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Evaluating the Properties of Gluten-Free Bread Produced Using Corn Sourdough Lactic Acid Bacteria

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ABSTRACT

Introduction: Celiac disease is an autoimmune intestinal disorder which occurs in susceptible individuals by eating gluten. Not only does gluten damages the small intestine villi in celiac patients also disrupts nutrients absorption as well. People with celiac disease are unable to tolerate the gluten protein which is present in wheat, barley, oat, and possibly rye. In this study the corn sourdough lactic acid bacteria were evaluated to produce the gluten-free bread, moreover the combination of rice and corn flour were investigated whether can improve the quality and organoleptic characteristics of bread or not.

Methods: In order to prepare corn sourdough lactic acid, *Lactobacillus plantarum* ATCC 20179 and *Lactobacillus fermentum* ATCC 9338 were used as starter cultures. The corn and rice flour were mixed with two different final concentrations of 5% and 10% of bacterial strains to evaluate their effects on nutritional value of breads. Physicochemical properties of breads were measured including, the moisture content; pH; Total Titratable Acidity (TTA); texture analysis; staling rate; the inhibitory activity of bacterial sourdough on bread mold growth; and organoleptic assessment of breads. All the treatments were performed independently and in triplicate. The results were statistically analyzed.

Results: The organoleptic characteristics of breads were improved in breads produced by sourdough lactic acid bacteria in compare with breads produced by rice and corn flour. The pH value of rice dough containing *L. plantarum* was more than corn dough containing equal ratio of *L. fermentum* and *L. plantarum.* Among the samples, the acidity of dough composed of *L. plantarum/L. fermentum* (1:1) was more than rice dough containing *L. plantarum,* corn dough containing *L. fermentum.* Samples with *L. plantarum* 10% and *L. fermentum* 10% showed significant differences in bread moisture compared to the other samples. For inoculated bread samples (with 5% sourdough), the required force in the third day was significantly different from the required force in the first day.

Conclusion: This study confirmed the importance of fortified breads made by sourdough lactic acid bacteria (5% and 10%) with a favorable impact on sensory and rheological characteristics of bread.

Keywords: Celiac bread, Corn sourdough lactic acid, Shelf-life

CITATION LINKS

[1] Current approaches to diagnosis and treatment of celiac... [2] The effect of dairy and rice powder addition on... [3] Gluten-Free Cereal Products and... [4] The widening spectrum of celiac ... [5] Food allergies and intolerances: An update. Nutr... [6] Extra intestinal manifestations of celiac... [7] Crust and crumb characteristics of ... [8] Impact of sourdough on the texture of... [9] Glutenin macro polymer: A gel formed by glutenin... [10] Viscoelastic properties of wheat gliadin and... [11] Evaluation of contributions from wheat protein... [12] Wheat protein composition and properties of... [13] First-degree relatives are frequently affected in coeliac... [14] Coeliac disease and oats: A systematic... [15] Lactobacilli in sourdough fermentation. [16] Effect of single strain and traditional mixed... [17] Sourdough bread production with Lactobacilli... [18] Staleness of bread. Sensory perception... [19] Effects of sourdough and enzymes on staling. [20] Application of selected starter cultures for... [21] Potential of lactic acid bacteria to inhibit rope spoilage... [22] Health Benefits of Rice Bran-A... [23] Antifungal activity of Wickerhamomyces anomalus ... [24] Modificatin of gluten-free sorghum batter... [25] Application of Kluyveromyces marxianus, Lactobacillus... [26] The effect of storage time on textural and crumb... [27] Wheat sourdough fermentation: Effects of... [28] Characteristics of bread making doughs: Influence... [29] Quality evaluation of the sourdough rye... [30] Application and stability of natural antioxidants in... [31] Application of nano-encapsulated olive leaf...

