

Critical quality attributes of Iranian 'Taftoon' breads as affected by the addition of rice bran sourdough with different lactobacilli

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RESEARCH ARTICLE

Abstract

Contribution effects of three probiotic bacteria of *Lactobacillus acidophilus*, *Lactobacillus plantarum*, and *Lactobacillus casei* in the fermentation of rice bran (RB, 5-15%) sourdough on the physical (colour and firmness), chemical (pH and total titrable acidity; TTA), antimicrobial and sensory characteristics of Taftoon bread (TB), an Iranian flat bread, were studied. The results showed that *L. acidophilus* with inducing a more TTA led to the production of TBs with lower firmness degree than two other strains. An increase in RB amount of sourdough from 5 to 15% led to a firmer texture with lower lightness. The TB obtained from RB sourdough of *L. plantarum* had higher anti-mould activity than the doughs fermented by *L. casei* and *L. acidophilus*. Sensory evaluation revealed a better taste for the control bread than TBs formulated with the different sourdoughs. TBs with 5% RB sourdough had higher overall acceptability than 10 and 15% RB sourdoughs. The highest staling during 3 days storage was also found for TBs with 15% sourdough fermented with *L. casei*.

Keywords: Taftoon bread making, lactic acid bacteria, sourdough, rice bran

1. Introduction

Sourdough is used as a leavening agent in bread making from ancient time. Nowadays, it is used in modern baking technology because of its importance in increasing the quality and shelf life of bread. However, sourdough fermentation improves the bread flavour due to the formation of acid and flavour precursors like amino acids and volatile compounds (Arendt *et al.*, 2007). Sourdough also makes the dough softer and less elastic because of the solubilisation and degradation of gluten in the result of amylolytic, proteolytic and other enzymatic activities during acidification steps of the sourdough (Katina *et al.*, 2005). The acidic condition decreases inter and intra-molecular sulfide bonds and increases solubility of proteins and gluten chains and thus provides better conditions for proteolysis and improvement of gas holding capacity of gluten (Thiele *et al.*, 2002). Sourdough also by the decreasing final pH of dough to an optimum level of 5.5 improves CO₂ production by the yeast (Holmes and Hoseney, 1987). A significant retardation in bread staling can happen by releasing organic

acids from structure of starch-protein and increasing protease and amylase activities. Meanwhile, alpha amylase with producing low-molecular weight dextrins can prevent from starch crystallisation and bread staling (Siljestrom *et al.*, 1988).

Dietary fibres have a lot of important roles in body such as reducing cholesterol content of blood and preventing of cardiovascular diseases and colon cancer (Rahaie *et al.*, 2012). Recently, consumers are interested to increase intake of dietary fibre in their daily food diets. Rice bran (RB) with 27% dietary fibre is a suitable case to add in the various bread formulations (Sangnark and Noomhorm, 2004). RB is rich in minerals, proteins, fatty acids and dietary fibres and it can be used for the enrichment of different foods. Addition of dietary fibres to dough has many effects on dough rheology as its extensibility can decrease by the increasing water absorption (Gómez *et al.*, 2003). Bagheri and Seyedein (2011) found that the dough development time of Farinogram decreases with the addition of RB. However, Park and Han (2010) showed that fermented

RB had a different manner as both the water absorption and extensibility of dough decreased with increasing of fermented RB. The RB addition also led to produce breads with low volume and acceptability (Abdul-Hamid and Luan, 2000). This component with absorbing water maintains moisture content in bread structure and increases staling time during storage period.

Mould spoilage due to low water activity in flat breads is the most important spoilage in bread and can cause many economic losses. For this reason, use of anti-mould compounds in bread formulation is unavoidable. Since the researches recently have been showed the adverse health effect of these compounds, thus investigation of natural anti-mould compounds can be very interesting (Plessas *et al.*, 2011). However, lactic acid bacteria (LAB) in sourdough with producing organic acids, CO₂, hydrogen peroxide, diacetyl, low-molecular weight peptides and other metabolites can prevent from mould spoilage (Digaitiene *et al.*, 2012).

