

Influence of pumpkin cellulose addition on conformational transformations in the structure of wheat flour dough and bread

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Abstract

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Introduction. The aim of the present study was to determine the effect of pumpkin cellulose addition to wheat flour on conformational transformations in the structure of dough and bread.

Materials and methods. The granulometric composition, functional and technological properties, and amino acid composition of pumpkin cellulose were compared with those of premium grade wheat flour. The influence of pumpkin cellulose in combination with phospholipids on conformational transformations in the structure of dough and bread was studied by method of infrared spectroscopy in the range of near-infrared regions.

Results and discussion. It was found that 96% of the particles of wheat flour of the premium grade passed through a sieve with holes of 132 microns, the remaining 4% – through a sieve with holes of 260 microns. Pumpkin cellulose was much coarser, because all 100% of its particles remained on a sieve (hole size 670 microns). Moisture binding capacity of pumpkin cellulose was 3.6 times higher, and moisture retaining capacity was 2.8 times higher than of wheat flour due to the presence of a significant amount of fibers. The amino acid score of lysine (the limiting amino acid in wheat flour) was 0.44. The amino acid score of methionine (the limiting amino acid in pumpkin cellulose) was 3.16, and the amino acid score of lysine was much higher than in wheat flour 3.49. Partial replacement of wheat flour with pumpkin cellulose (5–15%) increased this indicator for lysine by 6.5–15.2%. It was found that infrared spectra of dough samples after kneading (control sample and sample with the partially flour replacement by pumpkin cellulose) practically overlapped throughout the range of wavelengths. During the fermentation process conformational changes of functional groups occurred intensively as well as changes in structural and mechanical properties. The dough ball of the control sample thinned faster. Shape-retaining ability improved with increasing replacement percentage of wheat flour with pumpkin cellulose.

Conclusions. The partial replacement of wheat flour with pumpkin cellulose enhanced the biological value of bread and changed the structural and mechanical properties improving shape-retaining ability of dough but decreasing dimensional stability of bread.

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Introduction

The modern food industry is characterized by the production of food with useful properties having health benefits (Ivanov et al., 2021). Simultaneously, taking into account ecological and economic requirements, it is promising to convert the waste of food processing into valuable products using them as additives to increase the nutritional value of food (Dora et al., 2020; Jin et al., 2018; Shevchenko et al., 2023; Stabnikova et al., 2021). Thus, it was relevant to include in the recipes of bread pumpkin processing products with a high content of dietary fibers, which are generated during production of puree, juice, candied fruit and pumpkin oil (Jacobo-Valenzuela et al., 2011; Shevchenko et al., 2023).

Study of the influence of pumpkin residue powder (5 – 20%) and pumpkin pomace (10 – 50%) on the quality of wheat bread showed that an initial increase in the addition of pumpkin residue (5%) indicated an increase in bread volume, which began to decrease in case of higher amounts (10 – 20%) (Ozola et al., 2015). Sensory evaluation (appearance, surface, crust, porosity, texture, crumb, taste and aroma) of wheat bread with pumpkin by-products showed high consumer acceptance, except for the sample with 50% pomace addition. The total content of carotene in bread increased due to the addition of pumpkin by-products (Ahmed et al., 2011). For the production of wheat bread, it was recommended to add from 5% to 10% of pumpkin powder and up to 30% of pumpkin pomace to the dough.

The addition of pumpkin flour to the recipe of wheat bread affected its antioxidant activity and total phenolic content. Bread was made by partially replacing wheat flour with pumpkin flour in amounts from 5% to 20%. Bread enriched with 20% pumpkin flour had the highest antioxidant activity measured by ABTS scavenging activity – 81.74% compared to the control sample – 76.59%. The highest phenolic content – 5.39 mg/g (calculated as gallic acid equivalent) was observed in bread enriched with 20% pumpkin flour, compared to 1.38 mg/g in the control sample. That is, the antioxidant activity of bread increased significantly (Wahyono et al., 2020).

