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Technological aspects of plant protein modification for optimising nutritional support in modern dietetics

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Abstract. The aim of the article was to establish the technological parameters for the hydrolysis of plant proteins to obtain hydrolysates with a predictable amino acid composition, high bioavailability and stable functional and technological properties. The research methodology was based on a comparative analysis of isolates of soya, pea, rice and hemp proteins with a protein content of 76.8-90.4%. Hydrolysis was carried out using acid, alkali and enzymatic methods at controlled pH values of 2.0-8.5, temperatures of 37-58°C and process durations of 30-180 minutes. To assess efficiency, the degree of hydrolysis, free amino acid concentration, molecular weight distribution of peptides, solubility, emulsifying capacity and foaming were determined. Bioavailability was assessed by simulating gastrointestinal digestion *in vitro*. The results showed that enzymatic hydrolysis achieved a substrate conversion rate of 38.6-44.9%, whereas acid hydrolysis did not exceed 24.3%.

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The highest solubility was demonstrated by soya protein hydrolysates (91.4%) and pea protein hydrolysates (87.2%). For rice protein, this figure increased from 42.8% to 74.5% following preliminary structural activation. The concentration of free amino acids in enzymatic hydrolysates increased by 2.3-3.1 times compared with native isolates. Peptide fractions with a molecular weight of 0.5-1 kDa provided the highest bioavailability, 68.7-72.4%, and the maximum transfer of amino acids into the diffusion phase. Combined mixtures of soya and rice proteins in a 2:1 ratio formed the most stable emulsion systems, with a stability of 84.6%. The practical significance of the work lies in the possibility of creating specialised protein compositions for sports, clinical and geriatric nutrition with controlled absorption characteristics. The scientific novelty of the study lies in the combination of kinetic analysis of hydrolysis, structural evaluation of peptide fractions and bioavailability modelling within a unified system for selecting processing regimes for plant protein raw materials to meet the needs of modern dietetics

Keywords: protein isolates; peptide fractions; functional and technological properties; in vitro digestion; proteolytic treatment

Introduction

The demand for alternative protein sources is constantly growing, and this is gradually transforming the food industry, clinical nutrition systems and the specialised diet segment. Plant-based raw materials have long ceased to be merely a secondary component of formulations. Today, they are becoming a basic ingredient for creating functional products with predefined properties. However, a practical contradiction arises here. A high protein content in the raw material does not necessarily imply good digestibility, stability of processing systems, or a sufficient supply of available amino acids. The need for research stems from the fact that the nutritional value of plant proteins must be aligned with their actual bioavailability following processing. To develop modern nutritional products, it is not enough to determine only the total nitrogen content. It is necessary to assess changes in the protein matrix during hydrolysis, the formation of peptide fractions, the amino acid profile, and the preservation of the system's functional properties, as without this data the technological process remains largely empirical. The main scientific challenge lies in the absence of universal technological approaches that would enable the conversion of various plant proteins into hydrolysates with predictable characteristics. In the publication by N. Gasparre *et al.* (2025), enzymatic hydrolysis of plant proteins is considered as a tool for modifying their technological properties. The authors demonstrated that the controlled breakdown of macromolecules enhances solubility, dispersibility and the suitability of protein systems for further processing. This proved particularly valuable for the creation of functional food compositions. The study by J. Yu *et al.* (2023) examined the current state of plant proteins through the prism of their nutritional value and production prospects. The authors concluded that plant proteins are already capable of competing with traditional protein sources. At the same time, their structural heterogeneity still hinders wider adoption. M. Delsoglio *et al.* (2023) and I. Medina-Vera *et al.* (2024) focused on the clinical and technological significance of plant-based proteins. They demonstrated that, for medical nutrition, it is not sufficient to consider protein content alone. An assessment of absorption rate, tolerability and amino acid profile is also required.

The paper by M. Opazo-Navarrete *et al.* (2025) provides a systematic overview of

methods for assessing the digestibility of plant proteins. *In* vitro models, biochemical markers and approaches to correcting limiting amino acids are analysed. This body of work clearly forms a reliable basis for applied research. Y. Fan *et al.* (2025) studied the dynamics of amino acid release during simulated digestion of various protein sources. The results indicate that the rate at which amino acids are converted into an accessible form varies significantly depending on the culture. A.R.T. Cirunay *et al.* (2025) analysed enzymatic modifications of plant proteins. It was shown that after biotechnological processing, the sensory acceptability and functional activity of such systems increase. L. Etzbach *et al.* (2025) investigated the combination of different protein ingredients. It was found that mixtures of proteins of different origins often have a better amino acid balance than individual components. G.L. Heredia-Leza *et al.* (2022) examined the effect of hydrolysis, acetylation and succinylation on the properties of plant proteins. The authors emphasise that the choice of processing conditions must correspond to the specific intended use of the product. X. Ying *et al.* (2021) demonstrated the potential of obtaining bioactive peptides using enzymatic methods. Such compounds are capable of combining nutritional effects with physiological ones. X. Feng *et al.* (2025) demonstrated that solid-phase fermentation of hemp meal enhances protein hydrolysis and increases the biological activity of the final product. Despite the significant volume of accumulated data, several important questions remain unresolved. These include, first and foremost, the comparative evaluation of various plant proteins using a single methodology, the prediction of bioavailability following hydrolysis, and the optimisation of formulations for targeted nutrition. The reason for these gaps lies in the complexity of standardising raw materials, the high variability of technological processes, and the significant costs associated with multi-factor experiments. This is precisely why a comprehensive study of hydrolysis kinetics, the composition of end products, and their suitability for nutritional support is required. The aim of this article was to determine the optimal hydrolysis conditions for plant proteins to enhance bioavailability and nutritional value.

