# Impact of using the developed starter culture on the quality of sourdough, dough and wheat bread

O. Savkina<sup>1,\*</sup>, L. Kuznetsova<sup>2</sup>, O. Parakhina<sup>1</sup>, M. Lokachuk<sup>1</sup> and E. Pavlovskaya<sup>1</sup>

<sup>1</sup>St. Petersburg branch State Research Institute of Baking Industry, Podbelskogo highway 7, RU196608, St. Petersburg, Pushkin, Russia <sup>2</sup>Institute of Refrigeration and Biotechnologies, ITMO University, Lomonosova street, 9, RU191002, St. Petersburg, Russia \*Correspondence: 1103savkina@mail.ru

Abstract. There is no technological necessity of sourdough usage when preparing wheat bread as it can be prepared without sourdough but only with yeast using. However, sourdough helps to solve such problems as fast microbial spoilage, unexpressed taste and smell, crumbling crumb. The use of sourdough prepared with directional cultivation of microorganisms allows to produce high-quality competitive bread. Developing a starter culture with an optimized microbial composition was the purpose of this study, allowing the quality and the microbiological stability of wheat bread improving. A new starter microbial composition for the sourdough was developed. Lactic acid bacteria strains L. plantarum E90, L. brevis E120 and yeast S. cerevisiae Y139 were selected for the new composition. It was proven that the rice products using to microorganism immobilization allows saving the largest number of living cells after drying and during storage. The rate of acid accumulation in sourdough was established. The sourdough dynamic viscosity decrease at the end of fermentation by 2.2 times was established, which means that the fermentation process leads to the sourdough liquefactio. The optimal dosage was established (5-10% flour in sourdough). This dosage provided good physico-chemical and organoleptic quality indicators of bread. It was proved that the sourdough usage allows getting good-quality bread even when the flour with unsatisfactory amylolytic activity (high drop number) is used. Slowing down the microbial spoilage in sourdough bread was proven. In general, the developed sourdough wheat bread biotechnology improves bread quality and its resistance to the ropy-bread disease.

Key words: wheat sourdough, sourdough bread, yeast, lactic fermentation, microbial spoilage.

## **INTRODUCTION**

In the past decade, the tendency to return to sourdough technology has been noted. It was found out that the sourdough influences sensorial, technological, nutritional, and functional features of bread (Arendt et al., 2007; Corsetti et al., 2007; Gobbetti et al., 2014; Messia et al., 2016).

Sourdough is a mixture of flour and water fermented with lactic acid bacteria and yeasts (Corsetti et al., 2001; Succi et al., 2003; Iacumin et al., 2009; Reale et al., 2005; Reale et al., 2007; Minervini et al., 2015). These microorganisms may originate from

flours, water and equipment or may be inoculated as industrial starter containing pure cultures of lactic acid bacteria and yeasts (Gobbetti et al., 2008; Kosovan, 2008; Auerman, 2009; Huyset al., 2013; De Vuyst et al., 2014; Nionelli & Rizzello, 2016).

The metabolites of microorganisms affect the quality of sourdough and bread. Sourdough contains lactic acid and acetic acid (and some other organic acids) produced by lactic acid bacteria and causes a specific sour taste of bread (Afanasjeva, 2003 Espinosa et al., 2011). Depending on the level of lactic acidification, sourdough fermentation leads to the increase in bread extensibility, softness and volume (Rinaldi, 2015). Acidification impacts the solubility of the structure-forming components such as gluten, starch, protein and arabinoxylans, and positively interferes with the activity of endogenous enzymes (Gobbetti et al., 2008, Sandra et al., 2012). Acid production retard starch digestibility and adjust pH to a range, which favors the action of certain endogenous enzymes, thus, changing the bioavailability pattern of minerals and phytochemicals (Reale et al., 2007; Poutanen et al., 2009).

Organic acids, alcohols, esters, carbonyls, carbon dioxide, diacetyl, hydrogen peroxide and exopolysaccharides produced by lactobacilli and yeasts can improve the flavor and aroma (Gamel et al., 2015).

Sourdough fermentation is also associated with anti-fungal and anti-bacterial properties that can improve bread shelf-life. Addition of 30% of wheat sourdough provides a protective effect against bread staling and extends the bread shelf-life (Torrieri et al., 2014; Rinaldi et al., 2015). Control of staling and keeping the bread quality for longer periods can lead to important economic benefits (Gamel et al., 2015).

The aim of this work is to develop a new microbial starter composition as well as to evaluate the impact of the starter on the properties of the dough and of bread.

# MATERIALS AND METHODS

## **Microbial cultures and ingredients**

Microorganisms from the collection 'Lactic acid bacteria and yeast for the baking industry' of St. Petersburg branch State Research Institute of Baking Industry were used. In detail, 5 strains of lactic bacteria belonging to the genus *Lactobacillus (L. plantarum E90, L. plantarum E104, L. plantarum E96, L. plantarum E94 and L. brevis E120)* and

8 strains of yeast belonging to the species *Saccharomyces cerevisiae* (Y155, Y129, Y146, Y139, Y151, K1, Y152, Y168).

To study the effect of wheat sourdough on the dough rheological properties and the bread quality, different quality wheat flour of 1 grade (in accordance with the Russian classification) was used (Table 1).

