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Mechanically Activated Enzymatic Hydrolysis of Pea Seeds and Its Effects on Bakery Products

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Abstract

Background and Objective: Plant-derived protein hydrolysates are efficient sources of easily digested water-soluble nutrients. Pea is a promising crop, which is rich in protein. In this study, combining mechanical pretreatment of pea seeds and their enzymatic hydrolysis into a single manufacturing stage has been suggested to increase yields of water-soluble substances. Resulting hydrolysates were used as ingredients for bakery products.

Material and Methods: In this study, mechanical pretreatment of pea seeds included two stages. At the first stage, feedstock was subjected to preliminary comminution to particles of < 2 mm using knife grinder. At the second stage, feedstock underwent mechanical treatments in presence of enzyme products using RM-20 centrifugal roller mill. Enzymatic hydrolysis of the mechanically treated pea seeds was carried out at 50 °C and 120 g. Dough for the bakery products was prepared using two methods of sponge-and-dough and straight-dough. Total proteins in the hydrolysates and end products were assessed using Bradford protein assay. Quantities of free amino acids were assessed using liquid chromatography-mass spectrometry and Agilent 1200 Liquid Chromatography System.

Results and Conclusion: In this study, the optimal hydrolysis time was reported as 4 h and the enzyme product concentration of 2%. The optimal time of mechanical pretreatment of the raw materials in centrifugal roller mill included 40 s at 1500 g. Resulting pea seed hydrolysates were added to bakery products. Sponge-and-dough method was reported as the optimal method of preparing doughs with the hydrolysates since the end products included the best sensory characteristics. Due to the cleavage of peptide bonds by the enzyme products, addition of the hydrolysates ensured higher contents of amino acids and short peptides in the end products.

Conflict of interest: The authors declare no conflict of interest.

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1. Introduction

Nowadays, there are steadily growing customer demands for healthy and safe food products. The major reason is that a large proportion of the world population supports rational approaches to health, using balanced diets [1,2]. It seems most reasonable to produce food products that are steadily demanded by customers. This first includes bread and bakery products [3]. As in enriching ingredients, it is rather important to add protein-rich components to the formulas [4]. Plant and animal-based feedstocks added to food products include various effects on their nutritional values. Animal-based foods contain cholesterol and saturated fatty acids, which negatively affect the human health by causing cardiovascular diseases and cancers [5]. Excessive intakes of animal proteins lead to bone demineralization as the pH balance shifts towards acidic values, thus resulting in development of osteoporosis [6].

Foods of vegetable origins do not contain cholesterol, while predominantly contain unsaturated fatty acids and are sources of vitamins, minerals and dietary fibers [4,7,8]. Furthermore, production of protein-rich plant-based materials is a less resource-intensive, more eco-friendly and accessible process, compared to production of protein-rich animal-based materials [9].

Legumes (majorly peas, soybeans, chickpeas and kidney beans) are commonly used to enrich food products in plant-based proteins [10-12]. Pea seeds are included in the formulas largely because of their chemical compositions, water retentions, emulsifying and gelation properties, textures and biological values [7,8]. However, use of plant-based proteins in nutrition includes several challenges. First, plant-based proteins are incomplete in terms of their amino acid (AA) composition. Second, these proteins can be digested by 62-80% because they contain antinutrients [13-16]. The following procedures are currently used to enhance the digestibility of plant-based proteins, including decreases in effects of antinutrients using physical and chemical methods [13,16], sprout of seeds [17] and enzymatic hydrolysis [15,18]. In this study, integral assays were used on raw materials (mechanical pretreatment and enzymatic hydrolysis). Normally, mechanical pretreatment of plant-based feedstocks increases its reactivity due the increases in surface areas during comminution, amorphization of ordered biopolymer regions and disordering of the cellular structure of the raw materials [19,20]. Therefore, rates of the subsequent hydrolysis increase and yields of the low-molecular-weight substances are further complete. However, mechanical activation of the intensify enzymatic hydrolysis has been well studied for cellulose and β-glucan [21,22] but not for proteins. Therefore, the aims of this study were to investigate physicochemical processes occurring during enzymatic hydrolysis of mechanically pretreated pea seeds and to develop technologies for the production of bakery products using the resulting hydrolysates.

2. Materials and Methods

2.1. Materials

The study included seed peas and protosubtilin G3× complex enzyme product (OOO PA Sibbiopharm, Berdsk, Russia). Prior to the experiments, pea seeds were subjected to coarse comminution to < 2 mm using cutting mill. In this study, the enzymatic complex was selected based on its catalytic activity profile and availability for further technological uses. This commercially available enzyme product included an enzymatic complex containing neutral and alkaline proteases and glycosidases (proteases of ~11,000 U g^{-1}; xylanase of up to 150 U g^{-1}; β-glucanase of up to 200 U g^{-1} and α-amylase of up to 300 U g^{-1}).

