Influence of rice protein concentrate on redistribution of structural groups in the production of wheat bread

Anastasiia Shevchenko, Svitlana Litvynchuk

National University of Food Technologies, Kyiv, Ukraine

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Corresponding author:

Anastasiia Shevchenko
E-mail: nastyusha8@ukr.net

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Abstract

Introduction. The aim of the research was to determine the effect of rice protein concentrate on conformational changes in the structural units of wheat dough and bread.

Materials and methods. Functional and technological properties of rice protein concentrate were determined by centrifugation method. Conformational transformations of structural groups in the dough after kneading and during the fermentation process and of bread were investigated by method of infrared spectroscopy in the near-infrared region.

Results and discussion. The protein content in rice protein concentrate was 6.6 times higher than that of premium wheat flour. Rice protein concentrate was coarser than wheat flour of the second grade, but finer than wholemeal wheat flour. This was evidenced by the fact that there was no residue on a sieve with holes of 670 μm, and the passage through a sieve with holes of 160 μm was greater than the standard value for wheat flour of the second grade, but less than the standard value for wholemeal flour. The moisture binding and fat binding capacity of rice protein concentrate was 4.3 and 1.7 times higher than that of wheat flour, and its moisture retaining capacity was 1.8 times higher. The infrared spectra of dough and bread showed that the difference in the chemical composition and functional and technological properties of wheat flour and rice protein concentrate affected the change of the basic structural units of dough and bread. The addition of rice protein concentrate delayed the development of the gluten network, but had a positive effect on the dough's ability to keep its shape. The addition of rice protein concentrate in amounts of 4-16% to the mass of flour had practically no effect on the digestion of bread.

Conclusions. The addition of rice protein concentrate with a higher protein content than wheat flour and with different fractional composition to the recipe of wheat bread affected the structural elements of the dough and the structural and mechanical properties of bread. In addition, it can significantly enrich bread with complete protein.

Introduction

One of the most important nutrients in food products is protein (Małecki et al., 2021). Its content in bread made from wheat flour is quite low. In addition, it is inferior in the composition of essential amino acids and is absorbed at a low level (Eleazu et al., 2014). Therefore, a promising direction is to enrich wheat bread with protein by adding raw materials with a high protein content to the recipe.

Animal sources of protein, such as casein, albumin, and whey proteins, were used in bakery production. However, animal proteins can be allergens. An alternative was plant proteins in the form of concentrates, isolates, hydrolyzates.

The use of rice protein as a hypoallergenic raw material was promising (Pantoa et al., 2020). Protein isolates obtained from various stabilized rice bran were analyzed. Bulk density was higher for isolates obtained from heat-stabilized bran, because the treatment positively affected the fat absorption capacity, while the water absorption capacity decreased due to configurational changes in the protein structure. The isolates had better foaming properties due to the flexible nature of the protein molecules with less intense disulfide bonding. The nitrogen solubility index had a curved pattern with the lowest value near the isoelectric point, which showed a tendency to increase the basic pH, and steamed protein isolates showed better gelling properties (Khan et al., 2011).

To obtain rice protein concentrate, alkaline extraction of rice bran was applied. It was established that the optimal extraction conditions were pH 11 and a duration of 45 min, which led to the extraction of 69.16% of the total protein. When adding 1-5% of the concentrate to the bread recipe, weight loss after baking was reduced compared to the control without rice protein concentrate. However, sensory evaluation showed that the addition of more than 1% protein worsened sensory parameters. An important characteristic of rice bran protein was that it contained a large amount of lysine, an essential amino acid that was limiting in wheat flour, so it was able to enrich the amino acid profile of bread (Jiamyangyuen et al., 2005).

The methods of electrophoresis and infrared spectroscopy were used to study the chemical composition, digestibility and structure of protein. It was found that during acetylation of rice protein concentrate, the protein content increased (80.90–83.10 g/100 g versus 74.20 g/100 g in the control without acetylation). It was found that during acetylation, the content of the main fractions of rice proteins (prolamin and glutelin) decreased. Using spectroscopic analysis, wave numbers corresponding to the presence of proteins or lipids, aromatic systems, and carbohydrates were observed. The use of acetic anhydride did not significantly change the digestibility of rice protein concentrate compared to the control sample. Acetylation of rice protein concentrate resulted in a significant increase in its emulsifying properties and water binding capacity, but had no effect on fat absorption capacity. An increase in protein solubility and decrease in foaming capacity were observed (Miedzianka et al., 2023).

