



THE USE OF SUNFLOWER LECITHIN IN THE TECHNOLOGY ON GLUTEN-FREE BREAD WITH ENZYMATIC MODIFICATION OF FLOUR STARCH

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Abstract: *The necessity of search of new ways to improve the quality of gluten-free bread for people suffering from celiac disease is considered in the article. As one of the directions of this problem solution the use of surface-active substances has been suggested. The efficiency of the use of sunflower skimmed lecithin for production of rice bread with the use of enzymatic modification of flour starch has been proved. Its influence on the intensity of fermentation in the rice dough, the activity of fermentative microflora and the change of specific volume in the process of maturation has been investigated. The positive influence of emulsifier in the technology of rice bread on the indicators as ready-made products, in particular specific volume, porosity and elastic properties of the crumb have been studied out. It was established that the addition of sunflower skimmed lecithin to vegetable oil in the process of production of rice bread using flour starch hydrolysis promotes the extension of its shelf life and freshness.*

Keywords: *celiac disease, rice bread, gluten, surface-active substances, lecithin, starch, enzymatic modification.*

1. Introduction

One of the most complicated tasks of food science is the development of baked goods for special use, which are recommended to be consumed by people suffering from certain diseases. The most emerging problem nowadays is the considerable spread of gastroenterological diseases. These ones include the celiac disease (gluten-sensitive enteropathy), caused by the violation of nutrients absorption in the small intestine and the damage of its mucous membrane due to consumption of gliadin and wheat glutenin, as well as prolamynes of rye barley and triticale [1-3]. Gliadin and glutenin are the main fractions of gluten, which play a role in structure-forming in the bread dough and take part in the formation of desirable volume and texture of dough system [4]. During baking, the protein substances are

denatured; as a result they create a dense framework of the goods and provide them with their high quality. Since going on a gluten-free diet during the whole life is the only method of treatment and prevention of celiac disease, the use of gluten-free cereals flour (corn, rice, buckwheat, amaranth etc) which due to their chemical composition do not contain proteins is important in the production of bread for special use. That is why baked goods “without gluten” have low, if compared with traditional wheat bread, organoleptic and structural-mechanical properties. The point of search for new ways of quality improvement of gluten-free bread is rather acute and relevant for scientists and specialists in the bakery industry. Rice flour is an alternative raw material for production of gluten-free bread, which

contains rather little amount of prolamine (1.5-3.5%) and does not take part in the formation of gluten complex. Only gliadin fractions are toxic, and as rice proteins do not contain them, it is not dangerous for the people suffering from celiac disease. Moreover, rice flour is a source of vegetable protein, complete for amino acid composition, it contains sodium, potassium, phosphorus, magnesium, vitamins B₁, B₂ and PP, high amount of easily digestible carbohydrates, has soft taste, white colour and hypoallergenic properties [5, 6]. Its use in bread baking is limited to form the dough with the necessary structural-mechanical properties and to ensure high quality of the goods [7]. The study of literature shows that in order to achieve desirable bread quality from rice flour, scientists suggested the use of hydrocolloids (xanthan gum and guar gum, carboxymethylcellulose, hydroxymethyl propyl cellulose) and emulsifiers (glycerol and diacetyl vinyl and fatty acid esters (DATEM), sodium stearyl alkylate, distilled monoglycerides) [6, 8, 9].

One of the directions of this problem is the modification of the main chemical components of rice flour with the help of enzymes. The use of transglutaminase as a formulation agent in the rice dough is suggested in the research [10], the addition of which leads to crosslinking of proteins that allows creating a gluten-like network. In the National University of Food Technologies a study was conducted, the results of which substantiated the feasibility of using the amylolytic enzymes, particularly α -amylase and glucoamylase in the rice bread technology [11]. It was established, that the use of rice flour starch fermentative modification in dough preparation promotes more intensive microbiological processes, which is manifested in activation of gas and acid accumulation. It is made up by increase in

the amount of nutrients for fermentative microflora of sugars, which appear as a result of flour starch hydrolysis under the influence of α -amylase and glucoamylase. Though, the use of amylolytic enzymes does not allow getting the bread with high specific volume and porosity, which is related to low gas-holding capacity of this type, resulting in the formation of carbon dioxide during fermentation and does not lead to the loosening of dough preparation. One of the ways of solving out this problem is the use of surface-active substances (SAS), which, when added to the dough come into contact with the starch fraction of flour, forming the complex compound, which play an important role in forming the structure of semi finished products and quality of finished products [12]. However, the use of SAS promotes gas-holding in the dough that, in turn, depends on its structural mechanical properties and the intensity of fermentation. Therefore, in order to improve the quality of gluten-free bread by using of fermentative modification of rice flour starch we suggested the addition of SAS to dough.

