The mechanoenzymatic method for enhancing the biological value of condensed cream soups

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Abstract. Condensed creamy pea soups of high biological value have been developed. A stage comprising enzymatic hydrolysis in combination with mechanochemical treatment of raw material was proposed as a method to increase the content of free amino acid and short peptides in the finished product. Evaluation of physicochemical quality parameters demonstrated that the samples had high nutritional and biological value.

1. Introduction
The demand for protein is an evolutionary feature of human nutrition. It is associated with the need to meet the physiological requirements for the intake of essential amino acids, the compounds having a direct impact on vital functions of the human body. Unlike fats and carbohydrates, proteins are neither accumulated in the organism nor synthesized from other nutrients. In other words, adequate intake of proteins is ensured only by nutrition [1].

All proteins in human diet can be divided into two main groups: plant- and animal-derived ones. Animal protein contains all essential amino acids. However, a person eating animal proteins with proper amino acid content also consumes saturated fatty acids and cholesterol, which increase the risk of developing lifestyle diseases such as obesity, diabetes mellitus, and cardiovascular disorders [2]. Plant-based protein foods contain much less saturated fat, while the content of polyunsaturated fatty acids is rather high. Polyunsaturated fatty acids are involved in maintenance of the cell membrane function and reduce the risk of atherosclerosis and cardiovascular disorders. For this very reason, one needs to maintain the balance between consumption of animal and plant proteins [3, 4].

An analysis of the current global trends demonstrates that the percentage of manufactured animal source foods is rather low (80% of all protein produced worldwide is plant protein and only 20% is animal protein). Among the plant-based sources of protein, 50% of protein comes from grains and 25% comes from grain legumes and oil-bearing crops. Therefore, plant-based raw materials are a promising protein source for solving the problem of protein deficiency in the population [5, 6].

All the existing cultivated plants are classified into the following groups: ornamental plants (rose); cereal grains (rice, corn, and wheat); legumes (beans, peas, and soybeans); starch crops (sweet and regular potatoes); sugar crops (sugar beet); oil-bearing crops (sunflower); fiber crops; cucurbit crops (watermelon); vegetable plants (tomatoes, cucumbers, and dill); fruit plants (pineapples and coconuts), and plants exhibiting a stimulant effect (tea, coffee, and poppy). Among these species, the legumes, grains, oil-bearing and nuciferous crops (the latter group belongs to the class of fruits) can be used as a source of plant protein.
This classification infers that there is a wide variety of raw materials based on plant proteins, which can be processed to manufacture functional and specialized food products. However, when searching for sources of plant protein, it is important to assess the economic expediency of using a certain crop as the finished product may be too expensive. Thus, protein content in algae can be as high as 50%, making them superior even to legumes. However, it is infeasible to use this crop in Russia, since it is non-traditional for Russian population and, consequently, economically unviable for systemic use in the diet.

It is a known fact that only 62–80% of consumed plant proteins is absorbed in the human body. Substances known as antinutrients are the reason for this level of protein absorption. The data on the effects of the main inhibiting substances of plant origin are summarized in table 1 [7].

| Antinutrient name | Effect on the human body |
|-------------------|--------------------------|
| Proteolytic enzyme inhibitors | react with enzymes to form stable compounds, thus impeding protein biosynthesis. |
| Phytic acid | impairs absorption of iron, zinc, calcium, phosphorus, and other minerals from the food product in which it is contained. |
| Stachyose | are not completely absorbed in the small intestine, resulting in excessive formation of intestinal gas. |
| Raffinose | Lectins | impair digestion, worsen nutrient absorption, and inhibit maltase and aminopeptidase. |

The problem related to insufficient absorption of plant proteins can partially be solved using the chemical method consisting in removal of nonprotein constituents with inhibitory properties from the raw material. In this method, protein concentrates and isolates are obtained, which differ in terms of such parameters as the procedure used for their production, degree of purification, the nature of raw material, and total protein content. Protein concentrate is a purified protein product with 56–66% protein content. In order to produce it, the raw material needs to undergo such stages as defatting, suspension, salt or alkaline extraction, centrifugation, precipitation, and fractionation. Today, two methods are utilized to produce protein concentrates: turbo-separation (the "dry" method) and extraction in a liquid medium (the "wet" method). The protein isolate is a maximally purified product with protein content > 90%. The conventional flowchart for production of a protein isolate involves protein extraction followed by addition of an acid (in order to ensure isoelectric precipitation of the protein), centrifugation, purification, and drying [8–11].

