Development of symbiotic starters of lactic acid bacteria, propionic acid bacteria and yeast for sourdough for bread and bakery products and their probation in industrial conditions

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Abstract. Sourdough starter development includes examination of potential strains of lactic acid bacteria (LAB), propionic acid bacteria and yeasts, selection of suitable strains, development of combinations and examination of the relationships between the strains in each combination, selection of symbiotic combinations and their industrial probation to determine their qualities. Three sourdough starter combinations from selected lactobacilli (homo- and heterofermentative species) or selected lactobacilli and propionic acid bacteria were developed. The dynamics of the concentration of viable cells of the strains and the sourdough acidity in the back-slopping process up to the 96th hour were monitored. The rheological properties of the starter sourdoughs were characterized. The main dough obtained with sourdough was stronger, more elastic, the pieces of bread were taller. The developed sourdough starters were tested in the production of wheat, wheat-rye, spelt and spelled bread. The finished bread loaves had softer and lighter crumb, and pleasant and characteristic lactic acid aroma. It has been shown that the different bread types obtained with symbiotic starter sourdoughs had longer shelf life and increased microbiological safety. The best starter combination as well as the optimum concentration of starter sourdough for prevention of bacterial and fungal spoilage for each bread type has been determined.

1 Introduction

Bread is one of the most consumed products worldwide with an annual production of 9 billion kg [1-4]. It is an important ingredient of the daily human diet in almost every country. It is a source of nutrients, especially carbohydrates, fiber, proteins and some minerals (magnesium, phosphorus, iron) [5]. The main raw materials for its production are flour, water, salt and yeast.

Traditional bread is produced by biological leavening, under the action of baker's yeast or by applying starter cultures of LAB, propionic acid bacteria and yeast [6-8] leading to observing several positive characteristics of the final bread [9]. Biotechnology for the development of starter sourdoughs offers several nutritional and functional advantages over the use of baker's yeast alone in the production of bread and bakery products [4, 10]. For this reason, fermentation with the participation of sourdough is a widely advocated method for bread production [4, 6, 7, 11]. The application of starter cultures in food production directly affects food quality criteria such as nutritional development, shelf life, production of digestible and palatable foods. The main characteristics of white bread obtained with sourdough are parameters such as high volume and shelf life, microbiological safety, good nutritional and sensory properties [4, 6, 7, 12]. Thus, the deterioration of bread quality caused by spore-forming bacteria and fungi is delayed [4, 6, 7, 13, 14]. The economic losses from fungal spoilage of bread are particularly significant. Therefore, various methods are used to prevent it, such as the addition of chemical preservatives, different packaging methods, etc., but the use of sourdough is an alternative method for bread production, that also improves the textural and aromatic properties of the finished bread [15, 16].

Sourdough bread is a traditional product with high nutritional qualities as a result of the positive interaction and biochemical reactions of yeast and LAB [4, 6, 7]. Application of sourdough fermented by LAB, propionic acid bacteria and yeast is an alternative to commonly used additives in bread production and a strong bioprotective agent [17].

Lactic acid, acetic acid and other metabolites, such as bacteriocins, which suppress the growth of unwanted pathogenic and saprophytic microflora accumulate during the growth of lactobacilli and propionic acid bacteria in sourdough. In order to be included in the composition of sourdough starters, it is of particular importance that the strains of lactobacilli and propionic acid bacteria accumulate high concentrations of viable cells in a short time to carry out a purposeful fermentation process.

The purpose of the present study was the development of symbiotic sourdough starters from lactobacilli (homo- and heterofermentative) or lactobacilli and propionic acid bacteria, obtaining starter sourdoughs and probation of the sourdoughs in the production of wheat bread, wheat-rye bread, spelled bread and spelt bread, selecting the best starter combination for each bread type as well as the optimal percentage of starter sourdough addition for the prevention of bacterial and fungal spoilage.

2 Materials and methods

2.1 Microorganisms

The research in the present work was carried out with 5 LAB strains - *Lactiplantibacillus paraplantarum* Ph3 (*Lpb. paraplantarum* Ph3), isolated from spontaneously fermented wheat flour sourdough, *Levilactobacillus brevis* Car (*Lvb. brevis* Car), isolated from spontaneously fermented corn flour sourdough, *Lacticaseibacillus casei* subsp. *rhamnosus* LBRC11 (*Lbs. casei* subsp. *rhamnosus* LBRC11), *Limosilactobacillus fermentum* LBRH10 (*Lmb. fermentum* LBRH10), *Fructilactobacillus sanfranciscensis* R (*Frb. sanfranciscensis* R), *Propionibacterium freudenrechii* subsp. *shermanii* NBIMCC 327 and yeast *Saccharomyces cerevisiae* (baker's yeast) (Lesaffre).

Lactobacilli and propionic acid bacteria influence the acceleration of bread preparation time and improve technological performance. Sourdough combinations for the production of bread from different flours were developed based on the previously selected (data not published) two-strain combination of *Lpb. paraplantarum* Ph3 (isolated from spontaneously fermented sourdough from wheat flour) and *Lvb. brevis* Car (isolated from spontaneously fermented sourdough from Khorasan flour) with the addition of *Lbs. casei* subsp. *rhamnosus* LBRC11, *Lmb fermentum* LBRH10, *Frb. sanfranciscensis* R and *Propionibacterium freudenrechii* subsp. *shermanii* NBIMCC 327. Three combinations were created. Combination 1 included *Lpb. paraplantarum* Ph3, *Lvb. brevis* Car, *Lbs. casei* subsp. *rhamnosus* LBRC11, *Lmb fermentum* LBRH10 in a ratio of 2:1:1:1. Combination 2 was composed of Combination 1 (*Lpb. paraplantarum* Ph3 : *Lvb. brevis* Car : *Lbs. casei* subsp. *rhamnosus* LBRC11 : *Lmb fermentum* LBRH10 = 2:1:1:1) and *Frb. sanfranciscensis* R in a ratio of 2:1. Combination 3 was a combination of Combination 1 (*Lpb. paraplantarum* Ph3: *Lvb. brevis* Car: *Lbs. casei* subsp. *rhamnosus* LBRC11: *Lmb fermentum* LBRH10 = 2:1:1:1), *Frb. sanfranciscensis* R and *Propionibacterium freudenrechii* subsp. *shermanii* NBIMCC 327 in a ratio of 2:1:1. According to [18] and [19] mixed culture bread has better appearance, golden and crispy crust, larger volume.

2.2 Nutrient media

A. MRS broth medium (De Man, Rogosa & Sharpe's medium). Merck.
B. MRS agar medium. Merck.
C. Saline solution Composition (g/dm^3): NaCl – 5; distilled water – 1dm^3. It wass sterilized for 20 minutes at 121°C.
D. LAPTg10 broth Composition (g/dm^3): peptone – 15; yeast extract – 10; tryptone – 10; glucose – 10. pH was brought to 6.6 – 6.8 and Tween 80 - 1cm3/1dm^3 was added. The medium is sterilized for 20 min at 121°C.
E. LAPTg10 agar Composition (g/dm^3): Medium LAPTg10-broth + 2% agar. The medium was sterilized for 20 min at 121°C.
F. Sterile skimmed milk with titratable acidity 16-18ºT. Composition (g/dm^3): skimmed milk powder (Scharlau). Sterilization - 15 min at 121°C.
G. Elective medium for *Propionibacterium* sp. Composition (g/dm^3): tryptone – 10; yeast extract – 10; Na-lactate (freshly prepared) - 10g. Na-lactate - 7g lactic acid had been neutralized with 3.1g NaOH crystals and the remaining salts had been dissolved in distilled water had been added to it; KH_2PO_4 – 2.5; $MnSO_4$ – 0.005; agar - 20; pH 6.8. Sterilization - 20 min at 121°C.

2.3 Cultivation and storage of the studied microorganisms

Depending on the objectives of the individual experiments, the studied strains of microorganisms were cultivated on liquid media (MRS-broth, LAPTg10-broth) and on solid media (MRS-agar and LAPTg10-agar) at a temperature of 30 or 37°Cand for different durations. All tested strains were subcultured from a single colony and cultured on MRS-broth medium for 24 h to avoid the possibility of contamination. The strains were stored as a stock culture in MRS broth with 20% v/v glycerol at -20°C.

2.4 Physicochemical and microbiological methods

2.4.1 Determination of titratable acidity

The titratable acidity was determined according to a standard protocol using the Torner's method according to BDS 1111:1980. [20].

