Study of factors affecting the pathogenic microbiota growth on the surface of thermophilic sourdough and development of methods for its inhibition

Lina Kuznetsova, *Olesia* Savkina*, *Maria* Burykina, *Marina* Lokachuk, *Olga* Parakhina, *Elena* Pavlovskaya and *Natalia* Lavrenteva

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

Abstract. Sourdough is an important ingredient in the breadmaking. In the Baltic countries, Belarus, Ukraine and Russia, thermophilic rye sourdough are widely used, which are prepared using saccharified flour scald (scalded flour) and fermented at elevated temperatures (>35 ◦C). Pure cultures of L. amylolyticus 76 are widely used for thermophilic sourdough preparation. The aim of the research was to identify the causes of the spore-forming bacteria germination and the appearance of an unpleasant odor at the end of the first step of thermophilic sourdough preparation when thermophilic strain L. amylolyticus is used, as well as to develop methods for suppressing the development of unwanted microorganisms. Using the 16S rRNA sequencing method, it was established that the isolated bacteria belong to the species Bacillus licheniformis. Water at a temperature of 58-60 to 77-78 °C and steam inhibit the development of spore-forming bacteria, while water at a temperature above 97°C activates spores. The effect physiological activity of pure cultures L. amylolyticus 76 on the sourdough quality and the undesirable microflora growth was investigated. The influense of anaerobic conditions on the sourdough surface, for example, by creating a protective film of vegetable oil, also as acidifying of sourdough by lactic acid or glacial acetic acid was established.

1 Introduction

Sourdough is an important ingredient in the breadmaking. Sourdough is cereal flour and water mixter fermented by lactic acid bacteria and yeasts [1-4]. The sourdough microbiota can form spontaneously or be inoculated as starter microorganisms. Starter microorganisms are usually used as flavor carriers and texture improvers, or for their antifungal or health-promoting properties, in order to improve the performances and the properties of sourdough. Sometimes, other groups of microorganisms can grow in sourdough, whether or not added as starter or pure cultures, especially in several initial propagations [1-5]

* Corresponding author: 1103savkina@mail.ru

In the Baltic countries, Belarus, Ukraine and Russia, thermophilic rye sourdough are widely used, which fermented at elevated temperatures (>35 ◦C). This type of industrial sourdough is referred to as Type 3 sourdoughs. Type 3 sourdoughs are obtained through temperature-controlled fermentation of the flour-water mixture with specific strains of acid-tolerant LAB species followed by daily back slopping [1, 2]. Pure cultures of *L. delbrueckii* or *L. amylolyticus* from the collection 'Lactic acid bacteria and yeast for the baking industry' of St. Petersburg branch State Research Institute of Baking Industry are used for thermophilic sourdough (fermented scald) preparation for many decades [6-8]. Also this type of lactobacilli can be found in spontaneous thermophilic sourdough [1, 6-10]. For example, strain *L.amylolyticus 76* (formerly known as *L. delbrueckii*) was isolated in 1976 from the industrial sourdough of the Riga (Latvia) by Olga Afanasjeva. And since then it has been widely used in bakeries of Belarus, Ukraine, Russia, as well as at some bakeries in Estonia, Lithuania and Latvia. This strain gives thermophilic sourdough a unique aroma of ripe green apple or plum [6, 7].

In comparison with the sourdough in the EU, the major difference in the Baltic region and Russian rye sourdough is the scalding step in the process of flour-water mixture preparing [7-9]. The scald process is a heat treatment of flour with hot water (70-90°C) or steam until starch gelatinization. The initial scalded flour temperature is 64-67 °C, i.e. corresponds to the temperature at which the starch of both rye and wheat flour begins to gelatinize [7, 9, 11].

