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STUDY OF THE AMINO ACID PROFILE OF ALTERNATIVE PROTEINS (*HELIX POMATIA*, *LISSACHATINA FULICA*, *HELIX ASPERSA*) AND THEIR POTENTIAL APPLICATION IN A HEALTHY DIET: OPTIMIZATION OF A MODERN BRANDADE RECIPE

Integrating traditional European cuisine with alternative protein sources can strengthen food security and provide the population with healthy food. Previous scientific works indicate the nutritional and environmental potential of alternative proteins. However, there is a research gap regarding their practical integration into authentic European recipes to improve nutritional value, sensory characteristics, and promote healthy eating. This study focuses on the problem of improving cod brandade for healthy nutrition. One of the problematic aspects of traditional brandade is its high fat content, limited protein content, and the use of predatory fish. This raises questions about its compliance with healthy eating principles. The object of the study is the recipe and quality of cod brandade and its modifications with alternative protein. Four samples were developed using salted semi-finished products: A0 (control Gadus morhua), A1 (Helix pomatia), A2 (Lissachatina fulica), A3 (Helix aspersa), and four samples using salted and cooked semi-finished products (50 % w/w): F0 (control Gadus morhua), F1 (Helix pomatia), F2 (Lissachatina fulica), F3 (Helix aspersa). In the experimental samples, olive oil was replaced with pumpkin seed cake protein isolate (50 % w/w). Among the snails, the highest amount of essential amino acids was found in Helix pomatia (8.39 g/100 g), although this was 23.72 % lower than the control. The protein from the Helix pomatia sample had the highest potential biological value (87.01 %), which was 3.85 % higher than the control. The chemical composition of the brandade changes significantly. The control sample (A0) is distinguished by a higher fat content, having the highest value at 29.96 %, which is 14.46 % more than A1. Samples with snails have a higher protein content, with A1 having the highest at 35.20 %, which is 15.86 % more than A0. Sample A1 achieved the highest sensory score of 8.6, surpassing A0 by 0.4 points. Texture and emulsion homogeneity indicators were better in the experimental samples due to the protein isolate. The obtained results highlight the significant potential of using alternative proteins for modernizing European cuisine and creating innovative food products for healthy nutrition.

Keywords: *European cuisine, fish products, snail fillet, essential amino acids, food industry by-products, pumpkin seed meal protein isolate, future food.*

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1. Introduction

The rapid growth of the global population poses a serious challenge to the global food system. Projections indicating that the world population will reach nearly 9 billion by 2050 necessitate not only a significant increase in food production but also the search for alternative protein sources [1]. This challenge prompts intensive research and development in the field of alternative food technologies [2]. Traditional animal husbandry, despite its importance, faces limitations related to sustainable development and environmental impact. The impacts of livestock farming include high greenhouse gas emissions, extensive land and water use, and a significant contribution to deforestation and biodiversity loss [3]. This motivates scientists and producers to seek innovative alternatives, and one promising direction is the farming of edible invertebrates [4]. One prospective area for increasing the production of alternative proteins from invertebrates is the wider use of freshwater mollusks and snails, which have long been consumed in Europe [5].

Snails belong to invertebrates and are considered a sustainable source of alternative protein. Heliciculture is attractive due to its efficiency and minimal resource requirements, and their farming is environmentally sustainable [6]. Furthermore, invertebrates are not only tasty but also nutritious, aligning with current healthy eating trends. Research confirms that snails are a source of high-quality alternative protein, vitamins, and minerals [7]. Another novel ingredient for modeling and improving traditional food formulations is pumpkin seed cake protein isolate. It is a local, accessible resource, making it particularly attractive for use in Ukraine. Pumpkin seed cake protein isolate has significant advantages over traditional soy and pea protein isolates regarding protein functional properties and the content of all essential amino acids [8]. A significant advantage of producing pumpkin protein isolate is its environmental friendliness and sustainability, which involves processing pumpkin seed cake or meal, considered food industry by-products [9]. The addition of pumpkin seed protein isolate can significantly enrich the nutritional value of dishes and improve texture [10, 11]. Alternative proteins from snails and pumpkin seeds are valuable dietary products that meet current healthy eating trends.

Healthy eating is not just a trend but the foundation of quality of life and longevity [12]. Modern trends in this area increasingly focus not only on the composition of products but also on their planetary impact. Interest in sustainable food sources that minimize negative environmental impact and satisfy people's desire for healthy food is growing [13]. Consumers are becoming more conscious and demanding, choosing products whose production adheres to the principles of sustainable development [14]. Traditional fish dishes have always held an important place in the diets of many nations, providing the body with valuable protein, fatty acids, and micronutrients [15]. Particular attention in this regard is given to fermented food products, used to improve the taste and biological value of alternative proteins, or for preserving fish and seafood [16]. However, predatory fish species and seafood are associated with high food safety risks. Studies reveal contamination of predatory fish and seafood with heavy metals, metalloids, and microplastics, especially in wild-caught fish [17]. During the preservation of fish and seafood, in addition to the initial contamination of the fish, additional harmful substances are formed during processing [18]. There are numerous preservation methods which have varying impacts on quality, food safety, and the environment [19]. Chemical pollution of the seas by persistent organic pollutants (POPs), metals, and microplastics is toxic and negatively affects the state of aquatic ecosystems. These harmful compounds harm not only marine life but also humans who consume fish and seafood [20]. Therefore, without adherence to strict quality standards, producing healthy food from fish and seafood becomes increasingly complex, especially for developing countries. These challenges require decisive action from scientists and food businesses. Consequently, it is necessary to seek alternative protein sources and develop new formulations for traditional fish dishes.

