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ORIGINAL PAPER

Transformative shifts in dough and bread structure with pumpkin seed protein concentrate enrichment

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Abstract

Pumpkin seed protein concentrate, a promising raw material with high biological value, holds potential for incorporation into bread technology. Protein structural changes occur during the bread-making process. This study aimed to investigate how pumpkin seed protein concentrate incorporated at different concentrations (5, 10, and 20%) affected the structural, mechanical properties, and conformational changes in protein substances within dough and bread made from wheat flour, utilizing near-infrared reflection spectroscopy. The gas- and shape-holding capacities of dough enriched and unenriched by pumpkin seed protein concentrate resulted similar. This aspect is important for the impact of the final product as bread on the consumers. The near-infrared reflection spectroscopy analysis revealed that the enrichment of bread with pumpkin seed protein concentrate not only introduced higher protein and unsaturated fat content in bread but also reduced the level of lignin, enhancing the digestibility of the final product. A deeper investigation into the nutraceutical and nutritional value of this enriched bread as well as of physio-chemical features investigated in the present work could be useful for the introduction of a new functional food in the market. Moreover, the enriched bread was acceptable in terms of sensory quality analysis, especially using 10% pumpkin seed protein concentrate. Therefore, to better understand the market potentiality of the enriched bread proposed in this study, future research should investigate how consumer acceptability could increase with a higher concentration of nutritional components perceived as beneficial for a lifestyle by specific consumer segments.

Keywords Bread · Dough · Pumpkin seed protein concentrate · Near-infrared reflection spectroscopy · Protein · Structural properties

Introduction

In recent years, new trends have emerged in bread baking that see the use of alternative flours to wheat flour. The reasons for this change can be due to various economic, nutritional, and environmental issues. From an environmental point of view, plant-based food source can mitigate the environmental impact along with the shorter food supply chain and the use of native crops [1]. From a market perspective, there are several aspects to consider. On the one hand, producers might be interested in expanding their product range by introducing new items with diverse sensory and nutritional properties, thereby diversifying their product lines. On the other hand, they may aim to meet emerging nutritional trends, which have witnessed a reduction in the consumption of wheat in favor of functional products that have the potentiality to encourage consumers to alter their consumption patterns in line with these new trends. Similarly, the bakery products sector seems to follow the same

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trend. During the last few years, a trend of using non-traditional raw materials to increase the nutritional value of bakery products is increasing [2]. At a broader level, researchers have observed a growing trend among consumers, who are showing heightened interest and awareness regarding the connection between health and nutrition. People are becoming more thoughtful and open to embracing dietary changes focused on improving their health [3]. Hilton [4] highlighted the emergence of a convergence among product categories such as food, beverages, and pharmaceuticals, which is reshaping our perception of a healthy lifestyle. Consumers, once they identify the nutritional components from which they can benefit, prefer to incorporate them into their diet in a non-intrusive manner, primarily through regular consumption. In this regard, pumpkin processing products deserve attention.

Pumpkin is an excellent source of fiber, calcium, magnesium, and antioxidants. To determine the optimum substitution of wheat flour with pumpkin flour in bakery products, various research studies have been conducted [5–10]. For instance, when replacing 5%, 10%, or 15% of wheat flour with pumpkin flour, the protein content increased from 7.46% to 7.75% due to the higher protein content of this raw material when compared with that of wheat flour [7].

Significant variations were observed in fat, fiber, ash, and sugar content, ranging from 24.43% to 30.36%, 1.57% to 2.49%, 0.90% to 1.78%, and 0.18% to 0.22%, respectively [7]. Antioxidant activity was assessed using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging, revealing that replacing 5% of wheat flour with pumpkin seed flour led to a 39.87% increase in antioxidant activity [10]. When substituting 25%, 50%, and 75% of wheat flour with pumpkin seed flour for making cupcakes with improved texture, cohesion, pH, and soluble solids, firmness increased to 6.4 N, cohesiveness to 0.50, and chewiness to 311. Elasticity remained relatively consistent. The use of pumpkin seed flour impacted the specific volume of cupcakes, with the highest amount of pumpkin seed flour resulting in the lowest value [5]. The content of primary and secondary lipid oxidation products was measured for muffins with 17%, 33%, and 50% wheat flour replaced by pumpkin seed flour and stored for up to 4 weeks. This increase in lipid oxidation products was due to a higher content of linoleic acid, containing two *cis* double bonds that make it more sensitive to oxidation, in pumpkin seed flour and in experimental muffins than in control muffins [6]. The peroxide value increased to 11.5 meq/kg in the sample with 50% pumpkin seed flour by the third week of storage. This is due to the oxidative decomposition of linoleic acid. The same sample also exhibited the highest content of secondary lipid oxidation products [6]. The substitution of wheat flour with pumpkin powder (5%, 10%, 15%, and 20%) in biscuit recipes resulted in an increase in β-carotene content from 6.84 to 9.78 mg per 100 g of dry weight (DW) [8]. The density, ash content, and protein content of the baked biscuits increased, while moisture content and sample volume exhibited an opposite trend [8]. According to sensory evaluation, replacing 10% of wheat flour with pumpkin powder was deemed the most palatable [8]. Furthermore, substituting wheat flour with pumpkin seed flour contributed to reducing lipid peroxidation and extending the shelf life of bakery products due to the high antioxidant activity of this pumpkin by-product [10].

