UDC 664.6:579.674

APPLICATION OF BACTERIAL SOURDOUGH AND ENZYMES IN THE PRODUCTION OF BREAD FROM A MIXTURE OF RYE AND WHEAT FLOUR

https://doi.org/10.15673/fst.v17i4.2761

A. Korzhenivska[1], Postgraduate,
S. Danylenko[1,2], Doctor of Technical Science, Senior Researcher
S. Gunko[2], PhD, associate professor
G. Kozlovska[3], Candidate of Veterinary Sciences, Associate Professor
A. Lukianets[1], Postgraduate
[1]Department of Biotechnology Institute of Food Resources NAAS
E. Sverstyuka str., 4 a, Kyiv, Ukraine, 02002
[2]Department of Storage, Processing and Standardization of Plant Products
after prof. B.V. Lesik National University of Life and Environmental Sciences
of Ukraine, Heroyiv Oborony str., 13, Kyiv, Ukraine, 03041
[3]Department of Epizootology, Microbiology and Virology
National University of Life and Environmental Sciences of Ukraine,
Vystavkova Str., 16, Kiev, Ukraine, 03041

Correspondence:
S. Danylenko
E-mail: svet1973@gmail.com

Cite as Vancouver style citation

Korzhenivska A, Danylenko S, Gunko S, Kozlovska G, Lukianets A. Application of bacterial sourdough and enzymes in the production of bread from a mixture of rye and wheat flour. Food Science and Technology. 2023;17(4):4-13.
https://doi.org/10.15673/fst.v17i4.2761

Цитування згідно ДСТУ 8302:2015

Application of bacterial sourdough and enzymes in the production of bread from a mixture of rye and wheat flour / Korzhenivska A. et al. // Food Science and Technology. 2023. Vol. 17, Issue 4. P. 4-13.
https://doi.org/10.15673/fst.v17i4.2761

Abstract. Interest in sourdough rye-wheat bread with addition of enzymes is actively demonstrated by various manufacturers. Enzymes associated with the metabolic activity of sourdough microorganisms and exogenous enzymes deliberately added to the dough recipe are used in the production of these bakery products. Such combinations contribute to the improvement of both dough structure and the final product. The research investigated the influence of the combined application of the IPROVIT sourdough and enzymes α-amylase, glucoamylase and protease on the structural-mechanical properties of the dough and the quality of the resulting rye-wheat bread. Optimal concentrations of individual enzymes and their combinations were established: α-amylase – 0.35 cm^3, protease – 0.05 cm^3, glucoamylase – 3 cm^3, α-amylase+glucoamylase and α-amylase+protease in a 1:1 ratio. Adding enzymes at rational concentrations positively affected on the physical properties of the dough, making it elastic, shaping well into a ball and preventing spreading. Increased enzyme concentrations led to stickiness and eventual spreading of the dough. It was observed that the combined application of Lactobacillus plantarum, L. brevis, Lactobacillus paracasei ssp. paracasei bacteria and enzymatic preparations (α-amylase, protease, glucoamylase) of different actions positively affected the physical properties of the dough. The dough mixed well, did not stick to hands, maintained its shape, faster increased of volume and had moisture levels within the control range of 41–43%. The acidity of the resulting bread met the requirements of DSTU 4583:2005 and amounted to 8.9–10 degrees for all tested samples. Reduced acidity in the sample with the enzyme complex α-amylase+protease is explained by the fact that the amino acids formed as a result of the protease action were used by lactic acid bacteria as a source of nutrients. The combined application of lactic acid bacteria and enzymes positively contributed to intensifying fermentation processes, indicated by higher dough rising indicators, which were 3–9 min higher than the control. Through the use of enzymes with different actions, it was proven that the organoleptic characteristics of the bread improved, acquiring an attractive color, distinct taste and pleasant specific aroma with hints of nutmeg. The porosity of the experimental bread samples with the enzyme mixture was at the control level. Thus, the use of rye-wheat flour, sourdough and enzymes has a positive effect on the quality of bread and contributes to increasing its nutritional and biological value.

Keywords: Lactic acid bacteria, enzymes, physical properties of dough, quality of bread.

Introduction. Formulation of the problem

Lactic acid bacteria (LAC) are one of the most valuable and useful microorganisms that are widely utilize in the food industry [1,2].

Their application covers various areas of food production and provides many advantages that promoting to improvement of their quality, safety and ensure the provision of specific sensory characteristics.

One of the main functions performed by LAB in the production of food products is their participation in fermentation processes, where they play a main role in the transformation of sugar into lactic acid. Their action did not limited only acidification of the environment and also affords a specific taste to fermented products. Lactic acid, which formed at the same time, serves as a natural preservative that suppresses the growth of pathogenic microorganisms and as a result prevents spoilage of products [3]. In this way, we not only extend the shelf life of products, but at the same time we manufacture products without adding synthetic preservatives.

LAB play a main role in the production of milk processing products, in particular, in the production of various yogurts and cheeses [4]. The LAB strains of *Lactococcus* and *Lactobacillus* are able not only to ferment lactose, which gives products a characteristic spicy taste, but also to improve their texture and consistency. The compounds that are formed as a result of the vital activity of LAB have antimicrobial properties, which increases the safety and improves the hygienic properties of products.

Another advantage of LAB utilizing is their probiotic properties [5,6]. It is known that some strains of LAB, which belong to the genus of *Lactobacillus* and *Bifidobacterium*, have a positive effect on human health and improve immunity. Thanks to this, the production of functional and dietary food products and supplements that contain various strains of LAB is rapidly developing in the world today [7].

One of the areas of application of LAB in the production of bread, where they play a decisive role in the formation of both sensory and nutritional properties of the finished product [8]. An important function of lactic acid bacteria in the production of bread is their participation in the processes of fermentation and dough formation. The LAB strains of *Lactobacillus* and *Leuconostoc* convert sugar into lactic acid, which not only contributes to acidification, the development of the characteristic taste of sourdough, but also forms a characteristic organoleptic profile of local and traditional types of bread. Accumulation of lactic acid also plays an important role in improving the texture and volume of bread [9] and promotes to obtaining the desired crumb structure and improving whole the quality of product.