2.2. Mechanical pretreatment and enzymatic hydrolysis

Pea seeds with the enzyme were mechanically treated using RM-20 Activator Roller Mill (5.5 kW) (ISSCM RAS, Russia) equipped with a water-cooling system at a rotor speed of 1450 rpm [23]. The blend containing pea seeds and the enzymes was fed automatically at a rate of 3 kg h^{-1}. The enzymatic hydrolysis was carried out at 50 °C for 4 h. The ratio of the solid and liquid phases was 1:3 (m v^{-1}). For hydrolysis, 80 ml of water were added to 28 g of the pea seed flour. The resulting hydrolysate was dried using Iney 4 Laboratory Freeze Dryer (IBI RAS, Russia).

2.3. Production of dough semi-finished products

The resulting hydrolysate was added to the dough to activate fermentation of yeast dough and to increase biological values of the end products. Ingredients of the new bakery products were used, including first grade wheat flour, pea seeds, compressed yeasts, whey, butter, bran, carrots, flaxseeds, salt and sugar. Table 1 lists general characteristics of the feedstocks used in this study. To identify the optimal method for dough kneading, dough was prepared using two methods of sponge-and-dough and straight dough. Preparing dough using sponge-and-dough method involved two stages. At the first stage, the sponge starter was prepared. Baker yeasts were dissolved in whey at 30 °C. The resulting blend was mixed with pre-sifted wheat flour and sugar. Sponge starter was left to ferment at 32 °C for 2 h using proofing cabinet. Kneading was carried out in the second stage. Prepared sponge starter, pea seed hydrolysate, bran (or carrots) and salt were added to the wheat flour. To achieve gluten network development, dough was subjected to intensive mechanical treatment for 10 min using TAURO 22-2V Dough Mixer (TAURO, Italy). Butter was added to the dough at the end of kneading. The resulting dough was transferred into the proofing cabinet at 35 °C for 30 min. Then, dough was punched, divided into individual portions and shaped. Shaped products were left to prove at 35 °C for 20 min and baked at 190 °C for 5 min using combi steamer oven. Then, temperature decreased to 175 °C and products were baked for additional 5 min. For the yeast dough prepared using straight dough method, all ingredients were added during kneading. Other technological stages were similar to those for dough prepared using sponge-and-dough method. During the experiment, the optimal kneading technique was selected and developed for the formulation of new bakery products. Two samples of bakery products differing in ingredients were prepared as follows: Sample 1, a bakery product with bran; and Sample 2, a bakery product with carrots. Formulation of the bakery products is shown in Table 2. To justify how efficient action of the hydrolysate on fermentation was, increasing power of the semi-finished product was calculated using dough ball method and total insoluble solid content was assessed for various model samples of the sponge starter.

Table 1. Characteristics of the feedstocks

Component name	Key physicochemical characteristics	Country	Regulatory document for the manufacturing process
First grade wheat flour	Amount of gluten ≥ 28% Mass fraction of protein-10.3 w % Mass fraction of fat - 1.1 w % Mass fraction of carbohydrates-70.0 w %	Russia	National Standard GOST 26574-2017
Pea seeds	Mass fraction of protein - 22.0 w % Mass fraction of fat- 1.0 w % Mass fraction of carbohydrates - 62.0 w %	Russia	National Standard GOST 28674-2019
Protosubtilin G3h	11000 U g^{-1} of protease, ≤ 150 U g^{-1} of xylanase, ≤ 200 U g^{-1} of β-glucanase, ≤ 300 U g^{-1} of α-amylase	Russia	National Standard GOST 23636-90
Unsalted cheese whey	Mass fraction of protein ≥ 0.5 w % Mass fraction of fat - 0.09 w % Mass fraction of carbohydrates- 5.12 w %	Russia	National Standard GOST 34352-2017
Refined, deodorized sunflower oil	Mass fraction of water and volatiles ≤ 0.1 w % Mass fraction of fat – 99.9 w %	Russia	National Standard GOST 1129-2013
Fresh garden carrots	Mass fraction of protein - 1.3 w % Mass fraction of fat - 0.1 w % Mass fraction of carbohydrates- 6.9 w %	Russia	National Standard GOST 32284-2013
Baker's yeast "Lux Extra" (Saccharomyces cerevisiae)	Mass fraction of protein- 12 w % Mass fraction of fat - 0.4 w % Mass fraction of **c**arbohydrates -8.5 w %	Russia	National Standard GOST R 54731-2011
Granulated sugar	Mass fraction of sucrose ≥ 97 w %	Russia	National Standard GOST R 52305-2005
First grade salt	Mass fraction of sodium chloride ≥ 98.4 w %	Russia	National Standard GOST R 51574-2018
Wheat bran	Mass fraction of protein -15.1 w % Mass fraction of fat- 3.8 w % Mass fraction of carbohydrates - 23.0 w %	Russia	National Standard GOST 7169-2017