Researchers have evaluated the use of pumpkin seed protein concentrate and pumpkin protein isolate in bread production [11–13]. For example, pumpkin seed protein concentrate can be added to wheat flour up to 18%, and pumpkin protein isolate can be added up to 22% without negatively affecting dough or bread quality [11]. The addition of pumpkin proteins increased the protein content of bread by 11–38% and enhanced the content of essential amino acids (EAA) by 55–80% compared to the control bread [11]. Pumpkin seed protein concentrate was added at various levels to wheat flour to achieve mixtures with protein levels of 15%, 17%, 19%, and 21% [12]. As the protein level increased, dough stability time decreased and dough softening increased [12].

Organoleptic assessments considered crust color, breadcrumb color, breadcrumb texture, taste, and overall quality. Results indicated that up to 19% of pumpkin seed protein concentrate could be added to wheat flour without adverse effects on dough or bread quality [12].

More recently, pumpkin protein concentrate was utilized to enrich gluten-free bread with protein, affecting texture, volume, color, and visual aspects [13]. The inclusion of pumpkin protein concentrate decreased bread volume but had no significant impact on hardness and breadcrumb elasticity [13].

Despite existing literature on the visual quality, sensory profile, and physical characteristics of bakery products enriched with pumpkin protein concentrate, there has been no investigation into the conformational changes in the structure of protein substances in wheat flour dough and bread resulting from this enrichment. Therefore, this study aimed to examine the impact of adding pumpkin seed protein concentrate on the structural changes in the protein substances of wheat flour dough and bread.

Materials and methods

Preparation of flour, pumpkin seed protein concentrate, dough, and bread

The physicochemical composition, including total protein content, total fiber content, EAA composition, particle size, as well as the properties such as moisture binding and fat binding capacity, were assessed in both commercial wheat flour from “Kyivmlyn” LLC, Ukraine, and pumpkin seed protein concentrate from “Organic Oils” LLC, Ukraine. To produce the pumpkin seed protein concentrate, pumpkin seed meal was extracted with petroleum ether for 30 min (meal to solvent ratio 1:4 w/v) followed by extraction with 75% ethanol for 30 min (meal to solvent ratio 1:10 w/v). Both extractions were carried out at room temperature. The protein concentrate was dried using a vacuum oven (Type SPT-200, vacuum Dryer, Krakow, Poland) at 40 °C for 8 h. Dough samples were prepared by combining in turn the following ingredients in this order: 100 g of wheat flour, 1.5 g of salt (previously prepared a saline solution), 3 g of pressed baker's yeast (previously prepared a yeast suspension in the ratio of yeast to water—1:3 w/v), 3 g of sunflower lecithin, and varying amounts of pumpkin seed protein concentrate (5%, 10%, and 20% of the total wheat flour weight). Sunflower lecithin, as a source of phosphatidylcholine, participates in the formation of mucin and from the technological point of view it contributes to obtaining an elastic dough [14], thereby being advisable to add it in the recipe of dough. Mass of dough enriched with 5, 10 and 20% pumpkin seed protein concentrate was 158 ± 3, 168 ± 3, and 186 ± 3 g, respectively, while the mass of ready bread was 140 ± 3, 140 ± 3, and 164 ± 3 g, respectively. A control sample without any pumpkin seed protein concentrate was also prepared (Cnt). Mass of Cnt dough was 150 ± 3 g, while the mass of Cnt ready bread was 130 ± 3 g. After analyzing the dough samples, bread was produced using different concentrations of pumpkin seed protein concentrate (5%, 10%, and 20%). The dough was made in a single-phase method. At the first stage, the saline solution and yeast suspension were prepared to distribute the salt and yeast evenly in the mass of the dough. Then, all recipe components were mixed in two-speed dough kneading machine (KEMPER, Germany) for 10 min. After that dough was fermented at temperature 35 ± 2 °C, humidity 75–85%, during 3.5 h. Then, it was divided into portions of 300 g, put to the forms and left for proofing in resting cabinet, until getting ready for baking. After that, it was baked at 220 °C for 25 min in a ventilated oven (Sveba-Dahlen AB, Fristad, Sweden). The gas-holding and shape-holding capacities of the dough were examined. Spectroscopic profiles of the dough were analyzed immediately after kneading and after a 3.5-h fermentation period. Finally, spectroscopic analysis was conducted on the resulting bread.

Total protein and fiber content

The Kjeldahl method was employed to assess the total protein content in pumpkin seed protein concentrate. A gram of raw material was subjected to hydrolysis with 15 mL of sulfuric acid, which included two copper catalyst tablets, for a period of 2 h in a heat block set at 420 °C (FOSS A/S, Hillerød, Denmark). Following cooling, distilled H_2O was introduced to the hydrolysates before neutralization and subsequent titration, as outlined in Maehre et al. [15]. The total protein content was expressed as a percentage.

A collaborative study was undertaken to ascertain the total dietary fiber content in the wheat flour and in the pumpkin seed protein concentrate, utilizing the enzymatic–gravimetric method as described by McCleary et al. [16]. The total dietary fiber content was calculated by subtracting the weight of protein and ash from the weight of the residue. The total fiber content was expressed as a percentage.

EAA composition

The determination of EAA composition followed the methodology outlined by Huang et al. [17]. To determine amino acids, proteins were hydrolyzed, and their quantitative estimation was conducted using an automatic amino acid analyzer, T-339 (Mikro-techna Praha a.s., Praha, Czech Republic), employing polystyrene sulfonate ion exchange resins labeled as “Ostion LJ ANB” qualitatively and quantitatively within a Li-citrate buffer, utilizing a one-column mode.

