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Evaluation of Sourdough Effect on Iranian Barbari Bread Staling

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Abstract: The objectives of this research were to apply the sourdough *LAB* containing specific starter cultures for Barbari bread production and delay it's staling. For sourdough preparation, fresh microbial cells were collected by centrifugation from LAB primary cultures. Then equivalent 1.5% of flour (w/w) from this cell suspension with the same amounts of wheat flour and tap water and 0.25% (w/w) active dry yeast extract, containing *Saccharomyces cerevisiae* were mixed. The effects of fermentation time and it's levels (8, 16 and 24 h), fermentation temperature and it's levels (28, 32 and 36°C) and type of starter culture (*Lactobacillus sanfransicencis*, *Lactobacillus plantarum* and a mixture of both LAB) were analyzed in a completely randomized design with factorial experiment and 4 replications was conducted. Bread staling was determined by its crumb hardness and specific volume evaluations in 1, 24, 48 and 72 h after baking. Correlation between variables was obtained by multivariate regression. The results showed that, sourdough had significant effect ($p = 0.05$) on delay staling of Barbari bread in 1, 24, 48 and 72 h after baking in comparison with control sample. Moreover the produced sample with *Lactobacillus plantarum* (24 h fermentation time and 32°C fermentation temperature) had the maximum specific volume and the least staling, 72 h after baking.

Key words: Sourdough · lactic acid bacteria · fermentation · specific volume · staling

INTRODUCTION

Sourdough is a very complex biological system [1, 2] and an important modern fermentation method of cereal flours and water [3, 4]. The use of sourdough process as a form of leavening is one of the oldest biotechnological processes in food production [5]. Sourdough as an ancient way for improves flavour, texture and microbiological shelf life of bread, have been used for thousands of years and are "generally regarded as safe" [6]. Today, sourdough baking is an alternative to the use of additives [7].

Sourdough fermentation is based on lactic acid and alcoholic fermentation depending on the composition of microflora and fermentation conditions (fermentation temperature, time and dough yield) [8, 9]. These factors do not act separately but in an interactive way, adding to the complexity of the system [6, 10].

A common trend of sourdough fermentations is the unique symbiosis of certain hetero and homo-fermentative lactic acid bacteria with certain yeasts [11]. In the other hand, as typical sourdough often consists of both yeast and lactic acid bacteria, while the interaction of yeasts and lactobacilli is important for the metabolic activity of sourdough [12, 13]. *LAB* to yeast ratio in sourdoughs is generally 100:1. Whereas in the majority of fermented foods homofermentative *LAB* play an important role, heterofermentative *LAB* are dominating in sourdough, especially when traditionally prepared. The dominance of obligate heterofermentative lactobacilli in sourdoughs can be explained mainly by their competitiveness in and adaptation to this particular environment [13, 14]. The importance of antagonistic and synergistic interactions between lactobacilli and yeasts are based on the metabolism of carbohydrates and amino acids and the production of carbon dioxide [6, 15].

Sourdoughs have been classified into three types, based on the kind of technology applied for their production, as used in artisan and industrial processes [13]. Most traditional sourdoughs can be classified as type I. Strains of *LAB* are most frequently isolated from sourdoughs with longer fermentation times, or those doughs fermented at elevated temperature (Type II). Type III sourdoughs are special doughs initiated as souring enhancer by defined starter cultures for example

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L. plantarum, L. brevis and *Pediococcus pentosaceus* [2, 16].

Commercial sourdough processes do not rely on fortuitous flora but on the use of commercial starter cultures [6, 17]. Inoculation of the sourdough with starters increases the number of *lactic acid bacteria* to 10^7-10^8 cfu g^{-1}, which gives little possibility for growth of contaminating organisms. The range of commercial starter culture includes (1) pure starter cultures in powder form and (2) starter cultures that are active sourdoughs [18, 19].

