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Development of technology for producing protein hydrolysates from leguminous plants (peas) for sports nutrition

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ABSTRACT

In line with emerging trends, the sports nutrition industry actively seeks cost-effective and health-promoting protein ingredients to replace animal-derived products and transition toward more sustainable practices. Protein concentrates derived from legumes, including peas, have attracted consumer interest due to their rich amino acid profiles and favourable functional properties. Our research aimed to develop and optimise an enzymatic technology for producing protein hydrolysates from locally adapted pea varieties in Kazakhstan. Based on the research findings, optimal technological conditions for protein extraction from peas via enzymatic methods were established: pea flour-to-water ratio (hydromodule) 25%; duration of water extraction 24 hours; concentration of added pepsin enzyme 4.0%; pepsin fermentation time 24 hours; concentration of added pancreatin enzyme 4.0%; pancreatin fermentation time - 5 hours. A pilot batch of enzymatically hydrolysed pea protein concentrate was produced under laboratory conditions, and analyses were performed on the resulting concentrate derived from the *Aksary* pea variety. The protein concentrate contained 8.92% crude protein and 5.38 ± 0.02 mg/mL amino nitrogen. Notably, due to the amino acid L-arginine at 0.540±0.216% and lysine at 0.587±0.199%, the hydrolysate can be utilised as a component of sports supplements to support muscle mass development. This work was conducted as part of the project "Development of a technology for producing protein-vitamin concentrates based on plant raw materials for sports nutrition" within the framework of the scientific-technical program BR22886613, titled "Development of Innovative technologies for the processing and storage of agricultural crop production and raw materials". The project is funded under the 267-budget program "Enhancing the accessibility of knowledge and scientific research", subprogram 101 "Program-targeted financing of scientific research and activities" of the Ministry of Agriculture of the Republic of Kazakhstan for 2024–2026.

Keywords: highly digestible food products, pea hydrolysates, sports nutrition, protein concentrates, enzymatic hydrolysis

INTRODUCTION

Enzymatic hydrolysis involves enzymes that involve specific chemical bonds to transform complex compounds into simpler ones **[1]**. Enzymes have become essential tools in food biotechnology, catering to new consumer demands for food concentrates with easily digestible and high-quality protein additives.

Protein hydrolysates, produced through enzymatic hydrolysis, are complex mixtures of di- and tri-peptides formed by breaking primary proteins into smaller fragments via peptide bonds **[2]**. Scientific studies have shown that protein hydrolysates, predominantly composed of di- and tri-peptides, are absorbed more rapidly than native

proteins. This is because they require no digestion and are quickly assimilated into the body, stimulating the synthesis of skeletal muscle proteins **[3]** and **[4]**.

Peptide concentrates derived from protein hydrolysis serve as practical building blocks for athletes, essential for the growth and recovery of structural skeletal muscles following training and sporting events **[5]**, **[6]**. Numerous studies have revealed that athletes' total protein intake is 50–175% higher than that of the general population **[7]**, **[8]** and **[9]**.

With the global population projected to reach 9.5 billion by 2050, alongside climate change challenges like the greenhouse effect, there is an active pursuit of alternative protein sources for human consumption **[10]**. The production of animal-based proteins is both expensive and environmentally unsustainable. In contrast, plant protein production is associated with less deforestation and climate change impact, as it requires less land and emits fewer greenhouse gases than livestock farming **[11]** and **[12]**.

Recent research has shown that plant protein sources can stimulate protein synthesis to the same extent as animal protein sources, provided they meet the required amino acid intake levels **[13]**. The Food and Agriculture Organization (FAO) has recognised plants as a rich source of high-quality proteins, emphasising their nutritional value and the absence of cholesterol. Moreover, plant proteins are suitable for vegans and individuals who avoid animal-derived products for religious, medical or ethical reasons **[14]**.

Among alternative protein sources, legumes are notable for their high levels of globulins and albumins **[15]**. The FAO identifies ten major types of legumes: dry beans, soybeans, dry broad beans, dry peas, chickpeas, cowpeas, pigeon peas, lentils, Bambara groundnuts, vetches and lupins **[16]**. Of these, soybeans, peas, chickpeas, lentils, beans and peanuts are particularly rich in proteins, containing 30–35% protein **[17]**. In addition to carbohydrates, minerals and vitamins, legumes are associated with preventing chronic diseases due to their bioactive peptide content **[18]**. Consequently, legume protein hydrolysates are being extensively studied for their potential to produce peptides with biological properties **[19]** and **[20]**.

In particular, peas are among the legumes, one of the richest essential amino acids in the food industry **[21]** and **[22]**. Pea proteins have a well-balanced amino acid profile and primarily consist of globulins (50–85%) and albumins (15–25%) **[21]**, **[23]** and **[24]**. Pea albumins contain significantly more cysteine, methionine, tryptophan, and threonine, while globulins are predominantly rich in arginine, isoleucine, leucine and phenylalanine **[21]**. In addition to their nutritional value, epidemiological and clinical studies have shown that pea consumption positively impacts health by reducing LDL cholesterol, cardiovascular diseases, type 2 diabetes, and colorectal cancer risks **[25]** and **[26]**. Peas have versatile applications in food products, such as meat and dairy substitutes, baking flour, and food additives **[27]**.

Our research aims to develop and optimise enzymatic technology for producing protein hydrolysate from regionally adapted pea varieties of the Republic of Kazakhstan for use in sports nutrition. The study focused on determining the optimal enzyme concentration and fermentation duration to maximise hydrolysate yield, with amino nitrogen content as an indicative metric. Compared to chemical methods, enzymatic hydrolysis has advantages, such as milder conditions and higher product yield and quality. Furthermore, the resulting hydrolysates can contain bioactive peptides with potential antioxidant activity **[28]**.

