

# Influence of rice flour on conformational changes in the dough during production of wheat bread

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## Abstract

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**Introduction.** The aim of the work was to determine the influence of rice flour on the conformational transformations in the structure of the dough for the production of wheat bread, which contains lecithin.

**Materials and methods.** Chemical composition and fractional composition of proteins of rice flour were investigated. Conformational transformations of structural elements in dough and bread were investigated by infrared spectroscopy in the near-infrared region.

**Results and discussion.** In rice flour, the total protein content lower by 47%, and the dietary fiber content is 8.5 times lower than in premium wheat flour. The fractional composition of proteins showed a higher content of albumin, prolamin and insoluble proteins in wheat flour than in rice flour by 11, 90 and 75%, respectively. The content of globulins and glutelins is more in rice flour by 7 and 183%, respectively, but the composition of glutelins in the studied samples is different. In wheat flour, it is glutenin, which is a gluten protein, forming a heterogeneous mixture of polymers through disulfide bonds of polypeptides. In rice flour, oryzenin is a representative of glutelins. The infrared reflection spectra of wheat and rice flour showed a similar nature of the spectra: the extremes are observed at the same wavelengths, the spectra are located parallel to each other and differ only in the intensity of reflection. The spectrum of sunflower lecithin differs significantly due to its different chemical composition. Also, at some wavelengths on the spectrum of lecithin, extreme shifts in both short- and long-wavelength regions are visible. The secondary structure of gluten underwent changes in bread after exposure to temperature by promoting  $\alpha$ -helices and  $\beta$ -turns and contributed to the formation of disulfide bonds.

**Conclusion.** The conducted studies indicate the expediency of using rice flour in the technology of bakery products to replace wheat flour in order to minimize the fiber content in bread.

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## Introduction

The change in the environmental and economic situation in the world in recent years has significantly affected the structure of the population's diet and approaches to the production of food products (Ivanov et al., 2021). The increase in the incidence of alimentary and non-alimentary diseases, the rapid development of diseases of the gastrointestinal tract, including inflammatory bowel diseases (IBD), made it necessary to look for approaches to the development of dietary therapy for the prevention of these diseases. Need to investigate the possibility of using non-traditional raw materials increased in various branches of the food industry, in particular in the bakery industry, as one of the key ones (Forbes et al., 2017).

Most medical scientists prevent from using a large amount of dietary fiber in the diet of patients with IBD (Armstrong et al., 2021; Chiba et al., 2015), because they do not have the appropriate microorganisms to digest fiber (Armstrong et al., 2022). For this purpose, rice processing products are promising raw materials, in particular rice flour with a low dietary fiber content (Drobot et al., 2021).

Rice germ contains lipophilic antioxidants (tocopherols, tocotrienols and  $\gamma$ -oryzanol) and phenolic compounds. These substances protect from free radicals and extend the shelf life of products (Esa et al., 2013; Min et al., 2011).

Water- and fat-absorbing capacities, emulsifying and foaming capacity allow the use of rice by-products in bakery and confectionery industries. Defatted rice bran can be used to replace up to 10-20% of wheat flour in recipes of biscuits without adversely affecting their quality (Ashraf et al., 2012).

Rice flour is basically used in the technology of gluten-free bread in a mixture with starches, in particular potato and corn starch (Mykhonik et al., 2017). It was established that it is advisable to add rice flour to the recipe of products in the amount of up to 30% to replace starch. At the same time, the content of aroma-forming substances in bread increases by 12.7% compared to bread made from a mixture of starches. The nutritional value of bread is also significantly increased, in particular, the content of proteins increases by 2.5 times, the content of potassium, magnesium, iron and vitamins increases too.

The influence of various rice by-products (flour, wholemeal flour, and bran) on the rheological parameters and quality of bread was studied (Genevois et al., 2021). A three-component mixture scheme was used. The optimal proportion of the mixture is 45:35:20. The specific volume of bread was  $1.7 \pm 0.1$  cm<sup>3</sup>/g, hardness –  $0.23 \pm 0.01$ , color intensity –  $23.8 \pm 0.4$ . The use of different rice fractions improved product quality and nutritional profile.

The use of phospholipids with a high content of phosphatidylcholine is important in the diet of patients with IBD, which prevents damage to the upper and lower parts of the gastrointestinal tract, participates in the formation of the protective layer of intestinal mucus (Stemmel et al., 2014). Soy lecithin became widely used in the food industry, but a promising non-GMO alternative raw material is sunflower lecithin (Guitto et al., 2015, Shevchenko et al., 2021).

