Golovko, Tetyana; Golovko, Mykola; Vasilenko, Olha et al.

Article

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Tetyana Golovko,
Mykola Golovko,
Olha Vasilenko,
Fedir Pertsevoi,
Natalia Bolgova,
Vasyl Tischenko,
Vladyslav Prymenko

TECHNOLOGY OF PROTEIN ISOLATE FROM PEAS (*PISUM SATIVUM VAR. ARVENSE*)

The object of research is pea (Pisum sativum var. arvense) and pea protein isolate. The chemical composition of pea (Pisum sativum var. arvense) was studied to determine its potential as a new raw material for obtaining protein isolate by pH-shifting treatment. The obtained results confirm the effectiveness of using peas to obtain protein isolate for use in the food industry instead of soy protein isolate. Peas are low in lipids (1.61 % on dry matter), high in crude protein (19.21 % on dry matter), ash (3.41 % on dry matter) and minerals (Se, Fe, Zn, Mn, Cu, Mg, P). The ratio of essential amino acids to replaceable amino acids (0.78), which is higher than the amount recommended by the World Health Organization. Vegetable protein is an alternative to animal protein in the food industry. Protein isolate from peas was obtained by pH-shifting treatment and its chemical composition and functional properties were determined. After pH-shifting treatment, the chemical composition of protein and mineral substances did not show significant changes. The protein content of pea protein isolate was 82.2 %, and the yield of pea protein isolate was 5.6 %. To confirm the functional properties of pea protein isolate, the indicators of moisture-holding capacity (1.05 ml/g) and fat-holding capacity (0.82 ml/g) were investigated. Peas and protein isolate from it showed high functional properties and high-quality chemical composition of proteins and minerals for use in the technology of sausage products, which should be the focus of further research.

Keywords: *pea seeds, vegetables, protein isolate, pH-shifting treatment, sausage products, quality characteristics.*

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1. Introduction

Pea (*Pisum sativum var. arvense*) is a type of flowering plant from the legume family. The herbaceous plant widely grown in fields as a legume. Pea (*Pisum sativum var. arvense*) is used in the study, which has small beans (weight of 1000 seeds 150–300 g) and is excellent for replacing standard raw materials (soy and peanut). Before that, it was not used in the food industry to obtain protein isolate, but due to its high protein content, peas are suitable for high-tech processing.

In recent years, proteins of animal origin are gradually being replaced by proteins of vegetable origin, which is associated with high consumer demand and ecological problems of the environment [1]. Currently, a variety of proteins of plant origin, such as soy, wheat, corn, and cottonseed proteins, are widely used in the food industry as dietary supplements or food ingredients [2].

Legumes are edible seeds of legumes. Legumes are considered the second most important source of human nutrition after cereals [3]. Not only legumes, but also niche vegetable crops, such as pumpkin seeds, are used to replace traditional raw materials for the production of protein isolates, due to the high content of crude protein and the absence of allergens in their composition [4]. They are an inexpensive source of protein and other nutrients such as starch, dietary fiber, vitamins, minerals and polyphenols. In general, legumes are a good source of protein (20–40 %), especially in combination with cereals [5].

Proteins in the form of concentrates or isolates are used as a functional ingredient primarily to improve the nutritional quality and provide desired sensory characteristics such as structure, texture, taste and color of finished food products. Protein concentrates and isolates used in the food industry today are mainly derived from soy, whey, and wheat, but due to dietary restrictions and preferences, food manufacturers and consumers are looking for alternative protein [6]. Legumes can be considered the most suitable for the preparation of protein isolates due to their high protein content, low cost and wide acceptability [7]. Peas are a new raw material for obtaining protein isolates, as well as seeds of other vegetable crops, which are now widely used to optimize the production of protein isolates – pumpkin seeds [8] and hemp seeds [9]. Peas are one of the most widely cultivated and consumed legumes in the world [10]. Peas are the second most important leguminous crop grown on 25 million acres worldwide [11]. It is widely grown and consumed in Ukraine, China, the United

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States, Canada, and France [12]. Peas contain 20–25 % protein with a higher content of lysine and tryptophan than cereal grains. In addition, field peas contain 5–20 % less trypsin inhibitors than soy [13].