This work addresses the development of a novel sports nutrition product based on pea protein hydrolysates. The final product, derived from low-cost raw materials, has the potential to reduce Kazakhstan's reliance on imported goods, emerging as one of the first locally produced items in this market segment.

Scientific Hypothesis

The *Aksary* pea variety demonstrates significant potential as a raw material for producing high-quality protein hydrolysates. Its highly favourable amino acid composition makes it a compelling alternative protein source that can replace animal-based products. The developed enzymatic hydrolysis technology for pea flour can be utilised to produce locally sourced sports nutrition.

Objectives

Primary objectives:

1. Selection of the most effective raw material from two pea varieties for further research and development of a sports supplement.

2. Development of optimal technology for pea protein hydrolysate production: To optimize the enzymatic extraction process for obtaining high-quality and easily digestible protein hydrolysates from regionally cultivated pea varieties, specifically the *Aksary* cultivar.

3. Investigation of key process parameters: To determine the optimal technological conditions for enzymatic hydrolysis, including:

- The ratio of pea flour to water (hydromodule).
- Extraction and fermentation times.

- Effective concentrations of the enzyme's pepsin and pancreatin.

4. Evaluation of nutritional and biological properties: To analyse the amino acid composition, protein content, and bioactive properties of the pea protein hydrolysates for their suitability in sports nutrition, focusing on benefits such as muscle mass building, metabolism regulation, and cardiovascular health support.

5. Ensuring product quality and safety: To assess the microbiological purity and compliance of the produced protein hydrolysate concentrates with regulatory standards.

6. Development of practical applications: To produce experimental batches of pea protein hydrolysates and evaluate their potential for expanding the range of protein-vitamin products designed for sports and everyday nutrition.

7. Contribution to sustainable processing technologies: To utilise locally grown crops to develop innovative and sustainable technologies for producing high-value protein products, contributing to Kazakhstan's food industry and agricultural processing.

MATERIAL AND METHODS

The research was conducted at LLP "Kazakh Research Institute of Processing and Food Industry" of the Ministry of Agriculture of the Republic of Kazakhstan, in the laboratory of "Biotechnology, Quality, and Food Safety".

Samples

Samples description: To determine the physicochemical characterisation of the primary raw material, crushed seeds with a 1-2 mm particle size were taken as a sample. Figure 1 illustrates the visual appearance of whole and ground peas. Further, in determining the optimum fermentation parameters, yellowish-white coloured semi-viscous hydrolysates of pea flour were taken as samples. The amino acid composition of pea protein concentrate was determined in the sample as a thick yellow-coloured liquid.

Figure 1 Locally adapted pea varieties and their milling.
Note: A. *Zhasylai* variety; B. *Aksary* variety.

Samples collection: The primary raw material, pea flour, was stored in an airtight container at room temperature. Liquid samples were stored at low temperatures of 2-4ºC in sealed vials.

Samples preparation: Pea seeds were subjected to crushing with the help of a mill. Liquid samples were prepared by varying the ratios of pea flour to water (hydromodule) to optimize the extraction conditions. The tested hydromodules ranged from lower concentrations to the established optimal ratio of 25%, ensuring efficient protein extraction while minimizing the presence of insoluble residues.

Number of samples analysed: In summary, we analyzed a total of 37 samples.

Chemicals

In this work, pancreatin with an activity of 75 USP U/mg (Pancreatin 3X (3 NF/USP, RM7348), HiMedia, India) and pepsin with an activity of 1:1000 U (Pepsin GRM9155, HiMedia, India) were utilized. All chemicals used in this study were of analytical grade purity.

Animals, Plants and Biological Materials

Locally adapted pea varieties *Zhasylai* and *Aksary* (*Pisum sativum*) were purchased from the Kazakh Research Institute of Agriculture and Plant Growing (Almaty Region, Kazakhstan).

Instruments

Universal pH-meter Sartorius PB-11, P11, Germany,

Laboratory centrifuge TAGLER CM-12, Russia,
Rotary evaporator XD-52AA, China,
Capillary electrophoresis "Kapel 105M" (Lumex, Russia),
Laboratory dry-air thermostat TS-1/20 SPU, Russia,
Laboratory shaker PE-6500 without heating, Russia.

Laboratory Methods

The selected locally adapted pea raw material was analyzed for the following parameters: total protein content according to GOST 13496.4-2019 **[29]** and GOST R 51417-99 **[30]**; total fat content according to GOST 13496.15-2016 **[31]**; carbohydrates (sugars) content according to GOST 8756.13-87 **[32]**; moisture content according to GOST 13496.3-92 **[33]**; ash content according to GOST R 51418-99 **[34]**; energy value calculation according to the method of Skurikhin **[35]**; magnesium, calcium, iron, and zinc content according to GOST 32343-2013 **[36]**; zinc content according to GOST 32343-2013 **[36]**, GOST 30692-2000 **[37]**; soluble dry substances according to GOST R 51433-99 **[38]**; titratable acidity according to GOST 25555.0-82 **[39]**; crude fiber content according to GOST 13496.2-91 **[40]**; starch content according to GOST 26176-2019 **[41]**; vitamin A (β-Carotene) content according to GOST 13496.17-2019 **[42]**; vitamins B_1, B_2, B_3, B_6, B_9, C according to GOST 31483-2012 **[43]**; water-soluble antioxidants according to GOST R 54037-2010 **[44]**; nitrate content according to GOST 13496.19-2015 **[45]**; toxic elements: lead, cadmium according to GOST 30692-2000 **[46]**; arsenic according to GOST 26930-86 **[47]**; mercury according to GOST 26927-86 **[48]**; mycotoxins: aflatoxins B1 according to GOST 30711-2001 **[49]**; organochlorine pesticides (α-,β-,ϒ-HCH, DDT and its metabolites) according to ST RK 2011-2010 **[50]**.

