Research Article

The Impact of Different Lactic Acid Bacteria Sourdoughs on the Quality Characteristics of Toast Bread

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The effect of sourdough inoculated with three novel single strains of lactic acid bacteria (LAB) (*Lactobacillus casei* jQ412732, *Lactobacillus plantarum* jQ301799, and *Lactobacillus brevis* IBRC-M10790) as well as mixed strains was evaluated on the quality characteristics of Toast bread. Antifungal properties of sourdoughs due to organic acid production were measured by HPLC, and storability was evaluated by thermal and textural analysis in days 1, 3, and 6. Despite the impact of sourdough concentration on microbial preservation, no significant effect was observed in the case of enthalpy reduction. Mixed LAB strains showed the best results in reducing the enthalpy and hardness of bread as well as better microbial preservation by producing the highest amount of organic acids, justified by sensory panelists. Among single strains, *L. casei* gave better results in reducing hardness and staling rate of bread. Scanning Electron Microscopy micrographs of bread also showed the differences.

1. Introduction

The average bread consumption per capita in Iran is about 300 g per day, about five times as high as that of Europe. Therefore, it is the most important nutrition source for Iranian people [1]. Among different kinds of bread, the consumption of Toast bread is increasing steadily in Iran. Hence, the quality characteristics of this bread is considered as an important issue. Toast bread is an English style bread, which is the loafs baked in pans to give an even crust and square shape to the slices [2]. This kind of bread has a soft texture and is usually eaten after slicing and toasting.

The use of sourdough has a long tradition and still plays an important role in bread-making by clearly improving the properties of dough and bread. It is made traditionally by mixing flour, water, and salt, followed by spontaneous fermentation [3]. However, some types of sourdoughs require

the addition of baker's yeast (*Saccharomyces cerevisiae*) as a leavening agent [4]. Using different types of sourdoughs in bread has been widely studied by many researchers [3, 5–14].

Lactic acid bacteria (LAB) have been shown to have a positive effect on different characteristics of bread such as volume, texture, and staling rate [3, 8, 15] as well as increasing microbial shelf life [7] due to producing different metabolites.

Spoilage of bakery products is mainly due to the growth of molds [7] which may cause substantial economic loss in the baking industry as well as causing health problems due to the production of mycotoxins. Using chemical preservatives in bakery products is a common way for increasing their microbial shelf life, whereas the consumers are demanding for safe products with extended shelf life without chemical preservatives [16]. LAB have a long history of being used as biopreservatives. It is proved that using LAB sourdough is the best technique to keep the bread from mold spoilage

due to the action of lactic acid bacteria during fermentation [4, 17], and some of its effects have been attributed to the production of organic acids such as lactic and acetic acid [16, 18]. Furthermore, dough acidifying has been shown to have significant effects on the quality characteristics of bread such as texture and volume [15]. Among various LAB strains used in sourdough, it is proved that *Lactobacillus plantarum* and *Lactobacillus brevis* have beneficial effects in bread properties [3–5, 7, 10, 19]. Although they have showed to be dominating LAB species in the sourdough, Ventimiglia et al. [19] stated that *L. plantarum* is generally codominant with heterofermentative LAB. *Lactobacillus casei* is another strain that not only has dairy origin but also has been identified in sourdough microflora of traditional baking products [4, 20] as well as being used in sourdough media by many researchers [4, 21, 22]. Furthermore, some researchers have shown that this strain has the ability of producing exopolysaccharides [23, 24] and confirmed to have potential as sourdough starter culture [25]. However, little researches have been done on the use of these LAB strains in the sourdough of Toast bread.

The aim of this study was to investigate the effect of three novel LAB stains (*L. casei* jQ412732, *L. plantarum* jQ 301799, and *L. brevis* IBRC-M10790), isolated from Tarhana [26] in sourdough to improve textural and microbial characteristics of Toast bread during 6 days of storage by producing different metabolites as well as evaluating their synergistic or antagonistic effects in use with each other in sourdough and bread. Two former strains have been previously isolated from Tarhana by Settani et al., 2011 [27]. But to the best of our knowledge, these novel LAB strains which we have used in our study have not been used in any food matrix for verifying their metabolic effects.

2. Material and Methods

2.1. Materials. The flour utilized in this study for producing sourdough and bread was a commercial-type wheat flour (moisture, 14.18%; wet gluten: 30.2%; protein, 12.5%, ash, 0.66% (of dry basis); falling number, 364 seconds) obtained from Tehran Bakhtar Co., Tehran, Iran. Salt was obtained from local market. A typical compressed baker's yeast (*Saccharomyces cerevisiae*) was purchased from Iran Mayeh Co., Tabriz, Iran. *L. plantarum* jQ 301799, *L. casei* jQ412732, and *L. brevis* IBRC-M10790 were used as a sourdough starter, obtained from Tak Gen Zist Co. (Tehran, Iran).

2.2. Methods

2.2.1. Preparation of LAB Starters. LAB starters were received as a lyophilized form and then grown in selective media de Man, Rogosa, and Sharpe (MRS) broth (Fluka and catalogue number 69966) and incubated under anaerobic conditions (8% CO₂) at 37°C for 12 hr.

