

Full Length Research Paper

Evaluation of *Lactobacillus sanfransicencis* (ATCC 14917) and *Lactobacillus plantarum* (ATCC 43332) effects on Iranian Barbari bread shelf life

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The effect of sourdoughs, produced with *Lactobacillus sanfransicencis* (ATCC 14917) and *Lactobacillus plantarum* (ATCC 43332) at different fermentation time, fermentation temperature and type of starter culture on the staling and microbiological shelf life of Iranian Barbari wheat bread was studied. For statistical analysis a completely randomized design with factorial arrangement was used. The results showed that sourdough had significant effect ($p \leq 0.05$) on shelf life of Barbari bread in comparison with control sample. Moreover, the produced sample with *L. plantarum* at 24 h fermentation time and 32°C fermentation temperature had the maximum shelf life.

Key words: Sourdough baking, LAB starter, Barbari shelf life.

INTRODUCTION

A common trend of sourdough fermentations is the unique symbiosis of certain hetero- and homo-fermentative lactic acid bacteria (LAB) with certain yeasts (Gobbetti, 1998). The antagonistic and synergistic interactions between lactobacilli and yeasts had significant effects on sourdough potentials (De Vuyst and Neysens, 2005). Commercial sourdough processes do not rely on fortuitous flora but on the use of commercial starter cultures. Inoculation of sourdough with starters increases the number of lactic acid bacteria, which gives little possibility for growth of contaminating organisms (Stolz, 2003). Sourdough is capable of controlling and inhibiting spoilage organisms during fermentation, due to different factors especially low pH value and antimicrobial compounds produced with LAB (Simsek et al., 2006). Furthermore, LAB metabolites such as organic acids, exopolysaccharides and enzymes have anti-staling activities (Corsetti et al., 1998).

Barbari is a type of Iranian flat wheat bread that is made

into a variety of shapes and sizes, yeast leavened and often flavored with olive oil, which can be brushed on before baking. Some variations are also flavored with assorted spices or seeds. Barbari is often made into small, rounded rectangles, diagonally slashed several times, and resembling flattened buns, but some times made into larger flat rounds up to 3 cm in diameter (ISIRI, 2002).

Influence of sourdough on bread shelf life depends on fermentation conditions and the process utilized (Katina, 2005). Many researchers have studied the effect of sourdough on bread shelf life. For example, Corsetti et al. (1998) studied the effects of sourdough lactic acid bacteria on bread firmness and staling. Dal Bello et al. (2007) evaluated improvement of the quality and shelf life of wheat bread by fermentation with the antifungal strain *Lactobacillus plantarum*. Katina (2005) studied the effects of sourdough as a tool for improving texture and shelf life of wheat bread. Katina et al. (2006) evaluated the effects of sourdough and enzymes on staling of high-fiber wheat bread. Katina et al. (2002) and Mentis et al. (2007) studied the inhibitor activities of lactobacillus strains, isolated from sourdough, against rope-forming *Bacillus* strains. Simsek et al. (2006) isolate lactic starter cultures

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with antimicrobial activity for sourdough processes.

The present study was undertaken to evaluate the effects of sourdoughs at different fermentation conditions for extending Barbari bread shelf life.

MATERIALS AND METHODS

Bacterial strains and media

Two strains of LAB were used in this study. These starters were *Lactobacillus sanfranciscensis* (ATCC 14917) and *L. plantarum* (ATCC 43332) which were supplied from DSMZ company as vacuum dried cultures. Active dry yeast extract containing *Saccharomyces cerevisiae* was prepared from Iran Mellas Company. In order to activate LAB, *L. plantarum* was grown in modified MRS broth medium (Merck) at 36°C for 24 h with the addition of 0.5% (v/v) fresh yeast extract and a pH of 6.2 (De Man et al., 1960). *L. sanfranciscensis* was grown in Sourdough medium (Merck) at 30°C for 48 h with the addition of 2% (v/v) fresh yeast extract and a pH of 5.6 (Wagner, 2005).

