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STUDYING THE POSSIBILITY OF USING ENZYMES, LECITHIN, AND ALBUMEN IN THE TECHNOLOGY OF GLUTEN-FREE BREAD

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Науково обґрунтований комплексний підхід для технології хліба для хворих на целиакію шляхом застосування амілолітичних ферментів, лецитину та сухого яєчного білка.

Встановлено вплив амілолітичних ферментів на накопичення цукрів під час гідролізу крохмалю рисового борошна. Досліджено, що використання ензимів сприяє утворенню моно- та дисахаридів в кількості 5,5–6 %, які необхідні для інтенсифікації перебігу мікробіологічних процесів в тісті. Виявлено покращення газоутворення в тісті із застосуванням ферментативної модифікації крохмалю борошна при бродінні.

З метою підвищення показників якості тістового напівфабрикату та готових виробів обґрунтовано доцільність використання поверхнево-активних речовин. Визначено закономірності впливу соняшникового знежиреного лецитину на властивості тіста та якісні характеристики хліба з ферментами. Встановлено, що внесення фосфолітиду в тісто із застосуванням ферментативної модифікації крохмалю борошна сприяє покращенню газоутворення в ньому та зумовлює зростання питомого об'єму і пористості готових виробів.

Для забезпечення пористої структури м'якушки запропоновано додаткове використання в технології рисового хліба сухого яєчного білка. Встановлено, що попереднє відновлення білка при приготуванні тіста з ферментами та лецитином сприяє його кращому розрихленню, що забезпечує найвищі показники якості готових виробів.

Доведено, що застосування соняшникового знежиреного лецитину та збитого яєчного білка при замішуванні тіста з попередньо проведеним гідролізом крохмалю борошна α -амілазою і глюкоамілазою зумовлює суттєве підвищення його газоутримувальної здатності та сприяє збільшенню розпливання. Зважаючи на зниження в'язкості тіста з додаванням обраних сировинних компонентів, рекомендованим є виготовлення формового хліба.

Досліджено вплив застосування ферментативної модифікації крохмалю рисового борошна, лецитину та яєчного білку на процес клейстеризації водно-борошняних суспензій. Виявлені закономірності дозволяють прогнозувати збільшення терміну зберігання готового хліба

Ключові слова: безглютеновий хліб, рисове борошно, α -амілаза, глюкоамілаза, лецитин, яєчний білок

1. Introduction

Among the various environmental factors that influence the development of the organism, nutrition, the quality of which influences the human vital activity, is of the primary importance. One of the effective measures for correction of the food ration is the consumption of the products for special purposes, which are recommended for the prevention of a whole range of diseases of the population with alimentary disorders (iron-deficient and iodine-deficient anemia, osteoporosis, etc.). These also include the dietary products, taking into consideration the requirements of nutrition science to the diet of people with the diseases, which are associated with the intolerance to certain food components (celiac disease, phenylketonuria, diabetes, and allergies).

Celiac disease (gluten enteropathy) is a progressive autoimmune illness, 0.9–1.2 % of the population in the regions of Europe, North and South America, North Africa and the

Indian subcontinent suffer from [1, 2]. The sick have intolerance to some protein of crops, namely wheat gliadin and glutenin, rye secaline and barley hordein [3], which are combined by the general name “gluten”. In patients with celiac disease, consumption of gluten leads to the atrophy of villi and the damage to the mucous coat of the small intestine, accompanied by malabsorption of important nutrients. It can be assumed that the actual frequency of gluten-dependent diseases is much greater since allergy to crops and intolerance of gluten without celiac disease is becoming increasingly common. The only therapeutic measure for treatment of people suffering from gluten-dependent disease is keeping to a gluten-free diet throughout the whole life [4, 5].

Among the products produced from grain raw material, the manufacture of gluten-free bakery products is most challenging. This is due to the fact that the main role in shaping their quality is played by wheat enzymes, which during dough kneading produce gluten, which forms the frame

of the product. The need of the population of Ukraine for grain products that are necessary for people who suffer from celiac disease is met mainly due to the products of foreign origin. This enables the enterprises of the baking industry, including institutions of restaurant business, to introduce in the production the bakery products for special purposes. Given the fact that bread is the product of everyday use, the research aimed at the improvement and development of the technologies of gluten-free bakery products and new ways to ensure their high quality can be considered relevant.

2. Literature review and problem statement

The existing scientifically grounded technologies of gluten-free bread are based on the use of flours of cereal crops (corn, buckwheat, and rice) [6–8]. This raw material does not contain gluten proteins and is represented in the world market in a sufficient range. Organoleptic analysis of these types of flour showed that the most promising for the production of gluten-free bread is rice flour as it has neutral taste properties, white color and is hypoallergenic [9, 10]. However, this flour has low baking properties and does not ensure the formation of dough with the necessary structural and mechanical characteristics, which make it possible to obtain high quality bread with fluffed enough crumb.

An analysis of existing research indicates that to improve the quality of bread from rice flour, scientists [11, 12] proposed the use of hydrocolloids (xanthan and guar gum, carboxymethylcellulose, hydroxymethylpropylcellulose). This is associated with the fact that due to the high water-absorbing ability, food hydrocolloids affect the consistency of gluten-free dough, increasing its gas-retaining ability, improve the ability of dough pieces to retain the shape in the process of maturing and baking. However, it should be noted that these additives can make an improving effect on the structural and mechanical properties of gluten-free dough if it contains a sufficient amount of sugars that are the nutrition for yeast during fermentation. Rice flour is characterized by a small content of mono- and disaccharides (0.7 %), as well as the low activity of the own α - and β -amylases. That is why to solve this problem, researchers [13] substantiated the expediency of adding sugar, which results into a natural increase in the total volume of the released carbon dioxide.