In some technological recommendations it is noted that in order to prepare scald (saccharafied scalded flour), the flour must first be mixed with a small amount of warm water with a temperature of 50-60 °C, then, continuing kneading, the rest of the water with a temperature of 97-98 °C is added. Since hot (boiling) water reduces the activity of amylases, after reaching a temperature of 63-68 °C in the mixture, it is recommended to mix it with 5-10% of the flour (from its total mass in the scald) or with a similar amount of enzymatically active (unfermented) malt flour for better saccharification of the scald [7, 8]. In some bakeries and householders, as well as in research practice, boiling water (temperature 95-98°C) is used to prepare sweet (saccharified) scalded flour. The scald obtained by these methods are saccharified and cooled to necessary temperatures. Saccharafied scalded flour is used to the thermophilic sourdough refreshment (back-slopping).

It is difficult to ensure the sourdough quality in the first stage of preparation when pure cultures of *L.amylolyticus 76* are used, since it is highly dependent on the microflora present in the scalded flour and the temperature of sourdough. The long-term industrial experience has shown that when preparing sourdough using pure cultures of lactobacilli and heat-treated rye flour, extraneous unwanted microbiota (like spore-forming bacteria) is often found on the surface of the sourdough. This type of microbiota degrades the quality of the sourdough. Instead of the pleasant smell of a ripe green apple, thermophilic starter has an unpleasant stinky smell [6]. And it will determine which microorganisms end up in the mature sourdoughs, which depends on the competitiveness of the starter strain used for inoculation of the initial flour-water mixture and autochthonic flour strains [1, 3, 5, 12].

The development of the sourdough microbiota can be influenced by the scald temperature, the method of preparation of the scald, oxygen availability, pH, and the presence of preservatives such as organic acids [2, 13].

The aim of the research was to identify the causes of the spore-forming bacteria germination and the appearance of an unpleasant odor at the end of the first step of thermophilic sourdough preparation when thermophilic strain *L. amylolyticus* is used, as well as to develop methods for suppressing the development of unwanted microorganisms.

2 Materials and methods

To achieve the aim of the work, several scientific hypotheses were formulated, which causes the methods. To inhibit spore-forming bacteria grows follow methods can be used:

- pure cultures of *L. amylolyticus 76* in different physiological faze;
- heated flour and extruded flour usage;
- acidification of the scald;
- anaerobic condition on surface of scald.

2.1 Scald preparation and saccharification

When investigated influense of flour on the scald quality three type of flour from Russian local milling companies were used: native rye flour, heated rye flour and extruded rye flour (dry scald), obtained by thermoplastic extrusion. Flour quality indicators are presented in Table 1.

Table 1. Quality indicators of flour

Indicators	Wheat flour	
	Rye	Extruded
Moisture content, %	12.1±0.2 [a]	9.5±0.2 [b]
Falling number, s	248±10[a]	-
Ash content, %	1.88±0.03[a]	1.62±0.03[b]

a-b = Means ± SD within the same row with different lowercase superscript letters are significantly different ($p \leq 0.05$)

Scalds were prepared in laboratory by mixing flour with water heated to a temperature of 97-98 °C (boiling water). The ratio of flour and water was 1:2.5. Then the scald was cooled to a temperature of 63-65°C and 5% of unfermented barley m-alt was added into the scald. The scald was mixed throughout and left to cool down to 48-50°C temperature, which is optimal for scald saccharification [7-9]. The scalds prepared in this way were left for 120 min for saccharification at a temperature of 50°C.

2.2 Fermented scald preparation

Ready sweet scalds were used to prepare fermented scald.

Pure cultures of lactic acid bacteria *L. amylolyticus* 76 from the collection 'Lactic acid bacteria and yeast for the baking industry' of St. Petersburg branch State Research Institute of Baking Industry were used [6,14].

Strain was cultured in standard liquid medium MRS (BioMerieux, France). 1 ml of lactic acid bacteria cultural liquid was inoculated into 100 g mixture of sweet scald and was kept at 49±1°C. To prepare the sourdough, cultures were used after 24, 48 and 72 hours of growth. The titer of lactobacillus cells was 10^9 cells in 1 ml. The titratable acidity was calculated according to Di Renzo et al. [15].