Thus, the analysis of scientific literature confirms the existence of problems associated with the traditional fishing industry and the use of predatory fish in healthy diets. Simultaneously, it indicates the significant potential of snails and pumpkin seed protein isolate as alternative proteins for creating healthy and environmentally friendly products. Currently, there are no studies on adapting traditional cod brandade to new trends, such as the use of alternative proteins and meeting the requirements of a healthy diet. Although, this approach is used to improve other traditional dishes, including meat products and sauces.

The object of research is the formulation and quality characteristics of traditional cod brandade and its modifications using alternative protein sources. *The research hypothesis* is that the use of alternative proteins in the formulation will allow the creation of a new food product with improved quality characteristics for a wide range of consumers interested in healthy eating. *The aim of this research* is to develop and investigate innovative formulations of traditional brandade with greater accessibility for a healthy diet, where cod is replaced by alternative proteins, whose amino acid composition is determined and studied. Our research is focused on studying the feasibility of using snail fillet as an alternative to cod in the traditional dish brandade.

Despite the popularity of cod brandade, there is a research gap related to the use of alternative proteins (snail fillet, pumpkin seed cake protein isolate) for the creation of this dish. Previous research has focused mainly on the traditional formulation, overlooking the potential of other ingredients that could meet the requirements of traditional brandade in terms of chemical composition and sensory characteristics. Alternative proteins allow for the improvement of these characteristics and make brandade accessible to a wider range of consumers. Overall, the research aims to address current nutritional challenges by proposing an innovative approach to creating healthy products based on alternative proteins.

2. Materials and Methods

2.1. Materials and raw materials for research

Fresh snails of three species, *Helix pomatia*, *Lissachatina fulica*, and *Helix aspersa*, were purchased from a private farm in Ukraine. The subspecies of garden snail used in the study belongs to *Helix aspersa maxima*. Other recipe ingredients, such as fresh-frozen Atlantic cod (*Gadus morhua*), Extra Virgin olive oil, pumpkin seeds (*Cucurbita maxima*) of the *Red Kuri squash* variety, ground black pepper, and ground dried garlic, were purchased at a local market. All reagents used for laboratory research were of analytical grade. Deionized water was used in all experiments.

2.2. Preparation of snail and cod fillets

Fresh-frozen snails and cod were thawed in a refrigerator at 4 °C for 12 hours. To obtain snail fillets and cod fillets, they were prepared accordingly. Snails were prepared by removing mucus in an acidic water medium, followed by shell and viscera removal. Cod was prepared by removing residual viscera, rinsing with water, removing the head, and subsequent filleting.

2.3. Preliminary treatment of snails and cod by salting

Dry salting was performed by thoroughly covering the cod fillets and snail fillets with salt. They were then kept in a cool place ($t=8$ °C) for 24 hours. The salted cod and snails, cleaned of residual salt, were stored in a refrigerator ($t=4$ °C) for 72 hours before use in the brandade formulation. Before preparing the brandade, the snails and cod were soaked in water for 24 hours in a cool place ($t=8$ °C), with the water changed three times.

2.4. Preparation of cooked cod

Cod fillet was heat-treated by conventional pressure cooking ($t=115$–120 °C, $\tau=10$–15 min) until done. The cooked cod fillet was cooled to 20 °C before preparing the brandade.

2.5. Preparation of pumpkin seed meal protein isolate

Seeds were collected from *Red Kuri squash* (*Cucurbita maxima*) for further processing. The seeds were dried at room temperature for a week to reduce moisture content, and the husks were manually removed. Pumpkin seeds, preheated to 40 °C, were defatted using a screw oil press to obtain press cake, which was ground in a laboratory mill and sieved through a 1 mm sieve (18 mesh). Pumpkin seed cake protein isolate was obtained from the pumpkin seed cake (*Cucurbita maxima*) using acid-alkali extraction and pH-adjustment treatment to maximize the functional properties of the protein isolate [21].

2.6. Preparation of cooked-frozen snails

Snail fillets were heat-treated by conventional pressure cooking ($t=115$–120 °C, $\tau=7$–12 min) until done. After heat treatment and cooling, the cooked semi-finished product was packaged and rapidly frozen to a temperature of $t=-18$ °C. Before preparing the brandade, the cooked-frozen semi-finished products were thawed in a refrigerator at 4 °C for 12 hours.

2.7. Determination of the nutritional value of snail brandade

The protein content in the snail brandade was analyzed using a manual Kjeldahl apparatus [22]. Fat content was determined by the Soxhlet extraction method [23]. Ash and moisture content of the samples were measured according to standard laboratory methods of food chemistry [24]. Detailed determination of the qualitative composition of carbohydrates was not performed as the main components of the formulation contain almost no carbohydrates. Carbohydrates were determined by difference from other indicators. The energy value of the studied samples was calculated based on the macronutrient content (protein, fat, carbohydrates) according to generally accepted methods [24].

2.8. Determination of the amino acid composition of alternative proteins and cod

Dried and defatted samples of alternative proteins (snail fillet) and fish (Atlantic cod fillet) underwent acid hydrolysis in sealed tubes. Hydrolysis was carried out using 6N HCl at 115 °C for 24 hours. The amino acid composition of snails was determined by high-performance liquid chromatography [25]. Nine essential amino acids (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine), one essential amino acid for children (arginine), and two semi-essential amino acids (cysteine and tyrosine) were determined. Semi-essential amino acids are determined for calculating the amino acid score. The protein content of alternative proteins (snail fillet) and fish (Atlantic cod fillet) was analyzed using a manual Kjeldahl apparatus [22].

