Journal of Food Science and Technology (Iran)

Homepage:www.fsct.modares.ir

Scientific Research

Gluten free sourdough production by using millet, quinoa and amaranth flours and *Lactobacillus fermentum* and *Lactobacillus plantarum* starters

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ARTICLE INFO

Article History:
Received:2023/12/24
Accepted:2024/2/16

Keywords:

Starter,

Sourdough,

Gluten free,

Pseudo cereal.

DOI: **10.22034/FSCT.21.154.90.**

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ABSTRACT

Breads Produced by sourdough have a lighter texture and a higher quality of chewing. Due to the fact that sourdough prepared from wheat or rye flour cannot be used in the formulation of gluten-free products. Therefore, in the present study, millet, amaranth and quinoa flours and *Lactobacillus fermentum* and *Lactobacillus plantarum* starters were used individually and in combination in the sourdough formulation. Produced gluten-free sourdoughs were added to gluten-free bread dough in level of 10% and quantitative and qualitative characteristics of the produced bread samples were evaluated in a completely randomized design with factorial arrangement ($P \leq 0.05$). The results of evaluating the moisture content of the produced breads showed that two samples prepared from sourdough containing mixed starter and quinoa flour and the sample prepared from sourdough containing *Lactobacillus plantarum* starter and millet flour had the highest and lowest moisture content, respectively. Also, the samples containing sourdough prepared from quinoa flour had the highest amount of pH, acidity and organic acids. In the evaluation section of porosity and specific volume of the product, it was determined that the samples prepared from sourdough containing *Lactobacillus fermentum* starter and quinoa flour had the highest porosity and specific volume. Also, the texture evaluation results showed that the samples prepared from sourdough containing mixed starter and quinoa flour had the lowest hardness. On the other hand, the examination of the crust color values showed that the amount of L* and b* values increased by using sourdough prepared from quinoa flour in the gluten-free bread formulation. While the type of starter used had no significant effect on these values. At the end, by sensory evaluation, assigned the highest overall acceptance score to the sample prepared from sourdough containing mixed starter and quinoa flour.

1- Introduction

Celiac disease is a chronic autoimmune disorder caused by the ingestion of the gliadin component of gluten, found in wheat grain and rye prolamin (secalin), barley (hordein), and possibly oat (avidin), which have a similar amino acid composition to gliadin. It is one of the most common food allergies and is characterized by both intestinal and extraintestinal symptoms. Extraintestinal symptoms often make diagnosis of this disease difficult. The disease is characterized by damage to the small intestine. In addition to nutritional deficiencies, these patients are also at increased risk of developing malignancies such as intestinal lymphoma in the long term. The intestinal villi become shorter, wider, and flatter when exposed to gluten or its components, and their enzyme production is reduced and impaired [1].

As a result, these patients also suffer from allergies to other foods. Since the only effective treatment for these patients is a lifelong gluten-free diet, which can help improve their clinical condition, the demand for gluten-free products has increased in parallel with the increase in the number of patients with celiac disease or other gluten-related sensitivities. Therefore, the use of wheat flour and its derivatives is prohibited in the formulation of bread and other gluten-free products [2].

One of the main stages in the preparation of dough for the production of bread, which is the most important bakery product in the diet, is the fermentation, maturation, or rising of the dough. Although the use of baker's yeast Saccharomyces cerevisiae has become widespread due to its ease of use, breads produced by sourdough are far more flavorful. On the other hand, it is worth noting that in recent years, due to the increasing demand of consumers for natural, healthy and flavorful food, the production of sourdough breads has received attention and has been used as a means of improving the quality and taste of bread. In fact, the most noticeable change in dough caused by sourdough is the creation of its characteristic sour taste. According to available reports, at least half of the sour taste is due to the production of lactate by the genus Lactobacillus [3]. The bacteria and yeast present in sourdough produce leavening agents in this process, and the unique acidic flavor is also due to the activity of sourdough microorganisms. By using sourdough in bread production, it is possible to leaven bread dough with the addition of a small amount or no baker's yeast, the properties of the dough are improved, and the texture, flavor and taste of such bread will be superior to that of bread leavened with baker's yeast. In addition, the addition of sourdough extends the shelf life of bread. By definition, dough fermented by lactic acid bacteria and yeast is called sourdough [4]. In addition, the addition of sourdough extends the shelf life of bread and delays mold growth and ropy spoilage. These benefits are provided as a result of the synergistic mechanism of yeasts and lactic acid bacteria, which are the predominant microorganisms in natural sourdoughs [5].

Among the diverse species of Lactobacillus bacteria, those that grow in sourdough do not produce carbon dioxide; instead, they acidify and broaden the flavor with their activity. Hetero-fermentative lactic acid bacteria also produce a small amount of carbon dioxide, so the resulting bread is denser than breads made with baker's yeast. In general, sourdough breads have higher moisture content, a denser texture, and better chewability compared to breads made with baker's yeast. It is worth noting that in the present study, the Lactobacillus plantarum starter, unlike the Lactobacillus fermentum starter, was homofermentative and the product of its fermentation was specifically lactic acid. In fact, Lactobacillus fermentum is heterofermentative and produces a range of acids such as acetic and lactic acids [6]. On the other hand, in the past, sourdough was made from rye flour. However, in recent years, wheat flour has been used in most formulations, and for gluten-free products, the use of this type of sourdough is prohibited and gluten-free grains or pseudo-grains must be used, in the present study millet, amaranth and quinoa flour were used.