Most of the beneficial properties attributed to sourdough are determined by the acidification activity of *LAB*. In wheat fermentation, sourdough *LAB* fermentation creates an optimum pH for the activity of endogenous factors [20, 21] which improve dough properties and texture changes [22, 23], contributes directly to bread aroma and flavour [24], increases phytate breakdown, loaf volume and digestibility [25], delays starch retrogradation, bread firming and staling process [6, 26, 27], protects bread from mould and bacterial spoilage [28] and probably enhances the human tolerance to gluten [29, 30].

Preservation of foods by fermentation is a widely practiced and ancient technology [31]. There has been much interest in the potential application of lactic acid bacteria as a means of biopreservation, that is, control of one organism by another [5]. Sourdough capable to control and inhibition of spoilage organisms during fermentation, due to different factors especially low pH value [32, 33].

Furthermore, recent results demonstrate effectiveness of sourdough fermentation in improving the nutritional values and functional properties of cereal products [25, 34]. Sourdough fermentation can improve texture and palatability of whole grain, fiber-rich or gluten free products, stabilize or increase levels of various bioactive compounds, retard starch and improve mineral bioavailability [35, 36]. Meanwhile exopolysaccharides produced by sourdough *lactic acid bacteria* exert effects such as cholesterol lowering, immunomodulating, antitumoral and prebiotic activities [6, 25].

There has also been much progress in the development of tools that allow for the selection of key sourdough microorganisms for particular activities such as those concerned with enzymatic, antifungal, antimicrobial, nutritional and additive replacement aspects [37, 38].

Bread shelf life is limited by two main factors, including staling and microbial (fungi spoilage and ropiness) attack. Many researchers have been studied the effect of sourdough on bread shelf life. For example, Corsetti *et al.* [26] studied the effects of sourdough lactic acid bacteria on bread firmness and staling. Dal Bello *et al.* [27] evaluated improvement of the quality and shelf life of wheat bread by fermentation with the antifungal strain *Lactobacillus plantarum*. Katina [6] studied the effects of sourdough as a tool for the improving texture and shelf life of wheat bread. Katina *et al.* [4] optimized sourdough process for improved sensory profile and texture of wheat bread. Katina *et al.* [23] evaluated the effects of sourdough and enzymes on staling of high-fibre wheat bread.

The subjects of this study were application of sourdough *LAB* containing specific starter cultures for Barbari bread production and delay it's staling. Barbari is a type of Iranian flat wheat bread that is made into a variety of shapes and sizes. It is often made into small, rounded rectangles, diagonally slashed several times and resembling flattened buns. Barbari is also made into larger flat rounds up to 3 cm in diameter. Barbari is yeast leavened and it is often flavored with olive oil, which can be brushed on before baking. Some variations are also flavored with assorted spices or seeds [39].

To determine the effects of fermentation time and fermentation temperature and type of starter culture on Barbari bread staling, crumb hardness and specific volume of samples were evaluated in 1, 24, 48 and 72 h after baking.

MATERIALS AND METHODS

Raw wheat flour: Wheat flour was prepared from "Acee Ard Flour Mills Factory" (Iran). 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 (d.m.); and falling number, 460 s (based on AOAC and AACC standard methods).

Microorganisms and culture conditions: Two strains of *LAB* were used in this study. These starters were *Lactobacillus sanfransicencis* (ATCC14917) and *Lactobacillus plantarum* (ATCC43332) which supplied from DSMZ company (Germany) as vacuum dried cultures. Active dry yeast extract containing *Saccharomyces cerevisiae* was supplied from Iran Mellas company (Iran).

In order to activation of *LAB*, *Lactobacillus plantarum* was grown in modified MRS (De Man, Rogosa & Sharpe) broth medium (Merck, Germany) at 36°C for 24 h with the addition of 0.5% (v/v) fresh yeast extract and a pH of 6.2 and *Lactobacillus sanfransicencis* was grown

in Sourdough medium (Merck, Germany) at 30°C for 48 h with the addition of 2% (v/v) fresh yeast extract and a pH of 5.6 [4, 40, 41].

