributes of the breads produced during the storage time (means  $\pm$  standard deviation).**

| Bread type <sup>2</sup>   | Sensory characteristics <sup>1</sup> |                              |                                 |                                 |                                 |                               |                                 |                                 |
|---------------------------|--------------------------------------|------------------------------|---------------------------------|---------------------------------|---------------------------------|-------------------------------|---------------------------------|---------------------------------|
|                           | Crumb colour                         | Crust colour                 | Softness                        | Chewiness                       | Resilience                      | Porosity                      | Taste                           | Overall acceptability           |
| <b>1<sup>st</sup> day</b> |                                      |                              |                                 |                                 |                                 |                               |                                 |                                 |
| <i>Leu.</i> 1591          | 4.67 $\pm$ 0.49 <sup>a</sup>         | 4.73 $\pm$ 0.46 <sup>a</sup> | 4.80 $\pm$ 0.41 <sup>a</sup>    | 4.80 $\pm$ 0.41 <sup>a</sup>    | 4.73 $\pm$ 0.46 <sup>a</sup>    | 4.53 $\pm$ 0.49 <sup>a</sup>  | 4.07 $\pm$ 0.26 <sup>bc</sup>   | 4.61 $\pm$ 0.18 <sup>ab</sup>   |
| <i>Lb.</i> 20179          | 4.73 $\pm$ 0.46 <sup>a</sup>         | 4.87 $\pm$ 0.35 <sup>a</sup> | 4.73 $\pm$ 0.46 <sup>ab</sup>   | 4.73 $\pm$ 0.46 <sup>a</sup>    | 4.67 $\pm$ 0.49 <sup>ab</sup>   | 4.47 $\pm$ 0.46 <sup>ab</sup> | 4.13 $\pm$ 0.35 <sup>b</sup>    | 4.59 $\pm$ 0.29 <sup>abc</sup>  |
| <i>Lb.</i> 15996          | 4.60 $\pm$ 0.51 <sup>a</sup>         | 4.67 $\pm$ 0.48 <sup>a</sup> | 4.67 $\pm$ 0.49 <sup>ab</sup>   | 4.60 $\pm$ 0.51 <sup>abc</sup>  | 4.53 $\pm$ 0.52 <sup>abcd</sup> | 4.47 $\pm$ 0.52 <sup>ab</sup> | 3.93 $\pm$ 0.46 <sup>bcd</sup>  | 4.47 $\pm$ 0.25 <sup>abcd</sup> |
| Control                   | 4.80 $\pm$ 0.41 <sup>a</sup>         | 4.87 $\pm$ 0.35 <sup>a</sup> | 4.67 $\pm$ 0.49 <sup>ab</sup>   | 4.67 $\pm$ 0.49 <sup>ab</sup>   | 4.60 $\pm$ 0.51 <sup>abc</sup>  | 4.07 $\pm$ 0.46 <sup>bc</sup> | 4.80 $\pm$ 0.41 <sup>a</sup>    | 4.63 $\pm$ 0.27 <sup>a</sup>    |
| <b>3<sup>rd</sup> day</b> |                                      |                              |                                 |                                 |                                 |                               |                                 |                                 |
| <i>Leu.</i> 1591          | 4.53 $\pm$ 0.52 <sup>a</sup>         | 4.67 $\pm$ 0.49 <sup>a</sup> | 4.53 $\pm$ 0.52 <sup>abc</sup>  | 4.60 $\pm$ 0.51 <sup>abc</sup>  | 4.67 $\pm$ 0.49 <sup>ab</sup>   | 4.67 $\pm$ 0.49 <sup>a</sup>  | 3.67 $\pm$ 0.49 <sup>def</sup>  | 4.43 $\pm$ 0.29 <sup>abcd</sup> |
| <i>Lb.</i> 20179          | 4.60 $\pm$ 0.51 <sup>a</sup>         | 4.73 $\pm$ 0.46 <sup>a</sup> | 4.47 $\pm$ 0.52 <sup>abcd</sup> | 4.53 $\pm$ 0.52 <sup>abc</sup>  | 4.60 $\pm$ 0.51 <sup>abc</sup>  | 4.67 $\pm$ 0.49 <sup>a</sup>  | 3.73 $\pm$ 0.46 <sup>cdef</sup> | 4.41 $\pm$ 0.27 <sup>bcd</sup>  |
| <i>Lb.</i> 15996          | 4.47 $\pm$ 0.52 <sup>a</sup>         | 4.60 $\pm$ 0.51 <sup>a</sup> | 4.47 $\pm$ 0.52 <sup>abcd</sup> | 4.53 $\pm$ 0.52 <sup>abc</sup>  | 4.60 $\pm$ 0.51 <sup>abc</sup>  | 4.40 $\pm$ 0.51 <sup>ab</sup> | 3.87 $\pm$ 0.35 <sup>bcd</sup>  | 4.38 $\pm$ 0.34 <sup>cdef</sup> |