Protein concentrates and isolates are the purified forms of plant proteins neutral in both taste and aroma, so they can be used even at high doses. It is worth mentioning that plant-based raw materials utilized for protein production are much less expensive and more readily available than animal-derived raw materials. The costs of their storage and transportation are also lower. These factors are especially important for the countries having limited economical resources [9].

Today, protein derived from soybeans and wheat is a leader on the market of concentrates and isolates; however, there is also a gradually growing interest in pea protein. The key drivers of market growth are as follows: spreading vegetarianism, environmental safety of plant protein, as well as the fact that this type of protein is multi-purpose and functional. The key barriers for expansion of the market of protein concentrates and isolates involve allergies to protein and antinutritional properties of the resulting food products, which cannot be completely eliminated using the technologies discussed in this paper [6].

The germination method has proved to be a more efficient technique to enhance absorption of plant proteins. Germination is a transition between seed dormancy and sprout growth, accompanied by
modification of the chemical structure of compounds. Peptide zones become diffuse and eventually break down during imbibition and initiation of seed germination, while new ones are synthesized. As a result, availability of amino acids is enhanced. Enzymes present in the seeds cleave conjugated proteins, fats, and carbohydrates to simpler compounds that are needed for future plant development. For this reason, when a person eats sprouts, the substances supplied to his/her body are already processed and the impact of inhibitory compounds on the human organism is reduced [12]. However, seed germination under natural conditions is a rather labor-intensive and long-lasting process (taking up to 35 hours), which has not yet been brought to a large scale in Russia. The use of artificial enzymatic hydrolysis is an easier attainable technique for improving the nutritional value of legumes.

The objective of this study was to develop novel condensed cream soups using pea protein hydrolysate and to assess their quality.

2. Materials and methods
Pea seeds (grade I according to the State Standard GOST 6201-68) and Fidbest VGPro complex enzyme product manufactured by Sibbiopharm Ltd. were used as study objects.

At the initial stage, peas and the Fidbest VGPro complex enzyme product were sequentially subjected to mechanochemical disintegration on two mills (a rotor mill and a TM-30 centrifugal roller mill; "Novic" Ltd, Novosibirsk, Russia) in the conditions described earlier [13, 14]. Enzymatic hydrolysis of pea flour was carried out at the next stage. The hydrolysis was performed at 50°C, following the manufacturer's recommendations. Hydrolysis duration was determined experimentally according to saturation of the solution with water-soluble compounds.

The resulting hydrolysate was brought to a boil in order to inactivate the enzyme and subsequently added to the cream soups. Other ingredients of the soups were as follows: fresh carrots, cauliflower, turkey meat, and 10% dairy cream.

Protein quantification in the ready-to-use food products was performed using the Bradford protein assay. The calibration curve was plotted using 1 mg/ml bovine serum albumin.

Water-soluble compounds were quantified by exhaustive extraction in a Soxhlet extractor for 24 h. The contents of reducing sugars were determined refractometrically; free amino acids were quantified by chromatography/mass-spectroscopy on an Autoflex Speed system (Bruker Daltonics, Germany). The mineral composition of the samples was evaluated by atomic absorption spectroscopy. Other paragraphs are indented (BodytextIndented style).

3. Results and discussion
The nutritional value of peas is shown in Figure 1. The diagram demonstrates that this crop is a source of a wide range of valuable biologically active substances.
It is known that most of water-soluble highly absorbable compounds are contained within the cell. Mechanochemical treatment allows one to disrupt the cell wall and make the biologically active compounds available for the subsequent enzymatic hydrolysis. Addition of an enzyme during mechanochemical treatment is the most efficient way to reach the maximum degree of enzymatic hydrolysis as compared to other techniques. Mechanical activation of pea seed biomass gives rise to a reactive composite. Further enzymatic hydrolysis of this composite and exhaustive extraction allow one to obtain water-soluble compounds at high yields [15].

We determined the capacity of pea protein hydrolysate with respect to enzyme molecules. Test samples of pea flour in the presence of Fidbest VGPro enzyme product at different concentrations (0.5, 1, 2, and 3%) were subjected to hydrolysis. It was found that the amount of water-soluble compounds in the solution remained unchanged at enzyme concentrations higher than 2%. Further addition of the enzyme had no effect on the rate of enzymatic reaction, since the substrate was completely filled with the enzyme at its concentration of ~ 2%. The experimental data are shown in Figures 2 and 3.