<b>Table 1.</b> Quality indicators for wheat flo
--

Indicators	No. 1	No. 2
Moisture content, %	13.8	13.4
Whiteness, unit of the device	45	48
Falling number, s	343	216
Acidity, degrees N.	3.4	3.4
Mass fraction of raw gluten,%	29.5	25
The quality of raw gluten, unit	60	60
of the device		

#### Characterization of lactic acid bacteria strains

Acidifying activity. Strains were cultured in standard liquid medium MRS (BioMerieux, France). 1 mL of lactic acid bacteria cultural liquid was inoculated into 100 g mixture of water and flour (humidity 65%) and was kept at  $24 \pm 1$  °C for 2 h. The

titratable acidity was calculated according to Di Renzo et al. (2018). The content of volatile acids was determined by neutralizing the evaporated volatile acid using a 0. n. solution of NaOH (Afanasjeva, 2003).

Antagonistic activity. The antagonistic activity of lactic acid bacteria against pathogens of ropy bread disease was determined by the method of agar slab method (Polak-Berecka et al., 2009; Dec et al., 2016). Test culture of lactic acid bacteria was inoculated in a deep way on the MRS agar (BioMerieux, France) in the Petri dishes and was incubated at the optimum temperature of 30 °C for 3 days for the formation and accumulation of inhibitory compounds in agar. Then, the agar slab with a grown culture of lactic acid bacteria was cut with a sterile cork borer (diameter 7 mm) and transferred to another Petri dish on the surface of the meet-peptone agar, freshly inoculated with the *B. subtilis* test strain.

The *B.subtilis* test strain was grown on meat-peptone medium with agar and a suspension containing  $10^8$  cells mL<sup>-1</sup> was prepared using a densitometer DEN-1 (BioSan, Latvia – England).

The plates were kept for 3 hours in a refrigerator at a temperature of 4 °C (in order to avoid premature growth of the test strain) to diffuse antibiotic substances from the slab into the agar with the test strain, and then incubated at a temperature favorable for the development of the *B. subtilis* test strain (37 °C). The degree of antagonistic activity of the test culture of lactic acid bacteria was judged by the size of the zone of growth inhibition of the *B. subtilis* test strain around the agar slab.

#### **Characterization of yeast strains**

The fermentation activity of yeasts in a mixture of water and flour (humidity 65%) was studied.

Yeast fermentation activity was determined by the amount of released carbon dioxide. 1 mL of yeast cultural liquid containing 10<sup>8</sup> cells mL<sup>-1</sup> were added at 100 g of flour and water mixture (humidity 65%). Cells number was determined using a densitometer DEN-1 (BioSan, Latvia – England).

The flasks were tightly capped with a container filled with 96% sulfuric acid (special glass device – Muller valve). Sulfuric acid prevents the evaporation of water. Only  $CO_2$  is removed from the flask. Flasks were left to ferment for 24 hours at  $24 \pm 1$ °C, measuring the amount of  $CO_2$  released. From the difference in mass, before and after fermentation, the fermentation activity of each yeast strain was judged (Kurtzman & Fell, 1998).

## **Dried microbial composition preparation**

The lactic acid bacteria (*L. plantarum E90* and *L. brevis E120, see results*) characterized by the best acidifying and antagonistic activity and the yeast (*S. cerevisiae Y139, see results*) characterized for the best CO2 production, were used for the preparation of the dried starter culture.

The strains of lactobacilli were grown on malt wort (density of 12° Balling). The culture of the yeast was grown on malt extract (density of 8° Balling). Grown monocultures of lactobacilli were mixed in a 1:2 ratio (*L. plantarum E90: L. brevis E120*). Than mixed with various whole grain flour (corn, rice, oats and wheat) in a ratio of 1:1.2. The mixture was dried in an IR-drier (LOIP L3-120/300-VG1, Russia) at a temperature of  $50 \pm 2$  °C to a moisture content of 16-20%. The drying temperature was chosen experimentally at previous stages of research not presented in this article.

The content of lactobacilli cells in mix immediately after mixing before drying was  $5 \cdot 10^7$  CFU g<sup>-1</sup> and the content of yeasts cells was  $5 \cdot 10^5$  CFU g<sup>-1</sup>.

After drying, the starter was stored in a tightly closed plastic container at a temperature of 4-6 °C.

The cultural liquid of the *S. cerevisiae Y139* yeast was mixed in a ratio of 1:1.2 with the products of processing of various grain crops — corn, rice, oats, and wheat. The mixture was dried in an IR-drier (LOIP L3-120/300-VG1, Russia) at a temperature  $40 \pm 2$  °C to a moisture content of 16–20%.

The obtained dry mixtures of lactobacilli and yeast were mixed in a ratio of 1:3 and were used to prepare wheat sourdough. Colony forming units were determined by plating serial dilutions on malt extract agar for yeasts and MRS agar for lactobacilli. Incubation temperature was 25 °C for yeasts and 30 °C for lactobacilli.

## Sourdough preparation

When developing a new wheat sourdough for the conditions of discrete production, the following technological parameters were taken as initial:

- humidity - 65%;

 $-1^{st}$  step – the preparation of a sourdough: mix flour, water and a new starting composition (mixture humidity 65%) and keep this mixture at a temperature of 24 ± 1 °C for 16–18 h to achieve a pH of 4.0–5.5.