The promising direction in the technology of production of bakery products for people with celiac disease is the application of the methods that make it possible to modify the properties of the main chemical components of gluten-free flour using enzymes [14]. In studies [7, 15], it was proposed to use transglutaminase as a structure-forming agent in the rice dough, the addition of which leads to cross-linking of proteins, which makes it possible to create the network that is similar to gluten. Despite the significance of the obtained results, to ensure the dough fermentation process by sugars, paper [16] also substantiated the expediency of sucrose introduction. However, as it is well known, its molecules during contact with water get covered by hydrate membranes, which increases the intermolecular volume and leads to the dough dilution at high concentrations of disaccharide.

The research in the area of the improvement of bread quality with the use of enzymes of amyolytic action that hydrolyze starch flour is currently actively developing. Enzymatic effect on starch contributes to an increase in the amount of sugars in the dough, which leads to the intensification of its

fermentation process, improvement of gas production during maturing at the early stages of baking. Rice flour is a promising raw material for the modification of its carbohydrate composition using these preparations, because it is characterized by high content of this polysaccharide (75...80 %). The choice of the enzyme is determined by the desirable carbohydrate composition of the finished product. Thus, α -amylase randomly hydrolyzes α -1.4-glycoside bonds in a molecule of amylose, resulting into formation of maltose and the products of incomplete hydrolysis of starch – dextrines. Unlike α -amylase, which can split only non-branched chains of starch molecules, glucoamylase is also capable to catalyze hydrolytic breaking α -1.6-glycoside bonds of the branched chains of starch amylopectin. It also converts dextrines, which are formed under the influence of α -amylase, into glucose [14, 17]. Obviously, the use of amyolytic enzymes will have positive effects not only on the intensification of microbiological processes in fermentation of dough, but also on prolonging the freshness-retaining term of the finished bread. This is due to the action of α -amylase, which leads to the accumulation of low molecular dextrines, which cannot be achieved by adding crystalline sugar. Thus, there are some grounds to believe that to increase the amount of sugars in rice dough, needed to ensure the fermentation process, it is appropriate to apply the enzymes with amyolytic activity.

Analysis of the literature data indicates the relevance of additional application of structure-forming agents in the technology of bread from rice flour [11–13, 15]. In the case of making gluten-free dough, the use of amyolytic enzymes without introducing an additive of the structure-forming action will not lead to a significant improvement of the quality of products, which is due to the low gas-retaining ability of such dough. One of the ways to overcome this problem may be the use of lecithins, the main technological features of which in food systems include emulsification, complexing with starch, interaction with proteins, changing viscosity and the modification of crystals [18]. In addition, lecithins are a valuable source of phospholipids.

Industrial production of lecithin is associated mainly with the transformation of soy phosphatide concentrate. Given the fact that practically all soy is genetically modified and may cause allergic reactions, it is relevant to search for the alternative surface-active substances (SAS) of natural origin, which have the status of completely harmless. Sunflower lecithin, which, unlike products of soybean processing, is environmentally friendly, does not contain genetically modified organisms, phytoestrogens and substances that cause allergic reactions, is becoming increasingly common in the modern food industry. Given the range of the values of hydrophilic-lipophilic balance (HLB) of emulsifiers, which are recommended to improve the bread quality [19], it is appropriate to use defatted lecithin with the HLB index within 7...8. The choice of this particular SAS is explained by a high content of phospholipids (96...97 %), the highest HLB index, compared with standard lecithin and phosphatide concentrate, as well as a powdery consistency, which greatly facilitates the process of dosage. In addition, the use of defatted lecithin will not have an inhibiting impact on the activity of microorganisms in dough due to lack of lysoforms of phospholipids, which are contained in the hydrolyzed lecithin.

One of the ways to create the necessary bread structures is the use of a mechanical way of leavening the dough. To improve the quality indicators of rice bread, dry egg albumen is the promising raw material. The use of natural

egg products considerably complicates the organization of production and dramatically enhances the microbiological hazard. At the same time, the application of dry egg products makes it possible to eliminate a number of operations and significantly simplify the process of preparing raw material for the production [20]. Egg products contribute to foam formation, dough leavening and the formation of porous fixed structure of finished bread, as well as enrich products with fully-fledged protein.

There are no data on the influence of amylolytic enzymes, sunflower defatted lecithin and dry egg protein on the progress of microbiological processes in the rice flour dough, its structural-mechanical properties and the quality of finished products. There are no technological recommendations related to the quantitative dosage of these ingredients, which causes the need for research in this direction.

3. The aim and objectives of the study

The aim of this study was to determine the possibility of using α -amylase and glucoamylase for accumulation of sugars to ensure the fermentation of rice dough, to substantiate the appropriateness of using defatted lecithin and egg albumen for bread preparation.

To accomplish the set aim, the following tasks were set:

- to determine the impact of the amylolytic enzymes on accumulation of sugars during hydrolysis of rice flour starch and to substantiate the quantitative composition of the selected enzymes to improve the intensity of rice dough fermentation;

- to explore the influence of sunflower defatted lecithin and dry egg albumen on the technological properties of dough semi-finished products and the quality of finished products, to establish the optimal dosage of these raw materials;

- to study structural-mechanical properties of rice dough with the use of the enzymatic modification of the starch of flour with SAS and egg albumen at the change of the specific volume and dough running during fermentation and to explore the impact of the selected improvers and raw materials on its carbohydrate-amylase complex.

4. Materials and methods to study the influence of enzymes, lecithin, and albumen on the quality of rice bread

4.1. The studied materials and equipment used in the experiment

Gluten-free bread was made according to the following recipe:

- rice flour – 100 kg;
- pressed baking yeast – 3 kg;
- table salt – 1.2 kg.

Weight fraction of moisture in the dough was 53 %. Dough semi-finished product and bread prepared with the use of this composition were used as control samples.

To perform hydrolysis of rice flour starch, we used the enzymes of amylolytic action, specifically, α -amylase of mycelium origin (activity is 5,000 SKB/g, optimal pH is 4.7, temperature is 37–40 °C) and glucoamylase (activity is 500 AMG/g, optimal pH is 3.0–5.5, temperature is 40–64 °C).