2.2.2. Sourdough Preparation. Sourdoughs were prepared in a spiral mixer (Diosna Co., Germany) by mixing 500 g wheat flour with 310 mL tap water, 10 g salt, 20 g yeast, and 120 mL LAB starter at the final concentration of 10⁷ CFU/g in sourdough for 2 min in slow speed and 6 min fast speed.

Prepared doughs were then put in the bowl, covered with a plastic, and held for 24 hours in the room temperature. Prepared sourdoughs coded as follows: SDT5Y, sourdough inoculated with *L. plantarum* jQ 301799; SDTD4Y, sourdough inoculated with *L. brevis* IBRC-M10790 and yeast; SDL14Y, sourdough inoculated with *L. casei* jQ412732; SD3BY, sourdough inoculated with *L. plantarum* jQ 301799, *L. casei* jQ412732, *L. brevis* IBRC-M10790, and yeast; SDY, control sourdough with yeast and without bacteria. In SD3BY group, LAB starter was equivalent of each culture, respectively.

2.2.3. Bread Preparation. All bread was prepared by the following formulation and procedure: 600 g of wheat flour, 1.08% of salt (w/w, flour basis), 2.4% of fresh yeast (w/w, flour basis), 1.8% of sugar (w/w, flour basis), and 420 mL of tap water were utilized for control bread. Bread samples were prepared using two different sourdough concentrations: 20 g/100 g of the dough (formulation 1) and 30 g/100 g of the dough (formulation 2). All ingredients were mixed and kneaded in a spiral mixer (Diosna, model SP12-SP160, Germany) for 2 min slow speed and 6 min fast speed. The prepared dough was divided into 650 g pieces and put in the pan and then into proofer (38°C, RH = 85%) for 45 min. Baking was carried out in a Miwe rack oven (Germany) in 180°C for 40 min. Bread was cooled after 3 hours in room temperature, sliced, packed in plastic bags, and stored at 25°C for further analysis. Packed bread coded as follows: TT5Y-20, Toast bread containing 20% of SDT5Y; TT5Y-30, Toast bread containing 30% of SDT5Y; TTD4Y-20, Toast bread containing 20% of SDTD4Y; TTD4Y-30, Toast bread containing 30% of SDTD4Y; TL14Y-20, Toast bread containing 20% of SDL14Y; TL14Y-30, Toast bread containing 30% of SDL14Y; T3BY-20, Toast bread containing 20% of SD3BY; T3BY-30, Toast bread containing 30% of SD3BY; TCY-20, Toast bread containing 20% of SDY; TCY-30, Toast bread containing 30% of SDY; TCY-0, Toast bread prepared without sourdough.

2.2.4. pH and TTA Determination. Ten grams of each sample was suspended in 90 mL distilled water and homogenized. pH value was recorded with a pH meter (Metrohm, Switzerland). Total titratable acidity (TTA) was expressed as the amount (mL) of NaOH (0.1 N) required to neutralize 10 g of sample [6]. Measurements were made in triplicate.

2.2.5. Determination of Organic Acids by HPLC. Lactic acid (LA) and acetic acid (AA) standards were obtained from Sigma-Aldrich, Germany. All other chemicals used were of chromatographic and analytical grade, obtained from Merck Millipore Co., Germany. Determination by HPLC was carried out according to the method described by Alfonzo et al. [28]. Briefly, 10 g of samples was homogenized by 90 mL distilled water. Then aliquots of 10 mL were added to 5 mL of 0.1 mM HClO₄ solution, centrifuged at 4000 ×g for 15 min. Then the supernatants were acidified to pH = 3 by the means of 0.1 mM HClO₄ and brought to the final volume of 25 mL with distilled water. After being left in ice for 30 min, the solutions were filtered through a 0.22 μm pore size cellulose acetate filter from Millipore (Madrid, Spain) and injected to the HPLC instrument (Dionex, USA). HPLC analysis was conducted on

ODS column (Shim-pack VP, 5 μm , 150 $\times$ 4.6 mm, Shimadzu, Japan) under the following conditions: isocratic mobile phase of 0.5 mM HClO_4 solution, flow rate of 0.5 mL/min, and column temperature of 35°C with an automated injection system. The injection volume was 100 μL . Detection was performed with a fluorescence detector (RF 2000, Dionex, USA) at 220 nm. Samples were run in duplicate.

2.2.6. Volume and Texture Analysis. Bread volume was determined by rapeseed displacement method based on American Association of Cereal Chemists (AACC), method number 10-05 [29]. For each sample, three measurements were carried out. Crumb hardness was measured using a texture profile analysis (TPA test) in days 1, 3, and 6 to assess the potential effects of the microorganisms used, on the hardness of bread during the shelf life by a Brookfield TPA, model CT3, USA, according to the AACC method number 74-10A [29]. Triplicate measurements for bread from each storage time were made.

2.2.7. Differential Scanning Calorimetry. Calorimetric analysis was used to study the evolution of bread during 6 days of storage by means of a DSC calorimeter (Setaram, 131, France). Bread samples were accurately weighed in aluminum pans (5 mg) and heated from 25 to 200°C at 5°C/min. An empty pan was used as reference. T_0 is initial transition temperature, T_p is peak transition temperature, and ΔH is melting heat of retrograded starch. All analysis was conducted in duplicate.