Millet is a cereal with spherical grains that come in yellow, white or brown colors. The millet sprout is large relative to the grain size, like corn. Although millet flour is gluten-free, it is a rich source of protein, essential amino acids, vitamins and minerals compared to other cereal grains. In addition, it is rich in dietary fiber, phytochemicals and micronutrients [7]. Pseudo-cereals, despite not belonging to the Poaceae family, share many similarities with true cereals. Amaranth, scientifically known as Amaranthus spp., has gained attention as a potential crop in semi-arid regions due to its remarkable adaptability to nutrient-deficient soils and tolerance to drought stress. Additionally, Amaranth boasts a higher protein content compared to most commonly consumed cereal grains [8]. Another promising pseudo-cereal, quinoa, thrives in arid and water-scarce environments. Its superior quality and well-balanced amino acid profile make it a highly desirable crop. Quinoa surpasses wheat in lysine content, making it an excellent choice for human dietary balance. Furthermore, quinoa excels in calcium, phosphorus, magnesium, potassium, iron, copper, and manganese compared to wheat, barley, and maize. It also exhibits higher levels of fat, carbohydrates, and vitamins compared to wheat [9, 10].

In this study, millet, amaranth, and quinoa flours were employed as the primary substrates for cultivating two lactic acid starters, *Lactobacillus* *fermentum* and *Lactobacillus plantarum*, in gluten-free sourdough. The impact of this novel sourdough on the technological, textural, visual, and sensory characteristics of gluten-free loaf bread samples was thoroughly investigated.

2- Materials and Methods

2-1- Materials

Rice flour was purchased from a reputable store under the brand name 111. Millet, amaranth, and quinoa grains were obtained from the Research Institute for Seed and Seedling Improvement and Preservation and stored at 4°C. *Lactobacillus fermentum* and *Lactobacillus plantarum* strains were procured from the Iran Research Center for Science and Industry (Tehran, Iran). Other materials required for the experiments including salt, oil, sugar, and egg white powder were procured from a reputable store, and dry and instant yeast from Razavi Yeast Factory (Mashhad, Iran), defatted active soy flour from Soya San Maxway Company, guar gum and xanthan gum from Rhodia (France) and stored in refrigerator (at 4°C). All chemicals were purchased from Merck, Germany.

2-2- Methods

2-2-1- Evaluation of Physicochemical Properties of Rice Flour and Other Grains

Millet, amaranth, and quinoa grains were cleaned, de-hulled, and ground into whole flour using a laboratory hammer mill. The flour was then sieved through a 100-mesh sieve to control particle size. For quinoa grains, due to the presence of saponins (bitter-tasting glycosidic compounds soluble in water found in the quinoa seed coat), they were washed with water and dried at room temperature before grinding [11]. The flour was then sieved through a 100-mesh sieve to control particle size. The physicochemical properties of the flours, including moisture, protein, ash, and fat, were determined according to standard methods developed by the American Association of Cereal Chemists (AACC [1], 2000) [12]. Moisture content was assessed according to standard 16-44, protein content according to 10-46, ash content according to 01-08, and fat content according to 10-30. Fiber content was determined using the method of Ranganayaki et al. (2012) [13]. Carbohydrate content was calculated by subtracting the sum of all other constituents from 100 [14].

2-2-2- Sourdough Preparation

For sourdough preparation, 100 g of each source (millet, amaranth, and quinoa) was mixed separately with 125 ml of water. Eight grams of Saccharomyces cerevisiae yeast and 10 g of sugar were added to the mixture. Then, 162 ml of a suspension of Lactobacillus fermentum and Lactobacillus plantarum bacteria, either individually or in combination, was added to the mixture and incubated at 25°C for 12 hours to prepare the sourdough. Finally, 10% of the sourdough was added to the gluten-free loaf bread formulation. The bacterial suspension was prepared by activating and culturing the starter in MRS broth in an incubator at 37°C for 24 hours. After bacterial growth, the cells were separated using a centrifuge. The McFarland method was used to standardize the cell count [15].

2-2-3- Dough Preparation and Gluten-Free Loaf Bread Production

Gluten-free loaf bread samples were prepared containing 100% rice flour, 10% sourdough, 1% dry yeast, 1% salt, 1% sugar, 1% oil, 0.5% egg white powder, 10% defatted active soy flour, 0.4% guar gum, 0.2% xanthan gum, and water (as required).
Continuing, the dry ingredients were mixed in a mixer (Diosna, Germany) for one minute, followed by the addition of water. Mixing was continued for ten minutes at low speed. Finally, the oil was added, and the dough was allowed to rest for 30 minutes at room temperature (25°C) in a bulk form. The dough was then divided into 80 g pieces and placed in greased aluminum cylindrical molds with a diameter and height of 80 mm. The molds were transferred to a proofing chamber (MIWE, Germany) with a temperature of 45°C and relative humidity of 80%. After 45 minutes, the molds were transferred to a hot air oven (MIWE, Germany). Baking was carried out at 230°C for 10 minutes. The loaves were then placed on a laboratory bench to cool to room temperature and stored in lightweight polypropylene bags until quantitative and qualitative tests were performed [16].

2-2-4- Evaluation of Quantitative and Qualitative Properties of Bread

2-2-4-1- Moisture

The moisture content of the bread samples was determined according to the method described in the section on evaluating the physicochemical properties of flour [12].

2-2-4-2- Acidity

To determine the titratable acidity of the bread samples (expressed as lactic acid), 10 g of bread was mixed with 90 ml of distilled water and homogenized. The resulting solution was filtered and titrated with 0.1 N NaOH. Acidity was calculated based on the amount of NaOH consumed [12].

2-2-4-3- pH

The pH of the bread samples was measured using a pH meter.

2-2-4-4- Organic Acids

The levels of organic acids such as lactic acid and acetic acid were determined using high-performance liquid chromatography (HPLC) (Waters, USA) equipped with an ultraviolet detector at a wavelength of 205 nm. To remove suspended particles, 10 g samples were first homogenized with 60 ml of cold distilled water, and the sample weight was adjusted to 100 g. Centrifugation was then carried out at 4000 × g for 5 minutes at 15°C. 20 ml of the supernatant was removed and mixed with 5 ml of Carrez I solution (0.25 M $ZnSO_4$) and Carrez II solution (0.25 M $ZnSO_4$). The resulting mixture was neutralized with 0.5 M NaOH until a final pH of 5.8 was reached. The volume of the mixture was adjusted to about 50 ml with distilled water and then filtered twice to remove suspended particles (first with Whatman No. 41 filter paper and then with 0.22 µm cellulose acetate filter paper). The filtered samples were used for injection into the HPLC system [17].