Sourdoughs preparation

Biomass from actively growing lactic acid bacteria culture was collected with centrifugation (5000 g, 15 min and 4°C) and re-suspended in sterile tap water that was immediately mixed with wheat flour until dough formation. Fresh cells were added to sourdough at a level of 10^8 cfu/ml. Equivalent 1.5% of flour, 0.25% active dry yeast extract, containing *S. cerevisiae* (w/w) from this cell suspension with the same amounts of wheat flour and tap water were mixed (Katina et al., 2002; Dal Bello et al., 2007). Wheat sourdoughs were prepared with the mentioned strains either as single starters, or in combination. An inoculum of lactic acid bacteria was added to the mixture of water and wheat flour. The mixture was allowed to ferment at three different fermentation temperatures (28, 32 and 36°C) and three different fermentation times (8, 16 and 24 h) without agitation for sourdough preparation.

pH and TTA measurement

Sourdough samples (10 g) were homogenized with 90 ml of sterile distilled water. The pH value was recorded and the acidity was titrated using 0.1 N NaOH to final pH 8.5. The total titratable acidity (TTA) was expressed in ml 0.1 N NaOH (Katina, 2005).

Raw wheat flour characteristics

Wheat flour was prepared from "Acee Ard Flour Mills Factory". The characteristics of the wheat flour used were: extraction rate, 86.5%; moisture, 13%; protein (N x 5.70), 12.5%; fat, 1.72%; ash, 0.75% of dry matter; and falling number, 460 s based on AACC standard methods (AACC, 1983).

Production of experimental breads

An amount of 0.4% NaCl, 0.5% active dry yeast extract, containing *S. cerevisiae* and 25% sourdough samples (w/w) were added to each 100 g flour and mixed at 60 rpm for 20–25 min. The amount of water was adjusted according to the water absorption (60%) determined by farinography (Clarke et al., 2004). The dough was left for bulk fermentation for 30 min at $30 \pm 1^\circ\text{C}$ and 75% relative humidity. At the end of the fermentation time, each piece was rounded before

moulded by hand. The moulded dough pieces were proofed for 1.5 h at $30 \pm 1^\circ\text{C}$ and 85% relative humidity before baking at $220 \pm 5^\circ\text{C}$ for 15–16 min in a heated oven and then cooled in aseptic conditions for 1 h. The twenty seven bread groups were produced and coded. T 28 was control sample. The control for the sourdough bread samples was wheat bread without sourdough (Katina et al., 2006).

Crumb hardness determination

Instrumental texture evaluation of crumb was performed using texture analyzer equipment (QTS Textural Analyzer CNS Farnell). Texture profile analysis (TPA) was carried out to evaluate crumb texture using a cylindrical aluminium probe (35 mm diameter) and a crosshead speed of 60 mm/min to compress a crumb samples to 50% of their original height. Measurements were carried out on two slices (10 ± 2 mm thickness) taken from the centre of the loaf. Maximum peak force was measured and taken as crumb hardness. Crumb hardness was measured at 1, 24, 48 and 72 h interval after baking to assess the potential staling of the breads (Bourne, 1978).

Microbiological shelf life evaluation

Microbiological shelf life of samples was determined by plating serial dilutions on to Plate Count Agar, at days 1 to 7 (Katina et al., 2002; Mentis et al., 2007). Bread specific volume was determined by rape seed displacement method (A-A-20126E METRIC). This parameter was used for correlation between staling and microbiological shelf life of Barbari bread in applied conditions.

Statistical analysis

For statistical analysis a completely randomized design with factorial arrangement and 4 replications was used. To study the relationship between bread hardness and microbiological shelf life with sourdough fermentation conditions, multiple linear regression, and to identify differences of evaluated parameters among bread types, least significant difference test (LSD) at a 95% confidence level ($p \leq 0.05$) were used. Finally regression models for estimation crumb hardness and microbiological shelf life based on applied conditions were exhibited.

