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## Technological aspects of rice gluten-free bread production

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### ABSTRACT

The article presents data on the study of the influence of hydrocolloids and protein additives on the technological aspects of gluten-free rice bread production. The method of full-factor experiment PFE 2<sup>3</sup> determined the optimal conditions for bread production – the amount of yeast 1.5% by flour weight, dough moisture 60%, duration of fermentation, and proofing 70 minutes. The prescribed amount of yeast, salt, agar, and gelatin was dissolved in water at 35 °C and mixed with the specified amount of rice flour. The dough was kneaded for 15 minutes. The dough was placed in the mould and left to ferment for 40 minutes and stand for 30 minutes at the temperature of 30 °C. After fermentation, the dough was divided into pieces weighing 50 grams, placed in baking tins, and baked for 35 – 40 minutes at the temperature of 180 °C. Since adding polysaccharides and protein improvers to the recipe of gluten-free dough to regulate its technological properties can significantly affect the intensity of fermentation and the activity of amylolytic enzymes of flour, studied the dynamics of carbon dioxide release gluten-free rice dough. It was found that additives of protein nature increase the amount of carbon dioxide accumulation in gluten-free dough by 33 – 44%. It is experimentally substantiated that the recommended duration of fermentation of rice flour dough with the addition of gelatin is 45 – 50 min, with the addition of agar 25 – 30 min, and the mixture of gelatin and agar 35 – 45 min. It is established that to achieve full readiness of bread based on rice flour, it is possible after 35 minutes of baking at 200 °C. When extending the duration of heat treatment, the quality of bread does not change, so long-term heat treatment is not economically feasible.

**Keywords:** gluten-free rice bread, gelatin, agar, hydrocolloids, gas-forming ability, a specific bread volume, porosity, fermentation rate, baking, shrinkage.

### INTRODUCTION

Dough-like masses are considered polydisperse systems consisting of solid, liquid, and gaseous phases. The solid phase is formed by starch, cellulose, and hemicellulose proteins. The liquid phase is a multi-component aqueous solution of organic and mineral substances of flour and recipe ingredients due to fermentation and capture of air bubbles [1]. During fermentation, basic biochemical and microbiological processes take place. Lactic acid bacteria produce lactic acid, which acidifies the environment, creating favourable conditions for developing yeast and suppressing other microorganisms whose products are toxic to yeast. Yeast enriches the environment with nitrogenous substances and vitamins necessary for the growth of bacteria. The main processes in the maturation of the dough are alcohol and lactic acid fermentation. As a result of these processes, the dough is loosened with carbon dioxide; saturation of the liquid phase with carbon dioxide with the formation of carbonic acid; increasing the acidity of the dough due to the formation of lactic, acetic, and other acids; lowering the pH of the dough; accumulation of flavouring and aromatic substances [2], [3], [4], [5], [6], [7]. Baking yeast ferments sugars in the following sequence: glucose, fructose, sucrose, and maltose. Yeast fermentation enzymes directly ferment only glucose, others after hydrolysis into monosaccharides [8].

In the process of life, yeast absorbs nitrogenous substances, mineral salts, and vitamins in the dough's liquid phase. Within 1 – 1.5 hours after kneading the dough, the yeast ferments its flour sugars feeds on other water-soluble dough substances. In the future, their activity depends on the accumulation of maltose, soluble nitrogen-containing compounds that are products of enzymatic hydrolysis of starch and dough proteins.

Thus, two processes coincide: the process of maltose formation and the process of fermentation of the dough microflora. The method of maltose formation should precede its fermentation [3], [4], [5]. The ripe dough must

contain at least 3% of fermentable sugars. This is the amount of sugars required for fermentation processes during the aging of the dough and for the reaction of melanoidin formation, which causes the colour of the crust [7].