Amino acid elution was performed sequentially using Li-citrate buffers with the following pH values: 2.75 ± 0.01, 2.95 ± 0.01, 3.2 ± 0.02, 3.8 ± 0.02, and 5.0 ± 0.2. Amino acids were detected at a wavelength of 560 nm through rectification with a ninhydrin solution using a photometer (Unicam SP 800, Uni-cam Instruments, Cambridge, Britain). The results were recorded as absorption peaks of ninhydrin-positive substances in the eluent. The correlation between the ninhydrin reagent and eluent was maintained at a ratio of 1:2.

The prototype was diluted in Li-citrate buffer adjusted to a pH of 2.2 ± 0.02 and introduced into an ion exchange column (liquid-column, Bio-Rad, Hercules, USA). The quantitative estimation of chromatograms in the preproduction model was compared to the Bio-Rad standard amino acids mixture (Bio-Rad, Hercules, USA).

The content of each amino acid (A_i), expressed as percentage in the analyzed solution, was calculated using the following formula:

$$A_i = \frac{M_i \cdot S_i}{S_i^3},$$

where M_i is molecular mass of each amino acid; S_i is a peak area of each amino acid on an aminogram from the investigated solution; S_i^3 is an area of peak of each amino acid on an aminogram from solution of standard mixture of amino acids which accords to one micromole.

Particles size

The particle size analysis of both wheat flour and pumpkin seed protein concentrate involved the use of sieves with various sieve fabrics (including metal woven and polyamide) and differing hole sizes. These sieves were specified as follows: N-067 with a hole size of 670 µm, N 27 with a hole size of 260 µm, N 33/36 (35) with a hole size of 220 µm, N 41/43 (38) featuring a hole size of 160 µm, and N 49/52 PA (43) with a hole size of 132 µm, as detailed in the study conducted by Patwa et al. [18].

Moisture binding and fat binding capacity

For moisture determination, the centrifugation technique was employed, following the approach described by Raikos et al. [19]. In this method, 0.5 g of the raw material was deposited into pre-weighed centrifuge tubes. Subsequently, 50 mL of distilled water was introduced into the tubes, which were then subjected to centrifugation at 3500 rpm for a duration of 10 min. After the excess water was drained, the raw material within each tube was dried and re-weighed. The moisture binding capacity (MBC, %) was computed using the following formula:

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

where m_1 is the weight of precipitate expressed in g; m_2 is the weight of the wheat flour or of the protein concentrate expressed in g.

To determine the fat binding capacity, the centrifugation method, as outlined in Raikos et al. [19], was employed. In this procedure, 0.5 g of the raw material were deposited into pre-weighed centrifuge tubes. Then, 50 mL of refined sunflower oil were introduced into the tubes, which were subsequently centrifuged at 3500 rpm for a period of 10 min. The fat binding capacity (FBC, %) was calculated using the following formula:

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

where $F1$ is the fat content in the flour or protein concentrate suspension expressed as % and $F2$ is the amount of fat released after centrifugation expressed as %.

Gas-holding and shape-holding capacity

The gas-holding capacity is defined by the specific volume of the dough during its fermentation. The alteration in the specific volume of the samples was measured in a 250 cm^3 measuring cylinder within a thermostat TC-80 (MICROmed, Shanghai, China) at a temperature of 30 °C, starting from the beginning of fermentation until the dough dropped, and was expressed in cm^3 per gram (cm^3 g^{-1}) [20].

On the other hand, the shape-holding capacity was characterized by monitoring the expansion of the dough ball during fermentation. A 100-g portion of dough was placed on a round glass surface, and the initial diameter of the ball was measured. The changes in its diameter were observed over a 180-min period at a temperature of 30 °C in the TC-80 thermostat (MICROmed, Shanghai, China).

Near-infrared reflection spectroscopy

The investigation of reflection intensity involved the use of an Infrapid spectrometer (Labor-Mim, Esztergom, Hungary) within the near-infrared range spanning from 1330 to 2370 nm. Smooth surfaces and shredded samples of both wheat flour and pumpkin seed protein concentrate, as well as doughs immediately after kneading and after a 3.5-h fermentation period, along with bread, served as subjects for studying the reflection spectra.

The spectrometer initially recorded the reflectance spectrum using a reference, denoted as I_0 (an integral component of the instrument). Subsequently, a reflection spectrum from the sample under examination, labeled as I, was obtained. These spectra are presented in terms of reflectivity, denoted as R and expressed in relative units, signifying the ratio of intensities ($I/I_0 = R$) as a function of wavelength in nm [21–23]. The reflection intensity was quantified using the relative reflection coefficient.

Bread quality evaluation

Expert assessors evaluated the quality of the bread by examining its adherence to established quality criteria and identifying any quality deficiencies present in the bread. Various attributes, including specific volume, shape retention, crust color, surface condition, crumb color, porous structure, crumb elasticity, aroma, taste, and crumb chewability were assessed using predefined quality ratings. These ratings were within a scale ranging from 1 to 5 for each quality parameter, with ratings of 4–5 signifying excellent quality, 3–4 indicating good quality, 2–3 denoting satisfactory quality, and 1–2 representing unsatisfactory quality, as indicated in the work by Sammalisto et al. [24]. During the sensory evaluation, the weighting factor for each parameter was also taken into account. Given that the sum of the weighting factors for all parameters equaled 1, experts assigned a rank to indicate the importance of each parameter in the evaluation of bread by consumers. Consequently, the higher the importance of a parameter according to the experts' evaluation, the greater its corresponding weighting factor.