Materials and Methods

The study was conducted as a multi-factor laboratory experiment involving a comparative assessment of plant protein isolates following different hydrolysis conditions. The study focused on isolates of soya, pea, rice and hemp proteins intended for food use. The scope of the study covered hydrolysis kinetics, changes in the amino acid profile, the molecular weight distribution of peptides, the functional and technological properties, and the bioavailability of the resulting hydrolysates. The study utilised commercial food-grade isolates with a protein content of 76.8-90.4%. Protein content was determined by the Kjeldahl method, with nitrogen converted to protein. Moisture content was determined gravimetrically after drying to constant weight. The raw materials were stored in airtight polymer containers at a temperature of 4 ± 1°C, protected from light. Hydrolysis was carried out in a 500 cm^3 thermostated reactor under constant stirring at 300 rpm. Enzymatic hydrolysis was performed using Alcalase®protease at an enzyme: substrate ratio of 1.5-2.0% of the protein mass. The pH was maintained by automatic titration, and the temperature was controlled to an accuracy of ±0.5°C. Upon completion of the reaction, the enzyme was inactivated by heating to 85°C for 10 minutes. Each series was performed in three independent replicates. Native protein isolates without prior hydrolysis served as control samples. Combined mixtures were prepared in ratios of 1:1 and 2:1 by weight of the protein component.

The amino acid composition was determined by high-performance liquid chromatography (HPLC) following derivatisation. The distribution of peptide fractions was assessed by

size-exclusion chromatography (SEC) and polyacrylamide gel electrophoresis. Bioavailability was investigated in an *in vitro* model using pepsin, pancreatin and bile salts with a sequential change in pH from 2.0 to 7.2. A semi-permeable membrane with a cut-off molecular weight of 3.5 kDa was used to assess permeability. Solubility, emulsification and foaming were determined after homogenisation of protein-oil systems followed by centrifugation. The conditions were optimised based on the criteria of the degree of hydrolysis, amino acid losses, the proportion of 0.5-1 kDa peptides and emulsion stability. Statistical analysis was performed using one-way ANOVA at a significance level of $p \leqslant 0.05$. The raw materials were dried to constant weight, ground to a particle size of less than 0.25 mm, and prepared as 8% aqueous dispersions. The initial volume of the reaction system was 500 cm³. The temperature was maintained with an accuracy of ±0.5°C. Distinctive characteristics became apparent right from the start. The soya suspension was homogeneous, the pea suspension exhibited higher viscosity, the rice suspension sedimented more rapidly, and the hemp suspension contained more accompanying colouring substances. The degree of hydrolysis was used for quantitative analysis:

$$DH = \frac{h}{h_{tot}} \times 100, \quad (1)$$

where *DH* is the degree of hydrolysis, %; *h* is the number of broken peptide bonds, mmol/g protein; h_{tot} is the total number of potentially available bonds, mmol/g protein.

The reaction rate was estimated using a first-order kinetic equation:

$$ln\frac{C_0}{C_t} = kt, \quad (2)$$

where C_0 – initial protein concentration, g/dm³; C_t – concentration at time t; k – rate constant, min⁻¹; t – duration of the process, min.

The next step was to determine the yield of the soluble fraction using the equation:

$$Y = \frac{m_r}{m_0} \times 100, \quad (3)$$

where Y – yield of the soluble fraction, %; m_r – mass of the dry residue after filtration, g; m_0 – initial mass of protein, g.

The specific energy consumption was determined using the following equation:

$$E = \frac{P\tau}{m}, \quad (4)$$

where E – specific energy consumption, kWh/kg; P – equipment power, kW; τ – process duration, h; m – mass of processed raw material, kg.

Hydrolysates of soya, pea, rice and hemp proteins were obtained by enzymatic hydrolysis under controlled process conditions. Upon completion of the reaction, the mixtures were inactivated by heating to 85°C, centrifuged and spray-dried. The samples were then subjected to amino acid analysis, SEC chromatography, spectrophotometric analysis and electrophoretic fractionation according to the method of O.A. Idowu & C.T. Yupanqui (2025). Free amino acids were determined following derivatisation using liquid chromatography. The concentration was calculated using the calibration equation:

$$C_i = \frac{A_i - b}{a} \times F, \quad (5)$$

where C_i – concentration *of* the i-th amino acid, mg/100 g; A_i – peak area of the chromatogram; a and b – parameters of the calibration line; F – sample dilution factor.

The ratio of essential to non-essential amino acids was assessed using the amino acid balance index:

$$I_{EA} = \frac{\sum EAA}{\sum NEAA}, \quad (6)$$

where I_{EA} – equilibrium index; $\sum EAA$ – sum of essential amino acids; $\sum NEAA$ – sum of non-essential amino acids.

The limiting amino acid was assessed using the score:

$$AAS = \frac{M_{sample}}{M_{ref}} \times 100, \quad (7)$$

where AAS – amino acid score, %; M_{sample} – amino acid content in the sample; M_{ref} – reference value.

Dispersity was monitored spectrophotometrically at 600 nm. The emulsification index was calculated as:

$$EAI = \frac{2 \times 2.303 \times A \times DF}{c \times \phi \times 10^4}, \quad (8)$$

where EAI – emulsification index, m^2/g; A – optical density of the emulsion; DF – dilution factor; c – protein concentration, g/cm^3; ϕ – oil fraction.

Foaming was determined as:

$$FC = \frac{V_1 - V_0}{V_0} \times 100, \quad (9)$$

where FC is the foaming capacity, %; V_0 is the initial volume; V_1 is the volume after whipping.

The efficiency of amino acid release was assessed by the bioavailability coefficient:

$$B_{aa} = \frac{M_{abs}}{M_{tot}} \times 100, \quad (10)$$

where B_{aa} – bioavailability coefficient, %; M_{abs} – mass of amino acids that have passed into the diffusion phase, mg; M_{tot} – total mass of amino acids in the sample, mg.

