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Evaluation of the physicochemical composition of aqueous plant extracts

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Abstract. Despite the wide availability of raw materials, scientific data on the physicochemical properties of aqueous extracts of many promising plants remain limited. Most studies focus on alcoholic or combined extracts, whilst aqueous extracts are often considered only in the context of traditional medicine. The aim of this study was to determine the protein and mineral composition of aqueous extracts from selected medicinal plants and to evaluate their potential as sources of functional ingredients for the food industry. Aqueous extracts were prepared from 12 types of plant materials, including *Laminaria* (Elamin), cherry leaves and twigs, liquorice root, rosemary leaves, dill (leaves and seeds), chamomile, angelica root, beggarticks, fireweed, and stevia, using the method developed by V. Filatov. The physicochemical analysis involved the determination of total protein, albumin, calcium, phosphorus, magnesium, potassium, and zinc using a biochemical analyser. The results demonstrated significant variability in protein (1.31-17.54 g/L) and mineral content depending on the botanical origin of the raw materials. The highest protein concentrations were found in extracts from rosemary, stevia, beggarticks, and fireweed leaves. Laminaria extract showed the highest potassium content, whilst rosemary extract had the highest zinc content. Chamomile, angelica, and fireweed extracts also exhibited high levels of essential microelements, particularly phosphorus and zinc. All samples showed significantly higher concentrations of bioactive compounds compared to tap water, confirming the efficacy of the extraction method. The findings support the feasibility of using such extracts to enrich functional dairy products

Keywords: protein content; mineral composition; functional ingredients; functional dairy products; plant enrichment

Introduction

In the context of growing demand for natural and safe ingredients in the food, pharmaceutical and cosmetic industries, aqueous plant extracts are of particular importance as sources of biologically active compounds. The effectiveness of aqueous extracts is largely determined by their physicochemical characteristics, such as pH, dry matter content, acidity, redox potential and polyphenol concentration. These indicators can vary significantly depending on the type of plant raw material, the morphological part of the plant and extraction conditions, which complicates the standardisation and prediction of their functional properties. In this context, the study of the physicochemical parameters of aqueous plant extracts is both relevant and necessary to justify their rational use in the development of functional products and biotechnological solutions. According to I. Matasar *et al*. (2021), the dietary intake of the examined population group was found to be unbalanced in terms of macroelement supply. The diets were deficient in calcium (Ca) and magnesium (Mg) and exhibited disturbances in the optimal ratio between macroelements, which may lead to the development of premorbid conditions and contribute to the onset of nutritional and diet-related diseases. Nutrition is essential for human development, especially in children. Proper nutrition is required for optimal growth to ensure adequate intake of macronutrients and micronutrients, which are vital for healthy childhood development. G. Savarino *et al*. (2021) note that key micronutrients in this regard include zinc, iron, vitamin D, and folic acid.

Recent scientific studies on the metabolism and biological role of zinc in the human and animal body have indicated that the biological function of zinc is largely realised through its participation in the synthesis and stabilisation of nucleic acids and proteins, energy metabolism,

cell proliferation and differentiation, and the maintenance of antioxidant status. D. Jin *et al*. (2024) demonstrated that zinc also plays a protective role in the presence of various pathogenic factors. R. Tedesco *et al*. (2021) reported that a total of 261 whey samples were collected and analysed from four locations in the Veneto region of north-eastern Italy. It was found that the whey samples contained a wide range of concentrations of 17 trace elements (0.06-1,530 µg/kg) and 14 rare earth elements (0.16-28.2 µg/kg), but none reached toxic levels. This provides valuable information about the quality of dairy products and, since it reflects local environmental conditions, also serves as an excellent indicator of their geographical origin. The concentrations of 12 non-essential trace elements (Pb, Cd, Hg, As, U, Cr, Sr, Be, Ni, Al, Sn, and Tl) and 8 essential trace elements (Fe, Cu, Mn, Zn, V, Se, Co, and Mo) were determined in 78 samples of raw sheep and goat milk.

In response to the growing demand for high-quality, safe, and nutritious food products, studies were conducted on the content of rare earth elements in 200 samples of Italian sheep cheeses – pecorino romano PDO and pecorino sardo PDO. It was found that a 100 g serving of both cheese types provides more than 30% of the recommended daily intake of calcium, sodium, zinc, selenium, and phosphorus, and more than 15% of the recommended daily intake of copper and magnesium. A. Mara *et al*. (2024) demonstrated that the mineral composition of both cheese types is consistent with current European standards for the biological value indicators of fermented dairy products. The human body is generally compatible with most plant-based preparations, as these compounds are frequently present in plant-derived foods. The therapeutic properties found abundantly in plants are naturally compatible with the human body.

Scientific interest in such extracts is driven by their high bioavailability, safety, and natural origin, which meet the requirements for ingredients used in functional foods, including dairy products. In this context, particular attention should be given to studying the protein and mineral composition of aqueous extracts, as this serves as the basis for further justification of their use in the production of probiotic, fermented milk, or immunostimulatory beverages. F. Abbassi *et al*. (2024) have shown that the protein composition of extracts depends on the plant species and processing conditions. Similar results were obtained for extracts of dandelion (*Taraxacum officinale*), rose hips (*Rosa canina*), plantain (*Plantago major*) and other medicinal herbs, where a protein fraction consisting of hydrophilic peptides and amino acid residues was detected. The mineral composition of extracts is also quite diverse. Studies by A. Chandran *et al*. (2024) and C. Munialo (2024) reported high concentrations of potassium, magnesium, calcium, iron, and zinc in aqueous extracts of amaranth (*Amaranthus* sp.) and fenugreek (*Trigonella foenum-graecum*). These elements not only contribute to the mineralisation of body tissues but also positively influence the enzymatic activity and immunomodulatory properties of final food products enriched with such extracts.

L. Yan & A. Kljakić (2023) reported that using a low-temperature extraction method under dark conditions allows for the preservation not only of macroelements but also of trace amounts of microelements – such as selenium, manganese, and cobalt – which play an important role in metabolism and cellular antioxidant protection. Several studies have also emphasised that aqueous extracts derived from non-toxic plants can be used as additives in fermented dairy products to enrich their amino acid profile and mineral composition (Damani & Topi, 2022; Yarmolinsky *et al*., 2024). Considering that information on the nutritional value of aqueous plant extracts is limited, conducting such a study is both scientifically and practically justified. The results obtained may not only expand the understanding of the nutritional potential of these plant-based substances but

also serve as a foundation for the further development of new food products with enhanced biological value.

The aim of this study was to examine the protein and mineral composition of several types of aqueous plant extracts as potential sources of minerals and proteins. To achieve this aim, the following objectives were set: to prepare aqueous extracts of selected medicinal plants using the method developed by Academician V. Filatov; to examine their protein and mineral composition and draw conclusions regarding their potential for use in the production of functional dairy products; and, based on the results of the mineral composition analysis, to outline directions for further scientific research.

Materials and Methods

The study was conducted from May to August 2025 in the laboratory of Smartbiolab LLC and the Institute of Food Resources of the National Academy of Agrarian Sciences of Ukraine. Extracts were prepared from the following plants: Elamin (kelp) *Laminaria*; liquorice root (*Glycyrrhiza glabra*); young cherry leaves (*Prunus cerasus*); young cherry branches; dill leaves (*Anethum graveolens*); dill seeds; rosemary leaves (*Rosmarinus officinalis*); chamomile flowers (*Matricaria chamomilla*); angelica root (*Angelica archangelica*); beggarticks leaves (*Bidens tripartita*); fireweed leaves (*Chamaenerion angustifolium*); stevia leaves (*Stevia rebaudiana*). The plant raw materials were collected and purchased at the Experimental Station of Medicinal Plants of the Institute of Agroecology and Environmental Management of the National Academy of Agrarian Sciences of Ukraine. The choice of plant raw materials was governed by their prevalence in Ukraine and their high content of biologically active compounds of various types, in particular polyphenols, essential oils, organic acids and minerals. The selected plants are readily available domestic resources and are characterised by antioxidant, antimicrobial and functional properties, making their use in food technologies appropriate. This allows for the justification of developing products from local plant raw materials. The plant extracts were prepared according to the method developed by Academician V. Filatov, who studied the effects of biogenic stimulators such as aloe on the body's defences and their role in regeneration and recovery processes (Kovalyov *et al*., 2014). Individual 50 g portions of plant material were covered with water to a total volume of 1 litre and infused for 7-8 days. The mixture was stirred six times during infusion, then heated in a water bath for 5-7 minutes. The aqueous mixture of herbs was filtered to separate the liquid and solid fractions. The filtrates were dispensed into 100 mL bottles and sterilised at 120°C for 15 minutes.

The plant materials were rinsed under running tap water. Leafy plant materials were air-dried and ground in a coffee grinder, whilst root materials were soaked in water for 24 hours before grinding, and their outer skins were removed prior to processing. Samples were prepared by weighing 25 g of plant material and adding distilled water up to the 1 dm^3 mark (925 mL). The mixtures were stirred in containers and left to stand undisturbed in a cool, dark place. Extraction of active compounds from the studied plant ingredients was carried out at a temperature of (12 ± 2)°C for 3-4 days. Upon completion of the extraction process, the solid plant material was separated from the liquid by filtration. The liquid portion was sterilised in an autoclave at (120 ± 2)°C for 15 minutes, cooled to (20 ± 2)°C, and then subjected to physicochemical analysis using a Biochemical Analyser RT-1904C (Rayto Electronics, China) in the laboratory of Smartbiolab LLC. The wavelength parameters used during the analyses were as follows: albumin – 620 nm; protein – 546 nm; potassium – 578 nm; calcium – 670 nm; magnesium – 546 nm; phosphorus (UV) – 340 nm; zinc – 546 nm. Statistical analysis of the data was performed using Microsoft Excel 2016. Each experiment was performed in triplicate

(n = 3). Results were considered statistically reliable at a significance level of $p \leq 0.05$.

Results and Discussion

The selected raw material covers a wide range of biologically active components of various nature, which allows for a comprehensive assessment of the physicochemical and functional characteristics of aqueous plant extracts. The evaluation of the protein and mineral composition of 12 samples of aqueous plant extracts revealed significant variability of parameters depending on the type of raw material. The physicochemical parameters of the plant extracts, expressed in grams per litre, are presented in Table 1.

Table 1. *Physicochemical indicators of plant extracts*

Extract name	Protein, g/L	Albumin, g/L	Calcium (Ca), mg/L	Phosphorus (P), mg/L	Magnesium (Mg), mg/L	Potassium (K), mg/L	Zinc (Zn), mg/L
Elamin	5.98	0.08	3.65	2.45	3.72	22.03	4.46
Liquorice root	3.73	0.50	2.82	0.64	3.51	4.19	1.62
Young cherry leaves	4.66	–	2.67	1.27	2.54	5.72	7.90
Dill leaves	5.04	0.15	4.48	2.73	3.43	6.82	8.92
Rosemary leaves	17.54	0.34	4.94	5.12	3.57	9.46	47.82
Chamomile flowers	3.17	0.57	3.62	7.17	3.73	3.94	11.15
Dill seeds	2.99	0.04	4.03	2.78	3.98	7.10	0.20
Young cherry branches	1.31	0.27	2.65	0.25	2.38	2.26	3.45
Angelica root	1.68	0.34	2.49	6.45	3.37	10.66	7.90
Beggarticks leaves	13.06	0.08	4.41	5.07	4.00	11.36	36.88
Fireweed leaves	8.77	0.31	3.80	4.46	2.54	5.46	35.67
Stevia leaves	13.06	0.42	3.89	7.37	4.13	6.62	38.50
Tap water	–	–	1.81	–	1.03	0.08	1.62

Source: compiled by the authors

From the data in Table 1 it is clear that the revealed trend of growth of the content of total plant protein in aqueous extracts depending on the type of plant raw materials indicates a significant influence of anatomical and morphological features of plants on the efficiency of extraction of protein components. The lowest values are characteristic of woody tissues and root raw materials, while a significantly higher protein content is inherent in leaves and generative organs, which is due to a more intensive metabolism and a higher concentration of water-soluble compounds. The maximum indicators were recorded in leaf raw materials, in particular in stevia, beggarticks and rosemary, which confirms its prospects as a source of plant protein for the creation of functional food products. The results obtained also indicate the feasibility of targeted selection of plant raw materials of leaf nature to increase the protein value of extracts and optimise the recipes of functional and fermented products. The highest protein concentration was found in: rosemary leaves (17.54 g/L), fireweed leaves (8.77 g/L), and stevia and beggarticks leaves (13.06 g/L each). The lowest

protein concentration was observed in cherry branches (1.31 g/L), angelica roots (1.68 g/L), and dill seeds (2.99 g/L). This may be due to lower extractability or a low content of protein compounds in the raw plant material.

According to G. Nieto *et al*. (2018), rosemary leaves contain significant amounts of proteins, flavonoids, and phenolic acids, which determine their high biological activity. Stevia is also known for its high content of protein compounds and amino acids (Žlabur *et al*., 2013; Ahmad *et al*., 2020). Albumin content (g/L): was absent in young cherry leaves. In ascending order, it was detected in dill seeds (0.04); elamin and beggarticks leaves (both 0.08); dill leaves (0.15); young cherry branches (0.27); fireweed leaves (0.31); rosemary leaves and angelica root (both 0.34); stevia leaves (0.42); liquorice root (0.50); chamomile flowers (0.57). Although albumin represents a small fraction of the total protein, its presence is important due to its high biological value. The highest albumin content was recorded in: chamomile flowers – 0.57 g/L, liquorice root – 0.50 g/L, stevia leaves – 0.42 g/L. At the same time, the albumin fraction was not detected in the leaves of young cherry, indicating a different structure of protein compounds. Thus, it can be concluded that most of the extracts can be used as raw materials for obtaining plant protein and albumin, which was absent in young cherry leaves.

According to the data in Table 1, a clear increasing trend in calcium (Ca) content in aqueous extracts is observed depending on the type of plant raw material. The lowest levels are characteristic of root and woody materials, while higher calcium concentrations are typical of leaves and seeds. This pattern can be explained by the physiological role of calcium in actively metabolising tissues, particularly in leaves, where it participates in cellular structure formation and metabolic regulation. The highest calcium content is observed in leaf-based raw materials, especially rosemary, dill, and beggarticks, indicating their potential as promising sources of mineral enrichment for functional food formulations. The lowest calcium content was found in the extract of angelica root (2.49 mg/L) and cherry branches (2.65 mg/L), while the highest level, among the edible raw materials used as the basis for extract preparation, was observed in dill leaves (4.48 mg/L); and among the wild plant extracts – in rosemary leaves (4.94 mg/L). Similar values were reported by R. Subramanian *et al*. (2012) and Radha *et al*. (2021), who investigated the calcium profile of aqueous extracts of medicinal plants, including dill (Anethum graveolens), with up to 4.2 mg/L Ca in hot infusions.

A similar trend is observed for phosphorus (P) content in aqueous extracts, which varies depending on the type of plant raw material. Lower phosphorus levels are characteristic of woody tissues and certain root materials, whereas a gradual increase is noted in leaves, seeds, and flowers. This distribution is determined by the biological role of phosphorus in energy metabolism and the synthesis of cellular components, which occur more intensively in metabolically active plant organs. The highest phosphorus content is found in leaf and flower materials, particularly in stevia, chamomile, and rosemary. Thus, particularly high values were found in chamomile flowers (7.17 mg/L) and stevia leaves (7.37 mg/L), while the lowest levels were observed in cherry branches (0.25 mg/L) and liquorice roots (0.64 mg/L). However, these concentrations are sufficiently high for effective use, for instance, in the fortification of dairy products intended for special diabetic nutrition. The same conclusion applies to the analysis of the mineral composition of other plant-based extracts. These results are also consistent with the findings of A. El Mihyaoui *et al*. (2022) and M. Saeedi *et al*. (2024), which reported that medicinal chamomile is rich in phosphorus.

A consistent increasing trend in magnesium (Mg) content in aqueous extracts is observed depending on the type of plant raw material. The lowest levels are typical of woody

tissues and some leaf materials with lower metabolic activity, while higher concentrations are characteristic of leaves, seeds, and flowers. This pattern is associated with the key role of magnesium in photosynthesis and enzymatic processes, leading to its accumulation in metabolically active plant organs. The highest magnesium content is observed in leaf-based raw materials, particularly stevia and beggarticks. Thus, the lowest magnesium content was found in extracts from young cherry leaves and fireweed leaves (2.54 mg/L each), while the highest levels were recorded in extracts from beggarticks leaves (4.00 mg/L) and stevia leaves (4.13 mg/L), respectively. The lowest potassium (K) content, in increasing order, was found in the following aqueous extracts (mg/L): young cherry branches (2.26), chamomile flowers (3.94), liquorice roots (4.19), fireweed leaves (5.46), young cherry leaves (5.72), stevia leaves (6.62), dill leaves (6.82), dill seeds (7.10), rosemary leaves (9.46), angelica roots (10.66), beggarticks leaves (11.36), and elamin (22.03). A significant accumulation of potassium was observed in elamin (22.03 mg/L), which substantially exceeded all other samples, while the lowest potassium level was found in cherry branch extracts – 2.26 mg/L.

A pronounced increasing trend in zinc (Zn) content in aqueous extracts is observed depending on the type of plant raw material. The lowest levels are characteristic of seeds and root materials, while a substantial increase is noted in leaves and flowers. This pattern is determined by the biological role of zinc as an essential trace element involved in enzymatic activity and metabolic regulation, leading to its higher accumulation in metabolically active plant tissues. The highest zinc content is found in leaf-based raw materials, particularly rosemary, stevia, and beggarticks. A high Zn content was characteristic of rosemary (47.82 mg/L), beggarticks (36.88 mg/L), and stevia leaves (38.50 mg/L), while the lowest concentration was recorded in dill seeds – only 0.20 mg/L.