Physicochemical and structural properties of concentrated rice bran protein and rice bran protein fractions - albumin, globulin, prolamin, glutelin, were studied by the method of multiple extraction. The amino acid composition of the concentrated protein was superior to soy protein isolates with similar solubility, emulsification and foaming characteristics. Dichroism spectra in synergy showed that the primary structures were in the form of β-helices and random helices with different denaturation temperatures (Wang et al., 2015).

Due to the influence of rice concentrates on dough and bread structure the aim of the research was to determine the effect of rice protein concentrate on conformational changes in the structural units of wheat dough and bread.

Materials and methods

Materials

Premium wheat flour and rice protein concentrate were materials for research.

Dough was prepared from wheat flour, pressed baker's yeast in the amount of 3% by weight of flour and salt in the amount of 1.5% by weight of flour (control sample). Samples were also prepared with the addition of 5% and 16% rice protein concentrate to the weight of wheat flour to provide 20 and 40% of the daily protein requirement.

Moisture

The moisture content in wheat flour and rice protein concentrate was determined using the SuperPoint moisture meter. After switching on the device there was selected the appropriate type of measuring sample or product on the LCD screen. Sample was put into the bowl of the device to fill it, the pressure cover of the pressurizes to the level until the pressure indicator is set to the level with the upper surface of the lid. It took 10 seconds to obtain the result of the measurements. Measurement is carried out with an accuracy of 0.5% with a range of moisture content measurement from 8 to 45% (Hetman et al., 2021).

Total protein content

Total protein content was measured by Kjeldahl method (burning sample with concentrated sulfuric acid) and titration of the obtained liquid. 1 g of raw material was hydrolyzed with 15 ml concentrated sulfuric acid during 2 h in a heat block at 420 °C. Two copper tablets were added as catalyst. After cooling before neutralization and titration, distilled H_2O was added to the hydrolysates. 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).

Fat content

Fat content in wheat flour and rice protein concentrate was by the Soxhlet method. The sample was placed in the extraction chamber. The solvent was put to the flask and when it was heated, the solvent was evaporated and moved up to the condenser. Then it was converted into a liquid and collected into the extraction chamber with the sample. When the solvent passed through the sample, it extracted fats and carried them into the flask. Extraction took 6–24 h hours. After completion of the extraction, the solvent was evaporated, and the amount of the remaining lipid was measured (López-Bascón and de Castro, 2020).

Size of the flour particles

The size of the flour particles was determined by sieving on sieves. Sieves of different sieve fabric and different hole sizes were used: N 33/36 (35) (220 μm), N 27 (260 μm), N-067 (670), N 49/52 PA (43) (132 μm), N 41/43 (38) (160 μm) (Patwa et al., 2014).

Moisture binding and retaining capacity

The centrifugation method was used for determination of moisture binding and retaining capacity. For determination moisture binding capacity, 0.5 g of raw material was placed in pre-weighed centrifuge tubes, 50 ml of distilled water was added, and tubes were centrifuged at 3500 rpm for 10 minutes. The excess water was drained, the raw materials in the test tubes were dried and weighed.

Moisture binding capacity (MBC) was calculated by the formula:

$$MBC=\frac{m_1}{m_2}\cdot 100,$$

where m_1 – weight of precipitate, g;

m_2 – weight of original flour, g.

For determination the moisture retaining capacity the same method was used. The study differed in that after adding water, the mixture was placed in a water bath for heating for 30 minutes.

Moisture retaining capacity (MRC) was calculated by the formula:

$$MRC=\frac{m_1 - m_2}{m}\cdot 100,$$

where m_1 – weight of tube with flour and water retained, g;

m_2 – weight of tube with flour, g;

m – weight of flour, g (Shevchenko and Litvynchuk, 2022).

Fat binding capacity

The centrifugation method was used for determination of fat binding capacity. The method of determination was the same as in case of the determination of moisture binding capacity, but instead of water, 15 ml of refined sunflower oil was added.