Statistical analysis

The results of the analyses conducted on wheat flour and pumpkin seed protein concentrate were compared using a two-tailed Student's *t*-test, with a significance level set at 0.05. As for the analyses of different doughs, a one-way analysis of variance (ANOVA) was applied, considering the various concentrations of pumpkin seed protein concentrate (0%, 5%, 10%, 20%) as the source of variation. Post hoc comparisons of all means were carried out using the Least Significant Difference (LSD) test ($p < 0.05$). The normality of the data was assessed using the Shapiro–Wilk test, while homoscedasticity was examined using Bartlett's test. Data are presented as mean values accompanied by their respective standard deviations (SD). The statistical analysis was conducted using GraphPad (GraphPad, La Jolla, CA, USA).

Results and discussion

Protein, fiber, amino acid composition, and functional properties of wheat flour and pumpkin seed protein concentrate

Total protein content in pumpkin seed protein concentrate (58.4%; data not shown in graph) was 5.1-fold higher than in wheat flour (10.3%) as reported by Litvynchuk et al. [9]. Fiber content (Fig. 1) was 2.3-fold higher in pumpkin protein concentrate than in wheat flour. It means that pumpkin seed concentrate resulted able to enrich the bread with the main macronutrients such as proteins and fibers.

Pumpkin seed protein concentrate reported high biological value, thanks to a higher presence of EAA than that found in the wheat flour (data reported by Litvynchuk et al. [9]). Table 1 reported the EAA content found in pumpkin seed protein concentrate.

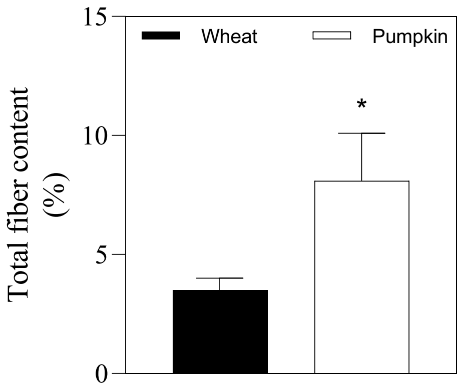


Fig. 1 Total fiber content (%) of wheat flour (closed bar) and pumpkin seed protein concentrate (open bar). Data were compared with Student's *t*-test ($p \leq 0.05$). Significance *$p \leq 0.05$

Table 1 The content of essential amino acids (EAA) in pumpkin seed protein concentrate

EAA (%)	Wheat flour (as reported by Litvynchuk et al. [9])	Pumpkin seed protein concentrate
Valine	0.42 ± 0.01	2.6 ± 0.1
Isoleucine	0.36 ± 0.01	2.8 ± 0.1
Leucine	0.71 ± 0.02	3.75 ± 0.1
Lysine	0.23 ± 0.01	1.42 ± 0.1
Methionine	0.40 ± 0.01	1.7 ± 0.1
Threonine	0.28 ± 0.01	2.1 ± 0.1
Tryptophan	0.13 ± 0.01	1.07 ± 0.1
Phenylalanine	0.52 ± 0.01	3.14 ± 0.1

The predominant EAA of pumpkin seed protein concentrate resulted the leucine. This was due to the high level of this amino acid in pumpkin seeds, which was already noted by other authors [25]. The lowest EAA content was reported for the tryptophan. This result agrees with amino acid profile analyzed by Nourmohammadi et al. [26] in hydrolyzed pumpkin (*Cucurbita pepo* L.) oil cake protein where tryptophan reported the lowest content (about 2%) among the EAA.

Moreover, granulometric composition of wheat flour and pumpkin seed protein concentrate was determined (Table 2). Particles of pumpkin seed protein concentrate were coarser than of wheat flour (Table 2). This is confirmed by the fact that more particles of pumpkin seed protein concentrate remain on sieves with larger holes than those of wheat flour. It can be due to the higher content of fiber in pumpkin seed protein concentrate than wheat flour (Fig. 1). Indeed, the granulometric fraction with the highest diameter of particles in the wheat flour is represented by bran that is rich in fiber [27] and the role of bran/fiber in interaction with starch have already been associated to influences on bread rheological features [27, 28]. However, utilizing the sieve with the smaller holes (132 μm), the percentage of the residue

Table 2 The percentage of residue of pumpkin seed protein concentrate and wheat flour on sieves with different holes diameter (670, 260, 220, 160, 132 μm)

Size indicators (no. of sieve)	Hole size (μm)	Wheat flour (%)	Pumpkin seed protein concentrate (%)
067	670	0.02 ± < 0.01	1.28 ± 0.01***
27	260	1.34 ± < 0.01	6.74 ± 0.01***
33/36	220	2.44 ± < 0.01	7.24 ± 0.01***
41/43 (38)	160	3.89 ± < 0.01	28.86 ± 0.10***
49/52 PA (43)	132	92.31 ± 0.10***	55.88 ± 0.10

Data were compared with Student's *t*-test ($p \leq 0.05$). Significance ***$p \leq 0.001$

of the pumpkin seed protein concentrate in the sieve was lower than that of the wheat flour (Table 2), likely due the lower presence of wheat flour in the enriched product and consequently of bran content, that was replaced by pumpkin seed protein concentrate, which predominantly comprises proteins with smaller particle sizes compared to both wheat flour and, in particular, wheat flour bran.