According to J. Suliburska & K. Kaszmarek (2012) and P. Konieczynski & M. Wesolowski (2015), the zinc content in rosemary extracts ranges from 20 to 35 mg/L. Stevia and beggarticks also exhibit a high zinc content (36.88-38.5 mg/L), consistent with the findings of A. Plaskova & J. Mlcek (2023), who emphasised the mineralising potential of plants in this group. An analysis was also conducted on the water used for extract preparation. Phosphorus was not detected in the water sample. The concentrations of four mineral elements – calcium (Ca), magnesium (Mg), potassium (K), and zinc (Zn) – were 1.81, 1.03, 0.08, and 1.62 mg/L, respectively. These findings are consistent with the conclusions of A. Tytarenko & E. Hryshyna (2011), who stated that water supplies only about 1.0-10% of the body's mineral requirements. For all parameters (with few exceptions), the extracts significantly exceeded the levels found in tap water, indicating the high extractive capacity of medicinal plant materials and their potential for nutritional enrichment. Studies by L. Oraon *et al*. (2017), S. El-Sayed & A. Youssef (2019), and A. Kandyliari *et al*. (2023) have confirmed that aqueous plant extracts can serve as an effective means of enhancing the nutritional and functional value of food products. Thus, the most nutritious extracts in terms of total protein and mineral content are those derived from rosemary, stevia, beggarticks, and fireweed. The extract from elamin showed the highest potassium content, while rosemary had the highest zinc concentration. A significant variability was observed among extracts of different botanical origins, allowing for targeted selection of raw materials depending on the desired nutrient enrichment profile of food products.

Conclusions

A comprehensive study of the physicochemical composition of 12 types of aqueous plant extracts prepared according to the method of Academician V. Filatov was conducted. The

results expand understanding of the protein and mineral content present in the analysed aqueous plant extracts. It was found that extracts from rosemary, stevia, beggarticks, and fireweed leaves contain the highest levels of total protein (8.77-17.54 g/L), indicating their potential as sources of protein components in food technologies. The data obtained allow for preliminary calculations to determine the optimal dosage of these extracts for enriching dairy products, taking into account the existing daily requirements of the human body for protein and minerals. Among the studied samples, the highest content of mineral elements (calcium, phosphorus, magnesium, potassium, and zinc) was recorded in extracts of rosemary, stevia, and beggarticks, which may enhance their biological value when incorporated into functional foods. Extracts from chamomile flowers, angelica root, and fireweed leaves demonstrated a high content of trace elements, particularly zinc (up to 47.82 mg/L), making them suitable for use as natural mineralising agents in dietary and infant nutrition. The established variability in the composition of the extracts makes it possible to purposefully select plant components according to the functional purpose of the food product. It is expected that dairy products enriched with essential plant extractives obtained from natural plant materials can be classified as functional foods. Further research should be aimed at studying the technological compatibility of plant extracts with dairy raw materials, their stability during storage, and the organoleptic evaluation of the final products.

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Conflict of Interest

None.

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Оцінка фізико-хімічного складу водних рослинних екстрактів

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Анотація. Незважаючи на широку доступність сировини, наукові дані щодо фізико-хімічних властивостей водних екстрактів багатьох перспективних рослин залишаються обмеженими. Більшість досліджень зосереджені на спиртових або комбінованих екстрактах, тоді як

водні екстракти часто розглядаються лише в контексті традиційної медицини. Мета цього дослідження полягала у визначенні білкового та мінерального складу водних екстрактів обраних лікарських рослин та оцінці їх потенціалу як джерел функціональних інгредієнтів для харчової промисловості. Водні екстракти були приготовлені з 12 видів рослинної сировини, включаючи *Laminaria* (Elamin), листя та гілки вишні, корінь солодки, листя розмарину, кріп (листя та насіння), ромашку, корінь дягелю, календулу, іван-чай та стевію, за методом, розробленим В. Філатовим. Фізико-хімічний аналіз включав визначення загального білка, альбуміну, кальцію, фосфору, магнію, калію та цинку за допомогою біохімічного аналізатора. Результати показали значну варіабельність вмісту білка (1,31-17,54 г/л) та мінералів залежно від ботанічного походження сировини. Найвищі концентрації білка виявлено в екстрактах із листя розмарину, стевії, календули та іван-чаю. Екстракт ламінарії мав найвищий вміст калію, тоді як екстракт розмарину – найвищий вміст цинку. Екстракти ромашки, дягелю та іван-чаю також продемонстрували високий рівень життєво важливих мікроелементів, особливо фосфору та цинку. Усі зразки показали значно вищі концентрації біоактивних сполук порівняно з питною водою, що підтверджує ефективність методу екстракції. Отримані дані підтримують можливість використання таких екстрактів для збагачення функціональних молочних продуктів

Ключові слова: вміст білка; мінеральний склад; функціональні інгредієнти; функціональні молочні продукти; рослинне збагачення

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A study of changes in the functional-technological properties of minced meat systems based on hake and broiler chicken meat mince

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Abstract. The relevance of the research stems from increased consumer interest in combined meat products, which necessitates determining the optimal formulation combinations of raw materials of different origins. The aim of the study was to determine changes in the functional-technological and rheological properties of minced systems depending on the ratio of meat and fish raw materials and the carrageenan content. Four formulations based on broiler chicken

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meat were investigated with the addition of 0 or 10% hake mince and 2.8-5.2% carrageenan. The samples were heat-treated to 70 °C followed by determination of moisture content, water-holding capacity, pH, plasticity, and rheological properties. The moisture content ranged from 71.21 to 74.85%. After heat treatment, moisture losses were 3.06-5.75%, with lower losses observed in samples containing higher levels of carrageenan, indicating the formation of a more stable gel structure. The highest water-holding capacity was observed in samples containing fish raw material. The pH increased to 6.21-6.32 with a further slight increase after heating. Rheological studies demonstrated pseudoplastic behaviour of the systems, with viscosity decreasing as the applied load increased. The addition of fish raw material increased the effective viscosity but reduced the yield shear stress. The obtained results confirm the feasibility of combining meat and fish raw materials with carrageenan to regulate the functional-technological properties and to form stable structures of minced systems. In addition, a decrease in plasticity after heat treatment and a change in yield shear stress were established, indicating the transition of the systems from a viscoplastic to an elastically structured state and the formation of a denser structure in the minced systems. The obtained results may be applied in the production of meat products with the addition of fish raw materials for the targeted regulation of functional-technological properties and the formation of a stable protein-polysaccharide structure of the finished product

Keywords: semi-finished meat products; hydrobionts; combined meat products; hydrocolloids; rheological properties; functional properties

Introduction

Modern approaches to the formation of food product assortments are increasingly focused on the scientifically substantiated combination of ingredients of different natural origins in order to achieve a balanced chemical composition, increase nutritional value, and improve the functional-technological properties of finished products. Such a strategy for the development of food technologies is based on the principles of rational nutrition, functional food design, and reducing the negative effects of excessive consumption of certain nutrients. According to the results of sociological studies conducted in European countries by researchers M. Banovic *et al*. (2022) and A.M. Barone *et al*. (2021), the majority of consumers positively perceive the creation of so-called hybrid products that combine meat, fish, and plant raw materials within a single formulation, particularly when familiar organoleptic characteristics are preserved.

This approach opens broad opportunities for the targeted regulation of the nutritional and biological value of products, particularly through optimisation of the amino acid composition of the protein fraction, reduction of the total saturated fat content, increase in the proportion of polyunsaturated fatty acids, as well as enrichment of products with biologically active components of natural origin. At the same time, the use of combined raw materials contributes to the improvement of technological indicators, including structural-mechanical and rheological characteristics, increased stability of emulsion systems, reduction of syneresis, and improvement of water- and fat-binding capacity, which is critically important for ensuring the stable quality of finished products throughout their shelf life. The development of the modern meat-processing industry is largely determined by the need to optimise product formulations in accordance with the principles of rational and functional nutrition, particularly balance in proteins, fats, carbohydrates, and micronutrients. Particular attention is also paid

to reducing the energy value of products without loss of their technological and consumer qualities. One of the effective approaches to such improvement, according to the research of S.Y.J. Sim *et al*. (2021), is the combination of raw materials of different origins, which makes it possible to purposefully regulate the nutritional and biological value of products, as well as to increase their functionality through the synergistic interaction of components within the food system.

According to OECD/FAO (2025), the production of meat and fish products demonstrated a stable growth trend during 2020-2025, driven both by increasing global demand for protein products and by the gradual expansion of the range of food products with added technological value. At the same time, according to the studies of researchers C. Barcenilla *et al.* (2022) and S. Sheffield *et al*. (2024), meat products remain one of the key and most accessible sources of complete protein, essential amino acids, B-group vitamins, and a number of minerals necessary for the normal functioning of the human body. As noted in the work of M.W.M. Kharl *et al*. (2025), fish is a valuable functional food product characterised by a high content of complete protein with high biological value, a significant amount of polyunsaturated fatty acids (particularly the omega-3 fraction), as well as a wide range of vitamins and minerals that positively affect metabolic processes and overall health. Owing to this, fish raw material is regarded as an important component for the creation of combined protein systems with increased nutritional value.

Particular attention among fish raw materials is attracted to hake, which, according to the studies of M.J. Santos *et al.* (2022), is one of the most widespread commercial fish species in Europe and has stable industrial significance. Its meat is characterised by a white colour, dense and fibrous consistency, low fat content, and favourable functional-technological properties, which determines the feasibility of its use in the production of combined meat-fish products. In addition, researchers R. Mendes *et al.* (2021) established that the physicochemical and structural properties of fish meat change significantly depending on storage conditions and methods, particularly chilling or freezing, which must necessarily be taken into account when designing processing technologies. In modern meat technologies, polysaccharides of natural and modified origin are regarded as functionally active ingredients capable of significantly influencing the structural-mechanical and rheological properties of minced systems. According to the findings of J. Shao *et al*. (2025), their action is due to the ability to form spatial hydrocolloid structures as a result of interaction with the aqueous phase, proteins, and lipids, thereby increasing moisture-retention capacity, stabilising the emulsion system, and reducing weight losses during heat treatment.

Studies conducted by N.E. Masiques *et al.* (2025) demonstrate that the use of hydrocolloid mixtures in meat products also significantly affects protein bioavailability and its subsequent digestion within the body. It was determined that κ-carrageenan and xanthan gum may modify protein digestion processes, influence the composition of the intestinal microbiota, regulate inflammatory pathways, and affect the intensity of oxidative processes in diets high in red meat. Such effects are associated both with changes in the structure of the food matrix and with the slowing down or modification of enzymatic protein hydrolysis in the gastrointestinal tract. The aim of the study was to determine changes in the functional-technological and rheological properties of minced systems based on broiler chicken white meat, hake mince, and carrageenan in various ratios.

Materials and Methods

The studies were carried out under laboratory conditions at the Department of Meat and Meat Products Technology of the National University of Food Technologies during 2025-2026. In

accordance with the developed experimental design, the experimental samples were formed by varying the content of structure-forming agents and the composition of the protein base, resulting in four formulation compositions. White meat of broiler chickens was selected as the main raw material for all samples. Carrageenan, previously hydrated at a ratio of 1:40, was used as the structure-forming component. According to the literature review data, the choice of carrageenan was due to its pronounced gel-forming ability. Its content in the formulations varied from 2.8% to 5.2%. The second variable component was fish raw material – hake mince, which was either excluded from the formulation (0%) or added in an amount of 10%. According to the analysis of literature sources, hake mince is one of the most widespread types of fish raw material, which makes the use of such raw material particularly relevant. As a result, four formulation variants were developed, differing in the combinations of carrageenan content and the presence of fish raw material. Such an approach made it possible to assess the effect of compositional changes on the functional-technological properties of the minced systems. The obtained formulations are presented in Table 1.

Table 1. *Formulation composition of minced systems with fish raw material*

Raw material	Sample 1	Sample 2	Sample 3	Sample 4
Broiler chicken fillet	94.8%	97.2%	84.8%	87.2%
Hake mince	0%	0%	10.0%	10.0%
Hydrated carrageenan	2.8%	5.2%	2.8%	5.2%

Source: compiled by the authors

Grinding of the meat raw materials was carried out using an HKN-22SS meat grinder. Mixing of the mince was performed in a Sirman IP 10 M meat mixer. The obtained minced systems were baked in a SIEMENS HB537A2S00 oven to an internal product temperature of 70°C. Moisture content was determined by the gravimetric drying method. For this purpose, a sample weighing 5.00 g (with an accuracy of 0.01 g) was taken and evenly distributed in a previously dried and weighed weighing dish. Drying was carried out in a drying oven at a temperature of 105 ± 2°C until a constant mass was achieved, which was monitored by repeated weighing at intervals of 30-60 minutes. Moisture content was calculated from the loss of mass after drying relative to the initial sample mass, and the result was expressed as a percentage. All measurements were performed in triplicate with subsequent averaging of the results to increase the reliability of the obtained data.

Water-binding capacity (WBC) was determined by the pressing method, which is based on the ability of the minced system to retain moisture under external pressure. For the analysis, a mince sample weighing 0.30 g was placed on filter paper and subjected to a standard load for 10 minutes. WBC was calculated in two ways: relative to the total moisture content in the sample (WBCa), which characterises the proportion of bound water; and relative to the sample mass (WBCm), which reflects the ability of the system to retain moisture per unit mass of the product. The obtained indicators make it possible to evaluate the structural-mechanical properties of the mince and the degree of water binding by protein components. Active acidity (pH) was determined by the potentiometric method using an MP511 laboratory pH meter. Prior to measurements, the pH meter was calibrated using standard buffer solutions with pH values of 4.00 and 7.00, and the electrode was cleaned and rinsed with distilled water between measurement series to prevent cross-contamination.

Effective viscosity and yield shear stress were determined using a Volarovich viscometer,

the operating principle of which is based on measuring the resistance of the material to shear under applied mechanical loading. Effective viscosity was determined as the ratio of shear stress to shear rate under specified flow conditions. Yield shear stress was established as the minimum stress required to initiate system flow. Measurements were carried out at a controlled temperature of 20 ± 1°C and within a range of regulated loads, which ensured reproducibility of the rheological characteristics. Each determination was performed in at least triplicate. The obtained indicators characterise the consistency of the mince, its plasticity, and its moulding ability, which are critically important for technological processing operations. Statistical processing of the results was carried out using determination of the mean value and standard deviation; the significance of differences between samples was assessed using Student's t-test at a significance level of $p < 0.05$

Results and Discussion

The developed minced systems, created using fish raw materials and specially selected structure-forming components, were subjected to comprehensive investigation according to the main physicochemical and rheological indicators. Such studies were carried out for a more detailed and objective assessment of their functional-technological properties, as well as to determine the stability of the formed structures in model systems. In order to establish the effect of heat treatment on the quality characteristics of the finished product, the obtained minced masses were formed into standard-shaped meat loaves. The samples were then baked under controlled temperature conditions until reaching a temperature of 70°C in the central part of the product, which ensured complete and uniform heat treatment. After cooling, the finished products were additionally analysed according to the main physicochemical indicators in order to assess the changes occurring in the structure and composition of the product during thermal processing.

The amount of bound moisture in minced systems, as well as its subsequent change after thermal treatment, is regarded as an important indicator of the level of structural organisation of the product. Variations and dynamics of this indicator make it possible to assess the effectiveness of interactions between ingredients, the degree of spatial network formation, and the overall stability of the system during technological processing. The results of determining the moisture content in the product are presented in Figure 1.

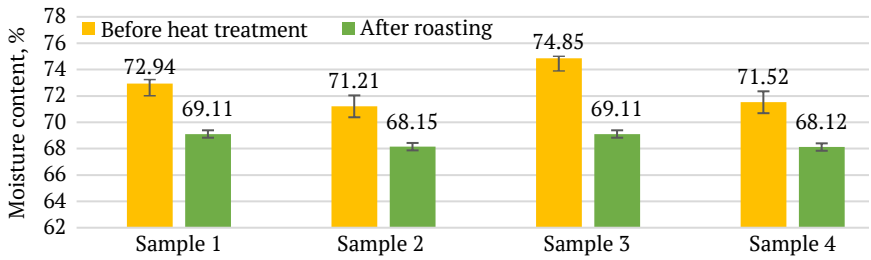

Figure 1. Changes in moisture content in minced systems before and after heat treatment
Source: compiled by the authors

According to the results presented in Figure 1, changes in moisture content in the developed minced systems showed that all experimental formulations had this indicator within the range of 71.21-74.85%. The highest moisture content was determined in Sample 3, which is explained by the higher content of hake mince. After heat treatment, the moisture con-

tent in the samples decreased by 3.06-5.75%. Lower losses were observed in the samples containing 5.2% carrageenan, indicating the formation of a stable gel matrix in these samples. Samples 3 and 4, which contained 10% hake mince, showed significant differences between each other, with decreases of 5.75% and 3.4%, respectively. This confirms the feasibility of enhancing structure formation in formulations containing fish raw material in order to ensure stability and reduce moisture losses. Similar trends are described in studies of hydrocolloid systems by T. Udo *et al*. (2023), where it was established that heat treatment promotes the formation of a denser spatial network, which partially displaces unbound moisture. As noted in the work of the research group led by M. Woo *et al.* (2025), the use of polysaccharides in meat products is effective for improving juiciness in low-fat products, which correlates with the obtained data. Lower moisture losses in samples with increased carrageenan content confirm its ability to form a stable gel matrix. Similar results were obtained in studies of meat-fish minced systems by A.M. Geredchuk *et al.* (2023) and N.M. Stukalska *et al.* (2024), where the addition of polysaccharides ensured increased water-retention capacity through the formation of a three-dimensional network that retains water in a capillary-bound state. In addition, increasing the content of fish raw material (hake) contributed to an increase in overall moisture content, which is explained by the higher hydration capacity of fish proteins compared with meat proteins. Similar conclusions were presented in the study by N. Bozhko *et al.* (2021), where it was shown that fish mince forms less dense but more moisture-saturated structures. In addition to assessing overall moisture content, determining the degree of moisture binding is also important. This indicator makes it possible to evaluate the stability of the formed dispersed system. The obtained results are presented in Figure 2.