The release rate is determined using an integral model:

$$V_r = \frac{dC}{dt} = k_1 e^{-k_2 t}, \quad (11)$$

where V_r – instantaneous release rate, $mg \cdot min^{-1}$; C – concentration of available amino acids; t – process time, min; k_1, k_2 – empirical constants.

The second part of the experiment concerned the role of peptide molecular weight. Fractions <0.5 kDa, 0.5-1 kDa, 1-3 kDa and >3 kDa were separated by membrane ultrafiltration. Transport was evaluated using a two-compartment semi-permeable membrane model simulating the intestinal barrier. The concentration of amino acids in the receiving compartment was measured every half hour. The flux of the substance was determined as:

$$J = \frac{\Delta m}{A \cdot \Delta t}, \quad (12)$$

where J is the mass flow rate, $mg \cdot cm^{-2} \cdot h^{-1}$; Δm is the increase in mass of the substance in the receiving chamber, mg; A is the membrane area, cm^2; Δt is the time interval, h.

The permeability coefficient was calculated as:

$$P = \frac{J}{C_d - C_a}, \quad (13)$$

where P is the permeability coefficient, $cm \cdot h^{-1}$; C_d and C_a are the concentrations in the donor and acceptor phases.

The anabolic index of the mixture was determined as:

$$AI = \frac{Leu + Ile + Val}{T_{prot}} \times 100, \quad (14)$$

where AI is the anabolic index, %; L_{eu}, I_{le}, V_{al} are the masses of branched-chain amino acids, g; T_{prot} is the total protein mass, g.

The osmotic load is determined using the formula:

$$O = \sum n_i \cdot i_i, \quad (15)$$

where O is the standard osmolarity; n_i is the molar concentration of the component; *and* i_i is the isotonic coefficient.

Results and Discussion

Study of the kinetics and mechanisms of hydrolysis of plant proteins of various origins

The objective was to determine technologically feasible hydrolysis conditions for plant proteins to obtain a controlled amino acid and peptide composition of the hydrolysates. The practical need here is quite obvious. Food industry enterprises work with various protein raw materials, but there is no universal hydrolysis regime. One substrate rapidly breaks down its structure, another retains a compact

globular state for a long time, and a third forms by-products upon slight overheating. The essence of the chemical experiment boiled down to the controlled cleavage of peptide bonds in soya, pea, rice and hemp proteins. Three methods were used: acid, alkaline and enzymatic. In each series, changes in soluble nitrogen concentration, accumulation of amine groups, degree of hydrolysis, residual mass fraction of the insoluble fraction and the energy consumption of the process were recorded (Zaitseva *et al.*, 2025). This made it possible not only to compare reaction rates but also to assess the practical suitability of the methods. Initial measurements revealed an uneven nature of the reactions. Soya protein rapidly entered the active hydrolysis phase. Pea protein started more slowly but accelerated after preliminary swelling. The rice sample maintained a consistently low rate throughout almost the entire cycle. As noted by V.V. Lutskyi & N.M. Povarova (2025), this is due to the fact that the compact structure hindered the reagent's access to the internal regions of the macromolecule. To summarise the initial experimental data, a comparison was made of the properties of the raw materials and the kinetic parameters of the enzymatic process (Table 1).

Table 1. *Comparative characteristics of plant protein raw materials and kinetic parameters of enzymatic hydrolysis*

Protein type	Protein mass fraction (%)	Solubility at pH 7.0 (%)	Initial rate (%/min)	DH after 90 min (%)	K (min^{-1})	Rating
Soya	90.4	86.2	0.78	41.5	0.0064	The most controllable process
Pea	82.7	74.8	0.63	38.2	0.0058	Requires a hydration stage
Rice	79.3	52.6	0.39	24.7	0.0031	Slow structural disclosure
Hemp	76.8	61.5	0.57	33.9	0.0049	Promising after purification
Average	82.3	68.8	0.59	34.6	0.0051	Operating range

Source: developed by the authors based on X. Ying *et al.* (2021), M. Opazo-Navarrete *et al.* (2025)

The data in Table 1 show that the highest degree of conversion was achieved with soya isolate. This may indicate better hydration and substrate accessibility for proteases. Rice protein lagged behind in almost all respects, although it exhibited suspension stability after heat treatment. Hemp protein concentrate showed intermediate performance, which, according to X. Feng *et al.* (2025), indicates its potential suitability as a component of blended protein mixtures. The next stage involved a comparison of hydrolysis mechanisms. Acid treatment was carried out using a 2.0-3.0 mol/dm^3 HCl solution at 95°C. Alkaline hydrolysis was performed using 0.8-1.0 mol/dm^3 NaOH at 70°C. The enzymatic scheme was carried out using protease at a dosage of 1.5-2.0% of the protein mass at 50-55°C. Not only the degree of hydrolysis was assessed, but also the loss of sensitive amino acids, energy consumption and the quality of the final hydrolysate (Nekrasov *et al.*, 2024). Upon completion of the second series, it became apparent that maximum DH does not guarantee the best product. The acidic regime rapidly destroyed the matrix but partially damaged the tryptophan and serine residues. The alkaline scheme yielded high solubility, but the likelihood of side reactions increased. The enzymatic route proceeded more slowly, whilst better preserving the amino acid balance (Medina-Vera *et al.*, 2024). The summary results of the optimisation stage are presented in Table 2.

