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SOURDOUGH FERMENTATION IN GLUTEN-FREE BREAD: A SHELF-LIFE IMPROVEMENT

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Abstract

Nowadays, the demand for high quality gluten-free (GF) products is growing, especially for bread. New approaches are necessary, such as finding new combinations of GF flours or different fermentation processes. Sourdough is one of the fermentation methods used in baking industry which showed an improvement to the quality of GF bread (texture, palatability, aroma, shelf life). The research aimed to improve the GF bread characteristics based on buckwheat flour, quinoa flakes and pea protein powder using two types of lactic acid bacteria (LAB). Three samples of GF formulation were developed: S1 with the addition of Lactobacillus plantarum (0.6%), S2 with Lactobacillus sanfranciscensis (0.6%) and a control without LAB. Breads were evaluated for their physico-chemical, microbiological and sensory characteristics. The best acceptance score was for S2 followed by S1 and control. The colour measurement showed similar values, while the crumb texture recorded diversified levels with S1 being the hardest. The microbiological analysis showed an increase in the bread shelf life from 3 days (control) to 7 days (S2). This study underlined that both shelf life and taste of GF bread have been improved by using LAB.

Key words*: gluten-free bread, lactic acid bacteria, sourdough, buckwheat, quinoa.*

INTRODUCTION

Celiac disease is an inflammatory condition induced by the ingestion of gluten to people who are genetically susceptible and affects approximately 1% of the global population (Catassi & Fasano, 2008). Celiac disease has no treatment yet, the only thing to keep the disease under control is following a strict gluten-free (GF) diet. Gluten is an essential element which forms the structure of wheat bread and confers high quality cereal-based goods. This also offers the dough its unique viscoelasticity, cohesiveness and elasticity, so, the gluten absence causes a liquid batter and several defects in baked products such as low loaf volume, dry crumbling texture, poor mouth feel and poor flavour (Gobbetti et al., 2008). Besides these, following a strict gluten-free diet leads to an unbalance input of the main nutrients (proteins, fats, carbohydrates) and the lack of some macro- and microelements and vitamins (Ozkan et al., 2012; Simpson & Thompson, 2012). The patience could also have a lack of dietary fiber, iron, unsaturated fatty acids, calcium and vitamins (such as B12, A, D, E, K) (Hopman et al., 2006). Therefore, researchers face multiple challenges to make gluten-free products and try in multiple ways to overcome any difficulties. One of these is the diversification of raw materials replacing rice flour and corn flour, which are the most used with others such as cereals (sorghum), pseudo-cereals (buckwheat, quinoa, amaranth), minor cereals (teff, millet) and legumes (soybean, chickpea, lentil, pea). Besides these, other ingredients are used to diversify and balance nutrients in gluten-free diet, such as: seeds flour (flax seeds, chia seeds, pumpkin seeds), nut flour (almonds, hazelnuts, chestnuts, walnut, cashew nut) and tubers flour (arrowroot, tapioca, jicama, taro, potato). Another issue in developing gluten-free products is the taste and the flavour. According to Kenny et al. (2001), the incorporation of dairy ingredients increased calcium content and protein efficiency ratio and also improved the flavour and the texture. The addition of milk

proteins in GF bread formulations showed an improvement in loaf volume and crumb texture and also delayed the staling of the bread (Ahlborn et al., 2005; Gallagher et al., 2003; Moore et al., 2004). The addition of protein from eggs is also used for improving the quality. The use of enzymes (cyclodextrin glycosyl, glucose oxidase) for improving the quality of GF bread has also been attempted. The addition of enzymes improved the functional properties of the flour and also, the crumb structure. However, these should be avoided or used in a low concentration. Dairy ingredients, due to the incidence of lactose intolerance, together with egg proteins intolerance, may cause allergies among celiac patients (Poulsen et al., 2001; Ojetti et al., 2005). In addition, consumers are skeptical about the use of enzymes or additives in food industry. These do not meet the consumers' requirements for natural products. Thus, the researchers had to find an alternative technology for producing high quality GF bread (Moroni et al., 2009). They discovered that sourdough solves many problems related to the production of high quality GF bread. Sourdough is a mixture of flour and water fermented with lactic acid bacteria (LAB) and yeasts (Hammes and Ganzle, 1998).