2.4.2 Determination of titratable acidity of sourdough/dough and bread

According to Vangelov and Karadjov [21].

2.4.3 Determination of the number of viable microorganisms

Appropriate tenfold dilutions in saline solution of each sample were prepared. 0.1 cm^3 from the last three dilutions were used for spread plating on or pour plating in the corresponding nutrient medium. The inoculated petri dishes and tubes were cultured for 3 d at the optimal temperature for growth of the respective microorganisms until the appearance of single colonies. The number of viable microorganisms was estimated based on the number of colonies.

2.4.4 Determination of the antimicrobial activity against saprophytic microorganisms

The agar diffusion method with wells was used to determine the antimicrobial activity of the four prepared starter sourdoughs. A dilution in a ratio of 1:1 of a sourdough:saline solution of each of the sourdoughs was prepared. The antimicrobial activity was tested against the following saprophytic test microorganisms: bacteria - *B. subtilis* ATCC 6051, *B. mesentericus*; yeasts - *Saccharomyces cerevisiae* ATCC 9763, fungi *Aspergillus niger* ATCC 16888, *Penicillium chrysogenum* ATCC 10106, *Rhizopus oryzae* ATCC 11145. A suspension of each of the test microorganisms (10^6 - 10^7CFU/cm^3) was used to inoculate a Petri dish with melted solid medium and after the hardening of the agar wells (7 mm) were prepared. 0.06 cm^3 of the dillusions were pipetted in the wells of the plates and the plates with the test microorganisms were incubated at 37 °C for 24 - 48 h, and then the inhibition zones in mm were reported [6,7].

2.5 Technological methods

2.5.1 Preparation of sourdough starters and their probation in production preparation of cell suspensions for inoculation of the flour/water mixture

10 cm^3 of MRS-broth were inoculated with the corresponding LAB strain (1%), followed by incubation for 24 hours at the optimal temperature for the growth of the strain – 30 °C or 37 °C. After incubation, the biomass was collected by centrifugation (6000 x g, 15 min, 4°C) and the pellet was resuspended to the initial volume with sterile saline. The resulting cell suspensions were used to inoculate the flour/water mixture to obtain the corresponding single strain sourdough. For the preparation of sourdough with a multi-strain combination, the 24-hour culture suspensions of the strains included in the sourdough combination were mixed and homogenized. They were then centrifuged and the pellet was resuspended to the initial volume of the mixed suspension with sterile saline solution and was used to inoculate the flour/water mixture to obtain multistrain sourdough combination.

2.5.2 Investigation of the reproductive capacity of the lactobacilli strains and the changes in the titratable acidity in starter sourdough

The ability of lactobacilli strains to grow in the flour/water foor matrix, to accumulate a high concentration of viable cells and to accumulate acids was determined by preparing single-strain sourdoughs with each of the studied LAB strains. The sourdough was prepared with the cell suspensions, the preparation of which is described above. The changes in the concentration of living cells in the sourdoughs and in the titratable acidity were monitored in the process of daily back-slopping for 96 h and incubation at the respective optimal temperature for the specific strain. The accumulation of biomass and the changes in the acidity of the single-strain sourdoughs under the following daily back-slopping scheme were investigated:

- day one - 44% flour: 56% tap water and 5% of the single strain cell suspension;
- second to fifth day: 25% sourdough from the previous day: 75% new flour/water mixture again in the ratio of 44%/56%.

The water temperature had to be around 40°C.

2.5.3 Probation of sourdough in bread production conditions

Main doughs with 5%, 7%, 10% of the starter sourdough with the corresponding multi-strain combination were prepared; the amount of included sourdough was determined according to the amount of flour. The following percentage ratios were used in the preparation of the main doughs: 1.5% salt, 2% baker's yeast, the corresponding percentage of sourdough and the corresponding amount of tap water (the amount of water was determined by the water absorption of the flour type used). The components were mixed with a dough mixer, first mixing slowly (1000 rpm) for 4 min, followed by rapid mixing (1400 rpm) for 10 min. The doughs were allowed to rest for about 10 min to improve their elastic properties. Rolls were formed and placed in the forms; then the doughs were left to leaven at high humidity (80±5 RH) and a temperature of 35°C for about 40-45 min. The leaven bread was baked at 225±5°C for 30 min. In production conditions, wheat bread, wheat-rye bread, spelled bread and spelt bread were baked with the corresponding percentage (5%, 7% or 10%) of sourdough with the respective strain combination. Besides, control breads from each flour type (bread without sourdough) were prepared. The baked bread loaves were cooled at room temperature for 120 min.

2.5.4 Determining the appearance of bacterial spoilage of baked bread

The determination of the occurrence of bacterial spoilage of the baked bread (roping of the bread) was carried out by 10 specialists by parallel storage of the baked bread with the corresponding percentage of starter sourdough at 37°C and at room temperature in the production laboratory. Bacterial spoilage was graded on a scale of I to IV, each grade corresponding to the following description: I – faint spoilage (pleasant fruity aroma); II – weak spoilage (clearly noticeable change in smell - acute); III – medium spoilage (moist, sticky environment, unpleasant smell); IV – strong spoilage (unpleasant smell, yellow-brown crumb, stringiness of the crumb)

2.5.5 Determining the appearance of fungal spoilage of baked bread

The determination of the occurrence of fungal spoilage of the baked bread was carried out by 10 specialists during parallel storage of the baked bread with the corresponding percentage of starter sourdough at 30°C and at room temperature in the production laboratory.

2.5.6 Processing of the results

Data from triplicate experiments were processed using MS Office Excel 2003, using statistical functions to determine the standard deviation and maximum error of estimate at $\alpha<0.05$ significance levels. Data presented are the arithmetic mean of three independent experiments and are presented as mean ± standard deviation (SD). Data were analyzed using one-way analysis of variance (ANOVA).

3. Results and discussion

3.1 Development of symbiotic starters for sourdough using lactobacilli and propionic acid bacteria for the production of wheat bread, wheat-rye bread, spelled bread and spelt bread

The ability of the strains from the composition of the combinations to grow in flour/water mixture was determined by preparing sourdoughs with each of the three combinations and esch flour type. The dynamics of the accumulation of cells and the changes in the titratable acidity of the sourdoughs during 96 h of daily back-slopping and cultivation at 30 ºC were monitored (Fig. 1, Fig. 2, Fig. 3, Fig. 4, Fig.5, Fig. 6 and Fig. 7).

The strains in all three combinations grew very well in the flour/water mixture, reaching 10^{13}-10^{14} cfu/cm^3 up to the 96th h (Fig. 1, Fig. 3, Fig. 5 and Fig. 6) and the acidity of the resulting sourdoughs increased up to 250°N (Fig. 2, Fig. 4 and Fig. 7). Corsetti et al. [22] showed that acidification of the medium is of particular importance for increasing the volume of bread and for retarding the retrogradation of starch. It is noteworthy that the growth dynamics of the lactobacilli strains from the composition of the Combinations demonstrated that the time to stabilize the sourdoughs was between 24-48 h, after which rapid growth and secretion of significant amounts of metabolites began. During fermentation, microorganisms generated volatile compounds, such as alcohols, acids, ketones and esters through the degradation of carbohydrates such as glucose, fructose and maltose. These metabolites were involved in the formation of the sensory quality of the food matrix [23, 24].