Sourdough preparation: Biomass from actively growing lactic acid bacteria culture was collected with centrifugation (5000g, 15 min and 4°C with Spectrafuge16M model) and resuspended 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^{-1} (Mac Farland method). Equivalent 1.5% of flour (w/w) from this cell suspension with the same amounts of wheat flour and tap water and 0.25% (w/w) active dry yeast extract, containing *Saccharomyces cerevisiae* were mixed by a mixture (mac. pan model) [6, 36].

Wheat sourdough was 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 total titratable acidity (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 TTA was expressed in ml 0.1 N NaOH [2, 6, 42].

Production of experimental breads: An amount of 0.4% NaCl and 0.5% (w/w) active dry yeast extract, containing *Saccharomyces cerevisiae* and 25% (w/w) sourdough samples 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. The dough was left for bulk fermentation for 30 min at 30±1°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±1°C and 85% relative humidity before baking at 220±5°C for 15-16 min in a SINMAG-heated oven and then cooled in aseptic conditions for 1 h. The twenty seven bread groups were produced and coded. T28 was control sample. The control for the sourdough bread samples was wheat bread without sourdough [6, 29, 34].

Textural and specific volume measurements: Instrumental texture evaluation of crumb was performed using a 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 days 0 and 3 after baking to assess the potential shelf life of the breads (staling) [26, 27].

Bread specific volume was determined by rape seed displacement method (A-A-20126E METRIC).

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 specific volume with fermentation conditions, multiple linear regression was used and regression models was exhibited.

RESULTS AND DISCUSSION

Sourdough TTA and pH: The TTA profile for the sourdoughs was also quite similar (starters interestingly continue to produce acid) and by increasing of TTA, the pH values were decreased. But the final pH value for the sourdough fermented with *L. sanfranciscensis* (8 h fermentation time and 28°C fermentation temperature) was significantly higher in comparison to that of *L. plantarum* sourdough (24 h fermentation time and 36°C fermentation temperature).

Produced sourdough using fermented *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. 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 (Fig. 1).

Other researchers [2, 4, 6, 12] reported the same results and most of the beneficial properties attributed to sourdough are determined by the acidification activity of LAB [15, 25]. 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 [6].