Lactic acid bacteria (LAB) represent a heterogeneous group of industrially important bacteria which contributes producing fermented food and beverages (Mozzi, 2016). LAB belongs to the *phylum Firmicutes*, class Bacilli, order *Lactobacillales* according to the current taxonomic classification. The most important advantage of using LAB is that these are recognized as being safe (Zamfir & Grosu, 2014).

LAB has been used empirically for preservation and production of fermented foods of plant or animal origin since ancient times and since 1930s, LAB started to be used as lactic starter cultures in the fermented food industry and later as probiotics (Mozzi, 2016).

De Vuyst & Vandamme shown the improvement of the shelf life of fermented foods due to lactic acid bacteria since 1994. The improvement is due to production of a large variety of compounds such as organic acids, ethanol, hydrogen peroxide, bacteriocins, antibiotic-like peptides. They act in a synergically way to prevent or remove microbial contamination. Besides antimicrobial effect, LAB also contributes to the improvement of organoleptic qualities offering a characteristic flavor to the fermented products. Furthermore, using beneficial microorganisms leads to increased health benefits (Leroy & De Vuyst, 2004; Mozzi, 2016).

Sourdough production and consumption have been documented Before Christ (Adrrario, 2002). Egyptians were the first who mixed flour and water, left it ferment and then added it to fresh dough before baking (Cappelle et al., 2013). After them, ancient greek took the technique in 800 BC (Moiraghi, 2002).

Sourdough is a mixture of flour usually from wheat (*Triticum* spp.) or rye (*Secale cereale* L.), water, salt with the addition of lactic acid bacteria (LAB) and yeasts which lead to the fermentation process. Besides wheat and rye other cereals such as maize (*Zea may*s L.), spelt (*Triticum aestivum* subsp. *spelta* L.), barley (*Hordeum vulgare* L.) and oat (*Avena sativa* L.) are used nowadays in baking industry (De Vuyst et al., 2017).

Therefore, this study aimed to investigate the influence of sourdough on gluten-free bread and the reaction of *Lactobacillus plantarum* and *Lactobacillus sanfranciscensis* to buckwheat dough.

MATERIALS AND METHODS

Materials

Both quinoa flakes and pea protein powder were bought from Paradisul Verde (Romania) while buckwheat flour was purchased from Eurokalis. The LAB starters (*Lactobacillus plantarum* and *Lactobacillus sanfranciscensis*) used in this study were provided by Millbo (Italy).

Bread formulations

The main ingredients used for developing gluten-free breads were quinoa flakes, buckwheat flour and pea protein powder. Besides these, two samples were prepared with LAB: sample 1 (S1) with *Lactobacillus plantarum* and sample 2 (S2) with *Lactobacillus sanfranciscensis*.

The bread developing process consisted of the following: day 1-mixing the ingredients (quinoa flakes, buckwheat flour and pea protein powder) for sourdough preparation. After that water and LAB starter were added to the mixture (Table 1). The composition was incubated at 35°C, 80% relative humidity (RH) for 24 h; day 2-weighing and mixing the ingredients for dough making (mixture, salt, yeast, sourdough) (Table 2). The ingredients were mixed using DOMOCLIP DOP150R stand mixer. In addition to S1 and S2, a control sample (without sourdough) was considered. The composition (500 g) is placed in trays covered with baking paper and then were proofed in a proofer (leavening cell M.C.E. Meccanica) at 35°C, 80% RH for 20 min. Four bread were made from each batch. In addition, there was an extra amount of dough left. The final step consisted in the bread baking in a preheated oven at 180°C for 30 min. After cooling, breads were stored in plastic bags at room temperature.

Table 1. The ingredients for sourdough preparation

Ingredients	S1	S2
Buckwheat flour	100 g	100 g
Quinoa flakes	50 g	50 g
Pea protein powder	50 g	50 g
Water	300 ml	300 ml
Lactobacillus plantarum	3 g	-
Lactobacillus sanfranciscensis	-	3g

Table 2. The main ingredients of bread formulations

Ingredients	Control	S1	S2
Sourdough	-	200 g	200 g
Buckwheat flour	500 g	500 g	500 g
Quinoa flakes	250 g	250 g	250 g
Pea protein powder	250 g	250 g	250 g
Water	1500 ml	1350 ml	1350 ml
Salt	20 g	20 g	20 g
Yeast	25g	25g	25g