The ability to form viscoelastic dough is affected by functional and technological properties of raw materials, such as ability to bind moisture and fat [29] as well as by their physicochemical characteristics. Properties of dough are important for obtaining high-quality bread with necessary structure [30]. Figure 2 reported the MBC and FBC percentages of wheat and pumpkin seed protein concentrate samples. MBC of pumpkin seed protein concentrate resulted significantly higher (+ 49.4%) than that of wheat flour. This was due to the protein fractional composition and higher fiber content present as hydrophilic constituents of this raw material, since these components of the concentrate had better adsorbing properties [31]. The hydroxyl group present in the fiber structure allowed a good interaction between the total fiber present in pumpkin seed protein concentrate and the water present in the dough. This could also be attributed to the substantial water-hydrating capability of pumpkin seed products, aligning with findings documented by El-Adawy et al. [32]. The FBC of pumpkin seed protein concentrate was notably lower (− 55%) compared to wheat flour, primarily because of the prevalence of a higher proportion of both hydrophilic and hydrophobic groups on the protein molecules' surfaces, as discussed by Atuonwu et al. [31] and also because of the higher hydrophobicity of protein fractions of pumpkin seed protein concentrate when compared to protein fractions of wheat flour.

Structural and mechanical properties of doughs

Gas-holding and shape-holding capacities of dough affect bread structure [33]. These parameters were not influenced by the addition of pumpkin seed protein concentrate (Fig. 3).

Indeed, no significant differences were found between dough with 100% wheat flour and dough with the addition of 5%, 10% and 20% pumpkin seed protein concentrate in terms of gas-holding capacity (2.08, 2.02, 1.94, and 1.86 $cm^3 g^{-1}$ in Cnt dough, 5%, 10% and 20% enriched dough, respectively) and shape-holding capacity (104, 102, 98, and 96 mm in Cnt dough; 5%, 10%, and 20% enriched dough, respectively). These results are important for the repeatability and the maintenance of the dough structure. In fact, the gas- and shape-holding capacities of dough are significantly influenced by the presence of gluten and its ability to form a protein network, thereby contributing to the typical volume of the final bread. Additionally, these parameters are also

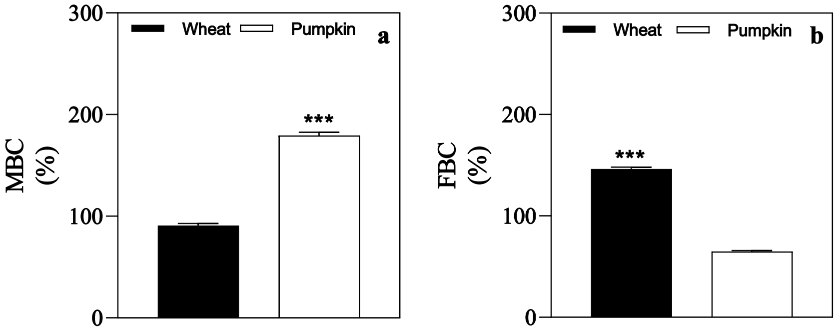


Fig. 2 Moisture binding capacity (MBC; **a**) and fat binding capacity (FBC; **b**) of wheat flour (closed bar) and pumpkin seed protein concentrate (open bar). Data were compared with Student's *t*-test ($p \leq 0.05$). Significance ***$p \leq 0.001$

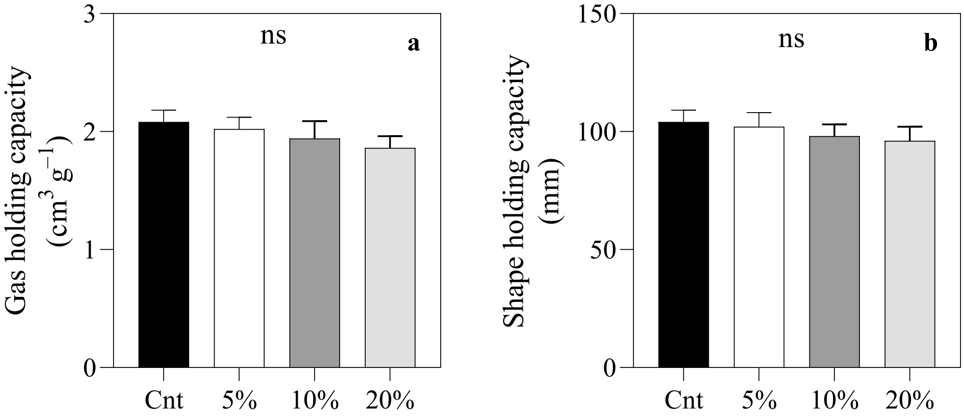


Fig. 3 Gas-holding capacity (**a**) and shape-holding capacity (**b**) of wheat flour dough (Cnt) and dough with the addition of 5, 10, and 20% of pumpkin seed protein concentrate. Means (± SD; $n = 3$) were analyzed following the one-way ANOVA with the different percentage of pumpkin seed protein concentrate as variability factor ($p < 0.05$). *ns* not significant

influenced by the fermentative activity of the yeast present in the dough. Notably, the concentration of gluten in bread enriched with 5%, 10%, and 20% pumpkin seed protein concentrate appeared sufficient to avoid significant differences, as did the fermentative activity of the yeast, which maintained a consistent production of CO_2 even when the growth substrate (wheat flour) was reduced. Nevertheless, despite the absence of significant differences, the reduction in wheat flour content and, consequently, gluten content led to a decrease in the specific volume of the enriched dough and of the final bread (Table 3) compared to the controls. This decrease followed a linear trend corresponding to the increase in the percentage of pumpkin seed protein concentrate enrichment.