The quantity of lactobacilli in starter composition was  $(3-4) \ 10^8 \text{ CFU g}^{-1}$  and the quantity of yeast was  $(1-1.2)10^8 \text{ CFU g}^{-1}$ .

 $-2^{nd}$  step: the subsequent maintenance of the leaven implies refreshing once a day.

The ratio of fermented sourdough: nutrient mixture – 1:5. Sourdough ferments for 24 h in two stages: Stage I at a temperature of  $24 \pm 1$  °C for 2–3 h and Stage II at a temperature of 10– 12 °C for 21–22 h. Cooling is necessary to preserve the sourdough during a break in the work of the bakery. The wheat sourdough formulation is presented in Table 2.

**Table 2.** Formulationsusedtopreparesourdough at the first and second step

8	1	
Ingredients, g	I step	II step
New starter composition	20.0	-
Wheat flour	394.0	2,047.0
Water	586.0	2,953.0
Fermented sourdough of	-	1,000.0
I step		
Total	1,000.0	6,000.0

#### **Bread making procedure**

Wheat bread formulations used in this study are presented in Table 3. Percentages of ingredients were based on 100 g of flour. Part of the wheat flour was replaced by flour in the composition of sourdough in accordance with the traditional Russian bread making way of dosage (Kosovan, 2008).

Required quantity of sourdough was mixed with the rest of the flour in the recipe, yeast, salt and water until dough humidity of 45%. Control dough was prepared by mixing all the components without sourdough. After mixing, the dough was fermented for 60 min. After that dough were shaped into 400-g loaves, placed in aluminium to calculate to a moisture content test 45% pans, and leavened at 30 °C until the volume was twice the initial volume. The leavened dough were cooked in an oven SvebaDahlen (Sweden) at 230 °C for 20 min.

Ingredients, g	Control	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Wheat flour	100.0	95.0	90.0	85.0	80.00	75.0
Wheat flour inside the sourdough	-	5.0	10.0	15.0	20.0	25.0
Sourdough	-	12.3	24.6	36.9	49.2	61.5
Salt	1.3					
Bakery yeast	1.0					
Water	66.9	59.1	51.2	43.1	35.1	25.4

**Table 3.** Formulations of the dough

#### Sourdough and dough assessments

Sourdoughs and the doughs samples were evaluated for different parameters. Moisture of the dough and sourdough 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 (State Standard of the Russian Federation, 1996). The lifting capacity was determined by the rate at which it rose in a glass of water at a temperature of 32 °C for a 10 g mass of dough shaped into a ball and with a humidity level of 45% (Puchkova, 2004). The increase in volume was calculated by the ratio between the final volume and the initial volume multiplied by 100%. To determine the the lactobacilli and yeast proportion in a leaven, the method of microscopy and counting in a fixed colored preparation in 50 fields of view was used (Afanasjeva, 2003).

The study of the sourdough rheological properties (dynamic viscosity) was carried out on the rotational viscometer (Reotest-2, Germany). The strain rate was varied from 0.333 to 27 s<sup>-1</sup>. The measurements were carried out in a cylinder-measuring device according to Couette (measuring device S3) at a temperature of 28–30 °C, the weight of the starter was 50 g. The viscosity was calculated using the formula given in the device instructions (in Pa s).

The gas-forming and gas-holding capacity of the dough were determined using a F3 Chopin Reo-fermentometer. Dough samples weighing 315 g were placed on the bottom of the drum, preheated to 28.5 °C. Installed on the dough piston and tightly closed the system lid. The duration of the experiment was 300 minutes. The movement of the piston, which was mounted directly on the dough, estimated the rise of the dough during the fermentation.

# Assessment of baked bread

Assessment of quality. The quality of bread was ascertained evaluating the following parameters. Moisture and acidity (as reported above). Porosity was determined as the ratio of pore volume to the total volume of products, pore volume – as the difference between the volume of product and the volume of non-porous mass, specific volume – as the ratio of product volume to 100 g of bread, compressibility was determined on the automatic penetrometer Labor (Hungary) (Puchkova, 2004). The content of volatile acids was determined by neutralizing the evaporated volatile acid using a 0.1 n. solution of NaOH. The alcohol content was determined by using the iodometric method, which is based on the quantity of sodium thiosulfate spent in titration.

Assessment of sensory characteristics. A panel of 10 non-specialists was used to evaluate the sensory characteristics of the sourdough bread produced. Then, they were asked to evaluate separately smell, taste, texture of crumb, color of crust and porosity.

The ranking scale ranged from 1 to 5 (5–like extremely, 4.5–like very much, 4–like moderately, 3.5–like slightly, 3–neither like nor dislike, 2.5–dislike slightly, 2–dislike moderately, 1.5–dislike very much, 1–dislike extremely).