2.2.8. Sensory Analysis. Sensory analysis of bread was carried out by 20 nontrained panelists according to the AACC method number 74-30 [29] with some modification. Panelists were asked to evaluate each loaf for appearance, color, texture, taste, staling, and overall acceptability in days 1, 3, and 6 after bread production, using a 9-point hedonic scale ranging from dislike extremely (1) to like extremely (9) for each sensorial characteristics. Each panelist was provided a piece of bread for judging appearance and color and a quarter of bread loaf for evaluating taste, texture, and staling in an odorless plastic, in room temperature.

2.2.9. Statistical Analysis. All statistical analyses were carried out using the SPSS software, version 23 (IBM, USA). Values are given as the means $\pm$ standard error (SEM). The significance of differences among means was determined by one-way ANOVA. The level of statistical significance was set at $P < 0.01$.

3. Results and Discussion

3.1. Effect of LAB Sourdough on the Volume of Bread. All bread samples containing LAB starter in the sourdough were significantly higher in volume comparing to the controls with and without sourdough ($P < 0.01$). The ability of gas retention in the dough can be improved in sourdough processing due to the increasing level of LAB activity during fermentation. The reason is that yeast fermentation runs faster in presence of heterofermentative LAB [12]. Bread samples produced without sourdough showed the lowest

FIGURE 1: The effect of starter culture and sourdough concentration on the volume of Toast bread.

volume among all the samples (Figure 1). Many researchers have shown that sourdough fermentation improves bread volume [10, 13, 14]. Gobbetti and Gänzle [30] stated that CO_2 production by baker's yeast is the main factor of increasing bread volume, but, in contrast, Clarke et al. [15] assumed that it may be associated with improving gas retention capacity of gluten network due to reduction of disulfide bonds by acidic condition in presence of sourdough which results in more network flexibility. Tamani et al. [6] stated that LAB cultures behave similarly in improving the volume of bread, but, in contrast, we observed different impacts of our used starters in combination of sourdough concentration. As shown in Figure 1, among the LAB used in this study, *L. casei* jQ412732 resulted in the highest volume of Toast bread significantly ($P < 0.01$) in both 20 and 30% of sourdough concentrations. Several studies showed that dough acidifying has a negative impact on the volume and texture of bread [3, 8, 31], assuming that samples containing LAB have less volume comparing to control. Since the acid content in the sourdoughs was different, better results with samples containing LAB may have additional metabolic reasons [3, 32]. Statistical analysis showed that, in contrast with the results reported by Torrieri et al. [3], in all bread samples except for TTD4Y, no significant differences were observed in the bread samples with different sourdough concentrations.

3.2. Antimold Effect of Lactic Acid and Acetic Acid Produced in LAB Sourdoughs. pH of both sourdough and bread with added LAB cultures decreased significantly comparing to the control (without LAB cultures) ($P < 0.01$). Various LAB starters show different range of pH and TTA. In both sourdough (Table 1) and bread (Table 2), samples containing three LAB had the lowest pH and highest TTA significantly ($P < 0.01$), representing the effect of lactic acid and acetic acid production by them clearly. Control sourdough and bread showed the highest pH and the lowest TTA. Similar data was reported by Palacios et al. [33].

During 6 days of storage, we checked all the samples twice a day for observing any spots of mold on the surface of the bread. Control bread samples without sourdough (TCY-0) were the first samples whose mold spots were observed after 72 h of storage. Compared to bread started with only baker's

TABLE 1: Acidification properties: pH and TTA, lactic acid, and acetic acid content of sourdoughs.

Sample code	pH	TTA (mL) of NaOH	Lactic acid (mg/100 g)*	Acetic acid (mg/100 g)*
SDTD4Y	5.11 ± 0.006 ^c	6.10 ± 0.098 ^c	968.708 ± 0.042 ^b	12.935 ± 0.052 ^b
SDT5Y	5.12 ± 0.006 ^c	5.91 ± 0.011 ^b	1001.038 ± 1.551 ^c	27.060 ± 0.072 ^d
SDL14Y	4.98 ± 0.014 ^b	6.91 ± 0.014 ^d	1347.229 ± 10.678 ^d	18.815 ± 0.102 ^c
SD3BY	4.02 ± 0.043 ^a	10.74 ± 0.009 ^e	2308.044 ± 7.112 ^e	35.865 ± 0.084 ^e
SDY	5.39 ± 0.003 ^d	2.10 ± 0.006 ^a	463.378 ± 1.995 ^a	3.649 ± 0.055 ^a

* Data are the mean of two replication analyses and standard error of the means (±SEM); different letters in each column indicate statistically significant differences; SDTD4Y: sourdough inoculated with *L. brevis* IBRC-M10790 and yeast; SDT5Y: sourdough inoculated with *L. plantarum* jQ 301799; SDL14Y: sourdough inoculated with *L. casei* jQ412732; SD3BY: sourdough inoculated with *L. plantarum* jQ 301799, *L. casei* jQ412732, *L. brevis* IBRC-M10790, and yeast; SDY: control sourdough with yeast and without bacteria.