2.3 Creation of anaerobic conditions on the scald surface

To create anaerobic conditions in order to inhibit the growth of spore-forming microflora in scald, its surface was covered with refined deodorized sunflower oil (Russia). Despite the fact that B. licheniformis are facultative anaerobes, the possibility of suppressing their growth by flooding the scald with a layer of vegetable oil was investigated. By analogy

with the cultivation of anaerobic microorganisms, the development of which is not accompanied by gas formation, under a layer of sterile vaseline oil [16].

2.4 Study on the effect of acidification

The effect of acids on the development of spore-forming bacteria was studied. When investigated influense of acidification on the scald quality only native rye flour were used (quality indicators are presented at Table 1). Sweet scald was prepared as it was mentioned above. Sweet scald was inoculated by pure cultures of *L. amylolyticus 76* (1ml of cultural liquid into 100g of scald) and mixed with acids. Acids were added into the scald in the following quantity (on 100 g of scald): 80% lactic acid was added in the amount of 0.1 and 0.2 ml; citric acid in amount 0.1 g and 0.5 g; ice acetic acid - 0.1 ml.

2.5 Study on the effect of water temperature and steam

Three types of scalds were prepared. Two was made in laboratory by mixing flour with water heated to a temperature of 97-98 °C (boiling water) and water at a temperature of 65 °C. The ratio of flour and water was 1:2.5. Then the scald was cooled or thermostated to a temperature of 63-65°C and 5% of unfermented barley malt. The scald was mixed throughout and left to cool down to (49 ± 2) °C temperature. The scalds prepared in this way were left for 120 min for saccharification at a temperature of 50°C.

One scald was prepared in the bakery enterprise using industrial machine. Rye flour and water (1:2,5) were added into the scalding machine. Temperature of mixture was adjusted to 64-68 °C by steam. The duration of heating was 30 min, and then the scald was mixed throughout and left to cool down to the temperature 48-50 °C for 60 min.

2.6 Fermented scald assessments

Scalds were evaluated for different parameters. Moisture of the scald was determined by drying it at a temperature of 130°C for a period of forty minutes in drier (SHS-1M, Russia). Acidity was determined by titration, using a 0.1 N. solution of NaOH [17]. To determine the lactobacilli content in a scald, the method of microscopy and counting in a fixed colored preparation in 50 fields of view was used [6]

The growth of spore-forming bacteria on the surface of fermented scalds was determined visually. From the colonies grown on the surface, fixed stained preparations were prepared and microscoped at a magnification of x 1500.

2.7 Assessment of organoleptic characteristics

Ten-member expert panel was used to evaluate the sensory (organoleptic) characteristics of thermophilic fermented scald. Experts evaluated surface appearance and odor. Odor was rated on a scale from 1 to 5: 1– dislike extremely; 2 – dislike; 3 - slightly dislike; 4 – moderately; 5 – very good). Surface appearance was rated on a scale from 1 to 5: 1– completely overgrown with foreign microbiota on the surface; 2 – strong growth of foreign microflora on the surface; 3 - several colonies on the surface diameter more than 2 mm; 4 – several colonies on the surface, diameter less than 2 mm; 5 – no growth of foreign microbiota.

2.8 Statistical analysis of the data

All of the experiments were carried out a total of five times. Statistical analysis was performed using Excell software. Comparison of the influence of factors was carried out by the method with significance tested at the 95% confidence level and differences among means were determined using the least significant difference and Duncan's test of two-factor analysis of variance with one repetition (ANOVA). The confidence intervals shown in the histograms and in the table reflect the accuracy of the used methods.

