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The development of gluten-free sourdough bread technology with rowan powder

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Abstract. A new form of technology was developed which focussed on gluten-free bread with gluten-free sourdough and rowan powder (from the botanical species *Sorbus aucuparia*). This new form of technology allows organoleptic characteristics to be improved, along with structure, texture, microbial spoilage resistance, and the shelf life of gluten-free bread. The gluten-free dry microbial composition with lactic acid bacteria was developed as a starter for sourdough. The lactic acid bacteria, *L. brevis E38*, was experimentally selected for dry microbial composition on the basis of its antagonistic activity against ropy bread disease pathogens (*B. subtilis* and *B. licheniformis*). The dependence was revealed of the accumulation of acetic acid and lactic acid in the sourdough on the microbial composition during fermentation. A gluten-free sourdough technology was developed which involved a new starter, rice, and soy flour at a ratio of 0.2:2:1. It was shown that the use of soy protein slows down the fermentation process in the sourdough. An increase – in acidity levels of between 7.5–9.5 times higher in the dough with sourdough and rowan powder when compared to dough without sourdough. Sourdough usage allowed compressibility of the crumb to be increased by between 1.8–2 times, with a specific volume of 19.0% and a porosity of 9.8% and 11.5%, and for the sensory characteristics to be improved as perceived by consumers. It was proved that microbial composition with a lactic acid bacteria, *L. brevis E38*, inhibits ropy disease and mould development in bread. The results of the present study showed that the addition of sourdough and rowan powder can be used to improve the quality of gluten-free bread.

Key words: gluten-free bread, lactic acid bacteria, dry microbial composition, rowan powder, quality, mould, ropy disease.

INTRODUCTION

The increased demand for gluten-free products proves that celiac disease is one of those food intolerances which is clearly prevalent across the world. However, the absence of gluten, which is necessary for the construction of the protein structure and crumb texture, makes it difficult to produce gluten-free bread. In addition, gluten-free

bread is easily subjected to microbial spoilage. Therefore, obtaining high-quality gluten-free bread is an important technological task.

Recent scientific studies are increasingly focusing on improving the formulation and technology of gluten-free bread. Many researchers have tried to improve the gluten-free quality of bread using gluten-free flour mixtures and starch (Clerici et al., 2009; Torbica et al., 2010; Sakac et al., 2011; Milde et al., 2012; Ozol et al., 2012; Ziobro et al., 2012; Korus et al., 2015), or additives such as hydrocolloids, gums, fibre, emulsifiers, or enzymes (Renzetti et al., 2008; Sciarini et al., 2010; Phimolsiripol et al., 2012; Tsatsaragkou et al., 2014; Różyło et al., 2017).

In traditional technology, the use of sourdough improves the traditional texture of bread, along with its flavour and shelf life. The use of sourdough in technology relating to gluten-free bread is of great interest. The amount of research being carried out on the use of gluten-free sourdough in bakery production has increased in recent years.

Sourdough was developed using buckwheat, oat, quinoa, sorghum, teff, and wheat flour along with the heterofermentative lactic acid bacteria, *Lactobacillus plantarum FST 1.7* (Wolter et al., 2014). But the studies did not provide any satisfactory results. Shelf life for sourdough breads was not prolonged by the addition of sourdough. The inferior aroma of bread that had been prepared using gluten-free flour was also not improved by the addition of sourdough. It can be assumed that the absence of a positive effect on the taste of gluten-free bread is associated with the use of a facultatively heterofermentative lactic acid bacteria strain which generally only produces lactic acid under normal conditions. Therefore, in this study we propose the use not only of facultatively heterofermentative strains but also of obligatory heterofermentative strains and their mixture with a homofermentative strain.

Recent studies also have confirmed the positive effects of adopting sourdough technology in gluten-free breadmaking (Mariotti et al., 2017). The sourdough was created using a stable association between *Lactobacillus sanfranciscensis* (an obligatory heterofermenatative strain) and *Candida humilis*.