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Introduction

Celiac is an intestinal lifelong disease caused by eating gluten which has been reported as one of the most common genetic disorders in the world. Celiac is a silent disease which usually occurs in adulthood and could not be diagnosed in most cases [1]. The complete and lifelong elimination of gluten from the diet is the only effective treatment for celiac disease; however the removal of gluten causes undesirable effects on dough properties [2]. Moreover, the gluten-free flour products have poor quality, mouthful, and inappropriate flavor [3]. Although gluten is essential for bread baking process, it could be toxic for patients with celiac disease. Not only does consumption of gluten by patients cause inflammation and damage in small intestine but also results in incomplete adsorption of essential materials such as iron, calcium, fat-soluble vitamins, which is sometimes followed by weight loss, diarrhea, anemia, fatigue, and bloating [4-6]. Due to the high amount of starch, the gluten-free breads are staled rapidly. As a consequence of lacking gluten much water is held in breads resulting in softer crumb and crust [7]. These mentioned problems inspired scientists to search for alternatives in production of gluten-free products. The gluten-free breads require a polymeric substrate, protein-based compounds, and hydrocolloids to pretend viscoelasticity characteristics of gluten. These types of doughs also need less mixing, fermentation and baking time compare to the common wheat doughs [8]. Gluten contains two types of proteins including gliadin monomers and glutenin polymers, which are usually present in equal amount in wheat [3]. While gliadin determines the extensional and viscous properties of dough [9], glutenin is responsible for dough elasticity [10]. Therefore, the use of higher amounts of gluten causes dough structure become strengthener [11], while the rheological properties of gluten are improved by adding reducing agents, oxidants, emulsifiers, lipids, or hemicelluloses [12]. Since the only way to treat patients with celiac disease is following a lifelong gluten-free diet [13], the derivatives of wheat, barley, and rye must be removed from a gluten-free diet and replaced by grains such as corn and rice. However the removal of oats from the diet is still open to be discussed [14]. Gluten-free breads are composed of starch; from corn, potato, and rice, flour of other gluten-free cereals; millet, buckwheat, etc., and additives such as sourdough and proteins like eggs, milk, and butter. The usage of sourdough can improve the quality of gluten-free breads by enhancing the minerals and reducing the phytase [15]. Sourdough has also considerable effects on the quality and durability of breads. Containing lactic acid bacteria, the sourdough has metabolites such as organic acids, exopolysaccharides, enzymes, and etc. [8]. The most common bacteria; over 50 different species, isolated from sourdough belong to the genus *Lactobacillus* which have been reported to be significantly effective on dough rheological properties [16]. Studies have been reported on the advantages of using individual species or a combination of species in quality of sourdough however with some disparity in specific volume and stiffness of bread crumb and crust [17] [18]. Another factor that affects the quality of bread is mold. The mold growth in bakery products is the most common type of spoilage causing economic losses. *Rhizopus stolonifer, Rhizopus nigricans, Penicillium expansum,* and *Aspergillus niger* are the most common known fungi spoiling bread. Spores could be destroyed industrially using infrared or microwave radiation; microbial inhibitors such as ethanol, benzoic acid, acetic acid, etc. packaging and chemical preservatives [8, 15, 17]. In the case of sourdough-produced breads, the shelf life is related to the lactic acid bacteria; for example, *Lactobacillus sanfranciscensis* has a wide antimicrobial effect even after baking. Contamination after cooking by molds and spores in the air is the main cause of bread spoilage and shelf life reducing [15].

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Objectives: The aim of the present study was to evaluate the usage of rice and corn flour alternatives for gluten and their impact on the physical, chemical, biological, and organoleptic properties of bread. The consumption of corn sourdough lactic acid along with rice and corn flour was also investigated in improving the quality and organoleptic characteristics of bread.