2-2-4-5- Specific Volume

To measure the specific volume of the bread samples, the volume displacement method with canola seeds was used according to AACC standard (2000) No. 10-72. For this purpose, within 2 hours after baking, a 2 × 2 × 2 cm piece of each bread sample was weighed using a digital balance with an accuracy of 0.001 g. Then, 250 ml of the volume of a graduated cylinder was replaced with canola seeds. Finally, the specific volume was calculated by dividing the weight by the volume [12].

2-2-4-6- Porosity

To measure the porosity, the center of the bread samples was imaged using a scanner with a resolution of 300 pixels. The resulting image was then imported into ImageJ software. With the 8-bit mode activated, grayscale images were created. To convert grayscale images to binary images, the binary mode of the software was activated. These images are a collection of bright and dark spots, and the calculation of the ratio of bright spots to dark spots is used as an indicator of the porosity of the samples. It is obvious that the higher this ratio, the more voids are present in the texture (higher porosity). In practice, this ratio was calculated by activating the Analysis mode of the software and the porosity of the samples was measured as a percentage [18].

2-2-4-7- Crust Color

The color test was performed using a HunterLab colorimeter. Before the test, the device was calibrated with white and black tiles and checked with a yellow tile. Color measurement in this device was based on the CIELAB system and measurement of the three values L*, a*, and b*. The L* value represents the lightness of the sample and its range varies from zero (pure black) to 100 (pure white). The a* value indicates the proximity of the sample color to green and red and its range varies from -120 (pure green) to 120+ (pure red). The b* value indicates the proximity of the sample color to blue and yellow and its range varies from -120 (pure blue) to 120+ (pure yellow) [19].

2-2-4-8- Texture Firmness

The texture firmness of the bread samples was evaluated one, three, and seven days after baking using a texture analyzer (QTS, UK). The maximum force required to penetrate a flat-ended cylindrical probe (2 cm diameter and 3.2 cm height) at a speed of 30 mm/min from the center of the bread, specifically the firmness index, was calculated. The starting point and target point were 0.05 N and 30 mm, respectively. In fact, the firmness was equal to the maximum force value in the force-deformation curve and was expressed in Newtons [16].

2-2-4-9- Sensory Evaluation

To evaluate sensory properties, including shape and appearance, crust characteristics, crumbliness and porosity, texture firmness and softness, and flavor (taste and aroma), were evaluated using the 5-point hedonic method (1: extremely undesirable, 2: undesirable, ... 5: extremely desirable). The relative importance of each attribute was assigned as follows: shape and appearance (4), crust characteristics (3), crumbliness and porosity (2), texture firmness and softness (3), and flavor (3). Each bread sample was evaluated by at least 10 panelists. Based on this information, the overall acceptability (bread quality score) was calculated using Equation 1.

Equation 1: $$Q=\frac{\Sigma(P\times G)}{\Sigma P}$$

where: Q = Overall acceptability, P = trait ranking coefficient, and G = trait evaluation coefficient

2-2-5- Statistical Design and Data Analysis Methods

The results of this study were analyzed using SPSS software version 16. For this purpose, a completely randomized design with a two-factorial arrangement was used, where the first factor was the type of flour used in sourdough preparation (millet, amaranth, and quinoa) and the second factor was the type of lactic starter culture (Lactobacillus fermentum and Lactobacillus plantarum alone or in combination). Gluten-free loaf bread samples were prepared in triplicate and the mean quantitative and qualitative properties of the bread were compared using Duncan's test at the 5% significance level ($P<0.05$). Finally, SigmaPlot software was used to create the graphs.

3- Results and Discussion

3-1- Physicochemical Properties of Flours Used in Sourdough Preparation and Gluten-Free Bread

The results of the evaluation of the physicochemical properties of the flours used in the gluten-free sourdough formulation and gluten-free loaf bread are presented in Table 1.

Table 1- Physicochemical characteristics of Flour

Physicochemical characteristics (%)	Amaranth flour	Quinoa flour	Millet flour	Rice flour
Moisture	13.8 ± 0.24	10.2 ± 0.03	9.80 ± 0.32	10.1 ± 0.12
Protein	14.2 ± 0.39	15.4 ± 0.53	10.9 ± 0.41	9.72 ± 0.06
Ash	1.97 ± 0.00	2.17 ± 0.36	1.29 ± 0.03	0.82 ± 0.01
Fat	3.60 ± 0.12	3.15 ± 0.01	2.54 ± 0.01	1.51 ± 0.01
Fiber	1.93 ± 0.03	6.11 ± 0.16	4.16 ± 0.05	0.63 ± 0.02
Carbohydrate	65.59 ± 1.08	62.57 ± 1.66	71.03 ± 1.25	77.20 ± 2.63

Data is in the form of average ± Standard deviation.

3-2- Evaluation of Gluten-Free Loaf Bread Containing Sourdough

3-2-1- Moisture

The results of the effect of flour type (millet, amaranth, and quinoa) and lactic starter culture type (Lactobacillus fermentum and Lactobacillus plantarum alone or in combination) on the moisture content of gluten-free loaf bread showed that the effect of flour type and starters used on the moisture content of gluten-free loaf bread was significant (P<0.05), and the samples containing sourdough prepared from quinoa flour and combination starters had the highest moisture content (Figure 1).