It was found that the introduction of pumpkin puree in the amount from 5 to 25% in the recipe of wheat bread practically did not affect the amount of washed gluten from the dough. However, the compression strain of raw gluten was 68.5–94.7 units, which was worse than for samples without it. In bread with pumpkin puree the content of pectin was 0.03 mg/100g, while pectin was absent in the control sample. The content of vitamins and minerals increased, thereby increasing the nutritional value of bread (Bayramov et al., 2022).

As pumpkin products are rich in beta-carotene, a fat-soluble provitamin A carotenoid, it is recommended to add these raw materials to the food products in combination with lipids (Li et al., 2016). A significant content of them is in lecithin that makes it a valuable additive for enriching bread.

Raw materials introduced in bread influence its structure, so the aim of the present study was to determine the effect of pumpkin cellulose addition on conformational transformations of the structure of dough and bread made from wheat flour.

Materials and methods

Materials

Dough samples and bread were prepared from main components – premium wheat flour, salt and pressed baker's yeast. Sunflower lecithin was added in the amounts of 3% by weight of flour (Partridge et al., 2019). 5, 7, 10, 15% pumpkin cellulose was added to replace wheat flour. A sample without pumpkin cellulose was the control sample. All components were mixed and analyzed immediately after kneading and after 3.5 hours of the fermentation. Bread was obtained using a monophasic way of dough preparation.

Methods

Size of the flour particles

Sieve analysis was used for determining size of the particles of flour and cellulose. Sieves with different hole sizes were used: No 33/36 (35) (220 μm), No 27 (260 μm), No 067 (670), No 49/52 PA (43) (132 μm), No 41/43 (38) (160 μm). Sieves were sequentially placed one under the other from the sieve with the largest holes on top to the sieve with the smallest holes at the bottom. The raw material was loaded onto a sieve with the largest hole size and sieved using a vibratory drive device. The percentage ratio of the residue on the sieves and the passage through the sieves was determined (Patwa et al., 2014).

Moisture binding and retaining capacity

For determining 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:

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

where m_1 is weight of precipitate, g; m_2 is weight of the original flour, g.

For determining 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:

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

where m_1 is weight of tube with flour and water retained, g; m_2 is weight of tube with flour, g; m is weight of flour, g.

Fat binding and fat retaining capacities

The procedure for determination of fat binding and retaining capacities was the same as in the determination of moisture binding and retaining capacities, but instead of water, 15 ml of refined sunflower oil was added.

Fat binding capacity (FBC, %) was determined as the difference between the fat content in the flour suspension (F_1) and the amount of fat released after centrifugation (F_2):

$$\text{FBC} = F_1 - F_2,$$

Fat retention capacity (FRC, %) was determined as the difference between the fat content in the flour suspension (F_1) and the amount of fat released during heat treatment (F_{rel}) (Suriano et al., 2017):

$$\text{FRC} = F_1 - F_{\text{rel}}$$

Stability of emulsion

For determining the stability of emulsion, oil and water were mixed in a water bath and cooling. 4 calibrated centrifuge tubes (50 ml) were filled with the obtained emulsion and centrifuged at a frequency of 500 rpm for 5 minutes. Then the volume of the emulsified layer was determined.

The stability of the emulsion was calculated by the formula (Silva et al., 2016):

$$SE = \frac{V_1}{V_2} \cdot 100,$$

where SE is stability of the emulsion, %; V_1 is a volume of emulsified oil, ml; V_2 is a total emulsion volume, ml.

Emulsifying ability

7 g of sample was suspended in 100 ml of water in a homogenizer at a frequency of 66.6 rpm for 60 s. Then 100 ml of sunflower oil was added and the mixture was emulsified in a homogenizer at a frequency of 1500 rpm for 5 minutes. After that, the emulsion was put into 4 calibrated centrifuge tubes with (50 ml) and centrifuged at 500 rpm for 10 minutes. Then the volume of emulsified oil in the layer was determined.

Emulsifying ability was calculated by the formula (Silva et al., 2016):

$$EA = \frac{V_1}{V} \cdot 100,$$

where EA is emulsifying ability, %; V_1 is a volume of emulsified oil, ml; V is a total emulsion volume, ml.