Description of the Experiment

Study flow: Samples of regionally adapted pea varieties were collected and processed into flour under controlled conditions. The flour was then prepared for experiments by ensuring uniform particle size and proper storage. To select the most effective raw material, physicochemical characteristics were determined for two pea varieties: *Zhasylai* and *Aksary*. In the first phase of the research, experiments were conducted to optimise the process parameters for protein extraction. The ideal hydration ratio (25% pea flour) and extraction time (24 hours) were determined based on nitrogen yield and residue minimisation. Then, enzymatic hydrolysis was optimised in two steps. Hydrolysis with pepsin was carried out at a concentration of 4.0% for 24 hours, followed by hydrolysis with pancreatin at 4.0% for 5 hours. Both stages were optimised for maximum amino nitrogen content. In the next step, an experimental batch of pea protein hydrolysate (500 ml) and its concentrate (400 ml) was obtained using optimal parameters. Biochemical analysis confirmed the high protein content (8.92 %) and amino nitrogen (5.38 ± 0.02 mg/mL). Capillary electrophoresis revealed a rich amino acid profile, particularly L-arginine and lysine, promoting muscle growth and repair. Adjustments were made during extraction and hydrolysis to achieve optimal results. Statistical analysis validated the optimised process, confirming its suitability to produce high-quality protein hydrolysates for sports nutrition.

Quality Assurance

Number of repeated analyses: Each analysis had a threefold repeatability.

Number of experiment replications: Each experiment was conducted three times.

Reference materials: This study's laboratory equipment, methods, and tests were validated using certified reference materials and secondary standards to ensure accuracy and reliability. The Kjeldahl method determined and validated protein content analysis using certified reference protein samples (CRM) **[29]**. The formol titration method determined the amine nitrogen content according to GOST 29311-92 **[51]**. The sterility of the final product was determined by standard microbiological methods according to GOST ISO 11737-2-2011 **[52]**.

The capillary electrophoresis system (Kapel 105M) was calibrated for amino acid profiling using standard amino acid solutions from certified suppliers. For the analysis, 100 ml of the sample was used. The method is based on the decomposition of samples by acid or alkaline hydrolysis of amino acids into free forms, the formation of phenylisothiocarbamyl derivatives, followed by their separation and quantitative determination by capillary electrophoresis **[53]**. Data detection and processing were performed using the “Elforun” software. Analysis conditions: Buffer: β-cyclodextrin (Fluka, Cat. No. 28707); phenylisothiocyanate (ICN, Cat. No. 190264) was used for the formation of phenylisothiocarbamyl derivatives. Detection is carried out in the UV region of the spectrum at a wavelength of 254 nm. The total length of the capillary was 75 cm, the effective length was 65 cm, the internal diameter of the capillary was 50 μm, and the operating voltage applied to the electrodes was 25 kV, at 30°C.

These reference materials ensured the accuracy and reproducibility of the experimental results.

Calibration: The pH meter was calibrated immediately before analysis using standard titers. When determining amino acids on the capillary electrophoretic system (Kapel-105M), calibration was carried out using a mixture of amino acids from the kit LAA-21 (Sigma-Aldrich, USA).

Laboratory accreditation: The experiments were conducted in the laboratory accredited by the accreditation system of the Republic of Kazakhstan for compliance with the requirements of GOST ISO/IEC 17025-2019.

Data Access

The data supporting this study's conclusions are in a report drawn up in closed access accordance with the Interstate Standard GOST 7.32-2017.

Statistical Analysis

Variation and statistical analysis methods were employed to evaluate all experiments. Statistical calculations were performed using Microsoft Excel 2019, with a significance level of $p<0.05$ applied to determine statistically significant differences between groups **[54]**.

Each analysis was conducted with threefold repeatability, ensuring the precision and reliability of the data. Additionally, each experiment was replicated three times to validate the consistency and robustness of the results.

Variation analysis techniques accounted for the structure of the data, with the correct degrees of freedom applied for statistical tests. Repeated measures were not included in the study design, as analyses were conducted independently for each outcome. No additional covariate tests were performed, given the uniformity of controlled variables across experimental groups.

RESULTS AND DISCUSSION

The obtained research results for the selected regionally adapted raw material varieties are presented in Table 1.

Table 1 Research results of selected pea varieties.

Parameter	Sample No. 1 - *Zhasylay*	Sample No. 2 - *Aksary*
Protein, %	22.36±0.08	23.61±0.006
Fat, %	3.4±0.02	2.4±0.01
Carbohydrate, %	47.55±0.24	47.95±0.15
Moisture, %	10.49±0.05	10.80±0.05
Ash, %	2.9±0.03	3.0±0.04
Dry matter, %	89.51±0.05	89.20±0.05
Fiber, %	11.3±0.06	10.2±0.05
Starch, %	46.69±0.22	47.07±0.25
Energy value, kcal	280.15	295.77
Tit. acid,	0.46±0.005	0.44±0.005
Nitrate, mg/kg	43.20	45.96
Antioxidants, mg/g	0.25±0.0037	0.30±0.0024
β-carotene, mg/kg	11.49±0.05	7.42±0.03
Vit. B_1, mg/100g	1.20±0.24	1.16±0.23
Vit. B_2, mg/100g	0.29±0.12	0.23±0.09
Vit. B_3, mg/100g	9.61±1.92	0.33±1.86
Vit. B_6, mg/100g	0.410±0.08	0.38±0.07
Vit. B_9, mg/100g	0.019±0.004	0.021±0.004
Vit. C, mg/100g	Not detected	Not detected
Ca, mg/100g	118.78±1.43	115.37±1.38
Mg, mg/100g	117.44±1.41	1121.07±1.36
Fe, mg/100g	9.34±0.12	9.07±0.11
Zn, mg/100g	9.25±0.04	3.16±0.04
Pb, mg/kg	0.0012±0.0001	0.0014±0.0001
Cd, mg/kg	0.0009±0.00002	Not detected
As, mg/kg	Not detected	Not detected
Hg, mg/kg	Not detected	Not detected
Aflotaxin B1, mg/kg	Not detected	Not detected
HCH (iso.), mg/kg	Not detected	Not detected
DDT and meth, mg/kg	Not detected	Not detected

Note: ± – standard deviation.