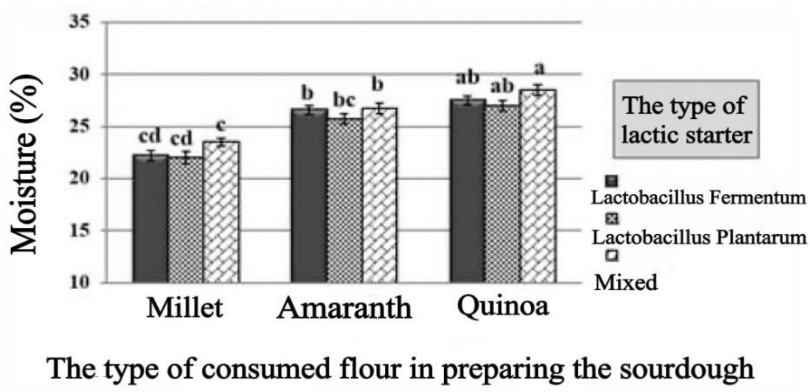

Figure 1- The effect of the type of consumed flour and the type of lactic starter in preparing the sourdough on moisture of molded gluten free bread (Similar letters have no significant difference in *p<0/05* statically)

The results also showed that the moisture content of the product was slightly higher when using sourdough containing the fermentum starter compared to the plantarum starter. In this regard, it can be stated that considering that some lactic acid bacteria can produce extracellular polysaccharides (EPS: Extracellular polymeric substances) such as dextrans, xanthan, glucan, fructan, and levan [20], in fermented samples containing lactic acid bacteria, the water absorption of the dough and consequently the moisture content of the final product is higher than the sample without sourdough. In this line, Fazel Tehrani Moghadam et al. (2021) investigated the possibility of producing celiac bread using lactic corn sourdough with Lactobacillus plantarum at two levels of 5 and 10% and observed that the moisture content of the product containing sourdough was higher than the samples without sourdough [21]. Also, Shin et al. (2010) stated that it is likely that increasing the amount of sourdough leads to the production of more free amino acids and increases the capacity of these compounds to bind with water [22]. On the other hand, it seems that quinoa flour, due to its higher protein content compared to amaranth and millet flours, was able to retain more moisture. In this regard, Jaldani et al. (2019) optimized the formulation of gluten-free cake containing rice, quinoa, and purslane flour and stated that the product containing quinoa flour had a higher moisture content than the control sample [14]. They stated that the increase in moisture content of the samples is in line with the increase in the addition of whole quinoa flour due to the large number of hydroxyl groups in its structure, which causes the absorption and retention of water molecules in the product during baking. On the other hand, Mahgoub & Omran (2022) studied the quality characteristics of flat gluten-free bread prepared from millet and rice flour and stated that with increasing the amount of millet flour in the formulation, the water holding capacity of the product decreased [23].

3-2-2- Acidity

The results showed that the effect of flour type and starters used on the acidity of gluten-free loaf bread was significant (P<0.05), with samples containing a combination sourdough having the highest acidity and samples containing sourdough with Lactobacillus plantarum strain having the lowest acidity. On the other hand, it was observed that the samples containing sourdough prepared from quinoa flour had the highest acidity (Table 2). One of the most important processes that occur during the fermentation of bread dough with sourdough is acid production and consequently a decrease in pH, which leads to increased porosity, inactivation of alpha-amylase enzyme, and increased softness of the texture (12). Based on the results, it was found that samples containing a combination sourdough had the highest acidity and samples containing sourdough with Lactobacillus plantarum strain had the lowest acidity. In this regard, it seems that the presence of two combined starters simultaneously in the sourdough has a synergistic effect and increases the activity of the bacteria used and ultimately increases the acidity of the product. The results also showed that the acidity of the product was slightly higher when using sourdough containing the fermentum starter compared to the plantarum starter, which is probably due to the higher initial resistance of Lactobacillus fermentum to Lactobacillus plantarum in adverse environmental conditions. In this regard, Noorbakhsh et al. (2017) investigated the comparative effect of applying sublethal stresses on the survival of Lactobacillus plantarum and Lactobacillus fermentum used as a co-culture in synbiotic yogurt and the consumer's digestive system, and their results showed that Lactobacillus fermentum is more resistant to adverse environmental conditions than Lactobacillus plantarum [24]. Also, Zhu et al. (2022) studied the characteristics of gluten-free sourdough fermentation using Lactobacillus plantarum strain and its effect on dough quality and nutritional value during the freezing process, and stated that the acidity of the dough increased during fermentation due to the activity of the respective strain and acid production [25]. On the other hand, it seems that quinoa seed flour, due to its higher protein content than other flours used, has a greater ability to maintain the balance of acids produced, and therefore the microorganisms present in the sourdough are under less stress (from acids produced) and therefore act more and increase the acidity of the product. The results of Wolter et al. (2014) support this [26]. Also, one of the important parameters in the use of microorganisms from carbohydrates present in flour is the enzymatic activity of the flour itself. In this regard, according to the research of Azizi et al. (2021), which studied the enzymatic activity of wheat, quinoa, and amaranth, it was found that the activity of alpha-amylase in quinoa is higher than amaranth, which can also lead to increased activity and increased acidity [8].

3-2-3- pH

The results of the effect of flour type and lactic starter type on the pH of gluten-free loaf bread showed that sourdough samples prepared from quinoa flour and containing Lactobacillus plantarum strain had the highest pH. Also, according to Iranian National Standard No. 2338 [27], the permissible pH of voluminous breads is between 5 and 6 (6), which according to the results, all produced samples were within the standard range (Table 2). As explained in the acidity evaluation section, it seems that the presence of two combined starters simultaneously in the sourdough has a synergistic effect and increases the activity of the bacteria used and ultimately decreases the pH of the product. The results also showed that the pH of the product was slightly lower when using sourdough containing the fermentum starter compared to the plantarum starter, which is probably due to the higher initial resistance of Lactobacillus fermentum to Lactobacillus plantarum in adverse environmental conditions.