During the fermentation of the dough, pentosans are depolymerised under the action of flour enzymes, and pentoses are formed, which participate in the melanoidin formation reaction. Dough proteins continue to swell during fermentation. Swollen proteins are more accessible to proteolysis. The redox potential is shifted towards enhancing the reduction processes in the yeast dough. As a result, the proteinase of the dough is activated, the oxidised part of the proteolysis activators (glutathione, cysteine) is restored, and the disaggregation of the protein molecule in the dough is deepened. The content of high molecular weight fractions decreases, and the content of lower molecular weight gliadin, albumin, and globulins increases.

The products of protein hydrolysis pass into the dough's liquid phase, nourish the dough's microflora, and form aromatic and colour compounds during the baking process [7], [8], [9].

As a result of proteolysis, the elasticity of the dough decreases, its elasticity improves, and specific structural and mechanical properties are formed. Biochemical processes in the dough intensify with increasing machining, increasing the dough's temperature. Their activity is influenced by the pH of the medium, the presence of activators and inhibitors of proteolysis, and the dough formulation.

In addition, during dough fermentation, the osmotic binding processes of water proteins, their swelling, and their increase in volume continue. Part of the proteins swells indefinitely, are peptised, and go into solution. This increases the content in the dough of the liquid phase and the dough thins.

Colloidal processes in the dough intensify with increasing structural and mechanical processing during kneading, acidity during fermentation, and fermentation temperature.

Thus, the analysis of the processes that occur during the dough's formation shows that the conversion of proteins and carbohydrates and their interaction with water fundamentally shape the quality of finished products. Therefore, when using proteins and hydrocolloids in gluten-free bread technology, it is necessary to consider changes in physicochemical, hydrocolloid, biochemical and microbiological processes, correct formulations, and justify technological modes of production.

The most common and widely used raw ingredients for gluten-free bread are rice flour and rice starch, corn flour and corn starch, potato, cassava, and wheat starch [10], [11], [12], [13]. Gluten-free cereal flour (sorghum, millet, oatmeal) is offered as an alternative raw material [14], [15]; gluten-free pseudo-grain flour (buckwheat, amaranth, quinoa); flour from roots and tubers (cassava, sweet potatoes); bean flour (soy, chickpeas, carob, beans, lentils, peas); other flour (flax, chestnut, banana, teffi, etc.) [16], [17], [18]. [19], [20], as well as flour mixtures.

The use of gluten-free flour raw materials to improve gluten-free bread's structural and mechanical properties [21] found that introducing flax, sorghum, sunflower flour, and quinoa flour reduces the irreversible relative deformation of dough by 36 – 68% and increase its elasticity. This reduces the relative plasticity - and increases the relative elasticity. In the presence of these additives, the amount of accumulated carbon dioxide in gluten-free dough increases by 10 – 30%.

Hydrocolloids are widely used as structuring agents to simulate the viscoelastic properties of gluten. These ingredients are usually used as a substitute for gluten due to their ability to thicken high water-binding and gelling properties. They can control the properties of the aqueous phase and stabilise the structure of emulsions, foams, suspensions, and multiphase systems [22]. Hydrocolloids increase the volume of the dough, stabilising its foam structure by increasing viscosity, flocculation, and coalescence. Hydrocolloids also prevent the effect of the aqueous phase on the foam structure, improving the stability of the liquid in the films surrounding the gas bubbles. Hydrocolloids can significantly affect the behaviour of the test, even if they are present in tiny quantities [23], [24].

Proteins are crucial in determining the structure of many foods, including gluten-free bread [25]. Due to their specific functional properties, proteins of animal origin are widely studied and proposed for use in food systems.

Scientists of Kharkiv National Technical University of Agriculture [26] found that using Na CMC at the concentration of 0.5% in the gluten-free unleavened dough is appropriate. The volume of bread increases compared to the control by 15%. The combined use of Na CMC and baking soda is inappropriate, as it leads to excessive loosening of the crumb structure and weakening its skeleton.

The positive effect of animal protein concentrates on bread's structural and mechanical properties is proved. For the use of concentrates of animal proteins (CoAP) as improvers of gluten-free unleavened dough, their concentration should be limited to 0.5 – 1.0% by weight of flour (higher concentrations lead to the slight deterioration of the structural and mechanical properties of the crumb, and there are inexpedient from the economic point of view). The proposed additives for improving the gluten-free yeast-free dough help improve the foam structure's porosity, forming the finely porous, uniform foam.