3 Results and discussion

The analysis of obtained results (presented in Table 2) showed that during cultivation of pure cultures *L. amylolyticus 76* the acid accumulations occurred in 48 hours and then it slowed down (Table 2). The cells count increased in pure culture at an incubation period of 24h, and then the increase in biomass was insignificant. Thus, the cultivation time of pure cultures has an impact on their physiological state. This confirms the data presented by other researchers. Vasudha and Hari, 2014 [18] observed for L. plantarum NCDC 414 that viable cell counts increased from 4 × 105 to 7 × 1010 CFUmL−1 in a 24 h. And significant lactic acid production was in the exponential (24h incubation) and stationary phases (48 h incubation). Rezvani et al , 2017 [19] showed that lactic acid production rate in exponential growth phase cold bi higher than the stationary phase for L. delbrueckii subsp. bulgaricus PTCC1737.

Studies on the effect of the duration of cultivation of LAB *L. amylolyticus* 76 on the development of spore-forming bacteria showed (Table 2) that when one-day old pure cultures of lactic acid bacteria with an acidity of 6.2 degrees were used, spore-forming bacteria did not develop on the surface of the fermented scald, the smell was pleasant, and its acidity was 13.7 degrees. When *L. amylolyticus* 76 cultivated for 72 h was used the surface of scald was overgrown with vegetative cells of spore-forming bacteria and had unpleasant odor, and its acidity was 13.2-13.4 degrees. It should be noted that after the removal of the film with colonies of spore-forming bacteria, the fermented scald had a characteristic smell, and during the further conduction of the breeding cycle, spore-forming bacteria did not develop. A similar picture was also observed when using *L. amylolyticus* 76 after 48 h of cultivation. Study has shown that optimal time for *L. amylolyticus* 76 cultivation before sending them to bakeries is 24 h.

Table 2. Biotechnological indicators of pure culture and thermophilic sourdoughs after first fermentation

Indicators	The indicators when pure cultures were cultivated for			
	0h	24 h	48h	72h
Pure culture *L. amylolyticus*				
Acidity , deg. N:	2.2±0,1^{a}	6.2±0,3^{b}	15.2±0.3^{c}	15.5±0.2^{c}
LAB, 10^6 CFU·g^{-1}	0.10±0.01^{a}	1021±21^{b}	1180±52^{c}	1208±52^{c}
Thermophillic sourdough (fermented scald)				
Acidity , deg. N:	-	13.7±0,3^{a}	15.0±0.3^{b}	13.2±0.2^{a}
Appearance of the sourdough surface	-	5.0±0.0^{a}	2.9±0.1^{b}	1.1±0.2^{c}
Odor	-	5.0±0.0^{a}	2.9±0.1^{b}	1.1±0.2^{c}

a-c = Means ± SD within the same line with different lowercase superscript letters are significantly different ($P \leq 0.05$)

Heating of flour at 150°C for 1 hour led to a decrease the content of colonies in flour in 10 times (Table 3). When using a 72-hours pure culture of *L. amylolyticus* 76, there was a

slight growth of unwanted microbiota on the surface of scald, and the unpleasant smell was less pronounced. The acidity of the thermophilic sourdough was lower than in sourdough made with native rye flour.

In the sourdough made from extruded flour, there was no growth of undesirable microbiota on the surface of the scald, and the acidity of the sourdough was slightly lower than in the scald made with native flour and heated flour. Obtained data confirms the data that flour affects sourdough microbiota because of its content of contaminating microorganisms [2, 4, 20].

Table 3. Quality of flour and sourdough

Indicators	Wheat flour		
	Rye	Heated rue flour	Extruded
Sporeforming bacteria, 10^2 CFU·g^{-1}	5.0±1.0 [a]	0.1±1.0[b]	not detected
Thermophillic sourdough (fermented scald) parameters			
Acidity , deg. N:	13.2±0.2[a]	12.5±0,2[b]	11.2±0.3[c]
Appearance of the sourdough surface	1.1±0.2[a]	2.8±0.2[b]	5.0±0.0[c]
Odor	1.1±0.2[a]	2.8±0.2[b]	5.0±0.0[c]

a-c = Means ± SD within the same line with different lowercase superscript letters are significantly different ($P \leq 0.05$)

Gram-positive rods with spores were found in the microbial unpleasant film formed on the surface of the scald. Using the 16SpRNA sequencing method, it was found that the isolated isolate can be attributed to the species *Bacillus licheniformis* - Gram-positive, mesophilic bacteria, facultative anaerobes. Bacillus species is unavoidable due to their occurrence as a part of the endophytic commensal microbiota of flour [21]. It is known that the main factors limiting or controlling the growth of spore-forming spoilage bacteria in food products are the pH value, as well as temperature-time heating, cooling, aerobic condition and storage [21, 22].