TABLE 2: Acidification properties: pH and TTA, lactic acid, and acetic acid content of Toast bread.

Sample code	pH	TTA (mL) of NaOH	Lactic acid (mg/100 g dry basis)*	Acetic acid (mg/100 g dry basis)*
TT5Y-20	5.63 ± 0.011 ^g	3.06 ± 0.026 ^c	494.127 ± 0.977 ^f	11.359 ± 0.038 ^g
TT5Y-30	5.50 ± 0.003 ^f	3.22 ± 0.009 ^d	541.597 ± 0.596 ^g	14.094 ± 0.033 ⁱ
TTD4Y-20	5.42 ± 0.003 ^e	3.86 ± 0.014 ^e	306.643 ± 2.306 ^d	3.008 ± 0.031 ^d
TTD4Y-30	5.38 ± 0.009 ^d	4.12 ± 0.063 ^f	464.853 ± 3.280 ^e	3.924 ± 0.055 ^e
TL14Y-20	5.30 ± 0.006 ^c	4.18 ± 0.009 ^f	618.581 ± 1.182 ^h	10.066 ± 0.030 ^f
TL14Y-30	5.13 ± 0.011 ^a	4.47 ± 0.026 ^g	690.368 ± 0.477 ⁱ	11.834 ± 0.113 ^h
T3BY-20	5.27 ± 0.009 ^c	4.85 ± 0.009 ^h	979.143 ± 2.137 ^j	15.172 ± 0.027 ^j
T3BY-30	5.20 ± 0.009 ^b	4.92 ± 0.017 ^h	1179.843 ± 5.434 ^k	16.980 ± 0.025 ^k
TCY-20	5.67 ± 0.009 ^h	2.69 ± 0.009 ^b	221.303 ± 1.345 ^c	1.435 ± 0.021 ^b
TCY-30	5.63 ± 0.017 ^g	2.77 ± 0.009 ^b	245.590 ± 2.344 ^b	2.252 ± 0.010 ^c
TCY-0	5.91 ± 0.009 ⁱ	2.36 ± 0.089 ^a	110.053 ± 1.653 ^a	0.973 ± 0.024 ^a

* Data are the mean of two replication analyses and standard error of the means (±SEM); different letters in each column indicate statistically significant differences; TT5Y-20, Toast bread containing 20% of SDT5Y; TT5Y-30, Toast bread containing 30% of SDT5Y; TTD4Y-20, Toast bread containing 20% of SDTD4Y; TTD4Y-30, Toast bread containing 30% of SDTD4Y; TL14Y-20, Toast bread containing 20% of SDL14Y; TL14Y-30, Toast bread containing 30% of SDL14Y; T3BY-20, Toast bread containing 20% of SD3BY; T3BY-30, Toast bread containing 30% of SD3BY; TCY-20, Toast bread containing 20% of SDY; TCY-30, Toast bread containing 30% of SDY; TCY-0, Toast bread prepared without sourdough.

yeast alone, the sourdough bread delayed fungal contamination until after 96 h of storage at room temperature. As expected, all the samples with 30% sourdough showed the higher content of lactic and acetic acid comparing to the same formulation with 20% sourdough. In accordance with Najafi et al. [34], higher replacement of sourdough in the current study with or without LAB resulted in the higher increase in the antimold activities of the bread. Because complicated interactions take place among different compounds produced during cell growth and may be their synergistic effect, exact mechanism of antimicrobials cannot be defined [35].

Both samples containing three LAB whether with 20 or 30% sourdough did not show any traces of mold during 6 days of storage. The obligatory heterofermentative LAB may produce different metabolites depending on their capacity to break down amino acids and to use various routes for pyruvate, external electron acceptors, and the oxygen supply [36]. Among metabolites, lactic and acetic acids, the main organic acids produced by LAB, are regarded as antifungal compounds in many scientific researches [5, 35, 37]. Statistical analysis of our study showed that the amounts of lactic and acetic acid were directly correlated with the TTA value of the bread ($r = 0.929$ and 0.922 , resp.; $P < 0.01$)

and adversely correlated with the pH level ($r = -0.903$ and -0.886 , resp.; $P < 0.01$) of the sourdough and bread. In line with our results, Gerez et al. [35] reported that the antimold effect of different LAB starters would be related to both the nature of organic acids produced and the low pH reached after fermentation, while Axel et al. [5] stated that acidification by lactic and acetic acids which results in pH drop may have a low preservative effect. As shown in Table 1, among three different LAB used in our study, *L. plantarum* jQ 301799 produced the highest amount of acetic acid in sourdough, but the highest content of lactic acid was produced by *L. casei* jQ412732. Comparing bread produced by such sourdoughs, samples containing 30% sourdough of each showed the longest antimold shelf life (delaying the growth of mold till 132 h).