Laboratory tests were conducted to analyse the physicochemical composition, biological and nutritional value, and safety parameters of the selected regionally adapted plant raw materials, specifically two pea varieties Zhayslay and Aksary.

Pea seeds typically contain 20–25% protein, 40–50% starch, and 10–20% fibre **[55]**. Similar results were observed in our study. Based on the research results presented (Table 1), it is evident that sample No. 2 Aksary pea variety, is the most suitable for further research, mainly due to its protein content, carbohydrate levels, energy value, antioxidant properties, and magnesium content. Magnesium plays a significant role in muscle mass formation for athletes and is beneficial for cardiovascular health.

In a study comparing protein content in peas, chickpeas, and lentils, the protein content in legumes ranged from 16.7% to 25.8%. The protein content of yellow peas was reported as 21.09 ± 0.28%, lower than lentils but significantly higher than chickpeas **[56]**. Pea protein differs from soy and other plant-based proteins due to its excellent digestibility, relatively low allergenicity, and lack of adverse health effects **[57]** and **[58]**.

Scientific studies have established that the effectiveness of enzymatic hydrolysis depends on various factors, such as enzyme type, enzyme-to-substrate ratio, reaction temperature, enzyme reaction time, and substrate characteristics **[59]** and **[60]**. Therefore, hydrolysis must be conducted under strictly defined and optimised conditions. The main criterion for selecting the optimal variant from the above conditions was the amine nitrogen content, determined according to GOST 29311-92 **[51]**.

The following optimal technological conditions for producing pea protein hydrolysates have been established:

1. Optimal ratio of pea flour to water (hydromodule). Various pea flour dilutions in water were tested in concentrations ranging from 5% to 30% (with a step of 5%). Constant extraction parameters for protein from plant raw materials included: pH 7.8–8.0, temperature +10–12°C, and extraction time 24 hours with periodic stirring. The research results for determining the optimal ratio of pea flour to water are presented in Figure 2.

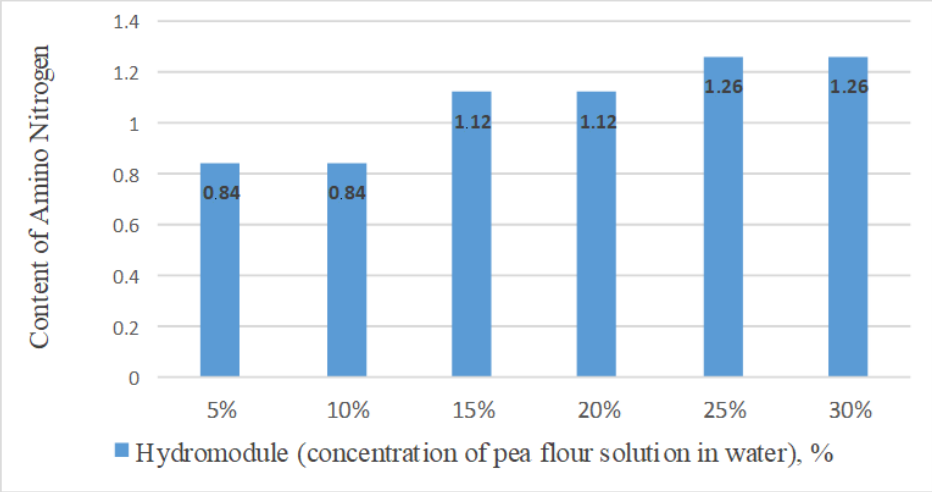

Figure 2 Results of determining the optimal ratio of pea flour to water (hydromodule).

Based on the presented results, it is evident that sample No. 5 (25%) is the most suitable in terms of amino nitrogen content (mg/ml). Additionally, it should be noted that concentrations of pea flour exceeding 25% result in a significant sediment of insoluble substances, which can complicate the extraction process.

2. Optimal time for aqueous extraction of pea flour proteins. After determining the optimal hydromodule, the extraction time for pea protein was optimised under the above constant conditions for durations ranging from 6 to 30 hours (with a step of 6 hours). The contents of the flask were stirred periodically every 2 hours. Aqueous extraction is a gentler, cleaner, and more sustainable method. Compounds such as starch and protein can be effectively separated from peas using only water. This method simultaneously extracts small soluble substances, achieving a higher % protein purity of 75% in the soluble pea concentrate **[61]**. The results for determining the optimal time for aqueous extraction of pea flour proteins are presented in Figure 3.

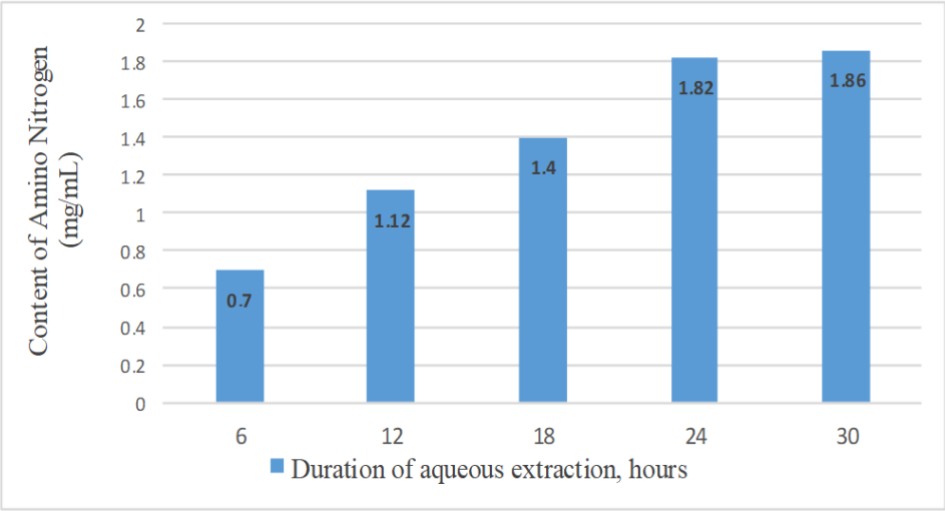

Figure 3 Results of determining the optimal time for aqueous extraction of proteins from pea flour.