Bender et al. (2017) have evaluated the performances of different *Lactobacillus spp*. strains which were applied in the fermentation of millet and buckwheat sourdoughs. They have established that the combination of four strains could be used as potential starter cultures for millet or buckwheat sourdough bread. They have shown that *Lactobacillus pentosus* and *Lactobacillus hammesii* positively influenced the crumb firmness of buckwheat and millet bread respectively, while *Lactobacillus paralimentarius* enhanced this property in both bread types. But in this research only one of the two *Lactobacillus sanfranciscencis* strains was able to improve all functional properties in both gluten-free breads (Bender et al., 2017).

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 using rowan powder which would improve the quality and microbial stability of bread.

MATERIALS AND METHODS

A characterisation of the ingredients

In this study, the lactic acid bacteria strains *Lactobacillus paracasei/Lactobacillus casei*, *Lactobacillus paracasei E3*, *Lactobacillus plantarum E4*, *Lactobacillus plantarum E5*, *Lactobacillus plantarum E36*, *Lactobacillus parabuchneri E7*,

Lactobacillus fermentum E28, and *Lactobacillus brevis E38* were used. The strains were taken from the collection at the St. Petersburg Branch of the State Research Institute of the Baking Industry.

The dry microbial composition was made by drying a mixture of gluten-free raw materials and pure cultures of lactic acid bacteria strains. A dry microbial composition was used as a starter for sourdough.

The powder from the fruit of the ordinary rowan (the botanical species *Sorbus aucuparia)* was used as an enriching additive.

The pathogen strains of *Bacillus subtilis*, *Bacillus licheniformis*, and *Penicillium chrysogenum* were taken from the collections of the St. Petersburg Branch of the State Research Institute of the Baking Industry, and were used to infect bread when testing microbiological stability.

The study of lactic acid bacteria strains

Strains were cultured in standard liquid medium MRS (BioMerieux, France). Lactic acid bacteria from the St Petersburg collection was inoculated into a liquid medium at the ratio of 1:100, and kept at 37 °C for forty-eight hours. An assessment of microbiological parameters was carried out by examining the following properties: the number of cells in the culture was determined by counting the colonies that had been grown on the MRS with agar, acidity levels were determined using a 0.1 n. solution of NaOH (Afanasjeva, 2003).

Antagonistic activity. lactic acid bacteria's antagonistic activity against ropy bread disease pathogens *B. subtilis* and *B. licheniformis* was investigated. A concentration of 0.1 cm^3 of the pathogen suspension with a cell content of 10^6 cells mL^{-1} was applied to the meat-peptone agar surface in Petri dishes and was distributed over the surface with a spatula, before being covered and left to undergo diffusion for thirty minutes. Then holes with a diameter of 7 mm were cut on the surface of inoculated meat-peptone agar by means of a sterile metal cylinder. A lactic acid bacteria culture of 0.1 cm^3 was inoculated into the holes with the contents of cells at 10^9 cells mL^{-1}. The Petri dishes were kept at 37 °C for twenty-four hours. After that the diameters of the pathogen were measured and no growth zones around the holes were found (Savkina et al., 2015).

Dried microbial composition preparation

A mixture of rice flour and soy protein isolate at a ratio of 2:1 was used to prepare a dry microbial composition. Lactic acid bacteria was grown as follows: a liquid medium of MRS was inoculated by means of lactic acid bacteria from the St Petersburg collection at a ratio of 100:1, and was kept at 37 °C for forty-eight hours. A culture fluid of strains *L. paracasei/L. casei, L. paracasei, L. plantarum* (starter 1), and *L. brevis E38* (starter 2) were mixed with the rice flour and soy protein isolate at a ratio of 1:1. The resulting mixture was granulated through a sieve with a mesh size of 1.5mm and dried in an IR drier (LOIP L3-120/300-VG1, Russia) at a temperature of no higher than 50 °C for between 60–90 minutes to a moisture content of not more than 14.0%.