As the tendency of eating healthily is growing, the use of lecithins is of perspectives, which do not only play the role of SAS, but also they are a valuable source of phospholipids from the physiological point of view. One of the most important functions of phospholipids is to provide and maintain protein-lipid metabolism in the organism. The main technological functions of lecithins in food systems are emulsification, interaction with proteins, change of viscosity and modification of crystals due to chemical structure of these compounds. Lecithin contains polar hydrophilic (negatively charged phosphoric acid residue and a cationic core group of choline) and nonpolar hydrophobic (higher

fatty acid radicals) groups, that are located in the opposite sides of molecule. Hydrophilic group provides with solubility in water, hydrophobic - in fat. This explains that in heterogeneous systems lecithin molecules are oriented at the boundary between two phases, reducing the surface tension and playing an important role as emulsifiers in foods. Taking into the consideration lecithin's technological functions and its beneficial properties, the use of the SAS is relevant to improve the quality of gluten-free bread [13, 14].

The degree of lecithin improvement in the production of bread is determined by the way of SAS production, on which the value value of the hydrophilic-lipophilic balance (HLB) and the content of phospholipids depend. As known, the emulsifiers with the index of HLB 6...14 have positive effect on the quality of bakery products [13]. The industrial production of lecithin is mainly due to the processing of soy phosphate concentrate, which is a byproduct of oil purification from concomitant substances. Taking into the consideration the fact that practically all soy is genetically modified and can lead to allergic reactions [14], the search for an alternative SAS of natural origin, which has the status of being completely harmless, is relevant.

Sunflower lecithin is widely used in modern food industry, which unlike soy processing products, is environmentally friendly product, does not contain genetically modified organisms, phytoestrogens and substances that cause allergic reactions. Considering the range of HLB emulsifiers values, which are recommended to improve quality of the bread, it is advisable to use low-fat lecithin with an HLB index of 7...8. The choice of this SAS is explained by high content of phospholipids, and as a result, high

emulsifying capacity, higher index of HLB, as compared with standard lecithin and phosphatidic concentrate, powdered consistency, which greatly facilitates the process of food additive dosing, as well as the absence of inhibitory effects on the activity of microorganisms of phospholipid lysosomes, which are contained in hydrolysed lecithin.

The purpose of this article is to research the possibility of using sunflower skimmed lecithin in order to improve the structural mechanical properties of rice dough with fermentative modification of flour starch and the quality of ready-made product.

2. Materials and methods

2.1. Materials

As the main raw material for bread preparation, rice flour was labeled as "crossed spike" with the following characteristics: the mass fraction of moisture was 11.5%, titrated acidity - 2.2 deg., ash content - 0.5%, protein content - 8%, mono and disaccharides - 0.7%, starch - 80.3%.

For the hydrolysis of rye flour starch amylolytic enzymes were used, particularly α -amylase with an amylolytic activity of 5000 SKB/g, which has an optimal pH of 4.7, the temperature of 37...40°C (Mühlenchemie, Germany), and glucoamylase with the activity of 500 AMG/g, an optimal pH - 3.0-5.5 and the temperature of 40-64°C (Germany).

Sunflower fat-free lecithin was used in the research (Scientific and Production Center "Dniprotehnologii", Ukraine), containing 96.5% of phospholipids insoluble in acetone and has the following characteristics: the mass fraction of moisture - 1.2%, the mass fraction of oil - 1.4%, acid number - 8.9 mg KOH/g, pH 1% aqueous solution - 6.7.

In the production of bread, other raw materials were used as well: pressed baking yeast, salt, citric acid, drinking water and sunflower oil.

2.2. Methods

Conducting enzymatic fermentation of rice flour starch

In order to provide with the optimal conditions for simultaneous action of α -amylase and glucoamylase, the ambient temperature of 40°C and pH 4.7 were set. To maintain the appropriate pH conditions, citric acid in the amount of 0.065% by flour weight was used. The enzymes were pre-dissolved in water at a temperature of 25-30°C at a ratio of 1:10. For enzymatic modification, the mixture of rice flour, citric acid, enzymes and water was prepared, which had been hydrolyzed in the thermostat at a temperature of 40°C for 2 hours before the accumulation of sugars 5.5-6%. The mass fraction of the moisture in the mixture was 65% [11].