Materials and Methods

The corn and rice flours were purchased from Tarkhineh and Nanavaran Companies, respectively. *Lactobacillus plantarum* (DSMZ, strain ATCC20179) and *Lactobacillus fermentum* (DSMZ strain ATCC9338) strains were purchased in lyophilized form from the microbiological laboratory (Department of Food Science, University of Tehran, Iran). Also, *Rh. stolonifer, Penicillium italicum,* and *A. niger* were purchased from plant protection group of University of Tehran. Salt, sugar, butter, and eggs were purchased from a local market.

Moisture Measurement: The moisture content was measured according to the AACC 44-16 (2000) standard guideline. About 5g bread was dried in oven (120°C) and cooled to the ambient temperature. The samples then were weighted, and the moisture percentage was calculated using Eq. 1 [18].

$$\text{Moisture (\%)} = \frac{\text{Weight of bread} - \text{weight of dried bread}}{\text{Weight of bread}} \times 100$$

(Eq. 1)

Microbial Inoculation: First, the lyophilized *L. plantarum* (10 CFU/ml) and *L. fermentum* (10 CFU/ml) strains were cultured on sterilized MRS agar (Safir Azma Kian Co.) and then inoculated in MRS Broth (Safir Azma Kian Co.), centrifuged and incubated at 37°C for 24 h. Two strains of *L. plantarum* were also used [18].

Providing Lactic Acid Source: About 150 g rice flour was mixed to 850 mL water, heated up to 55 °C in a water bath for 10 min, and then cooled to 37 °C . Similar process was applied for corn flour. Of each obtained dough (corn and rice), 200 g was separated in two times and mixed with the other one. Then 400 g of the mentioned dough was also separated. Of each of the rice and corn doughs, 100 g was kept as control, separately. In this way, *L. plantarum* strains in A and B forms (40 grams based on the dough weight; 20 grams A and 20 grams B) and *L. fermentum* strains (LBB) (40 grams based on the dough weight) were inoculated to the 400 g prepared mixed dough.

L. plantarum and *L. fermentum* strains in a 1:1 ratio were separately inoculated to the mixed dough, corn dough, rice dough. the meant; as a control. The samples were incubated at 30 °C for 16 h [19].

Microbial Count: The total bacterial population was counted at the beginning and the end of inoculation. In order to attain to 10^6 to 10^9 cfu/ml bacterial population *L. fermentum* and *L. plantarum* strains were cultured on MRS agar (Safir Azma Kian Co.), then the bacteria were kept in incubator at 37 °C for 48 h [18].Finally the bacterial suspension were inoculated in milk and injected into the celiac bread buns.

Dough Preparation and Fermentation: The dough composed of 50% corn and rice flour was prepared as follows; 60 mL water, 40 mL milk, 2% butter, 4% salt (based on the flour weight), an egg, and sourdough (5 and 10%, respectively) were mixed and kept at room temperature for 15 min. For control, celiac bread was prepared without sourdough [18]. Then the prepared dough was divided into 200 g units and baked on a Teflon pan with mild temperature [18].

Physicochemical properties of breads: The physicochemical properties were included pH, TTA, texture analysis, and bread staling. The pH of breads was measured by mixing 10 g dough or milled bread with 90 mL distilled water and stirred for 15 min. Then the upper phase pH was measured [19]. This upper phase

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also subjected to measuring the TTA by titrating with 0.1 M NaOH to reach pH=6.4. The used amount of NaOH was reported as TTA [19]. For texture analysis of breads, samples were sorted from the bread crumb and cut into 2×2 cm pieces with a thickness of approximate 2 mm. Texture stiffness was analyzed according to the AACC74-09 method using texture analyzer (M350-Koopa, England). [18].

The staling of prepared breads was tested according to the AACC 74-09 method after 0, 24, 48, and 72 h. By staling, the bread crumbs become harder so that more force is required for tearing bread pieces, representing more staling [18].