Sourdough preparation

To prepare the sourdough, the rice flour and soy protein isolate or rice and soy flour at a ratio of 2:1 for both were mixed in a kneading machine, the Kitchen Aid KSM45 (USA), at a speed of 120 revolutions per minute for three minutes with water at a

humidity of 65 ± 0.5%. A dry microbial composition was added to an amount of 2.0% by weight of the mixture. The mixture was mixed and was held at a temperature of between 29–31 °C for between 18–20 hours.

Bread-making procedure

The gluten-free bread formulations used in this study are presented in Table 1. Percentages of ingredients were based on 100 g of gluten-free mixture amount, which include flour, salt, and sugar.

Table 1. Formulations used to prepare different gluten-free bread types

Ingredients, g	Control	Sample 1	Sample 2
Corn starch	57.8	47.5	51.5
Extrusion corn starch	10.0	10.0	-
Soy protein isolate, kg	9.7	9.7	9.7
Rice flour	19.7	-	-
Salt	0.8	0.8	0.8
Sugar	2.0	2.0	2.0
Rowan powder	-	-	6.0
Vegetable oil	3.8	3.8	3.8
Yeast	2.5	2.5	2.5
Sourdough	-	73.0	73.0
Water	until dough humidity achieved of 52% or 49%		

All of the components were mixed in a kneading machine, the Kitchen Aid KSM45 (USA), at a speed of 120 revolutions a minute for seven minutes. After mixing, the dough samples were shaped into 250 g loaves, placed in baking forms, and leavened at 30 °C until the volume was twice that of the initial volume. The leavened dough samples were cooked in an oven, a SvebaDahlen (Sweden), at a temperature of 210 °C for eighteen minutes.

The dry microbial composition and sourdough assessment

The mass proportion of moisture in the dry microbial composition and in the sourdough was determined by drying it at a temperature of 130 °C for forty minutes in a drier, the SHS-1M (Russia). Acidity levels were determined by means of titration, using an 0.1 n. solution of NaOH (State Standard of the Russian Federation, 1996). The lactic acid bacterial cell count was determined by growth on MRS with agar.

The content of acetic and lactic acid in the sourdough were studied using liquid chromatography on a chromatograph Shimadzu LC-10, Japan (State Standard of the Russian Federation, 2014).

An assessment of baked bread

An assessment of quality. An assessment was carried out on bread quality levels in relation to the following properties: organoleptic – appearance (shape, surface, crumb colour), condition of crumb (porosity and texture), and taste and smell; physical and physico-chemical - the mass proportion of moisture was determined by drying out at a temperature of 130 °C for 45 minutes in a drier (the SHS-1M, Russia), and acidity levels were determined by means of titration, using a 0.1 n. solution of NaOH (State Standard of the Russian Federation, 1996); porosity – this being determined as the ratio of pore

volume to the total volume of products; pore volume - the difference between the volume of the product and the volume of the non-porous mass; specific volume - as the ratio of product volume to 100 g of bread, with compressibility being determined on the automatic penetrometer, Labor (Hungary).

The content of gluten in bread was evaluated by means of an enzyme-linked immunosorbent assay.

Ropy disease assessment

In order to determine the effects of the starter on microbial resistance, the bread was infected with the bacteria, *B. subtilis* and *B. licheniformis*. To contaminate the bread, bread crumbs with spores were prepared in the following way: spore-forming bacteria on meat-peptone medium was added to the surface of the sliced gluten-free bread and cultured at a temperature of 37 °C for 96 hours or until signs of disease became apparent. Diseased bread was dried in an oven at a temperature of 50 ± 2 °C and milled to obtain crumbs. A total of 1% of infected crumbs were added while kneading the dough for gluten-free bread. The ready bread was stored at 37 °C prior to any appearance of symptoms of ropy disease (Afanasjeva, 2003).