Table 2. Formulation of the bakery products

Name of the feedstock and food products	Amount of feedstock and food products per serving, g	
	Gross weight, g	Net weight, g
	Stage 1. Preparing the sponge starter*	
First grade wheat flour	17.0	17.0
Whey	15.0	15.0
Granulated sugar	2.0	2.0
Baker's yeast	1.0-3.0	1.0-3.0
	Adding the dough components .۲Stage	
Wheat flour	44	44
Bran (for sample 1)	5.0	5.0
Carrots (for sample 2)	۷,۰	۷,۰
Vegetable oil	2.0	5.0
Salt	0.7	0.7
Pea seed hydrolysate	20.0-40.0	20.0-40.0

2.4. Sensory evaluation of quality of the baked products

Sensory evaluation was carried out by untrained evaluators using five-point scale (excellent, 5; good, 4; satisfactory, 3; and poor, 2) [24]. During the experiment, evaluators assessed the standard sensory quality parameters: appearance, color, flavor, odor and texture. Evaluators included experts in sensory evaluation of ready-to-use foods and beverages. Sensory evaluation forms were filled out during the experiment. The average score for each sample provided by each evaluator was eventually calculated. The overall evaluation score for the sample was calculated as the arithmetic mean of final evaluations.

2.5. Analysis of the chemical compositions of the baked products

Water-soluble substances were quantified using comprehensive extraction in Soxhlet apparatus for 24 h [25].

Contents of free AAs were assessed using liquid chromate-graphy-mass spectrometry (LC-MS) and Agilent 6410 QQQ Liquid Chromatography System (Agilent, USA) coupled with an Agilent 1200 Liquid Chromatography System (Agilent Technologies, USA) [26]. Mineral compositions of the samples were assessed using atomic absorption and atomic emission spectrometry methods [27]. Vitamin contents were analyzed using infrared (IR) spectroscopy and IR 4250 Spectrophotometer (Pacific Scientific, USA) [28].

2.6. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis

Vertical sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis of Laemmli protocol [29] was carried out to compare molecular weights (MW) of the protein mixtures in control samples with that of the hydrolysate. Polyacrylamide gel electrophoresis was carried out using 18% separation gels. Samples were treated for pre-denaturation using 1.4-dithiothreitol at 1:1 ratios. Protein markers were represented using unstained protein MW marker (Thermo Fisher Scientific, USA) with protein MWs of 14.5–94.6 kDa. The target protein zones in the gels were visualized using Coomassie stain (R250) as dark blue bands.

2.7. Protein quantification (Bradford protein assay)

Total protein contents in finished products were assessed using Bradford protein assay [30]. Visualization of the proteins was carried out post reactions between Coomassie brilliant blue G-250 and proteins in the solution. To quantitative assess of AA contents, bovine serum albumin (BSA) calibration curve was used and optical density (OD) of the solution was measured at 595 nm.

2.8. Quantitation of the amino groups (Ninhydrin reaction)

Terminal amino groups of the AAs and polypeptides were detected post reactions with ninhydrin and quantitation of the formed Ruheman's Purple. Quantities of the amino groups in the samples were assessed using investigation of the color intensity of the solutions using photoelectric colorimetry at 590 nm. Quantitative assess of the AA contents was carried out using glycine calibration curve [31].

2.9. Mathematical modelling

A two-factor experiment was carried out to assess effects of the quantities of yeasts (X_1) and hydrolysates (X_2) on gas-forming ability of the dough and dry insoluble residue contents. The variation levels are listed in Table 3. Regression equations were derived to predict the formation of water-soluble substances during fermentation of a semi-finished dough product using STATISTICA Software (StatSoft, Russia).

Table 3. Levels of the experiments

Level	Factor	
	$X_{2,r}$	$X_{1,r}$
1	1	20
2	2	30
3	3	40

2.10. Statistical analysis

Results were presented as the mean ±SD (standard deviation) of three replicates. Differences were significant at $p \leq 0.05$.