Dough preparation and bread baking

Pressed baking yeast (3%) was dissolved in water at a temperature of 26-32°C. Aqueous saline solution was prepared at a concentration of 1.2% at 30°C and filtered. On the basis of pre-prepared hydrolyzed semi-finished product with a humidity of 53% the dough was kneaded with the addition of a yeast suspension, a salt solution and a second part of the flour according to the formulation. Then dough preparations were formed and fermented them in forms in the cabinet for 45 minutes at a temperature of 32°C and relative humidity of 85%. It was baked for 25...30 minutes at a temperature of 180°C. The ready-made bread was cooled for 180 minutes at a temperature of 23-27°C.

Lecithin was added in the stage of dough kneading in the amount of 0.5%, 1.0% and 1.5% by flour weight. The control sample was prepared by using enzymatic

modification of flour starch without SAS addition.

Investigation on the change in the specific volume of dough, intensity of its fermentation and activity of fermentation microflora

Gas holding capacity of the dough was evaluated by the change of the specific volume of the samples in measuring cylinder by 250 cm³ in the thermostat at a temperature of 30°C from the beginning of fermentation till the moment of dough dropping and showed in cm³/g.

The intensity of alcoholic fermentation in the dough was determined by volumetric method on AG-1 device on the indicator of its *gas-forming ability*, i.e. the volume of CO₂ allocated, cm³/100g, at a temperature of 30°C during the dough fermentation.

Determination of *yeast lifting power* was carried out by the standard method, which is the determination of raising speed of dough, prepared by the full formulation in the conditions of thermostat and characterized by the time from the moment of putting the dough into the form until it touched the bar, i.e. the raise for the height of 70 mm.

The activity of lactic acid bacteria in the dough was determined by the time needed for the blue color of samples to disappear in the presence of methylene blue as an indicator.

Evaluation of bread quality

The determination was carried out not earlier than 3 hours after baking for all the samples.

Specific volume was determined as the ratio of volume they occupy, to their mass (cm³/g).

Bread porosity was determined by Zhuravlyov device. From a piece of crumb at a distance not less than 1 cm three slots were made by the cylinder of the device, after which they were weighed.

The porosity $\Pi(\%)$, was calculated by the formula 1:

$$\Pi = \frac{V - \frac{m}{\rho}}{V}, \quad (1)$$

V – the general volume of the slots, cm^3 ;

m – mass of the slots, g;

ρ – density of a porous crumb, g/cm^3 .

The density of a porous crumb of gluten-free bread was determined in the following way. The slot was made by Zhuravlyov device, carefully squeezed it with the wooden piston in the cylinder of the device in order to remove the pores. Then, the slot in the form of pressed cylinder was weighed and the height was measured.

The density of a porous crumb, g/cm^3 , was calculated by the formula 2:

$$\rho = \frac{m}{\pi \cdot r^2 \cdot h}, \quad (2)$$

m – mass of the slot, g;

r – radius of the slot, cm;

h – height of the slot, cm.

The determination of *bread crusting* was carried out using the following methods: measuring the degree of deformation of the bread crumb on the automated penetrometer of the company AR-4/1 and determining the fragmentation of the bread by the amount of crumbs resulting from the shaking in the vibration mixer.

3. Results and discussion

The dosing of skimmed lecithins in dough was done accordingly to the recommendations of literary sources and manufacturers, namely 0.1...0.5% to the mass of flour. However, such amount of SAS is intended to improve the quality of bread from wheat flour. Taking into consideration the complete lack of gluten,

in order to improve structural mechanical properties of rice dough and quality of ready-made products, an increased dosing of lecithin 0.5%, 1.0%, 1.5% to mass of flour was chosen. Dry and skimmed lecithin was added in the form of colloidal solution (suspension). Due to the results of research the influence of lecithin on the dough properties and the quality of rice bread were determined (*Table 1*).

The data obtained (*Table 1*) makes it possible to state that the positive effect of lecithin studied is to increase the specific volume of bread, to improve the structure of the crumb; its porosity becomes more even and thin-walled. It is known that the volume increase of bread as a result of adding SAS is explained only by the increase of gas-holding capacity of the dough in the stage of fermentation. However, lecithins of natural origin have also functional properties, namely their phospholipid content improves permeability of the membranes of yeast cells, which promotes more effective access to food - sugars, amino acids, and vitamins. Thus, the formation of gas in the dough during the period of its maturation increases to 21.2...22.4% when added sunflower skimmed lecithin as compared with the control.