Other studies [27] show that introducing the solution of xanthan in the solution of gelatin structures increases the thermal stability of the flour whipped semi-finished product during heating. This is probably due to the

redistribution of associated and non-associated hydroxyl groups, forming many intermolecular hydrogen bonds. The catalytic effect of the enzyme transglutaminase in the gelatin-xanthan system on the interaction of lysine amino groups with the  $\gamma$ -carboxamide group of peptide-linked glutamine residues is also proven. This effect provides a higher level of cross-linking of macromolecules of the protein framework and significantly slows down the dehydration of the semi-finished whipped flour product.

Thus, the combined use of hydrocolloids and protein supplements in gluten-free bread technology is justified and needs more in-depth research.

### Scientific Hypothesis

The study aims to optimise the impact of protein and polysaccharide nature additives as structurants in their everyday use in the technology of gluten-free rice bread. Several interrelated tasks have been solved to solve the formulated goal:

- to determine rational technological parameters by conducting the full-factor experiment PFE 2<sup>3</sup>;
- to study the influence of the amount and type of additive-structuring agent on the structural and mechanical properties of gluten-free bread;
- to study the peculiarities of microbiological processes in the dough with additives;
- to conduct a comprehensive assessment of the quality of finished bakery products.

## MATERIAL AND METHODOLOGY

### Samples

Sample 1	2.5% of yeasts; 60% of water; 90 min of fermentation and proofing.
Sample 2	1.5% of yeasts; 58% of water; 70 min of fermentation and proofing.
Sample 3	2.5% of yeasts; 60% of water; 70 min of fermentation and proofing.
Sample 4	2.5% of yeasts; 58% of water; 70 min of fermentation and proofing.
Sample 5	1.5% of yeasts; 58% of water; 90 min of fermentation and proofing.
Sample 6	1.5% of yeasts; 60% of water; 90 min of fermentation and proofing.
Sample 7	1.5% of yeasts; 60% of water; 70 min of fermentation and proofing.
Sample 8	2.5% of yeasts; 58% of water; 90 min of fermentation and proofing.

### Chemicals

Agar	TM "Vprok", Ukraine.
Gelatin	TM "Deco", Ukraine.

### Animals and Biological Material

Rice flour	TM "World's Rice".
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### Laboratory Methods

The volume of the finished products was measured with the volume meter. Baking was defined as the difference between the weight of the dough and hot bread and was expressed as the percentage by weight of the dough. Drying was defined as the difference between hot and uncooled bread as a percentage of the weight of hot bread.

Gas-forming ability and the rate of gas formation were determined in parallel with the degree of loosening of the dough [28, 29]. The calculation of dry matter consumption for fermentation was performed in terms of glucose by the amount of CO<sub>2</sub> released during fermentation, using the equation of glucose fermentation of Gay-Lussac [30].

Loss of carbon dioxide during fermentation was calculated by formula 1:

$$B_{CO_2} = \frac{\int_a^b (F(x) - f(x)) dx}{\int_a^b F(x) dx} \times 100 \quad (1)$$

Where:

$B_{CO_2}$  – loss of carbon dioxide during fermentation (%),  $a$ ,  $b$  – values of the integration limit;  $F(x)$  is the area of the curved trapezoid on the segment  $[a, b]$ , limited by the equation of gas-forming capacity of flour and  $OX$  axis;  $f(x)$  is the area of the curvilinear trapezoid  $[a, b]$ , limited by the gas holding capacity equation and  $OX$  axis.

The change in the volume of the dough during fermentation was determined using the measuring cylinder of 500 ml, which was placed 10 g of dough and kept at the temperature of 30 – 35 °C. The change in dough volume was recorded every 60 s for 60 min.

Organoleptic assessment of bread quality was determined by the scale of quality assessment adopted by the Central Laboratory of the State Commission. The moisture content of bread was selected in the oven "Brabender" according to GOST 21094-75. The volume of the finished products was measured with the volume meter.