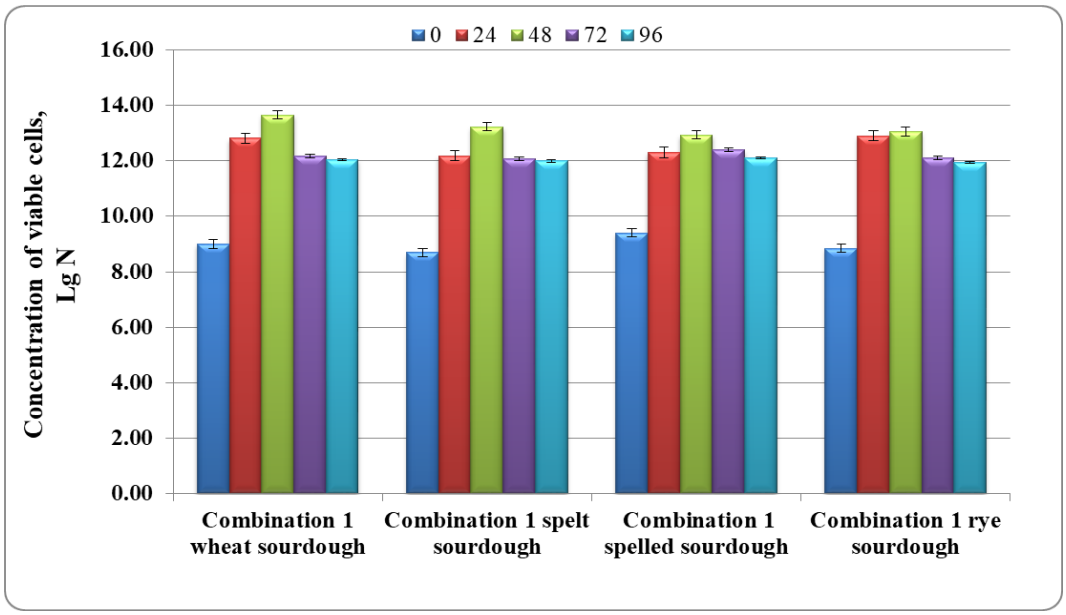

Fig. 1. Changes in the viable cell concentration of LAB in sourdough with Combination 1 during daily back-slopping for 96 h

Combination 1 - *Lbs. casei subsp. rhamnosus* LBRC11: *Lpb. paraplantarum* Ph3 : *Lvb. brevis* Car : *Lmb. fermentum* LBRH9 - 1: 2: 1: 1

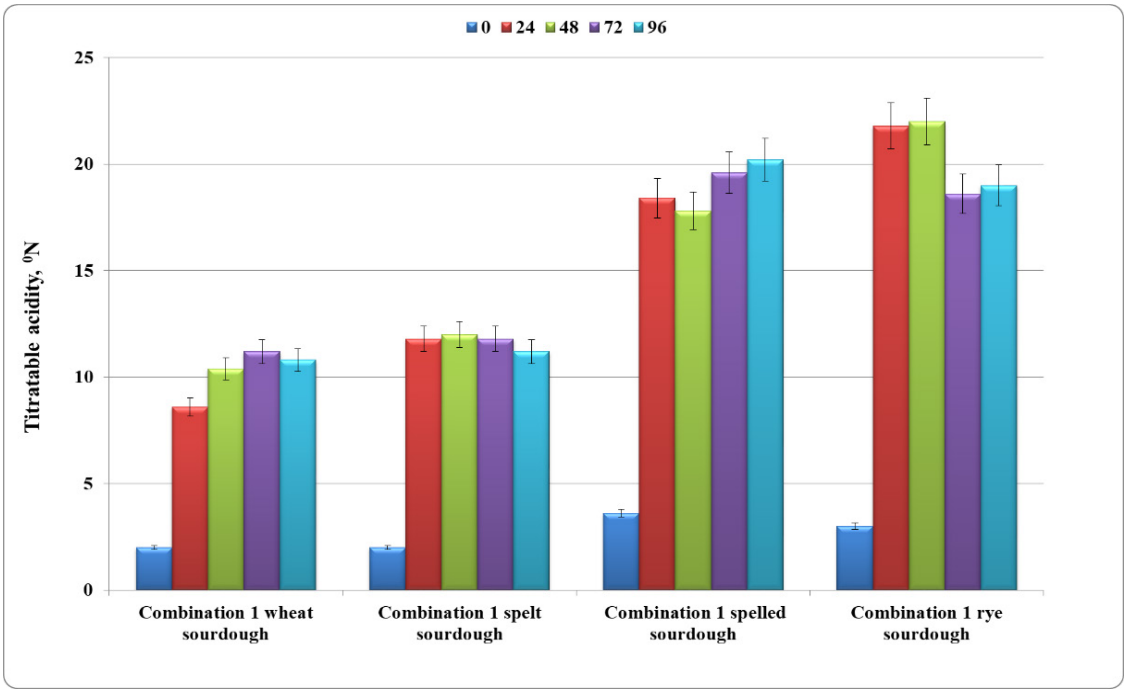

Fig. 2. Changes in the titratable acidity in sourdough with Combination 1 during daily back-slopping for 96 h
Combination 1 - *Lbs. casei subsp. rhamnosus* LBRC11: *Lpb. paraplantarum* Ph3 : *Lvb. brevis* Car : *Lmb. fermentum* LBRH9 - 1:2:1:1

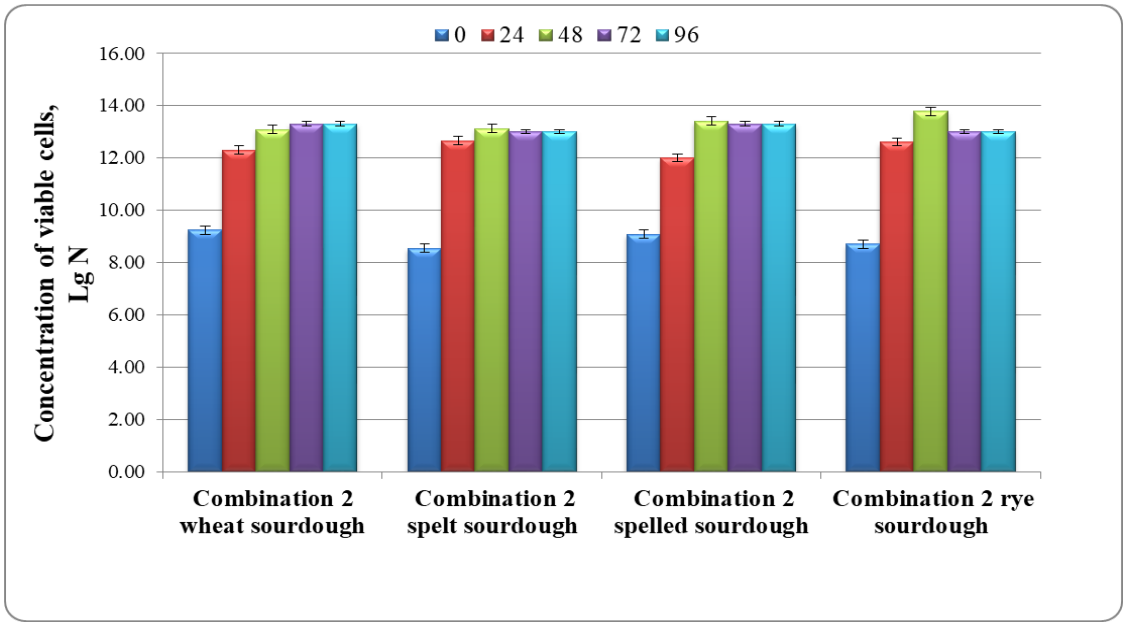

Fig. 3. Changes in the viable cell concentration of LAB in sourdough with Combination 2 during daily back-slopping for 96 h
Combination 2 - Combination 1 : *Frb. sanfranciscensis* R = 2:1

The aroma of all starter sourdoughs was typical, characteristic, strong, clearly pronounced lactic acid aroma without any unpleasant side aroma. All Combination microorganisms grew equally well. A slight acetone-like aroma was added to the typical sourdough aroma of Combination 3.

All three combinations in spelled and spelt flour formed richer, denser and stronger sourdough aroma. All combinations grew best in spelled and spelt flour, i. e. they accumulated the highest acidity and developed the richest aroma. The weakest aroma was observed in wheat flour breads. Combination 1 and Combination 2 differed slightly in aroma, but were both strong and pleasant.

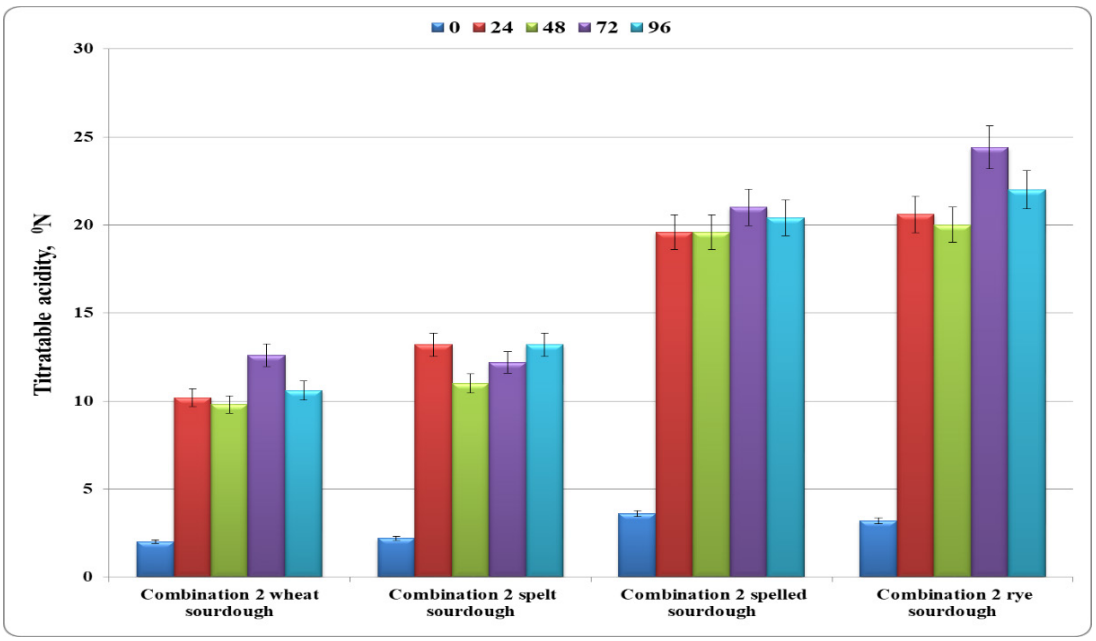

Fig. 4. Changes in the titratable acidity in sourdough with Combination 2 during daily back-slopping for 96 h
Combination 2 - Combination 1 : *Frb. sanfranciscensis* R = 2:1