3. Results and Discussion

Numerous studies have verified that pretreatment of the feedstocks efficiently intensifies the subsequent stage of enzymatic hydrolysis. This technique involves exposure to microwave radiation [31], hydrostatic pressure [32] and ultrasound [33]. In this study, effects of the mechanical pretreatment of pea seeds on the subsequent enzymatic hydrolysis were investigated. It is reported by the studies that mechanical pretreatment (activation) of plant materials with no added enzyme products (enzymes are added immediately before the hydrolysis and do not undergo mechanical pretreatments) significantly decreases the hydrolysis rate, compared to that the system of plant materials and enzyme treated simultaneously does [34]. In the present study, the optimal hydrolysis parameters were reported based on the accumulation rates of water-soluble compounds as follows: enzyme concentration, 2 wt. %; hydrolysis duration, 4 h (Fig. 1); and temperature, 50 ºC. Further added enzymes included no effects on the rate of enzymatic reaction since the substrate surface was completely filled with enzyme molecules at a enzyme concentration of 2 wt. %. The resulting hydrolysate was added to the dough to enrich the bakery products in micronutrients.Figure 2 shows the quantitative relationships between the quantities of proteins and amino nitrogen in the samples. Protein contents in products containing the hydrolysate were lower, while quantities of amino nitrogen were higher than those in control samples. Decreases in protein concentrations during enzymatic hydrolysis were expected, described in literatures previously [35]. Figure 3 shows an electropherogram of the proteins in the finished bakery products. The MWs of proteins in hydrolysate-containing samples differed from those in control samples; described previously by Gavrilova et al. [36].

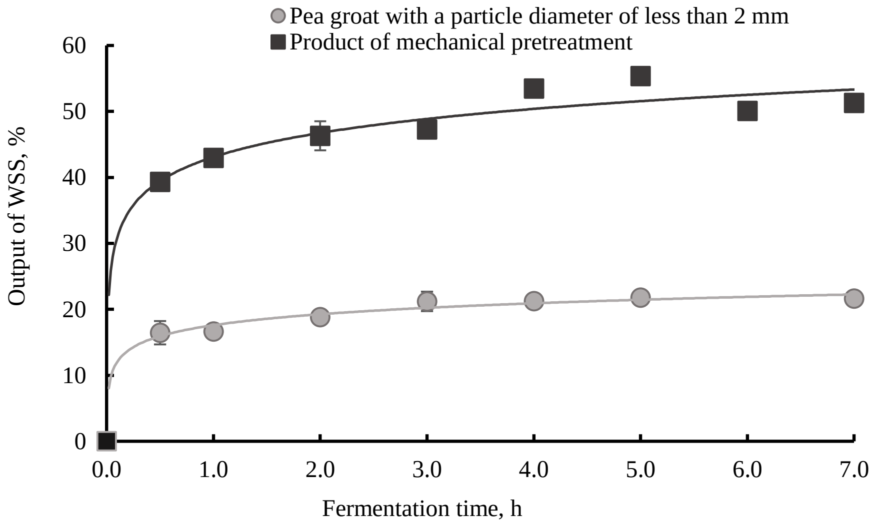

Figure 1. Outputs of the water-soluble substances (WSS) in the hydrolysate depending on the fermentation time (the lower curve is pea groat with a particle diameter of less than 2 mm, the upper curve is the product of mechanical pretreatment)

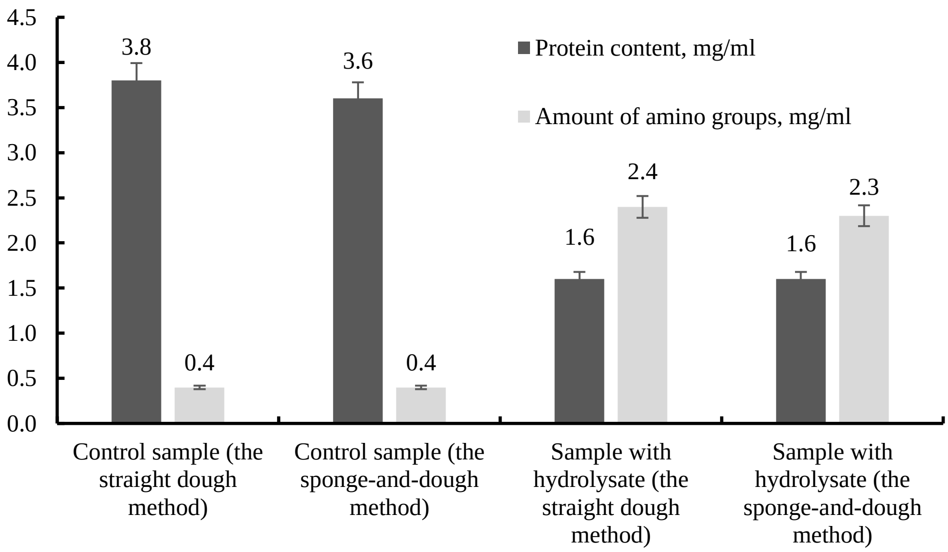

Figure 2. Quantities of the proteins and amino nitrogen in the end products. The figure schematically shows significant differences ($p < 0.05$)

Samples with the hydrolysate contained fewer polypeptides with less high-molecular-weight and more low-molecular-weight polypeptides.