A comprehensive product quality assessment was performed using quality methods [31], [32].

### Description of the Experiment

**Sample preparation:** The dough was kneaded as follows. The prescribed amount of yeast, salt, agar, and gelatin was dissolved in water at 35 °C and mixed with the specified amount of rice flour. The dough was kneaded for 15 minutes. The dough was placed in the mould and left to ferment for 40 minutes and stand for 30 minutes at the temperature of 30 °C. After fermentation, the dough was divided into pieces weighing 50 grams, placed in baking tins and baked for 35 – 40 minutes at the temperature of 180 °C.

**Number of samples analyzed:** 8.

**Number of repeated analyses:** 3.

**Number of experiment replication:** 3.

**Design of the experiment:** In the course of experimental research and production tests, the following products were selected as objects, the obligatory general quality indicators of which corresponded to the indicators of the current normative documentation: rice flour, agar; gelatin; gluten-free bread; gluten-free dough.

The dough was kneaded as described according to conditions of FFE 2<sup>3</sup> (Table 1). The volume of the finished products was measured with the volume meter. Gas-forming ability and the rate of gas formation in the semi-finished product were determined in parallel with the degree of loosening of the dough.

The scale of quality assessment adopted by the Central Laboratory of the State Commission determined the organoleptic assessment of bread quality.

### Statistical Analysis

For statistical analysis, absolute error was established by formula 2:

$$\Delta y_i = y_i - y_a \quad (2)$$

Where:

$y_i$  – value of measured parameter;  $y_a$  – average value of measured parameter.

The next stage was calculation of dispersion – mean square of deviation of a random parameter from an average value of measured parameter by formula 3:

$$S_{(y_i)}^2 = \frac{\sum_{i=1}^n (y_i - y_a)^2}{n-1} \quad (3)$$

Standard deviation was calculated by formula 4:

$$S_{(y_i)} = \sqrt{S_{(y_i)}^2} \quad (4)$$

And standard deviation of average result (formula 5):

$$S_{(y_a)} = \frac{S_{(y_i)}}{\sqrt{n}} \quad (5)$$

Checking the reliability of obtained results was made according to the Student's Criteria  $t_a$  for the number of conducted experiments  $f = n - 1$  with the selected reliable probability  $\alpha = 0.95$  by formula 6

$$\varepsilon = t_a S_{(y_a)} \quad (6)$$

The establishment of reliable interval was conducted by formula 7:

$$y_a \pm \varepsilon \quad (7)$$

### RESULTS AND DISCUSSION

To find the optimal modes of gluten-free bread production, we planned the full-factor FFE 2<sup>3</sup> [28], [29], [30], [31], [32] experiment. The factors of variation were selected, the amount of yeast, % ( $X_1$ ), the amount of water, and % ( $X_2$ ) the duration of fermentation and proofing, min. ( $X_3$ ). The conditions of the experiment are presented in Table 1.

The specific volume was chosen as the criterion of optimality for implementing the full-factor experiment, which more fully characterises bread quality. In our opinion, determining the specific volume of bread is the most informative way to establish the influence of optimisation factors on making bread [1, 2, 5]. The maximum value of the particular volume of bread is chosen as the maximum developed porosity of products is reached at such value. The extreme criterion of optimality is achieved because after the maximum value of the specific volume of bread, with further movement in the direction of the optimization vector, the dough loses the ability to form a coherent structure. The results of trial laboratory baking are shown in Figure 1.

**Table 1** Conditions for the full-factor experiment FFE 2<sup>3</sup>.

Sample	Factor of variation		
	X <sub>1</sub>	X <sub>2</sub>	X <sub>3</sub>
1	2.5	60	90
2	1.5	58	70
3	2.5	60	70
4	2.5	58	70
5	1.5	58	90
6	1.5	60	90
7	1.5	60	70
8	2.5	58	90



Sample 1



Sample 2



Sample 3



Sample 4



Sample 5



Sample 6



Sample 7



Sample 8

**Figure 1** The results of trial laboratory baking of gluten-free rice bread.

Additionally, baking and shrinkage were determined. The results are presented in Table 2.