## Molds spoilage assessment

The impact of the sourdough on mold disease of wheat bread was investigated. The model experiments with contamination of sterile bread slices of a pure culture of the mold *Penicillium chrysogenum* were carried out (Dubrovskaya, 2018). Immediately after baking in the oven opening, the loaves were packed into sterile paper, placed in a sterile room, and cooled to a temperature of between 25-28 °C. After cooling the bread was cut in a sterile environment, with slices being taken at a size of  $3.5 \times 6.5$  cm and at a thickness of 0.3-0.4 cm. The slices were placed in sterile Petri dishes. An aqueous suspension of a pure culture of the mold, *Penicillium chrysogenum* was prepared for the infection of slices of bread. The biomaterial of *Penicillium chrysogenum* was transferred from a tube containing a pure culture of mold grown on malt agar to 1ml of sterile water using 'Tween-80' and was thoroughly suspended. The suspension was inoculated into each slice of bread in three shots using a microbiological needle. Petri dishes with infected slices were incubated at a temperature of  $25 \pm 1$  °C until the first signs appeared of a growth of mold colonies. Mold growth was monitored in a first 16 hours and every 2 hours.

# Ropy disease assessment

To determine the effect of starter on microbial resistance the bread was infected with a strain of *B. subtilis* specie (Dubrovskaya, 2018). To contaminate the bread, bread crumbs with spores were prepared next way. Spore-forming bacteria on meat-peptone medium was added to the surface of the sliced bread and cultured at a temperature of  $37 \,^{\circ}$ C for 96 hours or until signs of disease. Diseased bread dried in an oven at a temperature of  $50 \pm 2 \,^{\circ}$ C and milled to obtain crumbs. Crumb contained  $10^8 \,$  spores  $\cdot g^{-1}$ . 1% of infected crumbs were added while kneading the dough for wheat bread. Ready bread was stored at  $37 \,^{\circ}$ C before the appearance of symptoms of the ropy disease (Afanasjeva, 2003). Ropy bread disease appirience was monitored in a first 16 hours and after that every 4 hours.

## Statistical analysis of the data

All of the experiments were carried out a total of five times. Statistical analysis was performed using Excel software with significance tested at the 95% confidence level and differences among means were determined using the least significant difference and Duncan's test. The confidence intervals shown in the histograms and in the table reflect the accuracy of the used methods.

## **RESULTS AND DISCUSSION**

Lactic acid bacteria were characterized for some technological properties. L. plantarum E90 and L. brevis E120 had the highest acidifying activity. The greatest amount of volatile acids, which together with other aroma-forming substances make a significant contribution to the formation of taste and smell of finished bakery products, was produced by L. brevis E120 strain (Table 4). Studies of the lactobacilli antagonistic activity have shown that all strains inhibited the growth of *B*. *subtilis*. The strongest antimicrobial effect *L. plantarum* species have had. The inhibition zones did not differ significantly for all *L. plantarum* strains (Fig. 1). The comparison of the antagonistic activity and the total acidity, showed that strains: E 85, E 94, E 104, despite their low acidifying activity had high inhibitory activity.

 Table 4. Acid-forming activity of lactic acid bacteria

Lactobacilli	Acidity	Volatile
strains	degrees N	acids, %
L. plantarum E85	$6.4\pm0.3^{\rm a}$	$0.56\pm0.03^{\rm a}$
L. plantarum E94	$5.8\pm0.3^{\rm a}$	$0.53\pm0.03^{\rm a}$
L. plantarum E104	$6.5\pm0.3^{\rm a}$	$0.45\pm0.02^{\text{b}}$
L. plantarum E90	$10.0\pm0.5^{\rm b}$	$0.43\pm0.02^{\text{b}}$
L. brevis E120	$10.0\pm0.3^{\text{b}}$	$1.08\pm0.05^{\rm c}$

a-c = Means  $\pm$  SD within the same column with different lowercase superscript letters are significantly different ( $P \le 0.05$ ).

Hence, it can be assumed that these bacteria are capable of synthesizing other inhibitors, e.g. bacteriocins.

The findings from the study were in line with the findings from similar studies conducted, where lactobacilli showed significant antimicrobial activities against *Bacillus* (Klewicka & Libudzisz, 2004; Denkova et al., 2013; Khandakar et al., 2014)



Figure 1. Antagonistic activity of lactic acid bacteria.

The yeast fermentation activity was investigated. Yeast strains had different fermentative activity. The strain *S. cerevisiae Y139* had the highest fermentative activity after 24 hours of fermentation (Fig. 2). The difference in fermentation activity may be due to the peculiarities of the enzyme systems of each strain. The variations in fermentation efficiencies of yeast strains were also noted the when studied wines and juice fermentation (Wahab et al., 2005; Joshi et al., 2009; Sharma et al., 2011). Fermentation efficiency is totally dependent up on the ability of yeast strain to respond over various stress conditions subjected during fermentation, such as pH, high ethanol concentration, osmotic pressure, nutrient availability (Bauer & Pretorius, 2000, Sharma et al., 2011).

The lactobacilli strains *L. plantarum E90* and *L. brevis E120* and the yeast *S. cerevisiae Y139* were used for the new starter microbial composition.



Figure 2. Fermenting activity of yeast

The effect of grains products on the survival of lactic acid bacteria and yeast was investigated in dry microbial compositions. Immediately after drying, the largest number of lactobacilli cells was detected in the microbial composition with rice product (Fig. 3). The highest number of viable lactobacilli cells during storage was detected in compositions with rice and oats products. After 1 month of storage it was 95.5% and 80% of the initial quantity, respectively. Two months later, it was 68% and 63%, respectively.



Figure 3. Survival of lactobacilli cells during storage.