Table 2- The interaction of the type of consumed flour and the type of lactic starter in preparing the sourdough on the rate of acidity, PH, and organic acids of molded gluten free bread

The type of consumed flour in preparing the sourdough	The type of lactic starter	Acetic acid (gram in 100 gram of sample)	Lactic acid (gram in 100 gram of sample)	pH (–)	Acidity (Milliliter)
Millet	*Lactobacillus Fermentum*	0.23 ± 0.01^{b}	0.79 ± 0.05^{c}	5.3 ± 0.05^{c}	4.5 ± 0.1^{c}

	Lactobacillus Plantarum	0.14 ± 0.01c	1.08 ± 0.03b	5.4 ± .06c	4.1 ± 0.2d
	Mixed	0.26 ± 0.00ab	1.12 ± 0.04b	5.1 ± 0.04d	4.8 ± 0.1bc
Amaranth	*Lactobacillus Fermentum*	0.25 ± 0.02ab	0.87 ± 0.06bc	5.5 ± 0.10b	4.8 ± 0.1bc
	Lactobacillus Plantarum	0.16 ± 0.01bc	1.13 ± 0.05b	5.6 ± 0.05b	4.4 ± 0.1c
	Mixed	0.30 ± 0.01a	1.25 ± 0.04ab	5.3 ± 0.03c	5.2 ± 0.2b
Quinoa	*Lactobacillus Fermentum*	0.31 ± 0.02a	0.94 ± 0.04bc	5.6 ± 0.02ab	5.1 ± 0.0b
	Lactobacillus Plantarum	0.19 ± 0.01bc	1.24 ± 0.03ab	5.8± 0.05a	4.6 ± 0.1bc
	Mixed	0.33 ± 0.01a	1.41 ± 0.04a	5.5 ± 0.07b	5.7 ± 0.1a

Data is in the form of average ± Standard deviation.
Different letters in each column have significant difference in *p<0/05* statically.

3-2-4- Lactic Acid and Acetic Acid

Based on the results, it was found that the independent and interactive effects of flour type and starters used had a significant effect on the organic acid content of gluten-free loaf bread (P<0.05), with samples containing a combination sourdough having the highest lactic acid and acetic acid content and samples containing sourdough with Lactobacillus fermentum strain having the lowest content of these acids (Table 2). This is because the plantarum starter, unlike the fermentum starter, is homofermentative, and its fermentation metabolites are specifically lactic acid [6]. In this regard, Zare et al. (2021) investigated the possibility of improving the quantitative and qualitative properties of toast bread using sourdough prepared from kombucha beverage and soy milk and Lactobacillus fermentum and Lactobacillus plantarum starters and stated that the lactic acid content of the product was higher when using sourdough containing the fermentum starter compared to the plantarum starter [28].

3-2-5- Specific Volume

The results of the evaluation of specific volume showed that the samples containing sourdough prepared from combined starters had the lowest specific volume and the samples containing sourdough prepared from quinoa flour had the highest specific volume (Figure 2).

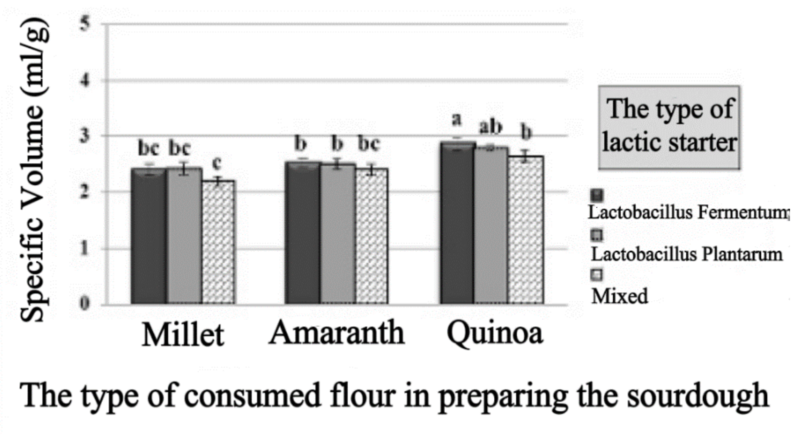

Figure 2- The effect of the type of consumed flour and the type of lactic starter in preparing the sourdough on the rate of specific volume of molded gluten free bread (Similar letters have no significant difference in *p<0/05* statically)

In relation to the decrease in specific volume of the product with the use of combined sourdough, it can be said that the presence of two combined starters simultaneously in the sourdough has a synergistic effect and increases the activity of the bacteria used. The results also showed that the specific volume of the product was slightly lower when using sourdough containing the plantarum starter compared to the fermentum starter. In bakery products, gluten protein is the main component responsible for creating the air bubble holding network, and since this component is not present in gluten-free loaf bread. Therefore, the starch present in the flours used can act as an important factor in creating texture [29]]30]. Here, it seems that with the use of sourdough containing combined starters in the formulation, because this combination probably has more enzymatic activity compared to other treatments, part of the starch in the product dough is converted to simpler sugars during the fermentation process and its gas holding capacity is reduced, which ultimately leads to a decrease in the volume of the final product. Also, Diowksz, & Sadowska (2021) also studied the effect of adding sourdough (containing Lactobacillus plantarum and Lactobacillus brevis) and transglutaminase on the quality of gluten-free bread prepared from buckwheat and stated that the specific volume of the product decreased with the addition of sourdough in the formulation [31]. In this regard, they stated that the activity of lactic acid bacteria and yeasts probably reduced the specific volume of gluten-free bread prepared from buckwheat. Also, Moroni et al. (2011) and Scarnato et al. (2016) stated that the pH decrease caused by the activity of lactic acid bacteria limits alcoholic fermentation caused by the activity of yeasts and limits the production of carbon dioxide [32] [33].