Determining Inhibitory Activity of Bacterial Sourdough on Mold Growth: After 16 hours, inoculated sourdough was centrifuged with 6000 rpm for 20 h, and the supernatant was filtered and sterilized. About 20% of the obtained solution volume was moved to PDA (Safir Azma Kian Co.) medium and mixed. After mixing, 15 mL of the solution was poured into 90 mL containers. After setting the medium, a circle with a diameter of 5mm was removed from the growing mycelium and put in the middle of the plate and incubated at 25°C . After 72 h, the samples were analyzed in terms of the colony diameter. Images were taken from the surfaces of the plates containing *P. italicum* and *A. niger* using a 5-megapixel camera and the percentage of plates surfaces covered by molds was determined using MATLAB software (ver. 7.8.0) [20]. The images were converted from the RGB mode to Grayscale. The imellipsa and roicolor commands were applied to calculate the plates surfaces and the surfaces covered by mold, respectively. By dividing the calculated amounts, the percentage of covering was obtained. Finally, the percentage of inhibition was calculated by Eq. 2 [20]:

$$\text{Inhibition (\%)} = \frac{\text{\% mold surface} - \text{\% blank mold surface}}{\text{\% blank mold surface}} \quad \text{(Eq. 2)}$$

Mold Formation Assessment: To determine the inhibitory activity of sourdough on bread mold formation, 102 mL of the desired mold solution in distilled water was prepared, which was counted using hemocytometer slides, of which 2 mL was distributed on the bread spices (10×10 cm). The samples were transferred to polyethylene bags incubated at 20 °C , and controlled every 24 h, until the first mold formation sign was revealed [21].

Data Analysis Method: Data analysis was performed using SPSS software (ver. 2) based on the completely randomized design in three iterations, using GLU (General Linear Model) [22]

Findings

pH Changes in Dough: Fig. 1. shows the pH values of samples. Controls pH values were about 4.2-4.6, while pH values of samples were varied 2.5-3.8. The pH value of rice dough containing *L. plantarum* was more than corn dough containing equal ratio of *L. fermentum* and *L. plantarum*. Also, *L. fermentum* (50/50) and *L. plantarum* (50/50) had the lowest pH values. The higher pH level in control groups was due to the absence of sourdough.

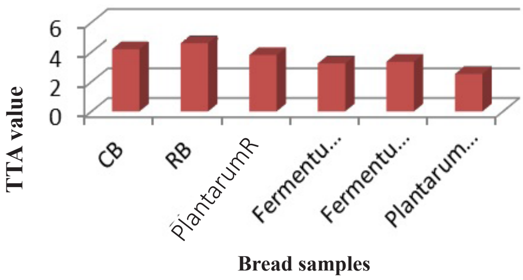

Figure 1) Changes in dough pH values. CB: corn control; RB: rice control. CB: Corn Blank and RB: Rice Blank.

TTA Change in Dough: s shown in Fig. 2, the acidity of samples and controls was about 3.8-6.5 and 0.8-1.3, respectively. Among the samples, the acidity of dough composed of *L. plantarum/L. fermentum* (1:1) was more than rice dough containing *L. plantarum,* corn dough containing *L. fermentum,* and rice *L. fermentum* (50/50), and the corn *L. plantarum* had the lowest acidity. The lower acidity in

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samples might be due to absence of sourdough.

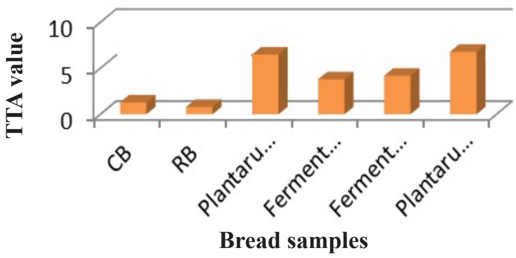

Figure 2) Change in TTA in dough. CB: Corn Blank and RB: Rice Blank.