RESULTS AND DISCUSSION

Sourdough TTA and pH

Produced sourdoughs fermented with *L. plantarum*, *L. sanfranciscensis* and mixture of these LAB were compared to control sample. By increasing fermentation time and temperature in all of mentioned sourdoughs, TTA were increased and pH values were decreased. The sourdough prepared with *L. plantarum* (24 h fermentation time and 36°C fermentation temperature) had a significantly higher TTA and lower pH than the others. The TTA profile for the sourdoughs was quite similar (starters interestingly continue to produce acid). But the final pH value for the sourdough fermented with *L. sanfranciscensis* (8 h fermentation time and 28°C fermentation temperature) was significantly higher than the others (Figure 1).

Most of the beneficial properties attributed to sourdough

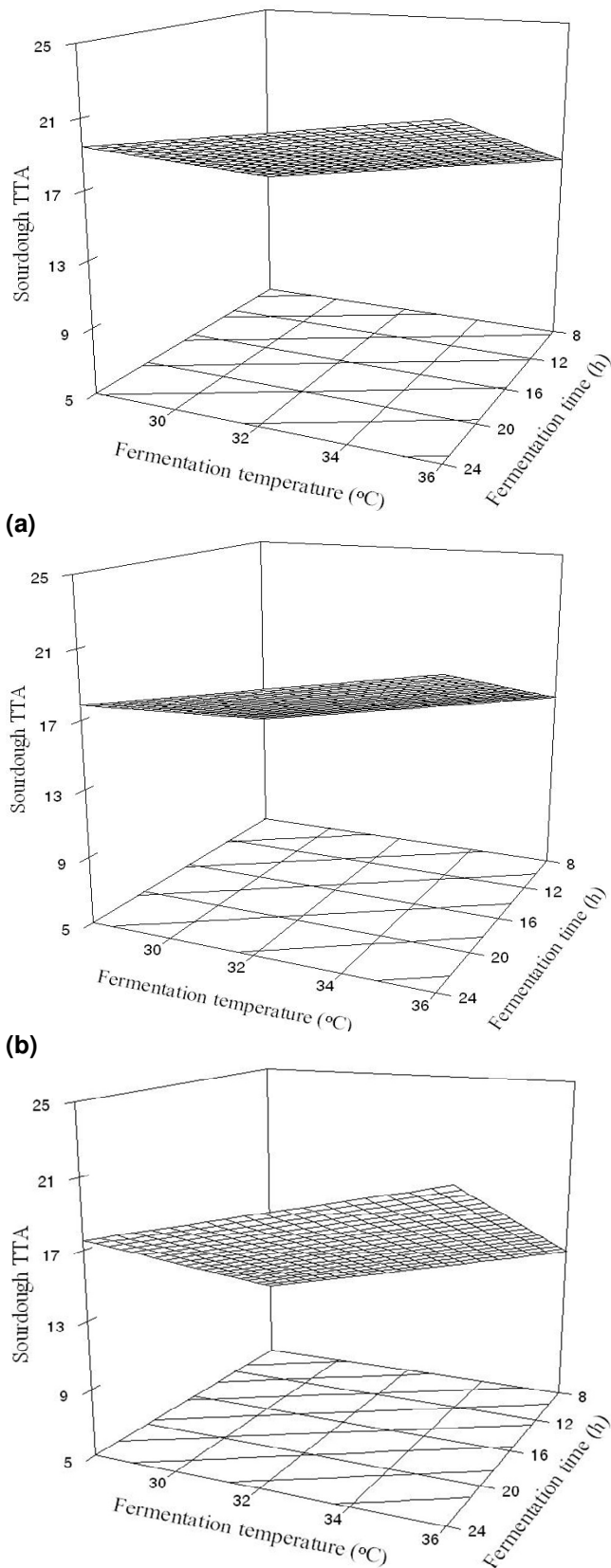


Figure 1. Influence of fermentation time and temperature on sourdough total titratable acidity (TTA), fermented with *L. plantarum* (a), mixture of LAB (b) and *L. sanfranciscensis* (c).

are determined by the acidification activity of *LAB*. The acid production depends on factors such as fermentation temperature, time and dough yield. In general, a higher temperature, a higher water content of sourdough and the utilization of whole meal flour enhances the production of acids in wheat sourdoughs (Gobbetti, 1998; Thiele, 2003; Katina, 2005).