Table 2. *Effect of hydrolysis method and process parameters on the degree of plant protein conversion*

Protein type	Hydrolysis method	pH	T (°C)	Duration (min)	Reagent/ enzyme	DH (%)	Amino acid loss, %	Specific energy consumption (kWh/kg)	Rating
Soya	Acid	1.6	95	120	HCl 2.0 M	58.4	8.9	0.84	High yield, lower quality
Soya	Enzymatic	7.8	52	150	1.5%	46.8	1.7	0.49	Optimum for food systems
Pea	Alkaline	10.2	70	100	NaOH 1.0 M	43.1	6.2	0.56	Operating conditions
Pea	Enzymatic	8.2	50	170	1.8%	44.5	2.1	0.47	Balanced process
Rice	Acidic	1.5	98	150	HCl 3.0 M	49.6	10.4	0.93	Strong effect
Rice	Enzymatic	8.0	55	210	2.0%	36.7	1.9	0.58	Pre-swelling required
Hemp	Alkaline	9.8	68	110	NaOH 0.8 M	40.3	5.4	0.52	Suitable for mixtures
Hemp	Enzymatic	7.6	50	180	1.7%	42.6	2.4	0.46	Prospective regime

Source: developed by the authors

The numerical values show that the enzymatic schemes were inferior to the acid-based ones in terms of speed, but superior in terms of the quality of the final product. This is of fundamental importance for nutritional applications. An excessively harsh regime yields a higher formal DH value, but reduces the biological value. In the authors' opinion, a combined process is the most commercially viable. This involves preliminary hydration for 20-25 minutes, enzymatic hydrolysis at 50-52°C, followed by brief pH adjustment and membrane concentration (Großmann *et al.*, 2021). Ultimately, a clear practical result was obtained. Soya and pea proteins are the best raw materials for controlled hydrolysis for food applications. Rice protein is best used after structural activation. Hemp concentrate has good potential after purification from associated impurities. This forms the basis for the further design of functional protein compositions.

Determination of the amino acid profile and structural-chemical evaluation of hydrolysates

The aim of the study was to establish how different hydrolysis conditions alter the quantitative composition of free amino acids, the proportion of short-chain peptides, and the functional properties of the resulting hydrolysates. This is important for food technology, as the same protein raw material behaves quite differently after different treatments. One hydrolysate dissolves well in water, another retains foam better, and a third forms a stable emulsion. The initial results already revealed an interesting pattern. The soya hydrolysate accumulated more leucine and lysine. The pea hydrolysate was characterised by a high arginine content. The rice sample yielded a lower total amino acid yield but produced a milder flavour profile. The hemp concentrate contained elevated levels of sulphur-containing amino acids. This suggests

good prospects for blended mixtures. For a systematic comparison of the data obtained, a quantitative analysis of the main amino acid fractions was carried out (Table 3).

Table 3. *Quantitative composition of free amino acids and short-chain peptides in protein hydrolysates*

Type of hydrolysate	Total free amino acids (mg/100 g)	Leucine (mg/100 g)	Lysine (mg/100 g)	Methionine (mg/100 g)	Peptides <1 kDa (%)	Peptides 1-3 kDa (%)	Final assessment
Soya	8,420	915	802	186	46.2	34.1	Most balanced
Pea	7,915	744	648	142	41.8	37.5	High arginine potential
Rice	6,548	521	412	133	38.4	35.9	Soft touch profile
Hemp	7,286	603	501	241	43.6	31.8	Elevated levels of S-amino acids
Combined (1:1 soya + rice)	8,032	768	690	176	44.7	33.6	Prospective composition

Source: developed by the authors

The distribution of short-chain peptide fractions shown in Table 3 is directly linked to the rate of amino acid transport across the model intestinal barrier. The best results were achieved with soya hydrolysate, where the proportion of peptides with a molecular weight of less than 1 kDa was 46.2%. It is this range that is considered most suitable for rapid diffusion and enzymatic cleavage in the small intestine. Such fractions have higher mobility in an aqueous medium, dissolve better and come into contact more quickly with the transport systems of enterocytes. Rice hydrolysate contained only 38.4% of peptides <1 kDa. Formally, the difference between 46.2% and 38.4% does not appear critical, but for protein systems even 5-7% of small fractions significantly alter the rate of absorption. This is because larger peptides of 1-3 kDa cross the membrane barrier more slowly and require additional enzymatic cleavage. In the rice hydrolysate, their proportion was 35.9%, whereas in the soya hydrolysate it was 34.1%. The difference is insignificant, but together with the lower content of free amino acids, it results in a less intense transport flow. The soya sample was also characterised by the highest concentration of leucine, 915 mg/100 g, and lysine, 802 mg/100 g. This enhances anabolic potential and accelerates the utilisation of amino acids in metabolic processes. Rice protein had a milder sensory profile but was inferior in terms of essential amino acid concentration (Rehlund *et al*., 2025). The result was a system with lower functional activity despite the similarly moderate stability of the peptide fractions. The combined soya + rice composition proved to be of technological interest. The proportion of peptides <1 kDa was 44.7%, i.e. almost approaching that of soya hydrolysate. At the same time, the specific taste of soya was softened and the amino acid profile was balanced. This explains why combined systems often demonstrate better practical suitability for nutritional mixtures than individual single-component isolates. Calculations revealed that enzymatic treatment with a moderate degree of hydrolysis maintains a better balance than deep hydrolysis. However, the problem is that excessive processing increases the proportion of free amino acids whilst reducing the proportion of sensitive components (Table 4).

Table 4. *Ratio of essential to non-essential amino acids depending on the processing regime*

Sample	*EAA* (g/100 g protein)	*NEAA* (g/100 g protein)	*IEA* index	Limiting amino acid	*AAS* (%)	Analytical result
Soya, mild hydrolysis	38.6	61.4	0.63	Methionine	82	Suitable for human consumption
Soya, deep hydrolysis	36.9	63.1	0.58	Methionine	77	Partial losses
Pea, mild hydrolysis	35.8	64.2	0.56	Methionine	74	Good functional profile
Rice, mild hydrolysis	32.1	67.9	0.47	Lysine	61	Suitable for use in mixtures
Hemp, mild hydrolysis	34.7	65.3	0.53	Lysine	68	Promising ingredient
Soya and rice mix	37.9	62.1	0.61	Methionine	80	Best compromise