Corsetti et al. [37] showed that, among compounds identified, acetic acid was one of the main organic acids responsible for preventing mold spoilage, and lactic acid did not show a significant effect even in high concentrations. Le Lay et al. [18] confirmed the result due to the fact that nonantifungal LAB strains are also capable of producing high amounts of lactic acid. The effect of acetic acid is attributed to its ability to denature protein, neutralize electrochemical

potential of plasmic membrane, and increase its permeability which leads to bacteriostasis and death of the organism [38]. But in accordance with Batish et al. [38] which demonstrated the synergistic effect of lactic and acetic acids and their ability to extend the lag phase of sensitive organisms, our statistical results showed also a positive correlation between these two acids produced by all the strains used in this study. Some other researchers also confirmed the synergetic effect of organic acids together and with other bioactive compounds in molds growth inhibition [5, 17, 18, 37]. Lactic acid and acetic acid content of Toast bread are presented in Table 2.

3.3. Effect of LAB Sourdoughs on Thermal Properties of Toast Bread. Fresh and stored bread crumb samples were analyzed for amylopectin retrogradation enthalpies using DSC. Mean values of initial transition temperature (T_0), peak transition temperature (T_p), and melting heat of retrograded starch (ΔH) measuring in days 1, 3, and 6 are summarized in Table 5. A major endothermic transition was observed. ΔH gives an overall measure of crystallinity [39]. TCY-0 and TCY-20 showed the highest enthalpy among all the samples in the first day of storage significantly ($P < 0.01$). The decrease of ΔH in TCY-30 as well as samples containing LAB sourdough may be attributed to partial hydrolysis of starch due to the production of organic acids by LAB [40]. Organic acids due to pH changes affect the protein and starch fractions by increasing protease and amylase activities due to reducing pH [9, 15]. Optimum activity for proteolytic enzymes can be obtained in the pH of 4-5, and for the amylolytic enzymes the optimum pH is 3.6-6.2 [41]. Enzyme activities enhance phase separation of amylose and amylopectin [42], which may have resulted in structural changes in the dough and finally bread firmness and staling [10, 15, 43]. In contrast with Torrieri et al. [3], sourdough concentration did not show any significant effect on the change of enthalpy ($P > 0.05$) except for the control samples, while strain type had a significant effect on the change of enthalpy ($P < 0.01$). Enthalpy differences indicated a lower crystallinity in samples containing *L. casei* jQ412732 as well as samples containing the combination of three LAB strains both at 20 and at 30% sourdough concentration on the first day of storage. Sourdough bread inoculated with *L. plantarum* jQ 301799 and *L. brevis* IBRC-M10790 showed a significant higher enthalpy (Table 3) and an approximately similar pattern. In line with ΔH , T_p was also increased in those samples. High degree of crystallinity, which provides structural stability (double helix length), results in high transition temperatures [39].

Storage time had a significant effect on ΔH as well as T_p ($P < 0.01$). However, no significant changes were observed in T_0 . Similar results have been reported by Torrieri et al. [3] and Barber et al. [40]. For all samples ΔH increased significantly during 6 days of storage. The maximum enthalpy change was observed in TCY-0, which can be concomitant with the maximum rate of starch gelatinization. All LAB sourdoughs delayed the retrogradation processing of the Toast bread. Acidification due to the sourdough fermentation has been claimed to affect moisture redistribution during storage [10]. A good correlation between the rate of starch retrogradation and firmness of bread crumb was observed in all the samples

containing LAB sourdough. Such a correlation was also demonstrated by other researchers [6, 40, 42]. Tamani et al. [6] suggested that higher levels of exopolysaccharides produced by LAB may have resulted in a greater water absorption, leading to the softer crumb structure of the bread.

3.4. Effect of LAB Sourdoughs on the Texture of Bread.

Hardness of bread was measured in days 1, 3, and 6 after production. Mean values for crumb hardness are shown in Table 4. As predicted, in all the samples hardness 1 was higher than hardness 2. But no significance changes were observed in the rate of hardness 2 to hardness 1. TCY-0 showed the highest level of crumb hardness in the first day after production. Using 30% sourdough gave less hardness in all the samples significantly, except for the control ($P < 0.01$). Among all samples, the lowest hardness was observed in T3BY-30. This influence has been assumed to be due to the acidity-induced activation of proteolytic enzymes present in wheat flour, which solubilizes gluten and decreases the hardness [15]. Proteolysis liberates water from the gluten network, allowing the activity of amylase and other enzymes presented in the dough to be increased [44]. Furthermore, lactic acid mainly produced by heterofermentative LAB may be responsible for more elastic gluten structure [45]. Results of this study affirms the hypothesis that, by presence of other metabolites produced by LAB, the deteriorative impact of acidity on the texture of the bread may be covered [3, 32]. Among three single strains used in the sourdoughs, *L. casei* jQ412732 showed the best effect in reducing hardness. Considering the results of acidity, this supports the hypothesis that biological acidification is attributed to the structural changes of the dough [15]. Thiele et al. [9] also believed that pH is one of the most important determinative factors in this regard, as well as the consumption of amino acids by fermentative microflora.