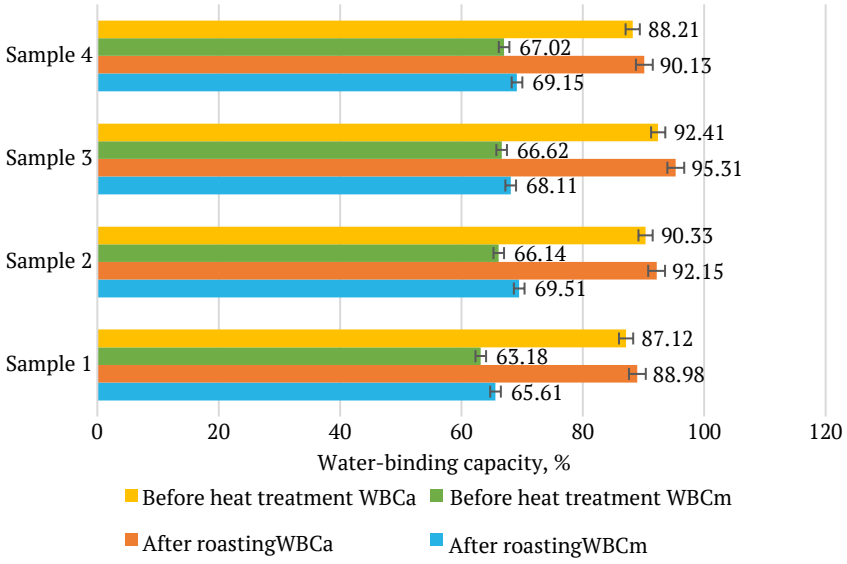

Figure 2. Water-binding capacity of the developed minced systems

Source: compiled by the authors

In accordance with the planned research programme, the water-binding capacity (WBC) of the obtained minced systems and its changes after roastingwere determined. The calculation

was carried out relative to the total moisture mass (WBCa) in the experimental sample and relative to the sample weight (WBCm) used for the analysis. The results for Sample 1 indicate the possibility of obtaining sufficiently high values with minimal addition of the structure-forming polysaccharide to broiler chicken meat mince. Sample 2 increased the WBCm values by 3.9% and WBCa by 3.17% due to the higher hydrocolloid content. After roasting, the studied indicators decreased, indicating the formation of a dense gel matrix capable of retaining moisture. Sample 3 demonstrated the highest WBCa value at 95.31%, despite moderate WBCm values of 68.11%. However, the combination of the polysaccharide-based hydrocolloid and fish raw material allowed only a slight decrease in the indicators by 2.92% and 1.49%, respectively. The highest amount of bound moisture in the finished product confirms the possibility of forming a thermally stable system as a result of the synergy between meat and fish proteins with the carrageenan-based hydrocolloid. As noted in the study by T.M. Prylipko *et al*. (2024), the combination of fish mince and plant protein components makes it possible to obtain improved structural characteristics of the product due to enhanced water binding. The obtained results of combining hake mince and broiler chicken meat indicate that the addition of fish raw material does not have a pronounced effect on water-binding capacity. The decrease in WBC values after heat treatment is consistent with the findings of T. Udo *et al.* (2023), who associate this with the transition of the system into a more ordered gel state, in which part of the water becomes less available for determination.

The obtained data show that the combination of fish raw material and hydrocolloid provides better stability of the indicators after heating. This is explained by the synergy between fish myofibrillar proteins and the polysaccharide matrix, which has been confirmed in studies of combined minced systems by N. Bozhko *et al.* (2021) and A.M. Geredchuk *et al*. (2023). At the same time, an excess of carrageenan (Sample 4) leads to a decrease in the efficiency of water binding after heat treatment, which is consistent with the findings of L. Montes *et al.* (2022) regarding possible oversaturation of the system with hydrocolloid and the formation of a fragile structure. Determining changes in active acidity during the development of a combined product makes it possible to assess the interaction of ingredients of different origins. The results of the conducted studies are presented in Figure 3.

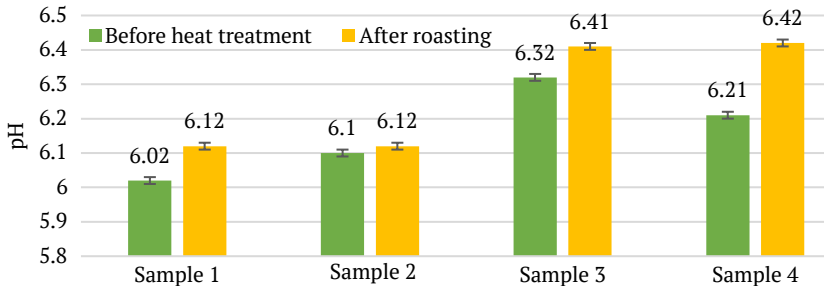

Figure 3. Changes in active acidity in minced systems before and after heat treatment
Source: compiled by the authors

The active acidity values in combined meat-fish systems primarily depend on the quantitative ratio of the raw materials. In the minced systems before heat treatment, a clear pattern was observed: the addition of hake meat contributed to a shift in pH towards the

alkaline range (6.21-6.32), which is explained by the specific biochemical composition of fish tissue and its higher intrinsic acidity level. After heat treatment to 70°C, a further increase in pH values was recorded in all experimental samples by 0.02-0.21 pH units, which is the result of thermal denaturation causing the disruption of hydrogen bonds. Considering the previously presented data, it can be stated that an increase in pH in the model minced systems promotes the formation of a stable gel matrix and improves moisture binding. The increase in pH after heat treatment is associated with protein denaturation and the destruction of hydrogen bonds, leading to the release of functional groups. A similar effect was described by T. Udo *et al.* (2023) for protein-polysaccharide systems, where heating alters the charge of protein molecules and promotes their interaction. Importantly, the increase in pH correlates with improved water-retention capacity, which is confirmed both by the obtained results and by literature data indicating that moving away from the isoelectric point enhances protein hydration. An important indicator for evaluating combined minced systems is the determination of rheological characteristics. Such indicators reflect the features of the structural organisation of the minced system and its behaviour under mechanical loading. One of the key indicators is plasticity, which determines the ability of the system to undergo deformation without destruction of the structure. The results of the conducted studies are presented in Figure 4.

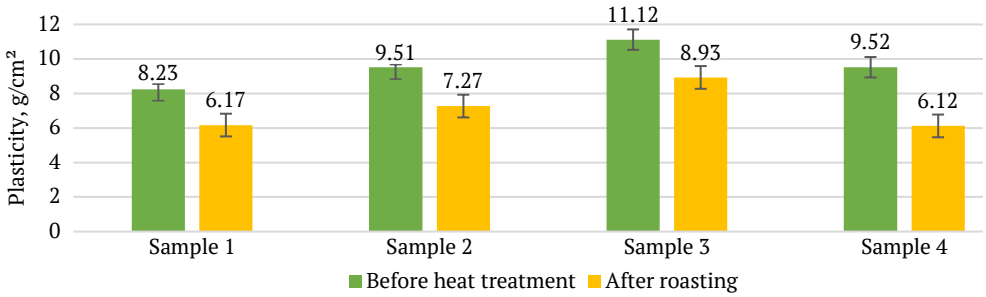

Figure 4. Plasticity of the studied minced systems

Source: compiled by the authors

Changes in plasticity in combined minced systems largely depend on the formulation composition and the state of moisture. According to the results presented in Figure 4, increasing the amount of hydrocolloid made it possible to increase the plasticity of the mince by 1.28 g/cm³. The increase in plasticity in Sample 3 correlates with the high moisture content in this sample and indicates greater flowability of the mince containing hake and a lower amount of hydrocolloid. After heat treatment, the plasticity of all experimental samples decreased by 2.06-3.4 g/cm³. Such a change is caused by the transition of the system from a viscoplastic state to an elastically mechanical state with the formation of a spatial framework that resists deformation. As can be seen from the obtained data, increasing the amount of carrageenan-based hydrocolloid intensifies the reduction in plasticity after heat treatment, despite the presence of fish raw material. The combination of 2.8% hydrocolloid and 10% fish raw material made it possible to maintain plasticity within 8.98 g/cm³, allowing the formation of a tender structure. At the same time, increasing the hydrocolloid proportion to 5.2% resulted in an excessively dense structure and a brittle consistency.

In the study by T. Udo *et al.* (2023) on hydrocolloids, it was shown that heating causes

a transition from a viscoplastic to an elastically resilient state due to the formation of a three-dimensional network. The increase in plasticity with the addition of fish raw material is consistent with the results of N. Bozhko *et al.* (2021), where it was noted that fish proteins form a softer and more plastic structure compared with meat proteins. At the same time, increasing the concentration of carrageenan leads to the formation of a more rigid structure, which is confirmed both by the obtained data and by literature sources, particularly the studies of G. Kalsi *et al*. (2025). An excess of hydrocolloid may result in the formation of an overly dense network, reducing plasticity and making the product brittle. Evaluation of effective viscosity and yield shear stress makes it possible to determine the resistance of the obtained system to structural breakdown and the characteristics of its internal flow under applied mechanical stresses. Taking into account the findings of H. Bao *et al.* (2025), it was established that carrageenan significantly affects the rheological behaviour of meat systems through the formation of a gel matrix in interaction with muscle proteins, while its effectiveness largely depends on the presence of protein components, particularly fish raw material, which enhances structure formation and stabilises the system. The results of the studies are presented in Figure 5.

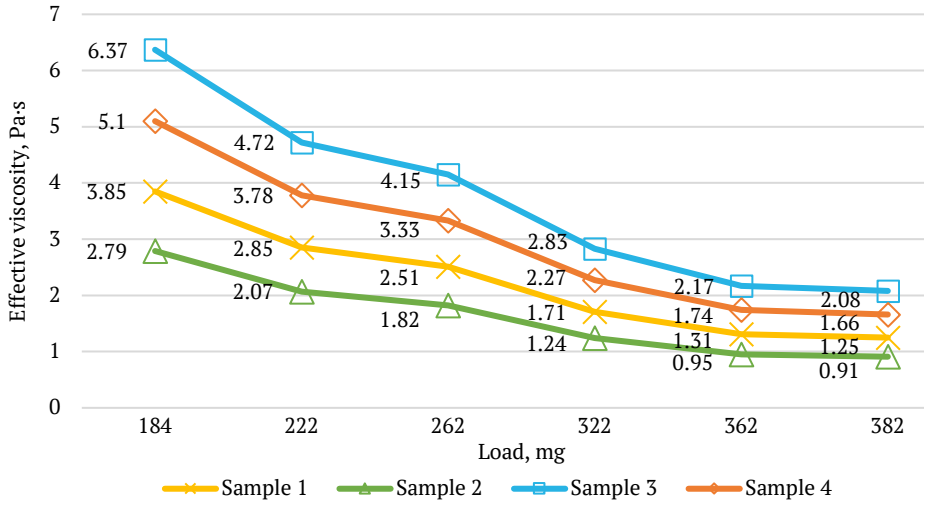

Figure 5. Effective viscosity of the developed minced systems

Source: compiled by the authors

In all samples, a decrease in effective viscosity was recorded with increasing load from 184 g to 382 g, which is typical for structured food dispersed systems and indicates the presence of pseudoplastic behaviour. This is associated with the destruction of the spatial structure of the protein-polysaccharide gel and the orientation of particles in the direction of flow. The lowest viscosity values were characteristic of Sample 2, whereas Sample 1 demonstrated slightly higher structural stability at low and medium loads, but also rapidly lost viscosity with increasing shear stress. This indicates that excessive addition of carrageenan without supplementary protein components does not ensure the formation of a more stable structural framework. In contrast, Samples 3 and 4, containing 10% hake mince, were characterised by significantly higher effective viscosity values throughout the entire load range. For example, at a load of 184 g, the values were 6.37 Pa·s and 5.10 Pa·s,

respectively, which is almost twice as high as the corresponding values in samples without fish raw material. This indicates enhanced interprotein interactions and the formation of a denser spatial matrix.

The increase in viscosity in samples containing fish raw material indicates strengthened intermolecular interactions. Similar results were obtained in the studies of N. Bozhko *et al*. (2021) and A.M. Geredchuk *et al*. (2023), where the addition of fish protein promoted the formation of more structured systems. At the same time, the low effectiveness of excessive carrageenan without a protein component confirms the findings of L. Montes *et al*. (2022), which state that protein-polysaccharide interaction is the key factor in the formation of a stable structure. The next stage of the research was the determination of yield shear stress, the results of which are presented in Figure 6.

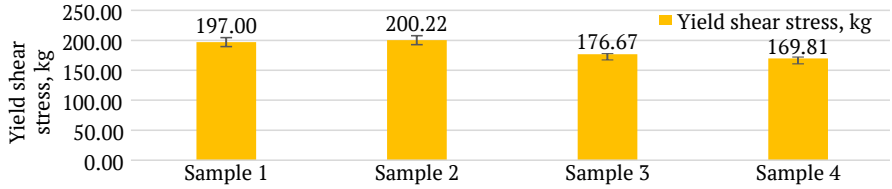

Figure 6. Yield shear stress of combined minced systems

Source: compiled by the authors

The obtained values of yield shear stress characterise the strength of the structural framework of the studied systems and their ability to resist destruction under external loading. The highest values were recorded for Sample 2 at 200.22 kg and Sample 1 at 197.00 kg, indicating a relatively stronger structure in systems without the addition of a fish component. This may be associated with a denser carrageenan gel matrix in the absence of additional protein-lipid phases that alter the nature of spatial interactions. In Samples 3 and 4, which contained 10% hake mince, a decrease in yield shear stress to 176.67 kg and 169.81 kg, respectively, was observed. This indicates a partial weakening of the structural framework, probably as a result of replacing part of the stronger muscle protein matrix of chicken with a more heterogeneous fish protein system that forms a less dense spatial network. This is consistent with the findings of G. Kalsi *et al.* (2025), where it was shown that carrageenan forms dense gels in the absence of external protein phases. The reduction in this indicator with the addition of fish raw material is explained by the more heterogeneous structure of the system. Similar results are described in studies of meat-fish minced systems by N.M. Stukalska *et al*. (2024) and H. Xie & L. Grossmann (2025), where the incorporation of fish protein reduces rigidity but improves textural characteristics.

Summarising the obtained results, it was established that the combination of broiler chicken meat, fish raw material, and carrageenan makes it possible to purposefully regulate the functional-technological and rheological properties of combined minced systems through the formation of a protein-polysaccharide spatial structure. The most pronounced structure-forming effect was observed in samples with an optimal content of carrageenan and fish raw material, which ensured a balance between viscosity, water retention, and the strength of the gel matrix. The obtained patterns confirm the possibility of controlled formation of the desired technological properties of combined minced systems and create a basis for further optimisation of formulations and technological production regimes.

Conclusions

As a result of the conducted study, it was established that the combination of broiler chicken white meat, fish raw material, and hydrated carrageenan significantly affects the formation of the functional-technological, structural-mechanical, and rheological properties of complex multicomponent minced systems. This effect is manifested both at the level of water-binding capacity and in the formation of a spatial protein-polysaccharide matrix, which determines product stability at all stages of the technological process, including preparation, heat treatment, and subsequent storage. It was established that the addition of carrageenan ensures the formation of a developed gel matrix that acts both as a structure-forming agent and as a stabiliser of the dispersed system. Through interaction with the protein components of the meat and fish raw materials, it increases the moisture-retaining capacity of the minced systems, reduces moisture and fat losses after heat treatment, and promotes a more uniform distribution of moisture within the structure of the finished product. The most stable moisture-retention values after roasting were characteristic of samples with an increased hydrocolloid content, indicating enhanced intermolecular interactions and more pronounced structure formation within the system, as well as the formation of a denser and more elastic gel.

It was demonstrated that the addition of 10% hake mince significantly alters the balance of structural interactions in the minced system. On the one hand, an increase in total moisture content and effective viscosity was observed, which is associated with the high water-binding capacity of fish proteins and their ability to form additional intermolecular bonds in the combined system. On the other hand, the incorporation of fish raw material resulted in a reduction in yield shear stress, which may indicate a certain weakening of the strength of the spatial protein framework compared with systems not containing a fish component, as well as a change in the nature of the structure from more elastic to more plastic. Overall, the obtained results indicate the feasibility and technological efficiency of using a combination of meat and fish raw materials with carrageenan for the targeted regulation of the functional-technological properties of combined minced systems. Such an approach makes it possible to develop products with predetermined structural-mechanical characteristics, increased stability of the emulsion and gel structure, and improved behaviour during heat treatment, which is important for achieving stable quality of finished products under industrial conditions. Prospects for further research include expanding the combination of carrageenan with other types of meat and fish raw materials of different functional compositions, as well as studying the influence of various heat-treatment methods (boiling, roasting, frying) on the formation of structural-mechanical and rheological properties of combined minced systems.