Microbiological analysis

Yeasts and molds

The method for number of yeasts and molds determination was performed according to SR ISO 21527-1: 2009. This method involves the determination of microbiological contamination with yeasts and molds of food products with water activity greater than 0.95. The analysis method was used to determine the microbial load of the packaged bread samples. Using a sterile pipette, inoculate 0.1 ml of the sample from the initial dilution of the sample into a Petri dish with Dichloran Rose-Bengal Chloramphenicol (DRBC) agar then a quantity of 0.1 ml of the 10^{-2} dilution was performed. The liquid was distributed on the surface of the Petri dish with agar using a sterile stick until the liquid is completely absorbed into the medium. A control dish, with an average of 15 ml, to check sterility was prepared. The inoculated plates were placed with the lid down in the thermostat at 25°C ± 1°C for 5 days.

Between two and five days of incubation, the colonies in each Petri dish were counted. After five days, those dishes containing less than 150 colonies were retained. The determination of the number of yeasts and molds per gram of product (N) after reading the colonies raised on selective media was performed by applying the formula:

$$N = \frac{\Sigma C}{(n_1 + 0{,}1 n_2) \times d}$$

in which:

- ΣC = sum of colonies counted in all retained dishes;
- n_1 = number of dishes retained at first dilution;
- n_2 = number of dishes retained at the second dilution;
- d = dilution from which the first counts were made.

The result is expressed as a number between 1.0 and 9.9 multiplied by 10^{X}. If there is no colony in the dishes corresponding to the initial suspension, where the initial product was solid, the number of yeasts and molds per gram of product is reported as less than 10. The following equipment was used to prepare the sample Automated Diluters- Dilumat START (US), Peristaltic homogenizer - Stomacher 400 Circulator (UK) and a bacteriological hood with vertical laminar flow-FASTER VS - 4 for Petri dish incubation.

Sensory analysis

Sensory analysis was performed by 18 people (11 females and 7 males, 25-60 years old) from National Institute of Research and Development for Food Bioresources - IBA

Bucharest. The sensory analysis followed the attributes such as exterior and interior appearance, aroma, taste and aftertaste using a descriptive test with a 5-point scale. Marks consisted on ratings between to 0 (unnoticed, slight, irregular) to 5 (intense attribute, colorful, flavoured). After each sample people cleaned up their mouth with water.

Colour measurement

Colour analysis of the bread crumb was performed using CM-5 Konica Minolta colorimeter. This method is based on the interpretation of three parameters such as parameter L* which measures the sample brightness on a scale from 0 to 100 (0 value represents black and value 100 represents white); parameter a* represents the sample color on the scale from pure green to pure red (the negative values are green, the positive values are red and 0 is neutral) and parameter b* represents the sample position on a scale from pure blue to pure yellow (the negative values are blue, the positive values are yellow and 0 is neutral).

Texture measurement

Textural analysis was determined using an Instron Texture Analyzer (5944, Illinois Tool Works Inc., SUA). First, 2 cm slices were cut from the middle of the bread. After that, the texture device was calibrated, then a test was ran by placing the bread slice sample on the platform of the texture analyzer. Determination were performed four times. This method consists of a cycle of compression in the middle of each slice of bread up to a distance of 50% from the height of the slice. The setting parameters were: compression speed: 12 mm/min; load cell: 50 N. The Bluehill 3.13 program calculated the texture parameter firmness (or hardness) which represents the maximum force (expressed in N) during the bread compression.

Physico-chemical analysis

Chemical composition of bread

Chemical composition of bread was determined according to the Association Official of Analytical Chemists (AOAC, 2005) methods for ash, fat, protein and total dietary fiber content. These analysis were performed to calculate the energy value. For the energy value calculation, the following conversion factors were taken into account: 9 for fat, 4 for carbohydrates, 4 for protein and 2 for fibre for kcal/100 g value, 17 for protein and carbohydrates, 37 for fat and 8 for fiber for KJ/100 g determination.