Fat binding capacity (FBC, %) was determined by the formula:

$$FBC = F_1 – F_2$$

where F_1 – fat content in the flour suspension, %;

F_2 – amount of fat released after centrifugation, % (Suriano et al., 2017):

Near-infrared reflection spectroscopy

Infrapid spectrometer (Labor-Mim, Hungary) was used to research the reflection spectra from shredded samples of dough and bread with a smooth surface in near infrared range from 1330 to 2370 nm. Firstly, the spectrometer recorded the reflectance spectrum from reference I0, 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/I0 = R), depending on the wavelength in nm. The intensity of reflection was measured in wheat flour, oat bran, lecithin, in dough after kneading and after 3.5 hours of fermentation and in bread. The reflection intensity was expressed through the recalculation of relative reflection coefficient to spectral index (Yip et. al, 2012).

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

Chemical composition of the recipe ingredients was the basis of the formation of the properties of the dough for bakery products (Table 1). The protein content of rice protein concentrate was 6.6 times higher than that of wheat flour. This indicated that such raw materials can significantly enrich products with protein.

Table 1

Chemical composition of rice protein concentrate and wheat flour of premium grade, % per 100 g of raw material

Indicator	Wheat flour of premium grade	Rice protein concentrate
Moisture, %	11.5±0.5	5.1±0.5
Protein, g	11.3±0.5	74.2±0.5
Fat, g	1.1±0.01	4.4±0.01

In addition to the chemical composition of raw materials, the properties of semi-finished products and bread were influenced by the granulometric composition and functional and technological properties of raw materials.

It was established (Table 2) that 96% of the particles of wheat flour of premium grade passed through a sieve with holes of 132 microns.

Table 2

Particle size of rice protein concentrate and wheat flour

Size indicators, No of sieve	Hole size, µm	Wheat flour, variety			Rice protein concentrate
		First	Second	Wholemeal	
The residue on sieve %, no more:					
No 33/36 (35)	220	2	-	-	18.4
No 27	260	-	2	-	4.9
No 067	670	-	-	2	-
Passage through a sieve, % no less:					
No 49/52 ПА (43)	132	80	-	-	4.08
No 41/43 (38)	160	-	65	35	56.2

Rice protein was coarser than wheat flour of the second grade, but finer than wheat wholemeal flour. This was evidenced by the fact that when examining the rice protein concentrate, there was no residue on a sieve with holes of 670 µm, and the passage through a sieve with dimensions of 160 µm was greater than the standard value for wheat flour of the second grade, but less than the for wholemeal flour.

Functional and technological properties of raw materials determined the behavior of dough during processing and characterized the ability to bind moisture and fat (Berton et al., 2002).These indicators influenced ability to form viscoelastic dough, which is important for obtaining high quality bread, and provided necessary structure and technological properties (Tsykhanovska, 2019).

It was found (Table 1) that the moisture binding and fat binding capacity of rice protein concentrate was higher. This was due to the fractional composition of the proteins of this raw material, since the proteins of the concentrate had better adsorbing properties.

Table 3

Functional and technological properties of rice protein concentrate and wheat flour

Indicator	Premium wheat flour	Rice protein concentrate
Moisture binding capacity, %	90.7±2.13	392±2.90
Moisture retaining capacity, %	148±2.89	272±2.46
Fat binding capacity, %	146±2.06	243±2.86

The increase in the moisture retaining capacity of rice protein concentrate compared to wheat flour was explained by the release of side polar groups of rice protein which had hydrophilic properties (Jayaprakash et al., 2022).

The difference in the chemical composition and functional and technological properties of wheat flour and rice protein concentrate should affect the change of the structural units of dough and bread with these components in the recipe. It was also known that the fractional composition of rice protein was significantly different from wheat protein, primarily due to the absence of gluten proteins in it. This will affect the formation of structural and mechanical properties of the dough and changes in its structural elements.

The analysis of the change and redistribution of structural groups after kneading the dough, after 3.5 hours of its fermentation and finished bread was carried out by the method of infrared spectroscopy by calculating the reflection coefficient on the spectral index (Figure 1 and Figure 2).