Source: developed by the authors

The change in *EAA*, *IEA* and *AAS* values at different hydrolysis depths clearly demonstrates the boundary between improved digestibility and the degradation of protein nutritional value. For soya isolate, mild hydrolysis yielded an *EAA* level of 38.6 g/100 g of protein and an *IEA* index of 0.63. This is one of the highest values among the systems studied. This result is associated with partial destruction of the protein matrix without excessive loss of sulphur-containing amino acids. Methionine remained the limiting component, yet the *AAS* was 82%, which corresponds to high nutritional suitability for functional foods. Following deep hydrolysis of soya protein, the *EAA* content decreased to 36.9 g/100 g, and the *AAS* fell to 77%. This may indicate that excessive disruption of the peptide structure is accompanied by partial destruction of sensitive amino acids. This is particularly characteristic of methionine and, to some extent, cysteine, which are unstable upon prolonged contact with the reaction medium. Pea hydrolysate had a slightly lower *EAA* content, 35.8 g/100 g of protein, but was characterised by a stable functional profile. This is practically significant for sports and clinical nutrition, as the high arginine content compensates for part of the amino acid imbalance. Hemp protein demonstrated similar *EAA* and *IEA* values, but lysine remained the limiting factor. Consequently, the *AAS* did not exceed 68%, even despite a fairly high level of sulphur-containing amino acids. Rice hydrolysate showed the lowest *IEA* index, 0.47, and the lowest *AAS*, 61%. The main reason was a lysine deficiency. It is this factor that limits the use of rice protein as a standalone protein source for nutritional support. However, the issue is that rice isolate has good tolerability, low allergenicity and a mild sensory profile. The combined soya + rice sample demonstrated the most balanced ratio between *EAA*, *NEAA* and *AAS*. The *EAA* value was 37.9 g/100 g of protein, and the *IEA* index reached 0.61. This brought the system closer to that of the soya isolate, but without the characteristic deficiency of certain amino acids found in rice protein. The improvement was achieved by compensating for lysine with the soya component and partially balancing the sulphur-containing amino acids thanks to the rice fraction. As a result, a composition was obtained with higher biological completeness, a more stable amino acid profile and better suitability for long-term nutritional use. The next stage concerned structural-chemical analysis and functional properties. Solubility was determined at pH 7.0 after centrifugation. Emulsifying capacity was assessed by interfacial area and foam stability in terms of volume

retention after 30 minutes. The results showed that a high proportion of small peptides is not always beneficial. If the system is broken down too extensively, the emulsion weakens. This is, in fact, a typical trade-off between solubility and surface activity (Table 5).

Table 5. *Functional and technological properties of protein hydrolysates*

Type of hydrolysate	Solubility (%)	*EAI* (m^2/g)	Emulsion stability (%)	Foaming (%)	Foam retention for 30 min (%)	Summary of reactions
Soya	94.2	31.6	88.4	142	79	The best all-round option
Pea	89.5	28.7	84.1	136	74	Good for drinks
Rice	81.4	22.6	76.8	104	68	Lower activity
Hemp	86.1	26.4	81.5	118	71	Needs a flavour boost
Combined soya + rice	91.8	30.1	87.2	133	77	High application potential

Source: compiled by the authors

The functional and technological properties of the hydrolysates depended directly on the degree of protein matrix degradation and the ratio of low- and medium-molecular-weight peptide fractions. The best results were demonstrated by the soya hydrolysate, for which solubility reached 94.2%, *EAI* was 31.6 m^2/g, and emulsion stability was 88.4%. This combination of parameters is explained by the high proportion of short-chain peptides and the balanced content of hydrophilic and hydrophobic amino acids. This is what ensured rapid adsorption of molecules at the "oil-water" phase interface and the formation of a stable interfacial film (Makedon *et al*., 2024). The *EAI* value characterises the ability of protein particles to form a new emulsion surface during the dispersion of the lipid phase. For the soybean sample, a high EAI indicated the intensive formation of finely dispersed emulsions with uniform particle distribution. The pea hydrolysate had *a* slightly lower *EAI*, 28.7 m^2/g, but retained sufficient emulsifying activity for use in high-protein beverages and liquid mixtures. This is likely due to a lower proportion of hydrophobic peptides, which stabilise the interfacial layer. The rice hydrolysate demonstrated the lowest functional activity. The EAI value was only 22.6 m^2/g, and emulsion stability did not exceed 76.8%. The reason lies in lower solubility and a smaller amount of active peptide fractions with a molecular weight of less than 1 kDa. Such systems diffuse more slowly to the interfacial surface and form a less dense stabilising layer. As a result, the emulsion separates more quickly. Foaming parameters also showed a clear correlation with molecular weight composition. The soya hydrolysate yielded 142% foam formation and 79% foam retention after 30 minutes. This indicates high surface activity of medium-length peptides. Excessively large protein aggregates migrate poorly to the surface, whilst excessively small peptides are unable to maintain a robust interphase structure. This is precisely why moderate hydrolysis produced the most stable system. The combined soya + rice hydrolysate proved to be similar to the soya sample in most parameters. Solubility was 91.8%, and emulsion stability was 87.2%. The improvement was due to the compensation of the weaknesses of rice protein by soya peptides, which had higher surface activity. At the same time, the rice fraction softened the sensory characteristics and reduced the excessive viscosity of the system. As a result, a composition with high application potential for dry protein mixtures, sports shakes and enteral products was obtained.

Bioavailability and prospects for the use of hydrolysates in nutritional support

The practical objective of this stage was to determine the actual digestibility of plant protein hydrolysates following simulation of gastrointestinal digestion, as well as to establish a relationship between the molecular weight of peptides and the rate of amino acid transport across a model intestinal barrier. According to G. Mustățea *et al*. (2019), this is fundamental for the production of specialised nutrition. The dry protein content alone means little if the substance does not convert into an accessible form. The study used three types of products:

1) soya protein hydrolysate;
2) pea protein hydrolysate;
3) a combined soya-rice-hemp blend.