Near-infrared reflection spectra of raw materials, doughs, and breads

Modifying the composition of recipe ingredients results in alterations in dough properties and a rearrangement of its structural components. To detect and analyze the constituents of dry powders, it is advisable to employ near-infrared reflection spectroscopy [34]. The utilization of this technology results a rapid and convenient way to compare the composition and the qualitative features of the main nutrients and components of the raw materials utilized in the present investigation. In this way, it could be easy to immediately understand which components are represented to a great extent in pumpkin seed protein concentrate and this could allow to conclude about the need to add pumpkin seed protein concentrate into bread recipe, depending on the purpose of such addition. The reflection spectrum of wheat flour, as reported by Litvynchuk et al. [9], exhibited greater reflection intensity in comparison to that of pumpkin seed protein concentrate. The obtained results from the reflection spectra of pumpkin seed protein concentrate (Fig. 4) revealed that the first pronounced minimum peak for pumpkin seed protein concentrate occurred at a wavelength of 1500 nm. The subsequent minimum peak was observed at 1720 nm, which was absent in the spectrum of wheat flour [9]. This particular extremum, related to lipid components and indicating the absorption of carbonyl esters of fats, was more pronounced for pumpkin seed protein concentrate. This might be attributed to its higher lipid content [6], particularly rich in unsaturated fatty acids [35]. At 2100 nm, the spectrum of pumpkin seed protein concentrate displayed a prominent maximum extremum, highlighting the significant protein content, which aligns with the data shown in Fig. 1. At 2180 nm, there was a minimal reflectance in the pumpkin seed protein concentrate spectrum, reflecting the absence of starch. At this wavelength, protein content is characterized, excluding the influence of starch. The disparity in reflection intensity at this wavelength compared to the data on wheat flour reported by Litvynchuk et al. [9] can be attributed to variations in the protein's fractional composition. Wheat flour contains gluten proteins, while pumpkin seed protein concentrate has minimal gluten proteins [36]. The final extreme in the pumpkin seed protein concentrate spectrum was observed at a wavelength of 2350 nm, indicating a high content of dietary fiber [37].

The analysis of the change and redistribution of structural groups of dough after kneading and after 3.5 h of its fermentation and of finished bread was carried out by the method of infrared spectroscopy by converting the reflectance coefficient to the spectral index (Figs. 5 and 6). For all spectra (both control samples and samples with pumpkin seed protein concentrate), there were maxima peaks at wavelengths of 1460 nm, 1770 nm, 1930 nm, 2100 nm, 2270 nm, and 2350 nm.

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

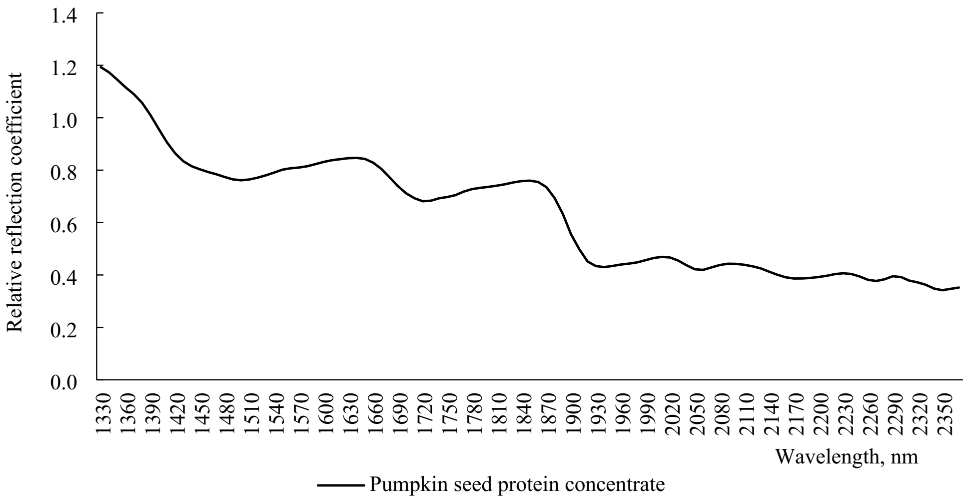


Fig. 4 Spectra of reflection of pumpkin seed protein concentrate

Fig. 5 Changes and redistribution of structural groups in dough after kneading and after 3.5 h of fermentation with 5, 10, and 20% pumpkin seed protein concentrate

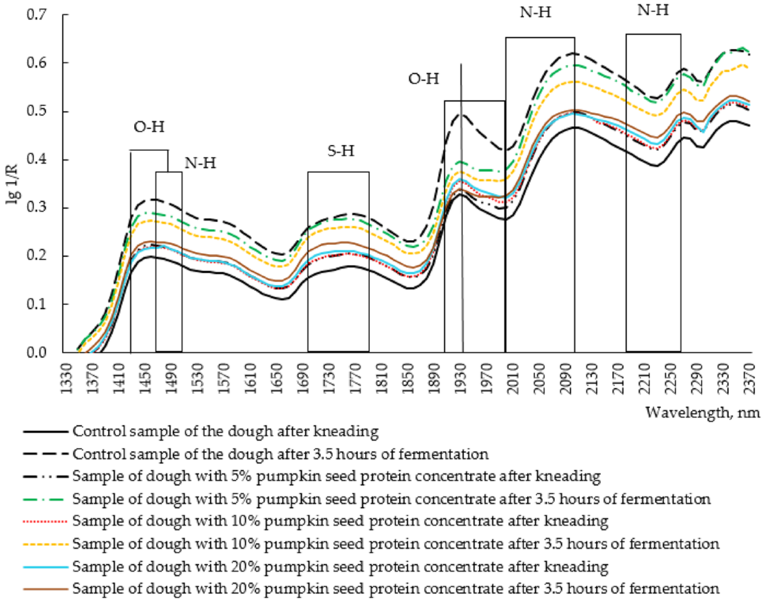
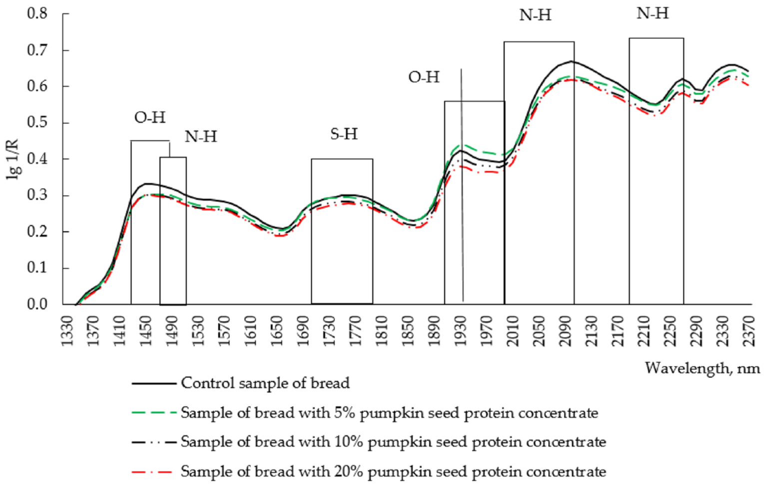