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Conflict of Interest

None.

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Дослідження зміни функціонально-технологічних показників фаршевих систем на основі фаршу м'яса хека та курчат-бройлерів

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Анотація. Актуальність досліджень зумовлена підвищеним зацікавленням споживача до комбінованих м'ясних продуктів, що зумовлює необхідність визначення оптимальних рецептурних поєднань сировини різного походження. Метою було визначення

змін функціонально-технологічних і реологічних показників фаршів залежно від співвідношення м'ясної та рибної сировини і вмісту карагенану. Досліджено чотири рецептури на основі м'яса курчат-бройлерів із додаванням 0 або 10 % фаршу хеку та карагенану 2,8-5,2 %. Зразки піддавали термообробці до 70 °С з подальшим визначенням вологи, вологоутримувальної здатності, pH, пластичності та реологічних показників. Вологовміст становив 71,21-74,85 %. Після термообробки втрати вологи були 3,06-5,75 %, менші втрати спостерігалися у зразках із вищим вмістом карагенану, що свідчить про формування більш стабільної гелевої структури. Найвищу вологоутримувальну здатність мали зразки з рибною сировиною. pH підвищувався до 6,21-6,32 з подальшим незначним зростанням після нагрівання. Реологічні дослідження показали псевдопластичну поведінку систем зі зниженням в'язкості зі зростанням навантаження. Додавання рибної сировини підвищувало ефективну в'язкість, але зменшувало граничне напруження зсуву. Отримані результати підтверджують доцільність поєднання м'ясної та рибної сировини з карагенаном для регулювання функціонально-технологічних властивостей і формування стабільних структур фаршевих систем. Додатково встановлено зниження пластичності після термічної обробки та зміну граничного напруження зсуву, що вказує на перехід систем від в'язко-пластичного до пружно-структурованого стану та формування більш щільної структури фаршевих систем у фарш. Отримані результати можуть бути застосовані у виробництві м'ясних продуктів з додаванням рибної сировини з метою цілеспрямованого регулювання функціонально-технологічних показників та формування стабільної білково-полісахаридної структури готового продукту

Ключові слова: м'ясні напівфабрикати; гідробіонти; комбіновані м'ясні продукти; гідроколоїди; реологічні показники; функціональні показники

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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

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Assessment of the toxicity of modified dried egg white

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Abstract. The relevance of this study stems from the need to develop safe and functionally effective protein carriers for the immobilisation of biologically active substances and microorganisms, which show promise for use in animal husbandry, the food industry and biotechnology. The aim of the study was to assess the toxicological properties of modified dried egg white by determining its acute toxicity, its effect on the haematological, biochemical and antioxidant parameters of the blood of laboratory animals, as well as its irritant effect on the ocular mucosa. The study utilised toxicological, clinical, haematological, biochemical and statistical research methods. The acute toxicity of modified dried egg white was investigated following intragastric administration to white mice at doses ranging from 100 to 5,000 mg/kg body weight, and its irritant effect on the ocular mucosa of California rabbits was assessed. It was found that even at the maximum dose of 5,000 mg/kg, no fatalities were observed, and short-term manifestations of depression and diarrhoea were transient and resolved completely without further intervention. Haematological, biochemical and antioxidant blood parameters were analysed and found to remain within physiological norms: at a dose of 2,000 mg/kg, haemoglobin concentration was 132 g/L, erythrocyte count was 5.7×10^{12}/dm³, total protein – 65.4 g/dm³, glucose concentration – 5.4 mmol/dm³, and total thiol groups – 16.3 µg/cm³; whereas at the maximum dose of 5,000 mg/kg, total protein reached 67.3 g/dm³, AST activity – 0.93 µmol/h/mL, ALT – 1.04 µmol/h/mL, and glucose concentration – 6.0 mmol/dm³, all

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within the physiological range. When assessing the irritant effect on the mucous membrane of the rabbits' eyes, only mild, transient clinical manifestations were observed, with no damage to the iris; whilst haematological, biochemical and antioxidant parameters also corresponded to physiological values, confirming the absence of haemotoxic, hepatotoxic, metabolic and pronounced oxidative effects of the modified dry egg white. The practical value of this work lies in the potential to use the results obtained to develop and implement safe protein carriers for biologically active substances and microorganisms in animal husbandry, the food industry, biotechnology and scientific research

Keywords: protein carrier; immobilisation; haematological parameters; biochemical parameters; laboratory animals; biotechnology

Introduction

The development of safe, biocompatible and functionally effective carriers for the immobilisation of biologically active substances and microorganisms is one of the priority areas in the development of modern food technology, biotechnology and animal husbandry. Such carriers ensure increased stability of active components, protection against adverse environmental factors, sustained release and preservation of biological activity, which significantly broadens the scope of their practical application. Of particular interest are materials of natural origin, which are characterised by high biocompatibility, biodegradability and safety, making them a promising basis for the creation of new functional delivery systems for biologically active compounds.

The work by T. Liu *et al*. (2024) notes that naturally derived protein matrices are among the most promising materials for the encapsulation of probiotics, enzymes, vitamins and other biologically active compounds. The authors found that the use of protein carriers provides a high level of protection for immobilised components during processing and storage, and also facilitates their controlled release in the required environment. Similar conclusions were reached by B. Ma *et al*. (2024), S. Razi *et al*. (2024) and Y. Tian *et al*. (2024), who demonstrated that delivery systems based on food proteins are characterised by high encapsulation efficiency, structural stability and the ability to maintain the biological activity of functional ingredients. The researchers emphasise that, thanks to their emulsifying, gelling and stabilising properties, chicken egg proteins are among the most promising natural materials for the creation of next-generation carriers. J. Su *et al.* (2020) and L. Song *et al*. (2022) note that, alongside the study of the material's physicochemical characteristics, it is necessary to determine its acute toxicity, its effect on the body's functional state, and any possible local irritant effects, as it is precisely these indicators that determine the potential for the further practical application of new protein systems. Further development of immobilisation technologies involves the use of chicken egg proteins as natural carriers of biologically active substances (Rostamabadi *et al.*, 2023). Y. Zhang *et al.* (2023) note in their review that ovalbumin and other egg proteins, owing to their amphiphilic nature, self-assembly capacity, gel-forming ability and biodegradability, are promising materials for the creation of nanoparticles, emulsions, hydrogels and aerogels designed to deliver biologically active compounds and probiotic cultures. At the same time, the authors emphasise the need for further study of their biological safety and behaviour in biological systems.

Analysing current trends in the development of protein-based delivery systems, J. Zang *et al.* (2023) and E. Armanu *et al*. (2025)

have shown that modified gels based on chicken egg proteins can be effectively used as carriers for nutraceuticals due to their high mechanical strength, stability and ability to ensure the controlled release of active components. At the same time, J. Lu *et al*. (2020) and T. Mehrotra *et al*. (2021) emphasise that the development of such systems requires optimisation of the structure of protein matrices to enhance the bioavailability of immobilised substances and preserve their functional activity. The safety of protein carriers is also attracting considerable attention from researchers. Thus, an analysis of the current scientific literature indicates that most studies are devoted to the development of protein-based delivery systems, the improvement of their structural characteristics, and the evaluation of the effectiveness of encapsulating biologically active substances. At the same time, the toxicological safety of modified dried egg white as a potential carrier for the immobilisation of biologically active components and microorganisms remains insufficiently studied. In particular, there is a lack of comprehensive studies that simultaneously assess acute toxicity, local irritant effects, and changes in haematological, biochemical and antioxidant parameters in laboratory animals, which necessitated the present study. The lack of information regarding the toxicological properties of modified dried egg white limits its practical application as a carrier for biologically active substances and microorganisms in food and biotechnological systems. Conducting a comprehensive assessment of its biological safety is a necessary prerequisite for the further introduction of such materials into production, as it allows the identification of potential risks to the organism and justifies the feasibility of their practical application.

The aim of the study was to experimentally substantiate the toxicological safety of modified dried egg white as a promising protein carrier for the immobilisation of biologically active substances and microorganisms through a comprehensive assessment of its acute toxicity, its local irritant effect and its impact on the haematological, biochemical and antioxidant parameters of laboratory animals.

Materials and Methods

The acute toxicity of modified dried egg white was investigated in male BALB/c mice weighing 21.5-22.0 g and aged 56-57 days. The mice were prepared for the experiment in accordance with the requirements of I. Kotsiumbas *et al*. (2006). The study was conducted in two stages. For each stage, four groups of mice were formed. Each group comprised six mice. The duration of the experiment was 14 days. During the first stage, the effect of low doses (100; 250; 500 and 1,000 mg/kg body weight) of modified dried egg white was investigated. During the second stage, the toxic effects of high doses of the carrier (2,000; 3,000; 4,000; 5,000 mg/kg body weight) were assessed. A solution of modified dried egg white was administered to the mice as a single intragastric dose using a metal probe with a welded lead tip to prevent damage to the oesophagus. The modified egg white was dissolved in a 1% starch solution. The animals were observed and housed in accordance with the recommendations set out by I. Kotsiumbas *et al.* (2006).

The irritant effect of modified dried egg white was investigated using California rabbits with a body weight of 2.7-2.8 kg. Three males were prepared for the experiment in accordance with the requirements of I. Kotsiumbas *et al*. (2006). A solution of modified dried egg white, at a dose of 2 drops, was instilled into the conjunctival sac of the left eye. The right eyes of the laboratory animals were used as controls. Observation of the rabbits over a 14-day period and determination of the carrier's toxicity level were carried out in accordance with the recommendations of W. Diener & E. Schlede (1999) and I. Kotsiumbas *et al.* (2006). The content of protein, low-molecular-weight and total sulphhydryl groups in the blood serum of rabbits

and mice was determined using the method of G. Ellman (1959); total protein was determined according to the method of O. Lowry *et al*. (1951), using the glucose oxidase-peroxidase method of P. Trinder (1969). Aminotransferase activity was investigated according to the method of S. Reitman & S. Frankel (1957). Haemoglobin content in the blood and erythrocyte count were determined according to the instructions of I. Tovstukha *et al*. (2025).

During the experiments, the air temperature in the laboratory animal quarantine rooms and procedure rooms was maintained at 17-18°C, relative humidity at 57-64%, and illuminance at the level of the laboratory animal cages was 305-320 lx. The animals had free access to standard pelleted feed and drinking water throughout the experimental period. All experimental studies were conducted in accordance with current methodological approaches and relevant requirements and standards, including ISO/IEC 17025:2017 (2017). Animal housing and all procedures were carried out in accordance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (1986) and Order No. 249 of the Ministry of Education and Science, Youth and Sport of Ukraine (2012). Statistical analysis of the research results was carried out using Microsoft Excel 2021 and Statistica 10.0 software. The arithmetic mean (M) and the standard error of the mean (m) were determined. The significance of differences between the indicators was assessed using the Student's t-test. A difference was considered statistically significant at $p < 0.05$.

Results and Discussion

The results of the acute low-dose toxicity study of modified dried egg white showed that a single intragastric administration to mice at doses ranging from 100 to 1,000 mg/kg body weight did not result in any fatalities in any of the test groups (Table 1). This indicates the absence of any marked acute toxic effects of the test substance when administered at low doses.

Table 1. *Results of acute low-dose toxicity testing of modified dried egg white in mice*

Group	Number of animals	Dose, mg/kg body weight	Number of dead animals	Mortality rate, %	Time of death	Adverse clinical signs
1st	6	100	0	0	–	Not specified
2nd	6	250	0	0	–	Not specified
3rd	6	500	0	0	–	Not specified
4th	6	1,000	0	0	–	Not specified

Source: compiled by the authors

During clinical observation of the mice, no adverse changes in the animals' general physiological condition were observed. All mice remained active, responded appropriately to external stimuli, and maintained normal mobility and coordination of movement. Feed and water intake were within physiological norms; no signs of central nervous system depression, convulsions, paresis, respiratory distress or gastrointestinal dysfunction were observed. When mice were administered modified dried egg white at doses of 100 and 250 mg/kg body weight, their clinical condition did not differ from the physiological norm. A similar pattern was observed when the dose was increased to 500 and 1,000 mg/kg body weight. The absence of adverse clinical signs, even at the maximum dose tested, indicates that the carrier is well tolerated by laboratory animals.

The results obtained provide grounds for concluding that modified dried egg white is characterised by a low level of acute toxicity within the range of doses tested. The results of the acute toxicity study of high doses of

modified dried egg white showed that a single administration of the preparation to mice at doses ranging from 2,000 to 5,000 mg/kg body weight did not result in the death of any animals in any of the test groups. All mice survived throughout the entire observation period, and the mortality rate was 0%. This indicates the absence of any pronounced acute toxic effects of the test preparation, even at high doses (Table 2).

Table 2. *Results of acute toxicity testing of high doses of modified dried egg white in mice*

Group	Number of animals	Dose, mg/kg body weight	Number of dead animals	Mortality rate, %	Time of death	Adverse clinical signs
1st	6	2,000	0	0	–	Not established
2nd	6	3,000	0	0	–	Not established
3rd	6	4,000	0	0	–	Temporary suppression
4th	6	5,000	0	0	–	Suppression, diarrhoea during the first 24 hours

Source: compiled by the authors

During clinical observation of animals administered 2,000 and 3,000 mg/kg body weight of modified dried egg white, no adverse clinical signs were observed. The mice remained active, responded appropriately to external stimuli, and consumed food and water in physiologically normal quantities. No signs of impaired coordination, convulsions, central nervous system depression or gastrointestinal disturbances were observed. When the dose was increased to 4,000 mg/kg body weight, temporary depression was noted in the animals. The mice exhibited reduced mobility and decreased activity during the first 24 hours following administration of the drug. However, these changes were transient and resolved without further intervention, indicating an adaptive response of the organism to a high toxicological load. In the group of animals administered the maximum dose of 5,000 mg/kg body weight, more pronounced clinical changes were observed in the form of depression and diarrhoea during the first day of the experiment. Despite this, no fatalities were recorded, and the clinical condition of the mice gradually returned to normal. A return to normal physiological activity and feed intake was observed as early as the second day of the study.

The results obtained indicate that the modified dried egg white is characterised by a low level of acute toxicity, even when administered at high doses. The absence of fatalities suggests that the median lethal dose (LD_{50}) exceeds 5,000 mg/kg body weight, which, in accordance with toxicological classifications, provides grounds for classifying the carrier under investigation as a low-toxicity or virtually non-toxic substance. These data confirm the findings of X. Lv *et al.* (2024), who note that the use of natural protein carriers in food and biotechnological systems requires a comprehensive assessment of their biological safety, including the determination of acute toxicity, effects on the body's functional state and possible local irritant effects, as it is precisely these indicators that determine the potential for the further practical application of new protein systems. The results of the analysis of haematological parameters in mice following the administration of high doses of modified dried egg white indicate that the test preparation had no significant adverse effect on the haematopoietic system. It was found that the haemoglobin content and erythrocyte count in the blood of animals in all experimental groups were within the physiological range for laboratory mice (Table 3).

Table 3. *Haematological parameters of the blood of mice following the administration of high doses of modified dried egg white*

Administered dose, mg/kg	Haemoglobin concentration, g/L	Erythrocyte count, 10^{12}/dm^3
2,000	132 ± 3.9	5.7 ± 0.32
3,000	128 ± 5.6	5.4 ± 0.28
4,000	135 ± 4.8	5.9 ± 0.33
5,000	137 ± 3.7	6.0 ± 0.31

Source: compiled by the authors

Following the administration of modified dried egg white to mice at a dose of 2,000 mg/kg body weight, the haemoglobin concentration was 132 g/L and the erythrocyte count was 5.7 × 10^{12}/dm^3. These results indicate a normal functional state of the haematopoietic system and an adequate supply of oxygen to the tissues. When the dose was increased to 3,000 mg/kg body weight, a slight decrease in haemoglobin concentration of 3.0% and in erythrocyte count of 5.2% was observed compared with the group administered 2,000 mg/kg of the preparation. These changes were minor and remained within physiological limits, indicating that no anaemia or suppression of erythropoiesis developed. When a dose of 4,000 mg/kg body weight was used, a tendency towards an increase in haematological parameters was observed. Haemoglobin levels were 2.2% higher, and erythrocyte counts were 3.5% higher, compared with mice administered the vehicle at a dose of 2,000 mg/kg body weight. A similar pattern was observed at the maximum dose tested – 5,000 mg/kg body weight.

The moderate increase in haematological parameters observed with the use of high doses of modified dried egg white is likely to be of an adaptive nature and is not associated with any toxic effects of the preparation. The absence of marked changes in haemoglobin levels and erythrocyte count indicates that the test preparation does not exert a pronounced haematotoxic effect on laboratory animals, even at high doses. The results of the study of serum protein metabolism parameters in mice following the administration of high doses of modified dried egg white indicate that the test preparation has no pronounced adverse effect on liver function or the body's protein metabolism. It was found that total protein content and aminotransferase activity in all experimental groups remained within the physiological range for laboratory mice (Table 4).