The pH and TTA Changes in Samples Inoculated with Bacteria: Comparing the two bacteria shown in Table 1, it was determined that *L. plantarum* had a considerable effect on decreasing pH probably due to the lack of lactic acid producing strains. The results showed when *L. plantarum* and *L. fermentum* controls were mixed with corn and rice flour, pH was reduced much effectively in dough samples in compare to when the same control samples were mixed with corn and rice flour, separately. The pH level was reversely related to the percentage of the lactic acid producing bacteria mixed with corn and rice flour. Increasing in the bacterial inoculum lead to reduction in pH level and correspondingly, increased the amount of TTA. Additionally, it was revealed that the sourdough acidity was higher than rice and corn flours acidity.

Table 1) pH and TTA changes in samples inoculated with bacteria.

Sourdough	pH	Acidity
Corn Blank (CB)	4.2	1.3
Rice Blank (RB)	4.6	0.8
L. plantarum Rice	8.3	6.5
L. fermentum Corn	3.24	3.8
L. fermentum 50/50	3.35	4.2
L. plantarum 50/50	2.53	6.8

One of the processes that occurs in sourdough during the fermentation is the production of acid and consequently, lowering the pH, increasing porosity, inactivating alpha-amylase enzyme, and producing soft tissue. By using higher temperatures in fermentation and more water content in sourdough, the production of acid increases during the sourdough fermentation [8,15,16].

Changes of pH in Bread Samples: The pH of control samples was about 6.81-6.86, while it was 6.43 to 6.47 in bread samples. Breads made of *L. plantarum* 5% sourdough had higher pH range in comparison to breads with *L. fermentum* (corn *L. fermentum* 5% - *L. fermentum* 5% and corn *L. fermentum* 10% - *L. fermentum* 10%) and *L. plantarum* (rice *L. plantarum* 10% and *L. plantarum* 5% - *L. plantarum* 10%). The breads containing *L. plantarum* 10% showed the lowest pH value, and the highest value was related to the samples without sourdough (Figure 3).

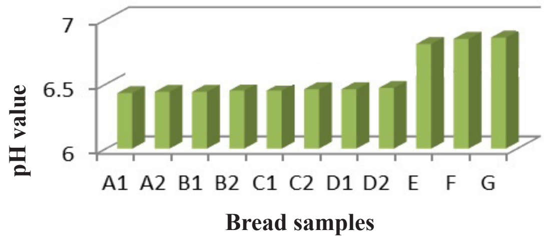

Figure 3) pH changes in bread samples A1: *L. plantarum* 10%, A2: *L. plantarum*, B1: *L. fermentum* Corn 10%, B2: *L. fermentum* 10%, C1: *L. plantarum* Rice 10%, C2: *L. fermentum* 5%, D1: *L. fermentum* Corn 5%, D2: *L. plantarum* Rice 5%, G: Blank (Corn & Rice), E: Blank (Corn) and F: Blank (Rice).

TTA Changes in Bread Samples: TTA value was varied from 0.8 to1.2 in different test samples and 0.65-0.8 for controls. The samples with *L. plantarum* 5% and *L. fermentum* 5% showed higher acidity than the others.

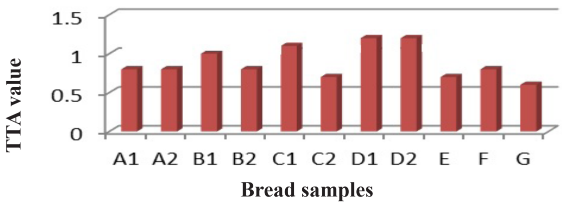

Figure 4) Changes in TTA in bread samples. A1: *L. plantarum* 10%, A2: *L. plantarum*, B1: *L. fermentum* Corn 10%, B2: *L. fermentum* 10%, C1: *L. plantarum* Rice 10%, C2: *L. fermentum* 5%, D1: *L. fermentum* Corn 5%, D2: *L. plantarum* Rice 5%, G: Blank (Corn & Rice), E: Blank (Corn) and F: Blank (Rice).