Additionally, it was verified that enzymatic hydrolysis stage was reasonably carried out since quantities of the free AAs in developed samples were higher than those in control samples (Fig. 4). It is reported that increased concentrations of free AAs in dough significantly change sensory perceptions of the bakery products (feeling unpleasant tastes), turning the crust too dark [37]. Therefore, complete sensory evaluation of the bakery products with the hydrolysate was a critical stage of the study. In the present study, quality sensory evaluation of the finished bakery products with the pea hydrolysate was carried out, which was prepared through various dough-mixing methods. Bakery products prepared using sponge-and-dough process and additional vigorous mechanical treatments were compared favorably with those prepared using straight dough method. Since these products were characterized by a higher porosity, moisture contents in soft parts of the products were lower and the product tastes were better. The average overall score of bakery products prepared using sponge-and-dough method was 4.7 (parameters such as appearance, color, flavor, odor and texture of the products were recorded during the sensory evaluation). The average overall score of samples prepared using straight dough method was 3.2 because of their poorly developed porosity and sticky crumb.

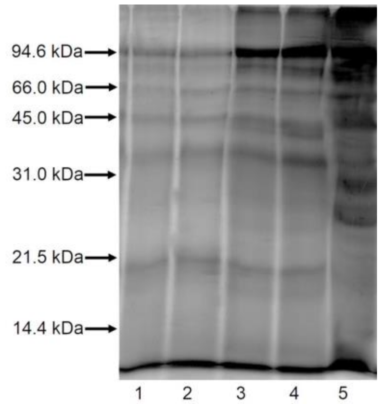

Figure 3. Electropherogram of the protein extracts in the bakery products. Lane 1, the hydrolysate-containing sample prepared using straight dough method; Lane 2, the hydrolysate-containing sample prepared using sponge-and-dough method; Lane 3, control sample prepared using straight dough method; Lane 4, control sample prepared using sponge-and-dough method; and Lane 5, extract from the first-grade wheat flour

Therefore, the sponge-and-dough mixing method was the most efficient method in this study. In fact, moisture content is a quality parameter of the yeast-fermented sponge doughs. In this study, moisture contents in dough samples with various quantities of the hydrolysate were assessed. When the hydrolysate content was 28% of the total composition, moisture content in dough was normal (37%). Concentration of the hydrolysate included no effects on the sensory quality parameters of the finished products. Figure 5 provides data on total contents of the water-soluble substances in control samples and those containing the hydrolysate.

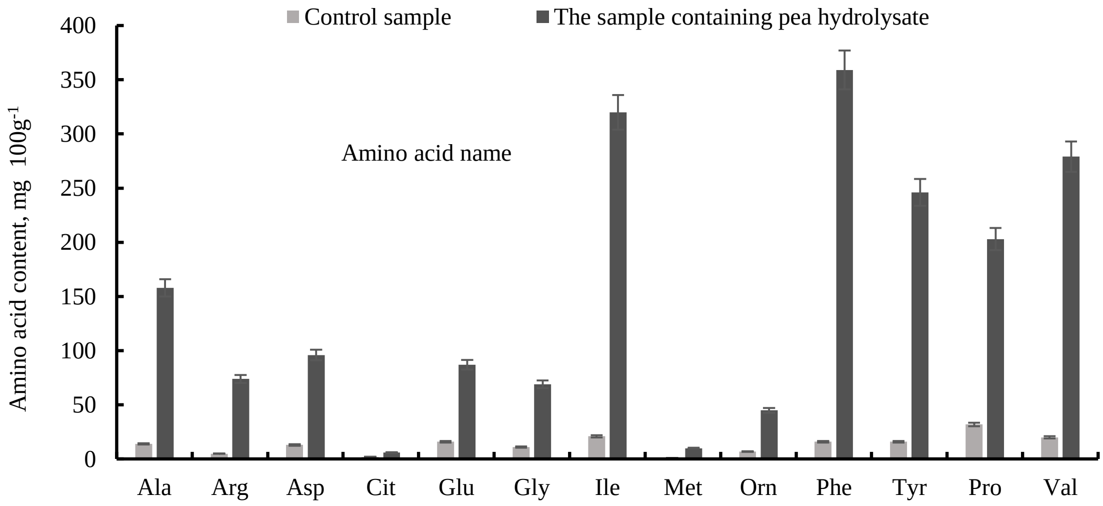

Figure 4. Amino acid composition of the finished products. The figure schematically shows significant differences ($p < 0.05$)