Table 4. *Protein metabolism parameters in the blood serum of mice following the administration of high doses of modified dried egg white*

Administered dose, mg/kg	Protein concentration, g/dm^3	AST activity, µmol/h/mL	ALT activity, µmol/h/ml
2,000	65.4 ± 1.16	0.81 ± 0.052	0.92 ± 0.031
3,000	66.3 ± 2.06	0.79 ± 0.015	0.94 ± 0.023
4,000	66.8 ± 2.33	0.84 ± 0.023	0.98 ± 0.011
5,000	67.3 ± 1.78	0.93 ± 0.049	1.04 ± 0.031

Source: compiled by the authors

Following the administration of modified dried egg white to mice at a dose of 2,000 mg/kg body weight, the total protein content in blood serum was 65.4 g/dm^3. AST activity was 0.81 µmol/h/mL, and ALT activity was 0.92 µmol/h/mL. These results indicate normal

protein metabolism and the absence of any impairment in hepatocyte function. When the dose of the drug was increased to 3,000 mg/kg body weight, a slight increase in total protein content of 1.3% was observed. AST activity remained virtually unchanged, whilst ALT activity showed a tendency towards a slight increase of 2.1% compared with the values in animals administered a vehicle dose of 2,000 mg/kg body weight. These changes were minor and showed no signs of a pathological nature. In the group of mice administered 4,000 mg/kg body weight of modified dried egg white, protein metabolism parameters increased within the margin of error compared with mice administered the lowest dose of the carrier. At the maximum dose studied – 5,000 mg/kg body weight – the highest protein metabolism indices were observed: total protein content was 67.3 g/dm^3, AST activity was 0.93 μmol/h/mL, and ALAT activity was 1.04 μmol/h/mL.

The observed moderate increase in total protein content and transaminase activity was dose-dependent; however, all parameters remained within the physiological range. The absence of a sharp increase in AST and ALT activity indicates that the modified dried egg white did not cause pronounced toxic liver damage or impairment of hepatocyte cell membrane permeability. The results obtained provide grounds for concluding that the carrier under investigation is well tolerated by laboratory mice, even at high doses. The observed changes in protein metabolism parameters are of an adaptive nature and do not indicate the development of marked hepatotoxic effects. The results of the study of thiol group content in the blood serum of mice following the administration of high doses of modified dried egg white indicate the absence of marked disturbances in the antioxidant status of the laboratory animals. It was found that the levels of protein-bound, total and low-molecular-weight (free) thiol groups in all experimental groups remained stable and fell within the range of physiological variations (Table 5).

Table 5. *Thiol group content in the blood serum of mice following the administration of high doses of modified dried egg white, μg/cm^3*

Administered dose, mg/kg	Protein-bound	Total	Low-molecular-weight (free)
2,000	14.6 ± 0.32	16.3 ± 0.22	1.7 ± 0.09
3,000	14.9 ± 0.26	16.6 ± 0.18	1.7 ± 0.07
4,000	15.2 ± 0.29	17.0 ± 0.35	1.8 ± 0.05
5,000	15.2 ± 0.30	17.1 ± 0.42	1.9 ± 0.11

Source: compiled by the authors

Following the administration of modified dried egg white to mice at a dose of 2,000 mg/kg body weight, the concentration of protein thiol groups in blood serum was 14.6 μg/cm^3, that of total thiol groups was 16.3 μg/cm^3, and that of low-molecular-weight (free) thiol groups was 1.7 μg/cm^3. These results indicate that the body's antioxidant system continued to function normally and that there were no signs of marked oxidative stress. When the dose of the preparation was increased to 3,000 mg/kg body weight, a slight increase in the content of protein-bound and total thiol groups was observed, by 2.0% and 1.8% respectively, compared with the values in mice administered the vehicle at a dose of 2,000 mg/kg. The content of low-molecular-weight thiol groups remained unchanged. These changes were not statistically significant and likely reflected the body's physiological adaptation to the administration of high doses of the preparation. In the group of mice administered 4,000 mg/kg body weight

of modified dried egg white, a further trend towards an increase in the content of thiol groups was observed. A similar pattern was observed at the maximum dose of 5,000 mg/kg body weight.

The observed moderate increase in thiol group content may indicate activation of the body's antioxidant system in response to the administration of high doses of modified dried egg white. At the same time, the absence of sharp changes in the indicators confirms that the test substance does not cause pronounced oxidative stress or disruption of the cellular redox balance. Thus, the results of the analysis of thiol groups in the blood serum of mice indicate the stability of the body's antioxidant status when high doses of modified dried egg white are administered and confirm the absence of any pronounced toxic effect on cellular metabolic processes. The results of the study of glucose levels in the blood serum of mice following the administration of high doses of modified dried egg white indicate that the test preparation has no significant adverse effect on carbohydrate metabolism in laboratory animals. It was found that glucose concentrations in all experimental groups remained within the physiological normal range for mice (Fig. 1).

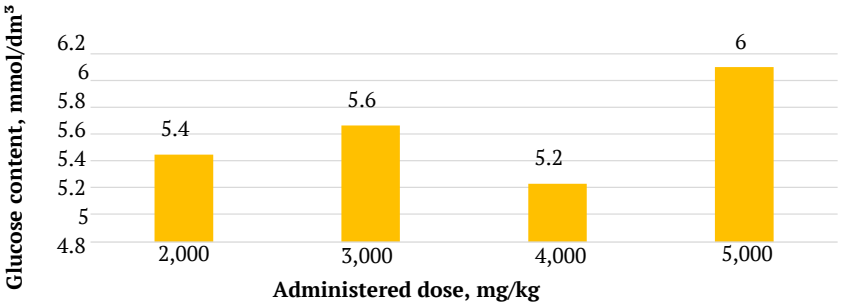

Figure 1. Serum glucose levels in mice following the administration of high doses of modified dried egg white, mmol/dm³

Source: compiled by the authors

Following the administration of modified dried egg white to mice at a dose of 2,000 mg/kg body weight, the serum glucose concentration was 5.4 mmol/dm³. This result indicates normal energy metabolism and an adequate supply of glucose to the body. When the dose of the preparation was increased to 3,000 mg/kg body weight, a slight increase in glucose concentration was observed. These changes were minor and showed no signs of being pathological. In the group of mice administered 4,000 mg/kg body weight of modified dried egg white, the glucose content was 3.7% lower compared with the group of mice administered the lowest dose of the vehicle. At the maximum dose studied – 5,000 mg/kg body weight – the highest serum glucose level was recorded, amounting to 6.0 mmol/dm³. Despite a slight increase in this parameter, it remained within the physiological range and did not indicate the development of marked disturbances in carbohydrate metabolism or toxic damage to the body. Thus, the results of the biochemical studies confirm the good tolerability of modified dried egg white and the absence of significant disturbances in carbohydrate metabolism, even when high doses of the preparation were used. It was found that, following administration of the feed additive into the conjunctival sac, the test animals exhibited only mild and transient clinical signs of irritation, which resolved within the first day of observation (Table 6).

Table 6. *Irritant effects of the feed additive on the ocular mucosa of rabbits (n = 3)*

Experimental animal	Observation time	Ocular discharge (0-3 points)	Conjunctival hyperaemia (0-3 points)	Conjunctival oedema (0-4 points)	Condition of the iris (0-2 points)
No. 1	1 hour	1	0	0	0
	24 hours	1	0	0	0
	2 days	0	0	0	0
	3 days	0	0	0	0
	14 days	0	0	0	0
No. 2	1 hour	2	0	0	0
	24 hours	1	1	1	0
	2 days	0	0	1	0
	3 days	0	0	0	0
	14 days	0	0	0	0
No. 3	1 hour	1	0	0	0
	24 hours	1	0	0	0
	2 days	0	0	0	0
	3 days	0	0	0	0
	14 days	0	0	0	0

Source: compiled by the authors

In rabbit No. 1, one hour after administration of the product, slight discharge from the eye was observed, which was assessed as 1 point. Similar changes were also observed 24 hours after administration of the feed additive. No signs of conjunctival hyperaemia, oedema or pathological changes in the iris were observed. By the second day of the experiment, the clinical signs of irritation had completely disappeared, and no pathological changes to the ocular mucous membrane were detected during the subsequent observation period. In rabbit No. 2, the reaction of the mucous membrane was slightly more pronounced. One hour after administration of the feed additive, moderate discharge from the eye was observed, rated at 2 points. After 24 hours, slight discharge, mild hyperaemia and oedema of the conjunctiva were recorded, corresponding to 1 point. On the second day of the study, discharge and hyperaemia were absent, although slight conjunctival oedema persisted. By the third day, all clinical signs of irritation had completely disappeared. The condition of the iris remained unchanged throughout the study period. In rabbit No. 3, only mild discharge from the eye was observed 1 and 24 hours after administration of the preparation, which was assessed as 1 point. No signs of hyperaemia, conjunctival oedema or damage to the iris were detected. From the second day of observation onwards, the ocular mucosa was in a physiological state.

The results obtained indicate that the feed additive under investigation did not cause any pronounced toxic or irritant effects on the mucous membrane of the rabbits' eyes. The clinical changes observed were mild and transient in nature, were not accompanied by damage to the iris or persistent pathological changes, and resolved spontaneously without further intervention. Thus, the results of the studies confirm the good local tolerability of the feed additive and indicate its low level of irritation to the mucous membrane of the rabbits' eyes. Given its low toxicity, the absence of any adverse effects on haematological and biochemical blood parameters, and its good tolerability by laboratory animals, the carrier under investigation may be a promising candidate for the immobilisation of biologically active substances, probiotic cultures and other functional components. The results obtained are consistent with the findings of C. Ferraro *et al*. (2024), who note that the introduction of new protein-based materials into food and biotechnology processes should

be accompanied by a comprehensive toxicological assessment, including the determination of acute toxicity, analysis of haematological and biochemical parameters, as well as studies of biocompatibility and possible local irritant effects. The results of the analysis of haematological parameters in the rabbits' blood indicate that the feed additive under investigation had no adverse effect on the haematopoietic system or the animals' general physiological condition. It was established that the haemoglobin content and erythrocyte count in the blood of all test rabbits were within the physiological range typical of clinically healthy animals (Table 7).

Table 7. *Haematological parameters of the blood of rabbits (n = 3)*

Experimental animal	Haemoglobin concentration, g/L	Erythrocyte count, 10^{12}/dm^3
No. 1	140 ± 2.98	6.4 ± 0.23
No. 2	137 ± 3.22	6.5 ± 0.23
No. 3	144 ± 4.22	7.0 ± 0.28

Source: compiled by the authors

In rabbit No. 1, the haemoglobin concentration was 140 ± 2.98 g/L, and the erythrocyte count was 6.4×10^{12}/dm^3. These results indicate that erythropoiesis is proceeding normally and that the body's tissues are receiving an adequate supply of oxygen. No signs of anaemia or suppression of haematopoietic function were detected. In rabbit No. 2, the haemoglobin concentration was 2.14% lower than that of the first rabbit. Despite a slight decrease in haemoglobin concentration compared with the other animals, the values remained within the range of physiological variation and did not indicate the development of pathological processes or toxic damage to the body. The highest haematological values were recorded in rabbit No. 3. The haemoglobin concentration in its blood was 2.8% higher, and the erythrocyte count was 9.3% higher than those of the first rabbit. The increase in these parameters compared with the other animals did not exceed the physiological norm and was likely due to the individual characteristics of the animal's organism.

The absence of sharp fluctuations in haemoglobin concentration and erythrocyte count indicates that the feed additive under investigation does not exert a pronounced haemotoxic effect and does not adversely affect the functional state of the haematopoietic system. The results also confirm the absence of disturbances in oxygen transport and the stability of the erythrocyte component of the blood in the test animals. Thus, the results of the haematological analyses of the rabbits' blood confirm the good tolerability of the feed additive under investigation and indicate that it is safe with regard to its effect on the haematopoietic system. The results of the study of protein metabolism parameters in rabbit serum indicate the absence of any marked adverse effect of the feed additive under investigation on liver function and protein metabolism in the animals. It was established that the total protein content and aminotransferase activity in all test rabbits were within the physiological normal range (Table 8).

Table 8. *Protein metabolism parameters in the blood serum of rabbits (n = 3)*

Experimental animal	Protein concentration, g/dm^3	AST activity, μmol/h/mL	ALT activity, μmol/h/mL
No. 1	70.1 ± 2.15	1.22 ± 0.45	0.85 ± 0.22
No. 2	65.4 ± 1.67	1.17 ± 0.23	0.91 ± 0.17
No. 3	68.3 ± 3.56	1.20 ± 0.16	0.94 ± 0.21

Source: compiled by the authors

In rabbit No. 1, the total protein concentration in the blood serum was 70.1 g/dm^3, which was the highest value among the laboratory animals. AST activity was 1.22 μmol/h/mL, while ALT activity was 0.85 μmol/h/mL. These results indicate normal protein metabolism and the absence of signs of toxic liver damage. In rabbit No. 2, a lower total protein concentration was established. AST activity was 1.17 μmol/h/mL, while ALT activity was 0.91 μmol/h/mL. Despite the slight decrease in total protein concentration compared with the other animals, the values obtained remained within the physiological range and did not indicate impaired protein-synthetic function of the organism. In rabbit No. 3, the total protein concentration and aminotransferase activity corresponded to physiological norms, indicating a stable functional state of hepatocytes and normal metabolic processes. The absence of a pronounced increase in AST and ALT activity in the blood serum of the rabbits indicates that the feed additive under study did not cause disruption of hepatic cell membrane permeability or the development of pronounced hepatotoxic effects. The slight variation in the parameters between the animals was probably due to individual physiological characteristics. The results of the study of thiol group concentrations in the blood serum of rabbits indicate a stable antioxidant status in the laboratory animals and the absence of pronounced oxidative stress following use of the carrier. It was established that the concentrations of protein-bound, total and low-molecular-weight (free) thiol groups in all rabbits remained within the physiological range (Table 9).

Table 9. *Thiol group content in the blood serum of rabbits, μg/cm^3, (n = 3)*

Experimental animal	Protein-bound	Total	Low-molecular-weight (free)
No. 1	11.3	13.5	2.2
No. 2	10.7	13.2	2.5
No. 3	10.5	12.9	2.4

Source: compiled by the authors

In rabbit No. 1, the concentration of protein-bound thiol groups was 11.3 μg/cm^3, total thiol groups 13.5 μg/cm^3 and low-molecular-weight (free) thiol groups 2.2 μg/cm^3. These values indicate the preservation of normal functioning of the body's antioxidant system and maintenance of cellular redox balance. In rabbit No. 2, the concentrations of protein-bound and total thiol groups were slightly lower than those in the first rabbit. The concentration of low-molecular-weight thiol compounds was the highest among the laboratory animals, at 2.5 μg/cm^3. These changes showed no signs of a pathological nature and were probably associated with individual characteristics of metabolism. Despite a slight decrease in some parameters in rabbit No. 3 compared with the other animals, the values remained stable and corresponded to physiological levels. The minor variations in thiol group parameters between the experimental rabbits did not indicate the development of pronounced oxidative stress or toxic effects of the feed additive under study on the animals. Thus, the results of the study of thiol groups in the blood serum of rabbits confirm the absence of an adverse effect of the feed additive under study on the antioxidant status of the organism and indicate its good tolerability by laboratory animals. The results of the study of glucose concentration in the blood serum of rabbits following exposure to modified dried egg white indicate the absence of an adverse effect of the preparation under study on carbohydrate metabolism in the animals. It was established that glucose concentrations in all experimental rabbits remained within the physiological range, indicating a stable state of energy metabolism in the organism (Fig. 2).

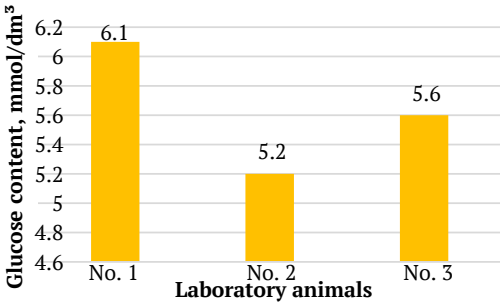

Figure 2. Serum glucose levels in rabbits following administration of modified dried egg white, mmol/dm³

Source: compiled by the authors

No statistically significant differences were found between the animals in terms of their blood serum glucose levels. The results obtained indicate the stability of carbohydrate metabolism in rabbits when a modified dry egg white is used. The absence of sharp changes in glucose concentration indicates that the feed additive under investigation does not have a pronounced toxic effect on the energy metabolism of laboratory animals. Thus, the results of the biochemical studies confirm the good tolerability of modified dried egg white by rabbits and indicate that the product has no adverse effect on carbohydrate metabolism parameters.

The research findings indicate a low level of acute toxicity of the modified dried egg white and its good tolerability by laboratory animals. The absence of fatalities in mice, even when high doses of the preparation of up to 5,000 mg/kg body weight were administered, indicates that the carrier under investigation can be classified as a low-toxicity or virtually non-toxic substance. These results are consistent with data from other researchers, who note that protein carriers of natural origin are characterised by high biocompatibility and low toxicity due to their natural origin and biodegradability (Ferraro *et al*., 2024; Li *et al*., 2024).

The absence of pronounced clinical signs of intoxication in animals when low doses of the preparation were used indicates that the modified dried egg white is well tolerated by mice. Even when high doses were administered, the clinical manifestations observed were short-lived and mild. The temporary suppression of activity and diarrhoea in animals at the maximum dose were likely associated with the body's adaptive response to a significant load of protein components, rather than with the development of toxic damage to organs and systems. Similar data on the safety of protein-based delivery systems are presented in the works of M. Fathi *et al*. (2018) and A. Martínez-López *et al*. (2020), which indicate that protein nanostructures and carriers do not induce pronounced toxic reactions in laboratory animals. The absence of significant changes in haematological parameters confirms that modified dried egg white has no adverse effect on the haematopoietic system. Haemoglobin levels and erythrocyte counts in the mice's blood remained within physiological ranges even when high doses of the preparation were administered. This indicates the absence of haematotoxic effects and disturbances in erythropoiesis. Such results are characteristic of natural protein carriers, which, according to E. Abeyrathne *et al*. (2013), are characterised by high biological compatibility and do not cause significant disturbances to the body's physiological state.