It was determined that the dosage of lecithin in the amount of 1.0% to the mass of rice flour in gluten-free bread technology improves gas formation of the dough and increases the specific volume of bread by 16.2% and porosity by 12.7% (Medvid et al., 2018).

However, the influence of the mixture of lecithin and rice flour on the technological process and the quality and nutritional value of bakery products was not investigated, so this direction is relevant.

The aim of the work was to determine the influence of rice flour on the conformational transformations in the structure of the dough for the production of wheat bread, which contains lecithin.

## Materials and methods

### Materials

Premium wheat flour, sunflower lecithin and rice flour were used for research.

Dough samples were prepared with the addition of pressed baker's yeast (3% by weight of flour) and salt (1.5% by weight of flour). Lecithin was added in the amount of 3% to the mass of flour. This dosage was chosen based on the recommendations for the daily norm of lecithin for people with IBD (Partridge et al., 2019). Wheat flour was replaced with rice flour in the recipe in the amount of 10%, 20%, 30%, and 40%. The control was a sample without additional raw materials.

### Total protein content

Kjeldahl method was used for determination followed by titration technique. 1 g of raw material must be hydrolyzed with 15 mL concentrated sulfuric acid for 2 h in a heat block at 420 °C with two copper catalyst tablets. After cooling before neutralization and titration, distilled H<sub>2</sub>O was added to the hydrolysate. The amount of protein was calculated taking into account the nitrogen concentration in the product. Data was expressed as g proteins per 100 g of flour (Shevchenko et al., 2022).

### Fiber

A collaborative study was conducted to determine the total dietary fiber (TDF) content in products, using enzymatic-gravimetric method. TDF was calculated as the weight of the residue minus the weight of protein and ash (Shevchenko et al., 2022).

### Protein fractional composition

During the sample preparation, rice flour was sifted through a mesh sieve to obtain a fine powder, and kept at 4 °C until further use. The raw material was defatted with pentane. The flour/solvent slurry was mixed at a 1:10 *w/v* ratio for 24 h, and then the solvent was removed by centrifugation. The mixture was dried and stored in airtight bottles at 4 °C until subsequent use. According to the Osborne differential extraction procedure rice proteins were fractionated from pentane defatted meal (Horax et al., 2010). The meal-water suspensions (20 g of meal into 100 mL of deionized water) were mixed for 2 h at room temperature and centrifuged at 20,000 *g* for 30 min to separate the supernatant from the pellet, and obtain the deionized water extract (DWE). The same conditions for extraction/separation were kept for the next protein extraction steps. The water extract pellet was put into 100 mL of 1 M NaCl solution and mixed as mentioned above. The supernatant obtained after centrifugation, was extracted in 100 mL of deionized water at pH 11 with 0.5 M NaOH solution, leading to the alkaline extract (AE). Each extraction was made twice. Pellets were washed twice after each extraction using 20 mL of solvent. It was made to collect the residual protein entrapped in the insoluble residues. Obtained extracts were precipitated for isolation by adjusting the pH of the obtained supernatant to the pH corresponding to the minimum of solubility (pH<sub>ms</sub>) determined from the turbidity experiment (Rezig et al., 2013). The pH was revised by 1 M HCl or 1 M NaOH solutions in acidic or alkaline pH, respectively. After centrifugation for 15 min at 15,000 *g*, the isolated protein precipitates were washed using deionized water twice at their respective pH<sub>ms</sub> and recentrifuged. The resulting protein fractions were resolubilized by providing the pH to 7.0, freeze-dried, and stored at 4 °C until further analysis (Rezig et al., 2015).

### Near-infrared reflection spectroscopy

Infrared spectrometer (Labor-Mim, Hungary) was used to research the reflection spectra from shredded samples and a smooth surface in near infrared range from 1330 to 2370 nm. Firstly, the spectrometer recorded the reflectance spectrum from reference I<sub>0</sub>, secondly a reflection spectrum from the researched sample. The spectra are represented as the reflectivity of R in relative units (the ratio of the intensities I/I<sub>0</sub> = R), depending on the wavelength in nm (Litvynchuk et al., 2022; Niewietzki et al., 2010; Yip et al., 2012). The intensity of reflection was measured in rice and wheat flours, in lecithin, in dough after kneading and after 3.5 hours of fermentation and in bread. The reflection intensity was expressed through the relative reflection coefficient.