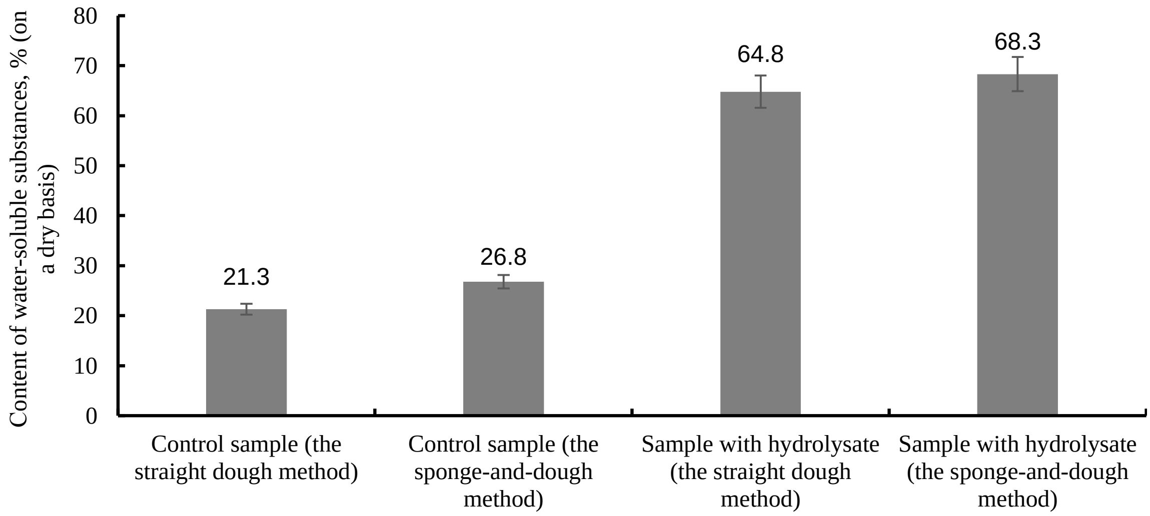

Figure 5. Total contents of the water-soluble substances (WSS) in the samples. The figure schematically shows significant differences ($p < 0.05$)

From physicochemical perspectives, the dough mixing method (sponge-and-dough or straight dough method) included no significant effects on compositions of the finished products. Mechanically pretreated enzymatic hydrolysis technology allowed 2.5-fold increases in quantities of the water-soluble substances as well as 6-fold increases in quantities of peptides and AAs. Figures 6A and 6B show nutritional values of the finished products. In general, developed samples are sources of Na, P and Fe. They can be used to enrich diets with thiamin, pantothenic acid, pyridoxine and biotin. Since the hydrolysate includes a large quantities of low-molecular-weight substances, it is a good substrate for feeding and reproduction of yeast cells. A comprehensive experiment was carried out to assess effects of two variable factors (quantities of yeasts and hydrolysates) on the gas-forming ability of dough and its total insoluble solid contents. Figure 7A shows results of the experiment as a regression plane. The regression equation for the relationships between three variables is written as follows:

$$Z = 8.13 - 1.02X - 0.07Y \qquad \text{Eq(1)}$$

Where, X was the quantity of yeasts (g); Y was the quantity of hydrolysate (g); and Z was the rising time of a dough ball (min). The regression equation for the calculation of total insoluble solid content when knowing the ratios between the quantities of hydrolysate and yeasts is written as follows:

$$Z = 15.06 - 0.06X - 0.9Y \qquad \text{Eq(2)}$$

Where, X was the quantity of yeasts (g); Y was the quantity of hydrolysate (g); and Z was the total insoluble solid content (g). Figure 7B demonstrates relationships between the parameters under consideration as a regression plane.

