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Evaluation of selected lactic acid bacteria as starter cultures for gluten-free sourdough bread production

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Abstract. Sourdough is one of the most promising technologies for gluten-free bread. The selection of appropriate starter cultures for the production of gluten-free sourdoughs is of a great importance, since not all microorganisms can adapt equally to the same raw material. The aim was to create a new starter microbial composition for gluten-free sourdough preparation, allowing improving the quality and the microbiological safety of gluten-free bread. Screening was conducted on 8 strains of lactic acid bacteria (LAB) and 5 strains of yeast previously isolated from spontaneously fermenting rice and buckwheat sourdoughs. The strain *S. cerevisiae* Y205 had the highest fermentative activity and alcohols content. The lactic acid bacteria *L. brevis* E139 and *L. plantarum* E138 were also experimentally selected for new gluten-free sourdoughs on the basis of acidity and volatile acids production and antagonistic activity. Two types of microbial composition were created and its influence on sourdough biotechnological indicators was studied. Sourdough with *L. plantarum* E138 had in 1.2 times lower titratable acidity, in 3.4 times lower volatile acids content compared to sourdough with *L. brevis* E139. Alcohol content was the same in both sourdoughs similarly to yeast cells amount. Sourdough dough proofing time increased in 1.2–1.3 times compared to the control. Sourdough did not affect the specific volume, porosity and compressibility of gluten-free bread, but its sensory characteristics were improved. Bread made with sourdoughs had more pronounced taste and flavor, brighter crust color and better texture compared bread without sourdough. The microbiological safety of sourdough gluten-free bread was also increased, especially when *L. brevis* E139 was used.

Key words: celiac disease, biotechnology, sourdough, lactic acid bacteria, yeasts, gluten-free bread.

INTRODUCTION

In recent decades, researchers have paid increasing attention to the development of gluten-free bread technologies. New ingredients and different techniques are being used to improve the quality of gluten-free products (Houben et al., 2012; Ziobro et al., 2012; Masure et al., 2016, Bender & Schönlechner, 2019; Azizi et al., 2020; Cappelli et al, 2020; Nissen et al., 2020; Puerta et al., 2021). With the growing interest of consumers in healthy lifestyle, the demand for clean label products is growing. This leads to increased research in the field of safe technologies without chemical additives.

Sourdough fermentation and new baking technologies have come into interest as they offer textural and sensory advantages without chemical compounds (Mariotti et al., 2017; Cappelli et al, 2020; Nissen et al., 2020; Puerta et al., 2021). Sourdough allows generating changes in the dough properties and bread quality (Arendt et al., 2011; Wolter et al., 2014; Cappa et al., 2016; Bender et al., 2018; Olojede et al., 2019; Cappelli et al, 2020).

Research shows that sourdough is a promising way for gluten-free bread making as it improves sensorial, nutritional and textural bread properties (Arendt et al., 2011; Nionelli & Rizzello, 2016; Olojede et al., 2019; Ren et al., 2020). It is well known that lactic acid bacteria (LAB) strains are able to improve the shelf life of bread since the acids formed during fermentation process lower the pH thus inhibiting the growth of spoilage organisms ((Zacharof & Lovitt, 2012; Khandakar et al., 2014; Axel et al., 2015; Hassan et al., 2015; Bender et al., 2018; Bartkiene et al, 2019). During sourdough fermentation organic acids and free amino acids, depending on the selected starter culture, are responsible for the increase in taste intensity (Houben et al., 2012; Wolter et al., 2014).

The application of sourdough offers improved textural characteristics and improved viscosity and elasticity in gluten-free batters, and effects are dependent on the amount of added sourdough and on the LAB strain used for fermentation (Arendt et al., 2007; Gobbettii et al., 2008; Houben et al., 2010; Jekle et al., 2010; Masure et al., 2016).

Gluten-free sorghum sourdough fermented with L. *plantarum* and *L. casei* or *L. fermentum* and *L. reuteri* enhanced its the nutritional value by inducing the degradation of polyphenols (Svensson et al., 2010).