### Statistical analysis

The data represents the mean of a minimum three replicates ± standard deviation (S.D.). Graphical presentation of experimental data was performed using standard statistical processing programs – Microsoft Excel 2010.

## Results and discussion

### Protein and amino acid composition of wheat and rice flours

The chemical composition of raw materials, especially the content and composition of proteins, plays a decisive role in determination of the properties of dough for bakery products (Amjid et al., 2013).

The total protein content in rice flour is 47% lower than in premium wheat flour. The content of dietary fibers is 8.5 times lower, which makes it a valuable raw material for the production of bread for patients with IBD (Drobot et al., 2021).

Fractional composition of rice flour proteins differ from wheat flour (Table 1).

**Table 1**

**Fractional composition of proteins of wheat flour and rice flour**

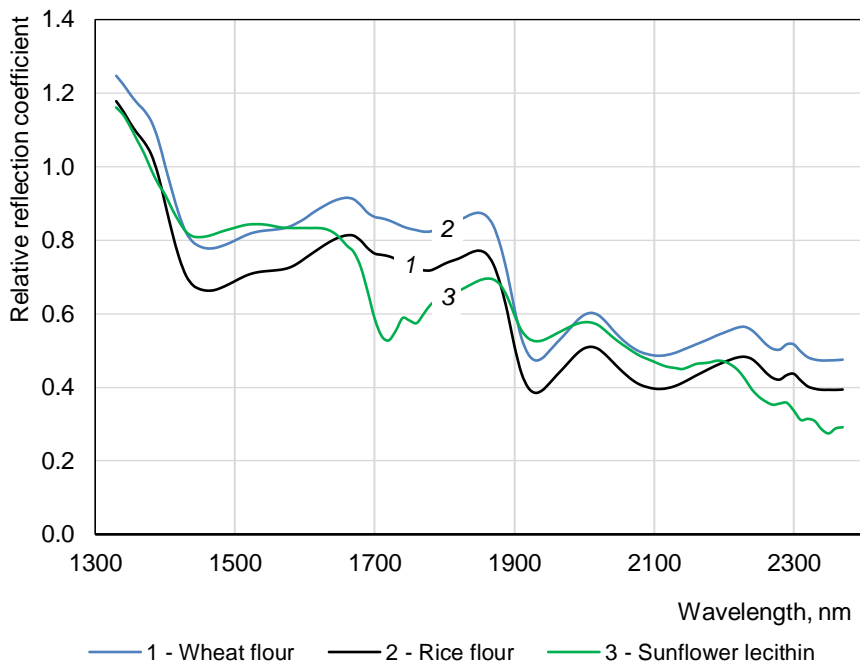
Mass fraction of proteins (g/100g)	Wheat flour	Rice flour
Albumin	5.4±0.21	4.8±0.21
Globulin	9.9±0.34	10.6±0.36
Glutelin	27.1±0.78	76.7±1.67
Prolamin	43.1±1.12	4.3±0.18
Insoluble proteins	14.5±0.46	3.6±0.15

A higher content of albumin, prolamin and insoluble proteins was determined in wheat flour. The content of globulins and glutelins is higher in rice flour, but the composition of glutelins in the studied samples is different. In wheat flour, it is glutenin, which is a gluten protein, forming a heterogeneous mixture of polymers through disulfide bonds of polypeptides (Huang et al., 2011). In rice flour, the representative of glutelins is orizenin, which does not have properties to form a dough frame (Jayaprakash et al., 2022).

The difference in the chemical composition of wheat flour, rice flour and lecithin should affect the change in the basic structural units of dough and bread with these components in the recipe. To identify and analyze these components, it is advisable to use the reflection spectrum in the near infrared region (Baslar et al., 2011).