Essential amino acid composition

Amino acid composition in pumpkin cellulose, wheat flour and bread was determined by ion exchange chromatography (Litvynchuk et al., 2022). The process consisted of two stages: hydrolysis of proteins and their quantitative estimation. For this purpose, automatic analyzer of amino acids T-339 (Mikrotechna, Czech Republic) was used. The elution of amino acids was conducted using Li-citrate buffers with pH 2.75 ± 0.01 ; 2.95 ± 0.01 ; 3.2 ± 0.02 ; 3.8 ± 0.02 ; 5.0 ± 0.2 in turn. For Amino detecting amino acids photometer Unicam SP 800 (Great Britain) was used at a wavelength of 560 nm. The process of rectification with a ninhydrin solution was applied. The results of detection were registered by the peaks of light absorption of ninhydrin-positive substances in an eluent. The ratio of concentrations of this substance in solution is direct with these peaks. For obtaining a comparison sample the prototype was diluted in Li-citrate buffer and inflicted on an ion exchange column. The content of every amino acid expressed per 100 g protein.

Amino acid score

Amino acid score was calculated as the ratio of a gram of the limiting amino acid in the food to the same amount of the corresponding amino acid in the standard protein:

$$EAA_{\text{score}} = \frac{EAA_{\text{lim}}}{EAA_{\text{FAO}}},$$

where EAA_{score} is amino acid score; EAA_{lim} is amount of the limiting amino acid in the sample, g; EAA_{FAO} is amount of the corresponding amino acid in the reference standard, the hen's egg protein, g (Caire-Juvera et al., 2013). The Food and Agriculture Organization (FAO) and World Health Organization (WHO) accepted the essential amino acid composition of the hen's egg protein as a reference standard (Lunven et al., 1973).

Shape-retaining capacity

Shape-retaining capacity was determined by dynamics of changes of spread of the dough ball during fermentation. Dough balls with the weigh of 100 g were placed on transparent glass surface at temperature of 30°C for 180 min. Diameter of dough ball was measured every 30 minutes (Arpul et al., 2015).

Near-infrared reflection spectroscopy

Spectra of dough and bread was determined by method of infrared spectroscopy in near infrared range from 1330 to 2370 nm. Infrapid spectrometer (Labor-Mim, Hungary) was used to obtain the reflection spectra from smooth surface of shredded samples. The process consisted of two stages: on the first stage, the spectrometer recorded the reflectance spectrum from referential sample, on the second stage a reflection spectrum from the researched sample. The intensity of reflection was measured in dough after kneading and fermentation and in bread (Shevchenko and Litvynchuk, 2022b). The reflection intensity was calculated as transformation of relative reflection coefficient to spectral index (Yip et al., 2012).

Statistical analysis

The data represents the mean of a minimum three replicates \pm standard deviation (S.D.). Graphical presentation of experimental data was performed using program Microsoft Excel 2010.

Results and discussions

Microbiological, biochemical processes in the dough, its structural and mechanical properties, structural changes are significantly influenced by the chemical composition and size of the components of the recipe. It was found that 96% of the particles of wheat flour of the premium grade passed through a sieve with holes of 132 microns, the remaining 4% – through a sieve with holes of 260 microns. Pumpkin cellulose is much coarser, because all 100% of its particles remained on a sieve with hole size 670 microns.

In the process of dough preparation, biochemical, colloidal processes and structure formation of the dough system take place. As a result the structural and mechanical properties of dough and bread are formed. A significant role in these processes belong to the ability of biopolymers of raw materials to absorb and retain moisture and fat, which is introduced with recipe components (Table 1).

Table 1
Functional and technological properties of premium wheat flour and pumpkin cellulose

Indicator, %	Wheat flour	Pumpkin cellulose
Moisture binding capacity	90.7 \pm 2.13	330 \pm 2.93
Moisture retaining capacity	148 \pm 2.89	415 \pm 3.02
Fat binding capacity	146 \pm 2.89	216 \pm 2.90
Fat retaining capacity	164 \pm 2.89	280 \pm 2.91
Emulsifying ability	36 \pm 0.50	27 \pm 0.52
Stability of emulsion	31 \pm 0.51	6.8 \pm 0.24