Hardness of all bread samples increased from day 1 to day 6 significantly ($P < 0.01$), but results showed that, in accordance with Torrieri et al. [3], bread containing sourdoughs inoculated with LAB starters was less rigid during 6 days of storage comparing to the control samples. Also, in agreement with Najafi et al. [34], results showed that crumb hardness of the samples was dependent to the strains used in the sourdough starter as well as sourdough concentration. Using a single strain of LAB gave different results from a combination of strains. Among all Toast bread, T3BY-30 showed the lowest hardness in the first day and also after 6 days of storage. The increasing process of crumb hardening which is an indication of staling was slower for such samples (Figure 2). Different metabolite patterns produced by three LAB complicated the direct comparison of the sourdough and bread characteristics with the single strain. However, the gas produced by baker's yeast could be retained better, giving higher volume, porous, and softer texture during the shelf life time [3, 32].

3.5. Effect of LAB Sourdoughs on the Organoleptic Properties.

Average values for sensory evaluation of different bread samples are shown in Table 5. In line with other studies, appearance and color of the samples containing LAB starters were significantly more preferred by the panelists [3, 32].

TABLE 3: Thermal properties of bread during storage: melting heat of retrograded starch (ΔH), peak transition temperature (T_p), and initial transition temperature (T_0).

Sample code	ΔH (J/g)			T_p (°C)			T_0 (°C)		
	Day 1	Day 3	Day 6	Day 1	Day 3	Day 6	Day 1	Day 3	Day 6
TT5Y-20	189.960 ± 8.034 ^b	274.691 ± 3.495 ^c	360.945 ± 11.489 ^b	74.638 ± 0.633 ^b	79.047 ± 0.275 ^b	81.529 ± 2.648 ^c	50.734 ± 0.118 ^b	52.651 ± 0.037 ^a	54.627 ± 0.638 ^a
TT5Y-30	180.371 ± 7.189 ^b	241.018 ± 6.296 ^b	342.854 ± 9.300 ^b	75.129 ± 3.195 ^b	78.542 ± 0.160 ^b	81.850 ± 0.226 ^c	50.428 ± 0.275 ^{ab}	51.642 ± 0.371 ^a	55.528 ± 0.039 ^a
TTD4Y-20	188.645 ± 7.537 ^b	269.734 ± 4.175 ^c	349.523 ± 5.829 ^b	75.537 ± 0.285 ^b	79.508 ± 2.175 ^b	81.096 ± 0.483 ^c	50.167 ± 0.158 ^a	53.758 ± 0.539 ^a	54.245 ± 0.044 ^a
TTD4Y-30	182.645 ± 9.537 ^b	238.705 ± 4.869 ^b	346.729 ± 9.582 ^b	75.923 ± 2.684 ^b	79.520 ± 4.295 ^b	81.042 ± 2.672 ^c	50.274 ± 0.150 ^a	53.759 ± 0.283 ^a	55.586 ± 0.013 ^a
TL14Y-20	127.980 ± 6.792 ^a	183.683 ± 3.440 ^d	265.109 ± 5.330 ^a	70.885 ± 0.205 ^a	73.015 ± 0.153 ^a	79.475 ± 2.104 ^b	50.230 ± 0.063 ^a	51.930 ± 0.104 ^a	56.005 ± 0.141 ^a
TL14Y-30	114.586 ± 6.552 ^a	159.898 ± 9.200 ^a	248.969 ± 5.863 ^a	70.135 ± 0.291 ^a	71.530 ± 0.514 ^a	81.395 ± 1.159 ^c	50.170 ± 0.040 ^a	51.580 ± 0.150 ^a	55.490 ± 0.159 ^a
T3BY-20	122.603 ± 4.421 ^a	175.478 ± 3.374 ^a	245.189 ± 3.143 ^a	71.165 ± 0.309 ^a	79.525 ± 0.522 ^b	80.037 ± 0.073 ^b	50.605 ± 0.037 ^{ab}	51.600 ± 0.104 ^a	56.025 ± 0.107 ^a
T3BY-30	122.793 ± 4.018 ^a	170.645 ± 6.524 ^a	268.940 ± 5.881 ^a	70.720 ± 0.110 ^a	71.680 ± 0.242 ^a	75.325 ± 0.234 ^a	50.505 ± 0.095 ^{ab}	51.560 ± 0.162 ^a	56.005 ± 0.066 ^a
TCY-20	206.381 ± 13.699 ^c	466.506 ± 13.266 ^d	520.281 ± 5.046 ^c	76.720 ± 0.387 ^b	93.965 ± 1.308 ^d	95.870 ± 0.023 ^d	50.555 ± 0.794 ^{ab}	57.590 ± 2.188 ^a	62.660 ± 0.185 ^b
TCY-30	181.734 ± 10.270 ^b	290.183 ± 6.505 ^c	358.675 ± 13.052 ^b	76.705 ± 0.707 ^b	80.675 ± 0.395 ^b	81.950 ± 0.063 ^c	50.800 ± 0.023 ^b	51.035 ± 0.032 ^a	55.605 ± 0.188 ^a
TCY-0	205.634 ± 8.532 ^c	489.649 ± 5.592 ^c	591.294 ± 10.649 ^d	78.643 ± 0.475 ^c	86.639 ± 0.269 ^c	96.638 ± 0.114 ^d	50.756 ± 0.175 ^b	51.649 ± 0.047 ^a	54.726 ± 0.027 ^a

*Data are the mean of three replication analyses and standard error of the means (±SEM); different letters in each column indicate statistically significant differences; TT5Y-20, Toast bread containing 20% of SDT5Y; TT5Y-30, Toast bread containing 30% of SDT5Y; TTD4Y-20, Toast bread containing 20% of SDTD4Y; TTD4Y-30, Toast bread containing 30% of SDTD4Y; TL14Y-20, Toast bread containing 20% of SDL14Y; TL14Y-30, Toast bread containing 30% of SDL14Y; T3BY-20, Toast bread containing 20% of SD3BY; T3BY-30, Toast bread containing 30% of SD3BY; TCY-20, Toast bread containing 20% of SDY; TCY-30, Toast bread containing 30% of SDY; TCY-0, Toast bread prepared without sourdough.