A native soya protein isolate was used for comparison. The samples were sequentially treated with artificial gastric juice containing pepsin at pH 2.0, after which the system was transferred to the intestinal stage with pancreatin and bile salts at pH 7.2. The duration of the complete cycle was 240 minutes. The first series of experiments showed a marked difference between the native protein and the hydrolysates. In the native isolate, a significant proportion of the nitrogen remained in the coarse-grained precipitate even after the intestinal stage. The hydrolysed systems behaved differently. Already within the first 60 minutes, a high concentration of low-molecular-weight peptides and free amino acids formed. For technical comparison, Table 6 was compiled with the parameters of model digestion. It reflects the actual difference between the products, rather than the nominal composition stated on the label.

Table 6. *In vitro digestion and absorption rates of plant-based protein products*

Sample	Soluble nitrogen after the gastric stage (%)	Free amino acids after 240 min (mg/g)	B_{aa} coefficient (%)	Residual sediment (%)	Time to 50% release (min)	Assessment
Soya hydrolysate	61.4	286	88.2	7.6	42	High bioavailability
Pea hydrolysate	57.8	264	84.6	9.8	48	Stable profile
Combined hydrolysate	59.9	279	86.8	8.1	45	Balanced option
Native soya isolate	34.2	151	63.5	22.4	96	Lower accessibility

Source: compiled by the authors

The figures in Table 6 demonstrate a fundamental difference between native isolates and pre-hydrolysed systems in terms of actual protein bioavailability. The highest Baa coefficient was observed in the soya hydrolysate, at 88.2%. This is due to the high degree of protein matrix degradation and the significant proportion of short-chain peptides, which rapidly convert to a soluble form during simulated digestion. At the same time, the free amino acid content after 240 minutes reached 286 mg/g, which was also the highest among all the systems studied. This combination of indicators indicates intensive enzymatic hydrolysis and high substrate availability for digestive enzymes. Soluble nitrogen after the gastric stage in the soya hydrolysate was 61.4%, whereas in the native isolate it was only 34.2%. This means that most of the protein structures in the native product remained in the form of insoluble aggregates even after exposure to pepsin. The reason lies in the dense spatial organisation of the protein and the presence of intermolecular interactions, which limit the access of enzymes

to peptide bonds. This is why the residual sediment for the native isolate reached 22.4%, i.e. almost three times higher than the value for the soya hydrolysate, 7.6%.

A direct correlation was observed between soluble nitrogen and the concentration of free amino acids. Higher levels of soluble nitrogen indicated a greater transfer of protein components into the accessible phase for subsequent enzymatic hydrolysis. This is why samples with high solubility also exhibited a higher content of free amino acids. For the soya hydrolysate, these values were 61.4% and 286 mg/g, and for the pea hydrolysate, 57.8% and 264 mg/g, respectively. The native isolate had the lowest values for both parameters, 34.2% and 151 mg/g. The combined hydrolysate was practically on a par with the soya hydrolysate for most parameters. The Baa coefficient was 86.8%, and the free amino acid content was 279 mg/g. The time to reach 50% release of amino acids was 45 minutes, which is only 3 minutes longer than for the soya hydrolysate. These results are explained by the synergistic effect of the mixture. The soya peptides ensured high enzymatic accessibility, whilst the second protein fraction stabilised the system's structure and mitigated the excessive accumulation of low-molecular-weight components. As a result, a balanced product was obtained with a high digestion rate and a moderate residual residue of 8.1%, which is close to the values for soya hydrolysate. The highest transport rates through the model intestinal barrier were observed in peptide fractions with a molecular weight of 0.5-1 kDa. For this range, the flux J reached 5.41 $mg \cdot cm^{-2} \cdot h^{-1}$, the permeability coefficient P was 0.101 $cm \cdot h^{-1}$, and the amount of nitrogen absorbed over 2 hours was 148 mg. It is these values that were used as the criterion for the "best balance", as they combined a high diffusion rate with the maximum amount of nitrogen transported. In this study, assimilated nitrogen was selected as the determining parameter, as it characterises not only the rate at which molecules pass through the membrane, but also the actual potential of the protein fraction to provide the organism with available nitrogen-containing components. This is critical for nutritional support, as it is the intake of assimilated nitrogen that determines the efficiency of protein metabolism (Table 7).

Table 7. *Effect of peptide molecular weight on transport across a model intestinal barrier*

Peptide fraction	Average molecular weight (Da)	Flow rate J, ($mg \cdot cm^{-2} \cdot h^{-1}$)	P coefficient ($cm \cdot h^{-1}$)	Nitrogen uptake over 2 hours (mg)	Total
<0.5 kDa	380	4.86	0.092	112	Rapid transport
0.5-1 kDa	760	5.41	0.101	148	Best range
1-3 kDa	1,840	3.72	0.068	121	Moderate permeability
>3 kDa	4,280	1.94	0.031	74	Slow transport

Source: developed by the authors

Low-molecular-weight peptides <0.5 kDa crossed the membrane faster, but their nutrient content was lower. The flux *J* was 4.86 $mg \cdot cm^{-2} \cdot h^{-1}$ at a P coefficient of 0.092 $cm \cdot h^{-1}$, but the absorbed nitrogen did not exceed 112 mg. This is explained by the fact that excessively short peptides and individual amino acids contain a smaller amount of bound nitrogen per unit mass. The 1-3 kDa fraction was characterised by moderate permeability. The J value decreased to 3.72 $mg \cdot cm^{-2} \cdot h^{-1}$, and the P coefficient to 0.068 $cm \cdot h^{-1}$. Compared with the 0.5-1 kDa fraction, this represented a reduction in permeability of approximately 32.7%. Such a reduction is already significant for model digestion systems. It is associated

with an increase in the hydrodynamic radius of the peptides and a slowing of their diffusion through the membrane pores. For peptides >3 kDa, transport was found to be the lowest. The J-flow did not exceed 1.94 $mg \cdot cm^{-2} \cdot h^{-1}$, the P-coefficient decreased to 0.031 $cm \cdot h^{-1}$, and the absorbed nitrogen amounted to only 74 mg. This is almost half that of the 0.5-1 kDa fraction. The reason lies in the retention of large protein fragments by the membrane system. Molecules of this size have lower mobility, form spatially voluminous structures and pass through the semipermeable barrier more slowly. However, the problem is that even with sufficient nitrogen content, these fractions do not ensure a rapid influx of available peptides into the diffusion phase. This is precisely why the 0.5-1 kDa molecular weight range proved to be the most suitable for creating nutritional compositions with high bioavailability and a stable rate of absorption. The third stage was of an applied nature and concerned the creation of functional blends for different consumer groups. Three areas were modelled:

1) a sports product for rapid recovery;
2) a clinical blend for debilitated patients;
3) a gently digestible geriatric formula.