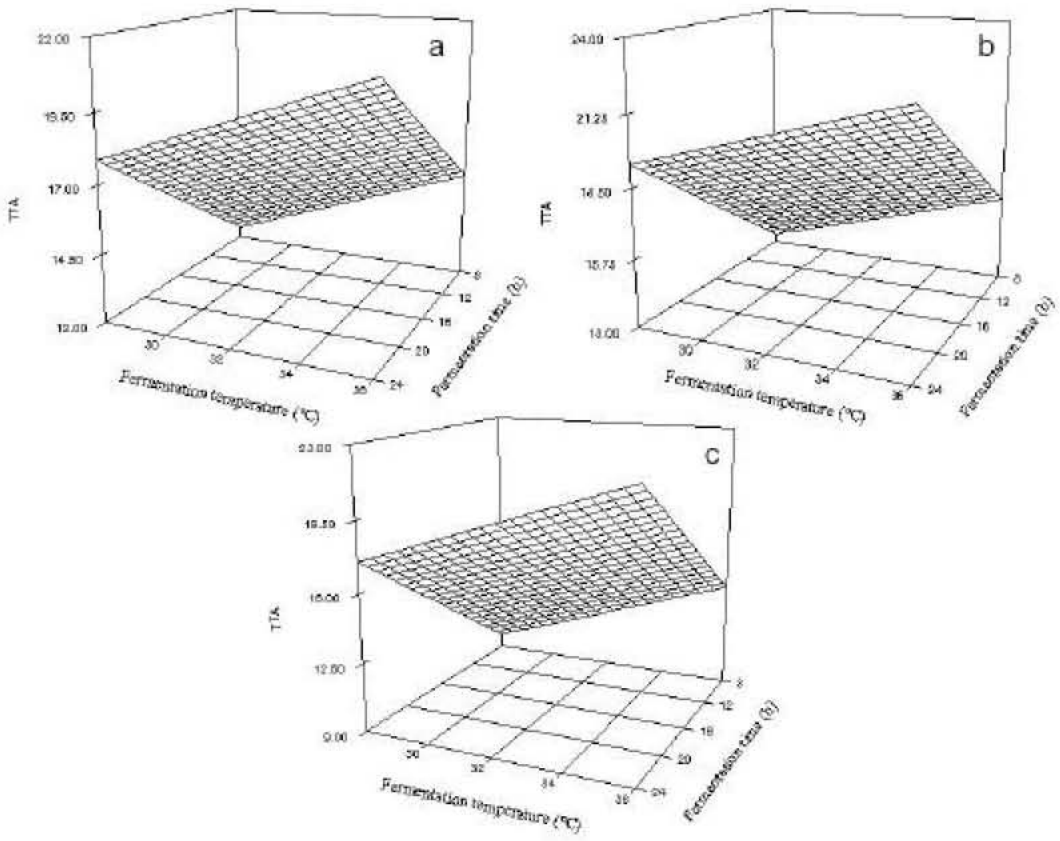

Fig. 1: Influence of fermentation time and temperature on sourdough Total Titratable Acidity (TTA), fermented with *L. plantarum* (a), mixture of *LAB* (b) and *L. sanfransicencis* (c)

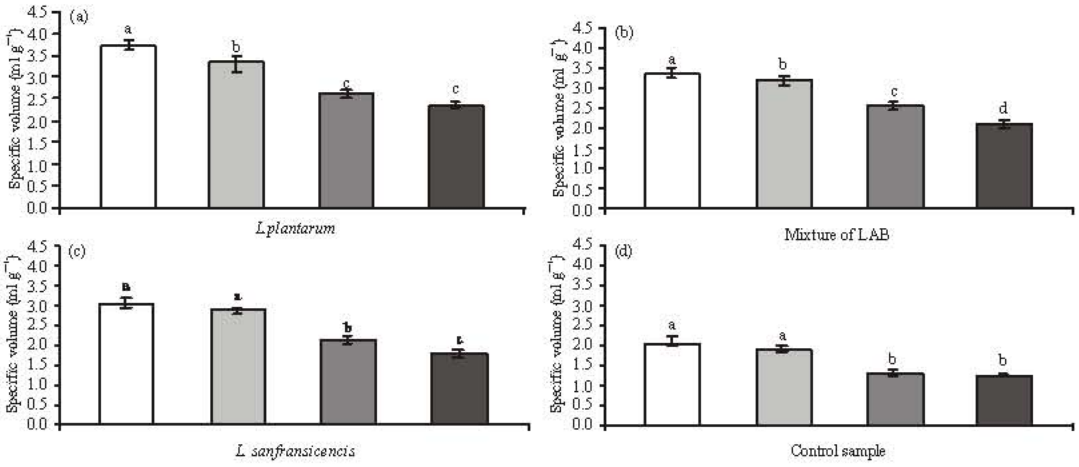

Fig. 2: Comparison between bread specific volume of samples in time intervals 1 (a), 24 (b), 48 (c) and 72 (d) hours after baking

Bread specific volume: The control sample, non-fermented with sourdough had the lowest specific volume and all the other treatments, significantly improved (P=0:05) specific volume in comparison with control sample in time intervals 1 h (a) except for the produced sample with *L. sanfransicencis*, 24 h (b), 48 h (c) and 72 h (d) except for the produced sample with *L. sanfransicencis*, after baking (Fig. 2). According to the results of this work, utilization of sourdough effectively improved specific volume in Barbari bread.

By increasing fermentation time and temperature in all of the mentioned sourdoughs, bread specific volume was increased but fermentation time is more effective than fermentation temperature. The highest bread specific volume was obtained in produced sample with *L. plantarum* (24 h fermentation time and 32°C fermentation temperature).