Crumb hardness

The sourdough prepared with *L. plantarum* (24 h fermentation time and 32°C fermentation temperature) had the most effect on improving crumb texture (measured as hardness of crumb). Among all of samples (1 h after baking), the control had the hardest crumb. After storage for 24, 48 and 72 h, the breads baked with sourdoughs were so softer than the control sample. With LAB fermented sourdough Barbari bread, the intensity of crumb hardness highly correlated to the level of sourdough TTA (Figure 2).

The effect of sourdough on softness improvement was partly due to a higher specific volume. Significant correlation coefficients were established between specific volume and softness (Maleki et al., 1980). Also it is reported that volume improvement is the main reason for a better shelf life in sourdough breads. The influence of sourdough on bread volume has been proposed to be mainly due to enzymatic reactions taking place during fermentation (Corsetti et al., 1988; Clarke et al., 2004; Katina et al., 2006).

By increasing fermentation time in all of the sourdoughs, crumb hardness was decreased and by increasing fermentation temperature, except for the produced sample with mixture of LAB, crumb hardness was decreased (Figure 3).

Bread becomes stale largely due to physicochemical changes that occur in the starch-protein matrix of the bread crumb. The acidification of the sourdough and the partial acidification of the bread dough will impact on structure forming components like gluten, starch and arabinoxylans. The swelling of gluten in acid is a well known effect (Corsetti et al., 1998, 2000). Acids strongly influence the mixing behavior of doughs. Doughs with lower pH values require a slightly shorter mixing time and have less stability than normal doughs (Arendt et al., 2007). Fundamental rheological evaluation of acid effect on gluten systems model indicated that both softness and elasticity of gluten were increased (Clarke et al., 2004).

Further to the direct impact of low pH on dough characteristics, secondary effects of acidification and fermentation time, other effects include changes in the activity of cereal or associated bacterial enzymes. Wheat flour proteases have optimal activity around pH 4. In addition, proteolytic enzymes with acidic pH optima in vital wheat gluten have been detected (Thiele et al., 2004).

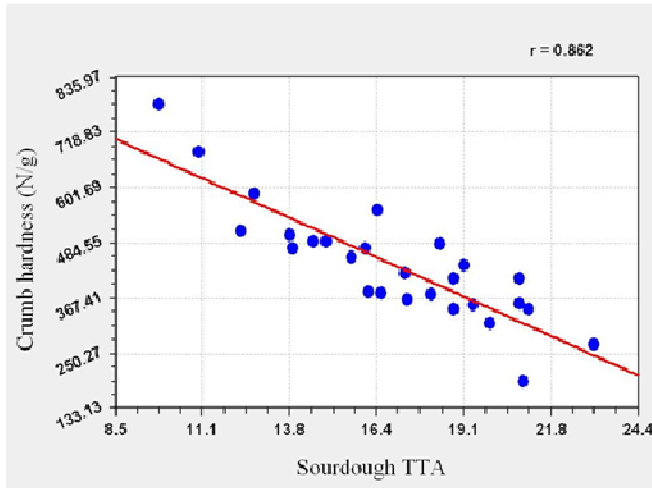


Figure 2. Correlation between sourdoughs TTA with bread hardness.

According to these results, the influence of sourdough on bread softness during storage is depended on fermentation conditions (temperature and time) and starter culture. Also the best treatment for delaying Barbari bread staling was the sourdough prepared with *L. plantarum* (24 h fermentation time and 32°C fermentation temperature).