Lactic acid bacteria not only take an active part in the formation of taste and texture of bread, but also prevent its staling [10] and extend the shelf life [11-13]. The lactic acid that formed during the fermentation of the dough, suppresses the growth of mould microorganisms [14-15] and in combination with the yeast in the sourdough, it has an antifungal effect against *Aspergillus flavus* [16]. It is act as a natural preservative that improves the hygienic properties of bread.

The metabolic activity of LAB contributes to the enrichment of the nutritional profile of bread, due to the fact that they participate in the breakdown of complex carbohydrates and proteins and increase the bioavailability of basic nutrients [17]. This increase in nutrient availability is consistent with current trends in producing foods that not only have a variety of flavor profiles, but also have a positive impact on the health of consumers.

In summary, it should be noted that lactic acid bacteria are an important component in bread production technology and offer many advantages that go out the boundary of the simple fermentation. They have a significant impact on the formation of taste properties, bread texture, shelf life and nutrient content [18], that makes LAB an indispensable component of the modern bread production process, which ensures a stable symbiotic relationship between microbiology and production technology.

One of the technological aspects of LAB utilizing in the production of bread, that some limits application, is the need to activate their vital activity in the dough system during fermentation. The researches about improving of activity of lactic acid bacteria in the bread production, especially rye and wheat-rye, impacts of such activation on the structure of the dough, its physical properties and indicators of the quality of finished bread are not systematize and complete and require further investigation.

Analysis of recent research and publications

Activation of the vital activity of lactic acid bacteria during bread production consists primarily in creating an environment favorable for their growth and metabolism. This is achieved due to utilize substances that provide the necessary nutrients and conditions for the development of bacteria.

For this purpose, so-called pre-sourdough are used, which are made from a mixture of flour and water. Them are fermented before adding to the dough. They contain natural populations of lactic acid bacteria that can significantly affect the taste and texture of bread [19].

Another influencing factor is the yeast that used in dough fermentation. They can be indirectly support the activity of lactic acid bacteria, creating an environment favorable for their growth [20]. As a result, yeast fermentation producing compounds that improve the taste and texture of bread, and also can be impact on the pH and redox potential that influence on the activity of lactic acid bacteria.

An important influencing factor is the quality and temperature of the water that used to prepare the dough. It can be affecting on the activity of lactic acid bacteria [21]. Water that is too hot or too cold can inhibit their growth, while water with the right temperature and mineral content can be create an optimal environment.

A positive effect of malt grain on the activation of the vital activity of LAB was established. They are containing significant amount of natural enzymes that capable of breaking down complex carbohydrates into simpler sugars and creating an easily accessible source of food for lactic acid bacteria [22].

Some authors believe that one of the most perspective ways increasing activity of the LAB is the application of enzymes [23]. Adding them to the dough recipe during fermentation will help break down starches and proteins into fermentable sugars and amino acids, respectively. This will indirectly support the activity of lactic acid bacteria.

Thus, in the publication [24] was established the positive effect of the addition of α-amylase, cellulase, glucose oxidase, maltogenic α-amylase, xylanase on the increasing the volume of bread, reducing the initial hardness of the crumb and staling.

Research on the effect of the use of xylanase, phytase, α-amylase, protease, cellulase, glucose oxidase and lipase enzymes in the production of bread showed good results in improving the nutritional and organoleptic indicators quality of product [25].

The investigations about application of α-amylase and hemicellulase in the production of bread from whole grain flour that contains a large amount of dietary fiber were conducted [26]. The authors established that the addition of optimal concentrations of the enzymes dramatically improved the gas-holding capacity of the dough, its specific volume and slowing its staling compared to bread without enzymes.

A positive effect of the application enzymes of α-amylase, xylanase, cellulase, glucose oxidase, lipase on the texture and sensory quality of bread after storage of the dough in the frozen state was established [27]. That treatment is also improving on the properties of the dough in terms of fermentation characteristics, frozen water content and microstructure. Except for α-amylase, other enzymes (especially xylanase) improved the sensory quality of bread and increased its overall score. Scanning electron microscopy revealed that freezing and frozen storage destroys the gluten structure of the dough, causing the starch granules to separate from the gluten. The inclusion of cellulase, xylanase and lipase in the recipe provided a more plastic and strong gluten frame in the frozen dough and glucose oxidase strengthened the gluten.

A good result was obtained with lipase and protease enzymes in the production technology of gluten-free bread with partial replacement of wheat flour by quinoa [28]. The application of these enzymes promoted to obtain a significant increase in the volume of bread, especially in the sample containing 15% quinoa flour and the best result of delaying its staling in the sample with 25% substitute.

Summing up, it should be noted that enzyme of various action effectively used in the technology of bread production and contribute to a significant improvement of both the structure of the dough and the finished product, but systematic and complete studies about their influence on the vital activity of lactic acid bacteria in the dough system and, as a result, on the quality of bread have not been conducted.

The **purpose** of the research is establishing the effect of complex application of *IPROVIT* sourdough and α-amylase, glucoamylase and protease enzymes on the structural and mechanical properties of the dough and the quality of the finished rye-wheat bread.

To achieve the purpose, the following **objectives** were formulating:

- establish optimal concentrations of enzyme and their combinations;
- determine the effect of synergism of *IPROVIT* sourdough lactobacilli and enzymes on the physical properties and quality of the dough;
- estimate the bread quality made with the use of sourdough and various combinations of enzymes.

Research materials and methods

Wheat and rye flour was used for research, the general characteristics of which are given in the Table 1.

Table 1 – Characteristics of rye and wheat flour that utilized in the researches

Indicator	Wheat flour the first grade of TM "Svoya linia"	Rye peeled flour of TM "Zernari"
Appearance	A homogeneous friable product with small shell parts	
Color	White, with a cream shade	White, with a slight grayish shade
Taste and smell	Characteristic to the crop from it was make. Foreign smells and impurities were absent	
Mass fraction of moisture, %	14.0	13.0
Proteins, g/100 g of flour	10.3	8.9
Carbohydrates, %	74.2	73.0
Fats, g/100 flour	0.9	1.2

The following enzymes and sourdough were used:

- food concentrated fungal α-amylase SQzyme FAL (*Aspergillus oryzae*) (EC 3.2.1.1). Activity 120,000 units/cm^3. (*China*, Suntaq company);
- liquid concentrated bacterial protease Maxazyme NNP DS (*Bacillus subtilus*). Activity 180,000 units/cm^3. (*Denmark*, DSM Food Specialties company);
- Glucoamylase. Activity 1500 units/g. (*Ukraine*, DP «Enzym»);
- *IPROVIT* sourdough is made from pure cultures of *Lactobacillus plantarum, L. brevis, Lactobacillus paracasei ssp. Paracasei.* 1 g of dry bacterial sourdough contains $5 \cdot 10^{10}$ CFU of lactic acid bacteria (*Ukraine*, Department of Biotechnology of the Institute of Food Resources of the National Academy of Agrarian Sciences);

- dry baking yeast "Lviv yeast" (*Ukraine*, PJSC «Enzym Company»).