In this study, RB was fermented with three different LAB of *Lactobacillus plantarum*, *Lactobacillus acidophilus* and *Lactobacillus casei* in combination with the bakery yeast in order to formulate traditional flat bread 'Taftoon'. Thus, the aim of this work was to improve quality attributes and shelf life of Taftoon bread (TB) as one of the most common breads consumed in Iran.

2. Materials and methods

Materials

A commercial blend of white wheat flour (WF) was used (Nanavaran Co., Tehran, Iran). Analysis of chemical components showed that the amounts of dry matter, ash and protein of WF and RB were 86.10, 89.80 and 0.59% and 8.12, 8.43 and 16.36%, respectively. Wet gluten content and gluten index of WF were 28.30 and 75.50%, respectively. Moreover, the fat and carbohydrate levels of RB were 19.20 and 46.15%, respectively.

Microorganism

The used LAB of *L. acidophilus*, *L. plantarum* and *L. casei* were grown in de Man, Rogosa and Sharpe broth medium (Merck Chemical Co., Darmstadt, Germany) at 37 °C for 24 h. The microorganisms were harvested by centrifugation at 4,000 rpm for 10 min, washed twice with saline solution and used for the sourdough production.

Bran fermentation

For the preparation of used sourdoughs, 300 g RB was mixed with 700 g of tap water, heated in a water-bath at 55 °C for 10 min and cooled to 37 °C. The microorganisms

were then added in an initial cell density of 10⁷ cfu/ml to the mixture and incubated at 37 °C for 16 h.

Bread making process

TB was made with mixing WF, tap water, salt and *Saccharomyces cerevisiae* yeast (1.7% and 1% w/w respectively on WF basis), and RB sourdough at 5, 10 and 15% w/w on WF basis. In brief, the ingredients were mixed using a mixer (M120; Escher Premium Spiral Mixers, London, UK) for 10 min at 25 °C, kneaded for 5 min, and then the resulted dough fermented at the room temperature for 1 h. The fermented dough was divided to 280 g portions, rounded and proofed for 10 min. The proofed dough opened and ducked in order to prevent the pocket formation during baking process. In the final step, they cooked in a traditional kind of oven called 'Tanoor'. Control bread was made with 1% baking soda on WF basis instead of adding sourdough. In this work, three types of sourdough incubated with three different LAB bacteria (*L. acidophilus*, *L. plantarum* and *L. casei*) were added at three concentrations of 5, 10 and 15% on WF basis. After the cooling process, all the breads were packaged in a polyethylene bags and kept at 20 °C.

pH and total titrable acidity

For the determining pH and total titrable acidity (TTA), 10 g of, powdered bread made from sourdough or dough was stirred with 90 ml distilled water for 10 min. The pH value was measured using a pH meter (MP230; MettlerToldo, Greifensee, Switzerland). TTA was determined by titration with NaOH 0.1 N until the pH reached to 8.5 and the amount of NaOH (ml) consumed expressed as a mean value of three replicates (Katina *et al.*, 2005).

Instrumental firmness quality analysis

The AACC method no. 74-09 (AACC, 2000) with some modifications was applied to analyse the breads texture using a universal testing machine (M350-10CT; Testometric, Rochdale, UK), equipped to a Kramer shear cell containing 5 blades. The constant force of 500 N with a speed 120 mm/min was used. One layer of bread was taken from the centre of the loaf with approximate thickness 1.5 mm and analysed to determine the maximum recorded force as the firmness. The firmness evaluated on days 1, 2 and 3 for breads packed in polyethylene bag and stored at 20 °C.

Determination of antifungal activity

To determine antifungal activity of three bacteria presence in sourdough fermentation, 10×10 cm slices of bread samples were inoculated with a suspension of 10² conidia/ml *Aspergillus niger*, *Penicillium italicum* and *Rhizopus stolonifer*. The slices were packed in polyethylene

bags, incubated in 20 °C and inspected until observation of fungal contamination (Coda *et al.*, 2011).