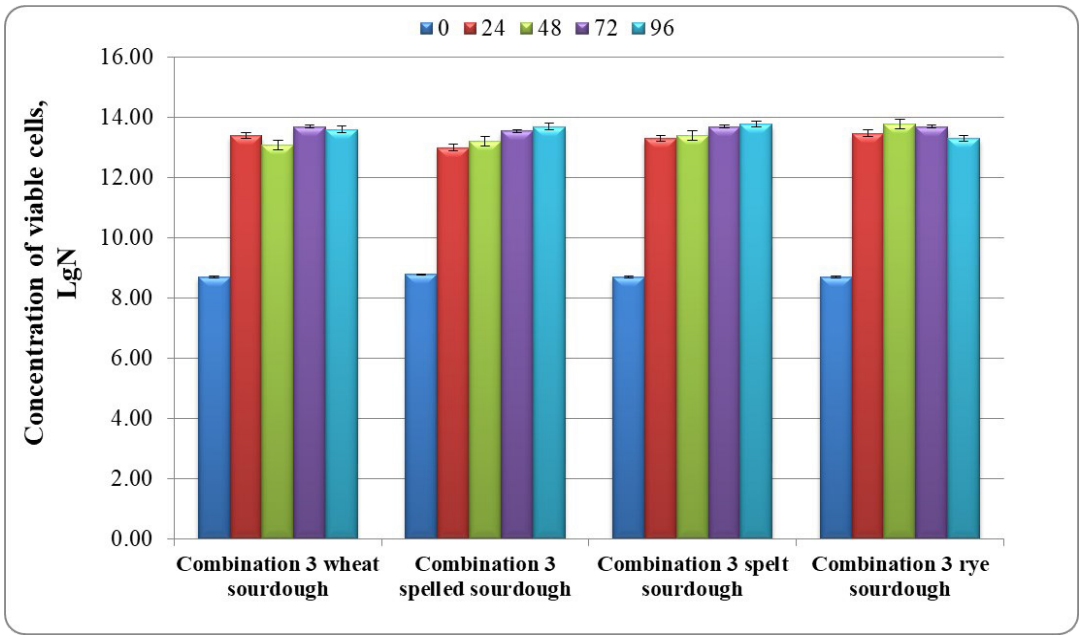

Fig. 5. Changes in the viable cell concentration of LAB in sourdough with Combination 3 during daily back-slopping for 96 h
Combination 3 - Combination 1 : *Frb. sanfranciscensis* R : *Pr. shermanii* 582D - 2:1:1

The antimicrobial activity of the sourdoughs at the 96th hour against saprophytic microorganisms was determined (Table 1). All twelve sourdoughs did not suppress the growth and development of baker's yeast, but they had antimicrobial activity towards the saprophytic microorganisms included in the study. The highest antimicrobial activity was reported in Combination 3 for all flour types. To a large extent, the observed inhibition was due to the metabolites produced by the lactobacilli and/or lactobacilli and propionic acid bacteria in the composition of the symbiotic starters. This is consistent with the studies of [22, 25-28], which proved that sourdough bread has a higher acidity due to the lactic acid and acetic acid secreted by the lactobacilli [29-32]. They also associate the antimicrobial activity of sourdough with the cyclic dipeptides, phenyllactic acid [29, 30] and and hydroxy fatty acids [30-32] formed.

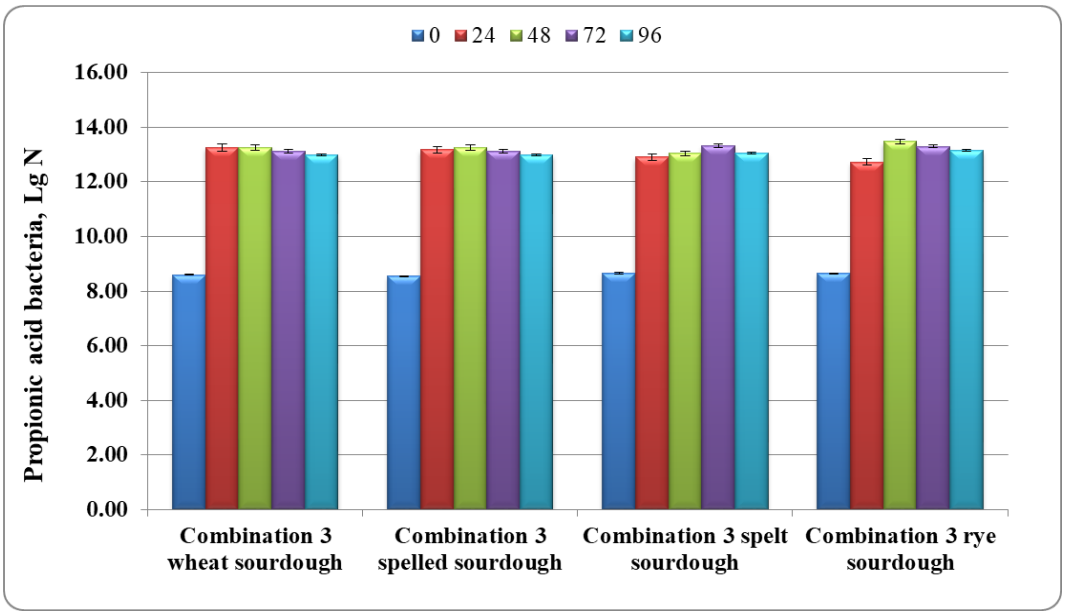

Fig. 6. Changes in the viable cell concentration of propionic acid bacteria in sourdough with Combination 3 during daily back-slopping for 96 h
Combination 3 - Combination 1: *Frb. sanfranciscensis* R : *Pr. shermanii* 582D - 2:1:1

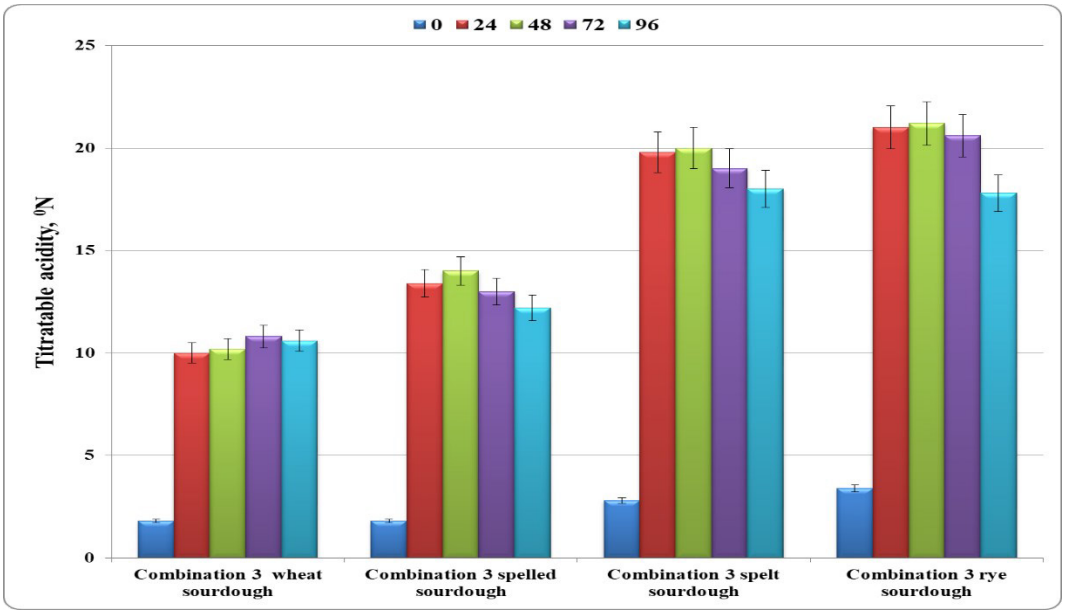

Fig. 7. Changes in the titratable acidity of sourdough with Combination 3 during daily back-slopping for 96 h
Combination 3 - Combination 1 : *Frb. sanfranciscensis* R : *Pr. shermanii* 582D - 2:1:1

3.2 Probation of starter sourdoughs in production conditions

Main dough was prepared and bread was baked using all 96-hour sourdoughs with all combinations. The starter sourdough was included in the following percentages: 5%, 7% and 10% of the corresponding sourdough with the respective combination. In terms of technology, all three sourdough starter combinations were successfully used in bread production. No undesirable processes that would hinder the production have been observed. Sourdough gave stability, strength and elasticity to the main dough during the technological process and fermentation.

