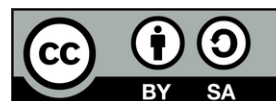




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Research Article

Design of food emulsions with the addition of encapsulated rice and pea proteins, and thyme oil extract

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Abstract

The use of plant proteins as sources of essential nutrients in the development of new products for people with specific dietary needs is of growing interest to the food sector. The aim of the present research was to design emulsion products i.e. mayonnaises supplemented with encapsulated rice and pea proteins and enriched with thyme oil extract as a source of bioactive compounds. The physicochemical and microbiological characteristics of the products were monitored during refrigerated storage at 4 °C for 20 days. The values of the emulsifying activity index and the emulsion stability index showed that the emulsion base was stable during the storage period. Regarding their microbiological quality, they retained a low number of mesophilic aerobic and facultative anaerobic microorganisms, yeasts and fungi, which were within the permissible limits (5×10^2 cfu.g⁻¹) on the 15th day of storage in all samples. The results from the sensory evaluation showed very good acceptance by the consumers. Therefore, the newly designed mayonnaises enriched with rice and pea proteins, and thyme as a source of bioactive compounds can be considered as products with improved quality and functional features, which can be used by people with specific dietary requirements.

Keywords

Plant proteins, rice protein, pea protein, food emulsions, mayonnaise

Abbreviations

EAI – Emulsifying activity index; ESI – emulsion stability index; FC – Foaming capacity; FS – Foam stability;
PP – Pea protein; RP – Rice protein

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Introduction

Nowadays, there is a growing belief among consumers that food plays a significant role in determining health. This has led to an increased demand for food that aligns with this perspective. As a result, the food industry is actively seeking new ingredients, formulations, and technologies to meet this demand. Specifically, product formulations are becoming simpler, with fewer additives and a focus on natural ingredients, giving rise to the concepts of clean label and healthy products (Cabrita et al. 2023).

The use of plant-based proteins in the food industry has increased due to their functionality, mainly as emulsifiers. Plant proteins are being extensively researched for emulsion formulation because they are environmentally friendly, offer health benefits, and are more cost-effective compared to animal proteins. The demand for plant proteins is often driven by the growing interest in vegan diets and healthy eating among consumers (Karaca et al. 2015; Gomes and Kurozawa, 2023).

Plant proteins play a vital role as stabilizers in food emulsion systems. These proteins can create emulsions that are indistinguishable from those made with animal protein. The key to successful emulsion preparation lies in selecting the right emulsifier. Emulsifiers not only facilitate the formation of emulsions but also enhance their stability. The emulsifying properties of proteins, with their mix of hydrophilic and hydrophobic amino acids, make them highly effective in forming a layer at the oil-water interface. Additionally, colloidal dispersions such as emulsions with a size ranging from 1 to 1000 nm consist of a dispersed phase and a continuous phase (Isnaini et al. 2021).

The effectiveness of using a protein as an emulsifier depends on how processing parameters affect its structural, conformational, surface, and functional properties. These factors directly influence how proteins behave in food systems during production, storage, and consumption (Akharume et al. 2021; Paulo et al. 2020). It's important to note that the concentration of the emulsifier can impact the final products obtained in the form of emulsions (Francisco et al. 2020). The size of the peptide molecules also influences the emulsion systems properties. For example, the molecular weight of rice glutelin with 2% degree of hydrolysis affects the storage stability of the emulsions as the larger

peptides coat more effectively the oil droplets and stabilize them for longer periods of time (Yang et al. 2022).

Rice (*Oryza sativa* L.) is a highly valuable food source. Despite historical neglect, in recent years, the interest in rice protein processing has increased, particularly as an alternative to animal proteins, especially as an emulsifier. The industry's rapid adoption of rice protein is driven by its cost-effectiveness, health benefits, and potential as a functional ingredient (Gomes and Kurozawa, 2021). Notably, rice protein concentrate exhibits promising properties as an emulsifying agent. In this regard, some studies have revealed that rice protein concentrate has great potential as an emulsifier at low pH values, with differences in its structural characteristics between pH 2 and 8 affecting its emulsifying properties (Romero et al. 2012).