Functional and technological properties of raw materials, particularly the ability to bind and retain moisture and fat, form an emulsion were important (Berton et al., 2002). Due to these characteristics of the raw materials viscoelastic dough was formed, which provided structure and technological properties of dough to obtain high quality bread. Raw materials with less particle exchange usually have a higher index moisture binding capacity and moisture retaining capacity (Changgao et al., 2022). However, both indicators resulted significantly higher in pumpkin cellulose than in wheat flour because of the higher content of fiber present in pumpkin cellulose (Jurgita et al., 2014). Moisture binding capacity of pumpkin cellulose was 3.6 times higher, and moisture retaining capacity 2.8 times higher than of premium wheat flour. The increase of moisture binding capacity of pumpkin cellulose was explained by release of side polar groups of protein, areas of fibers, which had hydrophilic properties and soluble molecules (Qiao et al., 2019).

Meanwhile, values of fat binding and fat retaining capacities were higher for pumpkin cellulose than for wheat flour 1.5 and 1.7 times, respectively. It was explained by the higher content of hydrophobic polysaccharides in pumpkin cellulose. The different distribution of hydrophobic and hydrophilic particles in the composition of pumpkin cellulose compared to wheat flour caused higher values of fat binding and fat retaining capacities (Wang et al., 2017)

In the recipe of bakery products, fat components of plant or animal origin were used, which were difficult to be distributed evenly during the dough kneading process. The ability to form stable emulsions was characterized by emulsifying ability and stability of emulsion. The decrease of these properties for pumpkin cellulose was because it contained lignin, which promoted the formation of interpolymeric bonds between polysaccharides resulting in binding of protein molecules (Aminzadeh et al., 2017).

Pumpkin cellulose contained a large amount of dietary fiber and protein (Shevchenko et al., 2023). The content of essential amino acids (EAA) in pumpkin cellulose was significantly higher than in premium wheat flour (Table 2).

Table 2
Content of essential amino acids (EAA) in pumpkin cellulose and premium wheat flour

EAA	Content, g/100 g of raw material	
	Wheat flour	Pumpkin cellulose
Valine	0.42±0.01	1.64±0.01
Isoleucine	0.36±0.01	1.36±0.01
Leucine	0.71±0.02	2.57±0.02
Lysine	0.23±0.01	1.35±0.01
Methionine	0.40±0.01	0.78±0.01
Threonine	0.28±0.01	1.12±0.01
Tryptophan	0.13±0.01	0.72±0.01
Phenylalanine	0.52±0.01	1.86±0.01

The protein of wheat flour was not complete; therefore, to increase the biological value of bread, it was advisable to add pumpkin cellulose. The protein profile of bread with it will increase. The amino acid score, that is the percentage content of each amino acid in relation to its content in the protein taken as a standard, of the limiting amino acid in wheat flour, lysine, was 0.44. The amino acid score of every standard amino acid was 1. For methionine – the limiting amino acid in pumpkin cellulose it was 3.16, and the amino acid score of lysine was much higher than in wheat flour, 3.49. It was found that the amino acid score of the

limiting EAA in pumpkin cellulose was higher than 1. It indicated that the protein of it was complete. Therefore, pumpkin cellulose will increase content of essential amino acids in bread when it is added to the recipe compared to the control sample that was bread without pumpkin cellulose (Table 3).

Table 3
Content of essential amino acids (EAA) in bread with partial replacement of wheat flour with pumpkin cellulose

EAA	Content, g/100 g of bread				
	Control	Pumpkin cellulose to replace wheat flour, %			
		5	7	10	15
Leucine	0.71±0.01	1.14±0.02	1.28±0.02	1.48±0.02	1.76±0.02
Isoleucine	0.39±0.01	0.40±0.01	0.41±0.01	0.41±0.01	0.42±0.01
Methionine	0.31±0.01	0.42±0.01	0.46±0.01	0.52±0.01	0.59±0.01
Lysine	0.23±0.01	0.25±0.01	0.26±0.01	0.26±0.01	0.27±0.01
Phenylalanine	0.68±0.01	0.64±0.01	0.62±0.01	0.61±0.01	0.58±0.01
Threonine	0.28±0.01	0.47±0.01	0.54±0.01	0.62±0.01	0.75±0.01
Valine	0.43±0.01	0.70±0.01	0.80±0.01	0.93±0.01	1.11±0.01
Tryptophan	0.09±0.01	0.23±0.01	0.27±0.01	0.34±0.01	0.43±0.01

The amino acid score for all essential amino acids in bread showed that the limiting amino acid was lysine with score 0.46. Partial replacement of wheat flour with pumpkin cellulose (5–15%) increased lysine score by 6.5–15.2% (Table 4).