Protein isolates of various vegetable crops are widely used to replace traditional raw materials in recipes of yogurts [14] and sausages [15]. Pea proteins have been found suitable for preparing gluten-free muffins with characteristics comparable to those made using wheat gluten [16].

The most important properties of protein in food products are solubility, ability to bind water and fat [17], emulsification, foaming and whipping [18]. Functional and rheological properties of protein are related to molecular size, structure, protein charge distribution, protein molecules, as well as environmental factors and processing conditions [19, 20].

Proteins from different leguminous crops and varieties differ in physicochemical and structural characteristics, which affect their functionality and application in the food industry. The current study provides information on the relationship between protein characteristics and functional properties. Information about the characteristics of proteins will help determine their intended use in the food industry [21].

This research was aimed at studying and comparing the physicochemical and chemical properties of peas and protein isolate from them. The high demand for high-quality protein isolates requires new methods and new raw materials for production in order to increase the share of this product for use in food technology and public consumption.

2. Materials and Methods

Fresh pea seeds (*Pisum sativum var. arvense*), purchased at the local market in Sumy, Ukraine, were used for the experiments. Before the experiment, the pea seeds were stored at room temperature. Before conducting research, peas were mashed into a puree to a uniform consistency in a laboratory blender. All chemicals were of reagent grade.

Method of pH-adjusting treatment. The pH of the native suspension was adjusted to 9.2 (with solutions of 1 mol/l HCl or 1 mol/l NaOH). The pH was changed for 1 hour, and was changed to neutral pH. The mixture was dialyzed and then lyophilized. Samples were stored at 4 °C for testing.

Determination of amino acids. Acid hydrolysis of proteins and peptides was performed according to the method of Spackman, Stein, and Moore [22]. 10–20 mg of a powdered pea seed 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 [23]. 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 Fe, Zn, Mn, Se, Cu, K, P, Mg, Ca using an atomic absorption spectrometer according to Pineiro, Baeta, Pereira, Domínguez and Ricardo [24].

The moisture-retaining capacity (MRC) was analyzed according to the described method [25]. The weighed pea isolate was centrifuged (10,000×g) for 15 minutes and then filtered for 20 minutes. After that, the pea isolate was weighed again and appropriate calculations were performed. Fat-retaining capacity (FRC) was determined according to the described method [26]. The pea isolate was dried to a constant weight, mixed with organic solvents and filtered through a paper filter. The filtrate was analyzed using a refractometer.

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

3. Results and Discussion

The chemical composition of pea (*Pisum sativum var. arvense*) was studied to determine its potential as a raw material for obtaining protein isolate by pH-adjusting treatment. The obtained results confirm the effectiveness of using peas to obtain protein isolate. The moisture content of fresh peas was 75.6 %. It is also possible to use dried peas, but this processing technology will be more expensive. Peas have a low lipid content of 1.61 % on dry matter, which meets the maximum fat content for pH-adjusting processing and obtaining protein isolate. High crude protein content of 19.21 % on dry matter, which makes it possible to obtain a large amount of protein isolate from peas. The ash content is 3.41 % on dry matter (Fig. 1).

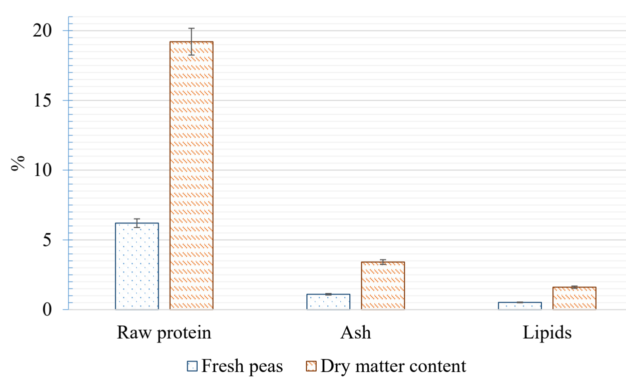

Fig. 1. Chemical composition of peas (*Pisum sativum var. arvense*)