The results of biochemical studies also confirm the absence of any marked toxic effect of modified dried egg white on liver function. Total protein levels and aminotransferase activity in the blood serum of mice remained within the physiological range. A slight dose-dependent increase in AST and ALT activity at the maximum dose was not accompanied by clinical signs of toxicity and was likely of an adaptive nature. It is known that slight fluctuations in transaminase activity may occur in response to an increased metabolic load without the development of structural damage to hepatocytes. Similar conclusions regarding the safety of protein carriers are presented in

the works of H. Cheng *et al.* (2023) and S. Wu *et al.* (2024), which emphasise the stability of the functional state of cells when using protein delivery systems.

An important indicator of the safety of the carrier under investigation is the stability of the antioxidant status in laboratory animals. It was found that the levels of protein-bound, total and low-molecular-weight thiol groups in the blood serum of mice remained stable and did not exceed physiological ranges. The results obtained indicate the absence of pronounced oxidative stress and disturbances in cellular redox balance. A slight increase in the concentration of thiol compounds when high doses of the preparation were used is likely associated with the activation of the body's compensatory antioxidant mechanisms. Similar patterns have been described in the work of J. Żur *et al.* (2016), which notes that immobilised protein systems are capable of maintaining a stable functional state of cells without the development of oxidative damage. The absence of marked changes in serum glucose concentration in mice indicates the stability of carbohydrate metabolism and the body's energy supply when using modified dried egg white. Even when the maximum dose was administered, glucose levels remained within the physiological range, confirming the absence of any adverse effect of the preparation on metabolic processes. This is an important criterion for toxicological safety, as disturbances in carbohydrate metabolism often accompany the development of intoxication and stress responses in the body.

The results of the assessment of the local irritant effect of modified dried egg white on the ocular mucous membrane of rabbits also deserve particular attention. It was established that only mild, transient signs of irritation were observed after administration of the preparation and that these resolved spontaneously during the first days of the study. The absence of damage to the iris, persistent hyperaemia or pronounced conjunctival oedema indicates a low level of local irritation caused by the preparation. The results obtained confirm the good local tolerability of the carrier under study and are consistent with data on the safety of natural protein-based systems reported by B. Ruan *et al*. (2018) and M. Maggonage *et al.* (2024). The results of the studies confirm the potential for using modified dried egg white as a protein carrier in biotechnology and the food industry. Given its low toxicity, the absence of any adverse effects on haematological and biochemical blood parameters, and its good tolerability by laboratory animals, the carrier under investigation may be promising for the immobilisation of biologically active substances, probiotic cultures and other functional components. The results obtained lay the groundwork for further research into the efficacy and functional properties of modified dried egg white within biotechnological systems.

Conclusions

Toxicological studies conducted on modified dried egg white indicate its low toxicity and good tolerability in laboratory animals. It was found that a single intragastric administration of the preparation to white mice at doses ranging from 100 to 5,000 mg/kg body weight did not result in any fatalities. Even at the maximum dose of 5,000 mg/kg, only transient depression and diarrhoea were observed, which resolved spontaneously without further intervention, indicating that the body's response was of an adaptive nature. The results of haematological studies showed that modified dry egg white has no adverse effect on the haematopoietic system of laboratory animals. Following administration of the preparation at a dose of 2,000 mg/kg, the haemoglobin concentration in mice was 132 g/L, and the erythrocyte count was $5.7 \times 10^{12}/dm^3$. In rabbits, the haemoglobin concentration ranged from 137 to 144 g/L, and the erythrocyte count was $6.4\text{-}7.0 \times 10^{12}/dm^3$, which corresponded to physiological norms and confirmed the absence of haemotoxic effects of the preparation.

Biochemical studies revealed that indicators of protein metabolism and liver function remained stable. At a dose of 2,000 mg/kg, the total protein content in the mice's serum was 65.4 g/dm^3, with AST activity at 0.81 μmol/h/mL and ALT activity at 0.92 μmol/h/mL, whereas at the maximum dose of 5,000 mg/kg, these values were 67.3 g/dm^3, 0.93 and 1.04 μmol/h/mL, respectively. The values obtained did not exceed physiological norms and did not indicate toxic damage to hepatocytes. An assessment of antioxidant status showed that the use of modified dried egg white did not induce the development of marked oxidative stress. Following administration of the preparation at a dose of 2,000 mg/kg, the content of protein thiol groups was 14.6 μg/cm^3, total thiol groups at 16.3 μg/cm^3, and low-molecular-weight thiol groups at 1.7 μg/cm^3, indicating that cellular redox balance was maintained and the body's antioxidant system was functioning stably. No adverse effect of the modified dried egg white on carbohydrate metabolism parameters was observed. The serum glucose concentration in the mice was 5.4 mmol/dm^3 at a dose of 2,000 mg/kg and 6.0 mmol/dm^3 at the maximum dose of 5,000 mg/kg, remaining within the physiological range and showing no evidence of impaired energy metabolism.

The results of the local irritation assessment confirmed that the preparation was well tolerated by the ocular mucosa of rabbits. The clinical signs of irritation observed were mild, short-lived and resolved completely within the first day of observation, without damage to the iris or the development of pathological tissue changes. The results obtained confirm the safety of modified dried egg white and justify its potential use as a protein carrier for the immobilisation of biologically active substances and microorganisms in animal husbandry, the food industry and biotechnology. Prospects for further research include investigating the chronic toxicity, allergenic and immunobiological safety of the modified dried egg white; studying the effectiveness of immobilising various biologically active compounds and probiotic microorganisms using it as a carrier; and assessing the stability, bioavailability and functional efficacy of the developed systems under conditions of practical application in animal husbandry, the food industry and biotechnology.

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Conflict of Interest

None.

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Встановлення токсичності модифікованого сухого яєчного білка

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Анотація. Актуальність роботи зумовлена необхідністю створення безпечних та функціонально ефективних білкових носіїв для іммобілізації біологічно активних речовин і мікроорганізмів, перспективних для використання у тваринництві, харчовій промисловості та біотехнології. Метою дослідження було оцінити токсикологічні властивості модифікованого сухого яєчного білка шляхом визначення його гострої токсичності, впливу на гематологічні, біохімічні та антиоксидантні показники крові лабораторних тварин, а також подразнювальної дії на слизову оболонку ока. У роботі використано токсикологічні, клінічні, гематологічні, біохімічні та статистичні методи дослідження. Було досліджено гостру токсичність модифікованого сухого яєчного білка за внутрішньошлункового введення білим мишам у дозах від 100 до 5000 мг/кг маси тіла та оцінено його подразнювальну дію на слизову оболонку ока кролів каліфорнійської породи. Встановлено, що навіть за максимальної дози 5000 мг/кг летальних випадків не спостерігали, а короткочасні прояви пригнічення та діареї були транзиторними й повністю зникали без додаткового втручання. Проаналізовано гематологічні, біохімічні та антиоксидантні показники крові, які залишалися в межах фізіологічної норми: за дози 2000 мг/кг вміст гемоглобіну становив 132 г/л, кількість еритроцитів – $5,7 \times 10^{12}$/дм^3, загального білка – 65,4 г/дм^3, концентрація глюкози – 5,4 ммоль/дм^3, загальних тіолових груп – 16,3 мкг/см^3, тоді як за максимальної дози 5000 мг/кг вміст загального білка досягав 67,3 г/дм^3, активність АсАт – 0,93 мкмоль/год/мл, АлАт – 1,04 мкмоль/год/мл, а концентрація глюкози – 6,0 ммоль/дм^3, не виходячи за межі фізіологічної норми. Під час оцінки подразнювальної дії на слизову оболонку ока кролів було виявлено лише слабко виражені транзиторні клінічні прояви без ушкодження райдужної оболонки, при цьому гематологічні, біохімічні та антиоксидантні показники також відповідали фізіологічним значенням, що підтверджувало відсутність гемотоксичної, гепатотоксичної, метаболічної та вираженої оксидативної дії модифікованого сухого яєчного білка. Практична цінність роботи полягає у можливості використання отриманих результатів для розроблення та впровадження безпечних білкових носіїв біологічно активних речовин і мікроорганізмів у тваринництві, харчовій промисловості, біотехнології та наукових дослідженнях

Ключові слова: білковий носій; іммобілізація; гематологічні показники; біохімічні показники; лабораторні тварини; біотехнологія

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Determination of the acute toxicity and irritant effects of a stabilised coenzyme-containing feed additive

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Abstract. The relevance of this study stems from the need to develop and provide toxicological justification for safe feed additives with antioxidant properties for use in animal husbandry. The aim of the study was to determine the acute toxicity and irritant effects of a stabilised coenzyme-containing feed additive supplemented with α-lipoic acid. The acute toxicity of the feed additive was investigated in Wistar rats following intragastric administration at doses ranging from 100 to 6,000 mg/kg, with a 14-day observation period. The irritant effect was evaluated in rabbits by instilling a suspension into the conjunctival sac of the eye, followed by the assessment of the main haematological and biochemical blood parameters. The results demonstrated the absence of mortality in rats administered the feed additive at doses up to 6,000 mg/kg body weight. The LD_{50} value exceeded 5,000 mg/kg, allowing the tested additive to be classified as a slightly toxic or practically non-toxic substance. At the highest doses, some animals exhibited transient gastrointestinal disturbances and mild depression, which resolved spontaneously without intervention. Haematological and biochemical blood parameters remained within the physiological reference range. No evidence of pronounced haemotoxic, hepatotoxic, or nephrotoxic effects was

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observed. Sulphydryl group levels indicated preservation of the antioxidant status of the organism. Evaluation of the irritant effect in rabbits revealed only slight transient ocular discharge and mild oedema of the conjunctival mucosa, both of which completely resolved by the second day of the study. No signs of hyperaemia or prolonged irritation were detected. The findings confirm the safety of the stabilised coenzyme-containing feed additive supplemented with α-lipoic acid and support its potential for further application in animal husbandry. The obtained results may be used to substantiate the safety and facilitate the introduction of the stabilised coenzyme-containing feed additive with α-lipoic acid into animal husbandry practice, as well as in the development of new functional feed products with antioxidant properties

Keywords: α-lipoic acid; laboratory animals; haemotoxicity; hepatotoxicity; sulphydryl groups; biochemical blood parameters; toxicological assessment; carbohydrate metabolism

Introduction

The intensive development of modern animal husbandry has been accompanied by an increasing demand for safe feed additives capable of supporting metabolic processes, enhancing the resistance of animals to adverse factors, and reducing the detrimental effects of oxidative stress (Hamlin *et al*., 2021). Particular attention has been directed towards biologically active compounds with antioxidant properties, among which α-lipoic acid occupies a prominent position. This naturally occurring sulphur-containing compound participates in the function of mitochondrial enzyme complexes and cellular energy metabolism. According to F. Superti & R. Russo (2024), α-lipoic acid exhibits pronounced antioxidant activity, influences redox processes, and contributes to the regulation of metabolic homeostasis.

Recent studies have identified α-lipoic acid as a compound capable of reducing oxidative stress and protecting cellular structures against damage induced by reactive oxygen species. F. Mozaffarian *et al*. (2022) demonstrated that α-lipoic acid exerted a protective effect against arsenic-induced oxidative stress in isolated rat liver mitochondria, highlighting its important role in maintaining mitochondrial function and protecting cells against the toxic effects of xenobiotics. Similarly, M. Nema *et al*. (2024) reported that α-lipoic acid alleviated the manifestations of acute deltamethrin toxicity in rats by improving biochemical parameters and reducing oxidative stress. The relevance of investigating α-lipoic acid as a component of feed additives is attributable to its potential to maintain the antioxidant status of the organism. In animal husbandry, antioxidant compounds play an important role in preserving the physiological condition of animals, particularly under the influence of technological, nutritional, and environmental stressors (Doboşi *et al*., 2022). O.E. Oke *et al*. (2024) emphasised that dietary modulation of the antioxidant defence system represents an important strategy for maintaining productivity, immune competence, and the health of poultry, especially under technological and environmental stress conditions.

The importance of α-lipoic acid in animal nutrition has also been highlighted by R.M. Anthony *et al*. (2021), who reviewed current evidence regarding its application as a nutraceutical feed supplement for dogs. The authors concluded that α-lipoic acid possesses high biological activity, participates in cellular energy metabolism, and contributes to the maintenance of antioxidant status in animals. These findings support the potential use of α-lipoic acid as a component of feed additives for various animal species. Nevertheless, the inclusion of biologically active substances in

feed additives requires comprehensive toxicological evaluation. Even naturally occurring compounds may induce functional alterations when administered at high doses or under specific conditions of use. C.M. Zwickl *et al.* (2022) emphasised that acute toxicity assessment is an essential stage in determining the hazard profile of chemical substances and that modern toxicological evaluation should integrate both experimental and predictive approaches. Consequently, the determination of the acute toxicity of feed additives is a necessary prerequisite for their practical application.

Particular importance should also be attached to evaluating the effects of the investigated preparations on haematological and biochemical blood parameters, as these reflect the functional status of the liver, kidneys, haematopoietic system, and protein metabolism. Accurate interpretation of these parameters in laboratory animals requires consideration of species-, age-, and sex-related differences. Recent studies have shown that the establishment of species-specific reference intervals is essential for the correct assessment of the physiological status of rabbits and for the interpretation of toxicological findings (Strong-Townsend *et al.*, 2024). Clinical investigations have likewise reinforced the scientific interest in α-lipoic acid as an antioxidant compound. A. Divković *et al.* (2023) evaluated the effects of α-lipoic acid on oxidative stress and lipid metabolism in women with low-grade cervical intraepithelial lesions. Although this clinical study was conducted in humans and is not directly related to feed additives, it further supports the scientific interest in α-lipoic acid as a biologically active compound with potential antioxidant properties.

Nevertheless, information regarding the toxicological characteristics of stabilised coenzyme-containing feed additives based on α-lipoic acid remains limited. In particular, insufficient data are available on their acute toxicity, effects on the clinical condition of animals, haematological, biochemical, and antioxidant blood parameters, as well as their potential irritant effects on mucous membranes. Thus, a comprehensive evaluation of the acute toxicity and local irritant effects of such a feed additive is of considerable importance for both toxicology and animal nutrition. An essential aspect of the safety assessment of novel feed additives is the investigation of their effects on the oxidative status of the organism. It is well established that disruption of the balance between the generation of reactive oxygen species and the activity of the antioxidant defence system may result in damage to cellular membranes, proteins, and nucleic acids. According to A. Divković *et al.* (2023), administration of α-lipoic acid was associated with improved antioxidant defence and a reduction in oxidative stress, further confirming its biological activity and potential for wider application. The authors also reported that α-lipoic acid is capable of modulating lipid peroxidation and maintaining the cellular redox balance.

Furthermore, contemporary toxicological studies emphasise the importance of evaluating the effects of biologically active substances not only on the clinical condition of animals but also on biochemical blood parameters, which serve as sensitive indicators of the functional status of the organism. Consequently, the determination of total protein concentration, transaminase activities, and indicators of nitrogen and carbohydrate metabolism constitutes an important component of the toxicological characterisation of novel feed additives. Such investigations provide a more objective assessment of the safety of these preparations and facilitate the prediction of potential risks associated with their practical application in animal husbandry. A new feed additive containing stabilised α-lipoic acid has been developed at the Research Institute of Food Technologies and Animal Husbandry of Bila Tserkva National Agrarian University. In accordance with current regulatory requirements, the introduction of new feed additives requires evaluation oftheir

toxic effects on animals (EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) *et al*., 2017).

The aim of the present study was to determine the level of acute toxicity and assess the irritant potential of a stabilised coenzyme-containing feed additive based on α-lipoic acid. The scientific novelty of the study lies in the comprehensive evaluation of the toxicological properties of this stabilised coenzyme-containing feed additive, including its acute toxicity, local irritant effects, effects on the clinical condition of laboratory animals, and its influence on haematological and biochemical blood parameters.

Materials and Methods

Preclinical studies evaluating the toxicity of the stabilised coenzyme-containing feed additive were conducted in the vivarium and laboratories of the Institute of Animal Husbandry and Food Technologies at Bila Tserkva National Agrarian University during 2024-2025. The acute toxicity of the stabilised coenzyme-containing feed additive supplemented with α-lipoic acid was investigated using 11-week-old female Wistar rats (non-pregnant) with a body weight of 192.0-197.0 g. The irritant effects of the feed additive were evaluated in male California rabbits weighing 2.6-2.7 kg. During the experiments, the air temperature in the quarantine and procedure rooms for laboratory animals was maintained at 17-18 °C, relative humidity at 57-64%, and illumination at cage level ranged from 305 to 320 lux.

The acute toxicity study of the stabilised coenzyme-containing feed additive supplemented with α-lipoic acid consisted of two stages: a preliminary experiment involving three groups of animals and an extended experiment involving five groups. Each group comprised five rats. Animals were allocated to experimental groups by random selection. Before the experiments, all laboratory animals underwent a quarantine period in accordance with the relevant requirements. With unrestricted access to fresh drinking water, the animals were fasted for 18 hours prior to the start of the experiment. Each rat was weighed individually before treatment. A suspension of the feed additive containing α-lipoic acid was administered as a single intragastric dose, either as a single administration or divided into fractions. The suspension was prepared using a freshly prepared 1% starch solution, with a final concentration of the active substance of 30 mg/cm^3. A stainless-steel gastric gavage tube was used for intragastric administration. The acute toxicity study was performed in two consecutive stages.