The use of lecithin positively influences the accumulation of acids in dough. After 45 minutes of maturation of dough preparations by adding SAS, titrated acidity increases by 0.7...0.8 deg., while a control sample of dough made from enzymatic modification of rice flour starch by 6 deg. The change of active acidity correlates with the increase of titrated one, which naturally decreases.

Table 1

The influence of sunflower skimmed lecithin on dough properties and quality of bread with enzymatic modification of flour starch

Indicators	Control sample (with enzymes, without SAS)	Samples with lecithin, % to mass of the flour		
		0.5	1.0	1.5
<i>Dough</i>				
Gas formation for 45 mins off fermentation, cm ³ /100 g of the dough	330	419	425	425
Titrated acidity, deg.				
- the initial one	2.6	2.6	2.6	2.7
- the final one	3.2	3.3	3.4	3.4
Active acidity, un. of the device				
- the initial one	4.75	4.74	4.73	4.71
- the final one	4.20	4.21	4.05	4.04
Specific volume (at the end of fermentation), cm ³ /g	1.32	1.45	1.55	1.57
<i>Bread</i>				
Specific volume, cm ³ /g	1.51	1.67	1.80	1.82
Acidity, degr.	2.8	2.8	2.9	2.9
Porosity, %	41.4	44.3	47.4	47.6

Lecithin in dosing of 1.0% to mass of flour causes the best improving action on the quality indicators of ready-made bread. In this case specific volume of the products is 16.2% as compared to the control sample. A similar pattern is observed in the study of the effect of SAS on porosity of rice bread, which increases by 12.7% as compared to the control.

The addition of lecithin in the amount of 1.5% to mass of flour does not lead to significant increase in quality of ready-made bread, which, obviously, is related to the increase of viscosity of dough due to the formation of protein-phospholipid complexes, which are decisive in the formation of its rheological properties [15]. It is known, that the use of skimmed lecithins contributes to the creation and stabilization of emulsions such as "oil in water", which is conditioned by the presence in their hydrophilic and hydrophobic groups of their molecules. Considering this, it was expedient to study

the effect of sunflower skimmed lecithin on the bread quality in the presence of vegetable oil. In this regard, the determination of the effect of lecithin dose chosen on dough quality and bread if sunflower oil addition is compatible was performed. SAS and vegetable oil were added after enzymatic modification of rice flour starch; the mixture was dispersed for 2-3 minutes, and the dough was kneaded after. With the help of trial laboratory baking the dosage of sunflower oil in a quantity of 3% to the mass of the flour was predetermined.

Further research on the effect of lecithin while adding it to change the specific volume of dough, using enzymatic modification of flour starch in the process of maturation, the properties of pressed baking yeast and lactic acid bacteria, quality indicators of ready-made products and on the process of getting stale is required.

The results of the change of specific volume of dough in the process of

fermentation are shown in the figure 1.

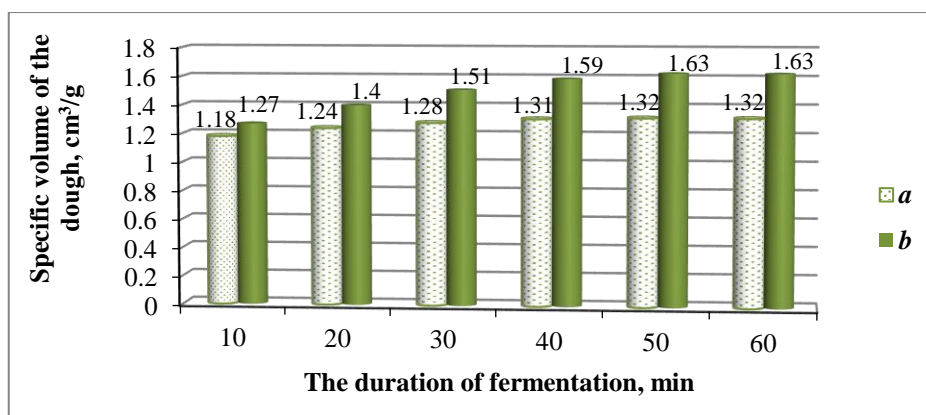


Fig. 1. The change of specific volume of the dough in the process of fermentation:
a - control (with enzymes); *b* - with enzymes and lecithin

The increase in specific volume of dough with enzymatic modification of flour starch by adding the lecithin is more intensive as compared to the control sample without SAS; this is explained by the technological functions of the improver in food systems. Thus, phospholipids of lecithin, due to their differentiated structure, are oriented to the boundary between two phases, reducing the surface tension, which contributes to the good formation of drops and protects them from coalescence. Emulsion becomes more stable, as coalescence is hampered by a rather strong energy barrier, which is created by emulsion pellicle on the surface of a drop of water. The emulsifier effects on water and fat molecules so that they repel from each other, or creates stable, often very viscous or even solid system of protective layers around the droplets. As a result of this process, phospholipids create specific barriers on the surface of the water droplets, due to which gas-holding ability of the dough is increasing, which leads to preservation of CO₂ oozed in the process of alcoholic fermentation of dough semi-finished products.