On the other hand, quinoa flour, due to its higher protein content than amaranth and millet flour, was able to retain more air bubbles. In this regard, Jaldani et al. (2019) optimized the formulation of gluten-free cake containing rice, quinoa, and purslane flour and stated that the product containing quinoa flour had a higher specific volume than the control sample [14]. They stated that the increase in the volume of enriched cakes is due to the creation of higher viscosity by quinoa flour due to improved water and gas distribution in the dough and trapping more gas bubbles in the cake.

3-2-6- Porosity

Based on the results, it was found that the type of flour and starters used had a significant effect on the porosity of gluten-free loaf bread ($P<0.05$), with the use of combined sourdough reducing the porosity of the product. Also, samples containing sourdough prepared from quinoa flour had the highest porosity (Figure 3).

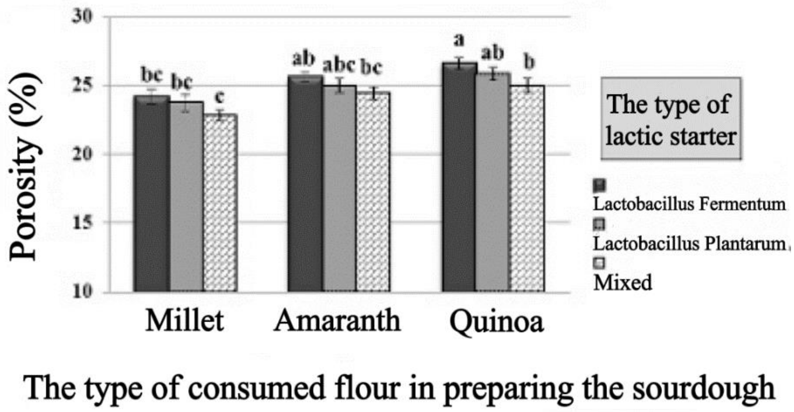

Figure 3- The effect of the type of consumed flour and the type of lactic starter in preparing the sourdough on the rate of porosity of molded gluten free bread (Similar letters have no significant difference in *p<0/05* statically)

One of the important parameters in bakery products is porosity, which generally refers to the pore structure in the core of the product and is considered one of the factors affecting the quality characteristics of the product [15]. In this line, Ayoubi (2018) stated that a porous structure is created through the expansion of air bubbles and an increase in volume during the baking process. Therefore, considering the relationship between specific volume and porosity of bakery products, it was expected that the use of combined sourdough would reduce the porosity of the product [34]. As mentioned in the evaluation of specific volume, it seems that with the use of sourdough containing combined starters in the formulation, because this combination probably has more enzymatic activity compared to other treatments. On the other hand, Elgeti et al. (2014) also studied the improvement of volume and texture of gluten-free bread using white quinoa flour and stated that with increasing the amount of quinoa flour in the formulation, the porosity of the product increases [35].

3-2-7- Crust Color

The results of the effect of the type of flour used (millet, amaranth, and quinoa) and the type of lactic starter (Lactobacillus fermentum and Lactobacillus plantarum alone and in combination) on the color values of the crust of gluten-free loaf bread are shown in Table 3.

Table 3- The interaction of the type of consumed flour and the type of lactic starter in preparing the sourdough on the crust color of molded gluten free bread

The type of consumed flour in preparing the sourdough	The type of lactic starter	Crust color (-)		
		L*	a*	b*
Millet	*Lactobacillus Fermentum*	41.4 ± 0.8[b]	8.54 ± 0.2[a]	24.3 ± 0.8[b]
	Lactobacillus Plantarum	41.3 ± 0.9[b]	8.50 ± 0.2[a]	24.6 ± 0.5[b]
	Mixed	41.4 ± 1.0[b]	8.52 ± 0.1[a]	24.6 ± 0.6[b]
Amaranth	*Lactobacillus Fermentum*	45.6 ± 1.1[ab]	8.58 ± 0.2[a]	24.8 ± 0.9[b]
	Lactobacillus Plantarum	45.3 ± 0.9[ab]	8.59 ± 0.2[a]	24.8 ± 0.7[b]
	Mixed	45.2 ± 1.0[ab]	8.58 ± 0.1[a]	24.7 ± 0.3[b]
Quinoa	*Lactobacillus Fermentum*	47.8 ± 0.12[a]	8.56 ± 0.1[a]	25.7 ± 0.8[a]
	Lactobacillus Plantarum	47.7 ± 2.2[a]	8.58 ± 0.2[a]	25.8 ± 1.2[a]
	Mixed	47.7 ± 0.6[a]	8.53 ± 0.2[a]	25.7 ± 0.9[a]

Data is in the form of average ± Standard deviation.

Different letters in each column have significant difference in *p<0/05* statically.

According to the results, it was found that only the type of flour used had a significant effect on the L* and b* values of the crust of gluten-free loaf bread ($P<0.05$), while the type of starters used did not have a significant effect on the amount of these parameters. Also, it was observed that the samples containing sourdough prepared from quinoa flour had the highest L* and b* crust values among the samples studied.

Here, it seems that quinoa flour, due to its higher protein content than amaranth and millet flour, was able to retain more moisture, which resulted in slower release of moisture from the inside of the product to the surface and the product surface had fewer wrinkles. In this regard, Purlis & Salvadori (2009) stated that changes in the surface of food are responsible for its brightness, and smooth and regular surfaces reflect more light than wrinkled surfaces and increase the L* value [36]. While the changes in the b* value can be attributed to the pigments present in these grains. In addition, it was observed that the type of flour and the type of starters used did not have a significant effect on the a* value of the crust of gluten-free loaf bread ($P<0.05$).

3-2-8- Bread Firmness

The results of the effect of the type of flour used and the type of lactic starter (in the preparation of sourdough) on the firmness of gluten-free loaf bread during storage showed that the samples containing sourdough prepared from combined starters and quinoa flour had the least firmness (Table 4).