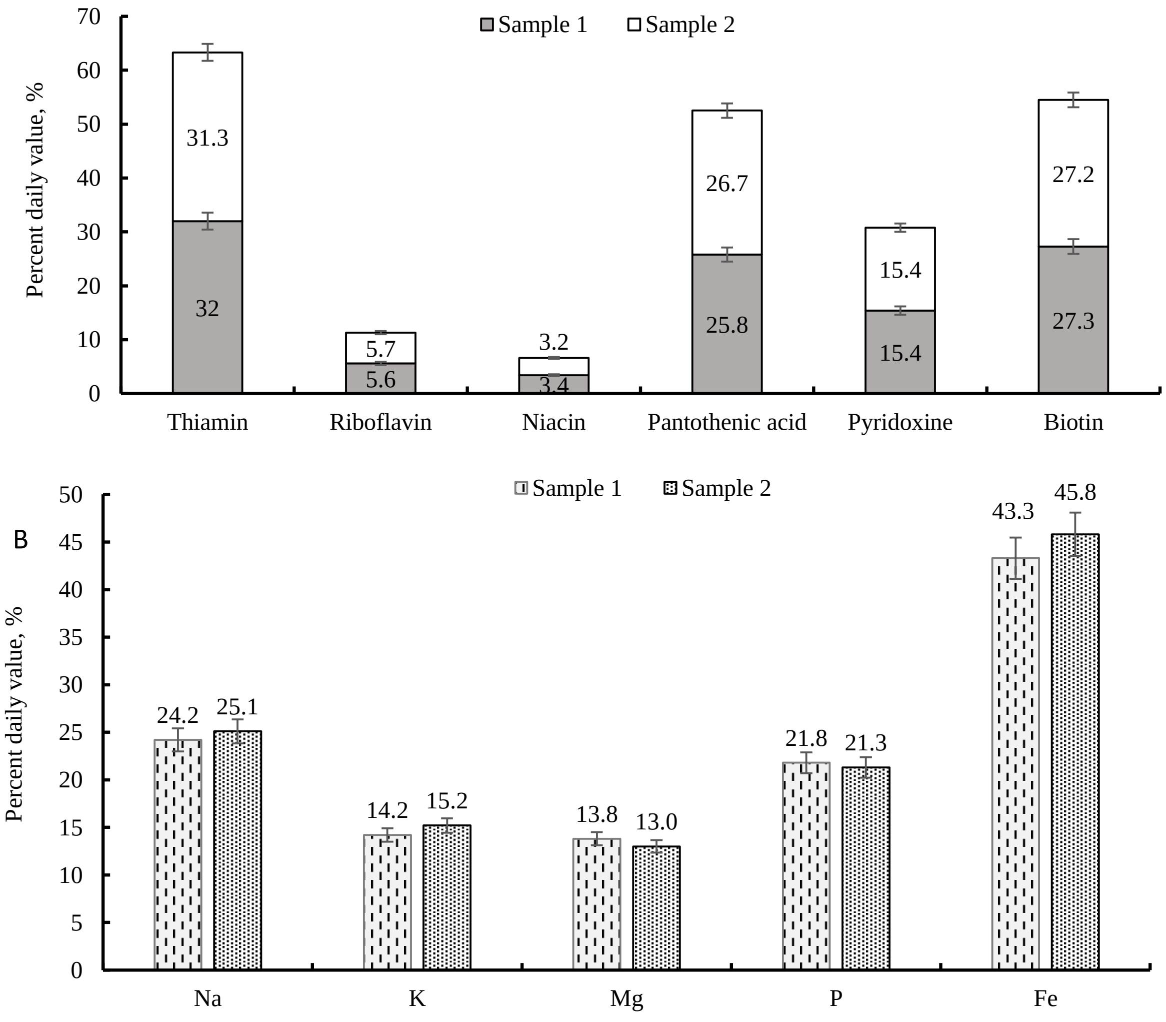

Figure 6. Daily values (%) of B vitamins (A) and minerals (B) (per 100 g of the product)

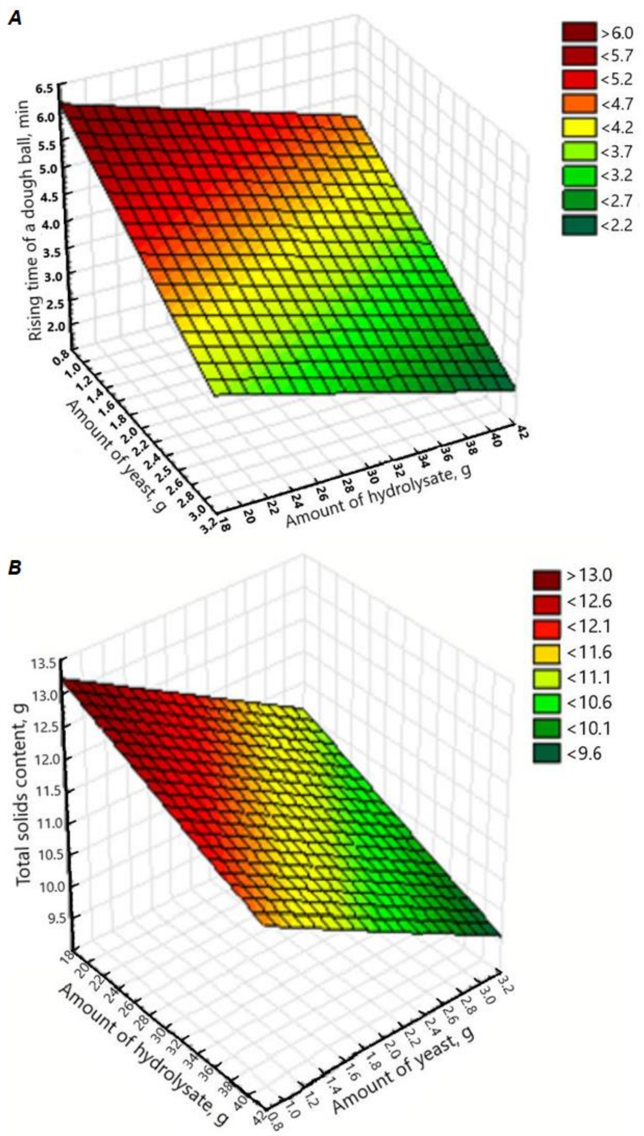

Figure 7. A) Rising time of a dough ball as a function of the quantities of added hydrolysates and yeasts; B) Regression plane for the effects of the quantities of yeasts and hydrolysates on total insoluble solid content