The data in the Figure 1 also show that the maximum amount of dough with lecithin addition in the presence of vegetable oil is reached in 45...50 mins of fermentation and is held for longer period in comparison to the control sample, which indicates the improvement of gas-holding capacity of the dough with SAS.

The effect of SAS on the fermentation activity of yeast was characterized by its lifting power (Figure 2) and it depends on the initial biological activity of saccharomycetes and adaptats to the anaerobic conditions of life in the dough system, as well as on its recipe components.

The analysis of obtained data has shown that the addition of lecithin in dough using enzymatic modification rice flour starch has a positive effect on yeast fermentation activity. This fact, evidently, is explained by the improvement of availability of sugars formed during the hydrolysis process, to the yeast cell due to the action of phospholipid component of SAS on its shell and the cytoplasmic membrane.

It also substantiates the more intense release of carbon dioxide in the test with

the addition of lecithin (Table 1). Thus, in the presence of SAS yeast lifting power increases by 15.6%, in comparison to the dough sample with enzymes without lecithin addition.

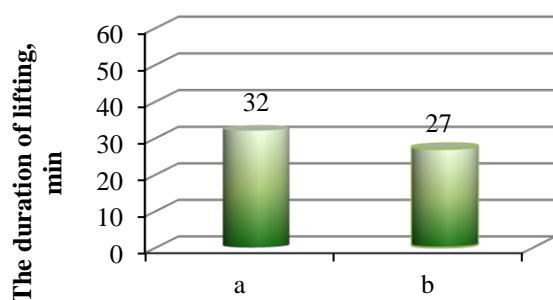


Fig. 2. The change of yeast lifting power
a - control (with enzymes), *b* - with enzymes and lecithin

While maturation of yeast dough, in addition to alcohol fermentation, there is also lactic acid one, which is due to the activity of homo- and heterofermentative lactic acid bacteria which convert sugars mainly into lactic acid, as well as to a small amount of volatile acids. For a clearer picture of the effect of SAS on the dough microflora, the activity of lactic acid bacteria in the samples was investigated in terms of the intensity of their discoloration in the presence of methylene blue immediately after mixing and in 45 minutes of fermentation (Figure 3).

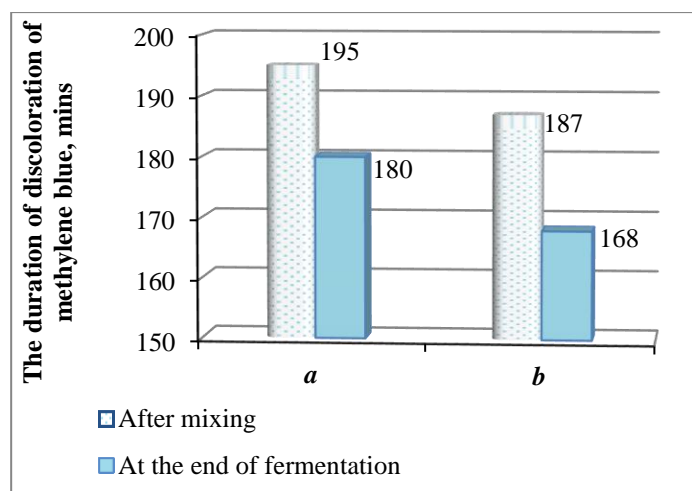


Fig. 3. The activity of lactic acid bacteria in the rice dough:
a - control (with enzymes), *b* - with enzymes and lecithin

As a result of the analysis of the time difference for discoloration of methylene blue by lactic acid bacteria for the investigated samples, it has been established that the vital activity of these microorganisms has a similar pattern with a change in the yeast lifting power.