2.9. Study of the amino acid composition of alternative proteins and cod

The amino acid score and its analysis for each essential amino acid were calculated based on the recommendations of the FAO/WHO scoring pattern [26], adopted for assessing protein quality in food products, using the formula:

$$S_i=(AP_i/RP_i)\cdot 100\ \%, \tag{1}$$

where S_i – amino acid score of the i-th essential amino acid of the protein, %; AP_i – content of the i-th essential amino acid in the alternative protein, g/100 g; RP_i – content of the i-th essential amino acid in the Scoring Pattern, g/100 g.

The Coefficient of Utility (a_j) of the j-th sample for assessing the biological value of alternative proteins from snail fillets (*Helix pomatia*, *Lissachatina fulica*, *Helix aspersa*) was calculated using the formula:

$$a_j=S_{\min}/S_i, \tag{2}$$

where $S_{\min}$ – minimum amino acid score (limiting amino acid), %; S_i – amino acid score of the i-th essential amino acid of the protein, %.

The Mean value of the coefficient of utility (A_j):

$$A_j=\sum a_j/n, \tag{3}$$

where n – number of essential amino acids.

The coefficient of rationality of amino acid composition (RC) was calculated using the formula:

$$RC=(S_{\min}\cdot\sum RP_i)/\sum AP_i, \tag{4}$$

where $S_{\min}$ – minimum amino acid score (limiting amino acid), %; RP_i – content of the i-th essential amino acid in the scoring pattern, g/100 g; AP_i – content of the i-th essential amino acid in the alternative protein, g/100 g.

The difference in amino acid score (D_i) for each i-th essential amino acid was determined by the formula:

$$D_i=S_i-S_{\min}, \tag{5}$$

where S_i – amino acid score of the i-th essential amino acid of the protein, %; $S_{\min}$ – minimum amino acid score (limiting amino acid), %.

The coefficient of difference of amino acid score (CD) was calculated by the formula:

$$CD=\sum D_i/n, \tag{6}$$

where n – number of essential amino acids.

The potential biological value (PBV) of the dietary protein was determined by the formula:

$$PBV=100-CD. \tag{7}$$

2.10. Sensory analysis of snail brandade

Sensory evaluation of all brandade samples was conducted using the scorecard method on a 9-point scale. Ten panelists, previously familiarized with the sensory evaluation method for fish brandade [27], were involved. Seven sensory attributes were evaluated: appearance, aroma, flavor, aftertaste, texture, emulsion homogeneity, and overall score. The overall score was calculated as the average of the other sensory attributes. Emulsion homogeneity was highlighted as a special sensory attribute, which at its maximum value should correspond to a brandade with an absolutely smooth, creamy texture.

2.11. Statistical analysis

Measurements were performed in quintuplicate ($n=5$) and results were presented as mean ± standard deviation, unless another number of replicates is indicated. Latin letters were used to denote statistically significant differences ($p<0.05$) between groups. Analysis of Variance (*ANOVA*) and Tukey's test were used to measure significant differences ($p<0.05$) between independent variables. Statistical analysis of the data was performed using R (4.4.2) with the RStudio Integrated Development Environment (2024.12.1+563).

3. Results and Discussion

For the study of cod brandade as part of European cuisine, it is important to adhere to traditional technology and formulation. Brandade is most common in Provençal cuisine (France) and Catalan cuisine (Spain). In French cuisine, brandade is mentioned in a cookbook that systematizes Provençal cuisine [28]. In Catalan cuisine, there are more ancient mentions of the brandade recipe, provided in a 14th-century medieval manuscript [29] and its modern scholarly edition and research [30]. Another example describing cod brandade is an old book from the Renaissance era [31]. This information about cod brandade became the basis for conducting research and determining its technology and formulation.

3.1. Formulation of brandade with snails for a healthy diet

To conduct the research and determine the formulation of control samples, common main ingredients for brandade from Provençal and Catalan cuisine were chosen, namely: salted cod, olive oil, and spices. Among the most common spices, garlic and pepper should be highlighted: black pepper in Provençal cuisine, and any pepper in Catalan cuisine. Although the availability of pepper in ancient times was more of a privilege for the wealthy, it is difficult to definitively call it a mandatory spice for brandade. Some ingredients mentioned in recipes as additional, but not common to both cuisines, are examples of improvements to traditional preparation approaches. The best brief description of the first brandade formulations would be: pounded fish with oil and spices. Spices are calculated separately in the formulation, as their amount can vary according to the preferences of cooks and consumers.

To study brandade from a food technology perspective, 8 samples were developed. Two control samples: A0, control with salted *Gadus morhua*, and F0, control with salted and cooked *Gadus morhua* (50 % w/w). Three experimental samples with salted semi-finished products: A1 with salted *Helix pomatia*, A2 with salted *Lissachatina fulica*, and A3 with salted *Helix aspersa*. Additionally, three experimental samples with a mixture of salted and cooked semi-finished products: F1 with salted and cooked *Helix pomatia* (50 % w/w),

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F2 with salted and cooked *Lissachatina fulica* (50 % w/w), and F3 with salted and cooked *Helix aspersa* (50 % w/w). Snails, which belong to invertebrates, and pumpkin seeds, which belong to plants, are used as alternative proteins.

Samples designated with the letter F were created based on a combination of salted and frozen semi-finished products from snails or cod, to make the formulation more accessible for people adhering to healthy diet principles in their nutrition. However, completely eliminating salted ingredients would result in a formulation that does not correspond to traditional brandade. Thus, sample F0 represents a traditional formulation adapted for a healthy diet. The addition of 30 % oil allows for the creation of a creamy consistency, which is the most traditional. The brandade formulation, including all 8 samples, is presented in Table 1.