Correlation between variables (TTA and fermentation time and temperature with specific volume) 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 (1-*L. plantarum*), (2-mixture of *LAB*) and (3-*L. sanfransicencis*) for bread specific volume estimation in applied conditions (TTA and fermentation time and temperature) were exhibited:

$$\text{Specific volume} = -1.33-(0.0716 \times \text{TTA}) + (0.163 \times \text{fermentation temperature}) + (0.0308 \times \text{fermentation time}) \quad R^2 = 0.909^{**} \quad (1)$$

$$\text{Specific volume} = -0.203 + (0.0458 \times \text{TTA}) + (0.0674 \times \text{fermentation temperature}) + (0.00505 \times \text{fermentation time}) \quad R^2 = 0.918^{**} \quad (2)$$

$$\text{Specific volume} = -0.152 + (0.00487 \times \text{TTA}) + (0.0681 \times \text{fermentation temperature}) + (0.0194 \times \text{fermentation time}) \quad R^2 = 0.948^{**} \quad (3)$$

Loaf specific volume is a primary quality characteristic of bread [22]. The application of sourdough has been reported to improve bread volume [2, 4, 6, 23] although a few reports indicated that the bread volume may be decreased by using sourdough [42]; it depends on the acidification rate and on the microbial strains utilized. Specific volume increasing can be due to various factors: (1) heterofermentative lactic acid bacteria have been reported to increase metabolic activity of yeast [17] and thus produce more carbon dioxide for leavening; (2) appropriate acidity might enhance the ability of gluten to retain Co_2 [2]; (3) accumulation of water-soluble pentosans may increase volume as a result of altered water distribution [6].

Crumb hardness: The sourdough prepared with *L. plantarum* (24 h fermentation time and 32°C fermentation temperature) had the most effect in 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 and the bread specific volume highly correlated to the level of sourdough TTA (r = 0.862 and 0.822 respectively, Fig. 3). The effect of sourdough on softness improvment was partly due to a higher specific volume. Significant correlation coefficients were established between specific volume and softness (Fig. 4) and it is reported that volume improvment is the main reason for a better shelf life in sourdough breads [6].

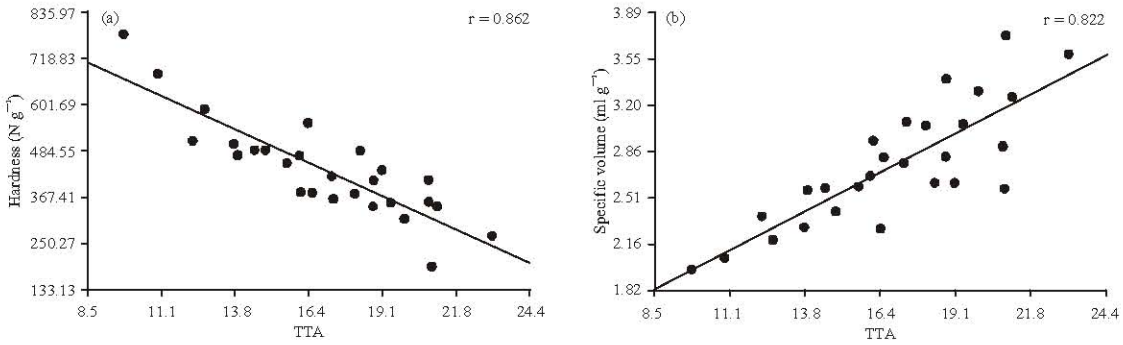

Fig. 3: Correlation between sourdough TTA with bread hardness (a) and specific volume (b)