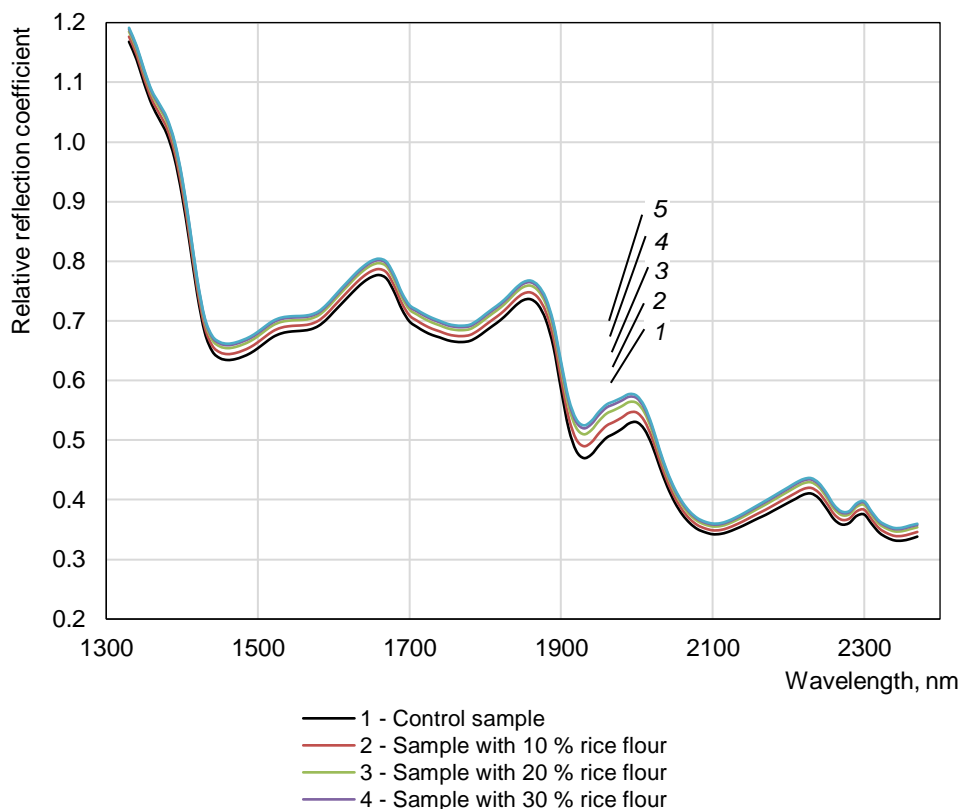
The infrared reflection spectra of wheat and rice flour showed a similar nature of the spectra: the extremes are observed at the same wavelengths, the spectra are located parallel to each other and differ only in the intensity of reflection (Figure 1).

Wheat flour has a higher relative reflection coefficient, so its spectrum is placed higher than the spectrum of rice flour. Sunflower lecithin has different chemical composition in comparison with flours, due to which its reflection spectrum differs from the reflection spectra of flour and has different shape with the appearance of additional extreme (at a wavelength of 1720 nm, a clear minimum of reflection intensity is observed in the spectrum of sunflower lecithin, which was not previously observed in flour spectra). Also, at some wavelengths on the spectrum of lecithin, extreme shifts in both short- and long-wavelength regions are noticeable. In particular, the reflection minimum of the sunflower lecithin sample compared to the flour samples shifted by 40 nm (from 2100 to 2140 nm). This indicates the absence of protein in its composition, which is present in flour samples (Beć et al., 2019).



**Figure 1. Infrared reflection spectra of raw materials**

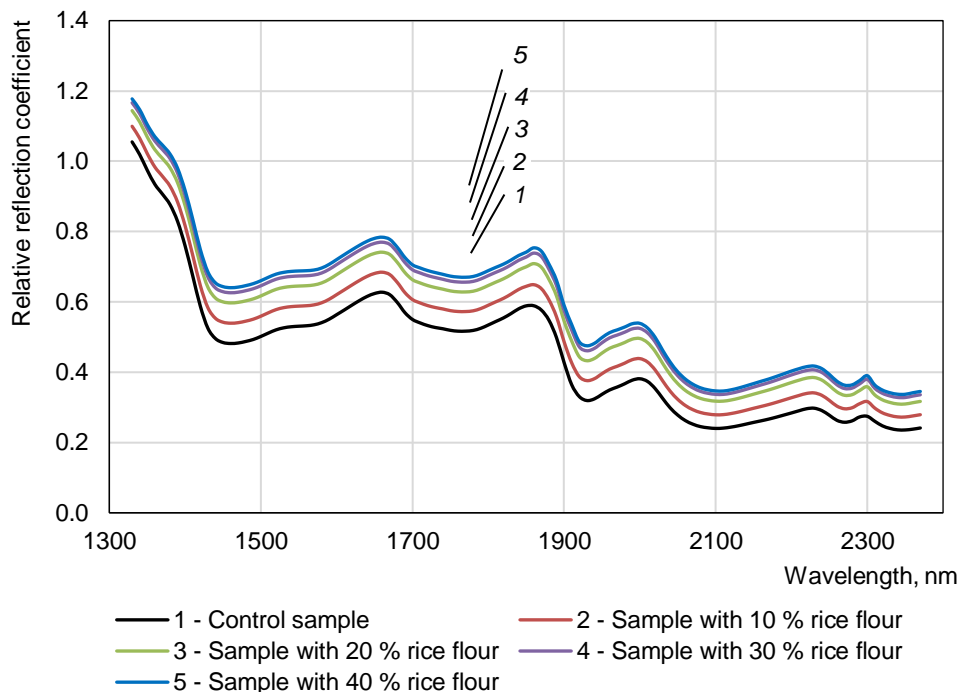
After kneading the dough, the spectra of the samples obtained with different replacement of wheat flour by rice differed slightly, although all extremes were the same (Figure 2).