The temperature optimum for vital activity of yeast during the dough fermentation is 28–32 °C, which is not

effective for the action of these enzymes, because this value is not included in the range of temperatures, in which amylases show their activity. Based on this, to ensure the optimal condition for simultaneous action of α -amylase and glucoamylase, we set the temperature of the medium of 40 °C and pH of 4.7. Originally, semi-finished product–hydrolysate from rice flour was prepared (that is, the enzymatic modification of flour starch was performed) at 40 °C, which ensured a more flexible hydrolysis of polysaccharide and makes it possible to enrich the medium with sugars. In order to maintain the appropriate pH conditions, we used citric acid in the quantity of 0.065 % of the flour weight. The enzymes were previously dissolved in water at temperature of 25–30 °C in the ratio of 1:10. To perform the enzymatic modification, the mixture of rice flour, citric acid, enzymes and water was prepared and subjected to hydrolysis in the thermostat at 40 °C until the accumulation of 5.5–6 % of sugars in it. The duration of hydrolysis and humidity of this semi-finished product was found experientially at the rate of formation of reducing sugars.

Based on the preliminarily prepared semi-finished product from rice flour, dough with humidity of 53 % with the addition of yeast suspension, salt solution and the other part of the flour was kneaded according to the recipe. Bread with the application of enzymatic modification of flour starch was prepared by the technology described in article [21].

We used defatted sunflower lecithin with the content of phospholipids of 96.5 %, which is a brown powder with a barely noticeable smell and taste of sunflower oil. Emulsifier was introduced after the enzymatic fermentation of rice flour starch, the mixture was dispersed for 2–3 min, then the dough was kneaded. For surface active substances to display their properties better, refined sunflower oil was used simultaneously with lecithin in the amount of 3 % of the flour weight.

Dry egg albumen, pasteurized with the content of protein substances of not less than 85 % was used as a structure-forming agent. It is not appropriate to introduce this raw material in the dry form because in the process of kneading dough in contact with water protein swells to form a viscous colloidal solution, which surrounds the yeast cells, which degrades their nutrition. Therefore, in order to improve the structural-mechanical parameters of the rice bread quality, given high foam-forming properties of egg albumen, it was used in the whipped form. For this purpose, dry albumen was reduced by water at the ratio of 1:7 at 30 °C and whipped for 5–6 minutes until the formation of stable foam used for kneading dough.

4.2. Methods to study the quality of rice bread with enzymes, lecithin, and albumen

In the course of the study, the influence of enzymes of amylolytic action on the content of mono- and disaccharides in model dough systems from rice flour was explored. The total content of sugars was determined by the Shorle method (iodometric), which is based on determining the amount of oxide copper before and after reducing the copper solution with sugar. This method is presented in more detail in [21].

The intensity of alcohol fermentation in the dough was determined by the volumetric method on the example of device AG-1 by its gas-producing capacity, that is, of the volume of released CO₂, cm³/100 g, at 30 °C during dough fermentation.

Total (titrated) acidity of dough was determined at the beginning and at the end of the fermentation process by titration

with 0.1 mol/dm³ NaOH solution of 5 g of the dough semi-finished product, ground with 50 cm³ of water in the presence of 1 % alcohol solution of phenolphthalein to pink coloration.

Active acidity was determined using laboratory pH-meter-ionometer “Expert-001” by the immersion of electrodes (chlorine-silver and glass) into the prepared sample of the dough semi-finished product [22].

Viscous-plastic properties of the dough were assessed by the change in the diameter of the dough ball (running) of the weight of 100 g at the temperature of 30 °C during its fermentation.

Gas-retaining ability of the dough was assessed according to the change of the specific volume of the sample in dimensional cylinder of 250 cm³ in the thermostat at the temperature of 30 °C from the beginning of fermentation until dough dropping and expressed in cm³/g.

The parameters of the process of rice flour starch gelatinization were determined by the amylograph made by “Brahbender” company (Sweden). The maximum viscosity of the water and flour suspension was determined by the height of the curve (amylogram) of viscosity of flour-water suspension, recorded by the automatic recorder. The temperature of the beginning of gelatinization (increase in its viscosity) was also recorded [22].

The bread quality was estimated by physical and chemical (specific volume, porosity, Ph) and organoleptic indicators (appearance, state of the crust surface, porosity structure, taste, and smell). Bread was analyzed not earlier than after 3 hours after baking.

Methods to study quality of the finished bakery products are described in more detail in paper [21].

The results of the experimental studies were statistically processed using the standard software package Microsoft Office.

5. Results of research into the impact of enzymes, lecithin, and albumen on the quality of rice bread

5.1. Research into the influence of amyolytic enzymes on accumulation of sugars in dough from rice flour

The degree of improvement of baked products quality depends on the ratio and the amount of added amyolytic enzymes. There are some recommendations as for the dosage of mycelium α -amylase and glucoamylase as improvers for wheat bread [17]. However, it is possible to assume that this dosage will not be effective in the case of the production of semi-finished products from rice flour. This is related to the fact that the rate of splitting amyolytic enzymes by enzymes of the starch of various crop grains is different due to the different dimensions and the shape of starch grains, as well as their structural features. The optimum amount of enzymes was determined by accumulation of mono- and disaccharides in the model dough system from rice flour without the addition of yeast, the humidity of which amounted to 53 %. Duration of hydrolysis was 3 hours at the temperature of medium of 40 °C. In this case, α -amylase was introduced in the quantity of 0.002 %, 0.005 % 0.007 % of the flour weight, glucoamylase was added in the amount of 0.03 %, 0.04 % and 0.05 % of the flour weight. Different dosage of these enzymes is explained by their different amyolytic activity. The results of determining the influence of amylases on the accumulation of sugars in the yeast-free rice dough are shown in Fig. 1.