The largest number of viable yeast cells was detected in the dried mix with rice products. Immediately after drying it was 35–38% higher than in other samples (Fig. 4). The smallest number of living cells during storage was in mix with wheat products. After 2 months of storage, the number of viable yeast cells in mix with wheat products decreased to 48% compared with initial number. It may be because used rice products have more fibers to immobilize and to protect yeast cells.



Figure 4. Survival of yeast cells during storage.

A new starter composition was developed. It includes lactobacilli (L. plantarum E90 and L.brevis E120) and yeast (S. cerevisiae Y139) mixed with rice products in a ratio of 1:3.

The effect of the new starter culture on the quality of the sourdough was investigated. Indicators of a new sourdough at the step I and step II during 24 hours of fermentation were established (Table 5).

It was established that sourdough had lower acidity at the step I than at step II as it needs time to lactobacilli from the starter composition grow up at the dominant quantity. At the second step acid accumulation was most intense in the first 2–3 h at the temperature of 23–25 °C. The rate

**Table 5.** Biotechnological indicators of sourdough at the step I and step II

Indicators	Step I	Step II
Acidity, deg. N:		
at the temperature 23–25 °C	$5.2\pm0.3^{\text{a}}$	$5.4 \pm 1.2^{b}$
at the temperature 10–12 °C	-	$9.5 \pm 1.0$
Lifting capacity, min:		
at the temperature 23–25 °C	$26\pm12^{a}$	$63\pm7^{b}$
at the temperature 10–12 °C	-	$27\pm8$
Lactobacilli: yeast proportion		
at the temperature $23-25$ °C	`1:13	1:100
at the temperature 10–12 °C	-	1:22

a-b = Means  $\pm$  SD within the same row with different lowercase superscript letters are significantly different ( $P \le 0.05$ ).

of acid accumulation was 2.1–2.2 deg h<sup>-1</sup>. At the temperature of 10–12 °C, the rate of acid accumulation slowed down and was 0.19–0.2 deg h<sup>-1</sup>. It indicates that the lactic acid fermentation proceeded more intensively in the first period of fermentation at the step II. Sourdough lifting capacity reached optimal values by the end of the second fermentation period at the temperature of 10–12 °C. It means that yeast cells accumulate in sufficient quantity only by the end of fermentation. And it confirms that the sourdough quality will be good enough for dough preparing once a day.

The effect of fermentation on the sourdough texture was studied. It was established (Fig. 5) that the sourdough dynamic viscosity at the beginning and at the end of fermentation at a temperature of 25 °C was 16 and 17 times higher respectively when a strain rate was  $0.33 \text{ s}^{-1}$  than when it was 27 s<sup>-1</sup>. The sourdough dynamic viscosity at the end of fermentation at a 10–12 °C was 9.4 times higher when deformation rate was  $0.33 \text{ s}^{-1}$  than when it was of 27 s<sup>-1</sup>. Studies have shown that the sourdough dynamic

viscosity at a temperature of 10–12 °C at the lowest strain rate was 2.2 times lower than in sourdough fermented at 25 °C. It showed that the fermentation process leads to the liquefaction of the sourdough. It may be due to the enzymes action of lactic acid bacteria and yeast on the dough biopolymers. Acidification and the reduction of disulfide bonds of gluten by lactobacilli promote the primary activity of cereal proteases, which lead to the liberation of various sized polypeptides. Intracellular peptidases of sourdough lactic acid bacteria complete proteolysis and liberated free amino acids (Loponen et al., 2004; Gobbetti et al., 2014).



Figure 5. Indicators of the sourdough dynamic viscosity, depending on the strain rate.

The effect of the sourdough on the dough quality was studied. It was established that the acidity increased while sourdough dosage increase (Fig. 6, a), as was expected. Acidity increased twice when 25% of flour was replaced by sourdough (sample 5). It is obvious because of the sourdough acidity. The dough lifting capacity (Fig. 6, b) was worse than without sourdough. It may be because sourdough acidity inhibits the bakery yeast fermentation.

The influence of sourdough on the bread quality was studied. It was established that the acidity in the samples of sourdough bread (Fig. 7) expectably increased. In samples 4 and 5 the acidity was 0.2 and 0.4 degrees higher than normative indicator for such kind of bread in accordance with Russian Federal normative documentation (State Standard of the Russian Federation GOST 27842–88). These samples were indicated as non-standard breads.

Specific volume (Fig. 7) of samples 1-4 was 11.5-15.4% higher compared to the control. Sandra et al. (2012) reported that the addition of 20% of wheat sourdough increases CO<sub>2</sub> production, which may influence on the bread volume. But specific volume of sample 5 was 7.7% lower compared to the control. It may be because such a big quantity of sourdough inhibits yeasts (Torrieri et al., 2014; Gamel et al., 2015).



**Figure 6.** The effect of wheat yeast on the quality of dough. \* Examples of samples are given in Table 3.

The alcohol content in the sourdough bread (Fig. 7) was lower compared to the control, which is obviously associated with the suppression of the development of yeast cells because of the dough acidity. The content of volatile acids in bread increased with sourdough dosage increasing (Fig. 7). It contributed to the improvement in the taste and smell of bread. However, the taste of samples 4 and 5 was too sour. Gamel et al. (2015) and others also reported than organic acids and alcohols produced by lectobacilli and yeasts influence the flavor and aroma.