The diagram shows that sample No. 4, with 24 hours of aqueous extraction of pea flour proteins, is the most suitable for amino nitrogen content. Based on the results, extending aqueous extraction beyond 24 hours is not beneficial, while extraction for less than 24 hours results in significant protein losses during the early stages of the process.

3. Optimal amount of added pepsin. Pepsin is an endopeptidase that hydrolyses proteins in the gastrointestinal tract with a broad range of substrate specificity. Pepsin preferentially cleaves aromatic residues such as phenylalanine, leucine, tyrosine, and tryptophan **[62]**. Constant fermentation conditions were as follows: fermentation temperature: 37 to 38°C in a thermostat; pH of the medium: 1.8–2.0, with the addition of a 1-normal solution of chemically pure hydrochloric acid; enzyme activity of the preparation: 1:1000 IU. To identify the optimal pepsin concentration, it was added to the pea aqueous extract in concentrations ranging from 1.0% to 6.0% (with a step of 1.0%), with an exposure time of 6 hours. The results for determining the optimal amount of added pepsin enzyme are presented in Figure 4.

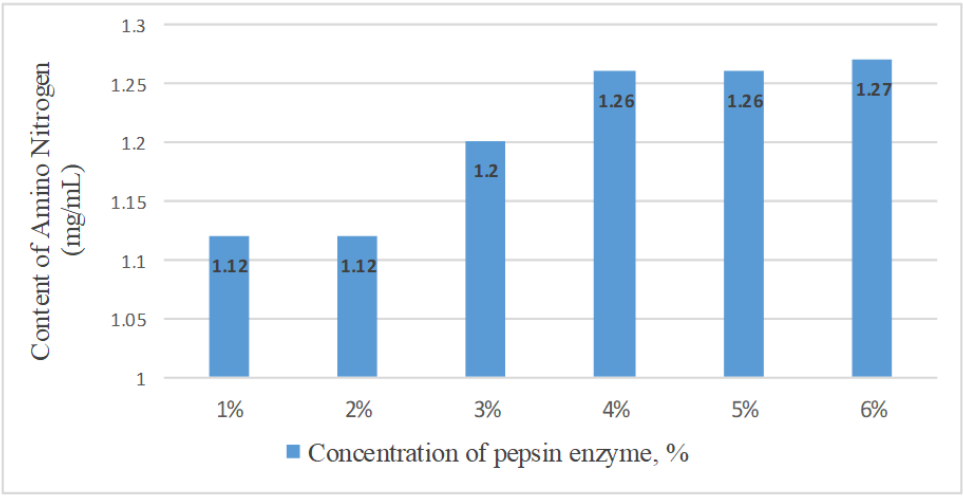

Figure 4 Results of determining the optimal concentration of pepsin to be added to the aqueous protein extract of peas during fermentation.

Based on the research results, sample №4, with 4.0% pepsin enzyme added to the pea protein extract during fermentation, was identified as the most acceptable regarding amino nitrogen content. For samples №5 and №6, containing 5.0% and 6.0% pepsin enzyme, respectively, the amino nitrogen levels were comparable to those of sample №4. Consequently, a 4.0% concentration of pepsin enzyme will be used for further experimental studies involving the fermentation of pea protein extract. Additionally, the studies revealed that plant proteins treated with proteases demonstrate good antioxidant activity, preserving the bioactive properties of peptides in the hydrolysates **[63]** and **[64].**

4. Optimal fermentation time using pepsin. After determining the optimal pepsin enzyme concentration, the optimal hydrolysis time with pepsin was tested under the above constant conditions, with durations ranging from 6 to 30 hours (with a step of 6 hours). The contents of the flask were stirred periodically every 2 hours. The results of these studies are presented in Figure 5.

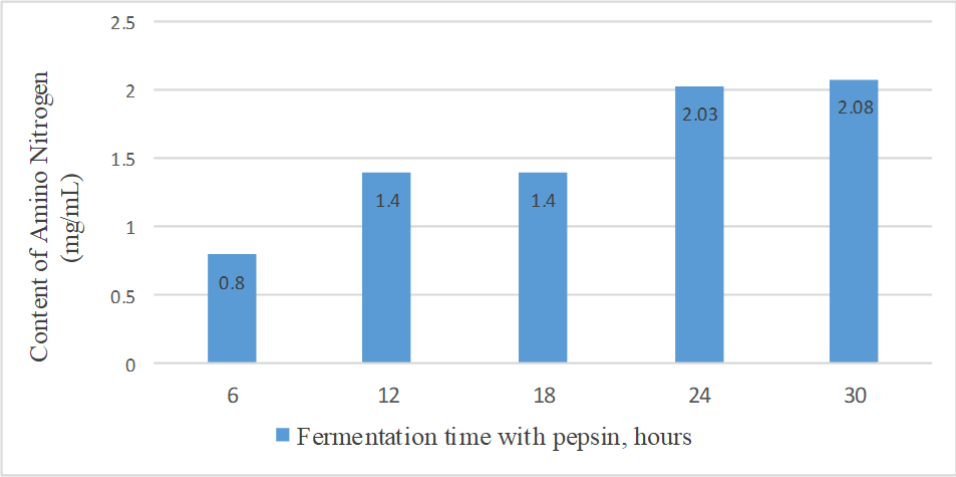

Figure 5 Results of determining the optimal fermentation time for pea protein extract with pepsin.