For determination optimal dose of enzyme preparing dough on the base of mixture of rye and wheat flour. The dough was prepared according to the following recipe: peeled rye flour – 100 g, wheat flour of the first grade – 100 g, sourdough – 0.12 g, yeast – 2.0 g, table salt – 3.6 g, sunflower oil – 4.0 g (Control) and different concentrations of enzymes were added. The dough was kneaded by the free-leavened accelerated method. The ready dough was kept for 2.5–3 hours at a temperature of 30–32°C.

The following enzyme concentrations were used:

- α-amylase: 1.4 cm^3 of enzyme solution (dilution 1:1000)/100 g of flour; 0.7 cm^3 of enzyme solution (dilution 1:1000)/100 g of flour; 0.35 cm^3 of enzyme solution (dilution 1:1000)/100 g of flour.
- protease: 0.5 cm^3 of enzyme solution (dilution 1:1000)/100 g of flour; 0.05 cm^3 of enzyme solution (dilution 1:1000) / 100 g of flour.
- glucoamylase: 1 cm^3 of enzyme solution (dilution 1:1000)/100 g of flour; 3 cm^3 of enzyme solution (dilution 1:1000)/100 g of flour.
- α-amylase and glucoamylase in a 1:1 ratio of their optimal concentrations.
- α-amylase and protease in a 1:1 ratio of their optimal concentrations.

The dough recipe for bread baking presented in the Table 2.

Table 2 – Bread recipe from the mixture of rye and wheat flour

The name of the raw material	K	Samples of bread with the addition of rational concentrations of enzymes				
		D1	D2	D3	D4	D5
Rye peeled flour, g	100	100	100	100	100	100
Wheat flour of the first grade, g	100	100	100	100	100	100
Sourdough, g	0.12	0.12	0.12	0.12	0.12	0.12
Yeast, g	2.0	2.0	2.0	2.0	2.0	2.0
Table salt, g	3.6	3.6	3.6	3.6	3.6	3.6
Sunflower oil, g	4.0	4.0	4.0	4.0	4.0	4.0
α-amylase	-		-	-	-	-
Protease	-	-		-	-	-
Glucoamylase	-	-	-		-	-
α-amylase + glucoamylase	-	-	-	-		-
α-amylase + protease	-	-	-	-	-	

* K – control; D1-D5 – experimental variants

The determination influence of semi-finished products that was made with additional of enzymes on the quality indicators of rye-wheat bread, trial baking was conducted according to the generally accepted methodology of scientific research in the laboratory of the Institute of Food Resources of the National Academy of Sciences.

The dough was kneaded by hand of the free-leavened accelerated method during 30 min with using previously activated bacterial sourdough for rye and a mixture of rye and wheat flour with adding enzymes in the concentrations that presented before.

Dough samples were formed by hand and placed in baking dishes. Semi-finished products were fermented for 60–90 min at a temperature of 31–33°C in the proofing cabinet at a relative humidity of 75–80% and a temperature of 30–32°C until the dough increased by 1.5 times. The readiness of dough samples was determined organoleptically. After fermentation, the dough was divided into pieces of the required weight and left until ready in a humidified thermostat at a temperature of 33–35ºC. The finished dough samples were baked at a temperature of 230–240°C for 40-45 min in a UNOX steam convection oven.

The quality of the bread was evaluated according physical and chemical indicators – moisture, acidity, porosity. Physico-chemical indicators were determined after 3 h after baking. Physico-chemical investigations of the quality of bread were carried out in accordance with the method:

- titrated acidity. 100 cm^3 of the original homogenate was clarified by centrifugation at 4000 rpm for 15 min, and 25 cm^3 of the supernatant was titrated at 20°C with using an automatic potentiometric titrator. The results were expressed as the volume (cm^3) of 0.1 M NaOH standard solution (Merck, Germany) required to titrate 10 g of starter to a final pH of 8.5 [29];

- the lifting force was determined by the floating ball method [29];

- moisture - by drying on the Chyzhova device to a constant weight at a temperature of 160°C [30];

- porosity was determined by Zhuravlev's device according to DSTU 7045:2009 Bakery products. Methods of determining physical and chemical parameters. The device consists of a metal cylinder with an inner diameter of 3 cm and a pointed edge on one side, a wooden sleeve and a wooden or metal pan with a cross wall and a slot for the protrusion of the metal cylinder at a distance of 3.8 cm from walls; the depth of the slot is 1.5 cm. A piece with a width of at least 7–8 cm was cut from the middle of the bread. From the pulp of this piece with the most typical porosity at a distance of at least 1 cm from the crust, samples were made with the cylinder of the device. The sharp edge of the cylinder was previously lubricated by vegetable oil. The cylinder was inserted with a rotary motion into the crumb of the piece. The cylinder filled with crumb was placed on the pan so that the rim of the cylinder fit snugly into the slot on the pan. Then the bread crumb was pushed out of the cylinder with a wooden sleeve about 1 cm and cut with a sharp knife near the edge of the cylinder. The cut piece of crumb was removed. The bread crumb that remained in the cylinder was pushed out with the sleeve against the wall and also cut near the edge of the cylinder. If the inner diameter of the cylinder is 3 cm and the distance from the wall of the pan to the slot is 3.8 cm, the volume of the sample of the crumb cylinder is 27 cm^3;

- organoleptic indicators – according to DSTU 7044:2009 Bakery products. The rules of acceptance, methods of sampling, methods of determining organoleptic indicators and weight of products;
- statistical processing of research results were performed by MS Office Excel software.