Moulds spoilage assessment

The impact was investigated in terms of the sourdough and rowan powder and its relation to mould disease in bread. The model experiments with the contamination of sterile bread slices from a pure culture of the mould *Penicillium chrysogenum* were carried out. *Penicillium chrysogenum* was used because the *Penicillium* species are by far the most common for bread. This strain was previously isolated from diseased gluten-free bread and was identified with the species according to cultural and biochemical characteristics. In addition it was contained in the St. Petersburg collection.

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 3.5×6.5 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 mould, *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 mould 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 ± 1 °C until the first signs appeared of a growth of mould colonies.

Statistical analysis of the data

All of the experiments were carried out a total of five times. The accuracy of the experimental data was evaluated by using mathematical statistical methods in Microsoft Excel (2010 version) at a theoretical frequency of 0.95. Results were given as mean ± standard deviation.

RESULTS AND DISCUSSION

Gluten-free bread is easily exposed to microbial damage (ropy disease and mould growth). The use of 6–10% rowan powder in the formulation for making gluten-free bread allows mould spoilage to be slowed down (Dubrovskaya et al., 2017). To inhibit ropy bread disease, it was suggested that a sourdough starter with lactic acid bacteria be developed, which has an increased level of antagonistic activity against ropy disease's causative agent.

The antagonistic activity of lactobacilli against *Bacillus subtilis* and *Bacillus licheniformis* was studied (Fig. 1). This showed that *L. brevis E38* suppressed the *Bacillus* growth more than other strains. The strains *L. paracasei/L. casei, L. paracasei E3*, and *L. plantarum E4* showed slightly worse results, but also had rather high levels of antagonistic activity.

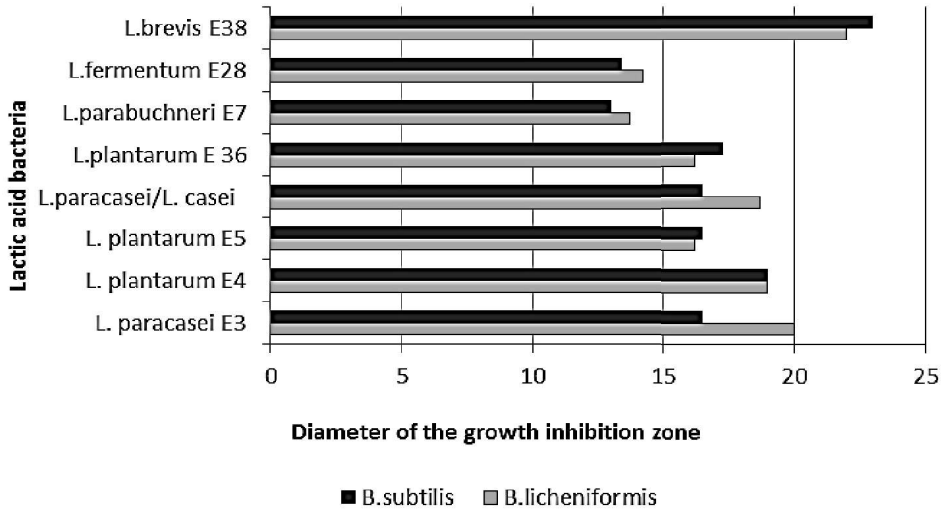

Figure 1. Antagonistic activity by lactic acid bacteria against *B. licheniformis* and *B. subtilis*.

All of the strains are obligatory heterofermentatives or facultatively heterofermentatives, so that they are able to produce organic acid (whether lactic or acetic), thereby having an antimicrobial effect. But investigated lactic acid bacteria had different levels of antagonistic activity. It was found that the species *L. brevis*, *L. paracasei/L. species casei*, *L. paracasei*, and *L. plantarum* had antagonistic activity which were between 30–46% higher than the *L. fermentum* and *L. parabuchneri* species. Thanks to this, the production of organic acids is not the only one mechanism of providing antagonism. Antimicrobial and antibiotic-like compounds – bacteriocins (lactocins) – can also play an important role (Reis et al., 2012).