It was determined that the duration of discoloration of the indicator for dough sample with the use of enzymatic modification rice flour starch with the addition of lecithin is lower by 6.7% after

the fermentation in comparison to the control, indicating an increase in the activity of lactic acid bacteria with the use of enzymes and SAS. The obtained research results are explained by the fact, that the presence of lecithin in dough and the hydrolysis of rice flour starch before kneading the dough promotes the activation of lactic acid bacteria due to the accumulation of simple sugars that are easily digested by microorganisms, resulting in more intensive allocation of

life products - organic acids, carbon dioxide, which provide with orientation and completeness of colloidal, physical and biochemical processes in dough semi finished products. So, the addition of SAS and vegetable oil in the rice dough with the use of enzymatic modification of flour starch promotes the activation of fermentation microflora, that leads to the intensification of processes of alcohol and lactic acid fermentation, as well as gas

retention capacity, which is expected to improve the porosity structure, and accordingly, the volume of ready-made bread. In the course of the further studies on the effect of lecithin added in the amount of 1.0% to the mass of flour in presence of vegetable oil on the indicators of bread quality using enzymatic modification of starch and the process process of getting stale was determined (Table 2).

Table 2

The effect of lecithin and vegetable oil on the rice bread quality with enzymatic modification of flour starch

Indicators	Control sample (with enzymes, without SAS)	Sample with enzymes, lecithin (1.0%) and vegetable oil (3%)
Specific volume, cm ³ /g	1.51	1.92
Acidity, degr.	2.8	3.2
Porosity, %	41.4	51.5
Fragility, %		
in 3 hrs	1.30	1.02
in 24 hrs	2.60	2.01
Deformation of bread crumb, un. of penetrometer		
in 3 hrs	59	72
in 24 hrs	46	61

It was established that the use of lecithin, when adding it in sunflower oil, leads to an increase in specific volume of the bread, improvement of crumb structure and porosity and its elasticity as compared with the control. Thus, porosity increases by 19.7%, specific volume of the bread - by 21.4%, the general deformation of the crumb increases by 18.1%. It can be explained by the capacity of oil to create thin fat pellicles between starch granules that make the dough more plastic, as well as increases its capacity to be stretched without rupture under the pressure of carbon dioxide, formed during fermentation. The obtained data correlate with the results of the research on the change of specific volume of dough in the process of fermentation indicating an

improvement in its gas-holding capacity (Figure 1).

It is known that during bread storage there occur changes in the structure of its protein-carbohydrate matrix due to the retrogradation of amylose and starch syneresis and the interaction of protein molecules with amylose and amylopectin with the formation of new bonds, as well as the redistribution of moisture between polymers, which leads to consolidation of the structure of the crumb and the change of its rheological properties. Considering this, it was determined the effect of raw material on the quality indicators of bread, that characterize its suitability for storage, namely fragility and elastic properties of the crumb.

It was found that the addition of lecithin and sunflower oil affects the process of bread staling, contributing to the preservation of its freshness. It can be seen from the Table 2 that the use of this raw material leads to less intensive increase of fragility and the reduction of general deformation of crumb of the rice bread during storage. It can be explained by the formation of complexes of starch polysaccharides with lipids, which contribute to the inhibition of the process of retrogradation of starch. Evidently, the

4. Conclusion

As a result of the theoretical and experimental studies, the feasibility of using SAS in the technology of rice bread with the use of enzymatic modification of starch flour has been substantiated. As an emulsifier, sunflower oil has been chosen as skimmed lecithin, which besides the technological function of SAS in the food systems, due to the high content of phospholipids has an important physiological significance. It was determined that the dosage of lecithin in the amount of 1.0% to the mass of flour contributes to the improvement of gas accumulation in the rice dough with the premade enzymatic hydrolysis has a positive effect on the porosity and specific volume of the ready-made bread.

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lipids create thin pellicles that wrap the particles of the polyesterized starch grains, which hinder the release of water in the process of bread storage. Besides, lecithin can create complexes with starch amylose; as a result the process of crystallization is slowing down.

Thus, the most positive effect on the quality of bread with the use of enzymatic modification of rice flour starch is observed when using sunflower skimmed lecithin when added to vegetable oil.

Considering the presence of a hydrophobic group in the molecule of lecithin, it is substantiated that the addition of SAS to the dough is compatible with sunflower oil in the amount of 3% by mass of flour. The use of phospholipid in the technology of gluten-free bread with the use of enzymatic modification of rice flour starch contributes to the improvement of vitality of dough fermentable microflora, increasing its gas-retaining capacity; as a result ready-made products are characterized by improved quality indicators. In addition, the use of lecithin and vegetable oil in the production of rice bread allows prolonging shelf-life and freshness.

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