In the production of protein isolate, the presence of essential amino acids in the raw materials plays an important role. In this study, 18 amino acids of pea protein were investigated (Table 1). The content of essential amino acids is 1.95 g/100 g of peas, replaceable – 2.48 g/100 g of peas. The total content of amino acids in this study was at the level of 4.43 g/100 g of peas. The ratio of essential amino acids to their total amount was 0.44. The ratio of essential to replaceable amino acids was 0.78, which is higher than the ratio recommended by the World Health Organization [27].

The mineral composition of fresh peas is given in Table 2. Based on the indicators of the recommended dietary norm [28], it can be concluded that peas have a high content of Mg, P, their ratio to the daily norm is 8.33–15.71 %. Among trace elements, peas have a high content of Se, Fe, Zn, Mn, Cu, their ratio to the daily norm is 3.57–21.78 %. Other elements are at a fairly low level or not detected in the samples at all, this situation requires special attention when creating balanced food compositions.

Table 1

Pea amino acids and their quantitative characteristics $n=3$, $\alpha<0.05$

Essential amino acids	Content, g/100 g	Non-essential amino acids	Essential amino acids
Arginine	0.43	Glutamic acid	0.75
Leucine	0.33	Aspartic acid	0.5
Lysine	0.32	Valin	0.24
Isoleucine	0.2	Serin	0.19
Phenylalanine	0.21	Alanine	0.24
Threonine	0.22	Glycine	0.19
Histidine	0.11	Proline	0.18
Methionine	0.09	Tyrosine	0.15
Tryptophan	0.04	Cysteine	0.04
In total	1.95	2.48	

Table 2

The content of micro- and macroelements of peas $n=3$, $\alpha<0.05$

Mineral elements	Contents	Recommended dietary norm, mg	% of the daily norm, which provides 100 g of peas
Microelements, mg/100 g			
Fe	1.57	15	10.47
Zn	1.54	11	14
Mn	0.46	2.3	20
Se	0.0025	0.07	3.57
Cu	0.196	0.9	21.78
Macroelements, mg/100 g		4700	5.43
K	255		
P	110	700	15.71
Mg	35	420	8.33
Ca	26	1000	2.6

Despite the small indicators of ensuring the daily norm of mineral substances, it should be taken into account that 100 g of peas is a small amount, taking into account the high moisture content (75.6 %). In Fig. 2, it is clearly visible that this amount is much higher if the calculation is made on dry matter.

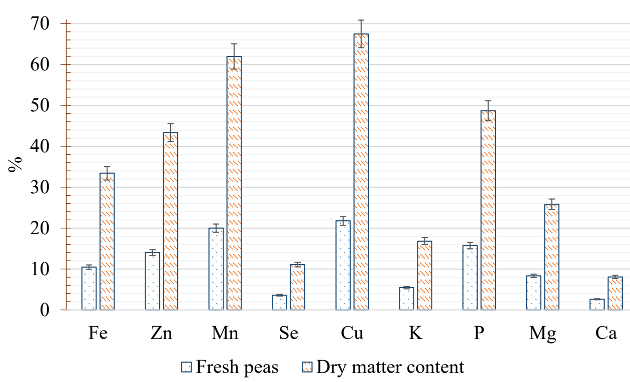

Fig. 2. Provision of the daily norm of micro- and macroelements with fresh peas

Analysis of the chemical composition of peas confirmed its potential as a raw material for obtaining protein isolate by pH-adjusting treatment. Prepared and crushed peas were subjected to acid-alkaline hydrolysis with pH-adjusting treatment. In the obtained samples, the protein content was 82.2 %, and the yield of protein isolate from fresh peas was 5.6 %. Studies have shown that there is a dependence on the content of crude protein in peas and protein in protein isolate, which affects its output.