Fig. 6 Changes and redistribution of structural groups in bread with 5, 10, 20% pumpkin seed protein concentrate



wavelength range of 1700–1790 nm can indicate the passage of the proteolysis process in the doughs [38]. Thus, functional sulfhydryl groups S–H (first overtone) and extrema were present in the spectra of all dough samples (Fig. 5). The spectral index of the control dough sample after kneading was 0.17 at the extrema, and that of dough samples with the addition of pumpkin seed protein concentrate was 0.20, 0.21, and 0.22 with the addition of 5%, 10%, and 20% of protein concentrate, respectively. This is explained by the fact that the proteins of the pumpkin additive affected the course of proteolysis [39]. During fermentation, the course of microbiological, biochemical and colloidal processes takes place in the dough [40]. The spectra of doughs shifted significantly upwards, especially the spectrum of control sample, which was 0.30, and that of enriched samples which was 0.27, 0.25, and 0.23 for 5%, 10%, and 20% pumpkin additive, respectively (Fig. 5). This could be because the pumpkin seed protein concentrate has higher MBC than wheat flour as reported in Fig. 2.

Protein structures, the main participants in conformational transformations in the dough system, were characterized by wavelength of 2100 nm (N–H deformation vibrations). It was established that the spectral index of the dough with the additive was 0.49, regardless of the dosage. At the same time, for the control sample it was 0.46. This might be caused by the little and not enough time passed for the beginning of the conformation of biopolymers [41].

After fermentation (3.5 h), the trend changed. The spectral indices of the control dough sample and samples with 5%, 10%, and 20% pumpkin additive were 0.62, 0.60, 0.56, and 0.48, respectively (Fig. 5). Such values are explained by the fact that pumpkin seed protein concentrate did not contain gluten proteins and did not participate in the gluten formation [42]. In addition, the inclusion of an additional source of protein as pumpkin seed protein concentrate led to changes due to the wedging of the new protein substances into the gluten network [43]. In particular, on the wavelength of 2180 nm, there was the minimum reflectance of pumpkin seed protein concentrate (Fig. 4). As at this wavelength, the protein content was characterized avoiding the influence of starch and since starch content in dough was high due to its presence in wheat flour, there was no peak on the spectra of dough.

During bread baking, high temperatures cause destruction of protein structures as reviewed by Bredariol and Vanin [44]. The spectrum of the control bread sample was higher than the spectra of the bread with the pumpkin additive, regardless of the dosage (Fig. 6). This is explained by the fact that the main properties of bread were determined by the content of globulins and glutelins in wheat flour and their properties to form gluten network.

In bread spectra, at a wavelength of 2270 nm (O–H valence vibrations/C–O valence combination) it was possible to analyze the content and the effect of lignin. Lignin is present in pumpkin seeds, but in smaller quantities than in cereals, especially in wheat [45]. During the production of pumpkin seed protein concentrate, lignin was removed. Since there was no lignin in the pumpkin protein concentrate, the spectrum of the bread with it was lower than the control sample. For this reason, the values of the spectral indices were 0.62, 0.60, 0.59, and 0.58 for control sample and samples with 5, 10, and 20% pumpkin seed protein concentrate, respectively. This aspect indicates a better digestibility of bread with the pumpkin seed protein concentrate addition by the body, because of the lower lignin content, traducing to a better digestibility of the bakery product [46]. Indeed, human beings firstly lack the specific enzymes needed to efficiently break down lignin [47]. Moreover, lignin chemical structure is complex and irregular, composed by phenolic compounds, and its complexity makes it resistant to enzymatic breakdown by the digestive enzymes present in the human digestive system [48]. Due to these factors, the reduced lignin content in the enriched bread contributes to improved digestibility.

Bread quality evaluation

Expert evaluation of products was carried out with the participation of tasters (Table 3).