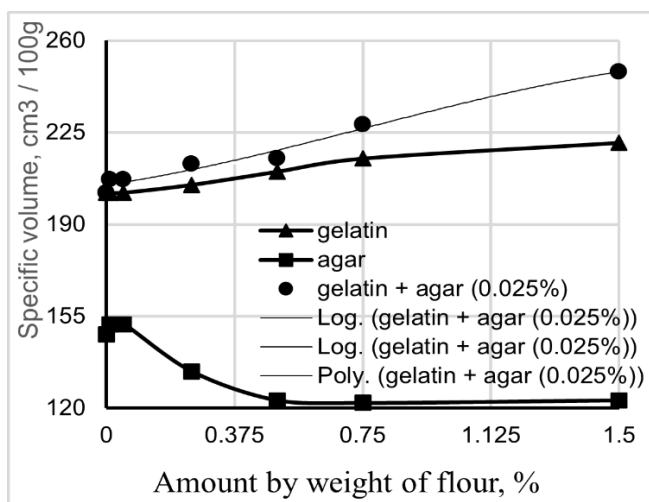
**Table 2** Physico-chemical parameters of gluten-free rice bread.

Sample	Specific volume, cm <sup>3</sup> /100g	Baked, %	Shrinkage, %
1	90	32.16	9.89
2	70	29.56	7.84
3	80	32.15	15.94
4	85	31.41	10.32
5	90	27.31	13.08
6	80	30.16	10.31
7	100	29.07	8.92
8	80	30.41	9.30

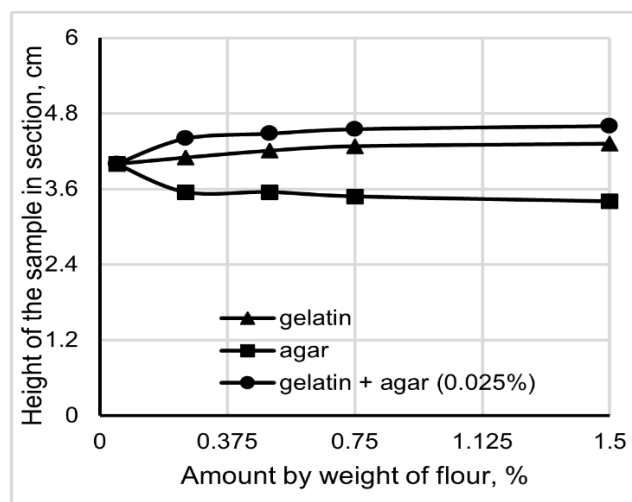
Note: (n = 3, p ≤ 0.05).

The results show that adding yeast in the amount of 1.5% by weight of flour is advisable, as variants of samples with such a concentration have well-developed porosity, higher specific volume, and lower shrinkage. The optimum humidity of the dough is 60%, because when the humidity is reduced to 58% (samples 2, 4, 5, 8), have a dense crumb structure and a lower specific volume (average 8%). Prolonged spawning time leads to the formation of the dense crumb structure in samples 1, 5, 6, and 8, so it is not advisable.

Thus, gluten-free bread of a certain level of quality can be obtained by adding yeast in the amount of 1.5%, dough moisture of 60%, and the duration of fermentation and proofing of 70 minutes. However, even under these technological regimes, the prototypes do not have such quality indicators that are fully able to satisfy consumers [6], [7], [9], [9], [10]. Therefore, the next step was to determine the potential of gelatin and agar as gluten-free bread improvers. We recommend using polysaccharides and protein structurants [11], [12]. To substantiate the type of additive and its concentration, the next stage of the study determined the effect of gelatin, agar, and their joint introduction on the specific volume and height of bread samples (Figure 2 and Figure 3).