To confirm the functional properties of pea protein isolate, indicators of moisture-retaining capacity and fat-retaining capacity were investigated. The process of changing the pH exposed the active sites and regions of the protein, resulting in a protein isolate with a compact structure that helps bind and retain moisture and fat. High moisture-holding capacity (1.05 ml/g) and fat-holding capacity (0.82 ml/g) allow the use of pea protein isolate in the technology of sausage products.

Protein isolate from pea (*Pisum sativum var. arvense*) is a new raw material for the food industry, which can replace a part of soy protein in the technology of meat products. It is best to use protein isolate from peas for the production of sausage products. The obtained research results were largely influenced by the variety of peas, when using a different variety, the results may differ. Martial law in Ukraine has slowed down research, and some laboratories and research institutes have been destroyed or stopped working. This situation led to the search for other places to conduct research, which made it difficult and only three repetitions were carried out, which reduced the accuracy of the experiments. Further research should be aimed at finding pea varieties with higher protein content and replacing soy protein with pea protein isolate in sausage recipes.

4. Conclusions

The chemical composition of peas (*Pisum sativum var. arvense*) was studied. It was established that the content of lipids in peas is 1.61 % on dry matter, crude protein – 19.21 % on dry matter, and mineral substances – 3.41 % on dry matter. It was determined that the ratio of essential to replaceable amino acids was 0.78, which is higher than the amount recommended by the World Health Organization. A protein isolate from crushed pea seeds was obtained with the help of pH-adjusting treatment, and its chemical composition and functional properties were determined. It was experimentally confirmed that the protein content of pea protein isolate was 82.2 %, and the yield of pea protein isolate was 5.6 %. To confirm the functional properties of pea protein isolate, the indicators of moisture-holding capacity and fat-holding capacity were investigated, which were 1.05 ml/g and 0.82 ml/g, respectively. Further studies of peas (*Pisum sativum var. arvense*) should be directed to the search for varieties with a higher crude protein content. The functional and technological properties of pea protein isolate should be investigated more widely. Researchers should pay attention to products using pea protein isolate, such as sausage products, to increase the biological value of these products and expand their range.

Conflict of interest

The authors declare that they have no conflict of interest in relation to this research, whether financial, personal, authorship or otherwise, that could affect the research and its results presented in this paper.

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Data availability

The manuscript has no associated data.

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Tetyana Golovko, *Doctor of Technical Science, Professor, Department of Meat Technology, State Biotechnological University, Kharkiv, Ukraine, ORCID: https://orcid.org/0000-0001-7059-3620*

Mykola Golovko, *Doctor of Technical Science, Professor, Department of Chemistry, Biochemistry, Microbiology and Food Hygiene, State Biotechnological University, Kharkiv, Ukraine, ORCID: https://orcid.org/0000-0002-1778-4847*

✉***Olha Vasilenko,*** *PhD, Associate Professor, Department of Occupation Safety and Physics, Sumy National Agrarian University, Sumy, Ukraine, e-mail: vasylenko.sumy@gmail.com, ORCID: https://orcid.org/0000-0003-1643-0702*

Fedir Pertsevoi, *Doctor of Technical Science, Professor, Department of Food Technology, Sumy National Agrarian University, Sumy, Ukraine, ORCID: https://orcid.org/0000-0002-3111-5017*

Natalia Bolgova, *PhD, Associate Professor, Department of Food Technology and Safety, Sumy National Agrarian University, Sumy, Ukraine, ORCID: https://orcid.org/0000-0002-0201-0769*

Vasyl Tischenko, *PhD, Associate Professor, Department of Food Technology and Safety, Sumy National Agrarian University, Sumy, Ukraine, ORCID: https://orcid.org/0000-0001-8149-4919*

Vladyslav Prymenko, *PhD, Associate Professor, Department of Management and Administrating, Dnipro Faculty of Management and Business of Kyiv University of Culture, Dnipro, Ukraine, ORCID: https://orcid.org/0000-0001-7856-6678*

✉*Corresponding author*