Wolter et al. (2014) have studied sourdoughs made with different gluten-free flours and wheat flour, fermented by *Lactobacillus plantarum FST 1.7*. It was found that dough made with sourdough had a lower strength and was softer. At the same time, the crumb porosity in baked bread was significantly higher. The studies have shown that the sourdough usage did not affect the shelf life and smell of gluten-free bread.

Mariotti et al (2017) obtained, in general, the promising results of adopting the sourdough technology in gluten-free bread making. Sourdough bread had a more uniform texture compared to yeast bread. However, sourdough bread had a lower specific volume and a denser crumb. So authors have recommended compressed yeasts and sourdough combination in order to obtain the best performances.

Bender et al (2018) have studied the effect of different strains of the genus *Lactobacillus on* the quality of the sourdoughs made from buckwheat and millet flour. Research has shown that the examined trains have a different effect on the quality of gluten-free bread. This shows the importance of the strains selection that allows improving the gluten-free bread quality.

The usage of LAB strains previously isolated from spontaneously fermenting wheat and rye sourdoughs in gluten-free sourdough technology ensured high quality of gluten-free bread when used together with the addition of rowan powder (Dubrovskaya et al., 2018).

Olojede et al. (2019) obteined good results when used *Pediococcus pentosaceus* SA8, 78 *Weissella confusa* SD8*, P. pentosaceus* LD7 and *Saccharomyces cerevisiae* YC1 previously isolated from spontaneously fermenting sorghum sponge (Ogunsakin et al., 2017; Olojede et al., 2019). The strains were selected on the basis of their functional properties. Sourdough from sorghum with these strains led to the development of bread

with improved rheology, texture and nutritional properties, thus confirming the functionality of the employed starter cultures.

All studies have shown the importance of selecting appropriate starter cultures for the production of gluten-free sourdoughs (Ogunsakin et al., 2017; Maidana et al., 2019; Olojede et al., 2019), since not all microorganisms can adapt equally to the same raw material. Microbial growth can be influenced by availability of carbohydrates, nitrogen sources, lipids and free fatty acids content, as well as the enzymatic activity, buffer capacity and the presence of growth factors (i.e. vitamins and minerals) in the substrate (Moroni et al., 2011; De Vuyst et al., 2014; Gänzle, 2014; Bender et al., 2018). However, the nature and quality of the raw material are not the only factors determining the dominant sourdough microbiota. Process parameters including temperature, dough yield, fermentation time and number of refreshment steps or interactions involving starter cultures, autochthonous strains and contamination microflora can influence the final composition of the sourdough (Arendt et al., 2007; De Vuyst et al., 2014; Gänzle, 2014; Zhang et al., 2019). These conditions contribute to the persistence of the dominating strains and can ensure a reproducible and controlled composition of the sourdough microbiota and ensure a constant bread quality.

Thus sourdough fermentation effectiveness in improving the quality of gluten-free bread depends on the strains of LAB and yeasts. The selection of appropriate starter cultures carefully chosen for specific gluten-free raw materials is of crucial importance. There is no substrate and activity limitation for the lactic acid bacteria in gluten-free flours, but its fermentation can differ between the strains used. There is always a need for a test of starter strain (Houben et al., 2012)

That is why the aim of this study was to investigate different strains of lactic acid bacteria, to create a new starter composition, and to develop gluten-free sourdough bread technology which would improve the quality and microbial safety of bread.

MATERIALS AND METHODS

Characteristics of ingredients

The study of lactic acid bacteria strains

The starter cultures of lactic acid bacteria used in this study were composed of: *Lactobacillus diolivorans* E133, *L. plantarum* E134, *L. plantarum* E135, *L. brevis* E136, *Pediococcus pentosaceus* E137, *L. plantarum* E138, *L. brevis* E139, *L. brevis* E140 previously isolated from spontaneously fermenting rice and buckwheat sourdoughs. The species of bacteria were previously determined based on the study of morphological, cultural and biochemical characteristics and by the method of 16S rRNA sequencing (Parakhina et al., 2020).