Water activity

The value of water activity (aw) for food is an essential criterion for the microbiological control of products. Water activity is defined as follows: when a hygroscopic material is placed in a closed chamber, a balance will be achieved between the material and air above it. Relative humidity, which occurs at a constant air temperature, corresponds to the value of water activity multiplied by 100 (aw = relative humidity (%)/100). Aquaspector AQS-2-TC was used for water activity determination. The sample was placed in a polypropylene box and was introduced in the special place of the device. The measured values of the samples were read to 3rd decimal place.

Moisture content

This procedure consists in sprinkling 5 grams of sample on the entire surface of the moisture analyzer tray without pressing. The equipment used was METTLER TOLEDO, model HE73 at 130°C.

pH values

WTW inoLab 7110, pH-meter and SenTix Sp-T 900 were used to measure the dough pH values. The pH-meter was calibrated using three standard pH buffer solutions (pH 4, pH 7 and pH 10).

Bread Volume

The volume was determined using the Fornet method as follows: the volume of rapeseed displaced by the bread product was measured and then it was reported per 100 g of product. After placing the sample the container was sealed and than basculated. The zero point position was verified 3 times and the maximum differences of 3 successive measurements have to be less than 30 cm^3.

The product volume (cm^3) at 100 g product was calculated using the formula:

$$V = \frac{V_1}{m} * 100 \text{ } (cm^3/100 \text{ g product})$$

where:
V_1 - the measured volume of the analyzed sample, in cm^3;
m - the bread sample mass in grams;
The result was calculated using one decimal and rounds up to a whole number (SR 91, 2007).

RESULTS AND DISCUSSIONS

Microbiological analysis

Yeasts and molds

Each bread sample was microbiological analyzed following the presence of yeasts and molds, a microbiological parameter specific to bakery products. The samples were analyzed at 24 h, 3 days, 5 days and 7 days after manufacture. The results of the initial microbiological analysis showed that the samples were compliant. At 24 h after manufacture, neither of bread samples showed yeasts and molds. On the 3rd day, the control presented a contamination of 6.2 x 10^2 cfu/g degree (Table 3), while the maximum limit allowed according to Order no. 27/2011 of ANSVSA is 1 x 10^2 cfu/g. S1 was compliant until the 5th day of storage. S2 recorded the most satisfactory results during the storage period (7 days shelf life). The differences between the samples and control are due to the lactic acid bacteria used in the formulations.

Table 3. The presence of yeast and molds

Bread samples	1 day	3 days	5 days	7 days
Control	<10	6.2 x 10^2	1.5 x 10^3	2.2 x 10^3
S1	<10	<10	2.0 x 10^1	1.2 x 10^2
S2	<10	<10	<10	<10

Sensory analysis

After performing the sensory analysis, the following results were obtained: the crust and crumb colour were similar, with small differences between them. Regarding the crust texture and crumb elasticity, no significant differences were registered. Considering the uniformity of the pores the most appreciated bread was S2 with the addition of *L. sanfranciscensis* sourdough followed by S1 with *L. plantarum*. Also, Jagelaviciute and Cizeikiene (2020) reported a higher porosity on sourdough bread. The following attributes were considered for flavor evaluation: yeast and cereal aroma. The cereal aroma registered a medium level. An important fact that can be observed is that the yeast flavour has low scores which means that it does not negatively influence the GF bread taste (Table 4). The results obtained for the sweet and salty attributes are similar. This fact demonstrates that the lactic acid bacteria used in this study do not influence the formulation for sugar and salt taste. However, we can observe that the LAB slightly influenced the sour taste (the most intense for S1). LAB improved the GB bread taste. The bitterness of quinoa flakes and the predominant taste of buckwheat were hidden due to the addition of LAB. For sensory analysis at the first bite, the following sensory attributes were taken into account: firmness, chewiness and moisture crumb. The results had similar values but it should be mentioned that a larger amount of water was added to develop the control because S1 and S2 compensated by adding liquid sourdough. Also, probably the differences would have occurred if all the samples had the same amount of water. The amounts of water were different in order to maintain the same texture of the dough. Moore et al. (2007) discovered that the chemical acidification led to a more fluid-like gluten-free dough than the control dough, whereas the use of a biological acidifier (dough obtained using sourdough) led to a significant increase in dough firmness (Moore et al., 2008). The following attributes, cohesiveness and mass adhesion were analyzed during chewing and referred to the mouth agglomeration degree and crumb teeth adhesion. All three samples had similar scores; moreover, the control and S2 had identical results. The last analyzed attribute was the aftertaste. All the results were similar but the highest value belonged to S2 (2.77). When people were asked which sample they preferred, 9 people have chosen S2, 5 people S1 and 4 of them answered the control is their favourite GF bread sample.