No fungal spores have been detected in all starter sourdoughs. In addition, the LAB from the composition of the Combinations inhibited the wild yeasts that get into the sourdough with the flour. This ability of theirs is of particular importance in sourdough fermentation and for the continuous back-slopping of sourdoughs for 6 months.

Table 1. Antimicrobial activity of the 96-hour sourdoughs against *B. subtilis* ATCC 6051, *B. mesentericus*, *Saccharomyces cerevisiae* ATCC 9763, *Aspergillus niger* ATCC 16888, *Penicillium chrysogenum* ATCC 10106, *Rhizopus oryzae* ATCC 11145

Combination / Flour	48th hour d_{well} = 6 mm					
	***Bacillus Subtilis* ATCC 6051**	***Bacillus mesentericus***	***Saccharomyces cerevisiae* ATCC 9763**	***Aspergillus niger* ATCC 16888**	***Rhizopus oryzae* ATCC 11145**	***Penicillium chrysogenum* ATCC 10106**
Combination 1 / wheat flour	10±0.68	14±0.17	-	10±0.21	15±0.23	10±0.32
Combination 1 / spelt flour	11±0.17	15±0.17	-	9±0.17	15±0.15	10±0.57
Combination 1 / spelled flour	12±0.23	12±0.23	-	10±0.29	9±0.12	9±0.25
Combination 1 / rye flour	10±0.12	15±0.15	-	10±0.12	10±0.20	10±0.17
Combination 2 / wheat flour	11±0.96	15±0.15	-	9±0.17	13±0.25	10±0.20
Combination 2 / spelt flour	13±0.25	12±1.11	-	8±0.20	14±1.15	11±0.51
Combination 2 / spelled flour	12±0.51	15±0.15	-	9±0.20	16±0.15	11±0.51
Combination 2 / rye flour	10±0.21	16±1.22	-	8±0.17	13±0.25	9±0.17
Combination 3 / wheat flour	11±0.96	15±0.15	-	9±0.17	15±0.25	9±0.25
Combination 3 / spelt flour	12±0.51	15±0.20	-	9±0.15	14±1.00	10±0.57
Combination 3 / spelled flour	11±1.22	15±0.20	-	9±0.17	14±1.00	11±0.12
Combination 3 / rye flour	13±0.25	14±0.12	-	9±0.17	15±0.23	10±0.21

All main dough parameters, respectively all bread parameters, in the preparation and evaluation of sourdough bread with all three starter combinations and all 4 flour types were monitored, in order to make sure that these amounts of starter sourdough addition did not negatively affect the rheological parameters of the dough and the technologies adopted by the manufacturer for bread production (Table 2, Table 3).

Table 2. Indicators characterizing dough rheology, taste and aroma of bread variants prepared with 96-hour starter sourdough. Starter sourdough percentage - 5%, 7% and 10% compared to the flour amount

Variant	**Bread Control**	**Bread 5% starter sourdough**	**Bread 7% starter sourdough**	**Bread 10% starter sourdough**
Main dough – moisture and rheology	Main dough without any sourdough is moister.	The main dough is a little more elastic with good rheological properties.	The main dough is more elastic with good rheological properties.	The main dough is stronger, more elastic with good rheological properties.
Fermentation time, min	60	60	60	60
Main dough pieces before baking	Relaxed to the sides.	Good shape stability.	Good shape stability.	More stable. Good shape stability.
Bread loaf appearance	Bread loaves are smaller in volume.	Bread loaves with 5% starter sourdough have good shape and volume.	Bread loaves with 7% starter sourdough have good shape and volume.	Bread loaves with 10% starter sourdough have the best shape and volume.
Softness of the crumb		The crumb is softer, more moist and lighter in colour than the control variant.	The crumb is softer, more moist and lighter in colour than the control variant.	The crumb is softer, more moist and lighter in colour than the control variant.
Bread aroma	Baker's yeast aroma	Weak, but pleasant lactic acid aroma.	Pleasant, characteristic lactic acid aroma.	Strong, pleasant, characteristic lactic acid aroma.

Regardless of the flour type and starter combination used, all doughs and respective bread loaves showed similar tendencies. Experimental data indicated an acceleration of the fermentation process. The application of starter sourdough influenced positively the moisture and rheology of the main dough. The main dough was more elastic with good rheological properties. The main dough pieces before baking of the dough with the inclusion of starter sourdough were characterized by good shape stability. Bread loaves with starter sourdough had good shape and volume. The crumb of the sourdough breads was softer, more moist and lighter in colour than the control variant. The finished bread had a softer and lighter crumb, with a pleasant and characteristic lactic acid aroma (Table 2). In wheat bread, in comparison with the control variant, the inclusion of 5% starter sourdough did not affect the time needed for the dough to rise. The increase in the percentage of starter sourdough addition, however, shortened the time needed for the rise of the dough. In wheat-rye bread prepared with sourdough with Combination 1, the addition of starter sourdough reduced this parameter, even when the percentage of addition was 5%. But in sourdough with Combination 3, the positive influence on the rise of the dough was observed at the

Table 3. Sourdough acidity, rise of the dough, bread loaf volume and bread acidity of bread variants prepared with 96-hour starter sourdough. Starter sourdough percentage - 5%, 7% and 10% compared to the flour amount

Variant	Sourdough acidity, °N	Rise of the dough, min	Bread loaf volume, cm^3	Bread acidity, °N
Wheat bread; Control		52	1720	1.2
Wheat bread; 5% wheat starter sourdough with Combination 1	10.8	52	1760	1.6
Wheat bread; 7% wheat starter sourdough with Combination 1	10.8	50	1780	1.8
Wheat bread; 10% wheat starter sourdough with Combination 1	10.8	50	1820	2.2
Wheat bread; 5% wheat starter sourdough with Combination 2	10.6	52	1760	1.8
Wheat bread; 7% wheat starter sourdough with Combination 2	10.6	50	1780	2.0
Wheat bread; 10% wheat starter sourdough with Combination 2	10.6	50	1820	2.6
Wheat bread; 5% wheat starter sourdough with Combination 3	10.6	52	1720	1.8
Wheat bread; 7% wheat starter sourdough with Combination 3	10.6	50	1740	2.4
Wheat bread; 10% wheat starter sourdough with Combination 3	10.6	50	1740	2.8
Wheat-rye bread; Control		48	1060	1.6
Wheat-rye bread; 5% rye starter sourdough with Combination 1	11.2	46	1120	2.6
Wheat-rye bread; 7% rye starter sourdough with Combination 1	11.2	45	1150	2.8
Wheat-rye bread; 10% rye starter sourdough with Combination 1	11.2	45	1150	3.0
Wheat-rye bread; 5% rye starter sourdough with Combination 2	13.2	48	1120	2.4
Wheat-rye bread; 7% rye starter sourdough with Combination 2	13.2	48	1180	3.0
Wheat-rye bread; 10% rye starter sourdough with Combination 2	13.2	46	1220	3.6
Wheat-rye bread; 5% rye starter sourdough with Combination 3	12.2	48	1160	2.6
Wheat-rye bread; 7% rye starter sourdough with Combination 3	12.2	56	1200	3.2
Wheat-rye bread; 10% rye starter sourdough with Combination 3	12.2	46	1220	4.0
Spelled bread; Control		60	1480	3.0
Spelled bread; 5% spelled starter sourdough with Combination 1	20.2	56	1530	3.4
Spelled bread; 7% spelled starter sourdough with Combination 1	20.2	56	1550	3.8
Spelled bread; 10% spelled starter sourdough with Combination 1	20.2	54	1580	4.0
Spelled bread; 5% spelled starter sourdough with Combination 2	20.4	56	1480	2.8
Spelled bread; 7% spelled starter sourdough with Combination 2	20.4	55	1480	3.6
Spelled bread; 10% spelled starter sourdough with Combination 2	20.4	55	1530	4.6
Spelled bread; 5% spelled starter sourdough with Combination 3	18.0	54	1480	3.2