Acidifying activity. LAB were cultivated in MRS liquid selective culture medium (BioMerieux, France). 1 mL of lactic acid bacteria liquid culture was inoculated into 100 g mixture of water and rice flour (moisture content 60%) and was kept at 24 ± 1 °C for 24 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 0.1 M 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 (de Man, Rogosa and Sharp) 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 meat-peptone agar, freshly inoculated with the *Bacillus subtilis* test strain from the collections of the St. Petersburg branch of Scientific Research Institute for the Baking Industry.

B. subtilis test strain was grown on meat-peptone medium with agar and a suspension containing 10^8 cells mL^{-1} 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.

The study of yeasts

Five strains of yeasts previously isolated from spontaneously fermenting rice and buckwheat sourdoughs were used in this study: *Kazachstania bulderi* Y202, *Candida humilis* Y 203 and Y 204, *S. cerevisiae* Y 205 and Y 206. The species of yeast were previously determined based on the study of morphological, cultural and biochemical characteristics and by the method of ITS sequencing (Parakhina et al., 2020).

The fermentation activity of yeasts in a mixture of water and rice flour (moisture content 60%) was studied.

Yeast fermentation activity was determined by the amount of released carbon dioxide. 1 mL of yeast liquid culture containing 10^8 cells mL^{-1} was added to 100 g of rice flour and water mixture (moisture content 60%). 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 ± 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).

Sourdough preparation

To prepare the starter culture, lactic acid bacteria were grown on MRS agar. Then an aqueous suspension with a cell titer of 10^9 CFU mL^{-1} was prepared using a densitometer DEN-1 (BioSan, Latvia - England). Yeast was grown on malt agar and then aqueous suspension with a cell titer of 10^8 CFU mL^{-1} was prepared. 2% LAB suspension and 4% yeast suspension were introduced into a mixture of water and rice flour with a moisture content of 57%. The dosages of pure starter cultures were established experimentally based on previous studies (data not shown). The initial content of examined LAB cell in sourdough was $2 \cdot 10^7$ CFU g^{-1} and the yeast cell content was $4 \cdot 10^6$ CFU g^{-1}.

Sourdough were fermented at a temperature of 28–30 °C for 18–20 h, and then the acidity, the content of LAB and yeast were determined.

The formulation of a gluten-free sourdough is presented in Table 1.

Then the obtained gluten-free sourdoughs were propagated by feeding with rice flour and water in a ratio of 1:1:1 every 16–18 h and fermentation at a temperature of 22–24 °C for two weeks. Biochemical indicators were evaluated at 12th backslopping.

Table 1. Formulations used to prepare sourdough

Ingredients, g	Sourdough I	Sourdough II
Suspension of *L. plantarum* E138, mL	2.0	-
Suspension of *L. brevis* E139, mL	-	2.0
Suspension of *S. cerevisiae* Y205, mL	4.0	4.0
Rice flour, g	50.0	50.0
Water, g	44.0	44.0
Total	100.0	100.0

Bread-making procedure

Gluten-free mixture including corn starch, extrusion corn starch, soy protein isolate, and rice flour were used as flour. Dough humidity was 52%. The compressed yeasts and sourdough combination was used in order to obtain the best performance (Mariotti et al., 2017; Dubrovskaya et al., 2018). The formulation of bread is presented in Table 2.

The dough was mixed in a mixer Kitchen Aid KSM45 (USA) at 120 rev min^{-1} for 7 min. 250 g dough pieces were placed in baking moulds and kept at 30 °C until the doubled in volume, then baked for 18 min in oven Sveba Dahlen (Sweden) at 210 °C with steam for 5 s at the beginning of baking.