The values of maxima at wavelengths of 1460 nm, 1770 nm, 1930 nm, 2100 nm, 2270 nm and 2350 nm were determined, which were the same for all spectra (both control samples and with the addition of 4% rice protein concentrate). The difference in the values of the relative reflection coefficient in the spectra of the dough of the control sample after kneading and the sample with the addition of 4% rice protein concentrate can be explained by the fact that the inclusion of an additional source of protein led to changes due to the wedging of the protein substances of the additive into the gluten framework, which already began to form by flour proteins.

The extremum at a wavelength of 1460 nm corresponded to valence vibrations of the O-H group. The S-H functional group was presented at a wavelength of 1770 nm.

The wavelength range of 1700-1790 nm indicated the passage of the proteolysis process in the dough. Thus, there were functional sulfhydryl groups S-H (first overtone) and gentle extrema were presented in the spectra of all samples. The spectral index of the control sample of the dough after kneading was 0.17 at the peak, and the sample with the additive was 0.20. This was explained by the fact that supplement proteins affected the course of proteolysis (Neji et al., 2023). During the fermentation process, the spectra of the dough in this range shifted significantly upwards, especially the spectrum of the control sample, the spectral index of which was 0.30, and for the sample with the additive - 0.24. This was explained by the fact that rice protein concentrate, due to its higher moisture binding and moisture retaining capacity, contributed to less dilution of the dough during the fermentation process, which ensured its better shape retention.

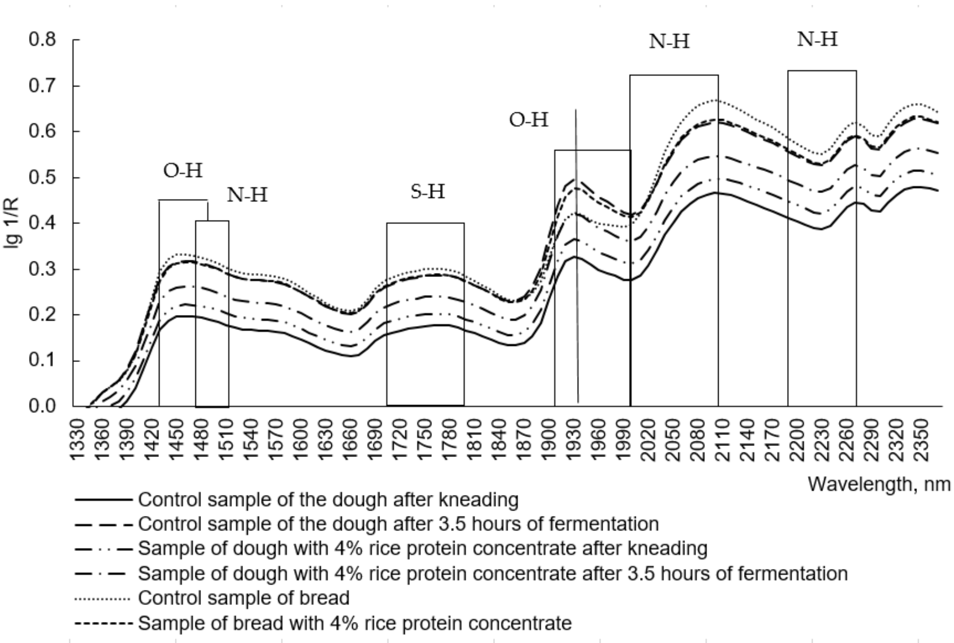

Figure 1. Changes and redistribution of structural groups in bread and dough with 4% rice protein concentrate after kneading and after 3.5 hours of fermentation