During the first stage (the preliminary experiment), the rats received the feed additive intragastrically at doses of 100, 500, and 5,000 mg/kg body weight. Progression to the second stage of the study was permitted only in the absence of mortality and pronounced clinical signs of toxicity, including prolonged depression, convulsions, impaired motor coordination, or persistent gastrointestinal dysfunction, within 24-48 hours after administration of the highest dose. Under these conditions, the maximum dose was considered sufficiently safe for the extended study, in which doses of 2,000, 3,000, 4,000, 5,000, and 6,000 mg/kg body weight were evaluated. The rats were observed for 14 days following intragastric administration of the feed additive. Throughout the observation period, the animals were examined daily for general clinical condition, locomotor activity, behavioural responses, motor coordination, responsiveness to external stimuli, feed and water consumption, coat condition, respiratory rate, the occurrence of diarrhoea, convulsions, paresis, and any other signs of intoxication. Body weight was recorded immediately before treatment and again at the end of the observation period. During the first 24 hours, the animals were monitored continuously, after which observations were conducted at 8-hour intervals. Standard compound feed was returned to the cages 6 hours after administration of the feed additive, while fresh drinking water was available throughout the study.

The assessment of the irritant effects of the feed additive suspension was performed using four clinically healthy rabbits that had completed the quarantine period. Two drops of the suspension were instilled into the conjunctival sac of the left eye of each animal using sterile pipettes. Following instillation, the nasolacrimal duct of each rabbit was occluded for 85-90 seconds. The right eye of each rabbit served as the untreated control. Ocular irritation was evaluated one hour after instillation based on the severity of ocular changes, including conjunctival hyperaemia, oedema (chemosis), ocular discharge, and corneal lesions. Each parameter was scored according to the recommendations of OECD Test Guideline 405 (OECD, 2023): 0 = no changes; 1 = slight changes; 2 = moderate changes; and 3-4 = marked or severe changes, depending on the parameter assessed. Ocular examinations were subsequently performed once daily for the following 13 days.

The acute toxicity of the stabilised coenzyme-containing feed additive supplemented with α-lipoic acid was determined in accordance with OECD Test Guideline 423, Acute Oral Toxicity – Acute Toxic Class Method (OECD, 2002). On Day 14 of the experiment, blood samples were collected from the animals for haematological and biochemical analyses. Blood sampling was carried out in the morning (08:00-10:00). In rats, blood was collected from the tail vein, whereas in rabbits it was obtained from the marginal ear vein. Between 2 and 4 cm^3 of blood was collected into sterile tubes. Following centrifugation, the serum was separated and used for subsequent biochemical analyses. All determinations were performed using standard reagent kits and laboratory equipment according to the manufacturers' protocols.

The total protein content in the animals' blood serum was determined using the method of O.H. Lowry *et al*. (1951). The content of protein, low-molecular-weight and total thiol groups in the blood serum was determined using the method of G.L. Ellman (1959). The urea content in blood serum was determined by the enzymatic urease method using the Humalyzer biochemical analyser, BioSystems, Cobas. Glucose levels were determined using the glucose oxidase-peroxidase method of R. Trinder (1969), whilst the activity of aminotransferases in the blood serum of the animals was investigated according to the method of S. Reitman & S. Frankel (1957). The haemoglobin content in the blood of rats and rabbits was determined by spectrophotometry in accordance with the recommendations of the Clinical and Laboratory Standards Institute (CLSI), specifically document H15, which specifies the cyanmethaemoglobin reference method as the standard for the quantitative determination of haemoglobin in blood. The method is based on the conversion of all forms of haemoglobin into stable cyanmethaemoglobin, followed by photometric measurement of the solution's optical density at a wavelength of 540 nm. Calibration and quality control were carried out in accordance with the requirements of CLSI C46 (CLSI, 2009), which ensures the standardisation of measurements and the reproducibility of results in clinical laboratory studies.

All experimental studies were conducted in accordance with current methodological approaches and in compliance with the relevant requirements and standards. Animal husbandry and all procedures were carried out in accordance with the provisions of the Regulations on the Conduct of Animal Experiments by Scientific Institutions (Law of Ukraine No. 249, 2012) and the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (1986).

Statistical analysis of the research results was carried out using Microsoft Excel 2021 and Statistica 10.0 software. The arithmetic mean (M) and the standard error of the mean (m) were determined. The significance of differences between indicators was assessed using the Student's t-test. A difference was considered statistically significant at $p < 0.05$.

Results and Discussion

The study was conducted in two stages – a preliminary experiment and a full-scale experiment – which allowed the potential toxicity of the feed additive to be assessed across a wide range of concentrations. According to the preliminary experiment, it was found that the administration of the feed additive at doses of 100 and 500 mg/kg body weight did not result in mortality among the experimental animals (Table 1).

Table 1. *Observation data for rats during the determination of the acute toxicity of the α-lipoic acid feed additive (preliminary experiment, n = 15)*

Number of rats in the group	Dose, mg/kg body weight	Number of rats that died		
		total	Expressed as %	Time of death recorded
5	100	0	0	-
5	500	0	0	-
5	5,000	0	0	-

Source: compiled by the authors

When rats were fed a dietary supplement at a dose of 100 t 500 mg/kg body weight, their behaviour was consistent with physiological norms. After the feed was placed in the cages, the animals consumed the feed and drank water periodically. Their response to external stimuli was appropriate. No disturbances in gastrointestinal function were observed.

Throughout the entire observation period, no clinical signs of intoxication, behavioural abnormalities or changes in physiological status were recorded in the rats. Nor was any time of death recorded for the animals, confirming the absence of acute toxic effects of the preparation even at the maximum dose used in the preliminary experiment – 5,000 mg/kg body weight. Gastrointestinal disturbances were observed in these rats during the first 24 hours of the study. Feed intake returned to normal on the second day of the experiment. The results of this preliminary experiment formed the basis for a full-scale study using even higher doses of the feed additive. In the full-scale experiment, α-lipoic acid was administered intragastrically to rats at doses of 2,000, 3,000, 4,000, 5,000 and 6,000 mg/kg body weight (Table 2).

Table 2. *Observation data for rats during the determination of the acute toxicity of the α-lipoic acid feed additive, (extensive experiment n = 25)*

Number of rats in the group	Dose, mg/kg body weight	Number of rats that died		
		total	Expressed as %	Time of death recorded
5	2,000	0	0	-
5	3,000	0	0	-
5	4,000	0	0	-
5	5,000	0	0	-
5	6,000	0	0	-

Source: compiled by the authors

The observational findings demonstrated that none of the rats died following administration of the investigated feed additive, irrespective of the dose administered. The mortality rate was 0% in all experimental groups, and no deaths were recorded throughout the observation period. These findings indicate that even the highest dose of 6,000 mg/kg body weight did not induce acute toxic effects in the laboratory animals. No behavioural or ethological

changes were observed in rats receiving doses of 2,000-3,000 mg/kg during the 14-day observation period. Clinical examination following administration of the feed additive revealed no alterations in behavioural responses, motor coordination, coat condition, respiration, or feed and water consumption. The animals remained active, responded appropriately to external stimuli, and maintained normal locomotor activity. The absence of central nervous system depression, convulsions, paresis, diarrhoea, or other signs of intoxication indicates good tolerability of the feed additive in rats. Gastrointestinal disturbances were observed in three animals that received 4,000 mg/kg of the feed additive. All rats administered doses of 5,000 or 6,000 mg/kg developed diarrhoea accompanied by mild depression during the first 24 hours of the experiment.

The absence of mortality in all experimental groups indicates that the median lethal dose (LD_{50}) of the investigated feed additive exceeds 5,000 mg/kg body weight. According to widely accepted toxicological classifications, these findings indicate that the preparation has a low level of toxicity and may be classified as a slightly toxic or practically non-toxic substance. Thus, the results of both the preliminary and extended experiments confirm the safety of the α-lipoic acid-based feed additive following a single intragastric administration to albino rats. The findings demonstrate the absence of acute toxic effects, which represents an important criterion in the safety assessment of feed additives and supports their potential for future application in animal husbandry. Following administration of the feed additive at a dose of 2,000 mg/kg body weight, the haemoglobin concentration in the blood of the rats was 138 g/L, which was within the physiological reference range. Administration of the feed additive at a dose of 3,000 mg/kg body weight did not result in a reduction in haemoglobin concentration compared with the animals receiving 2,000 mg/kg (Fig. 1).

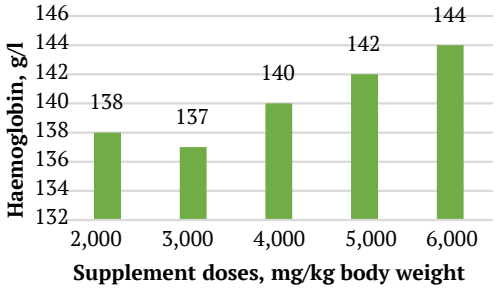

Figure 1. Haemoglobin concentration in the blood of rats administered different doses of the coenzyme-containing feed additive, n = 25
Source: compiled by the authors

Following administration of the feed additive at a dose of 4,000 mg/kg body weight, the haemoglobin concentration in the blood of the rats remained within the physiological reference range. The highest dose of the feed additive (6,000 mg/kg body weight) resulted in a 4.3% increase in blood haemoglobin concentration compared with the group receiving the lowest dose in the extended experiment. Overall, haemoglobin concentrations remained within the physiological range, indicating the absence of suppression of erythropoiesis, anaemic changes, or pronounced haemotoxic effects of the additive. The slight increase in haemoglobin observed at doses of 5,000-6,000 mg/kg may be regarded as a physiological variation rather than evidence of a toxic effect.

The indicators of protein metabolism likewise showed no signs of marked toxic injury. The total protein concentration increased gradually with increasing doses of the feed additive administered intragastrically to the rats. At a dose of 2,000 mg/kg body weight, the total protein concentration in the blood serum was 62.3 g/dm^3. Administration of 3,000 mg/kg resulted in a 2.0% increase in total protein concentration compared with the 2,000 mg/kg group; however, the difference was within the margin of experimental error. No statistically

significant increase in serum total protein concentration was observed in rats receiving 4,000 mg/kg of the feed additive. A tendency towards increased blood total protein concentration was recorded in animals administered 5,000 mg/kg of the feed additive (Table 3).

Table 3. *Protein metabolism parameters in the blood serum of rats during the determination of the acute toxicity of the coenzyme-containing feed additive (α-lipoic acid), n = 25*

Administered dose of the feed additive, mg/kg body weight	Total protein concentration, g/dm³	AST activity, μmol/h/mL	ALT activity, μmol/h/mL	Urea concentration, mmol/dm³
2,000	62.3 ± 2.31	1.02 ± 0.078	0.81 ± 0.0	4.8 ± 0.18
3,000	63.3 ± 1.43	1.05 ± 0.065	0.82 ± 0.0	4.7 ± 0.07
4,000	65.2 ± 1.56	1.12 ± 0.056	0.84 ± 0.0	5.0 ± 0.24
5,000	70.2 ± 2.45	1.21 ± 0.088	0.92 ± 0.0	5.1 ± 0.21
6,000	70.6 ± 2.65*	1.20 ± 0.094	0.93 ± 0.0	5.3 ± 0.28

Note: * refers to the lowest dose of the feed additive

Source: developed by the authors

At the dose of 6,000 mg/kg body weight, a statistically significant increase in serum total protein concentration was observed compared with rats receiving 2,000 mg/kg. However, the increase remained within the physiological reference range and should not be regarded as an adverse finding, as it was not accompanied by marked elevations in liver enzyme activities or serum urea concentration. AST activity in the serum of rats receiving the lowest dose of the feed additive was within the physiological range. Increasing the dose to 3,000 mg/kg body weight produced virtually no change in AST activity. Administration of the feed additive at doses of 5,000 and 6,000 mg/kg body weight resulted in a tendency towards increased AST activity compared with rats receiving 2,000 mg/kg. A dose-dependent trend was also observed for ALT activity, with higher doses of the feed additive being associated with increased serum ALT activity. Nevertheless, these increases remained within the physiological reference range and were not statistically significant.

This moderate increase in enzyme activity at the highest doses may reflect a degree of metabolic loading on the liver; however, it does not indicate pronounced hepatotoxicity, as the observed changes were minor and showed only a moderate dose-dependent pattern. Serum urea concentrations ranged from 4.7 to 5.3 mmol/dm³, indicating no substantial impairment of protein catabolism or renal excretory function. The slight increase in urea concentration observed at the dose of 6,000 mg/kg was not statistically significant compared with animals receiving the lowest dose of the feed additive and may have been associated with increased protein metabolism rather than representing an acute toxic response.

Serum sulphydryl group concentrations remained stable throughout the study. A dose-dependent increase in the concentration of total sulphydryl groups was observed with increasing doses of the feed additive. Administration of the feed additive at doses of 5,000 and 6,000 mg/kg body weight resulted in increases in total thiol group concentrations of 6.4% and 7.1%, respectively, compared with rats receiving 2,000 mg/kg. The differences between the groups represented a trend rather than a statistically significant effect (Table 4).

Table 4. *Sulphydryl group concentrations in the blood serum of rats, μg/cm3, n = 25*

Administered dose of the feed additive, mg/kg body weight	Thiol groups		
	total	protein-bound	free
2,000	15.4 ± 0.58	13.1 ± 0.36	2.3 ± 0.05
3,000	15.6 ± 0.36	13.5 ± 0.13	2.1 ± 0.03
4,000	16.1 ± 0.42	13.7 ± 0.23	2.4 ± 0.04
5,000	16.4 ± 0.28	14.0 ± 0.34	2.4 ± 0.03
6,000	16.5 ± 0.31	14.2 ± 0.38	2.3 ± 0.02

Source: compiled by the authors

Evaluation of the concentration of protein-bound thiol groups in the blood serum of the rats demonstrated a gradual increase with increasing doses of the feed additive. However, no statistically significant differences in sulphydryl (SH) group concentrations were observed between the experimental groups. The concentration of free sulphydryl groups ranged from 2.1 to 2.4 μg/cm^3 and remained essentially unchanged throughout the study. These findings suggest preservation of the antioxidant capacity of the blood serum and the absence of pronounced oxidative stress.

Blood glucose concentration decreased progressively with increasing doses of the feed additive. Following administration of the feed additive at a dose of 2,000 mg/kg body weight, the serum glucose concentration was 6.5 mmol/dm^3. Increasing the dose to 3,000 mg/kg resulted in a 1.5% reduction in serum glucose concentration compared with the group receiving 2,000 mg/kg. In rats administered 4,000 and 5,000 mg/kg of the feed additive, serum glucose concentrations decreased by 9.2% and 12.3%, respectively. Compared with the group receiving the lowest dose, these differences represented a trend rather than a statistically significant effect. A similar trend towards reduced serum glucose concentration was observed in rats receiving the highest dose of the feed additive. Nevertheless, glucose concentrations remained within the physiological reference range at all dose levels (Fig. 2). This trend may be attributable to the metabolic effects of α-lipoic acid, which is known to participate in the regulation of carbohydrate metabolism. As the reduction in glucose concentration was modest, it does not indicate the occurrence of an acute toxic response.

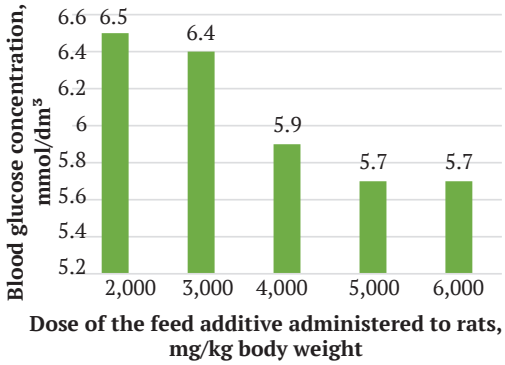

Figure 2. Blood glucose concentration in rats administered different doses of the coenzyme-containing feed additive supplemented with α-lipoic acid, n = 25

Source: compiled by the authors

The findings indicate that administration of the coenzyme-containing feed additive supplemented with α-lipoic acid to rats at doses of 2,000-6,000 mg/kg body weight did not result in marked pathological alterations in haematological or biochemical blood parameters. Thus, administration of the coenzyme-containing feed additive at doses of up to 6,000 mg/kg body weight did not induce pronounced adverse changes in the blood of the rats. These findings are consistent with those reported by R.M. Anthony *et al.* (2021), who described the

good tolerability of α-lipoic acid in animals and the absence of adverse effects on the principal physiological parameters when it is used as a component of feed additives. The evaluated haematological, protein metabolism, enzymatic, antioxidant, and carbohydrate metabolism parameters all remained within the physiological reference range. Collectively, these results indicate that, under conditions of acute administration, the investigated feed additive does not exhibit pronounced haemotoxic, hepatotoxic, or nephrotoxic effects.

Given the absence of evidence of systemic toxicity in the acute toxicity study in rats, the next stage of the investigation focused on assessing the local irritant effects of the coenzyme-containing feed additive. For this purpose, an experiment was conducted in rabbits, which are a widely accepted model for evaluating the local safety of novel feed additives and veterinary medicinal products. Following instillation of the investigated feed additive, all animals exhibited slight ocular discharge during the first day, which was assigned a score of 1 (Table 5).