Table 2. Bread formulation

Ingredients, g	Control bread	Sourdough bread	
	Dough	Sourdough	Dough
Corn starch	60.0	-	60.0
Rice flour	20.0	10	5.0
Extruded corn starch	10.0	-	10.0
Soy protein isolate	10.0	-	10.0
Vegetable oil	3.8	-	3.8
Yeast	2.5	-	2.5
Sugar	2.0	-	2.0
Salt	0.8	-	0.8
Sourdough	-	10	30.0
Water	93.4	10	77.2

Sourdough and dough assessments

Sourdoughs and the doughs samples were evaluated for different parameters. Acidity was evaluated by titration with 0.1 N sodium hydroxide solution using phenolphthalein as indicator (Puchkova, 2004). The content of volatile acids was determined by neutralizing the evaporated volatile acid using a 0.1 M solution of NaOH (Puchkova, 2004). The alcohol content was determined by using the iodometric method, which is based on the quantity of sodium thiosulfate spent in titration (Puchkova, 2004). Viable plate counts of lactic acid bacteria and yeasts in sourdough and the ratio between lactic acid bacteria and yeasts were studied. 10 g of sourdough were homogenized with 90 mL of sterile chloride (0.9% wt·wol^{-1}) solution. Serial dilutions were plated on MRS agar for determination of LAB. Plates were incubated at 30 °C under anaerobic conditions. An AnaeroGen System (Oxoid, UK) was used to maintain an anaerobic environment. The number of yeast in sourdough was estimated by using malt agar at 30 °C for 72 h. (Afanasjeva, 2003).

Assessment of baked bread

Assessment of quality. The quality of bread was evaluated the following parameters. Titratable Acidity (TTA) was determined by titration of crumb sample with 0.1 M sodium hydroxide solution using phenolphthalein as indicator (State Standard of the Russian Federation GOST 5670-96, 1996). Cells volume was calculated as the ratio of cells volume to the total bread volume. Bread specific volume was determined by a rapeseed displacement and calculated as the ratio of volume to 100 g of bread (Puchkova, 2004). The automatic penetrometer (Labor, Hungary) was used to estimate crumb compressibility (Puchkova, 2004). The volatile acids amount was estimated by determining the amount 0.1 M sodium hydroxide solution used for neutralization of the evaporated volatile acid. The alcohol content was determined by using the iodometric method, which is based on the quantity of sodium thiosulfate spent in titration.

Ropy disease and moulds spoilage assessment

To estimate sourdough performance on bread microbial resistance, the bread was infected with the spore-forming bacteria *B. subtilis* and moulds *Penicillium chrysogenum*. Both methods were described in details in previous study (Dubrovskaya et al., 2018).

Assessment of organoleptic characteristics. Ten-member expert panel was used to evaluate the bread sensory (organoleptic) characteristics. Experts evaluated taste and smell, texture of crumb (chewiness), color of crust and porosity. Each trait was rated on a scale from 1 to 5: 1– poor; 2 – dislike; 3 – slightly dislike; 4 – moderately; 5 – very good).

Statistical analysis

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

RESULTS AND DISCUSSION

Studies of the lactic acid bacteria antagonistic activity have shown that all strains inhibited the growth of *B. subtilis*. It may be stated that the size of inhibition zones depended on sensitivity of the target strain. The highest mean value of inhibition zones (14 ± 1 mm) were in case of *L. brevis* E139 and *L. plantarum* E138 (Fig. 1). Results evidenced that the antibacterial abilities of LAB were depended on the LAB

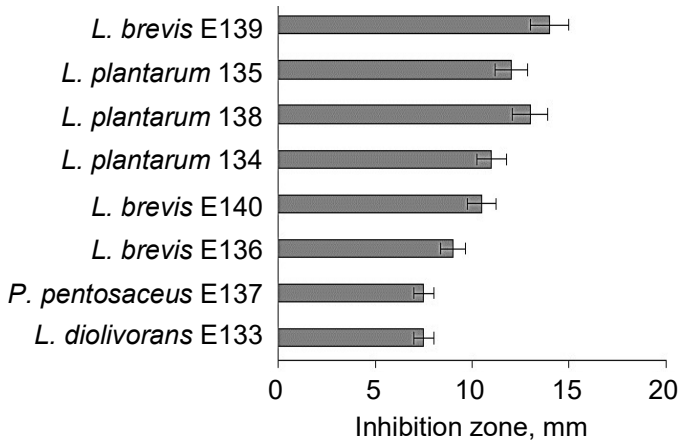

Figure 1. Antagonistic activity of lactic acid bacteria against *B. subtilis*.