The two control samples (A0, F0) allow for a comparison between two traditional recipes, but one of them (F0) contains a smaller amount of salted cod. In F0, the fish soaking time was halved to give the smaller portion of salted cod a more intense flavor. This was done to improve the traditional recipe by using less salted cod and replacing part of it with healthier fresh-frozen cod, while preserving the authentic flavor of brandade, which also makes F0 more accessible for a healthy diet. Also, salted semi-finished products are the source of salt in the recipe, so salt is not added separately.

Part of the olive oil in the experimental samples (A1–A3, F1–F3) was replaced with pumpkin seed meal protein isolate, produced from oil production by-products, namely the press cake. Pumpkin is also a more inherent and traditional ingredient in Ukrainian cuisine, although it was not grown in Ukraine in ancient times. Ground pepper in the experimental samples was replaced with ground dried garlic, which is not only found in traditional brandade recipes [28, 30], but is also a traditional spice in Ukrainian cuisine.

Additionally, the amount of ground pepper was reduced in the control samples (compared to some traditional recipes, focusing here on the garlic amount) to meet healthy diet requirements, which involves reducing the quantity of imported spices in the recipe. The developed brandade recipes allow for obtaining more precise results and identifying patterns in the research.

3.2. Technology of brandade with snails for a healthy diet

Analyzing ancient cod brandade recipes and their modern editions and research, it can be concluded that a generally accepted technology exists, common to both Provençal and Catalan cuisine [28, 30]. The technology for brandade with alternative proteins (*Helix pomatia, Lissachatina fulica, and Helix aspersa*), developed based on the analysis of ancient cookbooks, is presented in Fig. 1.

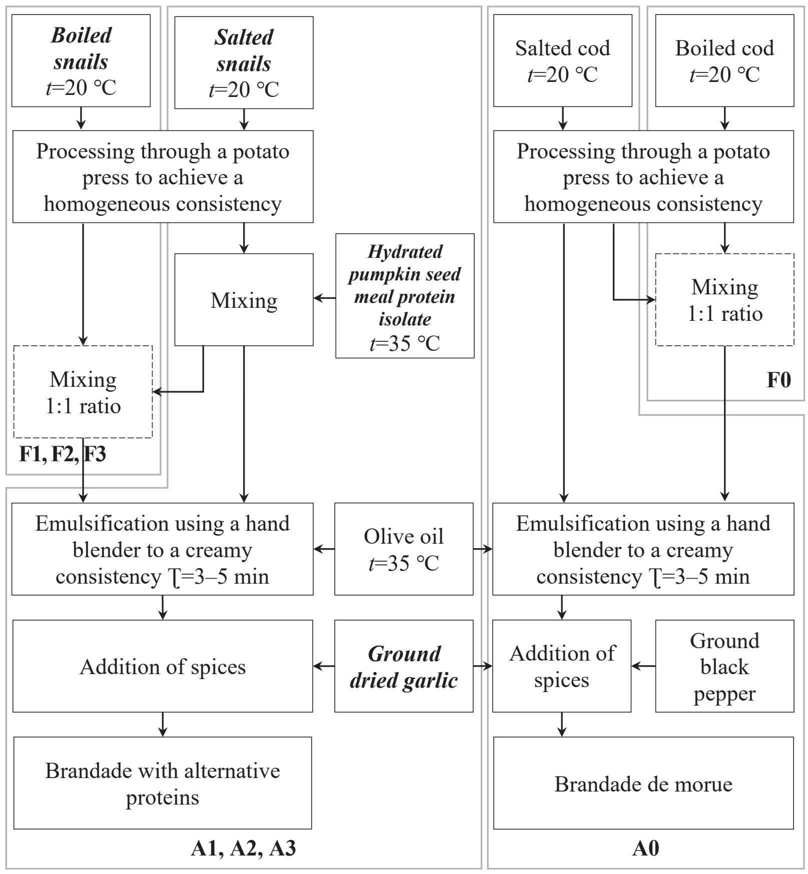

Fig. 1. Technology for brandade with alternative proteins

In Provençal cuisine (France) and Catalan cuisine (Spain), the technology for preparing cod brandade does not show significant differences. In ancient times, soaked and boiled salted cod was pounded in a mortar [29, 31]. The pounded fish was manually whipped with oil while adding spices. The consistency could vary and was not precisely described in ancient recipes; everything depended on the quality of the fish pounding [28, 30]. If the fish was insufficiently pounded in the mortar, the brandade's consistency was not homogeneous. Poor whipping led to further deviation from the desired consistency. That is, the cook had to pound the fish long and thoroughly and whip it with oil to obtain high-quality cod brandade. The developed technology for brandade with alternative proteins makes it possible to prepare the desired food product and compare it with the traditional one.

Table 1

Recipe of brandade with alternative proteins

Ingredients, g/100 g	Fish Brandade		Brandade with alternative proteins					
	Gadus morhua	*Gadus morhua*	*Helix pomatia*	*Lissachatina fulica*	*Helix aspersa*	*Helix pomatia*	*Lissachatina fulica*	*Helix aspersa*
	Control samples		Experimental samples					
	A0	F0	A1	A2	A3	F1	F2	F3
Salted snails	–	–	70	70	70	35	35	35
Boiled snails	–	–	–	–	–	35	35	35
Salted cod	70	35	–	–	–	–	–	–
Boiled cod	–	35	–	–	–	–	–	–
Olive oil	30	30	15	15	15	15	15	15
Pumpkin seed meal protein isolate	–	–	15	15	15	15	15	15
Ground black pepper	0.3	0.3	–	–	–	–	–	–
Ground dried garlic	0.7	0.7	1	1	1	1	1	1

3.3. Amino acid composition of alternative proteins (*Helix pomatia, Lissachatina fulica, Helix aspersa*)

Fillets of alternative proteins (*Helix pomatia, Lissachatina fulica, Helix aspersa*) were analyzed for the content of essential amino acids and arginine, an essential amino acid for children; the obtained data are presented in Table 2. The amino acid content is compared not only among the different snail species but also with the control sample of Atlantic cod (*Gadus morhua*).