Table 5. *Harmful effects of the feed additive on the ocular mucosa of rabbits, expressed as scores (n = 4)*

Effect of the feed additive	Eye examination during the experimental period					
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 14
			Rabbit I			
Discharge	1	0	0	0	0	0
Oedema	0	0	0	0	0	0
Hyperaemia	0	0	0	0	0	0
			Rabbit II			
Discharge	1	0	0	0	0	0
Oedema	1	0	0	0	0	0
Hyperaemia	0	0	0	0	0	0
			Rabbit III			
Discharge	1	0	0	0	0	0
Oedema	0	0	0	0	0	0
Hyperaemia	0	0	0	0	0	0
			Rabbit IV			
Discharge	1	0	0	0	0	0
Oedema	0	0	0	0	0	0
Hyperaemia	0	0	0	0	0	0

Source: compiled by the authors

A slight oedema of the conjunctival mucosa, corresponding to a score of 1, was also observed in one rabbit. No signs of hyperaemia were detected in any animal throughout the study period. By the second day of the experiment, all clinical signs of irritation had completely resolved. During the subsequent observation period – on Days 3, 4, 5, and 14 – no pathological changes of the ocular mucosa were observed. No ocular discharge, oedema, or redness was detected, indicating complete tissue recovery and the absence of prolonged irritant effects of the feed additive. The rabbits exhibited normal physiological behaviour throughout the study. Feed and water consumption resumed on the first day of the experiment after the animals had settled. The rabbits responded appropriately to sound, light, and tactile stimuli, and their respiratory rate remained within the normal physiological range while at rest.

These findings indicate that the α-lipoic acid-containing feed additive does not exert a pronounced irritant effect on the ocular mucosa of rabbits. The transient changes observed, consisting of slight ocular discharge and mild oedema, resolved spontaneously without intervention. Accordingly, the investigated feed additive may be regarded as safe with respect to its local effects on mucous membranes. On Day 14 of the experiment, haemoglobin concentration was determined in the blood of the rabbits. The highest value was recorded in Rabbit I (153 g/L), whereas the lowest was observed in Rabbit II (137 g/L). These findings indicate normal haematopoiesis and an adequate capacity for oxygen transport to the tissues (Fig. 3).

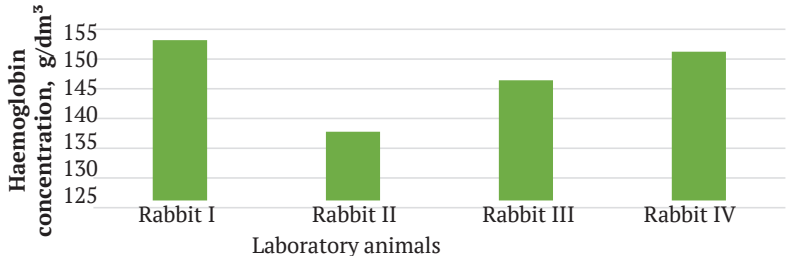

Figure 3. Haemoglobin concentration in the blood of rabbits following exposure to the irritant effects of the coenzyme-containing feed additive (n = 4)

Source: compiled by the authors

The absence of marked fluctuations in haemoglobin concentration confirms that the feed additive did not suppress erythropoiesis or induce the development of anaemic conditions. Analysis of the protein metabolism parameters showed that the highest total protein concentration was recorded in Rabbit I, with values remaining within the physiological reference range. The lowest value was observed in Rabbit IV (52.9 ± 2.32 g/dm³). The difference in total protein concentration between these animals was 7.5% and was not statistically significant (Table 6). These findings indicate a stable protein-synthesising function and the absence of disturbances in metabolic processes.

Table 6. *Protein metabolism parameters in the blood serum of rabbits during the assessment of the irritant effects of the coenzyme-containing feed additive supplemented with α-lipoic acid (n = 4)*

Laboratory animal	Total protein concentration, g/dm³	AST activity, μmol/h/cm³	ALT activity, μmol/h/cm³	Urea concentration, mmol/dm³
Rabbit I	57.2 ± 1.98	0.96 ± 0.054	0.86 ± 0.032	5.3 ± 0.23
Rabbit II	53.2 ± 2.24	1.05 ± 0.043	0.91 ± 0.025	5.4 ± 0.29
Rabbit III	56.3 ± 3.11	1.07 ± 0.054	0.91 ± 0.027	5.6 ± 0.19
Rabbit IV	52.9 ± 2.32	0.95 ± 0.013	0.88 ± 0.036	5.2 ± 0.07

Source: compiled by the authors

The activities of the enzymes AST and ALT also remained stable. AST activity ranged from 0.95 to 1.07 μmol/h/cm³, while ALT activity ranged from 0.86 to 0.91 μmol/h/cm³. The absence of increased transaminase activity indicates that the feed additive did not induce hepatotoxicity or impair the functional integrity of hepatocyte cell membranes. The serum urea concentration in the rabbits ranged from 5.2 to 5.6 mmol/dm³, corresponding to physiological

reference values and indicating normal protein metabolism and the absence of renal dysfunction. The highest value was recorded in Rabbit III (5.6 ± 0.19 mmol/dm³); however, it remained within the physiological range, and the difference from the lowest value was not statistically significant. The concentration of sulphydryl (thiol) groups in the blood serum was likewise stable. Total thiol group concentrations ranged from 16.2 to 17.1 μg/cm³. The difference between the maximum and minimum values was 5.5% and was not statistically significant (Table 7).

Table 7. *Sulphydryl group concentrations in the blood serum of rabbits, μg/cm³ (n = 4)*

Laboratory animal	Thiol groups		
	total	protein-bound	free
Rabbit I	17.1 ± 0.56	15.3 ± 0.43	1.8 ± 0.16
Rabbit II	16.9 ± 0.78	15.0 ± 0.39	1.9 ± 0.14
Rabbit III	16.2 ± 0.73	14.7 ± 0.29	1.5 ± 0.12
Rabbit IV	17.0 ± 0.43	15.1 ± 0.22	1.9 ± 0.09

Source: compiled by the authors

The mean concentration of protein-bound sulphydryl groups in the rabbits was 15.02 μg/cm³. Deviations from the mean value did not exceed 2.5%. The concentration of free thiol groups was within the physiological reference range. These findings indicate preservation of the antioxidant status of the organism and the absence of pronounced oxidative stress following exposure to α-lipoic acid. Blood glucose concentrations in the rabbits ranged from 5.9 to 6.5 mmol/dm³. These values reflect a stable state of carbohydrate metabolism and indicate that the feed additive had no adverse effect on the energy metabolism of the animals (Fig. 4)

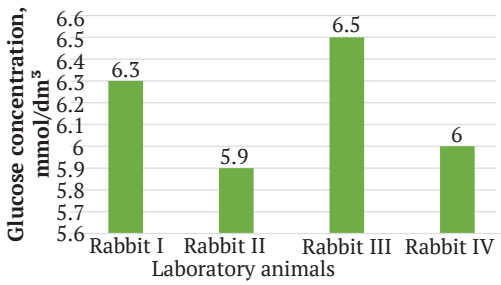

Figure 4. Blood glucose concentration in rabbits during the assessment of the irritant effects of the coenzyme-containing feed additive supplemented with α-lipoic acid (n = 4)

Source: compiled by the authors

The results of the haematological and biochemical analyses of rabbit blood performed during the assessment of the irritant effects of the coenzyme-containing feed additive supplemented with α-lipoic acid indicate that the investigated preparation had no adverse effect on the general physiological condition of the animals. The parameters measured were within the physiological normal range for rabbits and did not indicate the development of pathological processes or toxic injury to the organism. The results of the study into the irritant effect of a feed supplement containing α-lipoic acid on the mucous membrane of the rabbits' eyes indicate a low level of local toxicity. Thus, the results of biochemical and haematological studies confirm that the coenzyme-containing feed supplement with α-lipoic acid does not exert a toxic or pronounced irritant effect on the rabbits' bodies.

The results obtained indicate a low level of acute toxicity of the stabilised coenzyme-containing feed supplement with α-lipoic acid. The absence of fatalities in rats, even when administered the maximum dose of 6,000 mg/kg body weight, indicates good tolerability of the preparation by laboratory animals and is consistent with current understanding of the safety of α-lipoic acid. According to the data

from C.M. Zwickl *et al.* (2022), the assessment of acute toxicity is one of the key stages in the toxicological evaluation of new biologically active substances, and the absence of mortality at high doses indicates a low level of toxic risk associated with the test substance. These findings are consistent with the studies by E.J. Calabrese *et al.* (2023), which showed that α-lipoic acid is capable of reducing the toxic effects of deltamethrin in rats and helping to stabilise biochemical blood parameters. The authors attribute this to the antioxidant properties of α-lipoic acid and its ability to maintain the functional integrity of cell membranes. Similar results were also obtained by S. Rezaei Zonooz *et al*. (2021) and B. Skibska *et al.* (2023), who noted the protective effect of α-lipoic acid under conditions of oxidative stress induced by cypermethrin. The absence of marked changes in haematological parameters in the studies conducted indicates that the feed additive does not have a negative effect on the haematopoietic system. Haemoglobin levels in the blood of rats and rabbits remained within the physiological range, and minor fluctuations in these parameters showed no signs of haemotoxic effects. These results are consistent with the findings of M. Nema *et al*. (2024) and F. Superti & R. Russo (2024), which indicate that α-lipoic acid helps to normalise haematological parameters and mitigates oxidative stress-induced blood disorders caused by toxic agents.

The parameters of protein metabolism and the levels of AST and ALT in the blood serum of rats also did not indicate the development of any pronounced hepatotoxic effects. A slight increase in transaminase activity following the administration of high doses of the supplement was dose-dependent in nature, but did not exceed the limits of physiological variation. This is consistent with the findings of E.M. El-Mancy *et al*. (2022), who demonstrated that α-lipoic acid exhibits hepatoprotective properties and reduces the severity of oxidative stress and apoptosis in the livers of rats following exposure to cyclosporin A. Similarly, F. Mozaffarian *et al.* (2022) found that α-lipoic acid protects the mitochondria of rat livers from arsenic-induced oxidative damage.

The results obtained regarding the stability of sulphhydryl group levels in blood serum indicate that the antioxidant status of the laboratory animals' organisms is maintained. The observed trend towards an increase in the content of total thiol groups when high doses of the feed additive were used may be associated with the activation of the body's antioxidant system under the influence of α-lipoic acid. M. Nema *et al.* (2024) note that α-lipoic acid is a versatile antioxidant capable of functioning in both the aqueous and lipid environments of the cell, as well as participating in the regeneration of glutathione and other antioxidant compounds. The studies conducted revealed a trend towards a reduction in serum glucose levels in rats as the dose of the feed supplement increased. These findings can be explained by the role of α-lipoic acid in regulating carbohydrate metabolism and increasing tissue sensitivity to insulin. Similar properties of α-lipoic acid are described in the work of E.J. Calabrese *et al*. (2023) and A. Gezer *et al*. (2023), where the authors emphasise its important role in the regulation of energy metabolism and cellular respiration processes.

The absence of any marked irritant effect of the feed additive on the rabbits' ocular mucosa confirms its low local toxicity. Short-term manifestations in the form of mild discharge and slight oedema, which had completely disappeared by the second day of the experiment, do not indicate the development of persistent pathological changes. The biochemical parameters measured in the rabbits' blood were within the physiological reference ranges reported in recent studies on healthy rabbits (Strong-Townsend *et al.*, 2024; Khedr *et al.*, 2025), confirming the absence of any adverse effect of the feed additive on the animals' functional status.

The results obtained are consistent with current understanding of the biological properties of α-lipoic acid as a compound with antioxidant and cytoprotective effects. A. Divković *et al*. (2023) report that the use of α-lipoic acid helps to reduce the manifestations of oxidative stress and improve metabolic parameters, confirming the potential for using this substance in biologically active preparations. Thus, the results of the studies confirm the low toxicity and good tolerability of a stabilised coenzyme-containing feed supplement containing α-lipoic acid. The absence of marked pathological changes in clinical status, haematological, biochemical and antioxidant blood parameters indicates the potential for further study and practical application of the feed additive under investigation in animal husbandry.

Conclusions

The results of the present study demonstrated that the stabilised coenzyme-containing feed additive supplemented with α-lipoic acid is characterised by a low level of acute toxicity. Following a single intragastric administration to albino rats at doses ranging from 100 to 6,000 mg/kg body weight, no mortality was recorded, and the median lethal dose (LD_{50}) exceeded 5,000 mg/kg body weight. These findings support the classification of the investigated feed additive as a slightly toxic or practically non-toxic substance according to current toxicological classifications. Clinical observations of the laboratory animals showed that administration of the feed additive was not associated with significant disturbances in general physiological condition, behavioural responses, motor coordination, or central nervous system function. The animals remained active, responded appropriately to external stimuli, and maintained normal feed and water intake. Only at the highest doses of the additive (4,000-6,000 mg/kg body weight) did some rats exhibit transient gastrointestinal disturbances and mild depression during the first 24 hours of the experiment. These effects were temporary and resolved spontaneously without intervention.

The haematological findings indicate that the feed additive had no adverse effect on the haematopoietic system. Haemoglobin concentrations in both rats and rabbits remained within the physiological reference range, and the observed variations showed no evidence of haemotoxicity or the development of anaemia. The slight increase in haemoglobin concentration observed at the highest doses of the additive remained within physiological limits and was most likely associated with adaptive responses of the organism. Biochemical analyses of the blood serum likewise confirmed the absence of marked toxic effects of the investigated additive. Total protein concentration, AST and ALT activities, and urea concentration all remained within the physiological reference range. Although a tendency towards a moderate increase in transaminase activity was observed at the highest doses of α-lipoic acid, these changes were not accompanied by pathological alterations and did not indicate the development of significant hepatotoxic or nephrotoxic effects. Overall, the findings demonstrate good tolerability of the feed additive in laboratory animals.

Assessment of the antioxidant status showed that the concentrations of total, protein-bound, and free sulphydryl groups in the blood serum remained stable throughout the study. The moderate increase in thiol group concentrations observed following administration of the highest doses of the feed additive may indicate activation of the antioxidant defence system and further supports the antioxidant properties of α-lipoic acid. The absence of marked changes in sulphydryl group concentrations suggests preservation of cellular homeostasis and the absence of pronounced oxidative stress. Administration of the feed additive to rats did not cause significant disturbances in carbohydrate metabolism. Blood serum glucose

concentrations remained within the physiological reference range, although a tendency towards a moderate reduction was observed with increasing doses of α-lipoic acid. This finding is likely attributable to the well-established metabolic properties of α-lipoic acid and its role in the regulation of energy metabolism.

The results of the assessment of the irritant effects of the feed additive on the ocular mucosa of rabbits indicate a low level of local toxicity. During the first day following application, slight ocular discharge and mild oedema of the conjunctival mucosa were observed in some animals, each receiving a score of 1. These changes resolved completely by the second day of the experiment. No pathological alterations of the ocular mucosa were detected during the subsequent observation period. The absence of hyperaemia, prolonged irritation, or impairment of the general condition of the animals indicates good local tolerability of the investigated feed additive. Thus, the results of the toxicological, haematological, and biochemical investigations confirm the safety of the stabilised coenzyme-containing feed additive supplemented with α-lipoic acid following acute administration to laboratory animals. The investigated additive exhibited no evidence of pronounced haemotoxic, hepatotoxic, nephrotoxic, or irritant effects, supporting its potential for practical application in animal husbandry. Further studies should focus on the evaluation of the chronic toxicity of the feed additive, its effects on the productivity and antioxidant status of farm animals, and the assessment of its efficacy under different livestock production systems.

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Conflict of Interest

None.

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Встановлення гострої токсичності та подразнювальної дії стабілізованої коензимвмісної кормової добавки

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Анотація. Актуальність роботи зумовлена необхідністю розробки та токсикологічного обґрунтування безпечних кормових добавок з антиоксидантними властивостями для використання у тваринництві. Метою роботи було встановлення гострої токсичності та подразнювальної дії стабілізованої коензимвмісної кормової добавки з α-ліпоєвою кислотою. Гостру токсичність кормової добавки досліджували на щурах лінії Wistar після внутрішньошлункового введення у дозах 100-6000 мг/кг із 14-добовим спостереженням, а подразнювальну дію – на кролях шляхом внесення суспензії у кон'юнктивальний мішок ока з подальшим визначенням основних гематологічних і біохімічних показників крові. У результаті досліджень встановлено відсутність летальних випадків у щурів за введення кормової добавки у дозах до 6000 мг/кг маси тіла. Показник LD_{50} перевищував 5000 мг/кг, що дозволяє віднести досліджувану добавку до малотоксичних або практично нетоксичних речовин. За використання високих доз у окремих тварин спостерігали короткочасні розлади функції шлунково-кишкового каналу та незначне пригнічення, які самостійно зникали. Гематологічні та біохімічні показники крові залишалися у межах фізіологічної норми. Встановлено відсутність вираженої гемотоксичної, гепатотоксичної та нефротоксичної дії. Показники сульфогідрильних груп свідчили про збереження антиоксидантного статусу організму. Під час оцінки подразнювальної дії у кролів виявлено лише слабкі короткочасні виділення з ока та незначний набряк слизової оболонки, які повністю зникали на другу добу дослідження. Ознак гіперемії чи пролонгованого подразнення не встановлено. Результати дослідження підтверджують безпечність стабілізованої коензимвмісної кормової добавки з α-ліпоєвою кислотою та перспективність її подальшого використання у тваринництві. Отримані результати можуть бути використані для обґрунтування безпечності та впровадження стабілізованої коензимвмісної кормової добавки з α-ліпоєвою кислотою у практику тваринництва та при розробленні нових функціональних кормових засобів з антиоксидантними властивостями

Ключові слова: α-ліпоєва кислота; лабораторні тварини; гемотоксичність; гепатотоксичність; сульфогідрильні групи; біохімічні показники крові; токсикологічна оцінка; вуглеводний обмін

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