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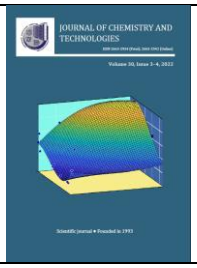

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CHEMICAL COMPOSITION OF VETCH SEEDS AND PROTEIN ISOLATE OBTAINED BY pH-SHIFTING TREATMENT

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Abstract

The chemical composition of vetch seeds (*Vicia sativa L.*) was studied to determine its potential as a raw material for obtaining protein isolate by pH-shifting treatment. Research results confirm the effectiveness of using vetch seeds to obtain protein isolate. Vetch seeds are low in lipids (2.08 % on dry weight), high in crude protein (30.12 % on dry weight) and rich in minerals (Zn, Mn, Cu, Mg and P). The ratio of essential amino acids to non-essential in vetch seeds (0.65). This is higher than the amount recommended by the World Health Organization. Plant protein is an alternative to animal protein in the food industry. Until now, vetch seeds (*Vicia sativa L.*) have not been widely used in the food industry due to the lack of industrial processing technologies. Protein isolate from vetch seeds was obtained by pH-shifting treatment. The chemical composition and functional properties of vetch seed protein isolate were determined. After pH-shifting treatment, the chemical composition of protein and mineral elements did not show significant changes. The protein content of the vetch seed protein isolate was 87.3 %, and the yield of the vetch seed protein isolate was 26.7 %. Indicators of water holding and fat binding capacity were investigated to confirm the functional properties of vetch seed protein isolate. The seeds of vetch and its protein isolate showed high functional properties and a high-quality chemical composition of proteins and minerals for use in the technology of sausage and confectionery, and as food for dietetic and vegetarian nutrition.

Key words: vetch seeds; essential amino acids; pH-shifting treatment; protein isolate; water holding capacity; fat binding capacity; plant protein; food additives; sausages; food products; confectionery; dietary and vegetarian nutrition.

ХІМІЧНИЙ СКЛАД НАСІННЯ ВИКИ ТА БІЛКОВОГО ІЗОЛЯТУ, ОТРИМАНОГО ШЛЯХОМ pH- КОРИГУЮЧОЇ ОБРОБКИ

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Анотація

Досліджено хімічний склад насіння вики (*Vicia sativa L.*) для визначення її потенціалу, як сировини для отримання білкового ізоляту pH-коригуючою обробкою. Показано, що насіння вики має низький вміст ліпідів (2.08 % на суху речовину), високий вміст сирого протеїну (30.12 % на суху речовину) та багате на мінеральні речовини (Zn, Mn, Cu, Mg та P). Співвідношення незамінних амінокислот до замінних (0.65), що вище за кількість, рекомендовану Всесвітньою організацією охорони здоров'я. Рослинний білок є альтернативою тваринного в харчовій промисловості. До теперішнього часу насіння вики (Vicia sativa L.) масово не застосовувалось в харчовій промисловості за відсутності промислових технологій переробки. Білковий ізолят з насіння вики отримували pH-коригуючою обробкою та визначали його хімічний склад та функціональні властивості. Після pH-коригуючої обробки, хімічний склад білка та мінеральних речовин не показав суттєвих змін. Вміст протеїну у білковому ізоляті насіння вики склав 87.3 %, а вихід білкового ізоляту з насіння вики – 26.7 %. Для підтвердження функціональних властивостей білкового ізоляту з насіння вики були досліджені показники вологоутримуючої та жироутримуючої здатності. Насіння вики та білковий ізолят з нього показали високі функціональні властивості та якісний хімічний склад білків і мінеральних речовин для використання у технології ковбасних та кондитерських виробів та як їжа для дієтичного та вегетаріанського харчування.

Ключові слова: насіння вики; незамінні амінокислоти; pH-коригуюча обробка; білковий ізолят; вологоутримуюча та жироутримуюча здатність; рослинний білок; харчові добавки; ковбасні вироби; продукти харчування; кондитерські вироби; дієтичне та вегетаріанське харчування.

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Introduction

Common vetch (Vicia sativa L.) – is a species of flowering plants of the legume family (Fabaceae). Vetch seeds are a potential source of protein [1] and can be used in food technology. Good adaptation to adverse environmental conditions contributes to vetch cultivation for seed production worldwide [2]. To improve existing technologies and overcome their shortcomings, new raw materials, specific to the region, are often used, for example, instead of sea mussels, scientists use freshwater mussels in regions far from the sea [3; 4]. The research uses vetch (Vicia sativa L.), which has small beans (weight of 1000 seeds 45–60 g) and is perfect for replacing standard raw materials. Before that, it was not used in the food industry and was grown only for animal feed, but due to its high protein content, vetch can be used in human nutrition. The quality of leguminous crops is now in the first place, and special attention is paid to their chemical composition [5–6].

