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RESEARCH ARTICLE

Study of the Functional Potential of Plant Proteins for Practical Application in Food System Technology

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ABSTRACT

In recent years, there has been an increased interest in the functional potential of plant proteins. The identification of the protein composition of raw materials is a crucial aspect, as it allows for the direct determination of the quality of the proteins in the final product. The main proteins found in legumes, particularly peas, are globulin and albumin. This study aims to analyze the molecular weight distribution of pea protein samples, compare it with the protein composition of pea flour, and identify potential trypsin inhibitors. The aim of the study was to assess the functional potential of plant proteins for application in food system technology.

INTRODUCTION

An important task of the food industry is the development of modern resource-saving technologies that improve production efficiency and product quality. At the same time, according to FAO/WHO forecasts, the global population is increasing, which necessitates the search for additional protein sources. According to expert estimates, the demand for animal protein in Russia reaches 2.9 million tons, while domestic production covers 1.9 million tons, imports account for 0.45 million tons, and the remainder constitutes a deficit.

Due to the imbalance of the amino acid composition of cereal crops, the search for ways to improve the quality of protein products through the use of alternative complete protein sources is of particular relevance. One of the promising directions is the development of technologies for deep processing of legume raw materials based on biotechnological innovations [5, p. 59].

Despite the high biological value and functional properties of soybean proteins, the genetic modification of soybean seeds has led to a negative consumer attitude toward products containing soy derivatives. In this regard, the use of other leguminous crops not subjected to genetic modification is of practical interest. In this context, peas represent a promising crop. Legumes have long been used in human diets, including among individuals who, for various reasons, do not consume meat. Peas, as an agricultural crop, attract increased interest in the development of technologies for producing protein concentrates, isolates, specialized flours, and related by-products [6, p. 24].

Legume proteins differ from cereal proteins by a higher content of several essential amino acids, including threonine, isoleucine, leucine, valine, lysine, and tryptophan. Among these, lysine occupies a special place; its concentration in pea protein is approximately 7 g per 100 g of product, making peas a valuable source of this amino acid [3, p. 6].

The results of previous studies [1, p. 28; 2, p. 21; 4, p. 26] confirm that pea protein isolate has high nutritional value and is of particular importance for individuals leading a healthy lifestyle. The isolate is widely used in the food industry due to its amino acid profile, resulting from the presence of a large number of amino acids and other nutritional properties. It should be noted that, unlike soy, the level of isoflavones, which negatively affect testosterone levels and behavioral responses, is relatively low; in addition, peas are considered non-allergenic products [10, p. 1835; 18, p. 7029].

Protein production processes (flour, isolates, concentrates) are divided into “dry” and “wet” methods. “Dry” processes include milling and air classification, which separates legume particles by size and density into two fractions: light (protein) and heavy (starch). The light (fine) fraction represents a protein concentrate, while the heavy (coarse) fraction is starch-rich. After primary fractionation of ground legume raw material, two main fractions are obtained: a light protein fraction and a heavy starch fraction. The heavy fraction is then subjected to repeated separation [7, p. 411; 13, p. 416].

Identification of the protein composition of raw materials is an important aspect, as it allows direct determination of the qualitative composition of proteins in the final product. The main proteins of legumes, including peas, are globulins and albumins. The principal components of the globulin fraction are well studied and include 11S globulin (legumin) and 7S globulin (vicilin) [14, p. 311; 15, p. 48].

Proteins contribute to emulsion stability (ES) by increasing the viscosity of the continuous phase and reducing the mobility of oil droplets in the system. A relationship between surface hydrophobicity, interfacial tension, and the emulsifying properties of pea proteins has been established [17, p. 301; 8, p. 357; 23 p.109980; 24 p. 233; 25 p. 356].

In addition, similar to hydrophobicity, the surface charge of proteins influences their adsorption at the phase interface. The charge must be sufficiently high to counteract attractive forces, thereby stabilizing electrostatic repulsion between oil droplets. At the isoelectric point, proteins have a net zero charge [16, p. 801; 11, p. 107].

Globulin 7S (both vicilins and convicilin) and globulin 11S exhibit potential allergenic properties, which are most pronounced for vicilin subunits (α + β + γ) and convicilin with molecular weights of 50 kDa and 70 kDa, as well as for vicilin-derived fragments obtained by proteolytic cleavage with molecular weights of 36, 32, 16, and 13 kDa. Pea albumins are a rich source of sulfur-containing amino acids; however, they are less studied and are characterized by significant heterogeneity, comprising several groups of proteins with molecular weights ranging from 10 to 100 kDa, including biologically active proteins such as enzymes, as well as protease inhibitors and lectins [12, p. 123; 9, p. 725; 20, p. 3315].