TABLE 4: Influence of LAB and sourdough on the hardness of bread samples*.

Sample code	Hardness 1 (N)	Hardness 2 (N)
TT5Y-20	28.422 ± 0.079 ^e	24.927 ± 0.096 ^d
TT5Y-30	26.342 ± 0.079 ^c	23.648 ± 0.096 ^c
TTD4Y-20	27.425 ± 0.079 ^d	25.891 ± 0.096 ^e
TTD4Y-30	26.593 ± 0.079 ^c	24.328 ± 0.096 ^d
TL14Y-20	28.672 ± 0.079 ^e	25.197 ± 0.096 ^e
TL14Y-30	21.732 ± 0.079 ^b	18.289 ± 0.096 ^b
T3BY-20	26.806 ± 0.079 ^c	24.532 ± 0.096 ^d
T3BY-30	17.062 ± 0.079 ^a	15.131 ± 0.096 ^a
TCY-20	36.149 ± 0.079 ^f	32.617 ± 0.096 ^f
TCY-30	39.579 ± 0.079 ^g	35.188 ± 0.096 ^g
TCY-0	46.735 ± 0.079 ^h	42.714 ± 0.096 ^h

*Data are the mean of three replication analyses and standard error of the means ($\pm$ SEM); different letters in each column indicate statistically significant differences; TT5Y-20, Toast bread containing 20% of SDT5Y; TT5Y-30, Toast bread containing 30% of SDT5Y; TTD4Y-20, Toast bread containing 20% of SDTD4Y; TTD4Y-30, Toast bread containing 30% of SDTD4Y; TL14Y-20, Toast bread containing 20% of SDL14Y; TL14Y-30, Toast bread containing 30% of SDL14Y; T3BY-20, Toast bread containing 20% of SD3BY; T3BY-30, Toast bread containing 30% of SD3BY; TCY-20, Toast bread containing 20% of SDY; TCY-30, Toast bread containing 30% of SDY; TCY-0, Toast bread prepared without sourdough.

FIGURE 2: The increasing process of hardness during 6 days of bread's shelf life. Differences between the hardness processing during 6 days of storage are represented as white bars.

Torrieri et al. [3] reported that the presence of EPS has also an effect on the color of the bread crust in terms of chromatic coordinates. However, Differences between samples in two latter attributes can be explained by the effect of baking process. Neither single strain type, nor sourdough concentration showed the significant effect on different attributes of the Toast bread ($P > 0.05$). In line with the results achieved by instrumental analysis, T3BY-30 was given the best score for texture and staling. Also, panelists preferred the taste of this sample better than the others. As discussed earlier in this article, samples inoculated with three LAB

had lower pH and higher TTA, in result of producing lactic and acetic acid by the bacteria. These two organic acids are responsible for creating desirable taste and odor in sourdough [46]. Also, Thiele et al. [9] showed that dough acidifying is a key factor for inducing proteolysis, and the latter has a main role in creating different flavor and odor in sourdough. Wang et al. [47] stated that engender of flavor and volatiles in sourdough is influenced by the activity of LAB, and each of them results in different flavor. In our study, T3BY-30 was the most acceptable sample in terms of overall acceptability.

3.6. Scanning Electron Microscopy (SEM). Figure 3 shows the scanning electron micrograph of control bread samples with two magnifications of $\times 150$ and $\times 550$. As expected and was shown in previous studies, starch granules were presented in the form of small and large spherical and ovoid granules [48]. Higher amounts of sourdough resulted in the larger holes and open structure (Figures 3(c) and 3(d)). This explains with the effect of higher acidity in such samples. In accordance with Clarke et al. (2000), the incorporation of either single or mixed strain of LAB led to significant changes in bread quality. It is hypothesized that biologically acidification of the system and pH variation may have indirectly affected the nature and degree of enzyme activity, leading to structural changes of the dough and bread [9, 15]. Li et al. [48] reported that the acid produced by *L. plantarum* may be helpful in developing gluten structure. But, in spite of having higher acidity, it seems that metabolites produced by LAB have covered some parts of surface structure (Figure 4). Observation of microstructure of bread by SEM confirmed the results of texture analysis. The microstructure of samples containing three LAB showed a weakened gluten network and more open structure, compared to the other samples.

4. Conclusion

This study demonstrated the positive effects of different LAB sourdoughs on quality characteristics of Toast bread comparing to the control. Considering the results, it can be concluded that synergetic effect of three LAB strains used in the sourdough had a significant role in improvement of volume, texture, staling rate, and microbial shelf life during 6 days of storage as well as sensory characteristics of bread. However, more researches are needed to investigate the type of metabolites involved, and the effect of each LAB on the quality characteristics of sourdoughs and different bread.