**Figure 2. Infrared reflection spectra of dough samples with rice flour (10, 20, 30 and 40% replacement) after kneading**

There is a direct regularity in the location of spectra of the samples: the control sample of the dough is the lowest, sample with 10% replacement is above it, samples with 20, 30, and 40% replacement are higher. The similarity of the obtained spectra indicated that the determining factor affecting the formation of dough properties is wheat flour, since its mass fraction remains the largest in the dough. However, the intensity of reflection of the dough with rice flour, regardless of the percentage content, tended to the spectrum of the control sample of dough, acquiring springy-elastic and visco-plastic properties, which in the control sample are provided by the content of glutenins and gliadins of wheat flour. The difference in the spectra near the wavelength of 1930 nm indicates the content of moisture in the samples (the control sample has the lowest intensity and a clear sequence is observed: the increase in the intensity of reflection by the samples with an increase in the replacement percentage). Since the dough samples were prepared for the experiment under the same conditions, it can be concluded that this trend is caused by the higher water absorption and moisture retention capacities of rice flour than wheat flour, which in turn is caused by the greater dispersion of its particles (Lapčíková et al., 2021).

After the end of fermentation and keeping of the dough (after 3.5 hours), the conformational changes of the structural substances of the dough were determined (Figure 3). All spectra of dough samples with different doses of rice flour changed their reflection intensity, but all extremes remained at the wavelength values observed in Figure 2.