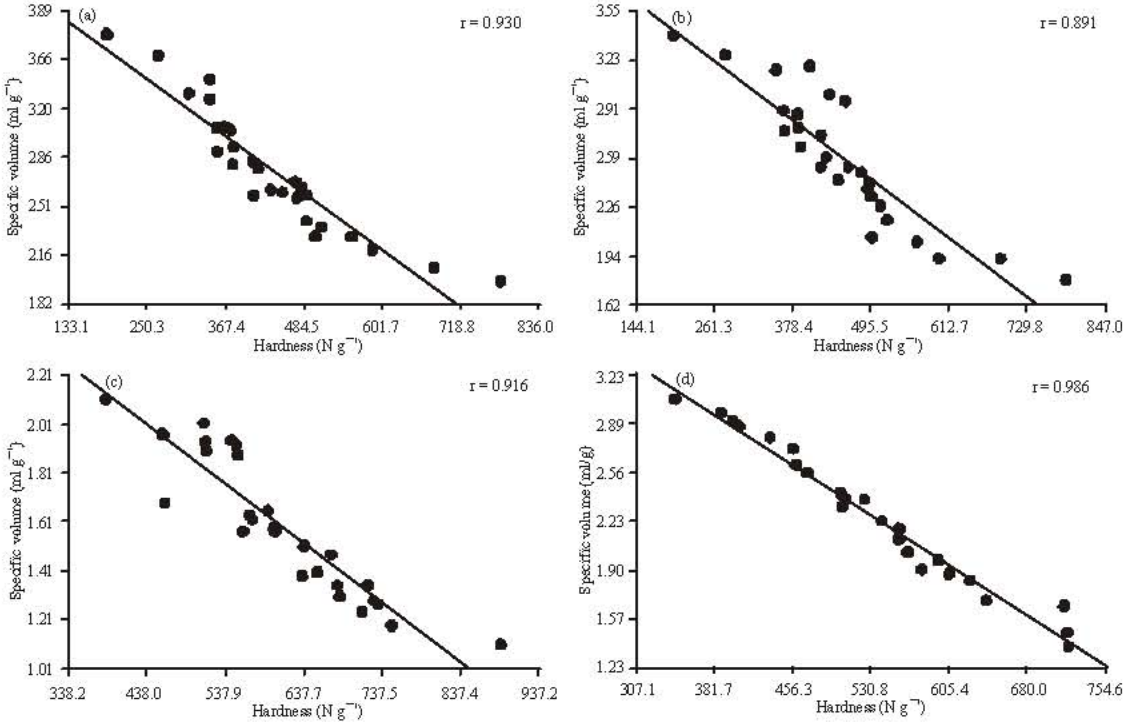

Fig. 4: Correlation between bread specific volume and hardness in time intervals 1 (a), 24 (b), 48 (c) and 72 (d) hours after baking

