# Development of gluten-free bread biotechnology using fermented scald

*Olga* Parakhina*, *Lina* Kuznetsova, *Olesia* Savkina, *Marina* Lokachuk, *Marina* Kostyuchenko, *Elena* Pavlovskaya and *Tamara* Gavrilova

St.Petersburg branch Scientific Research Institute for the Baking Industry, Podbelskogo highway 7, 196608, St.Petersburg, Pushkin, Russian Federation

**Abstract.** The article is devoted to the development of gluten-free bread technology with scalded flour, fermented by lactic acid bacteria and yeast isolated previously from samples of good quality gluten-free sourdough. The species affiliation of new strains of lactic acid bacteria and yeast isolated from samples of good quality gluten-free starter cultures was established. The antagonistic and acid-forming activity of lactic acid bacteria and the fermentation activity of yeasts were investigated. An increase in the content of volatile acids in fermented scald and the acidity of finished products was established when using scald fermented with a heterofermentative strain *L.brevis E139*. As a result of the research, a biotechnology of fermented scald was developed, which makes it possible to obtain gluten-free bread with increased physical, chemical, organoleptic quality indicators and safety for diet therapy for celiac disease. The influence of the sourdough with a new microbial composition on the physico-chemical, organoleptic quality indicators of finished products and their resistance to mold and potato disease was studied.

## 1 Introduction

In recent decades, researchers have paid increasing attention to the development of gluten-free sourdough bread technologies. Sourdough is a mixture of flour and water fermented with lactic acid bacteria and yeasts [1-5], which may originate from flours, water and equipment or may be inoculated as industrial starter cultures of lactic acid bacteria and yeasts [2, 3, 6].

The use of sourdough in bread making technology is one of the main tools for improving the physico-chemical and organoleptic (taste and smell) indicators of the quality of bakery products, increasing their microbiological stability and slowing down the staling process during storage [1-7]. The application of sourdough for gluten-free bread offers improved textural advantages and improved viscosity and elasticity in gluten-free batters, and effects are dependent on the amount of added sourdough and on the lactobacilli used for fermentation [7-12]

However, not every company, especially small bakeries, artisans, restaurants, home bakers, is convenient to start sourdough in production. Considering that the production of

* Corresponding author: o.parakhina@gosniihp.ru

gluten-free products is not mass-produced, the development of a less labor- and energy-intensive, but no less effective technology is relevant and timely.

The aim of the work was to develop a biotechnology of gluten-free bakery products with high consumer properties and resistance to microbial spoilage on fermented scald.

## 2 Materials and methods

The development of technology for the preparation of fermented gluten-free scald included the following steps:
- preparation of saccharified flour scald using green buckwheat flour and hot water;
- fermentation of saccharified flour scald with pure cultures of lactic acid bacteria (LAB) and yeast.

In the preparation of saccharified flour scald, the following were used as saccharifying agents:

- barley malt in the amount of 5% by weight of flour in scald;
- enzyme " Alphalift" (based on fungal α-amylase and xylanase) at a dosage of 0.36% by weight of flour/

Scalds were prepared by mixing green buckwheat flour and water at a temperature of 95±2°C at a ratio of 1:3. After cooling of scald to a temperature of 63-65°C the barley malt or Alphalift was added , scald were thoroughly mixed and then it was kept for in a thermostat at a temperature of 50 - 55°C to saccharify. The saccharified scalds were cooled to a temperature of 30-35°C and examined for the content of sugars using the Bertrand's method [13].

Lactobacilli *L.brevis E139* and *L.plantarum* E138 were added to saccharified scalds at a temperature of 32-34℃. Lactobacilli were added alone or in a mixture with yeast *S.cerevisiae* Y205. The dosage of lactobacilli and yeast was carried out in accordance with previous studies [14]. Samples of fermented scalds were kept at a temperature of 32-34℃ for 24 hours.

Then the sourdoughs (fermented scalds) were propagated by refreshing saccharified scald with in a ratio sourdough: saccharified scald - 1:3. Temperature of fermentation was 32-34℃ for 20-24 hours.