Table 4. The sensorial attributes analysed in GF bread sample

Sensorial attributes		Sample scores		
		234	**567**	**890**
EXTERIOR APPEARANCE	Crust colour	3.00	3.11	2.86
	Crust texture	3.02	3.16	2.80
INTERIOR APPEARANCE	Crumb colour	3.33	3.16	2.88
	Pores uniformity	2.93	3.50	4.21
	Crumb elasticity	2.61	2.27	2.63
AROMA/FLAVOUR	Cereal	2.61	2.94	2.63
	Yeast	1.16	1.22	1.36
TASTE	Sweet	0.88	0.94	0.91
	Salty	1.44	1.52	1.38
	Sour	0.61	2.16	1.80
	Bitter	3.25	1.50	0.91
FIRST BITE	Firmness	2.27	2.55	2.63
	Gumminess	2.33	2.38	2.50
	Crumb moisture	3.86	3.75	3.86
CHEWING	Cohesiveness	2.94	2.77	2.94
	Adherence	2.00	1.94	2.00
	Aftertaste	2.58	2.52	2.77

*The sample names have been modified for not influencing the tasters so it has been chosen 234 for control, 567 for S1 (*Lactobacillus plantarum*) and 890 for S2 (*Lactobacillus sanfranciscensis*).

Colour measurement

The color results of the analyzed samples showed similar values. The raw materials were the same for each formulation excepted the sourdough addition, so it proves that sourdough did not impact on the bread colour (Table 5).

Table 5. The colour of developed gluten-free bread

	L*(D65)	a*(D65)	b*(D65)
Control	59.05	1.88	13.06
S1	59.58	1.75	14.19
S2	60.34	1.71	14.88

Texture measurement

The results indicated an increase in GF bread hardness with the addition of sourdough. There was a significant increase between control (6.08 N) and the other samples which contain sourdough. S1 and S2 recorded values of 10.4 N and 9.2 N, respectively. Moroni et al. (2011) showed a substantial increase in crumb hardness and crumb chewiness with the amount of sourdough in buckwheat gluten-free bread. The values increased from 7.85 for control to 12.12, 13.01 and 15.94 for a sourdough addition of 20%, 35% and 50%, respectively. According to Rozyło et al. (2015a, 2015b), the crumb hardness in buckwheat and amaranth sourdough bread had a significant decrease. They also showed that the hardness of control rice bread was much lower compared to the bread with buckwheat and amaranth addition (Rozyło et al., 2016). In research presented by Novotni et al. (2012), breads with 15 and 22.5% sourdough had the lowest value of initial firmness and reduced firming compared to the control bread.

Physico-chemical analysis

Chemical composition of bread

The addition of sourdough did not greatly influence the physico-chemical properties, so the values were quite close. The energy value of S1 was the highest compared to control which was the lowest, but this difference is insignificant (Table 7).

Water activity

According to Flückiger and Cleven (1978), the level of water activity for white bread should be 0.92. Despite with the fact that the water activity result was higher (Table 6), the gluten-free bread samples showed good results in terms of contamination with yeasts and molds. Although water activity values were similar, the yeasts and molds concentration was different between samples. Control was first contaminated, on the 3rd day. It was noticed that the addition of lactic acid bacteria has slowed down the contamination process. *Lactobacillus plantarum* extended the shelf life up to 5 days and *Lactobacillus sanfranciscensis* up to 7 days. According to Moroni et al., (2011) the addition of 20% sourdough on gluten-free buckwheat bread positively influenced the staling rate. Otherwise, the incorporation of 50% sourdough led to a slight increase in staling rate during storage.