According to Table 2, almost all indicators of amino acid content for *Lissachatina fulica* were lower than in the other samples. This can be related to the lower protein content, but after statistical analysis, no significant differences were found ($p > 0.05$). Quantitatively, the protein content is almost the same as in *Helix aspersa*, while the amino acid content in the latter is higher. Therefore, this difference can be best explained by the fact that the farming conditions were most unfavorable specifically for *Lissachatina fulica*, which was ultimately reflected in the fillet quality and lower content of amino acids and protein. Although considering the larger sizes of *Helix aspersa* and *Lissachatina fulica*, this gives them economic advantages over the *Helix pomatia* snails, which were best in quality. The experimental snail samples had lower indicators of amino acid content (except for arginine and tryptophan) compared to the control sample *Gadus morhua*. Therefore, snail farming and adherence to optimal conditions are an important factor, especially considering the complexities of organic production as part of the food of the future. Additional studies of the amino acid composition according to FAO/WHO methodologies may reveal more patterns that are manifested in the obtained results.

3.4. Study of the amino acid composition of alternative proteins (*Helix pomatia*, *Lissachatina fulica*, and *Helix aspersa*)

For the correct evaluation of the amino acid score and its study, two semi-essential amino acids, cysteine and tyrosine, were additionally determined. According to FAO/WHO guidelines, for the correct assessment of the amino acid score, it is necessary to consider the total content of certain pairs of amino acids, which are presented in Table 3.

These data are necessary for the subsequent calculation of the amino acid score according to FAO/WHO recommendations, which allows for an objective assessment of the nutritional value of the studied alternative proteins in comparison with traditional sources, such as fish. Therefore, Atlantic cod (*Gadus morhua*) is used as the control sample.

The research results presented in Table 3 are necessary so that, when calculating the amino acid score, the sum of methionine with cysteine and phenylalanine with tyrosine is considered, in order to align the data with the FAO/WHO guidelines [26]. For methionine and cysteine, this approach is based on the fact that cysteine can partially spare methionine in the human body; therefore, their total amount is a more informative indicator of the sufficiency of sulfur-containing amino acids. For phenylalanine and tyrosine, this is explained by the fact that tyrosine can be synthesized from phenylalanine, and considering them together provides a more complete picture of the intake of aromatic amino acids.

Based on the obtained amino acid composition data (Table 2) for alternative proteins (*Helix pomatia*, *Lissachatina fulica*, *Helix aspersa*) and the control sample of Atlantic cod (*Gadus morhua*), the quality of their protein was analyzed, which is presented in Table 4. Arginine was not used for the calculations of amino acid score and protein quality as there are no data on its content in the FAO/WHO Scoring Pattern.

Table 2

Amino acid composition of alternative proteins (*Helix pomatia*, *Lissachatina fulica*, *Helix aspersa*)

Fillet samples, g/100 g	Protein	Essential amino acids*										ΣEAA**
		Arginine	Valine	Histidine	Isoleucine	Leucine	Lysine	Methionine	Threonine	Tryptophan	Phenylalanine	
Helix aspersa	15.68±1.37^{a***}	1.08±0.11^{a}	0.89±0.08^{a}	0.36±0.05^{a}	0.70±0.05^{a}	1.11±0.11^{a}	1.18±0.12^{a}	0.40±0.05^{a}	0.73±0.05^{a}	0.38±0.05^{a}	0.61±0.06^{a}	7.44
Gadus morhua	19.25±1.49^{ab}	1.22±0.06^{a}	1.14±0.10^{b}	0.81±0.06^{b}	1.04±0.03^{b}	1.55±0.14^{a}	1.90±0.14^{b}	0.58±0.05^{b}	0.95±0.11^{b}	0.33±0.05^{ab}	0.86±0.07^{a}	10.38
Helix pomatia	17.01±1.10^{b}	1.23±0.11^{a}	0.89±0.06^{b}	0.45±0.05^{b}	0.82±0.05^{c}	1.36±0.14^{b}	1.27±0.09^{b}	0.39±0.06^{b}	0.78±0.06^{b}	0.40±0.07^{ab}	0.80±0.09^{b}	8.39
Lissachatina fulica	15.39±0.98^{b}	1.20±0.07^{a}	0.69±0.08^{c}	0.43±0.08^{b}	0.60±0.04^{d}	0.99±0.09^{b}	0.81±0.06^{c}	0.27±0.02^{c}	0.59±0.06^{c}	0.47±0.07^{b}	0.59±0.05^{b}	6.65

Notes: * – arginine is considered essential for children; ** – ΣEAA includes the 9 essential amino acids (Val, His, Ile, Leu, Lys, Met, Thr, Trp, Phe) and arginine; *** – means in the same column with different letters are significantly different ($p < 0.05$)

Table 3

Content of semi-essential amino acids in alternative proteins (*Helix pomatia*, *Lissachatina fulica*, *Helix aspersa*)

Fillet samples, g/100 g	Semi-essential amino acids		Sum of amino acids according to FAO/WHO	
	Cysteine	Tyrosine	Methionine+cysteine	Phenylalanine+tyrosine
Gadus morhua	0.21±0.05^{a}	0.71±0.08^{a}	0.79	1.57
Helix aspersa	0.36±0.03^{a}	0.58±0.06^{b}	0.76	1.19
Helix pomatia	0.39±0.07^{a}	0.52±0.05^{b}	0.78	1.32
Lissachatina fulica	0.35±0.02^{b}	0.49±0.06^{b}	0.62	1.08

Note: * – means in the same column with different letters are significantly different ($p < 0.05$)