Table 4
Amino acid score of essential amino acids (EAA) in bread with partial replacement of wheat flour with pumpkin cellulose

EAA	Amino acid score				
	Control	Pumpkin cellulose to replace wheat flour, %			
		5	7	10	15
Leucine	1.11	1.76	1.98	2.28	2.70
Isoleucine	1.06	1.09	1.09	1.11	1.12
Methionine	0.97	1.31	1.43	1.59	1.82
Lysine	0.46	0.49	0.50	0.52	0.53
Phenylalanine	1.23	1.15	1.12	1.09	1.04
Threonine	0.77	1.28	1.45	1.68	2.01
Valine	0.93	1.53	1.73	1.99	2.37
Tryptophan	0.98	2.45	2.95	3.62	4.56

Nutrients of recipe components of dough and bread contained various functional groups, the change and redistribution of which was largely influenced by the protein composition of the recipe components and their properties. Chemical composition of wheat flour and pumpkin cellulose differed. It was assumed that pumpkin cellulose addition will affect the change in the structural units – OH, NH and SH groups in dough and bread. These units were analyzed in the near infrared region using the reflection spectrum (Baslar et al.,

2011). The results of research of dough and bread samples with the minimum researched replacement (5%) of wheat flour with pumpkin cellulose (Figure 1a) showed that spectra of control dough sample and samples with replacement after kneading and after fermentation, as well as bread samples had a similar character. However, the intensity of reflection was different.

It was found that samples of dough after kneading (control sample and sample with the replacement part of wheat flour by pumpkin cellulose) practically overlapped throughout the range of wavelengths except for the extremum at the wavelength 1930 nm where relative reflection coefficient was 0.47 and 0.49. Overlapping was explained by the fact that the biopolymers of the recipe components did not have time to interact. The difference on the wavelength 1930 nm was explained by the higher moisture binding capacity of pumpkin cellulose (Table 1) which required adding more water to the dough.

Since the proteins of the recipe components were involved in the formation of the gluten frame, they underwent changes during the formation and fermentation of the dough. The lowest extremum at a wavelength of 2100 nm characterized protein substances of the dough (Kröncke and Benning, 2022). The relative reflection coefficient of the control sample and the sample with replacement after kneading was 0.37.

During the fermentation process, conformational changes of functional groups occurred intensively, so spectra of the fermented dough were situated below. The intensity of reflection of the control dough sample was lower than of the sample with pumpkin cellulose. The relative reflectance of the control sample and sample with replacement at a wavelength of 2100 nm was 0.25 and 0.29. It meant that proteins of pumpkin cellulose did not participate in the formation of gluten. This was explained by the fact that its proteins had a globular structure, and pumpkin cellulose contained a large amount of dietary fibers. They were embedded in the gluten framework and delayed its development (Alfaris et al., 2022). That is why the structure of the protein matrix of dough with this component was less stable and more weakened.