Spelled bread; 7% spelled starter sourdough with Combination 3	18.0	52	1480	4.0
Spelled bread; 10% spelled starter sourdough with Combination 3	18.0	52	1520	4.6
Spelt bread; Control		54	1400	2.8
Spelt bread; 5% spelt starter sourdough with Combination 1	19.0	54	1440	3.6
Spelt bread; 7% spelt starter sourdough with Combination 1	19.0	52	1460	4.0
Spelt bread; 10% spelt starter sourdough with Combination 1	19.0	52	1480	4.6
Spelt bread; 5% spelt starter sourdough with Combination 2	22.0	54	1400	2.6
Spelt bread; 7% spelt starter sourdough with Combination 2	22.0	53	1420	3.6
Spelt bread; 10% spelt starter sourdough with Combination 2	22.0	53	1430	4.8
Spelt bread; 5% spelt starter sourdough with Combination 3	17.8	54	1400	3.8
Spelt bread; 7% spelt starter sourdough with Combination 3	17.8	52	1410	4.0
Spelt bread; 10% spelt starter sourdough with Combination 3	17.8	52	1440	4.8

inclusion of 7% starter sourdough, while in sourdough with Combination 2, this effect occured at the inclusion of 7% starter sourdough. In spelled bread with starter sourdough, the rise of the dough in comparison with the control decreased at 5% starter sourdough addition and the more the percentage of inclusionof the sourdough increased, the more the reduction of this parameter was. In spelt bread with staerter sourdough the rise of the dough decreased at 7% starter sourdough addition for all three starter combinations (Table 3). Regardless of the starter combination, the inclusion of starter sourdough led to increase in the bread loaf volume. In wheat bread with starter sourdough with Combination 1 or Combination 2 the volume of the bread loaves started to increase at 5% starter sourdough addition. In wheat bread with starter sourdough with Combination 3 the volume of the bread loaves started to increase at 7% starter sourdough addition. The inclusion of any percentage of starter sourdough led to the increase in bread acidity and the higher the percentage of starter sourdough addition was, the greater the increase in bread acidity was. For wheat bread sourdough bread acidity ranged from 1.6 to 2.8, for wheat-rye sourdough bread – from 2.4 to 4.0, for spelled sourdough bread – from 2.8 to 4.6 and for spelt sourdough bread – from 2.6 to 4.8. These observations are consistent with the observations of [18] and [19] who obtained mixed culture bread with better appearance, golden and crispy crust, higher specific volume. Furthermore, experimental data showed that this bread type was obtained at moderate titratable acidity and lower sourdough concentration. This is consistent with Crowley et al. [33] who obtained the highest specific volume of bread with a lower amount of sourdough.

Bread variants with all four flour types and all 3 symbiotic starter sourdoughs were prepared, varying the percentages of the 96-hour starter sourdough. The goal was to determine the best combination for the production of wheat bread, rye bread, spelt bread and spelled bread, as well as the optimum percentage of inclusion in the formulation of the main dough, in order to prevent fungal and bacterial spoilage of baked bread for as long as possible, without negatively affecting its organoleptic indicators (Table 4 to Table 11).

Baked loaves were stored at room temperature (20 - 25ºC) and at 37°C for 96 h to determine the occurrence of bacterial spoilage (Table 4 – Table 7) and at room temperature for at 168 hours to determine the occurrence of fungal spoilage (Table 8 - Table 11).

Simbiotic starter sourdoughs were added in amounts of 5%, 7% or 10% under non-sterile conditions and the storage at different temperatures of the baked bread was also carried out in non-sterile conditions, as close as possible to home storage conditions, in contrast to the experiments described by [34] which were conducted under aseptic conditions. Bacterial spoilage appeared in all control loaves at the 96th hour of storage at 37°C but it was not observed by the 96th hour neither in the control variants stored at room temperature, nor in the starter sourdough bread variants stored at 37°C or room temperature (Table 4, Table 5, Table 6, Table 7).

In wheat bread fungal spoilage occurred at the 120th h in the control variant and in the bread variants with 5% or 7% starter sourdough with Combination 1 or Combination 2 either at 30°C or at room temperature. When the percentage of starter sourdough addition increased to 10%, no fungal spoilage was established at both temperatures. In bread loaves with starter sourdough with Combination 3 no fungal spoilage was detected at any

percentage of starter sourdough (Table 8). The best starter combination for wheat bread was Combination 3 since even 5% starter sourdough inclusion prevented fungal spoilage at both temperatures.

Table 4. Bacterial spoilage caused by *Bacillus* sp. during storage of wheat bread at 37°C and at room temperature (RT)

Bread variant	Temperature	24 h		48 h		72 h		96 h	
		DS	Aroma	DS	Aroma	DS	Aroma	DS	Aroma
Combination 1 - *Lbs. casei subsp. rhamnosus* LBRC11 : *Lpb. plantarum* Ph2 : *Lvb. brevis* X4 : *Lmb. fermentum* LBRH9 = 1:2:1:1; Combination 2 - Combination №1 : *Frb. sanfranciscensis* R = 2:1; Combination 3 - Combination №1 : *Frb. sanfranciscensis* R : *Pr. shermanii* 582D = 2:1:1									
Control Wheat flour	37°C	-	No	-	No	-	No	I	Barely noticeable
	RT	-	No	-	No	-	No	-	No
Wheat flour Combination 1; 5 %	37°C	-	No	-	No	-	No	-	No
	RT	-	No	-	No	-	No	-	No
Wheat flour Combination 1; 7 %	37°C	-	No	-	No	-	No	-	No
	RT	-	No	-	No	-	No	-	No
Wheat flour Combination 1; 10 %	37°C	-	No	-	No	-	No	-	No
	RT	-	No	-	No	-	No	-	No
Wheat flour Combination 2; 5 %	37°C	-	No	-	No	-	No	-	No
	RT	-	No	-	No	-	No	-	No
Wheat flour Combination 2; 7 %	37°C	-	No	-	No	-	No	-	No
	RT	-	No	-	No	-	No	-	No
Wheat flour Combination 2; 10 %	37°C	-	No	-	No	-	No	-	No
	RT	-	No	-	No	-	No	-	No
Wheat flour Combination 3; 5 %	37°C	-	No	-	No	-	No	-	No
	RT	-	No	-	No	-	No	-	No
Wheat flour Combination 3; 7 %	37°C	-	No	-	No	-	No	-	No
	RT	-	No	-	No	-	No	-	No
Wheat flour Combination 3; 10 %	37°C	-	No	-	No	-	No	-	No
	RT	-	No	-	No	-	No	-	No

Degree of spoilage (SR): I - barely perceptible (pleasant fruity smell); II - weak (change in aroma – sharp aroma) ; III - medium (moist, sticky crumb; unpleasant aroma); IV - strong (disgusting with yellow-brown center)

In wheat-rye bread fungal spoilage occurred at the 96th hour in the control variant at 30°C, while in the control variant stored at room temperature and in the bread variants with 5% or 7% starter sourdough with Combination 1 or Combination 2 fungal spoilage was detected at the 120th h. When the percentage of starter sourdough addition increased to 10%, no fungal spoilage was established at both temperatures. But in bread loaves with starter sourdough with Combination 3 fungal spoilage occurred at the 96th h at 5% or 7% starter sourdough addition, and at the 120th h at 10% starter sourdough addition (Table 9). The best starter combinations for wheat-rye bread were Combination 1 and Combination 2 since at 10% starter sourdough inclusion no fungal spoilage was detected at both temperatures.

In spelled bread fungal spoilage occurred at the 120th h in the control variant and in all the bread variants with starter sourdough with Combination 1 or Combination 2 at both temperatures. In bread loaves with starter sourdough with Combination 3 fungal spoilage was detected at the 120th h at 30°C at 5% starter sourdough addition, while there were no signs of fungal spoilage at 7% or 10% sourdough addition at any temperature (Table 10). The best starter combination for spelled bread was Combination 3 since fungal spoilage was detected only at 5% starter sourdough addition at 30°C.