Colour measurement

For this analysis, images of the different samples were obtained using a scanner (HP Scanjet 5590; Hewlett-Packard Development Co., Palo Alto, CA, USA) with a resolution of 300 dpi and transferred to Photoshop CS5 (Adobe systems, San Jose, CA, USA). Three points of each image were analysed to obtain level of lightness (L) values of the samples (Yam and Papadakis, 2004).

Sensory evaluation

Sensory evaluation of the breads during the storage time carried out by 30 trained panellists consisting of graduate students and staff members of Tehran University's Food Engineering Department (Tehran, Iran) who were familiar with the characteristic qualities of produced breads. This evaluation was based on the firmness, chewiness and taste of breads using a five-point hedonic scale: 5 corresponded to 'most liked' and 1 to 'most disliked' (Abdul-Hamid and Luan, 2000).

Statistical analysis

Experiments were conducted in triplicate and data sets were subjected to the analysis of variance (ANOVA) by SAS, version 9.1.3 (SAS Institute Inc., Cary, NC, USA) software. The mean data were compared using the Duncan's multiple test range at a significant level of $P < 0.05$. The data were expressed as mean $\pm$ standard deviation.

3. Results and discussion

The pH values of different sourdoughs are shown in Figure 1A. pH of the sourdoughs incubated with *L. casei* was significantly higher than those fermented by *L. acidophilus*. pH and TTA values of the final doughs and breads are shown in Table 1. As a reliable principle, the pH and TTA values respectively decreases and increases by increasing sourdough concentration. pH of the control dough due to using baking soda was higher than the samples containing RB sourdough. However, the TTA of control bread because of the buffering properties of RB proteins was lesser than the samples with a same pH. *L. acidophilus* reduced pH more than *L. plantarum* and *L. casei*. All the samples except the dough prepared with 5% sourdough inoculated with *L. casei* significantly had higher pH values than the control dough.

Komlenić *et al.* (2010) showed that dropping pH by the releasing organic acids can be responsible for rheological changes of dough. The control bread baked without adding RB sourdough had the lowest firmness level. Sadeghi *et al.* (2008) also reported similar results. The results also

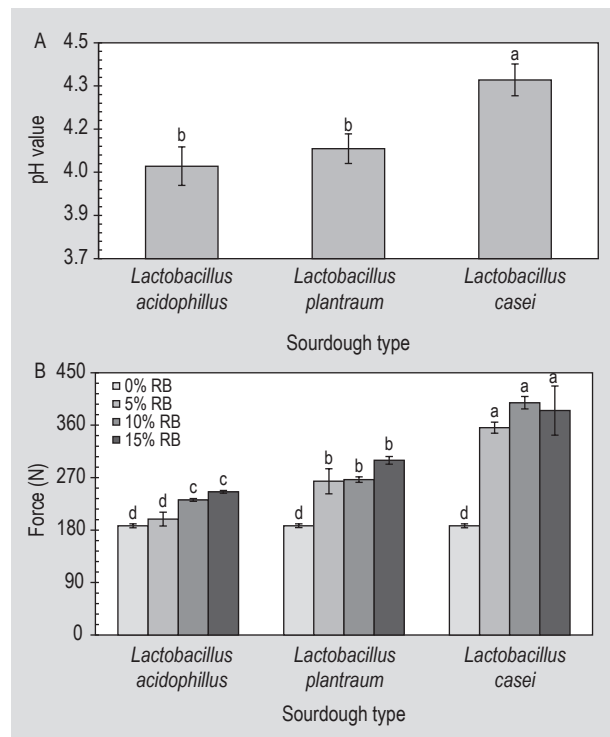


Figure 1. (A) pH and (B) firmness values of sourdoughs incubated with *Lactobacillus acidophilus*, *Lactobacillus plantarum* or *Lactobacillus casei*. Columns marked with the same letter are not statistically different at $P < 0.05$.