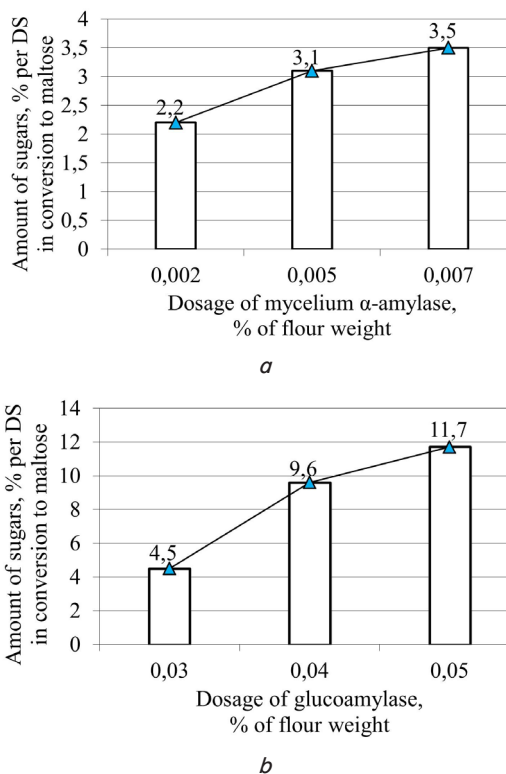


Fig. 1. Influence of enzymes on the accumulation of sugars in rice dough: *a* – α -amylase; *b* – glucoamylase

The results of the research (Fig. 1) revealed that the addition of α -amylase of mycelium origin in the amount of 0.005 % and 0.007 % of the weight of rice flour leads to the accumulation of sugars, respectively, 3.1 % and 3.5 %, which is by 29–37 % more than when using this enzyme in the amount of 0.002 % (Fig. 1, *a*). In this case, an increase in the dosage of α -amylase by more than 0.005 % does not lead to a significant increase in the amount of the resulting products of starch hydrolysis. Analysis of the influence of glucoamylase on the accumulation of sugars in the dough (Fig. 1, *b*) revealed that the fraction of the formed saccharides, when it was added to the amount of 0.04 % and 0.05 % of weight of flour, is 9.6 % and 11.7 % per DS, respectively, while the introduction of this enzyme in the amount of 0.03 % of the flour weight leads to the formation of 4.5 % per DS of sugars. An increase in the content of mono- and disaccharides in the dough semi-finished product of more than 6 % is technologically not desirable, because in this case, the vital activity of yeast cells is inhibited as a result of increasing osmotic pressure in liquid phase of the dough.

To determine the optimal dosage of enzymes, we took into consideration the fact that the whole cycle of bread preparation requires 5.5–6 % of sugars of the weight of dry substance of flour. We should note that the use of only α -amylase in order to enrich the dough semi-finished product with sugars is not advisable because of the considerable duration of the hydrolysis process. Thus, for the accumulation of saccharides to the above amount, the duration of fermentation will be over 3 hours. Therefore, based on the obtained results, it is technologically relevant to use simultaneously α -amylase in the amount of 0.005 % and glucoamylase in the amount of 0.03 % of the weight of flour, which will ensure their synergic effect. As a result, the enzymatic modification of rice flour starch will be more effective, and the amount of

accumulated products of its hydrolysis, specifically, mono- and disaccharides, will intensify the dough fermentation.

Taking into consideration the recommended temperature and acid intervals of the action of α -amylase and glucoamylase, specified by the manufacturer, the temperature of 40 °C and pH 4.7 are optimal for both enzymes. As the dough fermentation occurs at 28–32 °C, the application of enzymes at the stage of dough kneading will not be sufficiently effective. Therefore, for the purpose of accumulating the required amount of sugars within the entire cycle of bread preparation, it is advisable to prepare semi-finished product-hydrolysate from rice flour at 40 °C with subsequent kneading dough based on it.

There is a direct relationship between humidity of the environment and the effectiveness of the action of amylolytic enzymes on the substrate. In this regard, the dynamics of fermentative reaction at the accumulation of sugars over time during the preparation of the semi-finished product-hydrolysate from rice flour with its different humidity at the temperature of 40 °C (Fig. 2). The weight fraction of moisture was established based on the use of 100 %, 75 %, 50 % and 25 % of rice flour according to the recipe and was 53 %, 56 %, 65 % and 78 %, respectively. In order to create the optimum pH conditions (4.7) for both enzymes, citric acid in the quantity of 0.065 % of the weight of flour was used.

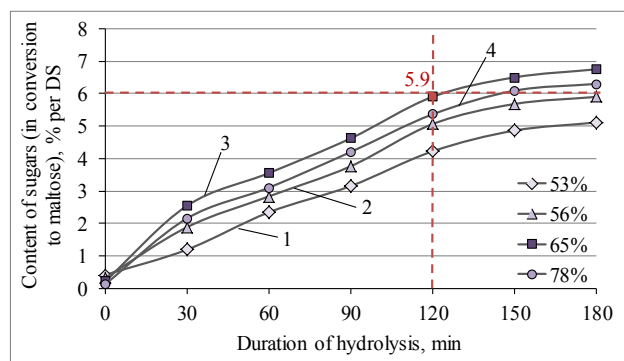


Fig. 2. Accumulation of sugars in semi-finished product-hydrolysate, depending on its humidity when using rice flour: 1 – 100 %; 2 – 75 %; 3 – 50 %; 4 – 25 % of its total weight

It was established that the use of 50 % of formulation amount of flour for making the semi-finished product-hydrolysate of humidity of 65 % (Fig. 2, curve 3) contributed to the best accumulation of sugars than when using 75 % and 100 % of flour (Fig. 2, curves 2 and 1). This is due to the fact that at an increase in weight fraction of moisture, the rate of the reaction catalyzed by enzymes increases. However, we should note the fact that a subsequent humidity increase up to 78 % is not advisable, because in this case, the amount of the accumulated products of starch hydrolysis decreases (Fig. 2, curve 4), which is obviously due to the violation of the ratio of enzyme to substrate.

As we know, the improvement of the fermentation activity of yeast and gas production in dough is observed at the addition of sugars up to 6 % [14]. The existence of their larger amount increases the osmotic pressure in the liquid phase of the dough and causes the plasmolysis of yeast cells (compression of the body of a living cell with the membrane exfoliation), which causes slowing down of alcohol fermentation, a decrease in gas production and as a result, and deterioration of dough leavening. Based on this, according to the data in Fig. 2, it is possible to determine that the optimal concentra-

tion of mono- and disaccharides for vital activity of yeast and improvement of gas-producing ability of rice flour is 5.9 % for DS. To accumulate this amount of sugars in the semi-finished product, the duration of hydrolysis is 2 hours (120 min) at humidity of 65 % under conditions of using 50 % of flour of the general formulation amount for its preparation.