### 2.1 Fermented scald and dough assessments

Fermented scald and doughs were evaluated for different parameters. Acidity was evaluated by titration with 0.1 N sodium hydroxide solution using phenolphthalein as indicator [15]. The content of volatile acids was determined by neutralizing the evaporated volatile acid using a 0.1 N. solution of NaOH [15]. The alcohol content was determined by using the iodometric method, which is based on the quantity of sodium thiosulfate spent in titration n [15]. To estimate the number of viable cells of microorganisms, 10g of fermented scald were homogenized with 90 ml of sterile chloride (0.9% wt·wol-1) solution. Serial dilutions were plated on MRS (de Man, Rogosa and Sharp) agar for determination of presumptive LAB. Plates were incubated at 30°C under anaerobic conditions. An AnaeroGen System (Oxoid, UK) was used to maintain an anaerobic environment. The number of yeast in sourdough was estimated by using malt agar at 30C for 72 h [16].

### 2.2 Bread-making procedure

Gluten-free mixture including from rice flour, green buckwheat flour, corn starch, potato starch, psyllium, hydroxypropyl methylcellulose and xanthan gum were used as flour.

Dough humidity was 52%. To do this, the dough was kneaded from a mixture of baking flour, consisting of rice flour (44%), green buckwheat flour (10%), corn starch (17%), potato starch (11%), tapioca starch (10 %), psyllium (5%), hydroxypropyl methylcellulose (2%), mono and diglycerides of fatty acids (0.5%) and xanthan gum (0.5%), with the addition of salt (1%), white sugar (3, 5%), baker's pressed yeast (2.5%), vegetable oil (5.0%) and water (107.0%). When mixing the experimental dough with the semi-finished product, 20% of green buckwheat flour was added from its total amount in the dough. Thus, the amount of flour (gluten-free mixture) and dough humidity was equal in all breads. The compressed yeasts and sourdough combination were used in order to obtain the best performance (Mariotti et al., 2017; Dubrovskaya et al., 2018).

The dough was mixed in a mixer Kitchen Aid KSM45 (USA) at 120 rev·min−1 for 7 min. 250g dough pieces were placed in baking forms and hold at 30°C until the duplicated in volume. Then dough was molded into dough pieces weighing 270 g, placed into molds and proofed in a proofer for 36–60 minutes at a temperature of 36–38°C and a relative air humidity of 75–85%. The spaced dough pieces were baked in a humidified baking chamber at 210°C for 25 min with steam supply for 5 s.

## 2.3 Assessment of baked bread

### *2.3.1 Assessment of quality*

The quality of bread was evaluated the following parameters. Titratable Acidity (TTA) was determined by titration with 0.1 N sodium hydroxide solution using phenolphthalein as indicator [17]. Cells volume was calculated as the ratio of cells volume to the total bread volume. Bread specific volume was determined by a rapeseed displacement and calculated as the ratio of volume to 100g of bread [15]. The automatic penetrometer (Labor, Hungary) was used to estimate crumbs compressibility [15]. The volatile acids amount was estimated by determining the amount 0.1 N sodium hydroxide solution used for neutralization of the evaporated volatile acid. The alcohol content was determined by using the iodometric method, which is based on the quantity of sodium thiosulfate spent in titration [15].

### *2.3.2 Gluten content assessment*

For the qualitative detection of gluten, an immunochromatographic test was used (“Hema”, Russia). During the test, gliadin is bound by specific antibodies deposited on the test strip and on the surface of the colored microparticles. As a result of their interaction, a complex is formed, visible in the form of a colored line.

During the analysis, 1 g of crushed crumb was added to the extraction tube up to the first division. Sample buffer was then added to the vial up to the "5 ml" mark, the vial cap was screwed on tightly and shaken vigorously for 40 s, and then the vial was left undisturbed on the table to settle the particles. Using a disposable pipette, one drop of the extract was transferred into a test tube for analysis and shaken for 5–10 s. Then, the test strip was immersed into the prepared sample up to the mark and held for 5–10 s. Lay the strip horizontally on a clean surface. The result was read 15 min after the strip was immersed in the sample. The test is considered positive if two lines appear on the test strip.

### *2.3.3 Ropy disease and moulds spoilage assessment*

To estimate bread microbial resistance to the microbial sspoilage, the bread was infected with the spore-forming bacteria B.subtilis and moulds Penicillium chrysogenum. Both methods described in details in previous study [14].