In spelt bread fungal spoilage occurred at the 96th h in the control variant at 30°C, while in the control variant stored at room temperature and in the bread variants with 5% starter sourdough with Combination 1 fungal spoilage was detected at the 120th h at both temperatures. With the increase in the percentage of sourdough addition fungal spoilage was observed only at the 120th h at 30°C, while no fungal spoilage was detected for 7% or 10% sourdough bread by the 120th h. In spelt bread with starter sourdough with Combination 2 no fungal spoilage was established at both temperatures. But in bread loaves with starter sourdough with Combination 3 fungal spoilage occurred at the 120th h at 5% or 7% starter sourdough addition at 30°C, while in all other variants with this combination there was no fungal spoilage (Table 11). The best starter combination for spelled bread was Combination 2 since no fungal spoilage was detected at any percentage of starter sourdough addition at any temperature. The optimal concentration of sourdough inclusion without

Table 5. Bacterial spoilage caused by *Bacillus* sp. during storage of wheat-rye bread at 37°C and at room temperature (RT)

Bread variant	Temperature	24 h		48 h		72 h		96 h	
		DS	Aroma	DS	Aroma	DS	Aroma	DS	Aroma
Combination 1 - *Lbs. casei subsp. rhamnosus* LBRC11 : *Lpb. plantarum* Ph2 : *Lvb. brevis* X4 : *Lmb. fermentum* LBRH9 = 1:2:1:1; Combination 2 - Combination №1 : *Frb. sanfranciscensis* R = 2:1; Combination 3 - Combination №1 : *Frb. sanfranciscensis* R : *Pr. shermanii* 582D = 2:1:1									
Control wheat-rye flour	37°C	-	No	-	No	-	No	I	Barely noticeable
	RT	-	No	-	No	-	No	-	No
Wheat-rye flour Combination 1; 5 %	37°C	-	No	-	No	-	No	-	No
	RT	-	No	-	No	-	No	-	No
Wheat-rye flour Combination 1; 7 %	37°C	-	No	-	No	-	No	-	No
	RT	-	No	-	No	-	No	-	No
Wheat-rye flour Combination 1; 10 %	37°C	-	No	-	No	-	No	-	No
	RT	-	No	-	No	-	No	-	No
Wheat-rye flour Combination 2; 5 %	37°C	-	No	-	No	-	No	-	No
	RT	-	No	-	No	-	No	-	No
Wheat-rye flour Combination 2; 7 %	37°C	-	No	-	No	-	No	-	No
	RT	-	No	-	No	-	No	-	No
Wheat-rye flour Combination 2; 10 %	37°C	-	No	-	No	-	No	-	No
	RT	-	No	-	No	-	No	-	No
Wheat-rye flour Combination 3; 5 %	37°C	-	No	-	No	-	No	-	No
	RT	-	No	-	No	-	No	-	No
Wheat-rye flour Combination 3; 7 %	37°C	-	No	-	No	-	No	-	No
	RT	-	No	-	No	-	No	-	No
Wheat-rye flour Combination 3; 10 %	37°C	-	No	-	No	-	No	-	No
	RT	-	No	-	No	-	No	-	No

Degree of spoilage (SR): I - barely perceptible (pleasant fruity smell); II - weak (change in aroma – sharp aroma) ; III - medium (moist, sticky crumb; unpleasant aroma); IV - strong (disgusting with yellow-brown center)

Table 6. Bacterial spoilage caused by *Bacillus* sp. during storage of spelled bread at 37°C and room temperature (RT)

Bread variant	Temperature	24 h		48 h		72 h		96 h	
		DS	Aroma	DS	Aroma	DS	Aroma	DS	Aroma
Combination 1 - *Lbs. casei subsp. rhamnosus* LBRC11 : *Lpb. plantarum* Ph2 : *Lvb. brevis* X4 : *Lmb. fermentum* LBRH9 = 1:2:1:1; Combination 2 - Combination №1 : *Frb. sanfranciscensis* R = 2:1; Combination 3 - Combination №1 : *Frb. sanfranciscensis* R : *Pr. shermanii* 582D = 2:1:1									
Control spelled flour	37°C	-	No	-	No	-	No	I	Barely noticeable
	RT	-	No	-	No	-	No	-	No
Spelled flour Combination 1; 5 %	37°C	-	No	-	No	-	No	-	No
	RT	-	No	-	No	-	No	-	No
Spelled flour Combination 1; 7 %	37°C	-	No	-	No	-	No	-	No
	RT	-	No	-	No	-	No	-	No
Spelled flour Combination 1; 10 %	37°C	-	No	-	No	-	No	-	No
	RT	-	No	-	No	-	No	-	No
Spelled flour Combination 2; 5 %	37°C	-	No	-	No	-	No	-	No
	RT	-	No	-	No	-	No	-	No
Spelled flour Combination 2; 7 %	37°C	-	No	-	No	-	No	-	No
	RT	-	No	-	No	-	No	-	No
Spelled flour Combination 2; 10 %	37°C	-	No	-	No	-	No	-	No
	RT	-	No	-	No	-	No	-	No
Spelled flour Combination 3; 5 %	37°C	-	No	-	No	-	No	-	No
	RT	-	No	-	No	-	No	-	No
Spelled flour Combination 3; 7 %	37°C	-	No	-	No	-	No	-	No
	RT	-	No	-	No	-	No	-	No
Spelled flour Combination 3; 10 %	37°C	-	No	-	No	-	No	-	No
	RT	-	No	-	No	-	No	-	No

Degree of spoilage (SR): I - barely perceptible (pleasant fruity smell); II - weak (change in aroma – sharp aroma) ; III - medium (moist, sticky crumb; unpleasant aroma); IV - strong (disgusting with yellow-brown center)

Table 7. Bacterial spoilage caused by Bacillus sp. during storage of spelled bread at 37°C and room temperature (RT)

Bread variant	Temperature	24 h		48 h		72 h		96 h	
		DS	Aroma	DS	Aroma	DS	Aroma	DS	Aroma
Combination 1 - *Lbs. casei subsp. rhamnosus* LBRC11 : *Lpb. plantarum* Ph2 : *Lvb. brevis* X4 : *Lmb. fermentum* LBRH9 = 1:2:1:1; Combination 2 - Combination №1 : *Frb. sanfranciscensis* R = 2:1; Combination 3 - Combination №1 : *Frb. sanfranciscensis* R : *Pr. shermanii* 582D = 2:1:1									
Control Spelt flour	37°C	-	No	-	No	-	No	I	Barely noticeable
	RT	-	No	-	No	-	No	-	No
Spelt flour Combination 1; 5 %	37°C	-	No	-	No	-	No	-	No
	RT	-	No	-	No	-	No	-	No
Spelt flour Combination 1; 7 %	37°C	-	No	-	No	-	No	-	No
	RT	-	No	-	No	-	No	-	No
Spelt flour Combination 1; 10 %	37°C	-	No	-	No	-	No	-	No
	RT	-	No	-	No	-	No	-	No
Spelt flour Combination 2; 5 %	37°C	-	No	-	No	-	No	-	No
	RT	-	No	-	No	-	No	-	No
Spelt flour Combination 2; 7 %	37°C	-	No	-	No	-	No	-	No
	RT	-	No	-	No	-	No	-	No
Spelt flour Combination 2; 10 %	37°C	-	No	-	No	-	No	-	No
	RT	-	No	-	No	-	No	-	No
Spelt flour Combination 3; 5 %	37°C	-	No	-	No	-	No	-	No
	RT	-	No	-	No	-	No	-	No
Spelt flour Combination 3; 7 %	37°C	-	No	-	No	-	No	-	No
	RT	-	No	-	No	-	No	-	No
Spelt flour Combination 3; 10 %	37°C	-	No	-	No	-	No	-	No
	RT	-	No	-	No	-	No	-	No

Degree of spoilage (SR): I - barely perceptible (pleasant fruity smell); II - weak (change in aroma – sharp aroma) ; III - medium (moist, sticky crumb; unpleasant aroma); IV - strong (disgusting with yellow-brown center)

Table 8. Fungal spoilage during storage of wheat bread at 30°C and at room temperature (RT)