The influence of the height of the vegetable oil layer on the surface of scald fermented by 72-h cultivated *L. amylolyticus* 76 was studied. It was found that at a layer height of up to 2 mm, spore-forming bacteria also developed on the surface of the fermented scald after 24 hours of fermentation. The acidity was 12.6 degrees, which is lower than in thermophilic sourdough made without oil (data on sourdough without oil presented at Table 2).

With an increase in the height of the vegetable oil layer to 5 mm, colonies of spore-forming bacteria did not develop on the surface of the scald, the smell pleasant, and the acidity was lower than in sourdough without oil on surface (11.7 degrees). Lavermicocca et al. (2016) and Pacher et al (2022) [21, 23] reported that *B. licheniformis* can show an anaerobic growth. This suggests that growth inhibition on the surface of the scald was not affected by anaerobic conditions, but by the oil itself.

Citric, lactic and acetic acids were used to create a low pH value. The results of the study showed (Table 4) that complete suppression of the growth of spore-forming bacteria was observed when 0.2 g of citric acid or 0.1 ml of 80% lactic acid or 0.1 ml of glacial acetic acid were added to 100 g of scald. At the same time, the pH in the control brew was 5.65, and in the experimental ones, acidified with citric, lactic, acetic acids - 4.49, 4.94 and 5.08, respectively.

When lactic and especially citric acid was added to the experimental scald, a decrease in the LAB cells number by the end of fermentation was observed compared to their number in the control scald and, as a result, a decrease in acidity.

In the scald with acetic acid, the values of acidity, pH and cell count were comparable to those in the scald without acidification.

It is known that growth slows down at pH 4.5 [21-23]. Our studies have shown that growth retardation is influenced not only by low pH, but also by the type of acid. Similar data were obtained by us in the study of the effect of acetic and lactic acid on the development of mold [14].

Table 4. Effect of acidification on thermophilic sourdough quality

Indicators	Wheat flour					
	Control	Citric acid		80% lactic acid		Acetic acid
	(without acidification)	0.1g/100g	0.2g/100g	0.1ml/100g	0.2ml/100g	0.1ml/100g
Acidity, deg. N:						
initial	2.2±0.1^{a}	3.4±0.3^{b}	5.0±0.3^{c}	3.0±0.2^{b}	3.7±0,1^{d}	3.5±0.3^{b,d}
In 24h	13.2±0.2^{a}	11.9±0.2^{b}	9.5±0.2^{c}	11.4±0.2^{d}	10.5±0.2^{f}	13.3±0.3^{a}
pH						
initial	5.7±0.1^{a}	5.1±0.3^{b}	4.5±0.2^{c}	4.9±0.2^{d}	4.6±0,1^{c}	5.1±0.3^{b}
In 24h	3.7±0.2^{a}	3.7±0.2^{a}	3.9±0.1^{a}	3.7±0.2^{a}	3.8±0.1^{a}	3.6±0.2^{a}
LAB, 10^{6} CFU·g^{-1}	1100±33^{a}	755±20^{b}	441±20^{c}	853±31^{d}	653±21^{f}	1265±21^{g}
Appearance of the sourdough surface	1.1±0.2^{c}		5.0±0.0^{c}			
Odor	1.1±0.2^{c}		5.0±0.0^{c}			

a-g = Means ± SD within the same line with different lowercase superscript letters are significantly different ($p \leq 0.05$)

The effect of water temperature and steam application was investigated. It was established that the method of scald preparing and the temperature of the water did not affect the acidity of the thermophilic sourdough at the end of fermentation (Table 5). When the water temperature was 77-78°C and 58-60°C or when steam was used, extraneous microbiota did not develop on the fermented scald surface. On the surface of scald prepared with water at a temperature of 97-98 °C, the germination of undesirable microbiota on the surface was noted. It is possible that such water temperature and the initial scald temperature have a beneficial effect on the germination of spores of bacteria of the genus Bacillus [21-23].