Correlation between variables (storage time, fermentation time and temperature with crumb hardness) was analyzed by multivariate regression and the best conditions for each starter culture were determined. These results were checked by backward step wise regression but all effective parameters survived. Equations 1 (*L. plantarum*), 2 (mixture of LAB) and (3 *L. sanfransicensis*) for crumb hardness estimation in applied conditions are:

$$(1) \text{ Hardness} = 1051.8 + (2.53 \times \text{storage time}) - (20.8 \times \text{fermentation temperature}) - (2.71 \times \text{fermentation time})$$

$$R^2 = 0.850^*$$

$$(2) \text{ Hardness} = 1014.6 + (2.29 \times \text{storage time}) - (18.3 \times \text{fermentation temperature}) - (1.64 \times \text{fermentation time})$$

$$R^2 = 0.876^{**}$$

$$(3) \text{ Hardness} = 1408.0 + (2.81 \times \text{storage time}) - (25.7 \times \text{fermentation temperature}) - (5.22 \times \text{fermentation time})$$

$$R^2 = 0.827^{**}$$

Microbiological shelf life

The control sample, non-fermented with sourdough, had the lowest microbiological shelf life. The produced sample with *L. plantarum* (24 h fermentation time and 32°C fermentation temperature) had the maximum microbiological shelf life (Figure 4). A selection of starters with antimicrobial potential will intensify the role of sourdough in preventing fungi and rope spoilages, as the combina-

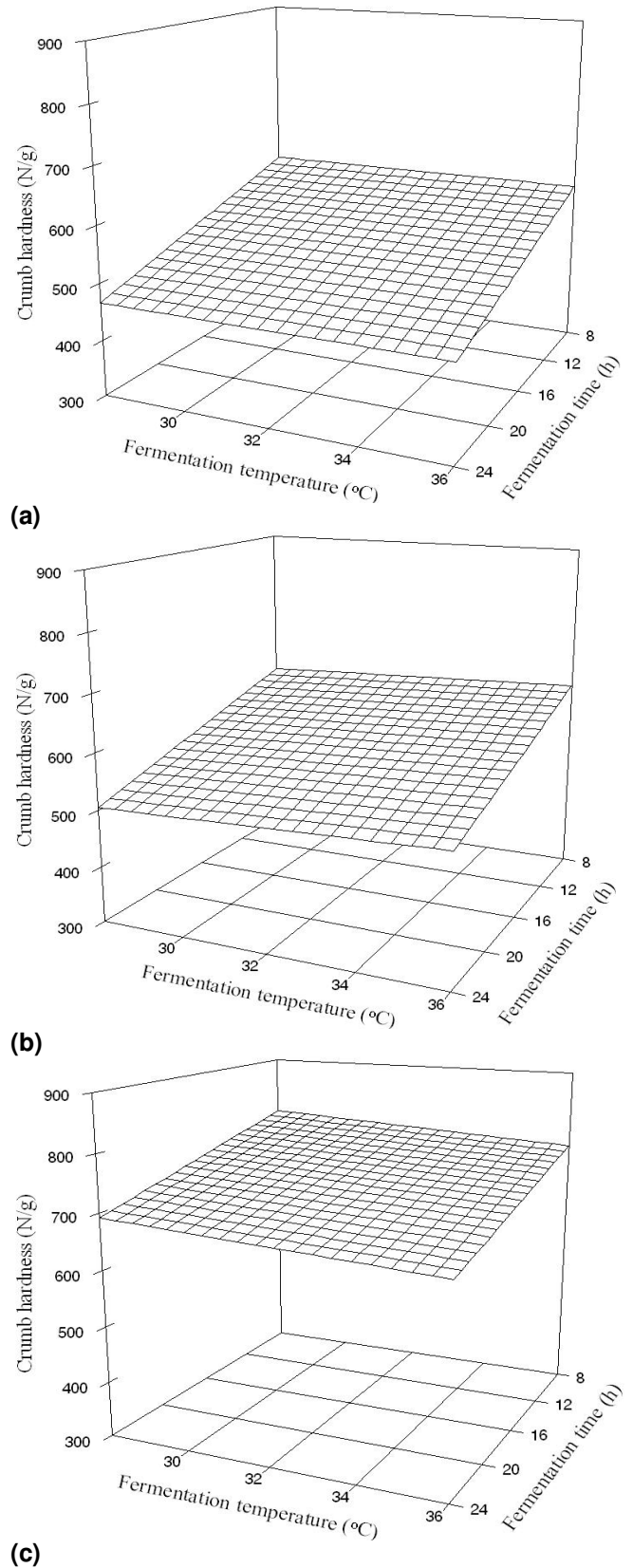


Figure 3. Influence of fermentation time and temperature on hardness of sourdough bread fermented with *L. plantarum* (a), mixture of LAB (b) and *L. sanfransicensis* (c), 72 h after baking.