According to the data presented, sample No. 4, with 24 hours of fermentation with pepsin, was the most suitable for amino nitrogen content. In previous studies, the degree of hydrolysis was reported to increase from 3.0% to 13.0% with longer pepsin treatment **[65]**. However, fermentation beyond 24 hours did not demonstrate economic feasibility or a significant increase in the yield of extractive substances. Therefore, in further experimental studies, 24 hours is the optimal fermentation time for pepsin extraction of pea protein.

5. Optimal amount of pancreatin. Following pepsin fermentation, the optimal fermentation conditions with pancreatin enzyme were investigated. Pancreatin is a serine protease that exhibits carboxypeptidase activity. It is a mixture of enzymes with tryptic, chymotryptic, and elastase-like activity, characterised by active centres that include peptide bonds formed by histidine, serine, and aspartate **[66]**. After the experimental mixture was optimally treated with pepsin, the effective amount of pancreatin to be added was determined. Constant fermentation conditions included: fermentation temperature: 43 to 45°C in a thermostat; pH of the medium: 8.0–8.2 (natural physiological pH); with the addition of a 1-N sodium hydroxide solution; enzyme activity of the preparation: at least 75 IU/mg. To identify the optimal concentration of pancreatin, it was added to the pea hydrolysate (after pepsin hydrolysis) in concentrations ranging from 1.0% to 6.0% (with a step of 1.0%), with an exposure time of 3 hours. The research results are presented in Figure 6.

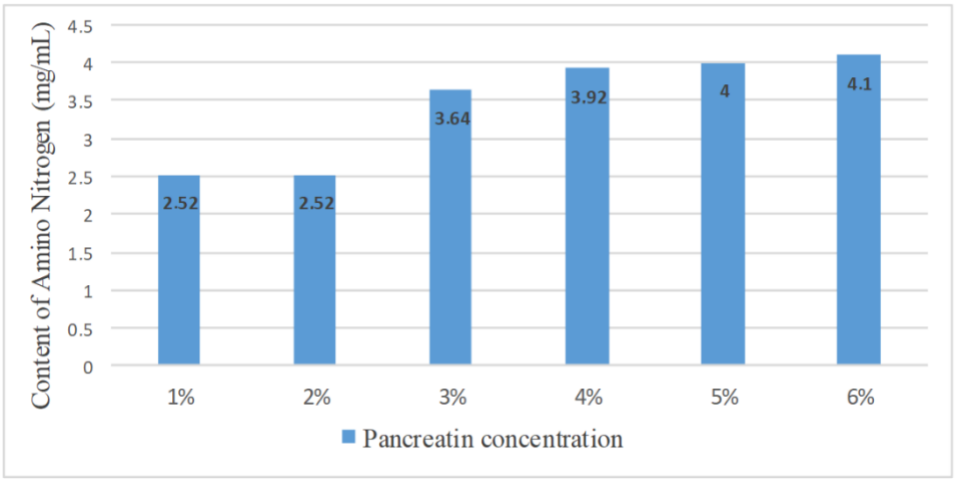

Figure 6 Results of determining the optimal concentration of pancreatin for addition to the aqueous protein extract of pea during fermentation.

Based on the experimental results, sample No. 4, with the addition of 4.0% pancreatin to the pea protein extract during fermentation, was identified as the most suitable in terms of amino nitrogen content. While samples No. 5

(5.0% pancreatin) and No. 6 (6.0% pancreatin) demonstrated slightly higher amino nitrogen levels compared to sample No. 4, the increased enzyme consumption beyond 4.0% and the marginal difference in extractive substance yield were deemed economically unfeasible.

6. Optimal fermentation time with pancreatin. After determining the optimal pancreatin enzyme concentration, the optimal hydrolysis time with pancreatin was tested under the above constant conditions, for durations ranging from 1 to 6 hours (with a step of 1 hour). The contents of the flask were stirred periodically every hour during this time. The obtained research results are presented in Figure 7.

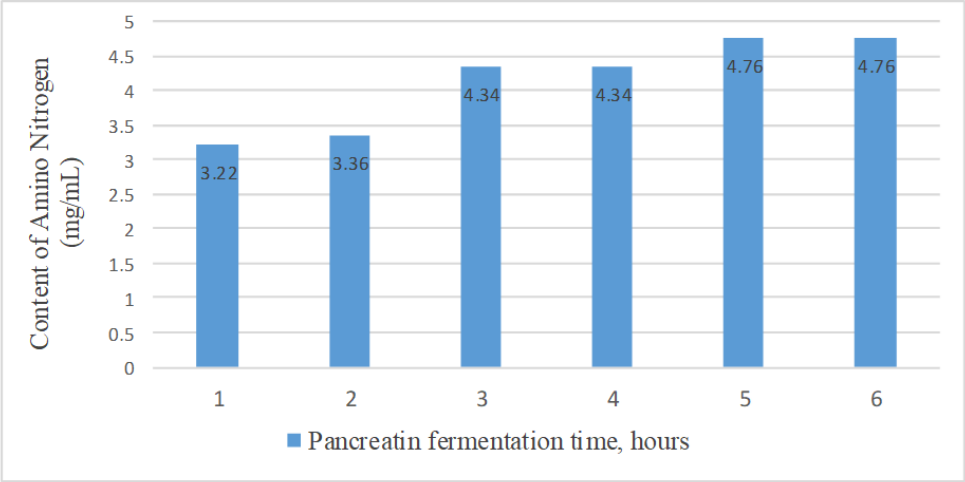

Figure 7 Results of determining the optimal fermentation time for extracting pea protein with pancreatin.