It is well known that legume seeds contain a wide range of compounds that serve as defense mechanisms against insect pests, including lectins, protease inhibitors (trypsin and chymotrypsin inhibitors), amylases, and secondary metabolites. Protease inhibitors are generally classified into serine, cysteine, aspartic, and metalloproteinase inhibitors. Kunitz and Bowman–Birk serine inhibitors are well characterized: Kunitz-type inhibitors are proteins of approximately 19 kDa with two disulfide bonds, whereas Bowman–Birk inhibitors are typically proteins of approximately 8 kDa with seven disulfide bonds.

This study analyzes the molecular weight distribution of pea protein samples, compares them with the protein composition of pea flour (used as a reference sample), and identifies proteins that are potential trypsin inhibitors.

MATERIALS AND METHODS

Molecular weight distribution was analyzed using one-dimensional electrophoresis. Sample preparation of pea protein included weighing 100 mg of the sample and adding 2000 μL of distilled water. After thorough mixing, the resulting suspension was centrifuged at 14,000 rpm for 10 min to precipitate insoluble components. The supernatant was then separated and mixed in a 1:1 ratio with protein buffer (1 mL of 10% sodium dodecyl sulfate (SDS), 250 μL of concentrated β-mercaptoethanol, 625 μL of 0.5 M Tris-HCl, 1.5 g of urea). Bromophenol blue was added to the solution until a dark coloration appeared, and the volume was adjusted to 5 mL with distilled water, followed by heating in a water bath at 100±2°C for 5 min.

Pea flour samples were also taken in an amount of 100 mg, mixed with 1000 µL of distilled water, and centrifuged at 14,000 rpm for 10 min. The supernatant was then mixed with protein buffer in a 1:1 ratio, bromophenol blue was added until a dark coloration appeared, the volume was adjusted to 5 mL with distilled water, and the samples were heated in a water bath for 5 min.

One-dimensional electrophoresis in polyacrylamide gel was performed using a vertical gel electrophoresis chamber (Helicon, Russia) filled with 12.5% polyacrylamide gel. A 6% stacking gel with wells for sample loading was layered on top. The sample volume loaded was 2 µL. The running buffer contained 25 mM Tris-HCl, 192 mM glycine, and 0.1% SDS. Electrophoresis was carried out under the following conditions: 60 V for the first 30 min, followed by 120 V until the dye front (bromophenol blue) reached the bottom of the gel [3, 4].

Visualization and image analysis were performed by staining proteins with Coomassie G-250 solution (10% acetic acid, 25% isopropanol, 0.05% Coomassie G-250). To remove unbound dye, the gels were washed in 10% acetic acid. One-dimensional electropherograms in a wet state were used for computer densitometry. Full digital images were obtained using a Bio-5000 Plus scanner (Serva, Germany) at 600 ppi in 2D-RGB mode. The resulting images were processed using a graphical editor. The visualized protein fractions were interpreted using the UniProt database [5].

RESULTS AND DISCUSSION

An electropherogram was obtained (Fig. 1).

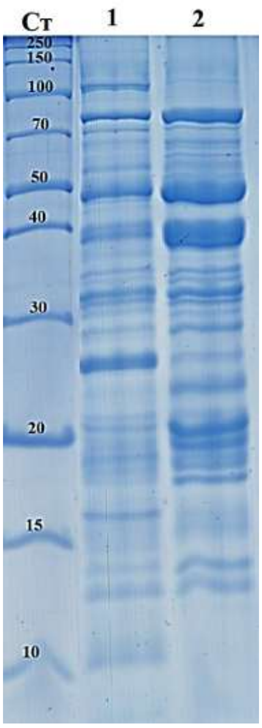

Figure 1. One-dimensional electropherogram of the samples (Coomassie G-250 staining). Designations: St – molecular weight marker; 1 – pea flour sample; 2 – pea protein sample.

A wide range of protein compounds with different molecular weights was identified in the electropherogram. Distinct major protein fractions were observed in the following molecular weight ranges: >70 kDa, 48–50 kDa, 37–40 kDa, 25 kDa, 23 kDa, and 19–20 kDa. Numerous minor bands corresponding to compounds in the ranges of 100 kDa, 70 kDa, 55–65 kDa, 50 kDa, 32–35 kDa, and 17–18 kDa were also detected.

Fractions in the molecular weight range of ~50–110 kDa may correspond to high-molecular-weight albumins; those in the range of ~20–35 kDa to medium-molecular-weight albumins; and those in the range of ~13–17 kDa to low-molecular-weight albumins.

Intense bands in the 32–35 kDa range may correspond to globulin fractions of pea proteins. In the electropherogram of the pea protein sample, intensely stained protein fractions were detected in the regions of ~80 kDa, ~45–46 kDa, ~38–40 kDa, and ~19–20 kDa.