### 2.4 Statistical analysis

Comparison of the influence of factors was carried out by the method with significance tested at the 95% confidence level and differences between means were determined using the least significant difference and Duncan's test of two-factor analysis of variance with one repetition (ANOVA). Each factor was analyzed at least in triplicate. Statistical analysis was performed using Excell software.

## 3 Results and discussion

Studies on the possibility of using the enzyme preparation Alfalift as a saccharifying agent showed that in the scald with its use in the amount of 0.36% by weight of flour, the sugar content was lower than in the scald sample saccharified with barley malt by 29%, while increasing the dosage Alfalift to 0.54% by weight of flour slightly increased the sugar content in scald, and therefore, and also, given the high cost of the enzyme, it is not economically feasible to use it in such a dosage (Fig. 1).

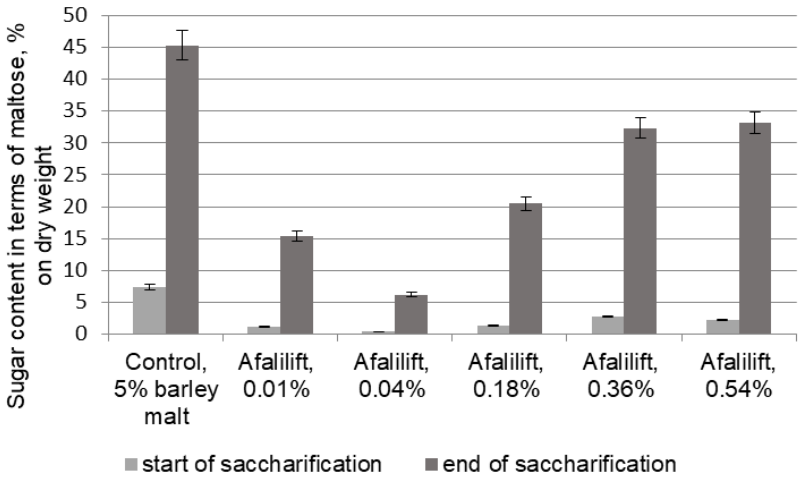


**Fig. 1.** Sugar content in scald in terms of maltose, % on dried weight

The content of sugars in saccharified scald directly affects the development of microorganisms in fermented scald, and their high content can have both stimulating and inhibitory effects, depending on the species and strain characteristics. Studies have shown (Fig. 2) that in scald saccharified with the Alphalift enzyme preparation in an amount of 0.36% by weight of flour, the content of sugars was lower than in the sample saccharified with barley malt.

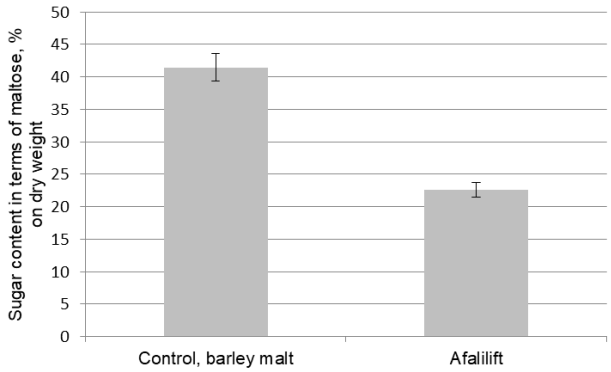


**Fig. 2.** The content of digestible carbohydrates in scald from green buckwheat flour, saccharified with barley malt 5% (1) and Alphalift 0.36% (2)

Studies on the effect of lactic acid bacteria and yeast on the biotechnological properties of sourdoughs showed that the best acid accumulation was observed in sourdoughs made on scald saccharified with Alphalift, despite the fact that the sugar content was less than in saccharified scald with barley malt (Fig. 3). At the same time, it should be noted that the samples of starter cultures fermented with a mixture of L.brevis E139 and L.plantarum E138 were characterized by a higher acid accumulation compared to the samples of starter cultures using monocultures.