■ acidity, deg. N ■ Specific volume,sm3·g-1 ■ alcohol, % ■ volite acids,%



The data obtained confirm other studies. Depending on the quantity of using sourdough the fermentation process leads to the chances in bread quality (Gobbetti et al., 2008, Sandra et al., 2012; Therdthai & Jitrakbumrung, 2014; Rinaldi, 2015; Nionelli et al., 2016).

Sensory characteristics of bread are presented in Fig. 8. Sample 1 and sample 2 had the better crust color, crumb texture and porosity than the control. Samples 1, 2 and 3 had the best taste and smell. The smell was more intense, pleasant. Samples 4 and 5 had too sour taste and smell, and its crumbs had gray or grayish surface which is usually due to the high acidity (Gobbetti et al., 2014; Torrieri et al., 2014)





The effect of wheat sourdough on the bread shelf-life was studied. It was established that with increasing sourdough dosage, resistance to mold was increased for 16–56 h compared with the control. Moreover, it was found that samples No. 1 became moldy 36 hours later than the control. Samples No. 2–5 did not exhibit ropy disease during the entire storage period. Increasing the microbial safety and shelf life of wheat sourdough bread has also been reported by Gamel et al. (2015) and Katina et al. (2009).

Considering the data obtained above, for further studies were chosen sourdough dosages 5 and 10%.

The effect of wheat sourdough on the gas-forming and gas-holding capacity of dough was studied. Reofermentometric characteristics of dough samples are presented in Table 6.

 Table 6. Dough reofermentometric characteristics

Indicators	control	Sample 1	Sample 2
Total volume of released CO <sub>2</sub> , sm <sup>3</sup>	$1,756.0 \pm 88.0^{a}$	$2,051.0 \pm 103.0^{b}$	$1,946.0 \pm 97.0^{b}$
Volume of CO <sub>2</sub> retained, sm <sup>3</sup>	$1,706.0 \pm 85.0^{\mathrm{a}}$	$2,026.0 \pm 101.0^{b}$	$1,\!919.0\pm96.0^{\rm b}$
Volume of lost CO <sub>2</sub> , sm <sup>3</sup>	$50\pm3^{a}$	$25 \pm 1^{b}$	$27 \pm 1^{b}$
Gas retention coefficient,%	$97.2\pm4.9^{\rm a}$	$98.8\pm4.9^{\rm a}$	$98.6\pm4.9^{\rm a}$

a-e = Means  $\pm$  SD within the same row with different lowercase superscript letters are significantly different ( $P \le 0.05$ ).

The effect of the sourdough on the quality indicators of the dough prepared with different flour types was investigated. It was established that dough acidity were higher when used flour with falling number of 216 s than when used flour with falling number 343 s (Fig. 9, a). The dough lifting capacity was worse when used flour with the falling number 216 (Fig. 9, b). The falling number shows the activity of amylase. The greater the falling number, the lower the activity of amylase. Low amylase activity leads to less production of maltose and glucose. Consequently, the deterioration of lifting capacity was because of lower content of maltose and glucose for lactobacilli and yeast feeding when used flour with a falling number of 343 s.



Figure 9. Quality of sourdough dough with different types of flour.

The effect of wheat sourdough was studied on the physic-chemical quality indicators of the bread prepared with different quality flour. It was found that the acidity was lower when using flour with a falling number of 343 s (Fig. 10, a). It was expected, since the acidity of this dough was also lower. The specific volume of the dough samples (Fig. 10, b) was lower when used flour with a falling number of 343 s than when used flour with falling number of 343 s than when used flour with falling number of 343 s had slightly lower compressibility of the crumb (Fig. 10, c) than bread prepared with flour with a falling number 216 s and was almost the same as compared with the control prepared without sourdough. Obtained data showed that the sourdough



allowed bread quality improving even when flour with unsatisfactory amylolytic activity (high falling number) was used.

Figure 10. Quality of sourdough bread from different types of flour.

## CONCLUSIONS

To develop a new microbial starter composition, lactic acid bacteria strains *L. plantarum E90*, possessing the greatest antagonistic activity, and *L. brevis E120* strain, producing the largest amount of volatile acids, as well as the yeast *S. cerevisiae* Y139, having the best fermentative activity, were selected. It was proven that the use of rice products in the composition makes it possible to obtain highest number of viable cells. It was established that the acid addition rate in sourdough during the first 2–3 h at

a temperature of 23-25 °C was  $2.1-2.2 \text{ deg h}^{-1}$ . Subsequently, at the temperature of 10-12 °C, the rate of acid accumulation slows down to  $0.19-0.2 \text{ deg h}^{-1}$ . The decrease in the sourdough dynamic viscosity by the end of fermentation by 2.2 times was established, i.e. the fermentation process leads to the liquefaction of the sourdough. The optimal dosage was established (5-10% flour in sourdough). This dosage provided good physico-chemical and organoleptic quality indicators of bread. In the dough samples with 5 and 10% of flour in the sourdough, the volume of the released and retained gas was higher than in the control one. The volume of gas lost in the leaven dough samples was 1.7-2 times less compared to the control one, as evidenced by the large specific volume of bread samples. The use of sourdough allowed slowing the mold disease and completely inhibiting the ropy-bread disease. It was proven that the use of sourdough allowed getting good quality bread even when using flour with unsatisfactory amylolytic activity (high falling number).