The antagonistic activity of the mixture of strains (*L. paracasei/L. casei*, *L. paracasei E3*, and *L. plantarum E4)* was higher, being comparable to *L. brevis E38* (data not shown). The increasing in antagonistic activity may be due to synergic action. Therefore, this symbiosis was used to develop the dry microbial composition.

Two types of dry microbial composition were created. *L. paracasei/L. casei*, *L. paracasei E*, and *L. plantarum E4* (starter 1), along with *L. brevis E38* (starter 2) were mixed with the rice flour and soy protein isolate at a ratio of 1:1 and these were subsequently dried.

Biotechnological indicators for two types of dry microbial composition are presented in Table 2. It was found that the number of cells of lactic acid bacteria in starter 2 were 4.85 times higher than in starter 1, although they had practically the same acidity levels. This may be due to competition for food between *L. paracasei/L. casei*, *L. paracasei*, and *L. plantarum.*

Table 2. Biotechnological indicators for two types of dry microbial composition

Biotechnological indicators	Starter 1	Starter 2
Mass proportion of moisture, %	14.0 ± 0.2	13.8 ± 0.2
Acidity, degrees N	9.0 ± 0.4	9.4 ± 0.4
Number of lactobacilli cells, CUFF g^{-1}	$(0.26 \pm 0.01) \times 10^9$	$(1.26 \pm 0.01) \times 10^9$

The number of cells in the starter is of great importance because this reflects an ability to develop in sourdough. That is why starter 1 was selected for further research.

During the sourdough development process, acid accumulation was studied within 36 hours at a temperature of 29–31 °C in two 65% moisture mixtures. This moisture content was chosen based on previous studies which are not listed here. One sourdough sample consisted of rice flour and soy protein isolate, and the other consisted of rice and soy flour at a ratio of 2:1. Rice and soy flour and soy protein isolate were taken from the basic recipe (Dubrovskaya et al., 2017). Starch was not used in the sourdough technology because it is less of a nutrient medium for lactic acid bacteria growth than the flour and the protein.

Besides this, *L. brevis E38* is an obligatory heterofermentative micro-organism, which ferments hexose into lactic acid, acetic acid (ethanol), and CO_2. Pentoses are fermented by them into lactic acid and acetic acid through the pentose-phosphate pathway. Therefore, not only were the acidity levels studied in the sourdoughs, but so was the content of lactic acid and acetic acid.

It was found that acidity accumulation was higher by between 25–48% in sourdough with soy flour than in sourdough with soy protein isolate (Fig. 2). This shows that soy flour is more nutritious for the development and life activity of lactic acid bacteria than soy protein.

As for the metabolite concentration found in the sourdoughs (Table 3), a higher lactic acid amount was produced in sourdough with soy protein isolate. The content of acetic acid was 2.7 times higher in sourdough with soy flour when compared to sourdough with soy protein isolate (Table 3). Higher acidity levels and a higher content of acetic acid can positively influence the taste of bread and increase its microbiological stability during storage.