Results of the research and their discussion

Determination of rational doses of enzymes

Preliminary studies of the influence of LAB on the properties of the dough according to the index of ball spreading showed that the addition of IPROVIT sourdough that based on LAB helps to reduce the ball spreading up to 5.0%, which indicates a slight decrease in the viscosity of the dough, that is, the additional of LAB can be contribute the improvement of the form stability of the dough [31]. The increase in dough viscosity may be due to the fact that in the presence of air oxygen, flour lipoxygenase converts unsaturated fatty acids into peroxide compounds. Peroxides can oxidize starch to form polyaldehyde starch, which can interact with gluten proteins, reducing the spread of the dough. With aim to obtain an additional amount of fermentable sugars and intensify the fermentation process, enzymes were used. In our research, 3 types of enzymes were used: α-amylase, glucoamylase and protease.

With aim to determine the rational dose of enzymes, dough samples of bread were made according to the recipe and solutions of EP were added according to the research scheme. The quality of the dough was assessed visually by its physical properties, characterized as follows: elastic, well formed into a ball; sticks to the hands, does not form well into a ball; spreading, does not form into a ball.

Alpha-amylase acts on starch, destroying α-1,4-bonds with the formation of maltodextrins and oligosaccharides. It hydrolyzes the polysaccharide chain of starch and other long-chain carbohydrates anywhere and leads to the formation of oligosaccharides of different lengths [32], therefore it is actively used to improve the quality of bread [33].

We used α-amylase in order to accumulate sugars, which should become an additional source of nutrients for the development of lactic acid bacteria and yeast and intensify fermentation processes. The dose of α-amylase was chosen based on the calculation of the destroying of 20%, 10% and 5% of all available starch, which corresponded to the amount of EP solution of 1.4 cm^3, 0.7 cm^3 and 0.35 cm^3/100 g of flour.

The dough stuck to the hands, the ball formed poorly and spread over time when we added α-amylase solution in the amount of 1.4 cm^3 to the dough. The addition of EP in amounts of 0.7 cm^3 and 0.35 cm^3 had a positive effect on the physical properties of the dough: it was elastic, formed well into a ball and did not spread. That is, based on the assessment of the physical properties of the dough, a preliminary conclusion can be made that these EP concentrations are optimal for use.

Glucoamylase is an enzyme that breaks down dextrin and starch molecules to form glucose. In addition to alpha-1,4-glycosidic bonds, glucoamylase hydrolyzes alpha-1,6-glycosidic bonds and has the ability to hydrolyze a highly polymerized substrate ten times faster than oligo- and disaccharides [34]. Considering this, the use of glucoamylase in the production of bread promotes active fermentation, especially in the first period, which affects the increase in the volume of bread [35].

The doses of 1 and 3 cm^3 solution of glucoamylase used by us were calculated to destroy 1.5% and 5% of all available starch. It was established that high quality indicators of the dough were ensured by a dose of 3 cm^3 of EP solution: the dough was well mixed, did not stick to the hands, kept its form and faster increase volume. That is, the ability of EP to quickly accumulate monosaccharides activated the action of lactic acid bacteria. A dose of 1 cm^3 of EP solution visually did not effects on the physical properties of the dough and it was similar to the control.

Proteolytic enzymes, affecting gluten proteins, cause changes in its physical properties and thus influence changes in the rheological properties of the dough, accelerating its ripening [36]. Enzymatic hydrolysis of proteins under the action of proteases catalyzes the breaking of peptide bonds with the formation of amino acids, which are a source of nutrients for the vital activity of lactic acid bacteria.

As a result of the addition of protease in the amount of 5 cm^3 of EP solution, the dough was difficult to mix, and a dose of 0.05 cm^3 of EP solution made it elastic, it formed a ball well and did not spread. That is, the optimal dose according to the organoleptic evaluation of dough samples was 0.05 cm^3 of protease.

Some of authors were established a significant synergistic effect on the quality of bread with the joint use of enzyme preparations with different effects [37]. Considering this fact, we also investigated the complex use of several EP at the same time: α-amylase + glucoamylase and α-amylase + protease. EP compositions were used in a 1:1 ratio their optimal concentrations, that were determined earlier.

As a result, we found that the addition of α-amylase and glucoamylase enzyme compositions made it possible to obtain a dough that was well mixed, well formed into a ball, did not stick to the hands and did not spread. But over time, drops of water formed on the surface of the dough as it stood. This is explained by the fact that the addition of α-amylase, that is already present in an active state in rye flour [38], contributes to its deeper hydrolytic destroying with the formation of low-molecular-weight dextrins and reduces its water-holding capacity.

The utilization of α-amylase and protease had a positive effect on the quality of the dough. It was as similar as possible to the control, since rye flour proteins in the dough do not form a gluten framework, swell easily, and a part swells indefinitely, peptizes and turns into a colloidal solution. The pentosans of this flour form viscous solutions. Therefore, rye-wheat dough, with the addition of more enzymes, makes the dough less springy and less elastic.

The results are given in Table 3 confirms our previous studies on the optimal dosage of enzyme preparations. The experimental variants were worse to the control by a maximum of 2% in terms of the moisture content of the dough, but were superior to it in terms of the physical properties of the dough.

Table 3 - Dough moisture with the addition of different concentrations of enzyme (n=3, p≤0.05)

Name of enzyme and its concentration	Humidity, %
Control	43±0.53
α-amylase: 1.4 cm^3 of enzyme solution/100 g of flour 0.7 cm^3 of enzyme solution/100 g of flour 0.35 $cm^3$of enzyme solution/100 g of flour	 38±0.25 42±0.35 41±0.26
Protease: 0.5 cm^3 of enzyme solution/100 g of flour 0.05 cm^3 of enzyme solution/100 g of flour	 37±0.48 42±0.52
Glucoamylase: 1 cm^3 of enzyme solution/100 g of flour 3 cm^3 of enzyme solution/100 g of flour	 44±0.65 43±0.55
α-amylase+glucoamylase: 0.35 cm^3 solution of amylase + 3 cm^3 solution of glucoamylase /100 g of flour	42±0.35
α-amylase+protease: 0.35 cm^3 solution of α-amylase + 0.05 cm^3 solution of protease/100 g of flour	41±0.42

Therefore, it was determined that the rational concentration of α-amylase was 0.35 cm^3, protease – 0.05 cm^3, glucoamylase – 3 cm^3, and EP compositions: α-amylase+glucoamylase and α-amylase+protease in a 1:1 ratio of their optimal concentrations.