**Figure 2** Dependence of specific volume of bread from the concentration and type of structure forming additives.



**Figure 3** The dependence of the bread sample height from the concentration and type of structure forming additives.

The following samples were used for the study: samples with the addition of gelatin in the concentration of 0 to 1.5% (because the introduction of gelatin to more than 1.5% causes the unpleasant odor and darkening of bread crumbs [13], [14], [15]; sample with the addition of agar in the concentration of 0 to 0.050%; sample with the combined use of agar and gelatin (where the amount of agar 0.025% was taken as the constant, as previous studies have shown that increasing the amount leads to the decrease in the specific volume of bread) [16], [17], [18], [19], [20]. Two circumstances can explain this: firstly, in minimal amounts, agar can increase the volume of bread, and secondly, the ability of mixed jelly to improve significantly at lower concentrations of components in comparison with single-component jelly.

The research results show that the use of agar alone harms both the specific volume of bread and the height of the sample. Thus, when the agar content increases to 0.08%, the specific volume and height of the sample decrease

by 16.9% and 15.0%, respectively. Thus, increasing the agar content by more than 0.025% by weight of flour is not considered appropriate. This result may be due to the agar's high-water absorption and water holding capacity, resulting in competition for water between biopolymers of flour and additives. The addition of gelatin slightly increases the indicators by 9.4% and 8%, respectively, to increase the specific volume and height of gluten-free bread when adding gelatin and agar. At the same time, with increasing the number of additives to the mass of flour, the results improve.

The addition of polysaccharide and protein improvers to the formulation of the gluten-free dough to regulate its technological properties can significantly affect the intensity of fermentation and the activity of amylolytic enzymes in flour [21], [22], [23], [24], [25], [26].

It should be noted that the technological stage of fermentation for the gluten-free dough is significantly reduced than for wheat, so studies of the dynamics of CO<sub>2</sub> release were performed for 70 min, corresponding to the established duration of fermentation during PFE. The results show (Figure 4) that adding polysaccharides and protein additives lead to the accumulation of carbon dioxide in gluten-free dough by 33 – 44%.

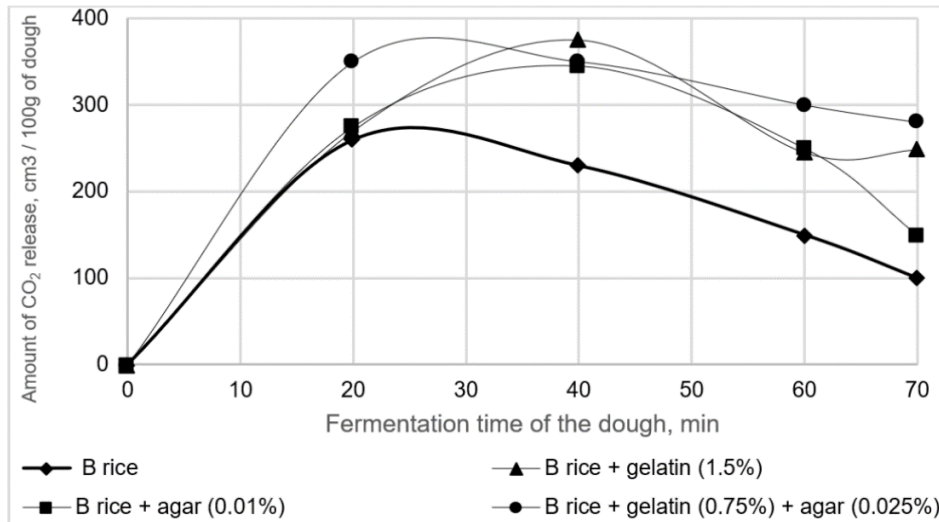


Figure 4 Amount of carbon dioxide released by gluten-free rice dough.

It is possible to assume that in the presence of supplements, the nutrition of yeast cells is improved (possibly by facilitating the transport of nutrients).

Also, research results show that adding additives leads to a slight slowdown in fermentation in the dough. In most cases, the peak of carbon dioxide accumulation is shifted by 15 – 20 minutes. In the case of using structurants, extending the fermentation to 40 minutes and settle to 30 minutes.