Pea (*Pisum sativum* L.) is a crop serving as an important source of nutrients, including proteins, starch, and fiber. Pea protein is considered a high-quality protein and a functional compound in the food industry due to its low allergenicity, high protein content, and a low price. In addition, pea protein possesses excellent functional properties (solubility, water and oil holding capacity, emulsion stability, gelation and viscosity), which make it a promising ingredient in the food sector. Pea protein is widely used as a food emulsifier, encapsulating agent, biodegradable natural polymer, and in the production of bakery, dairy, and meat products (Shanthakumar et al. 2022).

Thymus callieri Borbás ex Velen is an aromatic perennial herb native to the Rhodope Mountains and the Struma River Valley in Bulgaria, as well as the Crimean Peninsula and the North Caucasus region. It is recognized as a rich source of bioactive compounds, including polyphenols (e.g., flavonoids), organic acids, vitamin C, pigments, minerals (such as calcium, iron, and manganese), dietary fibers, tannins, and resinoid substances. These constituents contribute to the plant's notable antimicrobial activity and antioxidant properties (Parzhanova et al. 2023). In addition to its non-volatile constituents, *T. callieri*, like other members of the genus *Thymus*, produces essential oil in concentrations ranging from 0.15% to 1.5%. The quantity and chemical composition of the essential oil are influenced by species-specific traits and various environmental factors. The volatile compounds identified in *T. callieri* essential oil

belong to several chemical classes, including monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, oxygenated sesquiterpenes, aromatic compounds, and other minor constituents. The composition and complexity of these volatile components are closely linked to the biological activities of the essential oil and support its potential use in pharmacological, food, and cosmetic applications (Trendafilova et al. 2023).

The development of novel functional foods that address specific dietary needs or provide targeted health benefits is of growing significance to the food industry. Functional foods are generally defined as conventional foods that have been modified through the addition, enhancement, or removal of certain components to improve health outcomes. These modifications may include the enrichment of the product with bioactive compounds, the fortification with vitamins or minerals, or the reduction or elimination of ingredients associated with adverse health effects. Such foods are designed not only to provide basic nutritional value but also to exert a positive influence on physiological functions, thereby reducing the risk of chronic diseases or promoting general well-being (Granato et al. 2010). Therefore, the aim of the present research was to develop food emulsions (mayonnaise-type products) supplemented with encapsulated rice and pea proteins. These emulsion products were further enriched with *Thymus callieri* essential oil extract as a natural source of biologically active compounds. The resulting formulations were intended to serve as functional food products suitable for individuals with specific dietary requirements, contributing to both nutritional enhancement and potential health benefits.

Materials and Methods

Materials

Thyme. *Thymus callieri* Borbás ex Velen originating from the municipality of Dospat, Western Rhodope Mountains (GPS coordinates 41°66' N 24°16' E, altitude 1214 m) was used in the present study. Voucher specimen (SOM 176971) has been deposited in the Herbarium of the Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences in Sofia, Bulgaria. The physicochemical characteristics, the total polyphenolic content, the antioxidant activity and

antimicrobial activity of thyme were investigated in our previous study (Parzhanova et al. 2023).

Plant proteins. Two proteins - rice protein from Bulgaria (RP) with 81.88% protein content and pea protein (PP) from UK with 89.71% protein content were used in the study.

Ingredients for the extracts and emulsions. The following ingredients were used for the preparation of oil extracts and food emulsions: vegetable oil (refined sunflower oil-oleic type), Biser Oliva Ltd., Stara Zagora, Bulgaria (BC 01/2016); sterile skimmed dry cow's milk, UFT, Plovdiv, Bulgaria; salt (cooking, table, iodized), Lubex Ltd., Plovdiv, Bulgaria; citric acid (E330), Dr Oetker, Germany.

Methods

Thyme oil extraction. The oil was extracted according to the method described in our previous study using refined sunflower oil. The solid and liquid phase ratio was 1:20 (Parzhanova et al., 2023).