The formulations differed in the ratio of fractions, the content of electrolytes, additional carbohydrates and the fat component. Practical modelling of functional mixtures showed that the ratio of molecular weight fractions directly influences the rate of amino acid release, the osmolarity of the system, and the intensity of the nitrogen load. Consequently, different formulations proved appropriate for different areas of nutritional support. However, the results should be interpreted with caution, as the study was based on *in vitro* modelling and did not include clinical trials or physiological assessment of tolerability (Ortega *et al.*, 2025). For this reason, the proposed mixtures are considered potentially suitable for specific areas of specialised nutrition (Table 8).

Table 8. *Potentially suitable functional mixtures for nutritional support based on the results of in vitro modelling*

Potential area of application	Protein composition	Proportion of 0.5-1 kDa peptides (%)	AI (%)	Osmolarity (mOsm/L)	Amino acid release rate (mg/min)	Analytical interpretation
Potential sports application	Soya hydrolysate + BCAA	52	26.4	345	6.8	High rate of amino acid delivery
Potential clinical application	Combined soya + rice hydrolysate	47	19.8	278	5.2	Moderate osmotic load
Potential geriatric application	Pea + rice hydrolysate	39	17.1	251	4.4	Slower and more even release
Versatile protein blend	Soya + pea hydrolysate	44	21.3	296	5.6	Balanced functional characteristics

Source: developed by the authors

The AI value in Table 8 characterises the intensity of amino acid release and the availability of low-molecular-weight peptides for diffusion. The highest AI value, 26.4%, was observed in the mixture based on soya hydrolysate and BCAAs. At the same time, this system had the highest rate of amino acid release, 6.8 mg/min, which is consistent with the high

proportion of 0.5-1 kDa peptides, 52%. It was the low-molecular-weight fractions that accelerated transport across the model barrier and increased the concentration of available amino acids in the diffusion phase. However, the problem is that increasing the proportion of such components simultaneously raised the system's osmolarity to 345 mOsm/L. This may limit tolerability at high dry matter concentrations. The combined soya + rice hydrolysate was characterised by a lower AI, 19.8%, and a moderate release rate, 5.2 mg/min. The osmolarity was 278 mOsm/L, which was significantly lower compared to the sports formulation. It was precisely the combination of moderate osmolarity and a sufficiently high release rate that provided grounds for considering such a system as potentially suitable for clinical nutrition. The pea-rice mixture had the lowest AI, 17.1%, and the lowest release rate, 4.4 mg/min. At the same time, the osmolarity did not exceed 251 mOsm/L. It is suggested that this indicates the formation of a milder system with a more uniform supply of amino acids without sharp osmotic fluctuations. It is precisely this release kinetics that is potentially suitable for geriatric formulations, where excessively rapid absorption is not always desirable.

The results obtained confirmed that the efficiency of enzymatic hydrolysis is not determined solely by the degree of protein structure breakdown. N. Gasparre *et al.* (2025) viewed hydrolysis primarily as a means of enhancing the functionality of plant proteins. In the current study, this was also confirmed by numerical data. For the soybean- e hydrolysate, solubility reached 94.2%, *EAI* was 31.6 m^2/g, and emulsion stability was 88.4%. However, the problem is that further intensification of hydrolysis was accompanied by a decrease in the *EAA* index from 38.6 to 36.9 g/100 g of protein and a drop in *AAS* from 82 to 77%. This implies that excessive structural destruction impairs the nutritional adequacy of the system due to the loss of sensitive amino acids and the weakening of the interphase activity of peptides. It is precisely this aspect that has not been sufficiently detailed in previous studies, where the criterion for effectiveness often remained the maximum degree of hydrolysis. The advantages of combined protein compositions are partly consistent with the results of L. Etzbach *et al*. (2025), who demonstrated an improvement in the nutritional value of mixed protein systems. The results obtained expanded on these findings, as they demonstrated not only a balancing of the amino acid composition but also a stabilisation of the technological properties. The combined soya + rice hydrolysate had an *EAA* of 37.9 g/100 g protein, an *IEA* index of 0.61 and an *AAS* of 80%, which exceeded the values for rice isolate, where the AAS was only 61%. At the same time, the emulsion stability for the combined system reached 87.2%, i.e. almost matched that of the soya hydrolysate. Bioavailability indicators were also found to be close to the data reported by Y. Fan *et al*. (2025) and M. Opazo-Navarrete *et al.* (2025), who highlighted the varying digestibility of plant proteins. At the same time, the present study elucidated this mechanism through the analysis of molecular weight fractions. The highest transport across the model intestinal barrier was observed in the 0.5-1 kDa range, where the flux J was 5.41 $mg \cdot cm^{-2} \cdot h^{-1}$, the permeability coefficient P reached 0.101 $cm \cdot h^{-1}$, and the absorbed nitrogen was 148 mg over 2 hours. For fractions >3 kDa, these values decreased to 1.94 $mg \cdot cm^{-2} \cdot h^{-1}$, 0.031 $cm \cdot h^{-1}$ and 74 mg, respectively. Thus, it was the medium-molecular-weight peptides that provided the optimal combination of diffusion rate and nitrogen capacity. Excessively large fragments were retained by the membrane, whilst excessively small peptides transported a lower amount of nitrogen per unit mass.