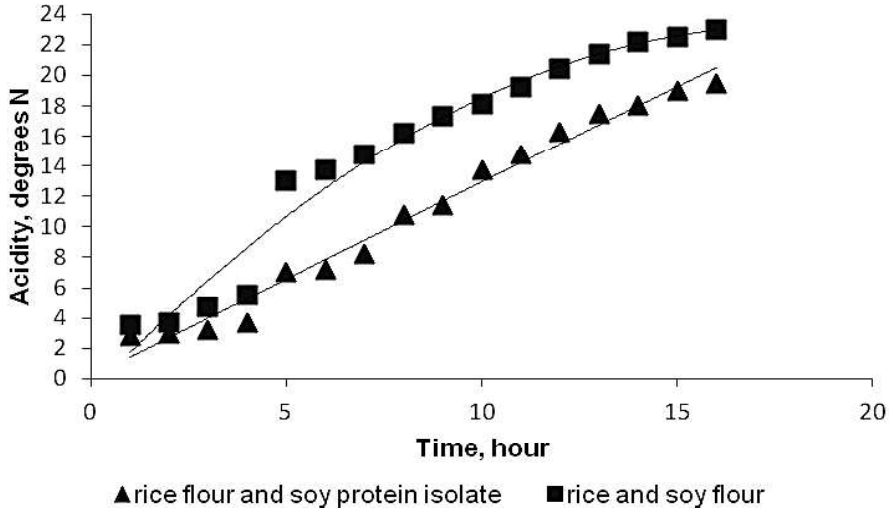

Figure 2. Acidity in two types of sourdough.

Therefore the use of a dry microbial composition with *L. brevis E38*, rice, and soy flour, at a ratio of 2:1 and with water to a moisture content of 65% may be recommended when it comes to preparing gluten-free sourdough.

Table 3 . Biotechnological indicators for two types of sourdough

Indicators	Sourdough with rice flour and soy protein isolate	Sourdough with rice and soy flour
Mass proportion of moisture, %	65.0 ± 1	65.0 ± 1
Acidity, degrees N	13.5 ± 0.5	14.5 ± 0.5
Temperature, °C	30 ± 1	30 ± 1
Duration of fermentation, hour	19 ± 1	19 ± 1
Lactic acid, g kg^{-1}	13.5 ± 2.7	9.7 ± 1.9
Acetic acid, g kg^{-1}	0.70 ± 0.14	1.90 ± 0.38

An investigation was carried out in terms of the influence exhibited by the sourdough on dough and bread quality. It was found that a sourdough and dough combination increases dilution and reduces viscosity (Fig. 3). The diluting effect of the sourdough may be related to the effect of lactic acid bacteria enzymes on the dough biopolymers. This permitted dough humidity to be reduced by 3% (from 52.0% to 49%). When the bread moisture content is reduced, gluten-free bread resistance to microbiological spoilage (mould) may be increased.

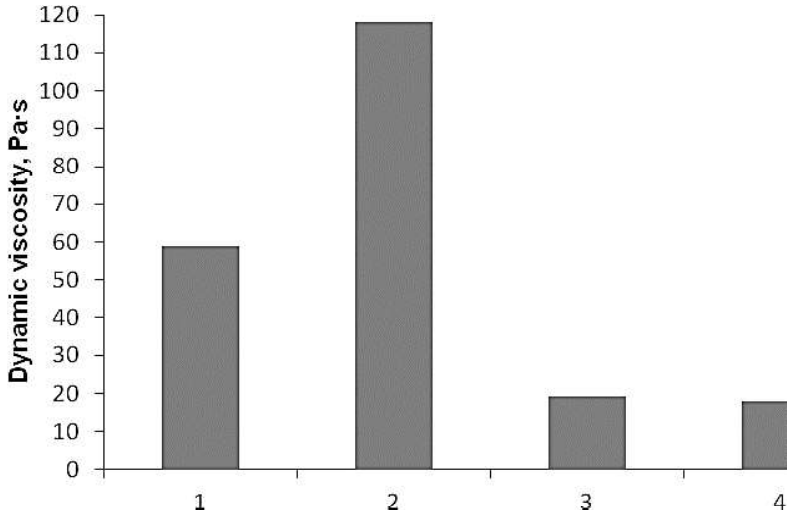

Figure 3. Dough dynamic viscosity at a shear rate of 9 s^{-1}. 1 – control, dough humidity 52%; 2 – control, dough humidity 49%; 3 – Sample 2, dough humidity 49%; 4 – Sample 2, dough humidity 49%.

It was found that sourdough and rowan powder usage increased the dough acidity levels (Fig. 4). Dough acidity in Fig. 4 represented a dough humidity of 49%.