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pH and TTA Changes in Bread Samples: The pH of bread samples ranged from 6.43 to 6.81 which were significantly different from controls. The *L. fermentum* and *L. plantarum* mixed with corn and rice flour, respectively, could decrease the pH of both bread and dough samples. Separately, bacteria were added to each of the flours and each of the bacteria was flour mixed separately, and rice and corn were added. Increasing in the sourdough amount was resulted in reduction of pH and increasing of TTA (Table 2). All comments related to changes in the acidity and pH of sourdoughs are also applicable to the acidity and pH of bread treatments.

Moisture Changes in Bread Samples: Moisture content in samples was varied from 32 to 42, while in control groups was about 35-38 (Fig. 5., Table 2). Samples with *L. plantarum* 10% and *L. fermentum* 10% showed significant differences compared to the other samples. By increasing the sourdough percentage, the moisture content and the shelf-life increased, while the staling decreased.

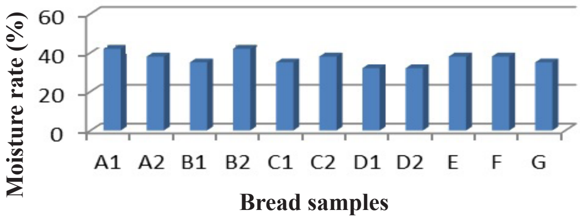

Figure 5) Changes in bread samples moisture in different treatments. A1: *L. plantarum* 10%, A2: *L. plantarum*, B1: *L. fermentum* Corn 10%, B2: *L. fermentum* 10%, C1: *L. plantarum* Rice 10%, C2: *L. fermentum* 5%, D1: *L. fermentum* Corn 5%, D2: *L. plantarum* Rice 5%, G: Blank (Corn & Rice), E: Blank (Corn) and F: Blank (Rice).

Texture Analysis and Staling Assessment For inoculated bread samples (5%), the required force in the third day was significantly different from the required force in the first day. In the case of 10% inoculation, there was no significant differences between the samples.

Table 2) pH, TTA, and moisture content changes in bread samples.

Treatments	Bread	Acidity	pH	Moisture
A1	*L. plantarum* 10%	0.8	6.43	42
A2	*L. plantarum*	0.8	6.44	38
B1	*L. fermentum* Corn 10%	1	6.44	35
B2	*L. fermentum* 10%	0.8	6.45	42
C1	*L. plantarum* Rice 10%	1.1	6.37	35
C2	*L. fermentum* 5%	0.7	6.22	38
D1	*L. fermentum* Corn 5%	1.5	6.46	32
D2	*L. plantarum* Rice 5%	1.2	6.47	32
G	Blank (Corn & Rice)	1.7	6.71	38
E	Blank (Corn)	0.8	6.25	38
F	Blank (Rice)	0.6	6.86	35

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Table 3) Effect of several sourdough on the bread samples staling.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Treatment	.000	10	.000	.000	1.000
Time	17.600	1	17.600	12.250	.001
Error	140.800	98	1.437		
a. R Squared = .111 (Adjusted R Squared = .011)					

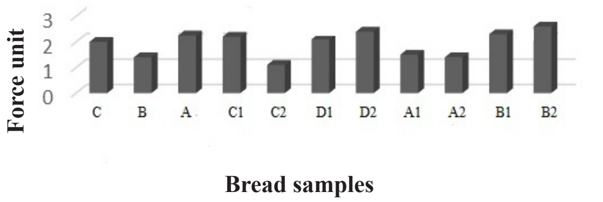

Figure 6) Texture analysis of the breads. C: Blank corn & rice:, B: Blank Rice, A: Blank corn, C1: *L. fermentum* 5%, C2: *L. fermentum* 10%, D1: *L. plantarum* 5%, D2: *L. plantarum* 10%, A1: *L. fermentum* corn 5%, A2: *L. fermentum* corn 10%, B1: *L. plantarum* rice 5%:, B2: *L. plantarum* rice 10%