species and strain (Khandakar et al., 2014; Dubrovskaya et al., 2018; Miadana et al., 2019; Bartkiene et al., 2020).

According to the lowest pathogen inhibition, *L. diolivorans* E133, *L. brevis* E136 and *P. pentosaceus* E137 were not used in further research. The acidifying activity of the chosen *Lactobacilli* was evaluated (Table 3).

The strains L. *plantarum* E138 and *L. plantarum* E135 had the highest acidity. The strain *L. brevis* E139 produced highest amount of volatile acids, most of which may be be acetic acid (Zalán et al., 2010). The antagonism activity could be due to the presence of organic acids, although it is possible to assume the production of bacteriocins (Zalán et al., 2010; Zacharof & Lovitt, 2012; Sadiq et al, 2019; Bartkiene et al., 2020).

Table 3. Acid-forming activity of lactic acid bacteria

Lactobacilli strains	Acidity, degrees N	Volatile acids, degrees N
L. brevis E139	6.2 ± 0.3^{a}	1.45 ± 0.13^{a}
L. brevis E140	7.2 ± 0.3^{b}	0.80 ± 0.11^{b}
L. plantarum 134	6.8 ± 0.2^{c}	0.95 ± 0.13^{c}
L. plantarum E138	8.1 ± 0.3^{d}	0.83 ± 0.11^{b}
L. plantarum E135	7.8 ± 0.2^{d}	0.90 ± 0.11^{c}

Mean values ± standard deviation (SD) within the same column with different letters are significantly different ($P \leq 0.05$).

Based on the data obtained, two strains (*L. brevis* E139 and L. *plantarum* E138) were selected for further research.

Yeast strains had different fermentative activity and produced different amount of alcohols (Figs 2, 3). Results confirm that the yeasts have different enzymatic activity (Okunowo et al., 2005; Joshi et al., 2009; Sharma et al., 2011) which may be due to the different ability of yeast strain to respond over various stress conditions subjected during fermentation, such as pH, high ethanol concentration, osmotic pressure, nutrient availability (Sharma et al., 2011; Bauer & Pretorius, 2000).

The strain *S. cerevisiae* Y205 had the highest fermentative activity and produced highest amount of alcohols after 24 hours of fermentation (Figs 2, 3). Therefore, this strain was selected for further research.

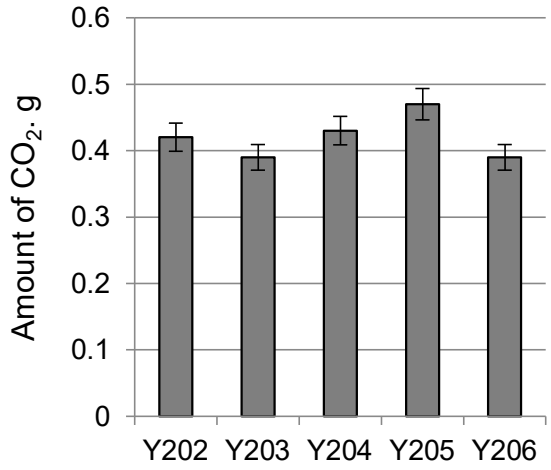

Figure 2. Fermenting activity of yeast.