Natural food components, which are presented in the form of functional powders with a wide range of applications, are now popular in food science. Among them, protein isolates obtained by pH-shifting treatment from oil crops [7], with high water holding capacity [8] and under certain conditions form gels that are soluble in various food media [9]. pH-shifting treatment is a new method of protein modification and improvement of its functional properties [7–9]. Interest in the nutritional value of vetch is confirmed by studies of acid hydrolysis of vetch to obtain starch [10] and its comparison with ordinary starch [11]. The production of starch and protein isolate from vetch creates prerequisites for its introduction into confectionery technology [12].

Various methods of drying and grinding can be used for the production of powders, preceding the process of obtaining protein isolates [13; 14] in order to use vetch as a food raw material even more efficiently. Powder from vetch was used to partially replace wheat flour in the technology of donut production [15]. To determine the required drying method, it is enough to conduct comparative studies of known drying methods and choose the best one [16].

Protein isolate from vetch seeds can be used for the production of yogurts with extended shelf life [17; 18]. Lupine [19], rice bran [20] and roasted barley [21] powder is used in yogurt technology and vetch can become their alternative.

In recent years, there has been a growing interest in plant-based diets to reduce the impact of climate change [22], and vetch can be a good substitute for animal protein. Protein isolate from vetch seeds is an excellent source of nutrients, micro- and macroelements for vegetarian and dietary food, due to its neutral taste and smell, it is well combined with other natural food ingredients to create balanced food compositions [23–26]. The high demand for high-quality protein isolates requires new methods and new raw materials for production, in order to increase the part of this product in food technology and public consumption.

Materials and Methods

Vetch seeds were used (Vicia sativa L.) for the experiments, that grown at the Sumy-Nasinnia agricultural company (Sad village, Sumy district, Sumy region, Ukraine) of the 2022 harvest. Before the experiment, vetch seeds were stored at room temperature. Before conducting research, vetch seeds were ground into powder with a particle size of 0.5 mm on a universal laboratory grinder. All chemicals were of reagent grade and used without further purification.

The method of pH-shifting treatment. The pH of the native suspension was adjusted to 9.5 (with solutions of 1 mol/l HCl or 1 mol/l NaOH). The pH was changed within 1 hour, and was changed to a neutral pH. The mixture was dialyzed and then lyophilized. Samples were stored at 4 ℃ for testing.

Determination of amino acids. Acid hydrolysis of proteins and peptides was carried out according to the method of Spackman, Stein and Moore [27]. 10–20 mg of a powdered vetch sample was transferred into 10 borosilicate ampoules with a diameter of 150 mm. 0.5 ml of 6 M HCl with 0.1 % phenol (w/v) was added to each ampoule. Amino acid samples were separated by ion exchange chromatography and determined by reaction with ninhydrin with photometric detection at 570 nm (440 nm for proline) on an automatic amino acid analyzer. In addition, nitrogen (N) was determined by the Kjeldahl micromethod in duplicate samples of dried seeds. Then the crude protein content was calculated by the coefficient of 6.25 [28].

Determination of lipid content was carried out by continuous extraction of lipids with a Soxhlet apparatus.

Determination of mineral substances. The seeds were washed with deionized water, dried at 75 °C, weighed and ashed at 480 °C in a muffle furnace. The ash was dissolved in 5 ml of 20 %

(v/v) HCl and diluted to a volume of 100 ml with deionized water. The solution was analyzed for K, P, Mg, Na, Ca, Zn, Mn, and Cu using an atomic absorption spectrometer SPECOL-11 according to Pinero, Baeta, Pereira, Domínguez, and Ricardo [29].

Water holding capacity capacity (WHC) was analyzed according to the described method [30]. Weighed vetch seed isolate was centrifuged (10 000 × g) for 15 minutes, and then filtered for 20 minutes. After this vetch seed isolate again weighed and carried out the corresponding calculations.

Fat binding capacity (FBC) was determined according to the described method [31]. The vetch seed isolate was dried to a constant weight, mixed with organic solvents and filtered through paper filter. Filtrate analyzed using a refractometer КФК-2МП.

Analysis of statistics. The results of the studies were expressed as the average value with the number of experiments n=3 and the standard error $\alpha \leq 0.05$.