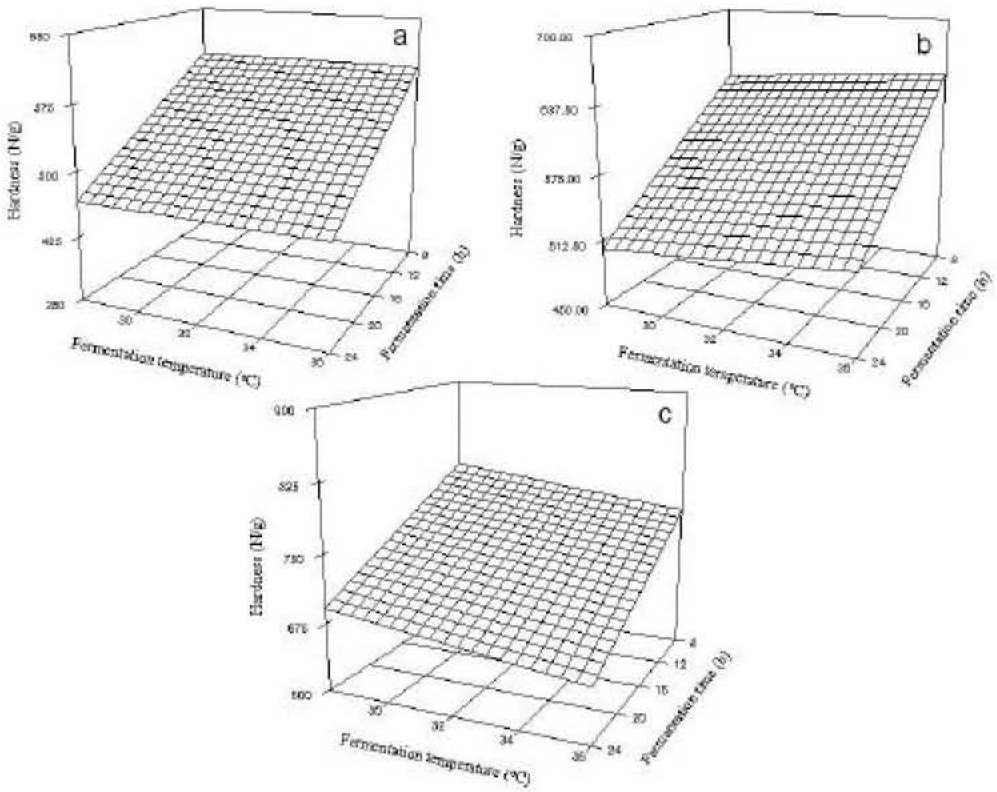

Fig. 5: Influence of fermentation time and temperature on hardness of sourdough bread fermented with *L. plantarum* (a), mixture of *LAB* (b) and *L. sanfransicencis* (c), 72 hours after baking

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 (Fig. 5). The highest bread specific volume was obtained in produced sample with *L. plantarum* (24 h fermentation time and 32°C fermentation temperature).

Correlation between variables (storage time and 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 stepwise regression but all effective parameters, survived. Equations (4-*L. plantarum*), (5-mixture of *LAB*) and (6-*L. sanfransicencis*) for crumb hardness estimation in applied conditions (storage time and fermentation time and temperature) were exhibited:

$$\text{Hardness} = 1051.8 + (2.53 \times \text{storage time}) - (20.8 \times \text{fermentation temperature}) - (2.71 \times \text{fermentation time}) \quad R^2 = 0.850^{**} \quad (4)$$

$$\text{Hardness} = 1014.6 + (2.29 \times \text{storage time}) - (18.3 \times \text{fermentation temperature}) - (1.64 \times \text{fermentation time}) \quad R^2 = 0.876^{**} \quad (5)$$

$$\text{Hardness} = 1408.0 + (2.81 \times \text{storage time}) - (25.7 \times \text{fermentation temperature}) - (5.22 \times \text{fermentation time}) \quad R^2 = 0.827^{**} \quad (6)$$

Bread becomes stale largely due to physicochemical changes that occur in the starch-protein matrix of the bread crumb [4]. 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 [22] and mild acid hydrolysis of starch in sourdough systems has also been hypothesized [34, 43]. 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 [6, 22]. Fundamental rheological evaluation of acid effect on gluten systems model indicated that both softness and elasticity of gluten were increased [11, 41].

Further to the direct impact of low pH on dough characteristics, secondary effects of acidification and fermentation time including changes in the activity of cereal or bacterial enzymes associated. Wheat flour proteases have optimal activity around pH = 4. In addition, proteolytic enzymes with acidic pH optima in vital wheat gluten have been detected [23, 38]. In the same vein, Thiele *et al.* [24] found a greater increase in the concentration of particular amino acids in an acidified relative to a non-acidified dough system during a 50 h fermentation period. These authors concluded that the most important factors governing the levels of amino acids in wheat dough is dough pH, fermentation time and the consumption of amino acids by the fermentative microbiota.

According to these results, the influence of sourdough on bread softness during storage was 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).

CONCLUSION

In this study, significant effect of sourdough process conditions on Barbari bread staling was clarified. Crumb firmness and bread specific volume values showed the opposite trend, with all sourdough breads showing a softer crumb than control sample on all evaluation times (1-72 h). Based on these results, sourdough processes for delaying Barbari bread staling were designed. Process requirements for optimum quality were strain-specific and different for textural improvement which should be taken in to account in designing future sourdough baking processes.

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