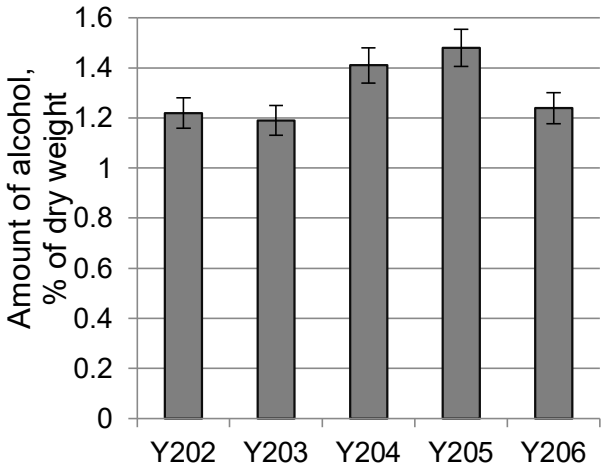

Figure 3. Alcohols production by yeast.

Two types of starter microbial composition were created: *L. plantarum* E138 and *S. cerevisiae* Y205 (starter I) and *L. brevis* E139 and *S. cerevisiae* Y205 (starter II).

The influence of two starter compositions on sourdough biotechnological indicators was studied (Table 4). When preparing the sourdough using pure cultures of LAB and yeast it was found that sourdough II had a lower acidity after fermentation, but the number of lactic acid bacteria was 3.6 times higher and yeast was 1.8 times less than in sourdough I. This may be due to the metabolic interaction of yeasts and LAB (De Vuyst et al., 2014; Carbonetto et al., 2020; Rogalski et al., 2020) Data obtained confirmed that the level of microorganisms competitiveness is strain specific (Carbonetto et al., 2020; Rogalski, et al., 2020).

The ability of LAB and yeast to grow together is very crucial in sourdough fermentation (Arendt et al., 2007, Wakil & Daodu, 2011; Arendt et al., 2011; Ogunsakin et al., 2017). Thus, the preferred yeast and LAB combination as metabolic partners should be studied during long-time propagation of sourdoughs.

Table 4. Biotechnological indicators of prepared sourdoughs after first fermentation

Indicators	Sourdough I	Sourdough II
Acidity, deg:	$7.3 \pm 0{,}3^{a}$	6.7 ± 0.2^{b}
LAB, CFU·g^{-1}	475 ± 21^{a}	1708 ± 52^{b}
Yeast, CFU·g^{-1}	63 ± 2^{a}	35 ± 2^{b}
Yeast/LAB ratio	1:7.5	1:48.8

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

Biotechnological indicators of sourdoughs were evaluated during multiple propagations. It was found (Table 5) that in 12th backslopping sourdough I made with *L. plantarum* 138 had lower titratable acidity, less volatile acids compared to sourdough II made with *L. brevis* E139. Alcohol content was the same in both sourdoughs similarly to yeast cells amount.

The LAB population was higher than the yeasts in both sourdoughs. This may be as a result of the fermentation conditions that tend to favour the growth of LAB than yeasts. This is in agreement with the report that LAB tend to dominate sourdough fermentations by the production of acid in the fermenting dough (De Vuyst et al., 2014; Ogunsakin et al., 2017; Go Carbonetto et al., 2020; Rogalski et al., 2020). The data showed that with long-term propagation, the cells amount of *L. brevis* E139 decreased compared to the amount after first fermentation of sourdough with pure cultures and remained approximately at the same level during multiple propagations. This indicates the stabilization of microflora.

Table 5. Biotechnological indicators for two types of sourdough during long-time propagation

Indicators	Sourdough I	Sourdough II
Acidity, degrees	7.5 ± 1.5^{a}	8.9 ± 1.5^{b}
Volatile acids acidity, degrees	0.85 ± 0.19^{a}	2.85 ± 0.37^{b}
Alcohol, % of dry weight	2.4 ± 0.4^{a}	2.4 ± 0.3^{a}
LAB, CFU·g^{-1}	770 ± 23^{a}	630 ± 20^{b}
Yeast, CFU·g^{-1}	43 ± 2^{a}	45 ± 3^{a}
Yeast/LAB ratio	1:77.9	1:14