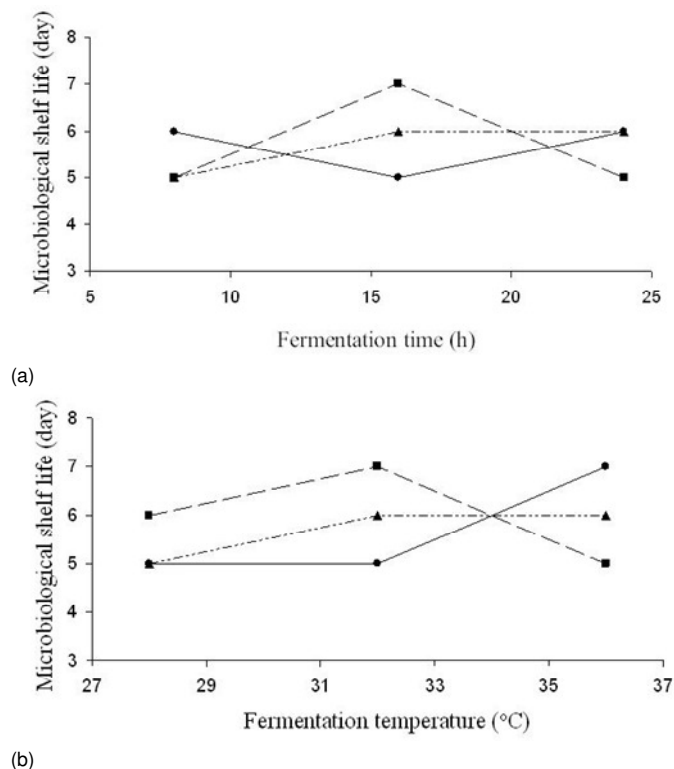


Figure 4. Evaluation of fermentation time (a) and temperature (b) effects on microbiological shelf life of samples (● *L. plantarum*, ■ mixture of LAB and ▲ *L. sanfranciscensis*).

tion of acids and other antimicrobial metabolites is effective also in wheat sourdough bread (Katina et al., 2002; Simsek et al., 2006).

Correlation between variables (bread specific volume, fermentation time and temperature with microbiological shelf life) was analyzed by multivariate regression and the best conditions for each starter culture were determined. These results were checked by backward stepwise regression but all effective parameters survived. Equations 4 (*L. plantarum*), 5 (mixture of LAB) and 6 (*L. sanfranciscensis*) for shelf life (correlation between staling and microbiological shelf life) estimation in applied conditions were:

$$(4) \text{ Specific volume} = (0.0105 \times \text{microbiological shelf life}) + (0.0108 \times \text{fermentation temperature}) + (0.0177 \times \text{fermentation time}) - 0.611$$

$$R^2 = 0.904^{**}$$

$$(5) \text{ Specific volume} = (0.0149 \times \text{microbiological shelf life}) + (0.0985 \times \text{fermentation temperature}) + (0.0145 \times \text{fermentation time}) - 0.663$$

$$R^2 = 0.918^{**}$$

$$(6) \text{ Specific volume} = (0.00309 \times \text{microbiological shelf life}) + (0.0724 \times \text{fermentation temperature}) + (0.0207 \times \text{fermentation time}) - 0.253$$

$$R^2 = 0.948^{***}$$

Conclusion

In this study, significant effect of sourdough process conditions on Barbari bread shelf life was clarified. All sourdough breads showing a softer crumb than control sample on all evaluation times (1-72 h) and many of these samples had higher microbiological shelf life. Based on these results, sourdough processes for delaying Barbari bread staling and improving its microbiological shelf life were designed. Process requirements for optimum quality were strain-specific and different for textural improvement should be taken into account in designing future sourdough baking processes.

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