A conclusion can be made from the results of these two experiments that the formation of water-soluble substances is intensified as the quantities of yeasts and hydrolysates in the model sponge samples increase. As a result, the gas-forming ability of doughs increases and the quantities of insoluble substances decrease. It is noteworthy that the gas retention capacity of the pre-ferments increases when the yeast concentration in the system does not change, while quantity of the hydrolysate increases. This indicates that expansion of the yeast cells as simple substances in pea hydrolysates provides a better nutrition for the cells. In contrast, sponge doughs containing the hydrolysate include flowable liquid textures. The more hydrolysate the pre-ferment contains, the lower its viscosity is. This occurs due to the greater contents of simple substances that are unable to retain water molecules. Similar findings were achieved by Thiele et al. [37] since the authors reported that partial replacements of wheat flour with hydrolyzed proteins decreased the water absorption. This phenomenon was attributed to decreases in MWs of the proteins and, therefore, decreases in hydrophilicity of the system.

4. Conclusion

In this study, the optimal hydrolysis time of mechanically pretreated pea seeds included 4 h and concentration of the enzyme product was 2%. The optimal time of mechanical pretreatment of the feedstocks included 40 s at 1500 g. The resulting pea seed hydrolysate was added to bakery products. The sponge-and-dough method was reported as the optimal method of preparing dough with the hydrolysate since the end products included the best sensory characteristics. Due to the cleavage of peptide bonds by the enzyme products, addition of the hydrolysate led to higher contents of AAs and short peptides in the end products. Furthermore, analysis of the nutritional values demonstrated that the developed products were sources of biotin, pantothenic acid, sodium and iron. In conclusion, these developed products can be used in diets of healthy people to increase digestibility of the plant-based proteins.

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6. Conflict of Interest

The authors report no conflicts of interest.

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آبکافت آنزیمی فعال مکانیکی دانه‌های نخود و اثر آن بر فرآورده‌های نانوایی

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- فرآورده‌های نانوایی
- آبکافت
- پیش‌تیمار مکانیمی
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چکیده

سابقه و هدف: هیدروزیلات‌های پروتئین گیاهی منابع کارایی برای هضم آسان مواد مغذی محلول در آب می‌باشند. نخود محصـولی امیدبخش، غنی از پزوتئین است. در این مزالعه، پیش تیمار تلفیقی مکانیکی دانه‌های نخودو آبکافت آنزیمی آن به تولید یک مرحله‌ای برای افزایش راندمان ترکیبات محلول در آب پیشنهاد شده است. هیدروزیلات‌های حاصل به‌عنوان مواد متشکله فرآورده‌های نانوایی مورد استفاده قرار گرفتند.

مواد و روش ها: در این مطالعه، پیش‌تیمار مکانیکی دانه‌های نخود دو مرحله‌ای بود. در مرحله اول، ماده اولیه به ذرات اولیه به اندازه‌ای کمتر از ۲ میلیمتر با چرخ چاقو خرد شد. در مرحله دوم، ماده اولیه در حضور فرآورده‌های آنزیمی و با استفاده از آسیاب غلتکی گریز از مرکز RM-20 تحت تیمار مکانیکی قرار گرفت. آبکافت آنزیمی دانه‌های نخودتیمار مکانیکی شده به‌میزان ۱۲۰ گرم و در درجه حرارت ۵۰ درجه سلسیوس انجام شد. خمیر فرآورده‌های نانوایی با دو روش اسفنج —و-خمیر و خمیر مستقیم تهیه شد. پروتئین تام هیدروزیلات‌ها و فرآورده‌های نهایی با روش سنجش پروتئین بردفورد اندازه‌گیری شد. میزان آمینواسیدهای آزاد با کروماتوگرافی مایع - طیف سنجی جرمی و سیستم کروماتوگرافی مایع اجیلنت ۱۲۰۰ ارزیابی شد.

یافته‌ها و نتیجه‌گیری: در این مطالعه، زمان بهینه آبکافت، ۴ ساعت و غلطت فرآورده آنریمی ۲ درصد بود. زمان بهینه پیش‌تیمار مکانیکی مواد خام در آسیاب غلتکی گریز از مرکز ۴۰ ثانیه در g ۱۵۰۰ بود. هیدوزیلات‌های حاصله دانه‌های نخودبه فرآورده‌های نانوایی اضافه شدند. با توجه به بهترین ویژگی حسی فرآورده‌های نهایی، روش اسفنج — و-خمیر روش بهینه تهیه خمیر گزارش شد. به علت شکستن پیوندهای پپتیدی توسط فرآورده‌های آنزیمی، افزودن هیدروزیلات‌ها محتوای بالاتر آمینواسیدهای و پپتیدهای کوتاه‌تر در فرآورده‌های نهایی را تضمین کرد.

تعارض منافع: نویسندگان اعلام می‌کنند که هیچ نوع تعارض منافعی مرتبط با انتشار این مقاله ندارند.