Results and their discussion

The chemical composition of vetch seeds (Vicia sativa L.) to determine its potential as a raw material for obtaining protein isolate by pH-shifting treatment. Research results confirm the effectiveness of using vetch seeds to obtain protein isolate. The vetch seeds have a low lipid content of 2.08 % on dry weight, which meets the maximum fat content indicators for pH-shifting processing and produce protein isolate. Vetch seeds have a high crude protein content of 30.12 % by dry weight, which allows a high yield of protein isolate. The ash content is 3.31 % on dry weight (Fig. 1).

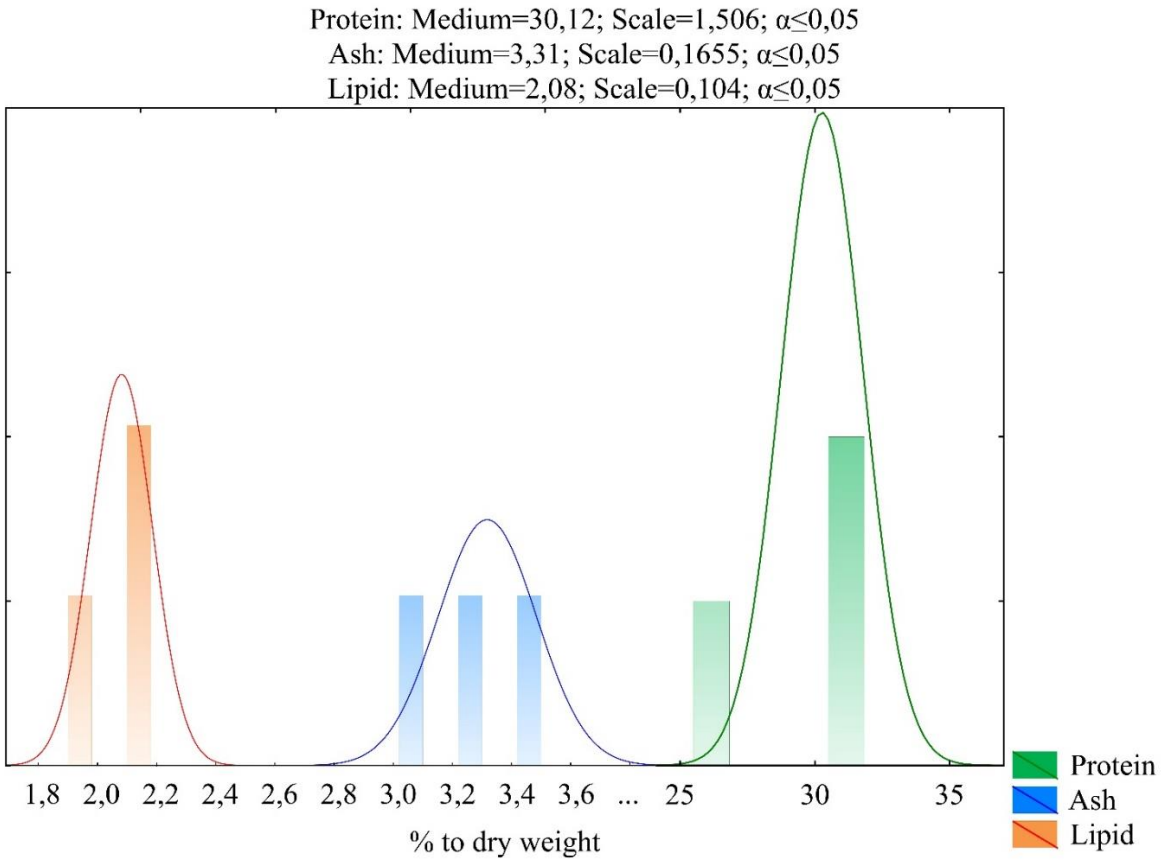

Fig. 1. General chemical composition of vetch seeds (Vicia sativa L.)

In the production of protein isolate, the presence of essential amino acids in raw materials plays an important role. This study examined 18 amino acids of vetch seed protein (Table 1). The content of essential amino acids is 11.24 %, non-essential - 17.37 %. The total content of amino acids in this study was at the level of 28.6 %. The ratio of non-essential amino acids to their total amount was 0.39. The ratio of essential to non-essential amino acids was 0.65, which is higher than the recommended ratio of WHO [32].