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

The effect of the sourdough on the dough quality was studied. It was established (Table 6) that dough

Table 6. Dough indicators and parameters

Indicators and parameters	Control bread	Sourdough bread I	Sourdough bread II
Acidity, degrees	2.6 ± 0.3^{a}	3.9 ± 0.5^{b}	4.2 ± 0.6^{b}
Proofing time, min	38 ± 2^{a}	46 ± 2^{b}	48 ± 3^{b}

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

acidity was 1.5–1.6 times higher in samples with sourdough. Proofing time before baking increased compared to the control when the sourdoughs were used, which is confirmed by other studies (Cappa, 2016). This may be due to the increase in the acidity of the dough, and as a consequence, with the suppression of the development of yeast cells, which, in turn, leads to an increase in the proofing duration (Afanasjeva, 2003).

Table 7. Indicators of gluten-free bread quality

Indicators	Control	Sourdough bread I	Sourdough bread II
Acidity, degrees	0.5 ± 0.1^a	1.3 ± 0.2^b	1.6 ± 0.2^c
Volatile acids acidity, degrees	0.31 ± 0.09^a	1.28 ± 0.19^b	1.83 ± 0.19^c
Alcohols, % of dry weight	1.1 ± 0.2^a	2.2 ± 0.2^b	2.2 ± 0.2^b
Cells volume, %	58 ± 2^a	58 ± 2^a	59 ± 2^b
Specific volume, $cm^3 \cdot g^{-1}$	1.3 ± 0.2^a	1.2 ± 0.2^a	1.2 ± 0.2^a
Compressibility, equipment units	15 ± 2^a	14 ± 2^a	15 ± 2^a

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

Estimation of bread quality has shown that sourdough bread had higher acidity and content of volatile acids than the control (Table 7). The high amount of volatile acids has a positive effect on taste and smell (Afanasjeva, 2003). Alcohol content was higher in sourdough bread. Cells volume, crumb compressibility, specific volume was the same in all samples. Thus, an improvement in the physicochemical properties of gluten-free bread was noted only in an increase in acidity. Other studies confirmed that gluten-free sourdough microbiota determines the bread properties in terms of acidification (Bender & Schonlechner, 2020).

Sensory characteristics of bread is presented in Fig. 4. Sample I and sample II, made with sourdough, had better taste and smell than control sample. The smell was more intense, pleasant. Experimental bread also had a brighter crust color with a brownish shade and better texture and chewiness of crumbs: crumbs were softer, more elastic and less crumbly compared the control. The facts that sourdough creates a stronger flavor and taste and gluten-free sourdough bread had softer and stales more slowly were observed by other researchers (Houben et al., 2012; Wolter et al., 2014; Cappa et al., 2016; Olojede et al., 2019).

Bread made with sourdough II had more pronounced taste and flavor may be due to the fact that *L. brevise* produced higher amount of volatile acids (Jekle et al., 2010; Khandakar et al., 2014; Wolter et al., 2014).

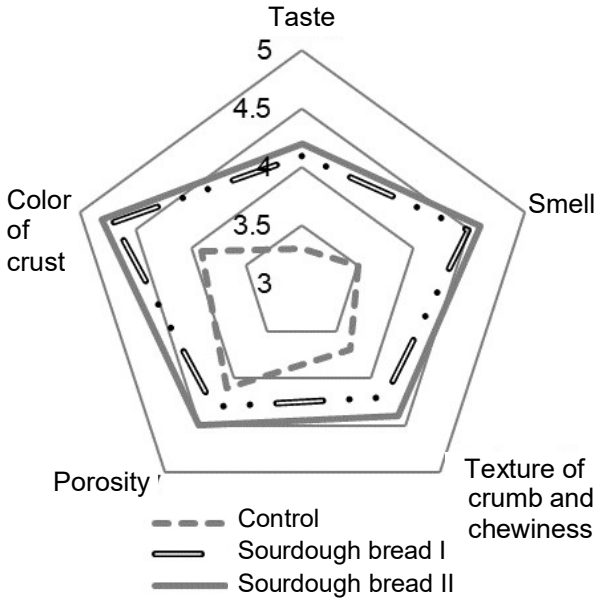

Figure 4. Sensory characteristics of gluten-free bread.