According to the research results presented in the diagram, sample No. 5, with a pancreatin fermentation time of 5 hours, was the most acceptable based on amino nitrogen content (4.76±0.02 mg/mL). Fermentation beyond 5 hours did not result in significant differences in the yield of extractive substances. Therefore, the fermentation time for the final stage of pea protein hydrolysis using pancreatin is 5 hours. In studies examining the effect of time on the degree of hydrolysis of mung bean protein hydrolysates, pancreatin demonstrated consistent growth in hydrolysis indicators with increasing time. The enzyme exhibits broad specificity, functioning as an endo- and exo-peptidase, producing highly efficient peptides **[67]** and **[68]**. The observed difference in amino nitrogen levels between hydrolysis with pepsin and pancreatin can be attributed to pepsin's more specific cleavage sites **[69]**.

Based on the obtained results, the optimal technological regime for enzymatic extraction of pea protein is as follows:

- Pea flour-to-water ratio (hydromodule): 25%
- Duration of aqueous extraction: 24 hours
- Concentration of added pepsin enzyme: 4.0%
- Pepsin fermentation time: 24 hours
- Concentration of added pancreatin enzyme: 4.0%
- Pancreatin fermentation time: 5 hours

The experiment aimed to simulate gastrointestinal conditions, creating an *in vitro* digestion model to develop a product capable of rapid absorption and facilitating efficient recovery for athletes.

Completion of fermentation processes. After pancreatin hydrolysis, the extract is filtered through a three-layer gauze filter or centrifuged (laboratory centrifuge TAGLER CM-12) at 1000–2000 rpm to remove the sediment. Then, the pH is adjusted to 5.0–5.2 using a 1 N hydrochloric acid solution (to stop the enzyme activity and prevent microbiological growth). If necessary, the extract is sterilised by filtration through a well-washed SF Zeitz filter plate. The concentration of the extract was carried out using a rotary evaporator (model XD-52AA, Stegler) at a temperature of 65±1.0°C and a vacuum pressure of 0.5 – 0.7 atm, until the content of soluble dry matter reached 22.0–23.0±0.01%. Subsequently, the samples were pasteurised at 90.0±1.0°C for 15 minutes (Figure 8).

Figure 8 Concentration of protein hydrolysate from *Aksary* pea flour.

A pilot batch of enzymatic hydrolysate of pea proteins (500 mL) and its concentrate (400 mL) was produced under laboratory conditions. The crude protein content in the concentrate of the enzymatic pea protein hydrolysate was 8.92%, and the amino nitrogen content was 5.38 ± 0.02 mg/ml.

The microbiological purity of the final product was also studied. The results of microbiological analysis (total bacterial count) of the concentrates of enzymatic hydrolysates of pea proteins are presented in Table 2. The results reflect the microbiological safety of the hydrolysates, ensuring suitability for consumption and compliance with safety standards.

Table 2 Results of microbiological analysis (total bacterial count) of the concentrates of enzymatic hydrolysates of pea proteins.

No.	Name of indicators, units of measurement	Permissible norms according to Normative documentation	Obtained results
1	Total Microbial Count, CFU/g (cm^3) no more	5x104	<2.0x101
2	Coliform Bacteria, per 1 g (cm^3)	Not acceptable	Not detected
3	E. coli, per 1 g (cm^3)	-	Not detected
4	Pathogenic Microorganisms incl. *Salmonella*, per 25 cm^3	Not acceptable	Not detected
5	Yeasts, CFU/g (cm^3) no more	10	<1.0x101
6	Molds, CFU/g (cm^3) no more	10	<1.0x101
7	*Staphylococcus aureus*, per 1 g (cm^3)	-	Not detected

The obtained pea protein hydrolysate concentrate meets the regulatory requirements according to the microbiological indicators presented in the table.

The nutritional value of proteins primarily depends on their ability to meet nitrogen and essential amino acid needs. An amino acid analysis was conducted on the produced pea protein hydrolysates. This analysis, performed via capillary electrophoresis, provides more detailed insights into the qualitative amino acid composition of the hydrolysates, enabling a better understanding of the biological value of the product for athletes. The results of the amino acid composition analysis of the enzymatic hydrolysates of pea protein, following the optimised technological regimes for pepsin and pancreatin hydrolysis, are shown in Table 3 and Figure 9. These results are crucial for evaluating the nutritional value of the hydrolysates and their potential application in sports nutrition.

As shown in Table 3, the obtained pea protein hydrolysate contains a rich amino acid composition. It was established that, due to the presence of the amino acid L-arginine (0.540 ± 0.216%) in combination with lysine (0.587 ± 0.199%), the hydrolysate can be used for muscle mass growth **[71]**. These amino acids stimulate the secretion of human growth hormone, which plays a role in regulating growth, metabolism, and muscle mass; weight correction – protein helps reduce ghrelin levels, a hormone that causes hunger; cardiovascular disease prevention – positively affects the balance of systolic and diastolic blood pressure, reduces inflammatory responses; detoxification – helps remove toxins and waste from the body; blood sugar regulation – protein helps minimise common symptoms of diabetes, such as fatigue, increased thirst, slow wound healing, and unwanted weight loss **[72]** and **[73]**. Protein sources containing arginine have recently gained popularity due to arginine's impact on preventing heart disease **[74]**.

Table 3 Results of the amino acid composition analysis of the concentrates of enzymatic hydrolysates of pea proteins.