Physicochemical characterization of proteins

Foaming capacity. Foaming capacity (FC, %) of the aqueous solutions of the proteins was assessed by manual stirring of a closed graduated cylinder containing 10 ml of the protein solution for 1 min (Cano-Medina et al. 2011; Ivanova et al. 2014). The concentrations of all the solutions were 5%, 10%, 15%, 20%, 25%, 30% and 35%. All tests were performed in duplicates. FC was determined by volume increase (%) immediately after whipping and calculated by the equation (1):

$$FC, \% = \frac{V_2 - V_1}{V_1} \times 100 \quad (1)$$

where V_2 is the volume of protein solution immediately after whipping, and V_1 is the volume of solution before whipping in ml.

Foam stability. The foam stability (FS, %) was characterized by the volume of entrapped air remaining in the foam after a certain period, $t > 0$. The FS was defined as the volume of the foam that remained after 30 min at room temperature and expressed as a percentage of the initial foam volume. Foam stability was determined as described

by [Marinova et al. \(2009\)](#) with minor modifications. FS was determined by the equation (2):

$$FS, \% = \frac{V_{foam}}{V_{foam 0}} \times 100 \quad (2)$$

where $V_{foam 0}$ is the volume of the formed foam (ml); V_{foam} is the volumes of the foam (ml) changed with time (t). FS over time was visually assessed by measuring the foam volume at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 and 30 min after stirring.

Sugar content. Sugar content of aqueous solutions of the proteins with different concentrations (5%, 10%, 20%, 25%, and 30%) was measured by an optical sugar content Brix refractometer-saccharometer (Hope Service Ltd.). The range of measurement of sugar concentration was from 0 to 32 °Brix, automatic temperature compensation (ATC) 20°C, resolution 0.2%, accuracy $\pm 0.2\%$ ([Bureau et al. 2009](#); [Jaywant et al. 2022](#)).

Alcohol content. The alcohol content of aqueous solutions with different concentrations of the precipitated proteins was measured by an optical

alcohol content Brix refractometer – alcohol meter (Hope Service Ltd.). The range of measurement of alcohol concentration was from 0 to 80% v/v alcohol content, ATC 20°C, accuracy $\pm 0.1\%$ ([Bureau et al. 2009](#); [Jaywant et al. 2022](#)).

Amino acid composition. The amino acid composition of the proteins was determined by high-performance liquid chromatography (HPLC) as previously described by [Tumbariski et al. \(2018\)](#).

Microbiological analyses. The microbiological analyses were performed in accordance with the Bulgarian State Standards (Table 1).

Preparation of calcium alginate microcapsules. The microcapsules for incorporation in the emulsions were prepared as previously described ([Tumbariski et al. 2024](#)). In the experimental procedure three types of alginate microcapsules were prepared: 1% sodium alginate (Sigma-Aldrich, Germany) + 0.5% ground thyme; 1% sodium alginate + 0.5% rice protein (RP) and 1% sodium alginate + 0.5% pea protein (PP).