In order to establish the recommended modes of dough fermentation, a study of the change in dough volume was conducted. It was found that adding additives slightly shifts the peak of the fermentation process (Figure 5).

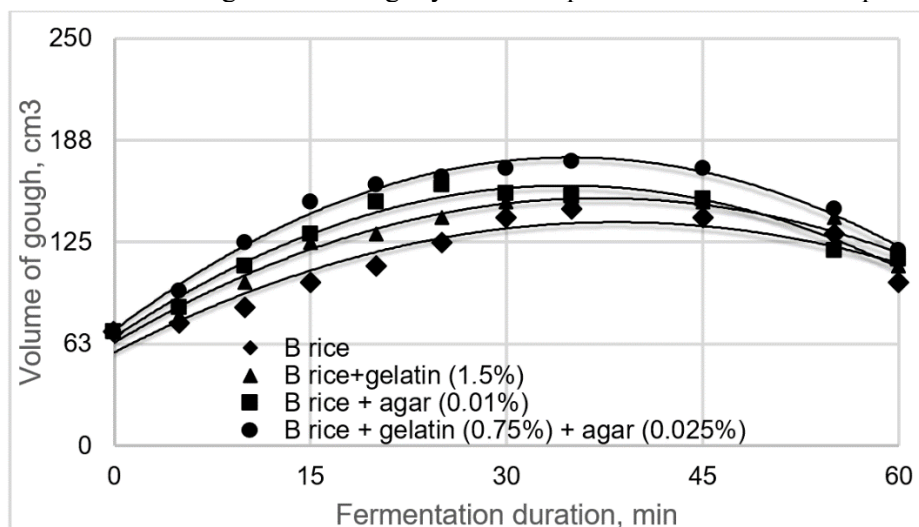


Figure 5 Changing the volume of gluten-free rice dough during fermentation.

Thus, it was determined that the recommended duration of fermentation of rice flour dough with the addition of gelatin is 30 – 35 min, with the addition of agar 25 – 30 min, with the addition of the gelatin mixture and agar 40 – 45 min. It should be noted that each sample was stand for 30 min after fermentation.

The increase in dough volume can be explained by the improvement of the rheological properties of the dough with additives and the higher ability of the dough to retain gas.

In addition, studies have shown that introducing additives changes the rate of accumulated CO<sub>2</sub> in the dough (Table 3).

**Table 3** Rate of accumulated CO<sub>2</sub> in the dough, ml CO<sub>2</sub>/min.

Composition of the sample	Fermentation duration, min								
	5	10	15	20	25	30	35	45	60
<b>B rice</b>	15.00	8.50	6.67	5.50	5.00	4.67	4.14	3.11	2.36
<b>B rice + gelatin (1.5%)</b>	16.00	10.00	8.33	6.50	5.60	5.00	4.34	3.33	2.55
<b>B rice + agar (0.01%)</b>	17.00	11.00	8.67	7.50	6.40	5.17	4.40	3.38	2.73
<b>B rice + gelatin (0.75 %) + agar (0.025%)</b>	19.00	12.50	10.00	8.00	6.60	5.67	5.00	3.78	2.64

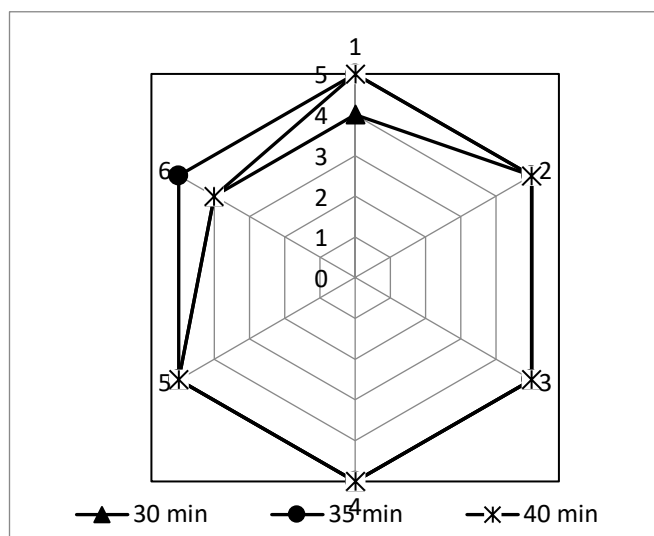
Note: (n = 3, p ≤ 0.05).