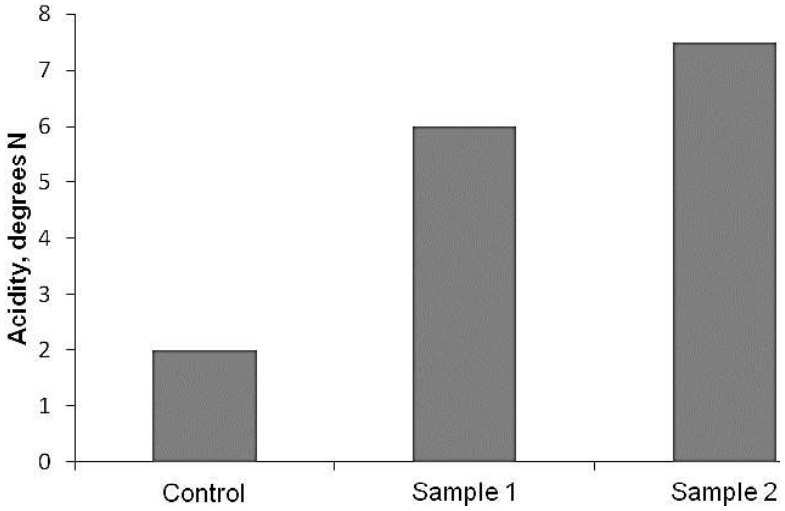

Figure 4. Dough acidity, degrees.

The bread quality indicators are presented in Table 4. It was found that bread with sourdough had an acidity level which was higher than the control by 7.5–9.5 times, while crumb compressibility was higher by between 1.8–2 times, the specific volume was higher by 19.0%, and porosity was higher by 9.8–11.5%. The improvement of gluten-free sourdough bread derives from the metabolic activity of lactic acid bacteria that

provide acidification and the production of exopolysaccharides and antimicrobial substances.

Table 4. Indicators of gluten-free bread quality

Indicators	Control	Sample 1	Sample 2
Mass proportion of moisture, %	51.7 ± 0.5	48.9 ± 0.5	49.1 ± 0.5
Acidity, degrees	0.3 ± 0.1	2.0 ± 0.5	2.8 ± 0.5
Porosity, %	61.0 ± 2	68.0 ± 2	67.0 ± 2
Specific volume, $sm^3 \cdot g^{-1}$	2.1 ± 0.1	2.5 ± 0.1	2.5 ± 0.1
Compressibility, equipment units	19 ± 4	39 ± 4	35 ± 4

Bread organoleptic characteristics are presented in Table 1 (Fig. 5). Sample 1 and Sample 2 had the better crust colour, crumb texture, and porosity than the control. Bread with sourdough and rowan powder (Sample 2) had the best taste and smell. The smell was more intense and pleasant, with fruity notes.

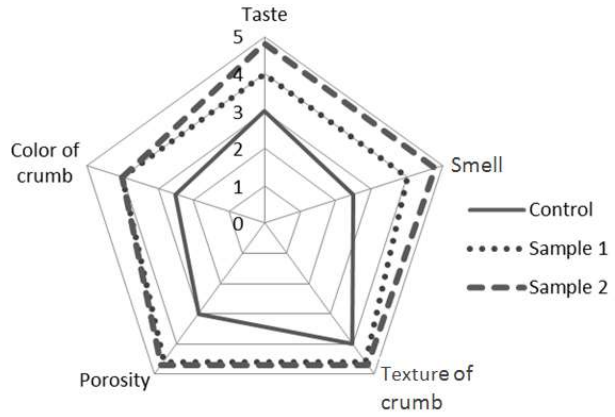

Figure 5. The influence of the sourdough and rowan powder on the organoleptic profiles of gluten-free bread.

The safety of bread with sourdough and rowan powder when it comes to providing nutrition for people who are suffering from coeliac disease was evaluated by means of analysing the gluten content in bread through an enzyme-linked immunosorbent assay. The content of immunoreactive gluten was less than 5 mg for each 1kg of bread, which meets the requirements of diet therapy in celiac disease.