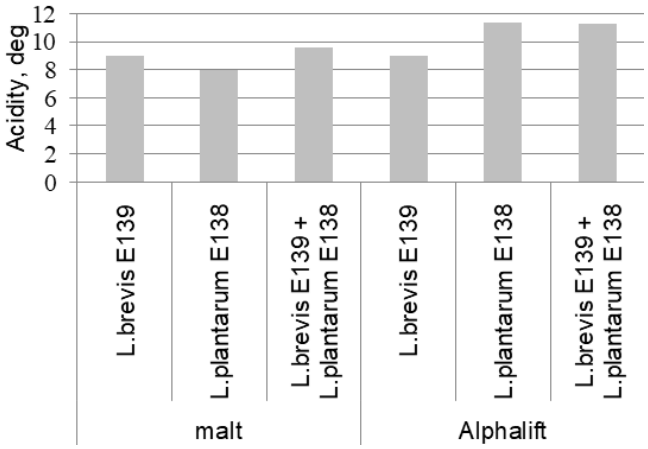


**Fig.3.** The influence of the type of LAB, the method of their introduction and the composition of the nutrition on the acid accumulation of starter cultures at the end of the breeding (a) and production (b) cycles

It is known that homofermentative types of LAB, which include *L. plantarum E138,* produce up to 10% of volatile acids, while obligately heterofermentative ones, incl. strain *L.brevis E139* produce in 2-3 times more volatile acids [16]. A similar pattern was observed in the study of gluten-free sourdoughs. Thus, the content of volatile acids in the samples of starter cultures fermented with *L.brevis E139* was 2.6-5.9 and 4.8-5.1 times higher compared to the rest of the samples (Figure 4). These sourdoughs had more pronounced odor.

Samples of sourdoughs bred on the *L.plantarum E138* strain and on a mixture of *L.brevis E139* and *L.plantarum E138* strains, regardless of the method of saccharification of the scald, had a more alcoholic smell, which is due to a lower content of volatile acids (Fig. 4) and a high content alcohol (Fig.5).

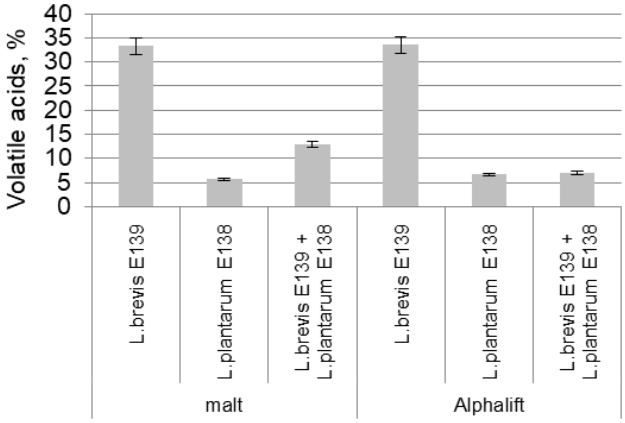


Fig. 4**.** The content of volatile acids in gluten-free sourdoughs

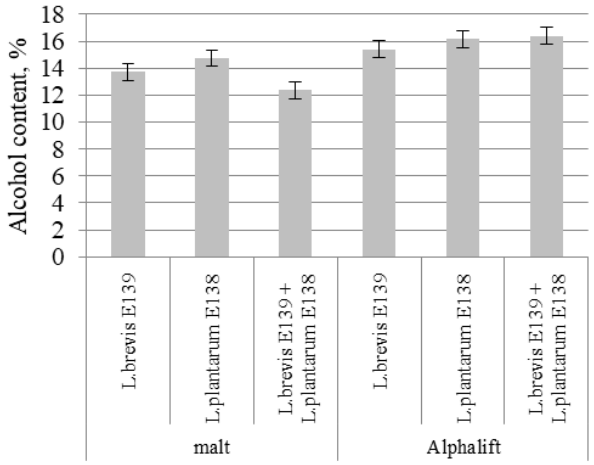


**Fig. 5.** Alcohol content in gluten-free sourdoughs

The study of the content of the number of microorganisms in the sourdough showed (Fig. 6, 7) that when using the obligate heterofermentative strain *L.brevis E139*, the number of lactobacilli and yeast cells was 2-3 times less, while volatile acids were contained 3-6 times more (Fig.6,7) than in sourdough based on *L. plantarum E138*, which is characterized by high alcohol resistance and can withstand alcohol concentrations up to 20% [10]. Thus, a sample of scalds saccharified with barley malt with *L. plantarum E138* differed in the maximum content of LAB cells and yeast, which was 1.2-5.5 and 1.4-5.9 times higher, respectively, compared to the rest of the samples, with At the same time, the content of volatile acids in this sample was minimal.