5. 2. Determining the influence of sunflower defatted lecithin on the indicators of the technological process and the quality of rice bread with the application of enzymatic hydrolysis of flour starch

Sugars, which were formed as a result of starch depolymerization during the application of amylolytic enzymes, are a source of nutrition for yeasts-saccharomyces, thereby improving the dough fermentation intensity and, accordingly, the quality of the finished products. However, such tendency is expected in the production of bread from gluten-containing wheat flour. During the preparation of rice dough, given the complete lack of gluten, it is necessary to use additionally the raw ingredients that are able to improve its structural-mechanical properties and ensure high quality indicators of finished products. We performed test baking to prove this hypothesis and assess gas production in dough during fermentation and specific volume of the finished bread and its porosity as the key quality indicators. Based on the obtained results, it was found that the application of enzymatic modification of flour starch in the technology of rice bread leads to an increase in specific volume and porosity of the finished products only by 15.4 % and 12.8 % compared with the control sample without additives, while gas production in the dough during fermentation increases by times.

That is why in the course of further research, we determined the influence of sunflower defatted lecithin on the improvement of physical and chemical properties of dough and the quality of rice bread (Table 1). To substantiate the optimum dosage of SAS, test laboratory baking was performed. During the studies, lecithin was introduced in the amount of 0.5 %, 1.0 % and 1.5 % of the flour weight. The sample with the application of enzymatic modification of flour starch was used as the control sample.

Based on the obtained results, it is possible to state the existence of the positive influence of sunflower defatted lecithin on the quality indicators of dough semi-finished product and finished bread with enzymes. Thus, gas production in the rice dough during its maturing increases by 21.2–22.4 % when adding SAS, compared with the control. The use of lecithin from sunflower has a positive effect on the acidity accumulation in the dough. After 45 minutes of fermentation of dough pieces with the introduction of emulsifier, titrated acidity increases by 0.7–0.8 degrees, while that of the control sample of the dough with the use of enzymatic modification of rice flour starch – by 0.6 degrees. An increase in titrated acidity correlates with the change in the active one, which naturally decreases. Obviously, the obtained data are explained by the improvement of accessibility of sugars formed during hydrolysis to a yeast cell due to the action of the phospholipid component of defatted lecithin on the membrane and the cytoplasmic membrane of yeast.

Analyzing the results of the research into the indicators of the quality of finished products, it was established that specific amount of bread increases by 9.5 %, 16.2 % and 17.0 % with the increasing dosage of lecithin. A similar pattern is also observed in determining the influence of SAS on the porosity of crumb, which increases by 6.5 %, 12.7 % and 13.0 %, respectively, compared with the control. Thus, lecithin demon-

strates the best improving action on the quality of rice bread in the dosage of 1 % of the flour weight, while its application in the amount of 1.5 % does not lead to a significant increase in specific volume and leavening the crumb of the products.

Table 1
Influence of sunflower defatted lecithin on dough properties and quality of rice bread

Indicators	Control samples (with enzymes)	Samples with enzymes and with the addition of lecithin, % of the weight of flour		
		0.5	1.0	1.5
Dough				
Gas production for 45 min of fermentation, cm ³ /100 g of dough	330	419	425	425
Titrated acidity, degrees				
– beginning	2.6	2.6	2.6	2.7
– ending	3.2	3.3	3.4	3.4
Active acidity, units of device				
– beginning	4.75	4.74	4.73	4.71
– ending	4.20	4.21	4.05	4.04
Specific volume (after 45 min of fermentation), cm ³ /g	1.32	1.45	1.55	1.57
Bread				
Specific volume, cm ³ /g	1.51	1.67	1.80	1.82
Acidity, degree	2.8	2.8	2.9	2.9
Porosity, %	41.4	44.3	47.4	47.6

As it is well known, the use of defatted SAS promotes the creation and stabilization of emulsions such as “oil in water”, which is caused by the existence of hydrophilic and hydrophobic groups in the composition of their molecule [23]. On this basis, using the test laboratory baking, we found that the introduction of sunflower oil in the amount of 2–3 % of the flour weight during the preparation of rice bread with improvers leads to an increase in specific volume of products, improvement of the structure of crumb porosity and its elasticity. Thus, in the presence of oil, porosity increases by 6.7–8.6 %, specific volume of bread increases by 7.5–9.4 % in comparison with the sample without adding it. That is why, in further research, we used sunflower oil in the amount of 3 % of the flour weight together with the emulsifier.

5. 3. Research into the influence of egg albumen on the indicators of the technological process and quality of rice bread with enzymes and lecithin

Porosity of bread depends on dimensions, uniformity and accumulation of air phase, which is formed by the accumulation of carbon dioxide during the fermentation of dough, as well as the presence of air, which gets during kneading. With the aim of improving the structure of porosity and specific volume of rice bread, we used dry egg albumen. Given the high foam-producing properties of egg albumen, it was previously reduced by water and whipped to the stable foam which was introduced when kneading dough. To determine the influence of protein on the basic quality parameters of rice dough and bread, laboratory test baking was performed (Table 2). The amount of dry egg albumen was 3 %, 4 % and 5 % of the weight of flour. For comparison, we used the sample with the application of enzymatic modification of rice flour starch with the addition of lecithin in the amount of 1 % of the weight of flour and vegetable oil.