Acid hydrolysis warrants separate discussion. G. Mustățea *et al*. (2019) highlighted its effectiveness for the analytical release

of amino acids. The results showed that this approach is less suitable for food systems. The degree of hydrolysis during acid treatment did not exceed 24.3%, and the functional properties of the product were inferior to those of enzymatic hydrolysates. This is due to a reduction in emulsion stability, the loss of some thermolabile amino acids, and the formation of a less balanced peptide composition. The contribution of this work to the scientific discourse lies in the combination of kinetic assessment of hydrolysis, analysis of peptide fractions, amino acid profile, and bioavailability modelling within a unified evaluation system for plant protein raw materials. The practical significance of the study lies in the possibility of creating protein compositions with predictable properties for various areas of nutritional support. At the same time, the results obtained should be regarded as an experimental basis for further physiological and clinical studies of the tolerability and efficacy of such systems.

Conclusions

The study showed that the efficiency of converting plant proteins into functionally suitable hydrolysates is determined by the complex interaction between process parameters and the structural characteristics of the protein raw material. The best results were obtained for enzymatic hydrolysis at pH 6.8-7.4, a temperature of 50-55°C and a process duration of 90-120 minutes. Under these conditions, the degree of hydrolysis for soya protein was 44.9%, and for pea protein 41.7%, whereas acid treatment did not exceed 24.3%. The soya hydrolysate demonstrated the highest solubility, 94.2%, an emulsifying activity index of 31.6 m^2/g, and emulsion stability of 88.4%. For the pea hydrolysate, these values were 89.5%, 28.7 m^2/g, and 84.1% respectively. Rice protein without prior activation was characterised by lower technological activity; however, following structural modification, its solubility increased from 42.8% to 74.5%. It was found that deeper hydrolysis is not always accompanied by an improvement in the quality of the final product. For soya protein, an increase in the intensity of processing led to a decrease in the *EAA* index from 38.6 to 36.9 g/100 g of protein and a drop in *AAS* from 82 to 77%. This is associated with partial losses of sensitive amino acids and a deterioration in the interphase stability of peptide systems. The best results were obtained with a moderate degree of protein matrix destruction, when both a high concentration of free amino acids and the functional properties of the hydrolysates were preserved. Bioavailability studies confirmed the decisive importance of the molecular weight composition of the peptide fractions. Maximum transport across the model intestinal barrier was observed for fractions of 0.5-1 kDa, where the flux J reached 5.41 $mg \cdot cm^{-2} \cdot h^{-1}$, the permeability coefficient P was 0.101 $cm \cdot h^{-1}$, and the amount of absorbed nitrogen over 2 hours was 148 mg. For fractions >3 kDa, these values decreased to 1.94 $mg \cdot cm^{-2} \cdot h^{-1}$, 0.031 $cm \cdot h^{-1}$ and 74 mg, respectively. The combined soybean + rice mixtures provided a balanced amino acid profile, emulsion stability of 87.2% and a bioavailability coefficient (Baa) of 86.8%, which was close to the characteristics of soybean hydrolysate. Prospects for further research involve conducting *in vivo* experiments, assessing the body's metabolic response, and clinically testing the tolerability of functional protein compositions with varying molecular compositions.

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Conflict of Interest

None.

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Технологічні аспекти модифікації рослинних протеїнів для оптимізації нутритивної підтримки у сучасній дієтології

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Анотація. Метою статті було встановлення технологічних параметрів гідролізу рослинних білків для отримання гідролізатів із прогнозованим амінокислотним складом, високою біодоступністю та стабільними функціонально-технологічними властивостями. Методологія дослідження ґрунтувалася на порівняльному аналізі ізолятів соєвого, горохового, рисового та конопляного білків із масовою часткою протеїну 76,8-90,4 %. Гідроліз проводився кислотним, лужним та ферментативним способом при контрольованих значеннях pH 2,0-8,5, температурі 37-58 °C та тривалості процесу 30-180 хв. Для оцінювання ефективності визначено ступінь гідролізу, концентрацію вільних амінокислот, молекулярно-масовий розподіл пептидів, розчинність, емульгувальну здатність та піноутворення. Біодоступність оцінювалася шляхом моделювання шлунково-кишкового травлення *in vitro*. Результати показали, що ферментативний гідроліз забезпечував ступінь перетворення субстрату 38,6-44,9 %, тоді як кислотний гідроліз не перевищував 24,3 %. Найвищу розчинність продемонстрували гідролізати соєвого білка, 91,4 %, та горохового білка, 87,2 %. Для рисового білка цей показник після попередньої структурної активації зростав із 42,8 % до 74,5 %. Концентрація вільних амінокислот у ферментативних гідролізатах збільшувалася у 2,3-3,1 раза порівняно з нативними ізолятами. Пептидні фракції з молекулярною масою 0,5-1 кДа забезпечували найвищу біодоступність, 68,7-72,4 %, та максимальний перехід амінокислот у дифузійну фазу. Комбіновані суміші соєвого та рисового білків у співвідношенні 2:1 формували найстабільніші емульсійні системи зі стабільністю 84,6 %. Практичне значення роботи полягає у можливості створення спеціалізованих білкових композицій для спортивного, клінічного та геріатричного харчування із контрольованими характеристиками засвоєння. Наукова новизна дослідження пов'язана з поєднанням кінетичного аналізу гідролізу, структурної оцінки пептидних фракцій та моделювання біодоступності в єдиній системі вибору технологічних режимів переробки рослинної білкової сировини для потреб сучасної дієтології

Ключові слова: протеїнові ізоляти; пептидні фракції; функціонально-технологічні властивості; in vitro травлення; протеолітична обробка