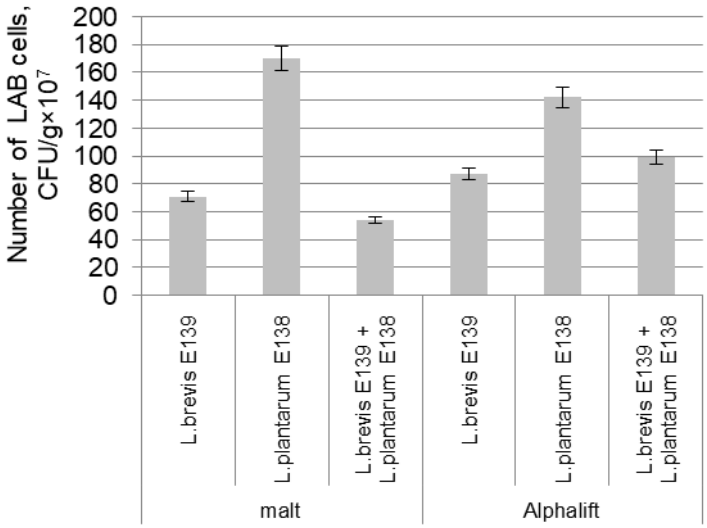


**Fig. 6.** Content of LAB cells in gluten-free starter cultures

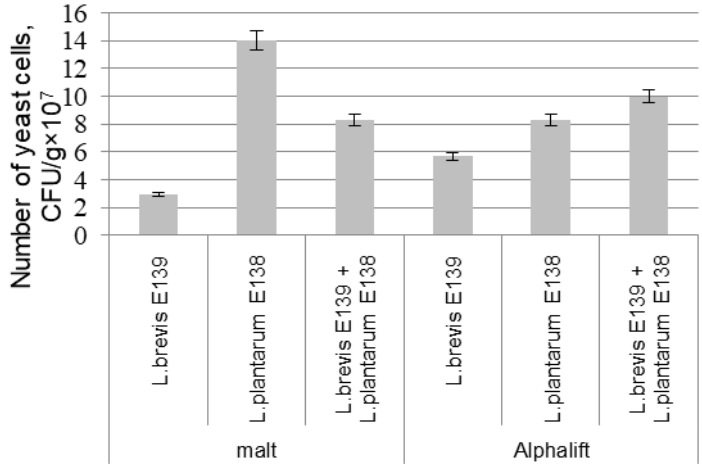


**Fig. 7.** Yeast cell content in gluten-free starters

The influence of sourdough containing 20% of green buckwheat flour from the total amount of the baking flour mixture in the dough on the quality of bread was studied.

The acidity of the sourdough bread was in 3.3-5.3 times higher compared with the control sample (Table 1). Samples of sourdough saccharified with barley malt and fermented with *L.plantarum E138* strain and a mixture of *L.brevis E139* and *L.plantarum E138* strains contributed to an increase in the specific volume of bread, which directly correlates with the compressibility of the bread crumb.

An analysis of the organoleptic indicators of bread quality showed that all samples were characterized by an even, rough upper crust of dark brown color. The porosity of the crumb of all bread samples was fine and uniform with separate large pores. At the same time, samples of bread of the control (1) and experimental variants on sourdough, in which barley malt was used during the saccharification of scalds (2, 3, 4), had a mushroom shape (Fig. 8).

The tasters noted an improvement in the taste and aroma characteristics of sourdough bread: the smell became more pronounced with a “butter shade”, a pronounced sourness

appeared when using 20% of the total amount of flour in the dough compared to the control sample.