Table 2

Influence of egg albumen on the properties of dough and quality of rice bread

Indicators	Sample with enzymes, SAS and oil	Samples with enzymes, SAS, oil and with the addition of egg albumen, % of the weight of flour		
		3.0	4.0	5.0
Dough				
Gas production for 45 min of fermentation, cm ³ /100 g of dough	422	420	419	417
Titrated acidity, degree				
– initial	2.9	3.0	3.1	3.2
– final	3.7	3.8	3.9	3.9
Active acidity, units of device				
– initial	4.65	4.16	4.10	4.05
– final	3.78	3.32	3.27	3.23
Specific volume (after 45 minutes of fermentation), cm ³ /g	1.65	2.05	2.11	2.10
Bread				
Specific volume, cm ³ /g	1.97	2.45	2.55	2.53
Acidity, degree	3.2	3.3	3.3	3.4
Porosity, %	51.5	64.0	66.5	65.0

Analysis of the conducted studies of regularities of changes in technological characteristics of dough semi-finished products indicates that when adding the egg albumen, the initial pH of the dough rises by 0.1–0.3 degrees. In this case, its accumulation in the process of fermentation takes place virtually identically in all the samples.

It should be noted that at an increase in the dosage of egg albumen, the intensity of fermentation in the dough slightly decreases, which is proved by a decrease in the amount of the released carbon dioxide within the period of maturation of dough pieces. Thus, the total gas production in samples with 3 % of protein to the weight of flour decreased by 0.5 %, with 4 % – by 0.7 %, with 5 % – by 1.2 %, which is obviously due to a decrease in the fermentation activity of yeast. This may be due to an increase in the viscosity of the colloidal solution of albumen, due to an increase in its concentration, as well as the deterioration of the access of nutrients to a yeast cell.

These results indicate that the addition of egg protein in whipped form has a more pronounced effect on the quality of finished products than defatted sunflower lecithin with vegetable oil without using it. It was established that the introduction of albumen in the amount of 3 % and 4 % of the weight of flour contributes to an increase in specific volume of bread by 24.4 % and 29.4 %, respectively. Porosity of the crumb at such dosage of this raw material ingredient grows by 24.2 % and 29.1 % compared with the control sample. The resulting dependence is explained by the fact that protein molecules having hydrophobic and hydrophilic groups in their composition, display surface-active properties, which contributes to the intensification of the action of lecithin and leads to the improvement of the quality of finished bread. In addition, the activation of the life activity of microflora at the beginning of baking ensures further leavening of the bread piece and its increase in volume.

If the amount of egg protein increases more than by 4 % of the weight of flour, specific volume of bread and porosity

somewhat decreased. Based on the received data, this dosage is optimal.

In addition to the physical-chemical quality indicators, organoleptic properties are the most important consumer properties of the product. The study of organoleptic parameters of quality indicate that the developed bread with enzymes, lecithin and protein has a smooth surface without tears and cracks, as well as the uniform fine, thin-walled porosity of the crumb. In addition, at the interaction of amino groups of protein and amino acids with carbonyl groups of saccharides, there occurs the Meyer reaction with the formation of melanoides, which give the baked rice bread golden crust, pleasant taste and aroma.

5. 4. Influence of amylolytic enzymes, sunflower defatted lecithin and egg albumen on the viscous-plastic properties of rice dough and its gas-retaining ability

The results of the test baking showed that the bread prepared based of semi-finished product-hydrolysate from rice flour and with additional using of SAS and albumen differs in quality indicators from the sample without additives. That is why it became necessary to determine the influence of this raw material on viscous-plastic characteristics of gluten-free dough, the changes of which can be understood from the indicators of dough balls running (Fig. 3). Enzymes, defatted sunflower lecithin and dry egg albumen were introduced in the optimal amount, which were substantiated in previous studies.

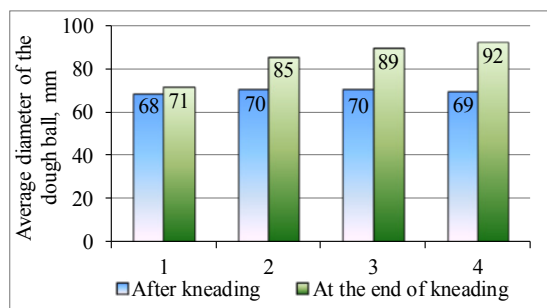


Fig. 3. Dough running: 1 – control (without additives); 2 – with enzymes; 3 – with enzymes and lecithin; 4 – with enzymes, lecithin and egg albumen

Research results indicate that during the period of fermentation of the dough with the use of improvers and proposed raw materials, running of dough pieces increases relatively to the control sample by 16.5–22.8 %. This is due to the low activity of enzymatic processes in rice dough without additives, as a result of which the fermentation process in it takes place with much less intensity.

An increase in running of the dough samples, prepared based of the semi-finished product-hydrolysate from rice flour, is explained by dehydration properties of sugars, formed as a result of hydrolytic decomposition of starch and depolymerization of the flour components during fermentation. High hydrophilicity of mono- and disaccharides causes the existence in the polymer structure of dough of the layers of concentrated solutions of sugars, which decrease the internal friction of the system, that is, its viscosity. This is also facilitated by the ability of vegetable oil, which is introduced with lecithin absorbing on the surface of starch grains and albumen micelles, to prevent swelling these colloids and increase the content of liquid phase of dough, due to which it is significantly diluted. The introduction of egg

albumen in the whipped state into the rice dough also leads to a decrease in its viscosity. This can be explained by the formation of the disperse system of gas-like phase, which is obtained by the mechanical distribution of the air in the reduced solution of egg albumen at whipping, which leads to an increase in its surface. Thanks to this, the system acquires a significant resource of free surface energy and becomes unstable.

High quality indicators of the finished products, in particular, specific volume and porosity of the crumb, largely depend on gas-retaining ability of dough, which is influenced by its structural-mechanical properties and fermentation intensity. The ability of dough samples with the use of enzymatic modification of flour starch and the examined raw material to retain carbon dioxide was estimated using the indirect method by the change in specific volume of dough in the process of fermentation. The results are presented in Fig. 4.