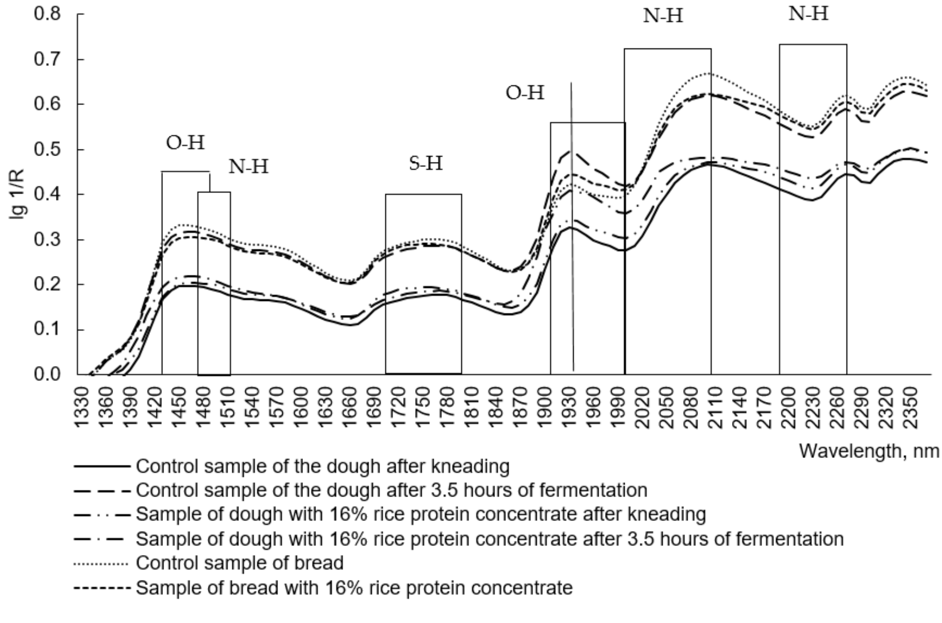

Figure 2. Changes and redistribution of structural groups in bread and dough with 16% rice protein concentrate after kneading and after 3.5 hours of fermentation

During bread baking, high temperatures caused destruction of protein structures (Zhou et al., 2021). The spectra of the bread samples, both control and with the additive, practically overlapped over the entire range of wavelengths. In the wavelength range of 1700–1790 nm, the spectral index of the peak value was 0.30, which coincided with the value of the control sample after fermentation. This was explained by the fact that the main properties of bread were determined by the characteristics of wheat flour and its properties, the share of which was predominant in the recipe.

To characterize protein structures, wavelengths of 1510 nm (N-H valence vibrations), 2060 nm, 2100 nm and 2180 nm (N-H deformation vibrations) were used. There were no peaks at wavelengths of 1510 nm, 2060 nm, and 2180 nm. At a wavelength of 2100 nm, the spectra of the dough after mixing showed that the higher the percentage of the additive was, the lower the spectral index was. So, when adding 4% of rice protein concentrate, it was 0.50, and in case of 16% - 0.47. At the same wavelength, the spectral index of the control sample was 0.46. After fermentation the spectral indices of the control sample, with 4% and 16% additives were 0.62, 0.55 and 0.48. Such values were explained by the fact that rice protein concentrate did not contain gluten proteins and did not participate in the formation of gluten (Ziobro et al., 2015). Instead, its protein substances formed complexes with flour proteins and delayed its development. Due to the properties of rice protein concentrate, the structure of the dough became looser, but the gluten was compacted.

The lignin content can be judged at a wavelength of 2270 nm (O-H valence vibrations / C-O valence combination). Since there was no lignin in rice protein concentrate, the spectrum of bread with it was a bit lower than spectrum of the control sample. This indicated practically the same digestion of bread by the body both with and without the additive (Thilagavathi et al., 2020).

Thus, the addition of rice protein concentrate with a higher protein content and different fractional composition to the recipe of wheat bread affected the structural elements of the dough as its shape-retaining ability improved.

Conclusion

- It was found that amount of protein and fat in rice protein concentrate was higher than in premium wheat flour 6.6 and 4 times.
- Granulometric composition of rice protein concentrate differed from wheat flour because 96% of the particles of wheat flour of premium grade passed through a sieve with holes of 132 microns and for rice protein concentrate this percentage was 4.08.
- Functional and technological properties of rice protein concentrate and wheat flour were characterized by moisture binding and fat binding capacity. For rice protein concentrate they were 4.3 and 1.7 times higher than for wheat flour, and imoisture retaining capacity was 1.8 times higher.
- The infrared spectra of dough and bread showed the difference in the values of the relative reflection coefficient in the spectra of the dough of the control sample and with the addition rice protein concentrate both after kneading and after fermentation. Rice protein concentrate caused delay of the development of the gluten network. At the same time there was positive effect on the dough's ability to keep its shape.

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