It was observed that some sensory characteristics of the control sample and the samples with different concentrations of pumpkin seed protein concentrate did not change, while for other panelists there was a significant variation in the product with increasing percentages of pumpkin seed protein concentrate. For example, bread-specific volume decreased linearly with the increase of percentage substitution of wheat flour with pumpkin seed protein concentrate (4.6, 3.5, and 3.0 $cm^3\ g^{-1}$ in 5%, 10%, and 20% enriched bread, respectively). This result is in line with the gas- and shape-holding capacities of dough (Fig. 2), despite dough results did not report significant differences. Moreover, porosity structure and the elasticity of crumb also confirmed the hypothesis posited for the results of dough gas- and shape-holding capacities that the reduction of wheat flour concentration did not reduce the fermentative yeast activity able to maintain the bread porosity structure and the gluten ability to maintain elasticity in the formation of this protein network. These results were in line with the bread form stability and surface condition that reported similar results among control and enriched samples, except for the 20% pumpkin seed protein concentrate enriched bread. Indeed, the product with a 20% concentration of pumpkin seed protein concentrate showed also changes in visual aspects such as the surface condition and the form stability with depressions on the upper crust, and the greenish-brown color of crust and crumb. The pumpkin taste started to be perceived at a concentration of 10% enrichment, while the pumpkin aroma at a concentration of 20% of pumpkin seed protein concentrate enrichment, even though the aroma values did not result significantly different between 10 and 20% enrichment. Finally, the chewability of crumb obtained the same results between enriched and control breads. In light of these results, the overall evaluation (Score) was similar between the control bread and those enriched with 5% and 10% pumpkin seed protein concentrate, while the bread enriched with 20% reported the worst final assessment.

Expert assessment and sensory indicators of the products indicate that to obtain bakery products of high quality, it is necessary to add up to 10% pumpkin seed protein concentrate to the recipe.

Table 3 Sensory assessment of bread with pumpkin seed protein concentrate using a 5-point scale considering the weighting factors of quality indicators

Indicators	Weighting factor	Control sample	Pumpkin seed protein concentrate (%)		
			5	10	20
Specific volume (cm^3 g^{-1})	0.15	4.6 ± 0.1a	4.6 ± 0.1a	3.5 ± 0.1b	3.0 ± 0.1c
Form stability	0.15	Convex upper crust	Convex upper crust	Convex upper crust	Upper crust with depressions
		5.0 ± 0.1a	5.0 ± 0.1a	5.0 ± 0.1a	4.0 ± 0.1b
Color of crust	0.05	Light yellow	Light yellow	Light brown	With greenish-brown color
		5.0 ± 0.1a	5.0 ± 0.1a	5.0 ± 0.1a	5.0 ± 0.1a
Surface condition	0.05	Smooth, without cracks	Smooth, without cracks	Smooth, without cracks	Uneven, with depressions
		5.0 ± 0.1a	5.0 ± 0.1a	5.0 ± 0.1a	4.0 ± 0.1b
Color of crumb	0.05	Light	Light	Light brown	With greenish-brown color
		5.0 ± 0.1a	5.0 ± 0.1a	5.0 ± 0.1a	5.0 ± 0.1a
Porosity structure	0.09	Even, small, thin-walled	Even, small, thin-walled	Even, small, thin-walled	Even, small, thin-walled
		4.8 ± 0.1a	4.8 ± 0.1a	4.8 ± 0.1a	4.8 ± 0.1a
Elasticity of crumb	0.12	Elastic	Elastic	Elastic	Elastic
		4.8 ± 0.1a	4.8 ± 0.1a	4.8 ± 0.1a	4.8 ± 0.1a
Aroma	0.11	Inherent in the product	Inherent in the product	Inherent in the product	With pumpkin flavor
		4.6 ± 0.1b	4.6 ± 0.1b	4.7 ± 0.1ab	4.8 ± 0.1a
Taste	0.13	Inherent in the product	Inherent in the product	With pumpkin taste	With expressed pumpkin taste
		4.8 ± 0.1a	4.8 ± 0.1a	4.8 ± 0.1a	4.8 ± 0.1a
Chewability of crumb	0.10	Good	Good	Good	Good
		5.0 ± 0.1a	5.0 ± 0.1a	5.0 ± 0.1a	5.0 ± 0.1a
Score (out of 5 points)		4.8 ± 0.2a	4.8 ± 0.2a	4.7 ± 0.1a	4.4 ± 0.1b

Means (±SD; $n = 3$) were analyzed following the one-way ANOVA with the different percentage of pumpkin seed protein concentrate as variability factor ($p < 0.05$). Different letters indicate significant differences between samples

Conclusions

In the present manuscript, the total protein content in pumpkin seed protein concentrate is 5.2-fold higher, and its fiber content is 2.3-fold greater than those observed in wheat flour. The high biological value of pumpkin seed protein concentrate can be attributed to the elevated presence of EAA, particularly leucine, exceeding the levels found in previous investigations on wheat flour. The gas- and shape-holding capacities of dough enriched with pumpkin seed protein concentrate exhibited no significant differences when compared to dough made from wheat flour. This factor bears significance for the marketability and repeatability of the final product, particularly bread. The near-infrared reflection spectroscopy analysis revealed that the enrichment of bread with protein concentrate from pumpkin seeds introduced higher protein and unsaturated fat content in bread but also reduced the level of lignin, enhancing the digestibility of the final product. Furthermore, sensory analysis revealed that the inclusion of pumpkin seed protein concentrate is well-received by consumers, especially up to a concentration of 10%. Future research should explore how consumer acceptance could further increase with higher concentrations of nutritional components perceived as beneficial for a lifestyle considered healthy by specific consumer segments.

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Data availability The authors confirm that the data supporting the findings of this study are available within the article.

Declarations

Conflict of interest The authors declare no conflict of interest.

Compliance with ethics requirements This study does not contain any studies with human participants or animal performed of any of the authors.

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