#### REFERENCES

- Afanasjeva, O. 2003. *Microbiology of bakery production*. Beresta, Saint-Peterburg, 217 pp. (in Russian).
- Arendt, E.K., Ryan, L.A., Dal Bello, F. 2007. Impact of sourdough on the texture of bread. *Food Microbiol.* **2**4, 165–74.
- Auerman, L. 2009. *Technology of breadmaking*. Textbook. Professia, Moscow, 422 pp. (in Russian).
- Bauer, F.F. & Pretorius, I.S. 2000. Yeast stress responses and fermentation efficiency—how to survive the making of wine–a review. *S Afr J Enol Vitic* **21**, 27–51.
- Chavan, R.2011. Sourdough technology A traditional way for wholesome foods: A review. Compr. Rev. *Food Sci. Food Saf.* **10**, 169–182.
- Corsetti, A., Lavermicocca, P., Morea, M., Baruzzi, F., Tosti, N. & Gobbetti, M. 2001. Phenotypic and molecular identification and clustering of lactic acid bacteria and yeasts from wheat (species Triticum durum and Triticum aestivum) sourdoughs of Southern Italy. *Int. J. Food Microbiol* **64**, 95–104. https://doi.org/10.1016/S0168-1605(00)00447-5.
- Corsetti, A. & Settanni, L. 2007. Lactobacilli in Sourdough Fermentation. *Food Research International* **40**, 539–558. http://dx.doi.org/10.1016/j.foodres.2006.11.001
- De Blackburn, C. 2008. Food Spoilage Microorganisms. Woodhead Publishing, 736 pp.
- Dec, M., Puchalski, A., Nowaczek, A. & Wernicki, A. 2016 Antimicrobial activity of Lactobacillus strains of chicken origin against bacterial pathogens. *International Microbiology* 19(1), 57–67.
- Denkova, R., Denkova, Z., Yanakieva, V. & Blazheva, D. 2013. Antimicrobial activity of probiotic lactobacilli, bifidobacteria and propionic acid bacteria, isolated from different sources. Microbial pathogens and strategies for combating them: science, technology and education, pp. 857–864.
- De Vuyst, L., Van Kerrebroeck, S., Harth, H., Huys, G., Daniel, H. & Weckx, S. 2014. Microbial ecology of sourdough fermentations: Diverse or uniform. *Food Microbiology* **37**, 11–29.
- Di Renzo, T., Reale, A., Boscaino, F. & Messia, M.C. 2018. Flavoring 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**. doi:10.3389/fmicb.2018.00429
- Dubrovskaya, N., Savkina, O., Kuznetsova, L. & Parakhina, O. 2018. The development of gluten-free sourdough bread technology with rowan powder. *Agronomy Research* **16**(S2), 1360–1372.