Table 4

Quality of snails protein (*Helix pomatia, Lissachatina fulica, Helix aspersa*)

Fillet samples	Quality indicators of alternative protein			
	A_j (3)	*RC* (4), %	*CD* (6), %	*PBV* (7), %
Gadus morhua	0.70	72.30	16.84	83.16
Helix aspersa	0.69	71.50	14.46	85.54
Helix pomatia	0.75	78.62	12.99	87.01
Lissachatina fulica	0.71	74.13	14.92	85.08

Helix pomatia has the highest A_j value, indicating better average utilization of amino acids in this protein compared to other samples. This means that the amino acid profile of *Helix pomatia* is more balanced and better meets the body's overall needs. *Helix aspersa* has the lowest A_j value, which may indicate a less optimal ratio of amino acids for effective use. Again, *Helix pomatia* demonstrates the highest *RC*. This highlights that the amino acid composition of this snail species is closest to the ideal amino acid profile recommended by FAO/WHO. *Helix aspersa* has the lowest RC, indicating a certain disproportion in its amino acid composition compared to the reference. The low *CD* value for *Helix pomatia* indicates a more uniform amino acid profile. This means there are no significant "bottlenecks" in the supply of essential amino acids, i. e., no single amino acid is excessively limiting relative to others. The higher *CD* value for *Gadus morhua* indicates a greater difference between the scores of individual amino acids, which may suggest the presence of a more pronounced limiting amino acid.

The protein from the *Helix pomatia* sample has the highest Potential Biological Value (*PBV*). This is also confirmed by the highest Coefficient of Rationality of Amino Acid Composition (*RC*) and the lowest Coefficient of Difference of Amino Acid Score (*CD*). This indicates its most balanced amino acid profile, which best corresponds to FAO/WHO recommendations. The Mean value of the coefficient of utility (A_j) is also the highest for this sample. The protein from the *Gadus morhua* sample has the lowest Potential Biological Value (*PBV*) among the studied samples. This is accompanied by the higher Coefficient of Difference of Amino Acid Score (*CD*) for *Gadus morhua*, indicating a less uniform amino acid profile compared to the other samples. According to the obtained data, the protein from *Helix pomatia* fillet shows the highest quality indicators among the studied samples, making it the most promising source of alternative protein in terms of amino acid composition and potential biological value. The protein from Atlantic cod (*Gadus morhua*) showed the lowest results according to these criteria. The protein and amino acid content in the alternative proteins directly influences the nutritional value of the brandade, as they are the main component of its recipe.

3.5. Nutritional value of brandade with snails

The nutritional value of brandade with snails for the experimental and control samples, according to the recipe (Table 1), excluding the water added during the hydration of the protein isolate, is presented in Table 5. This was done in order to correctly compare the nutritional value, as the control samples do not contain water added for hydration.

Analysis of nutritional value showed that replacing cod with snails significantly changes the composition of the brandade. Nutritional value is an important indicator when evaluating food products, alongside sensory evaluation. The control samples (A0 and F0), made with cod, are distinguished by higher fat content and energy value, whereas the experimental samples with different types of salted snails (A1, A2, A3) and salted and boiled snails (F1, F2, F3) have significantly higher protein content. The lowest protein content is observed in the control sample F0 with boiled cod, and the highest in sample A1 with salted *Helix pomatia*. Samples containing the protein isolate have less fat and more protein, which significantly improves the health benefits of consuming brandade and combine well with snails.

Besides the type of raw material, the processing method also affects the nutritional value. Samples with boiled-frozen snails (F1, F2, F3) demonstrate lower energy value and higher moisture content compared to samples with only salted snails (A1, A2, A3). Using snails and pumpkin seed meal protein isolate as alternative protein sources in brandade can significantly improve its dietary characteristics. This combination of ingredients leads to a reduction in fat content and an increase in protein content in the dish, making it healthier. The processing method of the recipe components is an additional factor that influences the final nutritional value of the brandade, allowing for its optimization. The improved nutritional value in the experimental samples must be combined with appropriate sensory characteristics inherent to brandade.

3.6. Sensory evaluation of brandade with snails

The results of the sensory evaluation of brandade with snails are presented in the form of a heptagonal radial diagram in Fig. 2. Emulsion homogeneity is used as an additional sensory attribute, which well characterizes traditional brandade. This is related to the fact that potato was often added in later recipes for cod brandade, which improved the texture of the brandade. However, potato was not included in the ancient brandade recipes, as it was not yet cultivated in Europe at that time.

The results of the sensometric evaluation of the brandade demonstrate a significant influence of the main ingredient and its preparation method on the final sensory characteristics of the dish. Brandade based on salted *Helix pomatia* (A1) received the highest overall scores, surpassing even the traditional version with salted cod (A0). Whereas thermal processing and the combination of salted and boiled snails (*F samples*) led to a decrease in sensory characteristics. Texture and emulsion homogeneity were better in all samples with pumpkin seed meal protein isolate, thanks to its emulsifying properties. The appearance of the experimental samples had lower scores than the control, due to the snails' color being different from cod. Although after salting, the color of cod becomes grayish and yellowish, it is not as intense as that of the snails, which affected the appearance. The high sensory evaluation scores and the protein composition of the snail brandade allow considering it as a ready-to-eat food after preservation.