**Table 1.** Quality indicators of gluten-free bread

| | control | Bread made with | | | | | |
|---|---|---|---|---|---|---|---|
| | | Malt | | | Alphalift | | |
| | | *L.brevis E139* | *L.plantarum E138* | *L.brevis E139 + L.plantarum E138* | *L.brevis E139* | *L.plantarum E138* | *L.brevis E139+ L.plantarum E138* |
| Acidity, degrees | 0,7± 0.1a | 2,4± 0.2$^{b}$ | 2,0± 0.2$^{c}$ | 2,2± 0.2$^{c}$ | 2,6± 0.2$^{b}$ | 2,2± 0.2$^{c}$ | 2,4± 0.2$^{b}$ |
| Cells volume, % | 72± 2$^{a}$ | 73± 2$^{a}$ | 71± 2$^{a}$ | 71± 2$^{a}$ | 68± 1$^{b}$ | 67± 2$^{b}$ | 68± 1$^{b}$ |
| Specific volume, sm3/g | 3,0± 0.2$^{a}$ | 3,0± 0.2$^{a}$ | 3,4± 0.2$^{b}$ | 3,2± 0.2$^{a}$ | 2,4± 0.2$^{c}$ | 2,7± 0.2$^{d}$ | 2,7± 0.2$^{d}$ |
| Compressibility, equipment units | 50± 2$^{a}$ | 53± 2$^{a}$ | 53± 2$^{a}$ | 50± 2$^{a}$ | 37± 2$^{a}$ | 36± 2$^{a}$ | 38± 2$^{a}$ |

Mean values ± SD within the same line with different letters are significantly different ($P \leq 0.05$)


**Fig. 8.** Appearance of samples of control bread (1) and experimental options for scalds, saccharified by:

- barley malt and fermented by monocultures *L.brevis E139* (2), *L.plantarum E138* (3), *L.brevis E139* and *L.plantarum E138* (4);

- Alphalift and fermented by monocultures *L.brevis E139* (5), *L.plantarum E138* (6), *L.brevis E139* and *L.plantarum E138* (7).

Since barley malt, which is a toxic ingredient for people with gluten-associated diseases, was used as a saccharifying agent in the preparation of saccharified scald, studies have been conducted on the safety of gluten-free bakery products. According to the Technical Regulations of the Customs Union TR CU 027/2012 “On the safety of certain types of specialized food products, including dietary therapeutic and dietary preventive nutrition”, the level of gluten in ready-to-eat products should be no more than 20 mg/kg. Qualitative determination of gluten (gliadin) in sourdough bread samples using test strips "Hema test gluten" showed that after immersing the test strips in a solution with an extract prepared from the crumb of bread samples, after 15 minutes of resting, only one red strip appeared , which confirms that the bread is gluten-free.

When mold was inoculated to the bread pieces, mycelium growth was observed on control bread after 37 hours, on bread with a monoculture of *L. plantarum E138* - after 41 hours, on bread with *L. plantarum E138* - after 60 hours.

The study of the effect of sourdoughs on the development of ropy disease showed that in the control sample of bread, the first signs of disease in the form of an unpleasant odor and stickiness of the crumb were observed after 24 hours. Sourdough bread did not get sick with ropy disease.

## 4 Conclusions

As a result of the comprehensive research, a technology for gluten-free fermented scald (sourdough) was developed, which makes it possible to obtain bread with improved physico-chemical and organoleptic characteristics, safe for diet therapy for celiac disease.

The use of barley malt or the "Alphalift" enzyme preparation for saccharification of scalds based on green buckwheat flour was experimentally substantiated.

It was shown that the use of the heterofermentative strain L.brevis E139 leads to an increase in the content of volatile acids in sourdough (fermented scald), an increase in the acidity of bread.

It was revealed that the use of sourdough starter, saccharified with barley malt and fermented by both the heterofermentative strain L.brevis E139 and the homofermentative strain L.plantarum E138, when kneading the dough, ensures the production of bread with a large specific volume and better crumb compressibility compared to sourdough bread, in which the scalds were saccharified with the enzyme preparation "Alfalift".

It was established that the addition of 20% green buckwheat flour when kneading dough with sourdough provides an improvement in the taste and smell of gluten-free bread.