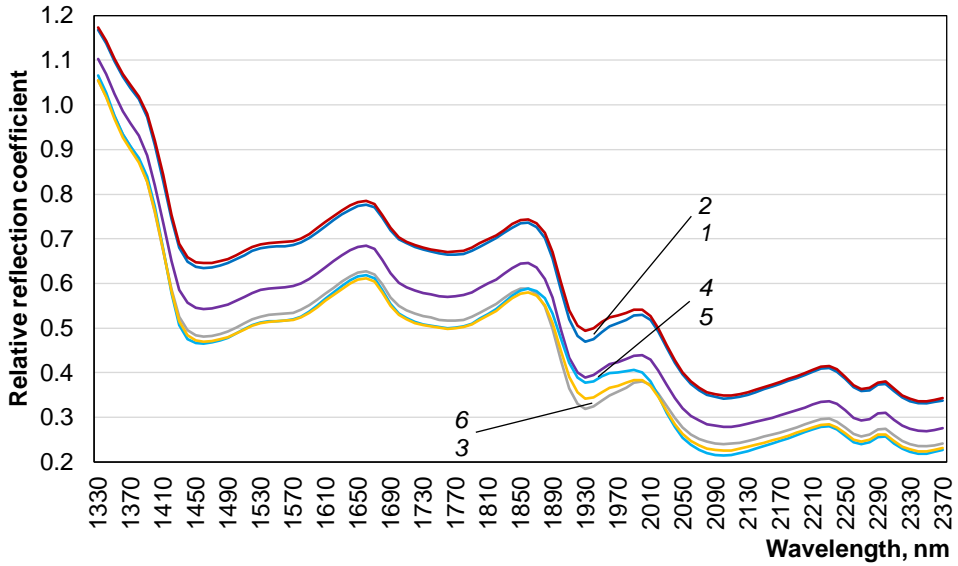
The infrared spectra of bread in terms of intensity practically coincided with the control sample of the dough after fermentation. High temperatures led to the destruction of macromolecules of protein, splitting peptide bonds (Zhou et al., 2021).

The spectra of dough and bread with 15% pumpkin cellulose had a similar character as when replacing 5% (Figure 1b).

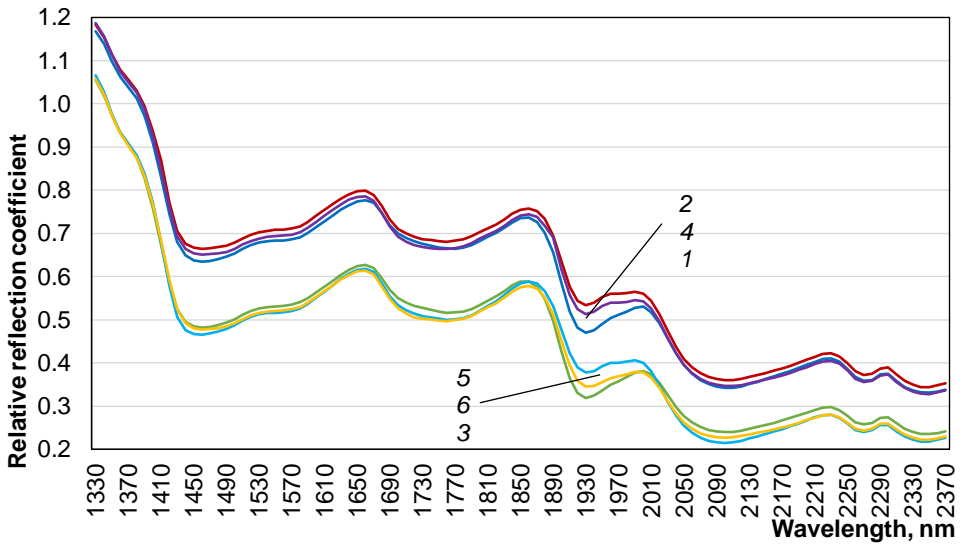
The spectra of the dough after mixing, as in the case of 5% pumpkin cellulose, had the highest relative reflectance in the entire range of wavelengths. However, due to the introduction of a large amount of dietary fiber, the development of the gluten framework of the dough was delayed, pumpkin cellulose significantly binded water and the fermentation process slowed down. It is very visible at the wavelength of 2100 nm because the relative reflectance of the sample of dough with 5% pumpkin cellulose after fermentation was 0.36, which is significantly higher than the control sample and the sample with 5% replacement.

The spectrum of the dough of the control sample after fermentation was close to the spectra of bread. The difference was observed at the wavelength of 1930 nm, which is because of the different moisture content in the samples. Technologically it confirms that the addition of 15% pumpkin cellulose will contribute more to less dilution of the dough ball during fermentation (Figure 2).