Factors such as moisture content, specific volume, and porosity are involved in the firmness of the texture of bakery products in the time period immediately after baking. However, the most important factor in increasing the firmness of the texture of these products during storage is the preservation and retention of moisture, as this has a significant impact on the staling of the product and the increase in its firmness during storage [37]. In this regard, Zeleznak & Hoseney (1986) also stated that the phenomenon of staling in bakery products such as bread is related to the moisture content and the performance of the water present in the core of these products [38]. Therefore, it was predictable that the samples containing sourdough prepared from combined starters and quinoa flour would have the least firmness and the firmness number reported by the texture analyzer, which shows the force required to compress the texture, would be lower. Since these samples had a higher moisture content. Of course, according to the results, it is observed that with the passage of time from 2 hours to 72 hours and finally 7 days after baking, the firmness of the texture increased, which indicates an increase in staling over time.

Table 4- The interaction of the type of consumed flour and the type of lactic starter in preparing the sourdough on the texture firmness of molded gluten free bread during storage time

The type of consumed flour in preparing the sourdough	The type of lactic starter	Firmness (N)		
		2 hours after baking	3 days after baking	7 days after baking
Millet	*Lactobacillus Fermentum*	1.0 ± 19.12^{abB}	3.0 ± 74.08^{aAB}	4.0 ± 97.14^{aA}
	Lactobacillus Plantarum	2.0 ± 02.15^{aB}	3.0 ± 83.10^{aAB}	5.0 ± 11.15^{aA}
	Mixed	1.0 ± 65.08^{bcB}	3.0 ± 58.12^{abAB}	4.0 ± 23.12^{bcA}
Amaranth	*Lactobacillus Fermentum*	1.0 ± 72.09^{abcB}	3.0 ± 31.15^{bAB}	4.0 ± 32.20^{bcA}
	Lactobacillus Plantarum	1.0 ± 76.10^{abcB}	3.0 ± 55.00^{abAB}	4.0 ± 68.10^{bA}
	Mixed	1.0 ± 59.13^{bcB}	3.0 ± 22.06^{bcAB}	4.0 ± 17.09^{bcA}
Quinoa	*Lactobacillus Fermentum*	1.0 ± 68.12^{bcB}	3.0 ± 26.08^{bcAB}	4.0 ± 27.08^{bcA}
	Lactobacillus Plantarum	1.0 ± 72.09^{bcB}	3.0 ± 40.06^{bAB}	4.0 ± 51.12^{bA}
	Mixed	1.0 ± 35.06^{dB}	3.0 ± 15.05^{cAB}	4.0 ± 03.10^{cA}

Data is in the form of average ± Standard deviation.
Different English lowercase letters in each column have significant difference in *p<0/05* statically.
Different English capital letters in each row have significant difference in *p<0/05* statically.

In addition, examination of the results of this section showed that adding sourdough containing Lactobacillus fermentum starter (compared to plantarum starter) to the formulation of gluten-free loaf bread reduces the firmness of the product's texture, which was also predictable based on the moisture and specific volume evaluation of the product. Due to the production of lactic acid and increased activity of the alpha-amylase enzyme, starch, the reversion of its crystalline state is the main cause of staling, is broken down and low molecular weight dextrins are produced, which itself can be effective in reducing bread staling. Wolter et al. (2014) also studied the effect of using sourdough containing Lactobacillus plantarum (at a level of 20% flour weight) on the baking and sensory properties of gluten-free breads containing quinoa and buckwheat flours and observed that the firmness of the product containing sourdough was lower than the samples without sourdough [26].
On the other hand, it was observed that the samples containing sourdough prepared from quinoa flour had the least firmness among the samples studied. As mentioned in the moisture evaluation section, it seems that quinoa flour, due to its higher protein content than amaranth and millet flour, was able to retain more moisture, and then the samples containing amaranth flour had less firmness than the samples containing millet flour. In this regard, Elgeti et al. (2014) studied the improvement of volume and texture of gluten-free bread using white quinoa flour and stated that with increasing the amount of quinoa flour in the formulation, the firmness of the product texture decreases [35].

3-2-9- Overall Acceptance in the sensory evaluation

The results of the evaluation of the sensory properties of gluten-free loaf bread showed that the use of sourdough prepared with combined starters and quinoa flour in the formulation of gluten-free loaf bread significantly increased the overall acceptance score of the final product and the satisfaction of the taste judges (P<0.05) (Figure 4).

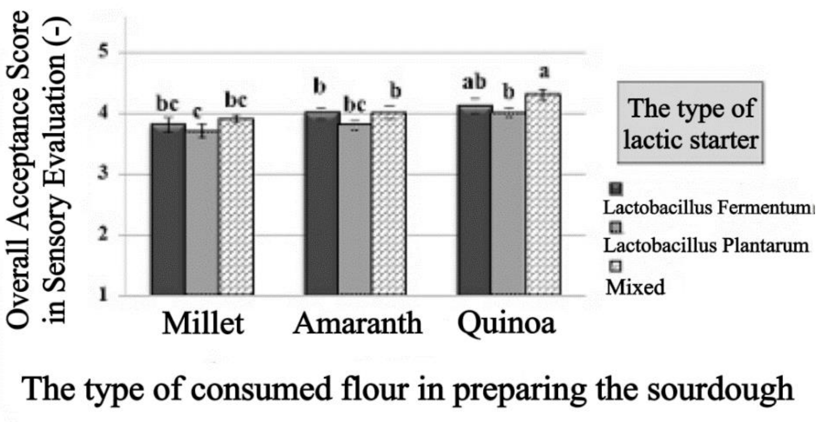

Figure 4- The effect of the type of consumed flour and the type of lactic starter in preparing the sourdough on the acceptance score of molded gluten free bread (Similar letters have no significant difference in *p<0/05* statically)

In general, the score for the form and shape of the product is determined by the sensory evaluators based on parameters such as symmetry or asymmetry, tearing or loss of part of the bread (crust and crumb), the presence of any holes or voids (tunneling phenomenon), etc. In this regard, the evaluators stated that the sample containing sourdough prepared from a combined starter and millet flour was given a higher score due to its uniform texture and symmetry, which was not unexpected given the results of the evaluation of specific volume, porosity, and firmness of the texture of the gluten-free loaf bread samples. Also, in the sensory evaluation of the crust characteristics, the samples were evaluated based on burning, unnatural color, wrinkles, and an abnormal surface. In this regard, the evaluators stated that the sample prepared from sourdough containing Lactobacillus plantarum starter and millet flour was given a lower score due to its dark color and somewhat wrinkled appearance.