Bread variant	Temperature	24 h	48 h	72 h	96 h	120 h
Combination 1 - *Lbs. casei subsp. rhamnosus* LBRC11 : *Lpb. plantarum* Ph2 : *Lvb. brevis* X4 : *Lmb. fermentum* LBRH9 = 1:2:1:1; Combination 2 - Combination №1 : *Frb. sanfranciscensis* R = 2:1; Combination 3 - Combination №1 : *Frb. sanfranciscensis* R : *Pr. shermanii* 582D = 2:1:1						
Control Wheat flour	30°C	No	No	No	No	Noticeable
	RT	No	No	No	No	No
Wheat flour Combination 1; 5 %	30°C	No	No	No	No	Excessive
	RT	No	No	No	No	No
Wheat flour Combination 1; 7 %	30°C	No	No	No	No	Excessive
	RT	No	No	No	No	No
Wheat flour Combination 1; 10 %	30°C	No	No	No	No	No
	RT	No	No	No	No	No
Wheat flour Combination 2; 5 %	30°C	No	No	No	No	No
	RT	No	No	No	No	Noticeable
Wheat flour Combination 2; 7 %	30°C	No	No	No	Noticeable	Excessive
	RT	No	No	No	No	No
Wheat flour Combination 2; 10 %	30°C	No	No	No	No	No
	RT	No	No	No	No	No
Wheat flour Combination 3; 5 %	30°C	No	No	No	No	No
	RT	No	No	No	No	No
Wheat flour Combination 3; 7 %	30°C	No	No	No	No	No
	RT	No	No	No	No	No
Wheat flour Combination 3; 10 %	30°C	No	No	No	No	No
	RT	No	No	No	No	No

Table 9. Fungal spoilage during storage of wheat-rye bread at 30°C and at room temperature (RT)

Bread variant	**Temperature**	**24 h**	**48 h**	**72 h**	**96 h**	**120 h**
Combination 1 - *Lbs. casei subsp. rhamnosus* LBRC11 : *Lpb. plantarum* Ph2 : *Lvb. brevis* X4 : *Lmb. fermentum* LBRH9 = 1:2:1:1; Combination 2 - Combination №1 : *Frb. sanfranciscensis* R = 2:1; Combination 3 - Combination №1 : *Frb. sanfranciscensis* R : *Pr. shermanii* 582D = 2:1:1						
Control	30°C	No	No	No	Noticeable	Excessive
wheat-rye flour	RT	No	No	No	No	Noticeable
Wheat-rye flour	30°C	No	No	No	No	Noticeable
Combination 1; 5 %	RT	No	No	No	No	Excessive
Wheat-rye flour	30°C	No	No	No	No	Excessive
Combination 1; 7 %	RT	No	No	No	No	Noticeable
Wheat-rye flour	30°C	No	No	No	No	No
Combination 1; 10 %	RT	No	No	No	No	No
Wheat-rye flour	30°C	No	No	No	No	Excessive
Combination 2; 5 %	RT	No	No	No	No	Noticeable
Wheat-rye flour	30°C	No	No	No	No	Noticeable
Combination 2; 7 %	RT	No	No	No	No	No
Wheat-rye flour	30°C	No	No	No	No	No
Combination 2; 10 %	RT	No	No	No	No	No
Wheat-rye flour	30°C	No	No	No	Noticeable	Excessive
Combination 3; 5 %	RT	No	No	No	No	Noticeable
Wheat-rye flour	30°C	No	No	No	Noticeable	Excessive
Combination 3; 7 %	RT	No	No	No	No	No
Wheat-rye flour	30°C	No	No	No	No	Noticeable
Combination 3; 10 %	RT	No	No	No	No	No

Table 10. Fungal spoilage during storage of spelled bread at 30 °C and at room temperature (RT)

Bread variant	**Temperature**	**24 h**	**48 h**	**72 h**	**96 h**	**120 h**
Combination 1 - *Lbs. casei subsp. rhamnosus* LBRC11 : *Lpb. plantarum* Ph2 : *Lvb. brevis* X4 : *Lmb. fermentum* LBRH9 = 1:2:1:1; Combination 2 - Combination №1 : *Frb. sanfranciscensis* R = 2:1; Combination 3 - Combination №1 : *Frb. sanfranciscensis* R : *Pr. shermanii* 582D = 2:1:1						
Control	30°C	No	No	No	No	Noticeable
spelled flour	RT	No	No	No	No	Excessive
Spelled flour	30°C	No	No	No	No	Noticeable
Combination 1; 5 %	RT	No	No	No	No	Excessive
Spelled flour	30°C	No	No	No	No	Noticeable
Combination 1; 7 %	RT	No	No	No	No	Noticeable
Spelled flour	30°C	No	No	No	No	Excessive
Combination 1; 10 %	RT	No	No	No	No	Noticeable
Spelled flour	30°C	No	No	No	No	Excessive
Combination 2; 5 %	RT	No	No	No	No	Excessive
Spelled flour	30°C	No	No	No	No	Excessive
Combination 2; 7 %	RT	No	No	No	No	Excessive
Spelled flour	30°C	No	No	No	No	Excessive
Combination 2; 10 %	RT	No	No	No	No	Excessive
Spelled flour	30°C	No	No	No	No	Noticeable
Combination 3; 5 %	RT	No	No	No	No	No
Spelled flour	30°C	No	No	No	No	No
Combination 3; 7 %	RT	No	No	No	No	No
Spelled flour	30°C	No	No	No	No	No
Combination 3; 10 %	RT	No	No	No	No	No

Table 11. Fungal spoilage during storage of spelt bread at 30°C and at room temperature (RT)

Bread variant	Temperature	24 h	48 h	72 h	96 h	120 h
Combination 1 - *Lbs. casei subsp. rhamnosus* LBRC11 : *Lpb. plantarum* Ph2 : *Lvb. brevis* X4 : *Lmb. fermentum* LBRH9 = 1:2:1:1; Combination 2 - Combination №1 : *Frb. sanfranciscensis* R = 2:1; Combination 3 - Combination №1 : *Frb. sanfranciscensis* R : *Pr. shermanii* 582D = 2:1:1						
Control	30°C	No	No	No	Noticeable	Excessive
Spelt flour	RT	No	No	No	No	Noticeable
Spelt flour	30°C	No	No	No	No	Noticeable
Combination 1; 5 %	RT	No	No	No	No	Noticeable
Spelt flour	30°C	No	No	No	No	Noticeable
Combination 1; 7 %	RT	No	No	No	No	No
Spelt flour	30°C	No	No	No	No	Noticeable
Combination 1; 10 %	RT	No	No	No	No	No
Spelt flour	30°C	No	No	No	No	No
Combination 2; 5 %	RT	No	No	No	No	No
Spelt flour	30°C	No	No	No	No	No
Combination 2; 7 %	RT	No	No	No	No	No
Spelt flour	30°C	No	No	No	No	No
Combination 2; 10 %	RT	No	No	No	No	No
Spelt flour	30°C	No	No	No	No	Noticeable
Combination 3; 5 %	RT	No	No	No	No	No
Spelt flour	30°C	No	No	No	No	Noticeable
Combination 3; 7 %	RT	No	No	No	No	No
Spelt flour	30°C	No	No	No	No	No
Combination 3; 10 %	RT	No	No	No	No	No

negatively affecting the volume and flavor profile of the resulting bread was 5% to prevent bacterial spoilage and 10% to prevent fungal spoilage.

The obtained results confirmed the conclusions of other authors' collectives. The inclusion of 10-15% or more sourdough in the main dough inhibits the growth of bacterial and fungal spores and ensures long shelf life of the baked products [34], and although acidification is necessary for optimal swelling of bread, for the control of enzyme activities, the elasticity of the medium and the long shelf life [35, 36], excessive acidification has an adverse effect on some rheological parameters [37, 38].

4. Conclusion

Symbiotic starter sourdoughs of lactobacilli or lactobacilli and propionic acid bacteria strains have been developed for use in the production of various bread types: wheat bread, wheat-rye bread, spelled bread, spelt bread. High concentrations of viable cells of lactobacilli or lactobacilli and propionic acid bacteria was found in all sourdoughs in the back-slopping process for 96 hours. Lactobacilli or lactobacilli and propionic acid bacteria in the composition of sourdough bread have been shown to inhibit the growth of spore-forming bacteria and mold spores.

The possibility of applying the symbiotic sourdough starters of lactobacilli or lactobacilli and propionic acid bacteria in the production of wheat bread, wheat-rye bread, spelled bread, spelt bread with improved taste and aroma has been revealed. Besides, the best starter combination for prevention of bacterial and fungal spoilage for each bread type has been selected, the optimum concentration of starter sourdough for prevention of bacterial and fungal spoilage for each bread type has been determined and the technological and organoleptic indicators of the produced bread have been improved.

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