Determination of the influence of the complex action of lactic acid bacteria and enzyme preparations on the quality of bread

With aim to determine the influence of the complex action of lactic acid bacteria and enzyme preparations on the technological process, after proofing and fermentation of the dough, bread was baked.

The results of research on bread quality indicators are given in Table 4. The appearance of finished bread is shown in Fig. 1.

The use of sourdough based on lactic acid bacteria has a number of advantages, namely, it improves the taste of bread, its volume and shelf life, especially when baking rye bread. Our research was focused on the interaction between the lactic acid bacteria of *IPROVIT* sourdough and enzymes of different actions. The addition of enzymes had a positive effect on the kinetics of acidification. The acidity of the dough for all samples was 8.9–10 degrees. The highest acidity was in the sample where the EP α-amylase + protease complex was used, which can be explained by the effect of protease, which contributes to the additional growth of this indicator due to the formation of amino acids as a result of the partial destruction of protein substances.

The acidity of the finished bread met the requirements of DSTU 4583:2005 and was 8.9–10 degrees for all experimental samples. The highest acidity was obtained in the control version – 11 degrees. The decrease in acidity in sample D5 is explained by the fact that the amino acids formed as a result of protease action were used by lactic acid bacteria as a source of nutrients. This fact confirms the effectiveness of enzymes as activators of the activity of LAB and improvers of bread quality.

The mass fraction of bread moisture for all variants was practically the same and ranged from 40.5% in sample D5 to 43.2% in sample D3. The addition of LAB and EP did not have any effect on the change of this indicator.

The complex application of LAB and EP positively contributed to the intensification of fermentation processes, which is evidenced by higher indicators of lifting power, especially in the variants of separate use of D1-D3 enzymes. They were 5–9 min higher than controls. EP complexes (variants D4-D5) also contributed to obtaining a higher value of this indicator, but the difference was only 2–3 min.

It is known that the porosity of rye-wheat bread should be at least 55–56%, and the higher it is, the longer they retain freshness. The results of the researches showed that the porosity of bread samples D4, D5 and control is at the level of 65%; for other variants it is slightly lower: D1, D2 – 55% and D3 – 59%. Test sample D4 without emptiness and seals.

The condition of the crumb in the D1 and D2 variants was better to the control in terms of quality, as wet spots were noted in certain places. Other variants D3-D5 were at the control level.

The use of both EP compositions (variant D4 and D5) improved the crumb structure: it was uniformly fine and more developed, compared to the control and other experimental variants (Table 4 and Fig. 1). These options also provided better results in terms of taste and aroma of the finished bread.

As can be seen in the photo, the specific volume of samples D1-D3 is lower compared to the control. The crumb of the bread has non uniformity porosity and seals in some places.

The results of the study of organoleptic indicators of the quality of experimental bread samples are presented in Table 5.

The complex application of LAB and EP improved the organoleptic and physico-chemical indicators of the quality of bread: the color and structure of the porosity of the bread crumb, as well as its taste and aroma.

The taste of bread is complex in nature, especially when sourdough is used, and the process of excessive acidity must be carefully controlled, which can give the finished product a sour or spicy taste, and create the conditions to enhance the taste of the toasted bread crust. The taste or smell defects any did not find during the researches. Thus, all bread samples met the requirements of DSTU 4583:2005.

The results showed that the addition of a mixture of enzymes in sample D4 improves the organoleptic indicators of product quality. For sample D5, the fermentation process accelerated. This affected the quality and appearance of the crust (Fig. 1, photo 3) due to the effect of protease on the protein, which catalyzes the biological reaction. The taste and aroma for samples D4-D5 is more pronounced. The crumb of the products is elastic, with a uniform fine developed structure of porosity.

Summarizing, we can conclude that the best effect on bread quality indicators is the complex use of LAB and EP compositions: α-amylase + glucoamylase (D4) and α-amylase + protease (D5), which ensure good dough forming ability and porosity structure of finished bread.

Table 4 – Quality indicators of bread with the addition of lactic acid bacteria and solutions of enzyme (n=3, p≤0.05)

Quality indicators	control	α-amylase (D1)	Protease (D2)	Glucomylase (D3)	α-amylase + glucoamylase (D4)	α-amylase + protease (D5)
Dough acidity, degrees	9.0±0.117	9.5±0.053	9.4±0.053	8.9±0.060	9.0±0.036	10±0.046
Lifting force, min	45±1.530	52±1.058	50±1.015	54±1.150	47±0.500	48±0.780
Bread acidity, degrees	11±0.280	10±0.166	10.5±0.288	9.9±0.133	9.3±0.089	9.5±0.100
Mass fraction of bread moisture, %	42.3±0.440	41.7±0.570	41.5±0.330	43.2±0.440	41.3±0.570	40.5±0.330
Porosity, %	65.0±0.880	55.0±0.670	55.0±0.880	59.0±1.150	65.0±1.21	65.0±0.880
The state of crumb	Elastic	Elastic, but wet places are visible		Elastic	Elastic	
Porosity structure	Uniform, fine	Uniform, fine moderately developed		Uniform, fine	Uniform fine, more developed	
Taste and aroma	Characteristic of bread from mixture of rye and wheat flour				More pronounced, with a pleasant taste and aroma of bread	