Discussion

One of the most important factors affecting the rheology of dough is the development of gluten network structure during the bread production. On the other hand, increasing temperature and fermentation time, especially while using *Lactobacillus* as starter culture increases acid production, lowers the order of gluten network structure, and subsequently, declines consistency and viscosity of dough. But excessive acidity could lead to tissue stiffness and premature staling of bread [6, 16, 22, 23]. The pH changes which observed in the present study can be supported by other studies. Clark and colleagues (2002) asserted that by increasing the temperature and duration of fermentation, pH and TTA will be increased, which can be attributed to the performance of microorganisms during the fermentation and bread making processes and producing some acid metabolites. Generally, by increasing the temperature and fermentation time and ash content of flour, the acidity and the formation of organic acids increase in sourdough. Hence pH can be considered as the most important factor affecting fermentation time [16].

The acidity changes and increasing the shelf-life of breads observed in this study were similar to other studies which conducted with other probiotic strains. In the study by Plessis and colleagues (2002), the sourdough was inoculated with *L acidophilus* and *L. sakei,* the results demonstrated that bread produced by sourdough including probiotic had higher acidity and shelf life[24]. Moreover, Haboubi and colleagues (2006) revealed that increasing the percentage of sourdough can lead to enhance the humidity and shelf-life of breads. Thus, the rate of bread staling was decreased [14]. Intermolecular bonds in the dough are non-covalent and continuously are broken down and reformed. Water absorption property of flour is an important factor in determining the properties of dough. While using a device for the production of dough, the use of yeast in dough production leads to a significant reduction in the water absorption. Adding sourdough reduces dough resistance compared to the control bread and bread that chemically is acidified [14, 24]. The results of texture analyzer and staling demonstrated that the required force for tearing bread pieces was increased due to staling. The effect of several sourdoughs prepared by *L. fermentum* and *L. plantarum* on bread samples staling revealed by increasing *L. fermentum* and *L. plantarum,* the staling process became slower and shelf life increased, which could be due to the breaking down of corn starch and rice flour by lactic

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acid bacteria. The similar trend in salting process of breads made from probiotic sourdough has been reported by Corsseti and colleagues (2007) [15]. Proteolytic and amylolytic ability of lactic acid bacteria and the ability to produce extracellular polysaccharides like starch and dextrin, which have the ability to interfere in starch retrogradation, improve the soft texture of the dough rheology. In addition, modifying flour polysaccharides during the fermentation prevents the interaction between the gluten and starch and leads to the production of different networks in produced breads. The mutual effect of lactic acid bacteria during the fermentation of sourdough impacts on dough rheology and bread volume [22, 23, 25]. Reducing the amount of protein polymers and comparative analysis of the gluten network in acidic conditions are other reasons for elasticity loss. The use of appropriate values in the final mixed dough creates sourdough optimal structure and maintains the carbon dioxide produced in the bread [22, 26]. evaluating the production of gluten-free sourdough and its effects on the quality and shelf life of bread without gluten, reported that the fermentation process by both *L. fermentum* and *L. Plantarum* species increased the elasticity, and breads containing sourdough exhibited the least fragile in the Days 2 and 5. According to the obtained results, all samples in both days showed statistically significant differences regarding bread staling and total acceptability. Scores showed that by increasing the percentage of sourdough, staling and total acceptability of all bread samples increased. Sourdough bread samples inoculated with *L. plantarum* and *L. fermentum* 5% and 10% had much better taste since the taste of bread is a function of sourdough percentage and the amount and type of starter culture; therefore, the increase in product acceptability is due to the increase in sourdough percentage. It could be concluded that an increase in the use of *L. fermentum* and *L. plantarum* (10%) inoculated with corn sourdough lactic acid significantly reduced the rate of staling. In other words, using sourdough delays staling phenomenon and increases shelf-life of breads. As it can illustrated from other studies the most important reason for the reduction of bread staling with sourdough processing is acid production, which increases the porosity, inactivates alpha-amylase enzyme, and increases tissue softness, while the use of higher fermentation temperatures and sourdough with high water content in full increases acid production during the sourdough fermentation [22, 23, 27]. Some lactic acid bacteria which are able to produce extracellular polysaccharides such as dextran, xanthan, glucan, fructan, and Levan, increase water absorption and prevent moisture transfer from crumb or crust. These compounds have a significant effect on the volume and shelf life of bread. On the other hand, regarding the production of lactic acid and increased activity of alpha-amylase enzyme, the change in starch crystalline form is the main cause of staling, decomposition, and conversion into dextrin with low molecular weight. This finding could be effectively used in reducing bread staling [21, 23, 26, 27]. Sourdough also increases water holding capacity, leading to water retention in the bread structure during the storage and delay in staling. In fact, lactic acid bacteria by deteriorating starch in rice and corn flour are liable to delay staling [11, 15]. As flavor and aroma are the most important parameters affecting product acceptability by consumers, breads produced by sourdough lactic acid bacteria can be more favorable due to producing metabolites involved in the development of bread flavor by lactic acid bacteria. Slightly sour flavor in