Table 5

Nutritional value of brandade with snails

Brandade samples		Chemical composition, %					
		Moisture	Protein	Fat	Ash	Carbohydrates	Energy value, kJ
A0	Control with salted *Gadus morhua*	$48.48 \pm 2.16^{a*}$	19.34 ± 1.49^{a}	29.96 ± 1.29^{a}	1.62 ± 0.40^{a}	0.6	1479.5
A1	Sample with salted *Helix pomatia*	45.98 ± 3.33^{ab}	35.20 ± 2.42^{ab}	15.50 ± 1.18^{a}	2.32 ± 0.50^{a}	1	1210.9
A2	Sample with salted *Lissachatina fulica*	47.10 ± 3.04^{ab}	34.20 ± 2.88^{ab}	16.18 ± 0.66^{b}	2.46 ± 0.61^{a}	0.06	1222.2
A3	Sample with salted *Helix aspersa*	46.28 ± 2.88^{ab}	34.64 ± 2.58^{bc}	16.34 ± 0.90^{b}	1.78 ± 0.61^{a}	0.96	1224.2
F0	Control with salted and boiled *Gadus morhua*	51.90 ± 3.16^{ab}	16.34 ± 1.16^{c}	29.26 ± 2.11^{b}	1.48 ± 0.29^{a}	1.02	1400.7
F1	Sample with salted and boiled *Helix pomatia*	51.42 ± 2.48^{ab}	30.48 ± 1.93^{c}	15.58 ± 0.85^{b}	2.02 ± 0.62^{a}	0.5	1130.1
F2	Sample with salted and boiled *Lissachatina fulica*	52.12 ± 4.91^{b}	29.04 ± 2.51^{d}	15.92 ± 1.60^{b}	2.06 ± 0.73^{a}	0.86	1119.6
F3	Sample with salted and boiled *Helix aspersa*	53.08 ± 3.58^{b}	29.32 ± 1.18^{d}	15.28 ± 0.87^{b}	1.84 ± 0.51^{a}	0.48	1096.4

Note: * – means in the same column with different letters are significantly different ($p < 0.05$)

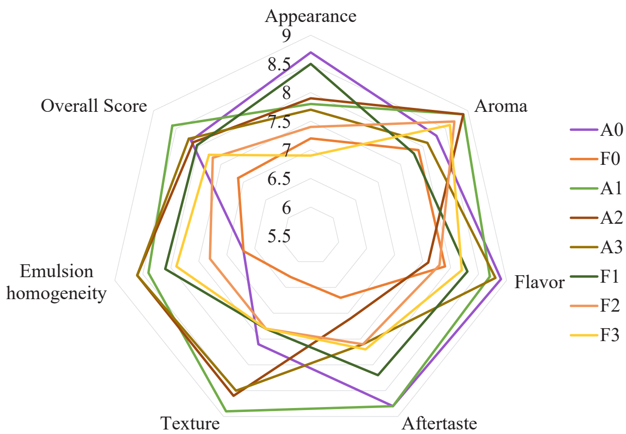

Fig. 2. Sensory evaluation of brandade with snails

3.7. Preserved brandade with snails

For preparing brandade for humanitarian purposes, special attention must be paid to packaging and the selection of the best recipe. To obtain preserved brandade with snails, it is best to use aluminum tubes, as this is the most convenient packaging. Also, this is made possible by the creamy texture of the snail brandade. The technology for preserved brandade with snails is shown in Fig. 3.

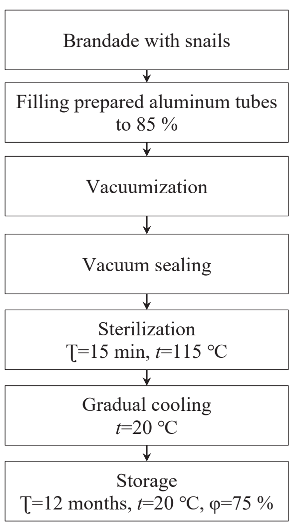

Fig. 3. Technology for preserved brandade with alternative proteins

The technology presented in Fig. 3 allows for the creation of an innovative protein brandade with snails, characterized by convenience in consumption and high nutritional value. Particular attention was paid to optimal packaging, which ensures ease of use and long-term preservation of the product's freshness. This preserved brandade, rich in alternative proteins, will be an excellent addition to crackers, biscuits (galettes), and other bakery products as a tasty and healthy spread, enriching the diet with valuable protein. Its high protein content contributes to enriching the diet with complete alternative protein, which is particularly valuable for a balanced diet.

Our results confirm that snail meat is a valuable source of protein, which can be used in the food industry. This aligns with studies indicating the high quality of snail protein [6]. It is important to note that the addition of pumpkin seed meal protein isolate significantly increased the nutritional value of the product, which underscores its potential as an innovative food ingredient. This can be compared with other studies where plant proteins were used to enrich food products [10, 11].

It should be noted that our study has certain limitations. Firstly, it is possible to use a limited number of snail species. Secondly, the sensometric evaluation was conducted by a small group of tasters, which may affect the overall objectivity of the results. Thirdly, the color of the snails, being different from cod, influenced the appearance scores, although this is not critical for brandade as a dish. Further research with a larger sample size and a greater variety of snail species could provide a more complete picture.

The conditions of martial law in Ukraine, introduced as a result of the full-scale invasion, significantly impacted the conduct of this research and, potentially, the obtained results. The research was conducted under conditions of limited access to resources, including laboratory equipment, reagents, and specialized literature. Socio-economic instability and the psychological pressure of wartime could have affected the researchers' productivity. The cost of imported ingredients was quite high, and the extended transportation time to Ukraine could have affected their quality. Legislative changes during wartime did not have a direct impact on this study.

Based on the obtained results, conducting a comparative analysis of snail brandade with other products based on alternative proteins (plant proteins, insects, algae), in terms of nutritional value, digestibility, and impact on human health, is a promising direction. It is also advisable to optimize the recipe by studying the influence of different processing methods for snails and other ingredients on the final characteristics of the brandade, and to expand the product range by developing new recipes with the addition of different ingredients to improve taste and nutritional value. Furthermore, it is important to study the potential of using snail brandade as a source of high-quality protein and other valuable nutrients to improve the diet of vulnerable population groups, especially in the context of achieving Sustainable Development Goal No. 2 "Zero Hunger".