## References

1. F. Minervini, A. Lattanzi, , M. De Angelis, G. Celano, M. Gobbetti. House microbiotas as sources of lactic acid bacteria and yeasts in traditional Italian sourdoughs. Food Microbiology 52, 66-76 (2015). https://doi.org/10.1016/j.fm.2015.06.009
2. L.De Vuyst, A. Comasio, A.Van Kerrebroeck. Sourdough production: fermentation strategies, microbial ecology, and use of non-flour ingredients. Critical reviews in food science and nutrition. 1-33 (2021).doi: 10.1080/10408398.2021.1976100.
3. L. De Vuyst, S.Van Kerrebroeck, F. Leroy. Microbial Ecology and Process Technology of Sourdough Fermentation. Advances in Applied Microbiology, 49–160 (2017). doi:10.1016/bs.aambs.2017.02.003
4. K. Arora, H. Ameur, A. Polo, R. Di Cagno, C. G. Rizzello, M. Gobbetti. Thirty years of knowledge on sourdough fermentation: A systematic review. Trends in Food Science & Technology (2020) doi:10.1016/j.tifs.2020.12.008
5. A. Comasio, M. Verce, S. Van Kerrebroeck, L. De Vuyst. Diverse microbial composition of sourdoughs from different origins. Frontiers in Microbiology 11 (2020) doi:10.3389/fmicb.2020.01212
6. M.Gobbetti, M.De Angelis, R. Di Cagno, M. Calasso, G. Archetti, C.G. Rizzello. Novel insights on the functional/nutritional features of the sourdough fermentation. Int. J. Food Microbiol 302, 103–113 (2018)
7. M. Gobbetti, M. Angelis, R. Cagno, C. Rizzello. Sourdough/Lactic acid bacteria, In E.K. Elke and F.D. Bello, (eds.). Gluten-Free Cereal Products and Beverages Academic Press. Burlington, MA, USA, 267–283 (2008).
8. M.C. Messia, A. Reale , L. Maiuro, T. Candigliota, E. Sorrentino, E. Marconi. Effects of pre-fermented wheat bran on dough and bread characteristics. Journal of Cereal Science 69, 138-144 (2016.)
9. J.Espinosa, J.Gloria, A. Angulo, R. Escobedo, J.Pérez, R. Rebollo, G. Domínguez. Sourdough and Bread Properties as Affected by Soybean Protein Addition, In T.B. Ng, (eds.). Soybean - Applications and Technology. InTech. Rijeka, Croatia, 387–402pp. (2011)

10. L. Nionelli, C. Rizzello. Sourdough-Based Biotechnologies for the Production of Gluten-Free Foods. Foods 5(3), 65 (2016).
11. A. O. Olojede, A. I. Sanni, K. Banwo. Rheological, textural and nutritional properties of gluten-free sourdough made with functionally important lactic acid bacteria and yeast from Nigerian sorghum. LWT- Food Science and Technology, 108875 (2019). doi:10.1016/j.lwt.2019.108875
12. H. G.Masure, E. Fierens, E., J. A. Delcour. Current and forward looking experimental approaches in gluten-free bread making research. Journal of Cereal Science 67, 92–111 (2016). doi:10.1016/j.jcs.2015.09.009
13. O. Savkina, L. Kuznetsova, M. Burykina, M. Kostyuchenko, O. Parakhina. The influence of the flour amylolytic enzymes activity, dosage of ingredients and bread making method on the sugar content and the bread quality.Agronomy Research 18(S3), 1873–1887(2020)
14. . Parakhina, M. Lokachuk, L. Kuznetsova, O. Savkina, E. Pavlovskaya , T. Gavrilova. Evaluation of selected lactic acid bacteria as starter cultures for gluten-free sourdough bread production. Agronomy Research 19(S3), 1260–1272 (2021)
15. Puchkova, L. I. 2004. Laboratory research methods for bread technology. GIORD, St. Petersburg, Russia, 259 pp. (in Russian).
16. O. Afanasjeva. Microbiology of bakery production. Beresta,Saint-Peterburg, 217 (2003). (In Russian).
17. State Standard of the Russian Federation GOST 5670-96. 1996. Bread, rolls and buns. Methods for determination of acidity, 5 pp (in Russian).
18. N. Dubrovskaya, L.Kuznetcova, O. Parakhina. Method for improving the microbiological stability of gluten-free bread. Bred making in Russia 4, 22-24 (2017)