No.	Component	Content., mg/l	Mass, fraction of amino acids in %
1	Arginine	23.0	0.540±0,216
2	Lysine	25.0	0.587±0,199
3	Tyrosine	91.0	2.135±0,640
4	Phenylalanine	8.50	0.199±0,060
5	Histidine	6.90	0.162±0,081
6	Leucine + isoleucine	12.0	0.282±0,073
7	Methionine	2.90	0.068±0,023
8	Valine	12.0	0.282±0,113
9	Proline	9.90	0.232±0,060
10	Threonine	8.60	0.202±0,081
11	Serine	9.80	0.230±0,060
12	Alanine	9.30	0.218±0,057
13	Glycine	9.30	0.218±0,074

Note: ± – standard deviation.

Figure 9 shows distinct peaks of amino acids identified in the concentrated pea protein hydrolysate obtained through enzymatic hydrolysis. Some studies suggest that hydrolysates appear to promote faster muscle recovery than intact proteins due to the increased bioavailability of amino acids obtained through *in vitro* digestion highlighting the potential benefits of enzymatically hydrolyzed proteins in enhancing nutrient absorption and improving recovery for athletes **[70]**.

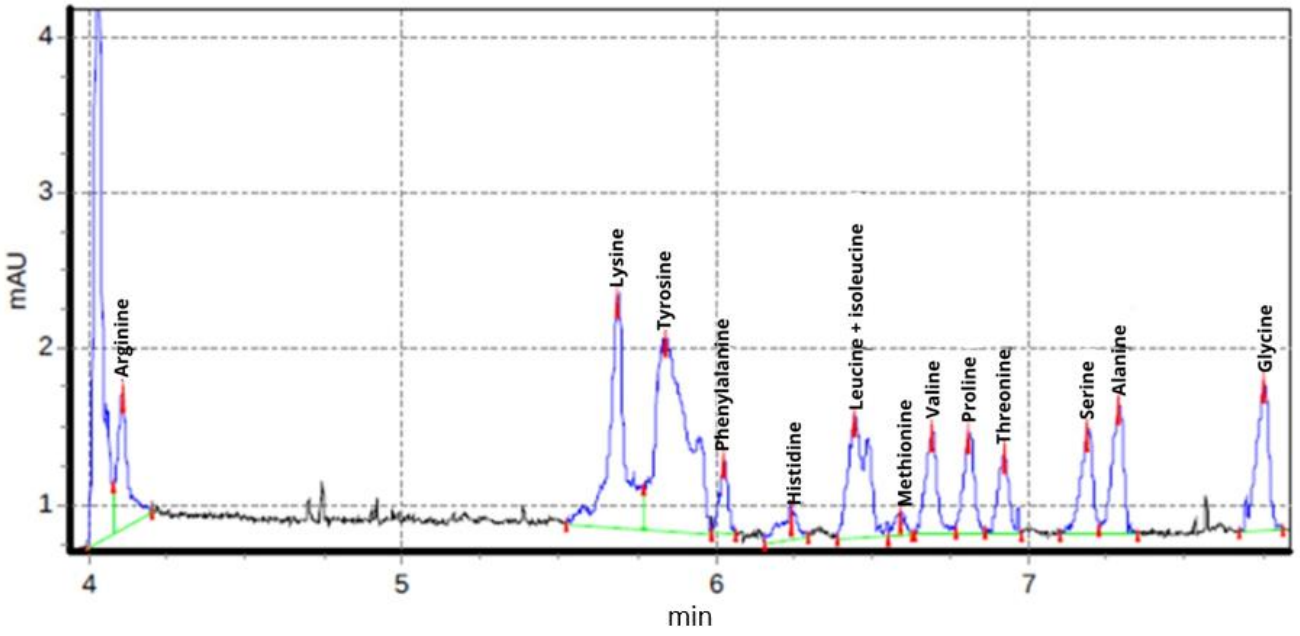

Figure 9 Results of amino acid composition analysis of pea protein enzymatic hydrolysate concentrates using the "Kapel 105M" capillary electrophoresis system.

The levels of most essential amino acids in the protein isolate were within the range of "standard protein" values **[75]**. The high lysine content in the protein is an essential factor, making pea hydrolysate a good complement to cereals that are deficient in lysine. The low methionine level is similar to other legume seed proteins **[76]** and **[77]**. Combining various plant proteins in one mixture is one strategy for formulating supplements with a more complete amino acid profile **[78]**, **[79]** and **[80]**.

Based on the above, it can be concluded that the amino acid composition of pea protein enzymatic hydrolysate concentrate can be used to expand the range of easily digestible protein-vitamin products for sports and everyday nutrition.

CONCLUSION

On the ground of the obtained research results, the optimal technological modes of pea protein extraction by enzymatic method were worked out: the ratio of pea flour and water (hydromodule) - 25%; time of water extraction - 24 hours; concentration of added enzyme pepsin - 4.0%; time of fermentation by pepsin - 24 hours; concentration of added enzyme pancreatin - 4.0%; time of fermentation by pancreatin - 5 hours.

An experimental batch of enzymatic pea protein hydrolysate (500 ml) and its concentrate (400 ml) was produced under laboratory conditions, and the obtained pea protein hydrolysate from the *Aksary* variety was analysed. At the same time, the crude protein content in the concentrate of the enzymatic hydrolysate of pea proteins was 8.92 %, and amine nitrogen was 5.38±0.02 mg/ml. According to microbiological indicators, the obtained concentrate of pea protein hydrolysate meets the regulatory requirements.

Amino acid analysis of the produced pea protein hydrolysates was carried out. It was found that pea protein hydrolysate contains a rich amino acid composition. Due to the content of amino acid L-arginine - 0.540±0.216%, in combination with amino acid lysine 0.587±0.199% in the composition, the hydrolysate can be used to build muscle mass.

Through enzymatic hydrolysis of leguminous crops, particularly from regional varieties of peas, it is possible to obtain easily digestible and high-quality protein hydrolysates suitable for developing sports nutrition products.

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AI tools were not used.

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