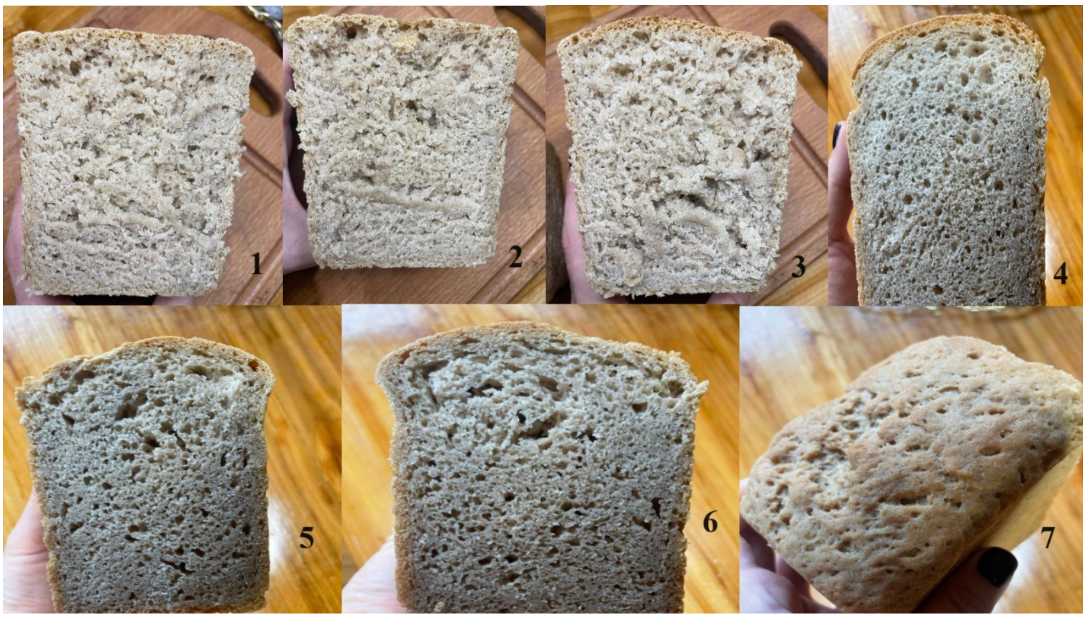

Fig. 1. Samples of bread with different enzymes:
1 – D1 (α-amylase), 2 – D2 (protease); 3 – D3 (glucoamylase), 4 – control, 5 – D4 (α-amylase + glucoamylase), 6, 7 – D5 (α-amylase + protease)

Table 5 – Organoleptic indicators of the bread quality made with the addition of enzymes

Indexes	Indicators				
	according to DSTU 7044:2009 [30]	control	D1	D3	D5
Form	Corresponds to the baking form, with a slightly convex upper crust without side protrusions	The form of the bread is correct, rectangular, not vague, without protrusions, corresponds to the type of product			
Surface	Corresponds to the type of product, without pollution, small cracks and undermining are allowed. Slight wrinkling is permitted for packaged products; for cut products with traces of cuts	Smooth, without large cracks and undermining			
Color	From light brown to dark brown, without burning	The crust is golden yellow, the top is light brown, without burning	The crust is golden yellow, the top is light brown, without burning	The crust is yellow, the top is light brown, without burning	The crust is yellow, the top is brown, without burning
The state of the crumb	Baked, without traces of non-knead	Baked, not sticky, not wet on the touch, without non-knead, after pressing the form is restore			
Taste and smell	Characteristic of this type of product, without foreign taste	The smell of wheat bread, without foreign taste and smell	The smell of wheat bread, with an intense nutmeg smell	The smell of wheat bread, with a pleasant and harmonious nutmeg, nutty smell	The smell of wheat bread, with a moderately pronounced taste and smell

Conclusion

The effectiveness of activating the vital activity of IPROVIT sourdough lactobacilli by enzyme preparations α-amylase, protease, glucoamylase and their compositions (α-amylase + glucoamylase and α-amylase + protease) at the production of rye-wheat bread was investigated.

On the basis of the obtained results, the expediency to addition into the dough from rye-wheat flour various EP in doses of cm^3/100 g of flour has been established: α-amylase – 0.35; protease – 0.05; glucoamylase – 3; α-amylase + glucoamylase and α-amylase + protease (in a 1:1 ratio of their optimal concentrations).

The complex utilization of IPROVIT sourdough and enzyme preparations with various effects had a positive effect on the physical properties of the dough: it mixed well, did not stick to the hands, kept its form, faster increase the volume and had moisture indicators at the level of control – 41–43%.

The organoleptic characteristics of bread are improved due to the use of EP with different action: it acquires an attractive color, pronounced taste and a pleasant specific aroma with tones of nutmeg. The porosity of experimental bread samples with a mixture of enzymes was at the control level. Adding to the recipe a complex of enzymes (α-amylase + protease) and IPROVIT sourdough accelerated and facilitated the processing of the dough, improved its elastic properties and prevented decrease of volume during baking.

Enrichment of the enzyme profile of fermented dough due to the accumulation of hydrolysis products will contribute to increasing the nutritional and biological value of the final product. The implementation of technology of rye-wheat bread on the base of sourdough with the addition of various enzymes will make it possible to expand the range of bread assortment, improve its quality and make it at enterprises of various capacities.

Further research will be aimed at measuring and analyzing of physico-chemical quality indicators, safety of these bread samples, as well as improving its recipes.

References

1. Bintsis T. Lactic acid bacteria: their applications in foods. J. Bacteriol. Mycol. 2008;6(2):89-94 https://doi.org/10.15406/jbmoa.2018.06.00182
2. Wang Y, Wu J, Lv M, Shao Z, Hungwe M, Wang J, et al. Metabolism characteristics of lactic acid bacteria and the expanding applications in food industry. Front Bioeng Biotechnol. 2021;9:612285 https://doi.org/10.3389/fbioe.2021.612285
3. Ayivi RD, Gyawali R, Krastanov A, Aljaloud SO, Worku M, Tahergorabi R, et al. Lactic acid bacteria: Food safety and human health applications. Dairy. 2020; 1(3):202-232 https://doi.org/10.3390/dairy1030015
4. Muir DD. Comparison ofthe sensory profiles of kefir, buttermilk and yogurt. Int. J. Dairy Technol. 1999;52:129-134 https://doi.org/10.15406/jbmoa.2018.06.00182