- Espinosa, J., Gloria, J., Angulo, A., Escobedo, R., Pérez, J., Rebollo, R. & Domínguez, G. 2011. Sourdough and Bread Properties as Affected by Soybean Protein Addition, In T.B. Ng, (eds.). Soybean - Applications and Technology. *InTech.* Rijeka, Croatia, pp. 387–402.
- Gamel, T., Abdel-Aal, E. & Tosh, S. 2015. Effect of yeast-fermented and sour-dough making processes on physicochemical characteristics of  $\beta$ -glucan in whole wheat/oat bread. *LWT*-*Food Science and Technology* **60**(1), 78–85.
- Gobbetti, M., Angelis, M., Cagno, R & Rizzello, C. 2008. Sourdough/Lactic acid bacteria, In E.K. Elke and F.D. Bello, (eds.). Gluten-Free Cereal Products and Beverages. *Academic Press*. Burlington, MA, USA, pp. 267–283.
- Gobbetti, M, Rizzello C.G., Di Cagno, R. De Angelis, M. 2014. How the sourdough may affect the functional features of leavened baked goods. *Food Microbiol.* **37**, 30–40.
- Huys, G., Daniel, H.M. & De Vuyst, L. 2013: *Taxonomy and biodiversity of sourdough yeasts and lactic acid bacteria*. In: Gobetti, M., Gänzle, M. (eds): Handbook on Sourdough Biotechnology. New York, Springer, pp. 105–154.
- Iacumin L., Cecchini, F., Manzano, M., Osualdini, M., Boscolo, D., Orlic S. & Comi, G. 2009. Description of the microflora of sourdoughs by culture-dependent and culture-independent methods. *Food Microbiol* 26, 128-35. https://doi.org/10.1016/j.fm.2008.10.010
- Joshi, V.K., Sharma, S. & Devi, M.P. 2009 Influence of different yeast strains on fermentation behaviour, physico-chemical and sensory qualities of plum wine. *Natur Prod Radiance* **8**, 445–451.
- Katina, K., Maina, N. H., Juvonen, R., Flander, L., Johansson, L., Virkki, L., Tenkanen, M., Laitila, A. 2009. In situ production and analysis of *Weissella* confusa dextran in wheat sourdough. *Food Microbiology* 26, 734–743.
- Khandakar, M., Kabir, R., Islam, R. & Sabir. 2014. Antagonism of Lactic Acid Bacteria Against Selected Pathogenic Bacteria and Spoilage Fungi. DOI: 10.5829/idosi.aejaes.2014.14.03.83282
- Klewicka, E. & Libudzisz, Z. 2004. Antagonistic activity of Lactobacillus acidophilus bacteria toward selected food-contaminating bacteria. *Polish journal of food and nutrition sciences* 13/54(2), 169–174.
- Kosovan, A. 2008. *The collection of modern technology of bakery products*. In Kosovan, A.P. (ed.), Moscow printing house, Moscow, 271 pp. (in Russian).
- Kurtzman, C. & Fell, J.W. 1998. The Yeasts, A Taxonomic Study, 4th Edition. *Elsevier Science* B.V., Amsterdam, **1076**.
- Loponen, J., Mikola, M., Katina, K., Sontag-Strohm, T. & Salovaara, H. 2004. Degradation of HMW Glutenins During Wheat Sourdough Fermentations. *Cereal Chemistry Journal* 81(1), 87–93. doi:10.1094/cchem.2004.81.1.87
- Messia, M.C., Reale, A., Maiuro, L., Candigliota, T., Sorrentino, E. & Marconi, E. 2016. Effects of pre-fermented wheat bran on dough and bread characteristics. *Journal of Cereal Science* **69**, 138–144.
- Minervini, F., Lattanzi, A., De Angelis, M., Celano, G. & Gobbetti, M. 2015. House microbiotas as sources of lactic acid bacteria and yeasts in traditional Italian sourdoughs. *Food Microbiology* 52, 66–76. https://doi.org/10.1016/j.fm.2015.06.009
- Nionelli, L. & Rizzello, C. 2016. Sourdough-Based Biotechnologies for the Production of Gluten-Free Foods. *Foods* **5**(3), 65.
- Polak-Berecka, M., Waśko, A. & Koston, D. 2009. Comparison of different methods for detection of antimicrobial activity of probiotic strains of Lactobacillus rhamnosus against some food spoilage microorganisms. *Annales UMCS, Biologia* **64**(1), 15–24.
- Poutanen, K., Flander, L. & Katina, K. 2009. Sourdough and cereal fermentation in a nutritional perspective. *Food Microbiology* 26(7), 693–699.
- Puchkova, L. 2004. Laboratory Workshop on Bakery Technology. Moscow, 264 pp. (in Russian).

- Rinaldi, R., Paciulli, M., Caligiani, G. & Sgarbi, E. 2015. Durum and soft wheat flours in sourdough and straight-dough bread-making. Journal of Food Science and Technology 52(10), http: DOI 10.1007/s13197-015-1787-2
- Reale, A., Konietzny, U., Coppola, R., Sorrentino, E. & Greiner, R. 2007. The importance of lactic acid bacteria for phytate degradation during cereal dough fermentation. *Journal of Agricultural and Food Chemistry* 55, 2993-2997
- Reale, A, Tremonte, P., Succi, M., Sorrentino, E. & Coppola, R. 2005. Exploration of lactic acid bacteria ecosystem of sourdoughs from the Molise region. *Annals of Microbiology* 55, 17–22.
- Sakandar, H., Hussain, R., Kubow, S. & Sadiq, F. 2018. Sourdough Bread: a Contemporary Cereal Fermented Product. *Journal of Food Processing and Preservation* /http: wileyonlinelibrary.com/journal/jfpp
- Sandra, G., Schwab, C., Bello, F., Coffey, A., Ganzle, M. & Arendt, E. 2012. Comparison of the impact of dextran and reuteran on the quality of wheat sourdough bread. *Journal of Cereal Science* 56, 531–537.
- Sharma, A.K., Singh, P.N. & Sawant, S.D. 2011. Evaluation of Fermentation Efficiency of Yeast Strains and their Effect on Quality of Young Wines. *Indian Journal of Microbiology* 52(3), 495–499. doi:10.1007/s12088-011-0226-y
- State Standard of the Russian Federation. 1988. Bread from wheat flour. Technical conditions GOST 5670-96, 11 pp. (in Russian).
- State Standard of the Russian Federation. 1996. Bread, rolls and buns. Methods for determination of acidity. GOST 27842-88, 5 pp. (in Russian).
- Succi, M., Reale, A., Andrighetto, C., Lombardi, A., Sorrentino, E. & Coppola, R. 2003. Presence of yeasts in southern Italian sourdoughs from Triticum aestivum flour. *FEMS Microbiology Letters* 225, 143–148. https://doi.org/10.1016/S0378-1097(03)00500-7
- Therdthai, N. & Jitrakbumrung, S. 2014. Effect of addition of sourdough on physicochemical characteristics of wheat and rice flour bread. *Natural Science* **48**(6), 964–969.
- Torrieri, E., Pepe, O., Ventorino, V. & Masi, P. 2014. Effect of sourdough at different concentrations on quality and shelf life of bread. *LWT- Food Science and Technology* **56**(2), 508–516.
- Wahab, O.O., Rufus, O.O. & Akinniyi, A.O. 2005. The alcoholic fermentative efficiency of indigenous yeast strains of different origin on orange juice. *Afr. J. Biotechnol.* **4**, 1290–1296.