showed that addition of RB sourdough increased the breads firmness. However, the breads prepared with *L. acidophilus* sourdough had less firmness than those prepared with *L. plantarum* and *L. casei*. This fact was probably due to the lowest pH value for sourdoughs inoculated with *L. acidophilus* (Figure 1A). The obtained findings in this study were in agreement with the results of Clark *et al.* (2002), who reported that lactic sourdough addition can reduce dough elasticity and can increase its softening degree. They demonstrated that more elasticity of a dough leads to the production of thicker breads (Clark *et al.*, 2002). These researchers reported that cereal proteases with an optimal activity in acidic conditions play an essential role in the rheological changes accrued during sourdough fermentation. Gocmen *et al.* (2007) also showed that the gluten degradation can significantly increase sourdough amount required to decrease the dough resistance to extension. The firmness increased with increasing the RB sourdough amount (Figure 1B). This result is in accordance with finding obtained by Katina *et al.* (2005), who found that the bread prepared with bran sourdough was firmer than white wheat bread.

LAB-sourdough by the reducing starch retrogradation was retarded staling with breaking gluten to smaller units (AACC, 2000; Corsetti *et al.*, 2000). This might be partly explained by the formation of low-molecular weight

Table 1. pH and total titrable acidity (TTA) values of the doughs and breads incubated with *Lactobacillus acidophilus*, *Lactobacillus plantarum* or *Lactobacillus casei*.¹

Bread sample	Rice bran (%)	Dough		Bread	
		pH	TTA (ml NaOH)	pH	TTA (ml NaOH)
<i>Lactobacillus acidophilus</i>	5	6.16±0.48 ^c	4.50±0.28 ^c	6.13±0.04 ^b	6.20±0.16 ^a
	10	5.91±0.12 ^c	6.00±0.42 ^b	6.05±0.09 ^b	6.50±0.04 ^a
	15	5.89±0.16 ^c	6.90±0.07 ^a	5.98±0.18 ^c	6.70±0.57 ^a
<i>Lactobacillus plantarum</i>	5	6.61±0.30 ^b	3.50±0.11 ^c	6.66±0.05 ^a	4.90±0.57 ^b
	10	6.61±0.13 ^b	3.70±0.71 ^c	6.54±0.08 ^b	5.00±0.11 ^b
	15	6.50±0.16 ^b	4.30±0.42 ^c	6.23±0.04 ^b	5.20±0.28 ^b
<i>Lactobacillus casei</i>	5	7.37±0.04 ^a	2.00±0.71 ^d	7.11±0.16 ^a	2.30±0.47 ^c
	10	6.60±0.05 ^b	3.10±0.00 ^c	6.58±0.00 ^b	3.00±0.07 ^c
	15	6.57±0.11 ^b	3.70±0.21 ^c	6.23±0.18 ^b	5.00±0.35 ^b
Control	0	7.30±0.35 ^a	2.10±0.21 ^d	5.87±0.50 ^c	5.20±0.14 ^b

¹ Values (mean ± standard deviation) followed by the same superscript letter in the same column are not significantly different ($P < 0.05$).

dextrins in acidic conditions, which have been postulated to interfere with the starch retrogradation process (Siljestrom *et al.*, 1988). The positive effect of sourdough on staling retardation has been also observed by Katina *et al.* (2005), who found bran bread staled slower than white wheat bread. They pointed out that the slow staling is due to the less starch content in bran bread and more swelling of its starch

granules. These researchers also declared that bran by more absorbing water reassociates the polymers structure so that the amylopectin crystallisation decreases water mobility (Katina *et al.*, 2005). In the current study, the control bread staled rapidly than the sourdough breads but there is not any specific order among the breads with different sourdough levels (Figure 2). However, the firmness of breads