Enzymatic hydrolysis lasting longer than 5 hours is inefficient, since the amount of water-soluble compounds remains virtually unchanged after this point. It is also worth mentioning that the rate and degree of hydrolysis of mechanically activated pea flour are higher than those for the non-activated sample.
The resulting pea hydrolysate was used to develop cream soups. The highest scores for the sensory quality parameters were assigned to sample 1 (creamy pea soup with vegetables) and sample 2 (creamy pea soup with turkey) (Figure 4).

Figure 4 Sensory profile of the samples.

The cream soup samples were freeze-dried. The main benefit of using this method for food products is that nutrients are not destroyed, since the samples are treated at low temperatures. Furthermore, it significantly reduces the risk of oxidation of unstable substances with atmospheric oxygen. The scanning electron microscopy data (Figure 5) show that the cream soup samples are lightweight, homogeneous and porous, and have a well-developed surface, thus indicating that proper freezing/drying modes have been selected.

The scanning electron microscopy image of cream soup lyophilizate.

Table 2 summarizes the physicochemical quality parameters of the reconstituted cream soups. The finished products are characterized by low acidity and low fat content.

Table 2. Physicochemical quality parameters of cream soups.

| Parameter      | Sample number |
|----------------|---------------|
|                | 1a            | 2b            | 3c            | 4d            |
| Dry solids, wt. % | 13.70         | 15.70         | 20.80         | 23.50         |
| Protein, wt. %   | -             | 13.60         | -             | 16.80         |
| Acidity, %       | 0.60          | 0.60          | 0.50          | 0.50          |
| Fat, wt. %       | 2.10          | 2.10          | 2.50          | 2.30          |
| Ash content, %   | 0.69          | 0.78          | 0.50          | 0.65          |

a sample 1 – creamy pea soup with vegetables (control sample);
b sample 2 – creamy pea soup with vegetables based on pea protein hydrolysate;
c sample 3 – creamy pea soup with turkey (control sample);
d sample 4 – creamy pea soup with turkey based on pea protein hydrolysate.

Figure 6 shows a diagram indicating changes in the yield of water-soluble compounds in cream soup samples before and after they were subjected to hydrolysis and mechanochemical treatment. It was found that the additional stage of pea flour treatment increases the yield of the water-soluble fraction approximately threefold. This indicates that high-molecular-weight compounds are converted into simpler substances and, consequently, their absorption is enhanced. In a similar fashion, the content of free carbohydrates is increased in soup samples based on pea protein hydrolysate (Figure 7). As a result, the glycemic index of the ready-to-eat meal also increases.
The increased amount of free amino acid in ready-to-eat soups also indicates that the enzyme product is efficient (Figures 8 and 9).

Figure 6. Changes in the contents of water-soluble compounds in the samples.

Figure 7. Changes in the contents of water-soluble carbohydrates in the samples.

Figure 8. Changes in the contents of free amino acids in the cream soup with vegetables.

Figure 9. Changes in the contents of free amino acids in the cream soup with turkey.

Figure 10 shows the data on the percent daily value of minerals for one serving of cream soup. The soup samples were found to be a valuable source of potassium and iron.
Figure 10. Mineral composition of the cream soups.

The ready-to-use lyophilisates of creamy pea soups were vacuumized and stored. Sensory evaluation showed that the dry soups had neither off-flavor nor off-aroma. They were easily reconstituted into the original state by adding water; the initial quality parameters of the soups were not altered.

The microbiological quality parameters of the reconstituted cream soups fall within the normal range.

4. Conclusions

Specialized cream soups based on pea protein hydrolysate have been developed in this study. The optimal concentration of the enzyme product has been identified. We have achieved the objective of increasing the contents of water-soluble compounds (including free amino acids, peptides, and simple carbohydrates) in the ready-to-serve soup as the mechano-enzymatic hydrolysis stage has been added to the preparation process. A rationale for using the freeze-drying stage to improve shelf life and make soup preparation more convenient has been presented. The recommended daily serving size of the reconstituted cream soup is 200 g. The developed soups consumed on a daily basis provide peptides, free amino acids, simple carbohydrates, and iron for the human body, while preventing allergic reactions and alleviating symptoms of gastrointestinal diseases. They also make sports training more efficient.

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