Table 6. The water activity values

Bread samples	1 day	3 days	5 days	7 days
Control	0.967	0.988	0.986	0.972
S1	0.985	0.987	0.985	0.989
S2	0.986	0.984	0.986	0.998

Moisture content

All the analyzed samples (gluten-free buckwheat bread) have a higher value of moisture content than normal bread (maximum 45%). The highest level was recorded for control (61.54%), followed by S2 (59.65%) and then S1 (58.03%). These high moisture values resulted due to the addition of a higher water content for a suitable dough developing.

pH value

The sourdough influenced the pH dough, this decreased compared to the control (6.00) to 5.65 for S1 and 5.59 for S2. According to Jagelaviciute and Cizeikiene (2020), the same trend was noticed for all the fermented sample. All the fermented sample (fermented chia dough; fermented hemp dough; fermented quinoa dough) have lower values than controls (control chia dough; control hemp dough; control quinoa dough;) with the following values 5.45, 5.21, 4.46 for fermented dough and 5.55, 5.75, 5.60 for control dough. According to another study on sourdough buckwheat bread, the pH also significantly decreased with the increasing level of sourdough (20%, 35%, 50%). After proofing, the control batter pH was 5.96 and the sourdough bread recorded lower values such as 5.15, 4.68 and 4.39, respectively (Moroni et al., 2011). Rozyło et al. (2016) also recorded differences between control and samples with sourdough. The pH decreased more with the amount of sourdough from 5.31 to control to 4.98 with 10%, 4.49 with 20%, 4.22 at 30% and 4.13 at 40%. Moore et al., (2008) reported a significant increase in crumb hardness during storage for all GF breads. However, the increase was higher for the chemically acidified and lower for *L. plantarum* and *L. sanfranciscensis* gluten-free breads, which means that sourdough helped in delaying the staling of gluten-free breads. Also, Corsetti et al. (1998) reported that fermentation by sourdough LAB, microbial hydrolysis of starch, and proteolysis influenced physicochemical changes during bread storage including a positive effect in delaying both bread firmness and staling.

Table 7. Chemical composition of gluten-free bread

Parameter	Control	1-*L. Plantarum*	2-*L. Sanfranciscensis*
Energetic value (kcal/100g)	**152**	**166**	**161**
Energetic value (kJ/100g)	**645**	**705**	**680**
Protein	12.74	12.69	12.70
Fat	1.18	1.26	1.30
Carbohydrates which:	22.28	25.49	24.12
-sugars	<1.0*	<1.0*	<1.0*
Fiber	0.77	1.14	0.75
Salt	1.20	1.20	1.10
Ash	1.49	1.39	1.48
Moisture	61.54	58.03	59.65

*quantification limit for sugars analysis
**all values are reported %

Bread volume

It has been noticed that LAB had a different influence on volume samples. S1 with the addition of *Lactobacillus plantarum* sourdough has a negative impact on bread volume compared to control. This decreased from 130 cm^3/ 100 g for control to 127 cm^3/ 100 g for S1. The greater volume was obtained for the bread S2 with *Lactobacillus sanfranciscensis* (135 cm^3/100) (Figure 1).

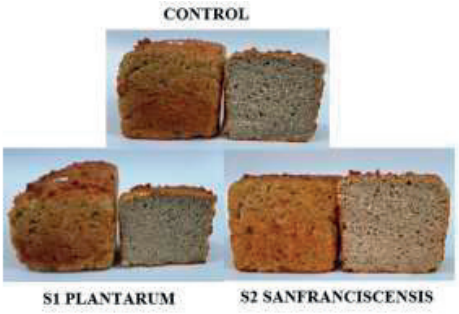

Figure 1. Gluten-free bread samples with buckwheat flour, quinoa flakes and pea protein

Rozylo et al. (2016) reported a volume increase from 167 cm^3/100 g for control to 170 cm^3/ 100 g for the sourdough sample. Novotni et al. (2012) and Moore et al. (2007, 2008) observed favourable changes in the volume of bread after sourdough addition.

CONCLUSIONS

The sourdough addition had a major impact on shelf life and taste of gluten-free bread. Thus, the bread shelf life was extended from 2 days (control) to 7 days (S2). Sourdough also improved the taste and flavour: reduced the bitter taste that comes from quinoa flakes and diminished the intensive buckwheat taste that felt too strong in case of control bread.

Thus, the use of *Lactobacillus plantarum* and *Lactobacillus sanfranciscensis* in bread formulations showed an improvement on both taste and extension of shelf life, but S2 with *L. sanfranciscensis* sourdough showed the best results and a better acceptance. Moreover, *Lactobacillus sanfranciscensis* had the best effect in increasing the gluten-free bread volume.

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