Table 5. Effect of water temperature on thermophilic sourdough (fermented scald) quality

	Industrial scald	Laboratory scald		
Temperature of water, °C	≥100 (steam)	97-98	77-78	58-60
Initial temperature of mixture,°C	64-67	76-78	60-62	47-49
Thermophilic sourdough (fermented scald) parameters				
Acidity, deg. N:				
initial	2.4±0.1^{a}	2.2±0.1^{a}	2.4±0.3^{a}	5.0±0.3^{c}
In 24h	10.5±0.2^{a}	13.2±0.2^{b}	14.2±0.2^{c}	16.2±0.2^{d}
pH:				
initial	4.0±0.1^{a}	5.7±0.1^{b}	3.7±0.3^{c}	4.0±0.2^{a}
In 24h	3.7±0.1^{a}	3.7±0.2^{a}	3.5±0.1^{a}	3.6±0.1^{a}
Appearance of the sourdough surface	5.0±0.0^{a}	1.2±0.2^{b}	5.0±0.0^{c}	
Odor	5.0±0.0^{a}	1.2±0.2^{b}	5.0±0.0^{c}	

a-d = Means ± SD within the same line with different lowercase superscript letters are significantly different ($p \leq 0.05$)

4 Conclusions

Study has shown that optimal time for *L. amylolyticus 76* cultivation is 24 h. Based on complex studies of the germination of spore-forming bacteria contained in flour on the surface of thermophilic fermented scald in the first phase of the breeding cycle, it was found that one of the main reasons for their activation with further germination of vegetative cells is the use of boiling water for preparation of brew. The use of water at a temperature of 58-60 to 77-78 ° C and steam used for the heat treatment of flour inhibits the development of spore-forming bacteria.

It was established that spore-forming bacteria germinated on the surface of thermophilic sourdough (fermented scald) belong to the species *Bacillus licheniformis*.

Heating the flour at 150°C and using extruded flour allows inhibition of spore-forming bacteria on the scald surface.

Low pH hasn't affected the spore-forming bacteria growth, but the type of acid has affected. Acidification of sweet scalded flour with 80% lactic acid or glacial acetic acid at the rate of 0.1 ml per 100 g of sourdough allows inhibiting the growth of spore-forming bacteria.

The influense of anaerobic conditions on the sourdough surface, for example, by creating a protective film of vegetable oil, was established. It was found that at a layer height of up to 2 mm, spore-forming bacteria also developed on the surface of the fermented scald after 24 hours of fermentation, while 5mm film inhibited the unpleasant microbiota grows.

The data obtained will allow the development of technological recommendations for bakeries using thermophilic fermented scald.

References

1. 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.
2. 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.
3. 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.
4. 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.
5. 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).
6. O. Afanasjeva. Microbiology of bakery production. Beresta,Saint-Peterburg, 217 (2003). (In Russian).
7. L. Kuznetsova. Production of custard breads using rye flour. Beresta, Saint-Peterburg (2003) (In Russian).