Additional Points

Use of sourdough in bread processing plays an important role in the quality characteristics of the bread. The current research investigates the potential use of sourdough in two concentrations (20 and 30%) inoculated with three novel single lactic acid bacteria (LAB) strains as well as mixed strains in attempt to improve the textural, sensorial, and shelf life of Toast bread. Bread inoculated with three LAB strains showed the best results in improving textural, thermal, and microbial properties comparing to the samples without sourdough or LAB due to metabolic reasons, indicating the

TABLE 5: Influence of LAB and sourdough on sensory attributes (mean value) of bread samples*.

Sample code	appearance	Color	Texture	Taste	Staling	Overall acceptability
TT5Y-20	7.40 ± 0.135 ^c	7.73 ± 0.116 ^b	5.62 ± 0.249 ^{b,c}	6.27 ± 0.207 ^{c,d}	5.52 ± 0.244 ^e	5.88 ± 0.205 ^{b,c,d}
TT5Y-30	7.48 ± 0.127 ^c	7.92 ± 0.112 ^{b,c}	5.78 ± 0.290 ^{b,c}	6.32 ± 0.225 ^{c,d}	5.40 ± 0.310 ^{d,e}	6.38 ± 0.199 ^{d,e}
TTD4Y-20	7.67 ± 0.108 ^c	8.27 ± 0.089 ^c	5.50 ± 0.260 ^b	6.42 ± 0.183 ^d	5.10 ± 0.251 ^{c,d,e}	5.63 ± 0.233 ^{a,b,c}
TTD4Y-30	7.20 ± 0.132 ^c	8.03 ± 0.106 ^{b,c}	5.65 ± 0.264 ^{b,c}	6.12 ± 0.217 ^{c,d}	5.40 ± 0.251 ^{d,e}	5.87 ± 0.236 ^{b,c,d}
TL14Y-20	7.58 ± 0.126 ^c	8.02 ± 0.102 ^{b,c}	5.07 ± 0.164 ^{a,b}	5.32 ± 0.149 ^a	4.75 ± 0.148 ^{b,c,d}	5.23 ± 0.127 ^a
TL14Y-30	7.47 ± 0.131 ^c	8.05 ± 0.090 ^{b,c}	5.82 ± 0.249 ^{b,c}	6.50 ± 0.177 ^d	5.48 ± 0.254 ^{d,e}	6.10 ± 0.208 ^{c,d}
T3BY-20	7.60 ± 0.141 ^c	7.97 ± 0.116 ^{b,c}	4.72 ± 0.231 ^a	5.48 ± 0.166 ^{a,b}	4.35 ± 0.216 ^b	5.12 ± 0.161 ^a
T3BY-30	7.60 ± 0.124 ^c	8.27 ± 0.098 ^c	6.83 ± 0.153 ^d	7.22 ± 0.160 ^e	6.42 ± 0.156 ^f	6.93 ± 0.157 ^e
TCY-20	6.15 ± 0.199 ^a	7.13 ± 0.212 ^a	5.22 ± 0.249 ^{a,b}	5.72 ± 0.281 ^{a,b,c}	4.55 ± 0.277 ^{b,c}	5.47 ± 0.220 ^{a,b}
TCY-30	6.92 ± 0.214 ^b	7.25 ± 0.200 ^a	5.67 ± 0.219 ^{b,c}	5.95 ± 0.258 ^{b,c,d}	5.20 ± 0.263 ^{c,d,e}	5.87 ± 0.235 ^{b,c,d}
TCY-0	6.10 ± 0.132 ^a	7.15 ± 0.196 ^a	4.89 ± 0.196 ^a	5.42 ± 0.178 ^a	4.01 ± 0.218 ^a	4.98 ± 0.221 ^a

*Data are shown as the means ± standard error (±SEM); different letters indicate statistically significant differences; TT5Y-20, Toast bread containing 20% of SDT5Y; TT5Y-30, Toast bread containing 30% of SDT5Y; TTD4Y-20, Toast bread containing 20% of SDTD4Y; TTD4Y-30, Toast bread containing 30% of SDTD4Y; TL14Y-20, Toast bread containing 20% of SDL14Y; TL14Y-30, Toast bread containing 30% of SDL14Y; T3BY-20, Toast bread containing 20% of SD3BY; T3BY-30, Toast bread containing 30% of SD3BY; TCY-20, Toast bread containing 20% of SDY; TCY-30, Toast bread containing 30% of SDY; TCY-0, Toast bread prepared without sourdough.

FIGURE 3: SEM micrographs of control Toast bread crumb (right side ×550, left side ×150); (a) and (b) TCY-20; (c) and (d) TCY-30.

FIGURE 4: SEM micrographs of LAB sourdough Toast bread crumb (right side $\times 550$, left side $\times 150$); (a) and (b) T3BY-20; (c) and (d) T3BY-30. Arrows indicate covered areas.

potential for these LAB, especially their synergistic effect in improving the quality characteristics of Toast bread.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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