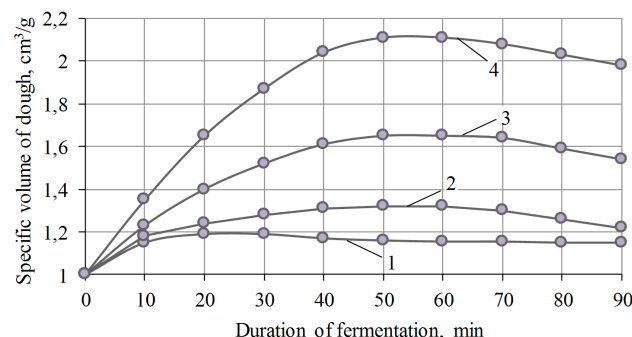


Fig. 4. Change in specific volume of dough in the process of fermentation: 1 – control (without additives); 2 – with enzymes; 3 – with enzymes and lecithin; 4 – with enzymes, lecithin and egg albumen

It was established that the maximum volume of dough semi-finished products with the introduction of lecithin with oil and protein is achieved after 45–50 min of fermentation and is retained over a longer period of time compared with the control sample and the sample with enzymes. The obtained data indicate the improvement of the gas-retaining ability of the dough with SAS and egg albumen.

Analysis of the results of the research into the change of specific volume of dough during maturing proved the regularities obtained as a result of the test baking (Tables 1, 2). Thus, the specific volume of the sample with the application of the enzymatic modification of rice flour starch (Fig. 4, curve 2) for 45 min of fermentation increased by 12 % compared with the control sample without additives (Fig. 4, curve 1). However, this increase does not exercise any significant influence on the volume output and porosity of the finished products. This tendency is expected, since rice dough is characterized by the lack of the gluten frame, which leads to the loss of carbon dioxide during maturing. When adding defatted sunflower lecithin with oil to the dough with the enzymes, its specific volume during fermentation increased more intensively (Fig. 4, curve 3) and increased by 29.7 % compared to the control sample. The dough with the introduction of whipped egg albumen is characterized by the largest value of the specific volume (Fig. 4, curve 4). Its use contributes to an increase in this indicator by 45 % and ensures the highest quality indicators of the finished bread.

5. 5. Determining the influence of amylolytic enzymes, defatted sunflower lecithin and egg albumen on the parameters of gelatinization of rice flour starch

Flour polysaccharides, the main of which is starch, play an important role in ensuring the quality and structure of bakery products. Its conversion, and primarily swelling and gelatinization, determines the course of physical-chemical and biochemical processes in dough and also ensures shaping the structure of crumb at baking.

Because dough is a multi-component system, other components, along with starch, take part in the formation of viscosity of flour-water system. That is why to determine the influence of the enzymatic modification of flour starch and the studied raw materials on the gelatinization process, we carried out amylographic research, the results of which are presented in Fig. 5.

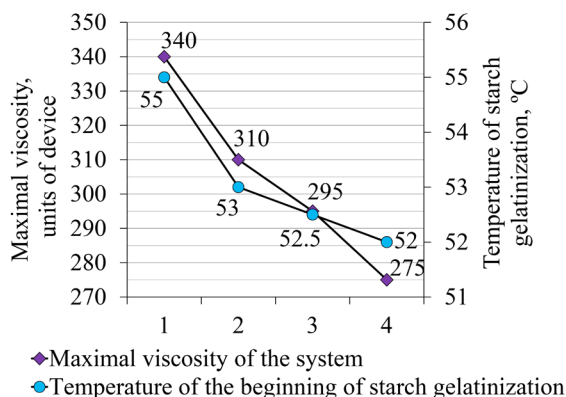


Fig. 5. Indicators of the process of gelatinization of water-flour suspensions: 1 – control (without additives); 2 – with enzymes; 3 – with enzymes and lecithin; 4 – with enzymes, lecithin and egg albumen

These results indicate that the use of the enzymatic modification of rice flour starch, SAS and albumen influence the viscosity of starch gluten. The active action of amylolytic enzymes in the system (Fig. 5, 2) promotes starch hydrolysis and, consequently, reducing its ability to raise viscosity during heating. This leads to a decrease in maximum viscosity of the suspension by 8.8 % compared with the control sample (Fig. 5, 1). The value of this indicator of the flour suspension with the introduction of lecithin (Fig. 5, 3) decreases by 4.8 % compared with the sample with enzymes without SAS (Fig. 5, 2).

A decrease in the maximum viscosity of the system is influenced by oil, which combines with flour proteins in non-polar groups and partially blocks the interaction of hydrophilic compounds with water. This results into an increase in the content of free water in the suspension.

Addition of egg albumen decreases the maximum viscosity of the suspension (Fig. 5, 4) by 11.3 % in comparison with the sample with the application of enzymatic modification of wheat starch without improvers and protein. The resulting pattern can be explained by the emergence of the additional amount of free water that forms as a result of denaturation of egg albumen during heating the aqueous-flour suspension. Thus, this process leads to a change in the spatial structure of a molecule, protein globula untwisting, the number of hydrophobic groups increases on its surface and the number of intra-molecular hydrogen bonds decreases, that is, hydrophilic properties decrease.

Analysis of the data in Fig. 5 shows that when applying the enzymatic modification of rice flour starch with the introduction of lecithin and egg albumen, the initial temperature of gelatinization decreases on average by 4–8 %. It should be noted that the change in temperature of starch gelatinization is an important indicator, which characterizes the process of its retrogradation. It is known that the lower the starch gelatinization temperature, the more slowly flour products get stale [24]. This makes it possible to assume that rice bread with the addition of the studied components will retain freshness somewhat longer.

6. Discussion of results of research into the influence of enzymes, lecithin and albumen on the quality of rice grain

When determining the impact of amylolytic enzymes on the process of hydrolysis of rice flour, as revealed by the obtained results (Fig. 1 a, b), an increase in the amount of the formed sugars with the increasing dosage of enzymes is natural. It should be noted that it is appropriate to use simultaneously α -amylase and glucoamylase for the accumulation of mono- and disaccharides in the amount of 5.5–6 %, which is necessary to intensify the process of dough fermentation. This is largely due to the duration of the process of hydrolysis of rice flour starch in the case of the independent application of α -amylase for more than 3 hours, which is economically inappropriate during implementation of such technology at the institutions of restaurant business and mini-bakeries. Joint application of these enzymes promotes better effectiveness of the process of the enzymatic modification of starch flour, due to which it takes 2 hours to accumulate the required amount of sugar during hydrolysis. In this case, the dosage of α -amylase is 0.005 % and that of glucoamylase is 0.03 % of the weight of flour. Comparison of the amount of enzymes determined in the studies (Fig. 1, 2) with their recommended dosage in case of application in the technology of wheat bread [17], indicates almost twice decreased addition. It is associated with the dimensions of starch grains that for rice are 5–6 μm , while for wheat – 25–35 μm . At a decrease of dispersion of starch grains, the starch attacking increases, since their relative surface of the contact with the enzyme increases, which leads to an increase in the number of products of hydrolysis. This means that the dosage of α -amylase and glucoamylase for conducting the enzymatic modification of flour starch depends on the type and origin of the substrate, that is, flour raw materials.