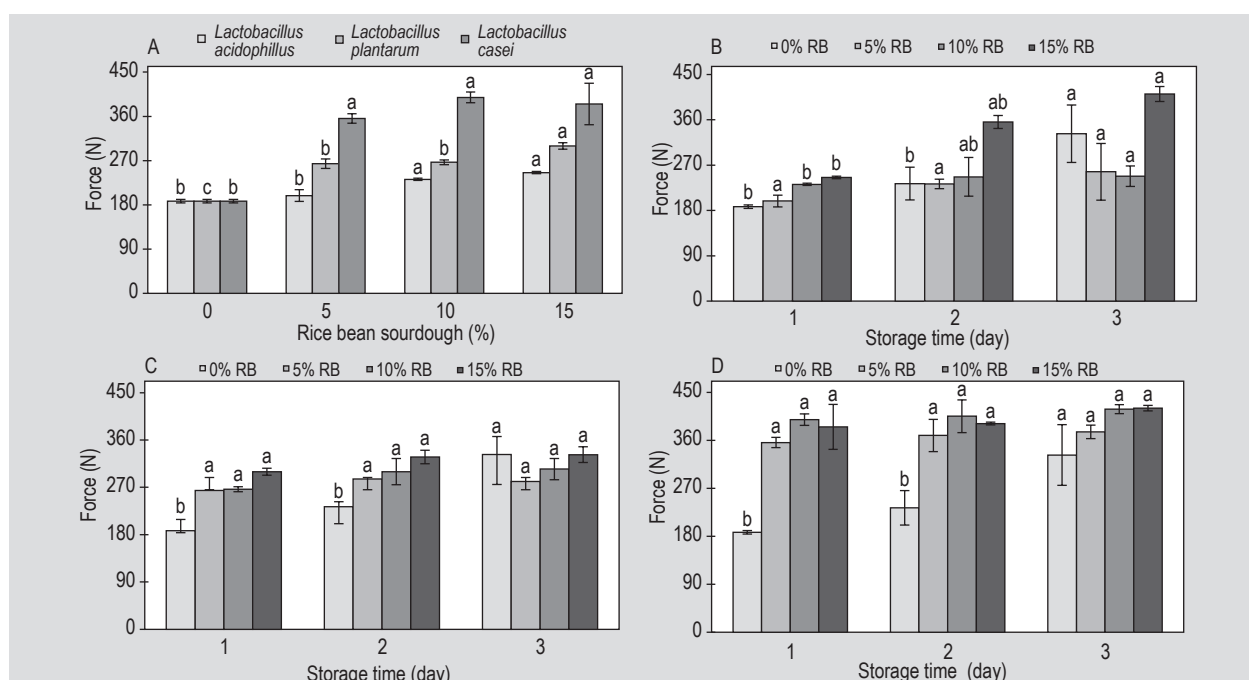


Figure 2. (A) Effect of different amounts of sourdough on the firmness of fresh breads, and staling level of the samples made with sourdoughs containing (B) *Lactobacillus acidophilus*, (C) *Lactobacillus plantarum* and (D) *Lactobacillus casei* during 3 days of storage (0% rice bran (RB; white column), 5% RB (light grey column), 10% RB (grey column) and 10% RB (black column)). Columns marked with the same letters are not significantly different ($P < 0.05$).

made from 15% sourdough inoculated with *L. acidophilus* seems to be more than 10 and 5%. It is probably because of the lower pH value. This staling accelerating factor can thus provide better conditions for amylolytic enzymes (Komlenić *et al.*, 2010). Comparison of firmness amounts of the breads during the storage revealed that this character in control bread on the third day of storage was significantly higher than first day. The firmness of breads prepared with *L. acidophilus* and *L. plantarum* sourdoughs during the storage significantly increased but a significant staling was observed for the breads prepared with *L. casei*.

Results of antifungal activities of the different breads are shown in Table 2. Contamination with *A. niger* occurred later with the increasing sourdough amount inoculated with *L. plantarum*. No significant difference in preventing spoilage was found for the breads obtained from 5 and 10% sourdoughs prepared with *L. acidophilus* and *L. casei*. An increase in mould inhibition was resulted with the increasing sourdough content upto 15%. The same findings observed in the case of *P. italicum* and *R. stolonifer*. Results of sensory evaluation in term of bread taste showed a high score for the control bread. It seems that the acidic taste had a negative effect for panellists' preference. The breads containing 5% sourdough had higher scores than 10 and 15% sourdoughs, respectively. In general, the breads made from 5 and 10% sourdough had a higher acceptable taste (Figure 3). The chewiness evaluation of breads showed that the lowest scores were belonged to the breads baked with 15% of RB sourdough due to RB effect on their firmness level. It can be concluded that the chewiness of breads decreased during storage time because of the staling phenomenon.