4. Conclusions

Based on the analysis of ancient recipes and the aim of the study, 8 samples were developed: 4 samples with salted semi-finished products: A0 (control with *Gadus morhua*), A1 (*Helix pomatia*), A2 (*Lissachatina fulica*), A3 (*Helix aspersa*), and 4 samples with salted and boiled semi-finished products (50 % w/w): F0 (control *Gadus morhua*), F1 (*Helix pomatia*), F2 (*Lissachatina fulica*), F3 (*Helix aspersa*). In the experimental samples, olive oil was replaced with pumpkin seed meal protein isolate (50 % w/w). The best brief description of the first brandade recipes is: pounded fish with oil and spices.

Analyzing ancient cod brandade recipes and modern research on them, it can be concluded that a generally accepted technology exists, in both Provençal and Catalan cuisine. The developed technology for brandade with alternative proteins allows for the preparation of the desired food product and its comparison with the traditional one.

According to the obtained amino acid composition data, almost all indicators of amino acid content for *Lissachatina fulica* were lower than in the other samples. This can be related to the lower protein content, but after statistical analysis, no significant differences were found ($p > 0.05$). Quantitatively, the protein content is almost the same as in *Helix aspersa*, while the amino acid content in the latter is higher. Therefore, this difference is most likely explained by the farming conditions being most unfavorable specifically for *Lissachatina fulica*, which was ultimately reflected in the fillet quality and lower content of amino acids and protein. The highest amount of essential amino acids among the snails was found in *Helix pomatia* (8.39 g/100 g), but this value was 23.72 % lower than the control (*Gadus morhua*).

The protein from the *Helix pomatia* sample had the highest *PBV* (87.01 %), which was 3.85 % higher than the control. This is also confirmed for the *Helix pomatia* sample by the highest Coefficient of Rationality of Amino Acid Composition (*RC*) – 78.62 % and the lowest Coefficient of Difference of Amino Acid Score (*CD*) – 12.99 %. This

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indicates its most balanced amino acid profile, which best corresponds to FAO/WHO recommendations. The Mean value of the coefficient of utility (A_j) – 0.75, is also the highest for this sample. The protein from the *Gadus morhua* sample has the lowest *PBV* – 83.16 %, among the studied samples. This is accompanied by the higher Coefficient of Difference of Amino Acid Score (*CD*) for *Gadus morhua* – 16.84 %, indicating a less uniform amino acid profile compared to the other samples. According to the obtained data, the protein from *Helix pomatia* fillet shows the highest quality indicators among the studied samples, making it the most promising source of alternative protein in terms of amino acid composition and potential biological value. The protein from Atlantic cod (*Gadus morhua*) showed the lowest results according to these criteria.

Analysis of nutritional value showed that replacing cod with snails significantly changes the composition of the brandade. Nutritional value is an important indicator when evaluating food products, alongside sensory evaluation. The control samples (A0 and F0), made with cod, are distinguished by higher fat content and energy value, whereas samples with different types of salted snails (A1, A2, A3) have significantly higher protein content. The control is distinguished by higher fat content, with A0 having the highest value at 29.96 %. The experimental samples with snails have higher protein content, with the highest value in A1 at 35.20 %. The lowest protein content is observed in the control sample F0 (16.34 %) with boiled cod. Samples with the protein isolate contain less fat and more protein, which significantly improves the health benefits of consuming brandade and combine well with snails.

The results of the sensometric evaluation of the brandade demonstrate a significant influence of the main ingredient and its preparation method on the final sensory characteristics of the dish. A1 received the highest sensory score (8.6), surpassing the most traditional A0 (8.2). The appearance of the experimental samples had lower scores than the control due to the snails' color being different from cod. Although after salting, the color of cod becomes grayish and yellowish, it is not as intense as that of the snails, which affected the appearance. Texture and emulsion homogeneity indicators were better in the experimental samples, thanks to the protein isolate.

The technology for preserved brandade with snails allows for the creation of an innovative protein brandade with snails, characterized by convenience in consumption and high nutritional value. Particular attention was paid to optimal packaging, which ensures ease of use and long-term preservation of the product's freshness. This preserved brandade, rich in alternative proteins, will be an excellent addition to crackers, biscuits (galettes), and other bakery products as a tasty and healthy spread, enriching the diet with valuable protein. Its high protein content contributes to enriching the diet with complete alternative protein, which is particularly valuable for a balanced diet.

Our obtained results demonstrate that snail meat, especially *Helix pomatia*, can successfully replace cod in brandade. It is found that brandade with snails not only meets the dish requirements according to organoleptic indicators, especially sample A1 based on salted *Helix pomatia*, but also has high nutritional value, particularly due to the addition of pumpkin seed meal protein isolate. This allows the product to be classified as a protein product. Key evidence includes the high sensometric analysis scores for sample A1, which surpassed even the traditional version with cod, as well as the confirmed emulsifying property of the protein isolate. The study showed that snail meat is a promising alternative to cod for preparing brandade. The new product, which we named "Brandade with snails", has high nutritional value, good organoleptic indicators, and can be used as a source of high-quality protein. The research results expand the possibilities for using alternative proteins in the food industry and can be useful for developing new food products, particularly for humanitarian purposes and specialized nutrition.

Conflict of interest

The authors declare that they have no conflict of interest regarding this research, including financial, personal, authorship, or any other nature, that could have influenced the research and its results presented in this article.

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Use of artificial intelligence

The authors confirm that they did not use artificial intelligence technologies in the creation of the presented work.

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