These fractions can be attributed to subunits of globulin proteins 11S (legumin) and 7S (vicilin). Legumin is a hexamer consisting of three acidic (α-subunit, ~40 kDa) and three basic subunits (β-subunit, ~20 kDa). Vicilin is a trimer with a molecular weight of approximately 150 kDa and includes three polypeptides of ~50 kDa, which undergo enzymatic hydrolysis into an α-chain (19–20 kDa), β-chain (13 kDa), and γ-chain (12–16 kDa). The α+β chains may form a subunit with a molecular weight of 30–36 kDa, which together with the γ-chain forms the 7S globulin. Protein fractions in the region of ~70 kDa may be identified as convicilin or vicilin fraction.

Table 1 presents trypsin inhibitor proteins characteristic of the genus *Pisum* (pea), identified using the UniProt database.

Table 1: Presence of proteins in pea protein samples

No.	Protein name	Gene name	Organism	Molecular weight, kDa
1	Trypsin/chymotrypsin inhibitor, seed IVB		*Pisum sativum* (garden pea)	7,86
2	Trypsin/chymotrypsin inhibitor, seed IVA	TI1236	*Pisum sativum* (garden pea)	10,54
3	Trypsin/chymotrypsin inhibitor	ti7V	*Pisum sativum* (garden pea)	12,53
4	Trypsin/chymotrypsin inhibitor		*Pisum sativum* (garden pea)	12,57
5	Seed trypsin/chymotrypsin inhibitor TI5-72	TI572	*Pisum sativum* (garden pea)	12,60
6	Trypsin/chymotrypsin inhibitor	ti1 KIW84_033621	*Pisum sativum* (garden pea)	12,67
7	Putative trypsin inhibitor	ti6	*Pisum sativum* (garden pea)	14,82
8	Pea protease inhibitor 2	PIP20-2 / FUC2	*Pisum sativum* (garden pea)	23,64
9	Pea protease inhibitor 1	PIP20-1 / FUC1	*Pisum sativum* (garden pea)	23,79

The trypsin/chymotrypsin inhibitor seed IVB with a molecular weight of 7.86 kDa and the trypsin/chymotrypsin inhibitor seed IVA with a molecular weight of 10.54 kDa were not detected in the pea protein sample.

Proteins in the molecular weight range (MW) from 12.53 to 12.67 kDa showed similar intensity in both the pea flour sample and the pea protein sample.

The putative trypsin inhibitor (14.82 kDa) was approximately threefold more abundant in the pea protein sample compared to the pea flour sample. The pea protease inhibitor 2 (23.64 kDa) and pea protease inhibitor 1 (23.79 kDa) were approximately twelvefold more abundant in the pea protein sample.

For clarity, the identified proteins are highlighted in Figure 2. Proteins No. 1 and No. 2, which were detected only in the pea flour sample, are marked in orange on the electropherogram. The integrated densities of the selected protein bands were determined using ImageJ software (Table 2).

Table 2: Peak areas of protein fractions

Molecular weight, kDa	Peak area of pea flour sample, a.u.	Peak area of pea protein sample, a.u.
12	3082,0	3195,5

14	1288,1	3820,5
23	685,5	8 794,5

The study revealed a wide range of protein compounds with different molecular weights. Regions corresponding to albumins, globulins, and legumin (~32–34 and ~38–42 kDa), as well as vicilin fractions (~30–36 kDa), were identified.

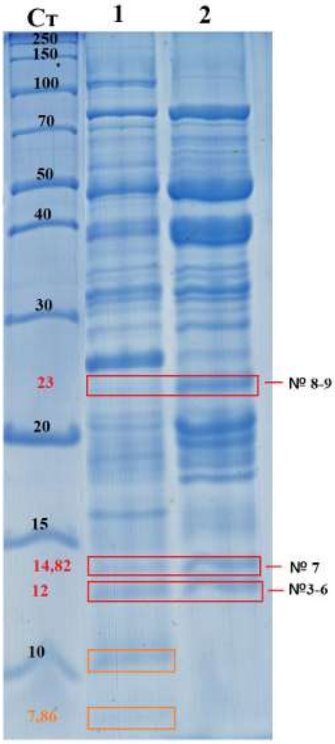

Figure 2: One-dimensional electropherogram with highlighted proteins. Note: proteins present only in pea flour are marked in orange, whereas proteins present in both samples are marked in red.

CONCLUSION

1. Trypsin inhibitors were tentatively identified in pea flour and pea protein samples: trypsin/chymotrypsin inhibitor seed IVB (7.86 kDa), trypsin/chymotrypsin inhibitor seed IVA (10.54 kDa), trypsin/chymotrypsin inhibitor (12.53 kDa), trypsin/chymotrypsin inhibitor (12.57 kDa), seed trypsin/chymotrypsin inhibitor (12.60 kDa), trypsin/chymotrypsin inhibitor (12.67 kDa), putative trypsin inhibitor (14.82 kDa), pea protease inhibitor 2 (23.64 kDa), and pea protease inhibitor 1 (23.79 kDa).
2. The results obtained in this study indicate the feasibility of further investigation and application of pea proteins in food system technologies.

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