The control bread was firmer at the first day of storage, but it became drier at the end of 48 h storage and therefore had the minimum score of firmness. The staling scores on the third day showed that the control and 5% sourdough breads were more staled (Table 2).

Results of colour analysis showed that the L-values decreased by increasing RB sourdough content. The maximum and minimum L-values were found for the control and 15% sourdough breads, respectively. Addition of RB is responsible for the breads' darkness. Nevertheless, no significant difference was observed between samples containing 5 and 10% sourdough (Figure 4).

4. Conclusions

In this study, the addition effect of RB sourdoughs fermented by three probiotic LAB on the critical quality attributes of TBs was studied. Results showed that the breads firmness significantly increased with increasing sourdough amount. Among three applied microorganisms, *L. acidophilus* seems was more effective than *L. plantarum* and *L. casei* to improve the bread quality. The results showed that application of RB sourdough retards staling process. Application of RB sourdough can reduce the negative effect of RB on the bread firmness. Use of RB sourdough can also omit the baking soda that was traditionally used in the TB formulation and therefore it improves the nutritional value of TB. Moreover, LAB-sourdough with the producing organic acids and antimicrobial compounds can effectively inhibit mould spoilage in the bread during storage.

Table 2. Chewiness and firmness quality scores and antifungal activities of the different formulations of Taftoon bread.¹

Bread type	Rice bran (%)	Chewiness, storage period (day)			Firmness, storage period (day)			Antifungal activity ²		
		1	2	3	1	2	3	<i>Penicillium italicum</i>	<i>Rhizopus stolonifer</i>	<i>Aspergillus niger</i>
Control		4.80±0.45 ^a	4.20±0.84 ^a	4.00±0.71 ^a	4.71±0.49 ^a	3.57±0.53 ^b	2.43±0.53 ^d	2±0 ^c	3±0 ^c	2±0 ^c
<i>Lactobacillus acidophilus</i>	5	4.40±0.45 ^a	4.20±0.45 ^{ab}	3.80±0.45 ^b	4.71±0.49 ^a	3.71±0.49 ^b	2.86±0.38 ^{cd}	3±0 ^b	2±0 ^c	4±0 ^{bc}
	10	3.60±0.55 ^b	3.20±0.45 ^{bc}	3.20±0.71 ^{bc}	4.57±0.53 ^a	3.86±0.38 ^b	3.57±0.53 ^b	4±0 ^b	3±0 ^c	4±0 ^{bc}
	15	3.40±0.45 ^b	2.80±0.45 ^c	2.80±0.55 ^c	4.57±0.53 ^a	3.71±0.49 ^b	3.14±0.69 ^c	5±1 ^b	6±0 ^b	6±1 ^b
<i>Lactobacillus plantarum</i>	5	4.00±0.71 ^{ab}	3.80±0.45 ^{ab}	3.80±0.84 ^{ab}	4.57±0.53 ^a	3.57±0.53 ^b	2.86±0.90 ^c	5±0 ^b	4±0 ^b	5±0 ^b
	10	3.20±0.45 ^b	3.20±0.45 ^{bc}	2.80±0.84 ^c	4.43±0.53 ^a	3.71±0.49 ^b	3.29±0.49 ^{bc}	8±1 ^{ab}	6±1 ^b	7±0 ^{ab}
	15	2.80±0.84 ^c	2.60±0.55 ^c	2.4±0.84 ^c	4.29±0.49 ^{ab}	3.57±0.79 ^b	3.29±1.11 ^b	11±0 ^a	12±1 ^a	10±2 ^a
<i>Lactobacillus casei</i>	5	4.00±1.00 ^a	3.80±0.45 ^a	3.40±0.55 ^{ab}	4.57±0.53 ^a	3.43±0.53 ^b	2.86±0.38 ^d	2±0 ^c	2±0 ^c	2±0 ^c
	10	3.00±0.71 ^b	3.00±0.71 ^b	2.60±0.55 ^{bc}	4.29±0.49 ^a	3.43±0.53 ^b	3.29±0.49 ^b	2±0 ^c	2±0 ^c	2±0 ^c
	15	2.60±0.55 ^{bc}	2.60±0.55 ^{bc}	2.40±0.55 ^{bc}	4.14±0.38 ^{ab}	3.57±0.79 ^b	3.29±0.76 ^{bc}	3±0 ^b	4±1 ^b	3±0 ^c