It was found that during the period of fermentation, the dough ball of the control sample thinned faster. This is explained by the fact that the content of fiber and pentosans in pumpkin cellulose increased viscosity of the dough system in samples with this recipe component (Apostol et al., 2020). Shape-retaining ability improved with increasing replacement percentage.



a



b

Figure 1. Reflection spectra of dough and bread:
a – with 5% pumpkin cellulose; *b* – with 15% pumpkin cellulose

- 1 - Control sample of the dough after kneading
- 2 - Sample of dough with 5% pumpkin cellulose after kneading
- 3 - Control sample of the dough after 3.5 hours of fermentation
- 4 - Sample of dough with 5% pumpkin cellulose after 3.5 hours of fermentation
- 5 - Control sample of bread
- 6 - Sample of bread with 5% of pumpkin cellulose

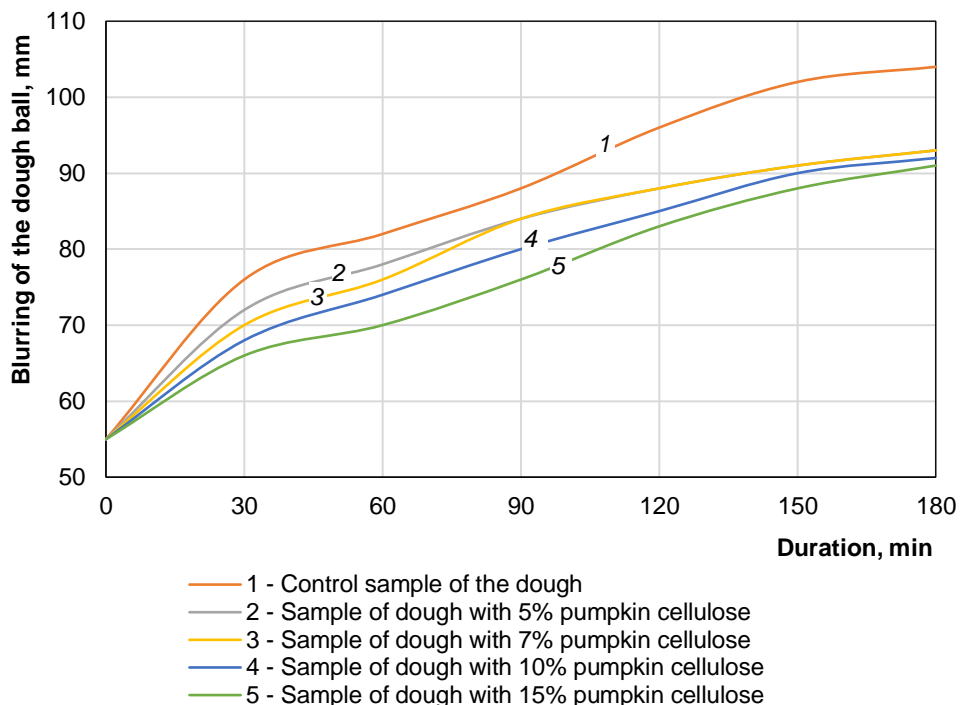


Figure 3. Dynamics of changes in the shape-retaining ability of the dough during fermentation

Thus, pumpkin cellulose improved the biological value of bread due to the higher content of dietary fiber, protein and complete amino acid profile. It also affected the structural and mechanical properties of dough and bread improving shape-retaining ability of dough but decreasing dimensional stability of bread.

Conclusions

1. The pumpkin cellulose had larger particle sizes than wheat flour because all 100% of its particles remained on a sieve with hole size 670 microns. At the same time 96% of the particles of wheat flour of the premium grade passed through a sieve with holes of 132 microns.
2. Pumpkin cellulose binds water 3.6 times better and retains water 2.8 times better than wheat flour.
3. The limiting amino in wheat flour is lysine with amino acid score 0.44. Addition of pumpkin cellulose in bread (5–15%) increased the amino acid score for lysine by 6.5–15.2%.
4. During the fermentation process in dough with pumpkin cellulose conformational changes of functional groups occurred more intensively than in samples without it.
5. Addition of pumpkin cellulose affected the structural and mechanical properties of dough and bread, improving shape-retaining ability of dough but decreasing dimensional stability of bread.

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