5. Gupta RK, Jeevaratnam K, Fatima A. Lactic Acid Bacteria: Probiotic Characteristic, Selection Criteria, and its Role in Human Health (A Review). International Journal of Emerging Technologies and Innovative Research (www.jetir.org). 2018;5(10):411-424
6. Ringø E, Van Doan H, Lee SH, Soltani M, Hoseinifar SH, Harikrishnan R, et al. Probiotics, lactic acid bacteria and bacilli: interesting supplementation for aquaculture. Journal of applied microbiology. 2020;129(1):116-136 https://doi.org/10.1111/jam.14628
7. Abedin MM, Chourasia R, Phukon LC., Sarkar P, Ray RC, Singh, SP, et al. Lactic acid bacteria in the functional food industry: biotechnological properties and potential applications. Crit Rev Food Sci Nutr. Published online July 5, 2023. https://doi.org/10.1080/10408398.2023.2227896
8. Hu Y, Zhang J, Wang S, Liu Y, Li L, Gao M. Lactic acid bacteria synergistic fermentation affects the flavor and texture of bread. J Food Sci. 2022;87(4):1823-1836 https://doi.org/10.1111/1750-3841.16082
9. Hajinia F, Sadeghi A, Sadeghi Mahoonak A. The use of antifungal oat-sourdough lactic acid bacteria to improve safety and technological functionalities of the supplemented wheat bread. Journal of Food Safety. 2020; 41(1): e12873 https://doi.org/10.1111/jfs.12873
10. Corsetti A, Gobbetti M, De Marco B, Balestrieri F, Paoletti F,. Russi L, et al. Combined effect of sourdough lactic acid bacteria and additives on bread firmness and staling. J Agric Food Chem. 2000;48(7):3044-3051 https://doi.org/10.1021/jf990853e
11. Katina K, Heiniö RL, Autio K, Poutanen K. Optimization of sourdough process for improved sensory profile and texture of wheat bread. LWT - Food Science and Technology. 2006;39(10):1189-1202 https://doi.org/10.1016/j.lwt.2005.08.001
12. Siepmann FB, Ripari V, Waszczynskyj N, Spier MR. Overview of Sourdough Technology: from Production to Marketing. Food Bioprocess Technol. 2018 ;11: 242-270 https://doi.org/10.1007/s11947-017-1968-2
13. Sun Lei, Xiangfei Li, Yingyue Zhang, Wenjian Yang, Gaoxing Ma, Ning Ma, et al. A novel lactic acid bacterium for improving the quality and shelf life of whole wheat bread. Food Control. 2020;109:106914. https://doi.org/10.1016/j.foodcont.2019.106914
14. Alkay Z, Kılmanoğlu H, Durak MZ. Prevention of Sourdough Bread Mould Spoliage by antifungal Lactic Acid Bacteria Fermentation . Avrupa Bilim ve Teknoloji Dergisi. 2020;(18):379-388. https://doi.org/10.31590/ejosat.646043
15. Cizeikiene D, Juodeikiene G, Paskevicius A, Bartkiene E. Antimicrobial activity of lactic acid bacteria against pathogenic and spoilage microorganism isolated from food and their control in wheat bread. Food Control. 2013;31(2):539-545 https://doi.org/10.1016/j.foodcont.2012.12.004
16. Juhui Jin, Thi Thanh, Hanh Nguyen, Sanjida Humayun, SungHoon Park, Hyewon Oh, et al. Characteristics of sourdough bread fermented with Pediococcus pentosaceus and Saccharomyces cerevisiae and its bio-preservative effect against Aspergillus flavus. Food Chemistry. 2021;345:128787 https://doi.org/10.1016/j.foodchem.2020.128787
17. Sen Ma, Zhen Wang, Xingfeng Guo, Fengcheng Wang, Jihong Huang, Binghua Sun, et al. Sourdough improves the quality of whole-wheat flour products: Mechanisms and challenges-A review. Food Chemistry. 2021;360:130038 https://doi.org/10.1016/j.foodchem.2021.130038
18. Antognoni F, Mandrioli R, Potente G, Saa DLT, Gianotti A. Changes in carotenoids, phenolic acids and antioxidant capacity in bread wheat doughs fermented with different lactic acid bacteria strains. Food Chemistry. 2019;292:211-216 https://doi.org/10.1016/j.foodchem.2019.04.061
19. Suo B, Chen X, Wang Y. Recent research advances of lactic acid bacteria in sourdough: Origin, diversity, and function. Current Opinion in Food Science. 2021;37:66-75 https://doi.org/10.1016/j.cofs.2020.09.007
20. Hu Y, Zhang J, Wang S, Liu Y, Li L, Gao M. Lactic acid bacteria synergistic fermentation affects the flavor and texture of bread. Journal of Food Science. 2022;87(4):1823-1836 https://doi.org/10.1111/1750-3841.16082
21. Siepmann FB, de Almeida BS, Waszczynskyj N, Spier MR. Influence of temperature and of starter culture on biochemical characteristics and the aromatic compounds evolution on type II sourdough and wheat bread. Lwt. 2019;108:199-206 https://doi.org/10.1016/j.lwt.2019.03.065
22. Kezer G. Functional Perspective on Sourdough Bread. Turkish Journal of Agriculture-Food Science and Technology. 2022; 10(8):1410-1414 https://doi.org/10.24925/turjaf.v10i8.1410-1414.4860
23. Dong Y, Karboune S. A review of bread qualities and current strategies for bread bioprotection: Flavor, sensory, rheological, and textural attributes. Comprehensive Reviews in Food Science and Food Safety. 2021;20(2):1937-1981 https://doi.org/10.1111/1541-4337.12717
24. Tebben L, Chen G, Tilley M, Li Y. Individual effects of enzymes and vital wheat gluten on whole wheat dough and bread properties. Journal of Food Science. 2020;85(12):4201-4208 https://doi.org/10.1111/1750-3841.15517
25. Dahiya S, Bajaj BK, Kumar A, Tiwari SK, Singh B. A review on biotechnological potential of multifarious enzymes in bread making. Process Biochemistry. 2020;99:290-306 https://doi.org/10.1016/j.procbio.2020.09.002
26. Matsushita K, Terayama A, Goshima D, Santiago DM, Myoda T, Yamauchi H. (2019). Optimization of enzymes addition to improve whole wheat bread making quality by response surface methodology and optimization technique. Journal of food science and technology, 56, 1454-1461 https://doi.org/10.1007/s13197-019-03629-5
27. Wang X, Pei D, Teng Y, Liang J. Effects of enzymes to improve sensory quality of frozen dough bread and analysis on its mechanism. Journal of food science and technology. 2018;55:389-398. https://doi.org/10.1007/s13197-017-2950-8
28. Azizi S, Azizi MH, Moogouei R, Rajaei P. The effect of Quinoa flour and enzymes on the quality of gluten-free bread. Food science & nutrition. 2020;8(5):2373-2382 https://doi.org/10.1002/fsn3.1527
29. Drobot VI. Laboratory workshop on the technology of bakery and pasta production. Kyiv: Education Center. Literature; 2006. (in Ukrainian).
30. Drobot VI. Handbook on the technology of bakery production. Directory: education. manual / 2nd ed., revision. and added Kyiv, 2019. (in Ukrainian).
31. Hrushkovska AO, Danylenko SH, Kryzhska TA, Khonkiv MO. The influence of lactic acid bacteria on indicators of rye sourdough.. Scientific notes of Taurida National V.I. Vernadsky University". Series: Technical Sciences. 2019; 30 (69):92-97. (in Ukrainian). https://doi.org/10.32838/2663-5941/2019.4-2/15
32. Tiwari SP, Srivastava R, Singh CS, Shukla K, Singh RK, Singh P, et al. Amylases: an overview with special reference to alpha amylase. J Global Biosci. 2015; 4(1): 1886-1901.
33. Chen Y, Eder S, Schubert S, Gorgerat S, Boschet E, Baltensperger L, et al. Influence of amylase addition on bread quality and bread staling. ACS Food Science & Technology. 2021;1(6):1143-1150. https://doi.org/10.1021/acsfoodscitech.1c00158
34. Soccol CR, Rojan PJ, Patel AK, Woiciechowski AL, Vandenberghe LP, Pandey A. Glucoamylase. In: Pandey, A., Webb, C., Soccol, C.R., Larroche, C. (eds) Enzyme Technology. Springer, New York, NY; 2006. https://doi.org/10.1007/978-0-387-35141-4_11
35. Han X, Wen H, Luo Y, Yang J, Xiao W, Ji X, et al. Effects of α-amylase and glucoamylase on the characterization and function of maize porous starches. Food Hydrocolloids. 2021;116:106661. https://doi.org/10.1016/j.foodhyd.2021.106661
36. Pourmohammadi K, Abedi E. Hydrolytic enzymes and their directly and indirectly effects on gluten and dough properties: An extensive review. Food Sci Nutr. 2021; 9: 3988-4006. https://doi.org/10.1002/fsn3.2344
37. Caballero PA, Gómez M, Rosell CM. Bread quality and dough rheology of enzyme-supplemented wheat flour. Eur Food Res Technol. 2007;224:525-534. https://doi.org/10.1007/s00217-006-0311-3
38. Wrigley C, Bushuk W. Chapter 7 - Rye: Grain-Quality Characteristics and Management of Quality Requirements. In Woodhead Publishing Series in Food Science, Technology and Nutrition, Cereal Grains (Second Edition), Woodhead Publishing; 2017:153-178. https://doi.org/10.1016/B978-0-08-100719-8.00007-3