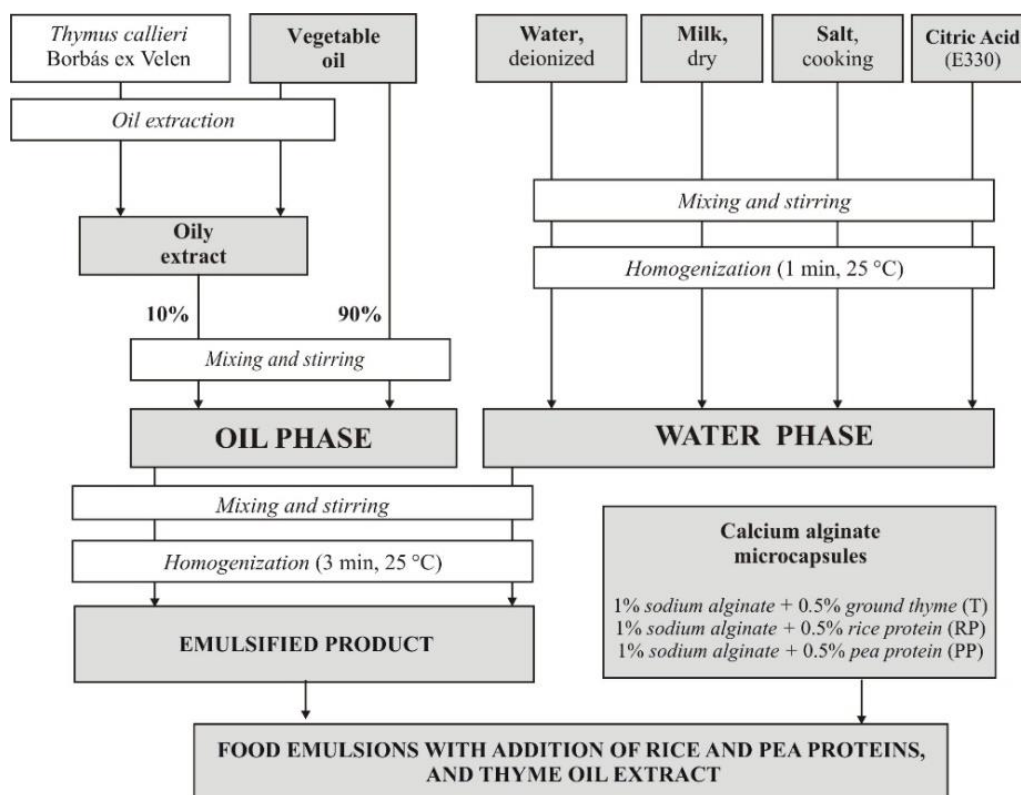


Figure 1. Technological scheme of food emulsion base with 70% fat content.

Table 1. Microbiological parameters and standards

Parameter	Culture medium / t°C	Standard
Total plate count (TPC)*	PCA / 30°C	BSS EN ISO 4833-1:2013/A1:2022
Yeasts and fungi*	DG18 / 25°C	BSS EN ISO 21527-2:2011
<i>Escherichia coli</i>*	TBX / 44°C	BSS EN ISO 16649-2:2014
<i>Salmonella</i> spp. *	XLD / 37°C	BSS EN ISO 6579-1:2017/A1:2020
<i>Staphylococcus aureus</i>**	Baird-Parker agar / 37°C	BSS EN ISO 6888-1:2021/A1:2023
<i>Enterobacteriaceae</i>*	VRBG / 37°C	BSS EN ISO 21528-2:2017
<i>Bacillus cereus</i>**	MYP / 30°C	BSS EN ISO 7932:2004/A1:2020
Sulfite-reducing bacteria*	ISA / 37°C	BSS EN ISO 15213-1:2023

*Pour-plating method; ** spread-plating method

Emulsion system preparation. The emulsion (mayonnaise) base with 70% fat content was prepared according to the recipe given in Table 2.

Table 2. Ingredients of food emulsion base with 70% fat content (per 100 ml)

Ingredient	Quantity
Oil phase	
Vegetable sunflower oil, ml	63.0
<i>Thymus callieri</i> oil extract, ml	7.0
Water phase	
Water, ml	30.0
Skimmed milk, g	5.0
Salt, g	1.0
Citric acid, g	0.3

The oil phase was prepared by stirring the oil extract of *T. callieri* (10%) in vegetable sunflower oil (90%), at 25 °C with stirring. The water phase was prepared by dissolving sterile skimmed milk, salt and citric acid in water (30°C) and homogenized for 1 min. The oil phase was added to the aqueous phase by continuous stirring by a laboratory homogenizer Polytron CH-6010 (Kinematica GmbH, Switzerland). To obtain an emulsion system with a uniform structure and better stability, the emulsion was subjected to additional homogenization at 25°C for 3 min.

The emulsion was divided into four aliquots in sterile containers. The first portion served as a control, while the other three portions were used for the incorporation of 10% of the respective calcium

alginate microcapsules (Fig. 1). Three different types of food emulsion were developed: sample T – food emulsion base with 70% fat content and encapsulated ground thyme (T); sample RP – food emulsion base with 70% fat content and encapsulated rice protein (RP); sample PP – food emulsion base with 70% fat content and encapsulated pea protein (PP). The samples were compared to the control main base for food emulsion.

Physicochemical characterization of emulsion base

Emulsifying activity and emulsion stability. The emulsifying activity index (EAI) and emulsion stability index (ESI) were determined according to [Murdzheva et al. \(2015\)](#) with some modifications. Aliquots of the emulsions (1 g) were pipetted from the bottom of the container at 0 and 5 min after homogenization and diluted 100-fold. The absorbance of the diluted solution was measured at 540 nm using a UV/VIS spectrophotometer Camspec M107 (Spectronic-Camspec Ltd., Leeds, UK). Absorbance values, measured immediately after agitation stopped (A_0) and 5 min after emulsion formation (A_5) were used to calculate EAI (3) and ESI (4).