¹ Values (mean ± standard deviation) followed by the same superscript letter in the same column are not significantly different ($P < 0.05$).

² Data show the delay time (day) for the mould growth.

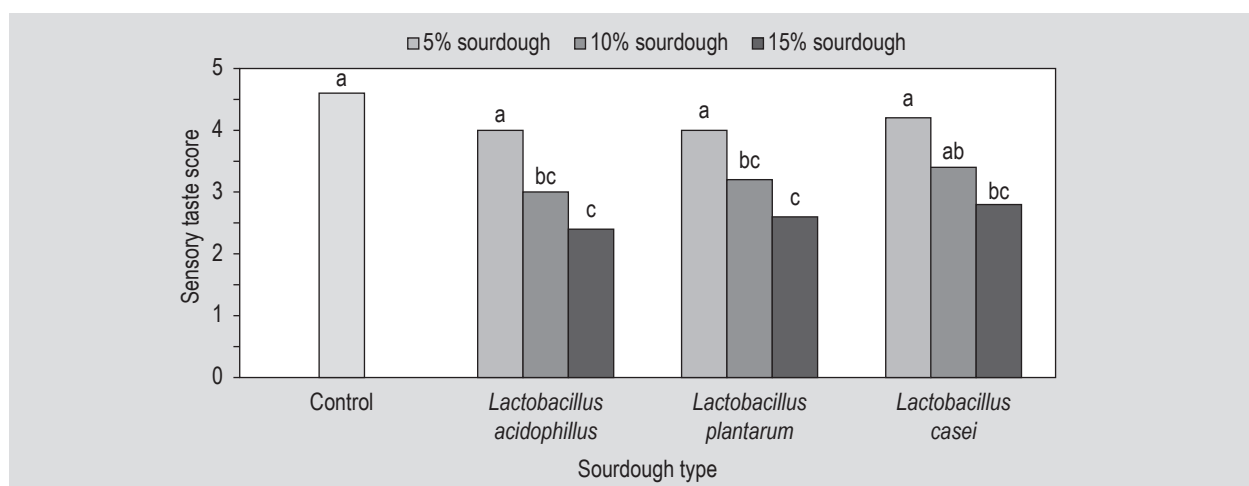


Figure 3. Sensory scores for taste of the breads made with different sourdoughs. Columns marked with the same letters are not significantly different ($P < 0.05$).

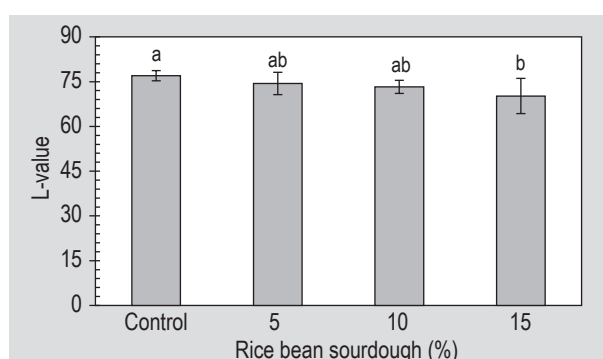


Figure 4. The lightness (L) value changes as a function of rice bran-sourdough concentration. Columns marked with the same letters are not significantly different ($P < 0.05$).

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