ЗАСТОСУВАННЯ БАКТЕРІАЛЬНОЇ ЗАКВАСКИ ТА ЕНЗИМІВ ПРИ ВИРОБНИЦТВІ ЖИТНЬО-ПШЕНИЧНОГО ХЛІБА

А. Корженівська[1], аспірант, *e-mail:* alin4yk557@ukr.net
С. Даниленко[1], доктор технічних наук, старший науковий співробітник, *e-mail:* svet1973@gmail.com
С. Гунько[2], кандидат технічних наук, доцент кафедри, *e-mail:* cgunko@gmail.com
Г. Козловська[3], кандидат ветеринарних наук, доцент, *e-mail:* kozlovska@nubip.edu.ua
А. Лук'янець[1], молодший науковий співробітник, *e-mail:* onisenkoalla627@gmail.com
[1]Відділ біотехнології Інститут продовольчих ресурсів Національної академії аграрних наук України, вул. Євгена Сверстюка, 4а, м. Київ, Україна, 02002,
[2]Кафедра технології зберігання, переробки та стандартизації продукції рослинництва ім. проф. Б.В. Лесика Національного університету біоресурсів і природокористування України вул. Героїв Оборони, 13, м. Київ, Україна, 03041
[3]Кафедра епізоотології, мікробіології та вірусології Національного університету біоресурсів і природокористування України вул. Виставкова, 16, м. Київ, Україна, 03041

Анотація. Інтерес до житньо-пшеничного хліба на заквасках з додавання ензимів активно виявляють різні виробники. Для його виготовлення використовуються ензими, пов'язані з метаболічною активністю мікроорганізмів закваски, а також екзогенні ензими, які навмисно додають у рецептуру тіста. Таке комбінування сприяє покращенню, як структури тіста так і готового продукту. У роботі досліджували вплив комплексного застосування закваски ІПРОВІТ та ензимів α-амілази, глюкоамілази та протеази на структурно-механічні властивості тіста та якість готового житньо-пшеничного хліба. Було встановлено оптимальні концентрації окремих ензимів та їхніх комбінацій: α-амілаза – 0,35 см^3, протеаза – 0,05 см^3, глюкоамілаза – 3 см^3, α-амілаза+глюкоамілаза і α-амілаза+протеаза у співвідношенні 1:1. Додавання ензимів у раціональній концентрації позитивно впливало на фізичні властивості тіста: воно було еластичним, гарно формувалось у кульку і не розпливалося. Збільшення концентрації ензимів приводило до того, що тісто прилипало до рук та з часом розтікалось. Виявлено, що комплексне застосування лактобактерій *Lactobacillus plantarum*, *L. brevis, Lactobacillus paracasei ssp. paracasei* та ензимних препаратів (α-амілаза, протеаза, глюкоамілаза) різної дії, позитивно впливало на фізичні властивості тіста: воно добре замішувалося, не прилипало до рук, утримувало форму, швидше підходило та мало показники вологості на рівні контролю – 41–43%. Кислотність готового хліба відповідала вимогам ДСТУ 4583:2005 і становила для всіх дослідних зразків – 8,9–10 град. Зменшення кислотності у зразку з комплексом ензимів α-амілаза+протеаза пояснюється тим фактом, що амінокислоти, які утворилися в результаті дії протеази були використанні молочно-кислими бактеріями, як джерело поживних речовин. Комплексне застосування молочнокислих бактерій та ензимів позитивно сприяло інтенсифікації процесів бродіння, про що свідчать більші показники підіймальної сили, які були на 3–9 хв вищі, ніж у контролі. Доведено, що за рахунок застосування ензимів різної дії покращуються органолептичні характеристики хліба: він набуває привабливого кольору, вираженого смаку, та приємного специфічного аромату із тонами мускатного горіха. Пористість дослідних зразків хліба з сумішшю ензимів була на рівні контролю. Таким чином, використання житньо-пшеничного борошна, закваски та ензимів позитивно впливає на якість хліба та сприяє підвищенню його харчової і біологічної цінності.

Ключові слова: молочнокислі бактерії, ензими, фізичні властивості тіста, якість хліба.