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the bread refers to the fermentation process and producing lactic and acetic acids. *Lactobacillus* invertase enzyme causes the hydrolysis of sucrose and increases the availability of sugar during the sourdough fermentation process. Increase in soluble carbohydrates by Maillard reaction causes the aroma and flavor in bread [26, 28]. Some studies reported that combination of yeast and lactic acid bacteria produces more flavor [5, 14, 26].

Plessis and colleagues studied the effect of sourdoughs inoculated with *L. saki* and *L. acidophilus* on bread. They found that sourdough bread inoculated by a combination of two microorganisms had more flavor and aroma compared with breads inoculated just by *L. acidophilus* or *L. saki* [25]. The volume of the inoculated sourdough lactic acid bacteria also play an important role in the quality of breads in order to prove the former claim Banue and colleagues (2011) applied sourdough 20% and 40% in producing breads. The results concluded that breads with sourdough 20% were better in terms of the quality than those made with sourdough 40%, and increasing the percentage of sourdough to 40% had a negative impact on crumb firmness. After 48 hours, breads with sourdough 20% showed better quality as a whole. The mold formation at 25 ºC was happened after 4 days. Due to the lack of sourdough, it was faster for the controls compared to the samples. In the case of 5% inoculated samples, the mold formation was faster than in 10% inoculated samples, but in the case of clean sourdough, mold formation was faster in 10% inoculated samples than in 5% inoculated samples. Gluten-free bread is maintained about 5-4 days at 4 °C in the refrigerator, and if kept in the freezer at -18 °C, shelf life mutually increases [28]. In their review about the use of *Lactobacillus* in sourdough fermentation, Corsetti and his colleagues (1998) isolated antifungal compounds of *L. sanfranciscensis.* In their study, they concluded that among the identified compounds, some of the metabolites were present in the sourdough lactic acid, which could have an important role in increasing the antifungal activities [15].

Conclusion

The effect of *L. fermentum* and *L. plantarum* with two concentrations of 5% and 10% was investigated on the stiffness and staling of celiac bread. According to this study results, it could be said that in general, the use of sourdough bread could improve bread quality and delay staling process as well. In addition, the use of sourdough lactic acid bacteria improves bread aroma and makes it to be customer-friendly. The use of higher levels of corn and rice flour could increase the quality of bread and decrease its staling. While cooking, the higher moisture content was preserved in celiac bread in comparison with the traditional bread making. Sourdough lactic acid bacteria affected and delayed the bread mold formation by producing organic acids and antimicrobial materials. Fermented sourdough affects all positive aspects including quality, flavor, shelf-life against microbial spoilage, and other useful properties of bread. Therefore, fermented sourdough could have a significant impact in the production of bread and in improving bread quality.

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Ethical Permissions: Since we used no animal model and used the isolates which were previously obtained from the clinical samples, we have no ethical code for this study.

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