In the sensory evaluation of the crumb and porosity of the samples, the presence of abnormal voids and excessive density and compactness of the texture in such a way that the porosity of the crumb of the samples was hidden from the consumer resulted in a deduction of points. In this section, the taste judges stated that the sample prepared from sourdough containing Lactobacillus plantarum starter and millet flour had more irregular holes and that these holes were not evenly distributed throughout the texture of the product, which resulted in the deduction of points for crumb and porosity of the mentioned sample.

In the sensory test to score the texture of the product, gooeyness or abnormal softness, excessive firmness, crispness, and brittleness resulted in a deduction of points. Therefore, based on the results obtained from the evaluation of the texture during each of the three evaluation time periods, it was expected that the taste judges would give a higher score to the sample prepared from sourdough containing a combined starter and quinoa flour, as this sample had less firmness than the other samples.

Another important parameter in sensory evaluation is the flavor of the final product, and having a desirable taste and aroma plays a key role in the acceptance of this product. In this regard, the sensory evaluators stated that the sample prepared from sourdough containing Lactobacillus plantarum starter and millet flour had a less pleasant taste in the mouth due to its firmer texture, and therefore the mentioned sample was given a lower score. Finally, from the total sensory characteristics scores, it was observed that the use of combined starters and quinoa flour in the formulation of gluten-free loaf bread significantly increased the overall acceptance score of the product ($P<0.05$).

4- Conclusion

Considering the importance of improving the quality characteristics of bakery products using modified fermentation methods and eliminating chemical compounds and additives from the formulation of this category of food, the production of gluten-free sourdough is one of the best and most practical methods for improving the quality characteristics of gluten-free products. For this purpose, high-nutritional value cereal and pseudo-cereal flours were used along with different lactic starters, and finally the gluten-free loaf bread sample prepared from sourdough containing a combination of two lactic starters, Lactobacillus fermentum and Lactobacillus plantarum, and quinoa flour was introduced as the best sample.

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مقاله علمی_پژوهشی

تولید خمیرترش بدون گلوتن با استفاده از آرد ارزن، کینوآ و آمارنت و آغازگرهای *لاکتوباسیلوس فرمنتوم* و *لاکتوباسیلوس پلانتاروم*

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اطلاعات مقاله

تاریخ های مقاله :

تاریخ دریافت: ۱٤۰۲/۱۰/۳

تاریخ پذیرش: ۱٤۰۲/۱۱/۲۷

کلمات کلیدی:

آغازگر،

خمیرترش،

بدون گلوتن،

شبه غلات

DOI:10.22034/FSCT.21.154.90.

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چکیده

نان‌های تولید شده از خمیرترش دارای بافتی سبک و کیفیت جویدنی بالاتری هستند. این در حالی است که در فرمولاسیون محصولات بدون گلوتن نمی‌توان از خمیرترش تهیه شده از آرد گندم و آردهای حاوی گلوتن استفاده نمود. از این‌رو در پژوهش حاضر از آرد ارزن، آمارنت و کینوآ و آغازگرهای *لاکتوباسیلوس فرمنتوم* و *لاکتوباسیلوس پلانتاروم* به صورت تک و ترکیبی در فرمولاسیون خمیرترش استفاده و به میزان ۱۰ درصد به خمیر نان قالبی بدون گلوتن افزوده شد و خصوصیات کمی و کیفی نمونه‌های تولیدی در یک طرح کاملاً تصادفی با آرایش فاکتوریل مورد ارزیابی قرار گرفت ($P\leq$۰/۰۵). نتایج ارزیابی میزان رطوبت نشان داد دو نمونه تهیه شده از خمیرترش حاوی آغازگر ترکیبی و آرد کینوا و نمونه تهیه شده از خمیرترش حاوی آغازگر *لاکتوباسیلوس پلانتاروم* و آرد ارزن به ترتیب از بیشترین و کمترین میزان رطوبت برخوردار بودند. همچنین در بین نمونه‌های تولیدی، نمونه‌های حاوی خمیرترش تهیه شده از آرد کینوا از میزان اسیدیته و اسیدهای آلی بیشتری برخوردار بودند. در بخش ارزیابی میزان تخلخل و حجم مخصوص بیشرین میزان به نمونه حاوی آغازگر فرمنتوم و آرد کینوآ اختصاص یافت. همچنین نتایج ارزیابی بافت نشان داد که نمونه‌های تهیه شده از خمیرترش حاوی آغازگر ترکیبی و آرد کینوآ از کمترین میزان سفتی بافت برخوردار بودند. از طرفی بررسی مؤلفه‌های رنگی پوسته نان نشان داد با استفاده از خمیرترش تهیه شده از آرد کینوآ در فرمولاسیون نان قالبی بدون گلوتن میزان مؤلفه‌های L^* و b^* پوسته افزایش یافت. درحالیکه نوع آغازگر مورد استفاده تأثیر معنی‌داری بر میزان این پارامترها نداشت. در نهایت بیشترین امتیاز پذیرش کلی به نمونه تهیه شده از خمیرترش حاوی آغازگر ترکیبی و آرد کینوا اختصاص داده شد.