In order to establish the effect of sourdough and rowan powder on ropy bread disease, laboratory baking was carried out with spore-infected breadcrumbs. Bread deposited in precipitating conditions at 37 °C and with a humidity of 70 ± 5%.

It was found that control samples are infected with the disease after a period of seventeen hours, while gluten-free sourdough bread (Sample 1) and bread with sourdough and rowan powder (Sample 2) are not infected at all with ropy disease during the entire storage period (Fig. 6). Therefore, the results of the research have shown that the use of sourdough in gluten-free bread allows the development of the *Bacillus* spore to be completely suppressed.

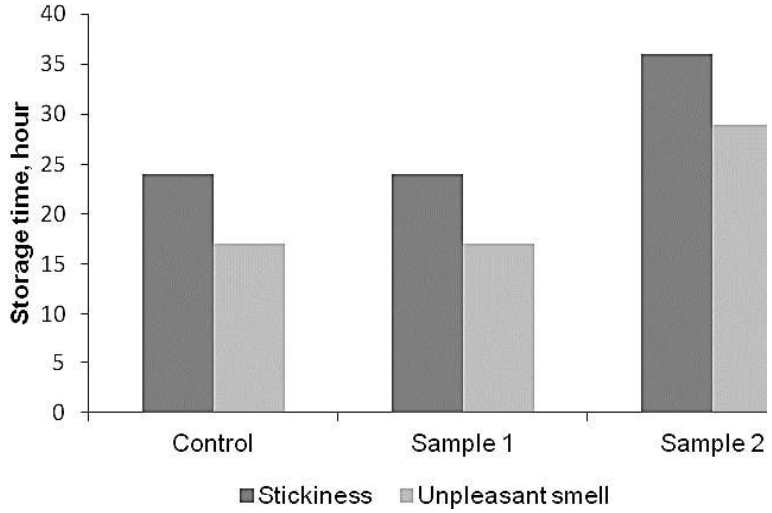

Figure 6. The influence of sourdough and rowan powder on ropy disease in gluten-free bread.

An investigation was carried out into the effect of the sourdough and rowan powder on gluten-free bread's resistance to the moulds. It was found that in the control bread slices were contaminated by *Penicillium chrysogenum*, and a growth of mould colonies was observed between 22–24 hours, while in samples 1 and 2 this was between 36–38 hours. The usage of sourdough with lactic acid bacteria *L. brevis E38* allowed the progression of gluten-free bread mould disease to be slowed down.

Therefore it was proven that the new gluten-free sourdough bread technology using *L. brevis E38* and rowan powder served to increase the microbiological resistance of bread against mould and ropy disease. It also improves the quality, taste, and smell of bread.

CONCLUSIONS

Studied here were the antagonistic activity of a strain of lactic acid bacteria, its biomass, and acidity accumulation during cultivation on an MRS liquid medium. The strain, *L. brevis E38*, was selected to create a dry microbial composition. The dependence was revealed of acetic acid and lactic acid accumulation in the sourdough during fermentation on the microbial composition.

A dry microbial composition was created as a gluten-free sourdough starter. A gluten-free sourdough technology was developed with a new starter, rice, and soy flour at a ratio of 0.2:2:1.

It was found that when using lactic acid bacteria, *L. brevis E38*, the dough liquefies due to the action of the lactobacilli enzymes on dough biopolymers.

It was also shown that sourdough and rowan powder which contained various organic acids and flavouring substances improves the sensory characteristics of gluten-free bread as perceived by consumers. The use of sourdough with a dry microbial composition and rowan powder allowed the compressibility of the crumb to be increased

by between 1.8–2 times, the specific volume by 19.0%, and the porosity by 9.8% and 11.5%.

It was found that sourdough which contained a large volume of lactic acid bacteria ($3.6 \cdot 10^9$), lactic acid (9.7 g kg^{-1}), and acetic acid (1.9 g kg^{-1}), helped to slow down the onset of spore-forming bacteria and mould growth in bread during storage.

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