Research into the influence of amylolytic enzymes on specific volume of rice bread and crumb porosity shows insignificant improvement in these quality indicators. Comparison of the obtained results with the data of determining gas-production in dough indicates a mismatch in the amount of released carbon dioxide during the fermentation and the quality characteristics of the finished bread. It is possible to explain the established dependence by the fact that only a part of carbon dioxide that is formed during maturity leads to leavening of dough pieces, the rest of it is lost and does not have any technological importance. This is caused by the absence in rice dough of the hydrated gelatin network, which usually forms the spatial structure of wheat flour dough. Considering this, the additional application of improvers is becoming necessary in order to improve the structural and mechanical properties of dough semi-finished product with the application of the enzymatic modification of flour starch,

and in particular, its gas-retaining ability. This complies with the scientific data, well known from papers [11–13], the authors of which, by the way, also argue about the feasibility of the joint use of sugar (sucrose) and structure-forming agents, which are able to simulate the gluten properties of dough. However, in contrast to the research results published in [11–13], in addition to sugars that have a positive impact on the intensity of dough fermentation, dextrines are also accumulated. These compounds increase the amount of bound moisture in the bread crumb and provide a pronounced effect on slowing down the process of starch retrogradation, which causes staling.

When adding lecithin with vegetable oil and albumen, during kneading the dough with the use of enzymatic hydrolysis of flour, its gas-retaining ability enhances, which is proved by the studies of changes in specific volume of dough during fermentation (Fig. 4). This is explained by the technological functions of these raw ingredients in food systems. Thus, phospholipids of lecithin due to their diphilic structure are oriented on the boundary separating the two phases by decreasing surface tension, which promotes good formation of drops and protection them from coalescence. The emulsion becomes more stable because coalescence is prevented by the strong energy barrier that is created by the emulsion film on the surface of a water drop. Emulsifier influences molecules of water and fat so that they repel each other, or creates a stable system of protective layers around the drops. As a result of this process, phospholipids form specific barriers on the surface of water droplets preventing their joining, which increases the gas-retaining capacity of the dough. This leads to saving the released CO₂ in the process of alcohol fermentation of dough semi-finished products. In addition, protein molecules having hydrophobic and hydrophilic additives in their composition, can also demonstrate surface active properties.

Thus, the improvement of gas-retaining and gas-producing ability of dough with enzymes, SAS and egg albumen helps to improve the quality indicators of finished products, in particular, specific volume and porosity (Table 2). The important role in ensuring high-quality characteristics of rice bread is played by the addition of egg albumen in the whipped form, which improves leavening of dough semi-finished products and denaturants during baking, partially compensating for the lack of gluten proteins, and to some extent serves as a frame of dough. Strengthening the protein network in dough as a result of albumen denaturation contributes to the fixation of bubbles of CO₂ and the air, formed during fermentation in dough base, resulting in increased specific volume and porosity of finished goods.

A decrease in dough viscosity during using raw ingredients, which was found as a result of the interpretation of findings of research into running of a dough ball during fermentation, shown in Fig. 3, can be regarded as an improving

effect. As a result of lower dough viscosity, air bubbles that are a part of the disperse phase are more expanded during baking. In this case, a semi-finished product settles less during baking and is characterized by higher values of specific volume and porosity, which is proved by quality indicators of finished products (Table 2). It should be noted that it is appropriate to bake pan bread applying these technological measures.

The results of amylographic studies (Fig. 5) are of particular interest. According to them, the use of enzymatic modification of rice flour starch and the introduction of lecithin with oil and egg albumen helps to reduce the initial temperature of gelatinization of starch polysaccharide. The obtained data make it possible to assume that the finished bread will slightly longer retain freshness due to slowing down the process of starch retrogradation. This assumption enables us to establish the direction for further research, which will be focused on determining the quality indicators of the products during storage and identifying the forms of moisture binding in the crumb.

7. Conclusions

1. Results of the study conducted revealed the appropriateness of hydrolysis of rice flour starch, using amylolytic enzymes in the technology of gluten-free bread. It was found that the dosage of α -amylase in the amount of 0.005 % and of glucoamylase in the amount of 0.03 % of the weight of flour contributes to the accumulation of sugars in the amount of 5.5–6 % that are essential for the intensification of the flow of the dough fermentation process. To perform a more complete hydrolysis of flour starch, we grounded the appropriateness of the preparation of the semi-finished product-hydrolysate of 65 % humidity from 50 % of rice flour of its formulation amount with subsequent mixing of dough based on it.

2. It is established that the introduction of lecithin in the amount of 1 % and dry egg albumen in the amount of 4 % of the flour weight to dough with the enzymatic modification of starch flour provides a positive impact on the quality of bread. Given the existence of hydrophobic group in the composition of the lecithin molecule, the appropriateness of introducing SAS into dough together with sunflower oil in the amount of 3 % of the weight of flour was indicated.

3. It was proved that the improvement of the bread quality with the additional use of lecithin with oil and whipped egg albumen is caused by a significant increase of gas-retaining ability of dough and a decrease in its viscosity. The research into the state of carbohydrate-amylase complex of rice dough revealed a decrease in the initial temperature of starch gelatinization, which can be a prerequisite for the extension of the term of freshness retaining by bread with the use of the technological measures and selected raw materials.

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