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Table 1

Amino acids of vetch seeds

n = 3, α≤0.05

Essential amino acids	Content, % of dry weight	Non-essential amino acids	Content, % of dry weight
Arginine	2.41	Glutamic acid	5.52
Leucine	2.13	Aspartic acid	3.7
Lysine	1.67	Valine	1.68
Isoleucine	1.27	Serine	1.38
Phenylalanine	1.22	Alanine	1.33
Threonine	1.05	Glycine	1.19
Histidine	0.7	Proline	1.19
Methionine	0.49	Tyrosine	0.88
Tryptophan	0.31	Cysteine	0.51
In total	11.24		17.37

The mineral composition of vetch seeds was studied in table 2. Based on the indicators of the recommended dietary norm [33] it can be concluded that vetch seeds are rich in Zn, Mn and Cu, their ratio to the daily norm is 38.48 % - 72.33 %. Among the macronutrients, vetch seeds have a high content of Mg and P, their ratio to the daily norm is 56.31 % and 32.86 %. The other mineral elements are found at a rather low level or not detected at all in the samples. The use of vetch seeds as food supplements requires special attention to balanced nutritional compositions, as their mineral composition is incomplete.

Table 2

Mineral composition of vetch seeds

n = 3, α≤0.05

Mineral elements	Contents	Recommended dietary allowance, mg	% of the daily norm, which provides 100 g of vetch seeds
Microelements, mg/kg			
Zn	42.33	11.0	38.48
Mn	16.64	2.30	72.33
Cu	5.99	0.90	66.5
Macroelements, g/kg			
K	9.83	4700.0	20.92
P	2.30	700.0	32.86
Mg	2.37	420.0	56.31
Na	1.24	1500.0	8.27
Ca	1.13	1000.0	11.25

Analysis of the chemical composition of vetch seeds confirmed its potential as a raw material for obtaining protein isolate by pH-shifting treatment. Prepared and ground into flour vetch seeds were subjected to acid-alkaline hydrolysis with pH-shifting treatment. After pH-shifting treatment, the chemical composition of protein and mineral elements did not show significant changes. In the obtained samples, the protein content was 87.3 %, and the yield of protein isolate from vetch seeds was 26.7 %. Studies have shown that there is a dependence on the content of crude protein in vetch seeds and protein in the protein isolate, which affects yield of vetch seeds protein isolate (Fig. 2).

To confirm the functional properties of the vetch seed protein isolate, the indicators of water holding capacity and fat binding capacity were investigated (Fig. 3).

The process of changing the pH exposed the active centers and areas of the protein, resulting in a protein isolate with a compact structure that helps bind and retain water and fat. High indicators of water holding capacity (1.2 ml/g) and fat binding capacity (0.9 ml/g) allow the use of vetch seed protein isolate in the technology of sausage and confectionery products.