8. A. Romanov, O. Ilyina, V. Iunihina, S. Kraus. Bread and bakery products. Raw materials, technologies, assortment: textbook. Moscow: DeLi plus, 538pp (2016.). (In Russian).
9. G Juodeikiene. Traditional Rye Sourdough Bread in the Baltic Region. In Kristbergsson, K., Oliveira, J. (ed.): Foods in Turkey: General and Consumer Aspects, Ney-York, Springer US, pp 173–187 (2016). doi:10.1007/978-1-4899-7648-2_12.
10. D. Cizeikiene, J. Jagelaviciute, M. Stankevicius, A. Maruska. Thermophilic lactic acid bacteria affect the characteristics of sourdough and whole-grain wheat bread. Food Bioscience 38, 100791 (2020). doi:10.1016/j.fbio.2020.100791.
11. Poutanen, K., Katina, K.& Heiniö R-L. 2014. Rye. In Zhou, W, Hui, Y.H., De Leyn, I, Pagani, M.A., Rosell, C.M., Selman, J.D. & Therdthai , N.(eds): Bakery Products Science and Technology, Second edition. John Wiley & Sons, Hoboken, New Jersey, pp.75-87.
12. R. Coda, Y. Xu, D. S. Moreno, D. Mojzita, L. Nionelli, C. G. Rizzello, K. Katina. Performance of Leuconostoc citreum FDR241 during wheat flour sourdough type I propagation and transcriptional analysis of exopolysaccharides biosynthesis genes. Food Microbiology 76, 164–72 (2018). doi: 10.1016/j.fm.2018.05.003.
13. M. Calvert, A. Madden, L. Nichols, N. Haddad, J. Lahne, R. Dunn, E. McKenney. PeerJ Publishing 9:e11389 (2021) https://doi.org/10.7717/peerj.11389.
14. O. Savkina, L. Kuznetsova, O. Parakhina, M. Lokachuk, E. Pavlovskaya. Impact of using the developed starter culture on the quality of sourdough, dough and wheat bread. Agronomy Research 17 (S2), 1435 – 1451 (2019) https://doi.org/10.15159/ar.19.138.
15. T. Di Renzo, A. Reale, F. Boscaino, M.C. Messia. Production in Kamut®, Quinoa and Wheat Doughs Fermented by Lactobacillus paracasei, Lactobacillus plantarum, and Lactobacillus brevis: A SPME-GC/MS Study. Frontiers in Microbiology 9 (2018). doi:10.3389/fmicb.2018.00429.
16. V. Zverev, M. Boychenko. Microbiology, virology: a guide to practical exercises. Moscow, GEOTAR-Media, 360pp (2015) (In Russian).
17. State Standard of the Russian Federation. 1996. Bread, rolls and buns. Methods for determination of acidity. GOST 27842-88, 5 pp (in Russian).
18. S.Vasudha, N. Hari. Un-structured kinetic modeling of growth and lactic acid production by Lactobacillus plantarum NCDC 414 during fermentation of vegetable juices. LWT –Food Sci Technol 59,1123–1128 (2014).
19. F.Rezvani, F. Ardestani, G. Najafpour. Growth kinetic models of five species of Lactobacilli and lactose consumption in batch submerged culture. Brazilian Journal of Microbiology 48(2), 251–258 (2017). doi:10.1016/j.bjm.2016.12.007.
20. D. Ercolini, E. Pontonio, F. De Filippis, F. Minervini, A. La Storia, M. Gobbetti, R. Di Cagno. Microbial Ecology Dynamics during Rye and Wheat Sourdough Preparation. Applied and Environmental Microbiology 79(24), 7827–7836 (2013). doi:10.1128/aem.02955-13.
21. N.Pacher, J. Burtscher, S. Johler, D. Etter, D. Bender, L. Fieseler, K.J. Domig. Ropiness in Bread—A Re-Emerging Spoilage Phenomenon. Foods 11, 3021 (2022). https://doi.org/10.3390/foods11193021.
22. C. De Blackburn. Food Spoilage Microorganisms. Woodhead Publishing, 736pp. (2008).

23. F.Valerio, M. Di Biase, V. M. T. Lattanzio, P. Lavermicocca. Improvement of the antifungal activity of lactic acid bacteria by addition to the growth medium of phenylpyruvic acid, a precursor of phenyllactic acid. International Journal of Food Microbiology, 222, 1–7 (2016). doi:10.1016/j.ijfoodmicro.2016.01.011.