The study results show that the highest rate of accumulated CO<sub>2</sub> in the dough can be achieved by making gelatin in combination with agar. At the same time, after 5 minutes of fermentation, the amount increases by 26% compared to the sample without additives, and after 10 minutes by 47%. After 35 minutes of fermentation, the rate of CO<sub>2</sub> begins to decrease: which coincides with the peak of reaching the maximum volume of the dough. Therefore, the addition of additives in the mixture significantly improves the gas-forming and gas-holding capacity of the dough, which allows prolonging the fermentation operation accumulate more gas in the dough and get bread with a higher specific volume and better organoleptic properties [27], [28], [29], [30].

At the next stage of the study, the bread quality was assessed depending on the baking duration [31], [32]. Based on the results of experimental research, quality stars were constructed, and the rational duration of baking was determined (Figure 6).

The assessment was performed according to the 5-point scale:

- crumb stickiness: 1 – sticky, viscous, 2 – sticky, 3 – sticky in the middle, baked from the edges, 4 – baked throughout, there is the slight stickiness, 5 – baked throughout, excessive no stickiness;
- crust stickiness: 1 – sticky, not separable from the form, 2 – sticky, partially separated from the form, 3 – completely separated from the form, but there is excessive stickiness, 4 – there is slight stickiness, 5 – no excessive stickiness;
- porosity: 1 – absent, 2 – slightly developed, 3 – developed from the edges of the crumb, absent in the middle, 4 – unevenly developed throughout the volume of the crumb, 5 – well and evenly developed;



**Figure 6** Stars of gluten-free bread quality based on rice flour (depending on the duration of heat treatment (1 – the stickiness of the crumb, 2 – the stickiness of the surface, 3 – porosity, 4 – the presence of crust, 5 – smell, 6 – the color of crust).



- Crust: 1 – completely absent, 2 – slightly developed in the upper part of the bread, 3 – developed in the upper part of the bread and absent in the lateral parts, 4 – unevenly developed over the entire surface of the bread, 5 – evenly developed over the entire surface of the bread.
- odor: 1 – side, unpleasant, 2 – not expressed, side, 3 – weakly expressed, characteristic, 4 – expressed, characteristic, 5 – intensely expressed, characteristic;
- crust color: 1 – pale or burnt, 2 – light beige or from yellow to brown, very uneven, 3 – yellow or intensely dark brown, not uniform enough, 4 – light golden or brown, fairly uniform, 5 – from golden to light brown, uniform.

The results show that the rice flour-based bread is fully baked after 35 minutes of baking at 180 °C. When extending the duration of heat treatment, the quality of bread does not change, so long-term heat treatment is not economically feasible.

Thus, based on the studies carried out, it can be argued that the introduction of additives of the polysaccharide and protein nature does not lead to significant changes in the rate of evaporation of moisture from the dough. Therefore, the increase in the duration of heat treatment is inappropriate.

### CONCLUSION

It is established that the optimal humidity of gluten-free rice bread is 60%. At such humidity, the porosity of the dough develops well, the specific volume increases, and the crumb's moisture remains sufficient. The study results show that adding polysaccharide and protein additives leads to an increase in the amount of carbon dioxide accumulation in gluten-free dough by 33 – 44%. It is experimentally substantiated that the recommended duration of fermentation of rice flour dough with the addition of gelatin is 45 – 50 min, with the addition of agar is 25 – 30 min, with the addition of the mixture of gelatin and agar is 40 – 45 min. It is established that to achieve full readiness of bread based on rice flour can be reached after 35 minutes of baking at the temperature of 180 °C.

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