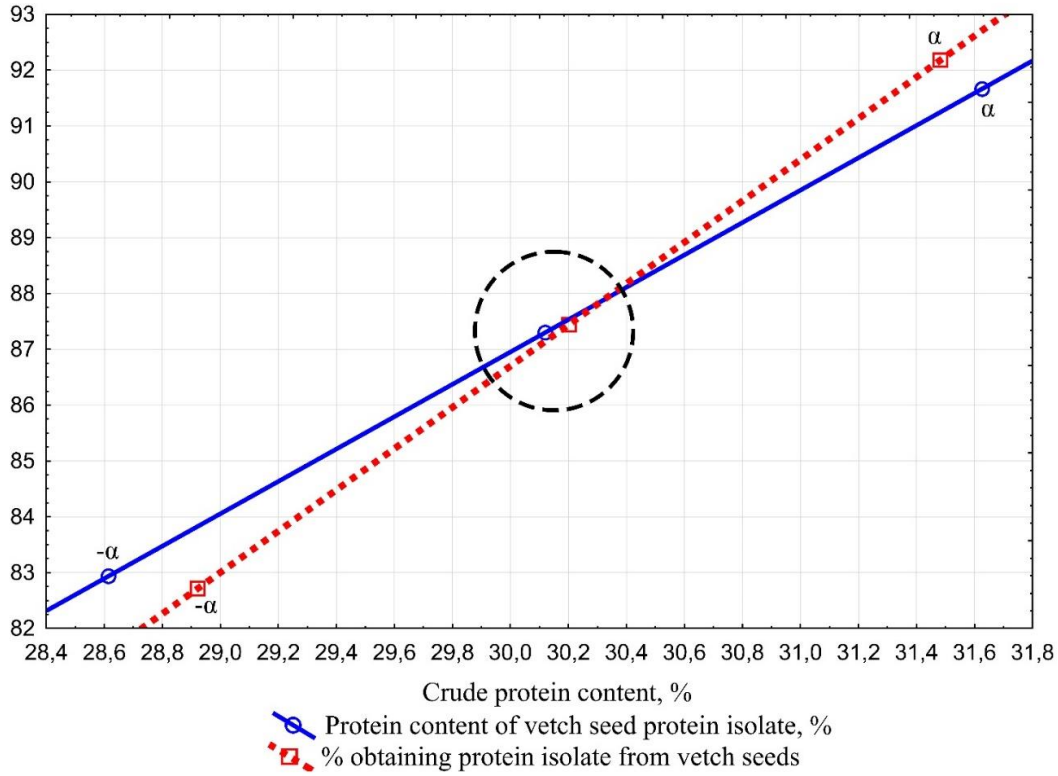

Fig. 2. The effect of crude protein content in vetch seeds on the yield of protein isolate

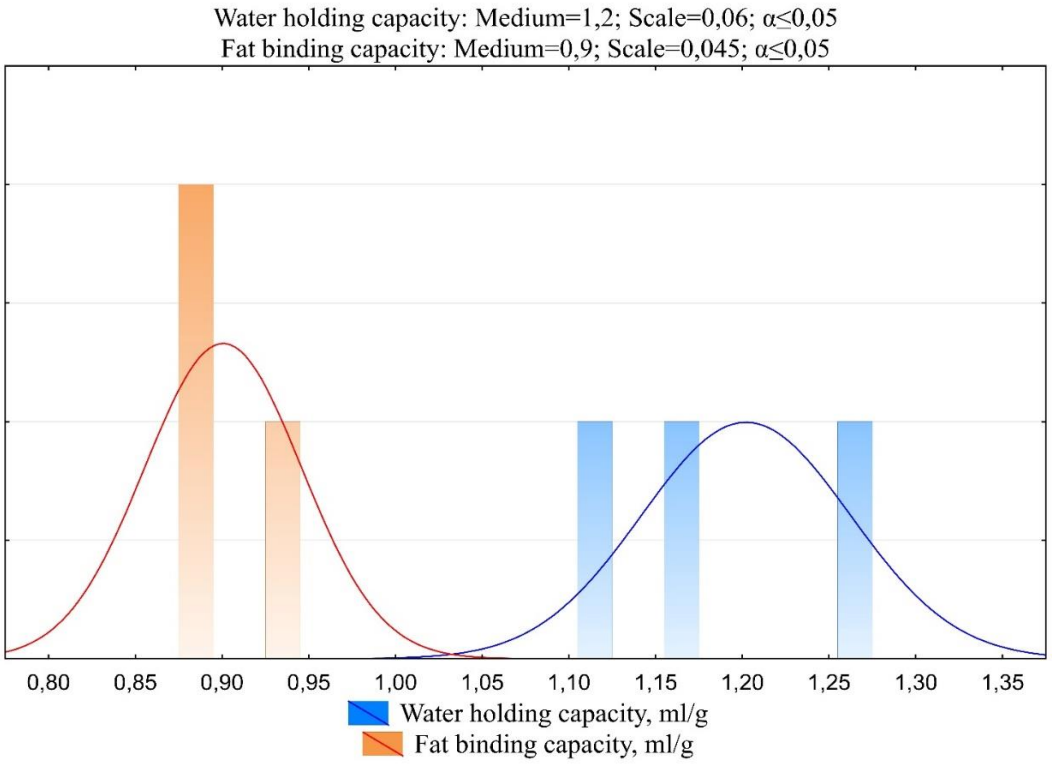

Fig. 3. Functional properties of vetch seed protein isolate

Conclusions

During the research, the chemical composition of vetch seeds (Vicia sativa L.) was determined. It was established that the content of lipids in vetch seeds is (2.08 % of dry weight), crude protein (30.12 % of dry weight) and minerals (3.31 % of dry weight). It was determined that the ratio of essential to replaceable amino acids was (0.65), which is higher than the amount recommended by the World Health Organization. Protein isolate from vetch seeds was obtained by pH-shifting treatment. The chemical composition and functional properties of vetch seed protein isolate

were determined. It was experimentally confirmed that the protein content of vetch seed protein isolate was 87.3 %, and the yield of vetch seed protein isolate was 26.7 %. Further studies of vetch seeds (Vicia sativa L.) should be directed to the search for vetch varieties with a higher crude protein content. The functional and technological properties of the vetch seed protein isolate should be investigated more widely. Researchers should pay attention to products using vetch seed protein isolate, such as sausages and confectionery, to increase the biological value of these products and expand their assortment.

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