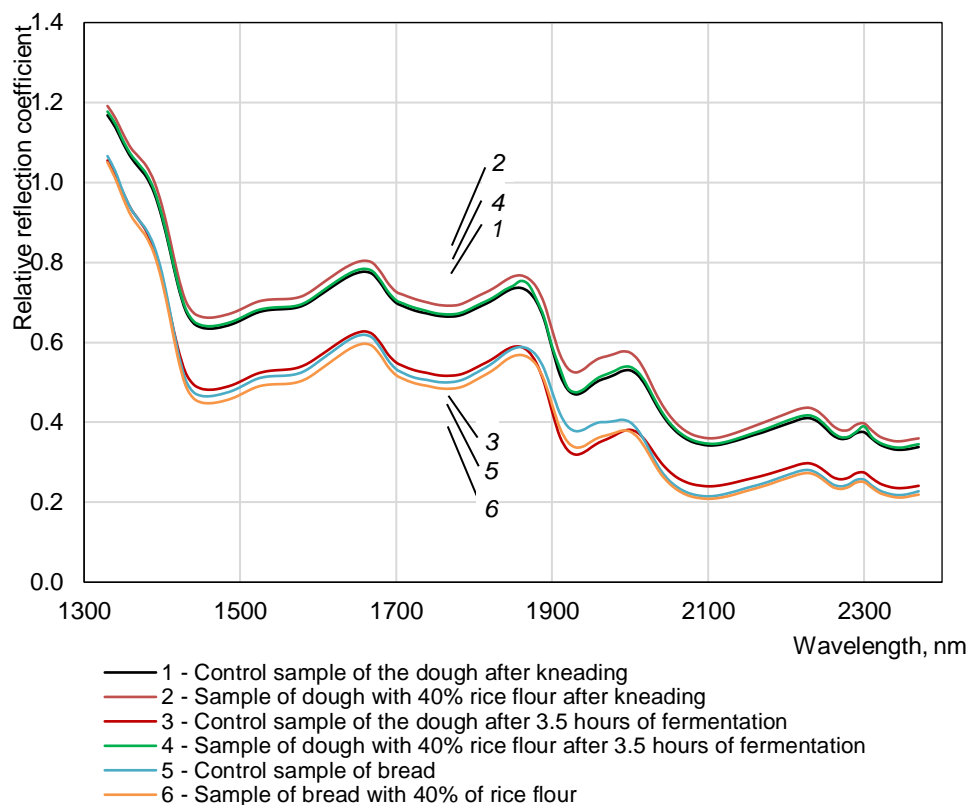


**Figure 3. Infrared reflection spectra of dough samples with rice flour (10, 20, 30 and 40% replacement) after fermentation**

In contrast to the spectra of the dough obtained after kneading, due to changes in the properties of the biopolymers of the raw material, in particular, the swelling of proteins and starch, during the fermentation process, the spectra changed the intensity of reflection and moved away from each other evenly, but kept their regularity of location. That is, with an increase in the percentage of replacement of wheat flour with rice, the reflection coefficient increased, and the spectrum was higher than the control sample of the dough. But in the dough samples with the replacement 30 and 40%, the spectra almost overlapped and their difference was barely noticeable. This indicates that structural changes are more active, although less, in samples with a lower percentage of replacement.

In the process of baking, under the influence of high temperatures, protein and other substances of the dough change, which affects the intensity of the reflection of the finished products. The analysis of the spectra of the control sample of dough and the dough samples with 40% replacement of wheat flour with rice flour (after kneading and after 3.5 hours of fermentation), as well as samples of bread (Figure 4), made it possible to conditionally divide them into two groups (three spectra in each group), since each of them has the same initial relative reflection coefficient.

The obtained spectra of the first group showed that the control sample of the dough after kneading and the sample of dough with 40% rice flour after 3.5 hours of fermentation are practically the same. The spectrum of the dough sample after kneading with 40% replacement with rice flour is close to them, but with a slightly higher value of the relative reflection coefficient.



**Figure 4. Infrared reflection spectra of dough and bread samples with rice flour (40% replacement)**

The spectra of the control sample of the dough after 3.5 hours of fermentation, the control sample of bread and bread with 40% replacement, which are separated into the second group, also conditionally match and overlap each other. However, the initial relative reflection coefficient has a lower value than that of the first group. This indicates that the conformational transformations in finished products have a similar character to transformations in dough with wheat flour after fermentation and are determined to a greater extent by the properties of wheat flour (Chompoorat et al., 2022). The secondary structure of gluten underwent changes in bread after exposure to temperature by promoting  $\alpha$ -helices and  $\beta$ -turns and contributed to the formation of disulfide bonds (Xiang et al., 2020). This indicates that the deformation of gluten with a lower content of intermolecular  $\beta$ -sheets, antiparallel  $\beta$ -sheets and  $\beta$ -turns, but a higher content of  $\alpha$ -helices compared to dough gluten (Verbauwhede et al., 2018) increases during the baking process.

The conducted studies indicate that in the technological process of breadmaking conformational changes of protein take place and the expediency of using rice flour in the technology of bakery products to replace wheat flour in order to minimize the fiber content in bread according to the recommendations of diet therapy for patients with IBD.

## Conclusion

1. The total protein content in rice flour is 47% lower than in premium wheat flour, and the dietary fiber content is 8.5 times lower.
2. Wheat flour has a higher content of albumin, prolamin, and insoluble proteins than rice flour by 11, 90, and 75%, respectively. The content of globulins and glutelins is more in rice flour by 7 and 183%, respectively. But the composition of glutelins in the studied samples is different. In wheat flour, it is glutenin, which is a gluten protein, forming a heterogeneous mixture of polymers through disulfide bonds of polypeptides. In rice flour, oryzenin is a representative of glutelins.
3. The infrared reflection spectra of wheat and rice flour showed a similar nature of the spectra: extremes have been observed at the same wavelengths, the spectra are located parallel to each other and differ only in the intensity of reflection. Sunflower lecithin has a minimum reflection intensity at a wavelength 1720 nm. Also, at some wavelengths on the spectrum of lecithin, extreme shifts in both short- and long-wavelength regions are noticeable.
4. The secondary structure of gluten underwent changes in bread after exposure to temperature by promoting  $\alpha$ -helices and  $\beta$ -turns and contributed to the formation of disulfide bonds.

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