The effect of new sourdoughs on the microbial spoilage of gluten-free bread was studied (Fig. 5). The mould colonies of *Penicillium chrysogenum* on bread slices made with sourdough I and sourdough II appeared 6 and 12h respectively later than on control slices. The ropy-disease was not found in samples II during storage period (72 h). Samples I became ropy 24 hours later than the control. The inhibition of rope disease and mold developmentcan be attributed to the presence of organic acids and antimicrobial compounds of sourdough-resident microorganisms. A large number of published papers attribute the antifungal activity of LAB to organic acids (lactic, acetic, etc.) produced during fermentation leading to increased acidity which later inhibit mold growth (Hassan et al., 2014; Khandakar et al., 2014; Wolter et al., 2014; Axel et al., 2015; Bartkiene et al., 2019).

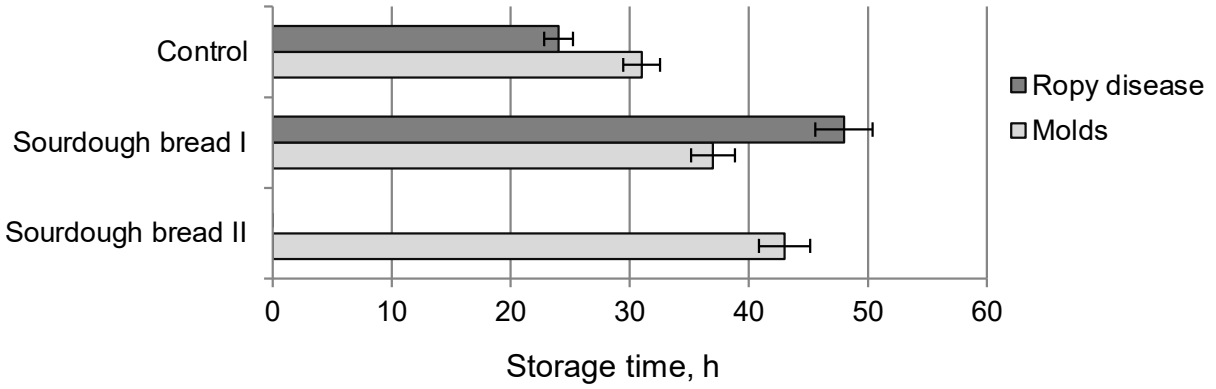

Figure 5. Microbial spoilage of gluten-free bread.

CONCLUSIONS

Based on their antagonistic and acidifying activities two LAB strains *L. brevis* E139 and *L. plantarum* E138 were selected to create new gluten-free sourdoughs as well as a yeast strain *S. cerevisiae* Y205 possessed highest fermentative activity. The influence of the LAB and yeast combination on the quality of gluten-free sourdough was studied. The starter microbial composition affected the sourdough and dough biochemical indicators in terms of cells content, leavening, acidification, volatile acids and alcohol content. New starter microbial composition usage contribute to the introduction of the dominating strains in the sourdough which allows ensuring a reproducible and controlled composition of the sourdough microbiota and ensure a constant bread quality.

The use of starter composition for sourdough preparation makes it possible to obtain bread with desired properties, taste and aroma characteristics. Sourdough did not affect the specific volume, porosity and compressibility of gluten-free bread, but its sensory characteristics were improved. Bread made with sourdoughs had more pronounced taste and flavor, brighter crust color and better texture compared bread without sourdough. Better results in flavor and antimicrobial activity of bread with *L. brevis* 139 compared to the bread with *L. plantarum* 138 may be due the higher production of volatile acids by *L. brevis* 139. Both of new starter composition may be recommended for applications in the gluten-free bread-making.

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