| Control                   | 4.60 $\pm$ 0.51 <sup>a</sup>         | 4.80 $\pm$ 0.41 <sup>a</sup> | 4.33 $\pm$ 0.49 <sup>bcd</sup>  | 4.47 $\pm$ 0.52 <sup>abcd</sup> | 4.53 $\pm$ 0.52 <sup>abcd</sup> | 3.87 $\pm$ 0.35 <sup>c</sup>  | 4.60 $\pm$ 0.51 <sup>a</sup>    | 4.42 $\pm$ 0.34 <sup>abcd</sup> |
| <b>5<sup>th</sup> day</b> |                                      |                              |                                 |                                 |                                 |                               |                                 |                                 |
| <i>Leu.</i> 1591          | 4.53 $\pm$ 0.52 <sup>a</sup>         | 4.60 $\pm$ 0.51 <sup>a</sup> | 4.33 $\pm$ 0.62 <sup>bcd</sup>  | 4.47 $\pm$ 0.52 <sup>abcd</sup> | 4.47 $\pm$ 0.52 <sup>abcd</sup> | 4.60 $\pm$ 0.51 <sup>a</sup>  | 3.53 $\pm$ 0.52 <sup>ef</sup>   | 4.29 $\pm$ 0.28 <sup>defg</sup> |
| <i>Lb.</i> 20179          | 4.47 $\pm$ 0.52 <sup>a</sup>         | 4.67 $\pm$ 0.49 <sup>a</sup> | 4.13 $\pm$ 0.52 <sup>cdef</sup> | 4.27 $\pm$ 0.46 <sup>cde</sup>  | 4.33 $\pm$ 0.49 <sup>abcd</sup> | 4.47 $\pm$ 0.52 <sup>ab</sup> | 3.73 $\pm$ 0.46 <sup>cdef</sup> | 4.20 $\pm$ 0.22 <sup>efgh</sup> |
| <i>Lb.</i> 15996          | 4.53 $\pm$ 0.52 <sup>a</sup>         | 4.67 $\pm$ 0.49 <sup>a</sup> | 4.00 $\pm$ 0.38 <sup>ef</sup>   | 4.07 $\pm$ 0.26 <sup>ef</sup>   | 4.13 $\pm$ 0.35 <sup>de</sup>   | 4.47 $\pm$ 0.52 <sup>ab</sup> | 3.73 $\pm$ 0.46 <sup>cdef</sup> | 4.11 $\pm$ 0.20 <sup>ghi</sup>  |
| Control                   | 4.67 $\pm$ 0.49 <sup>a</sup>         | 4.80 $\pm$ 0.41 <sup>a</sup> | 3.73 $\pm$ 0.46 <sup>fg</sup>   | 3.87 $\pm$ 0.35 <sup>fg</sup>   | 3.80 $\pm$ 0.41 <sup>ef</sup>   | 3.87 $\pm$ 0.35 <sup>c</sup>  | 4.53 $\pm$ 0.52 <sup>a</sup>    | 4.06 $\pm$ 0.19 <sup>hi</sup>   |
| <b>7<sup>th</sup> day</b> |                                      |                              |                                 |                                 |                                 |                               |                                 |                                 |
| <i>Leu.</i> 1591          | 4.60 $\pm$ 0.51 <sup>a</sup>         | 4.67 $\pm$ 0.49 <sup>a</sup> | 4.13 $\pm$ 0.64 <sup>cdef</sup> | 4.33 $\pm$ 0.49 <sup>bcd</sup>  | 4.27 $\pm$ 0.59 <sup>bcd</sup>  | 4.60 $\pm$ 0.51 <sup>a</sup>  | 3.47 $\pm$ 0.52 <sup>f</sup>    | 4.18 $\pm$ 0.29 <sup>fgh</sup>  |
| <i>Lb.</i> 20179          | 4.73 $\pm$ 0.46 <sup>a</sup>         | 4.86 $\pm$ 0.35 <sup>a</sup> | 4.07 $\pm$ 0.46 <sup>def</sup>  | 4.13 $\pm$ 0.35 <sup>def</sup>  | 4.20 $\pm$ 0.41 <sup>cd</sup>   | 4.67 $\pm$ 0.49 <sup>a</sup>  | 3.53 $\pm$ 0.52 <sup>ef</sup>   | 4.16 $\pm$ 0.23 <sup>gh</sup>   |
| <i>Lb.</i> 15996          | 4.40 $\pm$ 0.51 <sup>a</sup>         | 4.60 $\pm$ 0.51 <sup>a</sup> | 3.73 $\pm$ 0.46 <sup>fg</sup>   | 3.80 $\pm$ 0.41 <sup>fg</sup>   | 3.73 $\pm$ 0.46 <sup>f</sup>    | 4.40 $\pm$ 0.51 <sup>ab</sup> | 3.73 $\pm$ 0.46 <sup>cdef</sup> | 3.93 $\pm$ 0.25 <sup>ij</sup>   |
| Control                   | 4.53 $\pm$ 0.52 <sup>a</sup>         | 4.67 $\pm$ 0.49 <sup>a</sup> | 3.40 $\pm$ 0.51 <sup>g</sup>    | 3.60 $\pm$ 0.51 <sup>g</sup>    | 3.47 $\pm$ 0.52 <sup>f</sup>    | 3.93 $\pm$ 0.59 <sup>c</sup>  | 4.00 $\pm$ 0.53 <sup>bcd</sup>  | 3.78 $\pm$ 0.25 <sup>j</sup>    |

<sup>1</sup> Values within each column followed by the same superscript letters are not significantly different ( $P < 0.05$ ).

<sup>2</sup> *Leu.* 1591 = *Leuconostoc mesenteroides* ssp. *mesenteroides*, strain PTCC1591; *Lb.* 20179 = *Lactobacillus plantarum* ssp. *plantarum*, strain ATCC 20179; *Lb.* 15996 = *Lactobacillus delbrueckii* ssp. *lactobacillus delbrueckii*, strain DSM 15996.

bread. Also the presence of these bacteria in the sour dough that used in the production of breads can delay the growth of mould during storage.

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