The emulsifying activity index ($\text{m}^2\cdot\text{g}^{-1}$) and emulsion stability index (min) were calculated using the equations (3) and (4):

$$\text{EAI} = \frac{2 \times 2.303 \times A_0 \times D}{c \times l \times (1 - \varphi) \times 1000} \times 100 \quad (3)$$

$$\text{ESI} = \frac{A_0}{A_0 - A_5} \times t \quad (4)$$

where c is the initial concentration (1.0 wt%), ϕ is the volume fraction of the oil used in the emulsion (0.70), D is the dilution factor used (100), t is time (5 min) and A_0 and A_5 are the diluted emulsion absorbance at times (0 and 5) in min.

Sensory analysis. Twenty tasters over the age of 18 were invited to perform the organoleptic test. The sensory evaluation was performed by testing a small amount of the food emulsions spread on slices of bread. The emulsions were assessed according to the following seven sensory parameters: overall appearance, consistency, colour, odour, aroma, flavour and aftertaste using a five-point hedonic rating scale (1 – dislike extremely; 2 – dislike; 3 – neither like nor dislike; 4 – like and 5 – like extremely) (Tumbariski et al. 2024).

Statistical analysis. MS Office Excel 2010 software was used to process the data from triplicates and to determine the standard deviation ($\pm$ SD) and the maximum estimation error at significant level $p < 0.05$.

Results and Discussion

Physicochemical characterization of proteins

Foaming capacity, sugar content, and alcohol content. These parameters are important for the emulsions stability. The aqueous phase can contain biopolymers (polysaccharides and proteins), organic (ethanol, sugars, etc.) and inorganic (salts) components that can exert either a stabilizing or destabilizing effects (Pal et al. 2021). The behaviour of emulsions is determined by the three phases of the emulsion system: oil phase, interfacial phase and water phase (Leal-Calderon, et al. 2007). The interface can be composed of proteins, small molecules such as monoglycerides, phospholipids, fatty acids or a mixture of these three surfactants or solid particles (Fan et al. 2022).

The results in Table 3 demonstrate that the increase in the concentration of RP led to a decrease in their FC (%) due to the increase in water viscosity. In aqueous solutions containing the PP protein, an initial increase in the FC was observed. After a certain concentration (25%), the FC of the protein solutions decreased as the viscosity increased. The largest volume of foam was observed in pea protein. At concentrations of 10, 20 and 30%, the volume of the foam was almost three times more than those of

the protein solution. At the other concentrations, the volume of the foam was at least two times more than the volume of the protein solution. The rice protein showed lower FC in comparison with pea protein, but formed a higher viscosity solution with the most stable foam (Fig. 2A). The pea protein was characterized by the best foaming capacity, while the rice protein was characterized by the highest foam stability.

The sugar and alcohol content (Table 3) were proportional to the concentration of protein in aqueous solutions.

Table 3. Foaming capacity, sugar content and alcohol content of the aqueous solutions of the proteins

Concentration, %	Rice protein	Pea protein
<i>Foaming capacity, %</i>		
5	65.0	260.0
10	50.0	285.0
15	20.0	285.0
20	17.7	290.0
25	9.0	240.0
30	–	240.0
35	–	200.0
<i>Sugar content, %</i>		
5	–	9.0
10	1.0	11.0
15	1.6	15.4
20	2.0	20.0
25	3.2	24.0
30	–	28.2
35	–	29.0
<i>Alcohol content, %</i>		
5	–	23.0
10	1.0	29.0
15	2.0	45.0
20	4.0	80.0
25	7	> 80
30	–	> 80
35	–	> 80

The presence of ethanol in each protein sample was due to the isolation and processing of the raw plant materials. In this regard, the encapsulation of the protein is considered as a technological approach to avoid the potential negative impact of the sugars and alcohol on the emulsion stability.

Foam stability. The FS was monitored for 30 min. As seen in Fig. 2a and Fig. 2b, the foam volume of the aqueous solutions of both rice and pea proteins decreased over time.

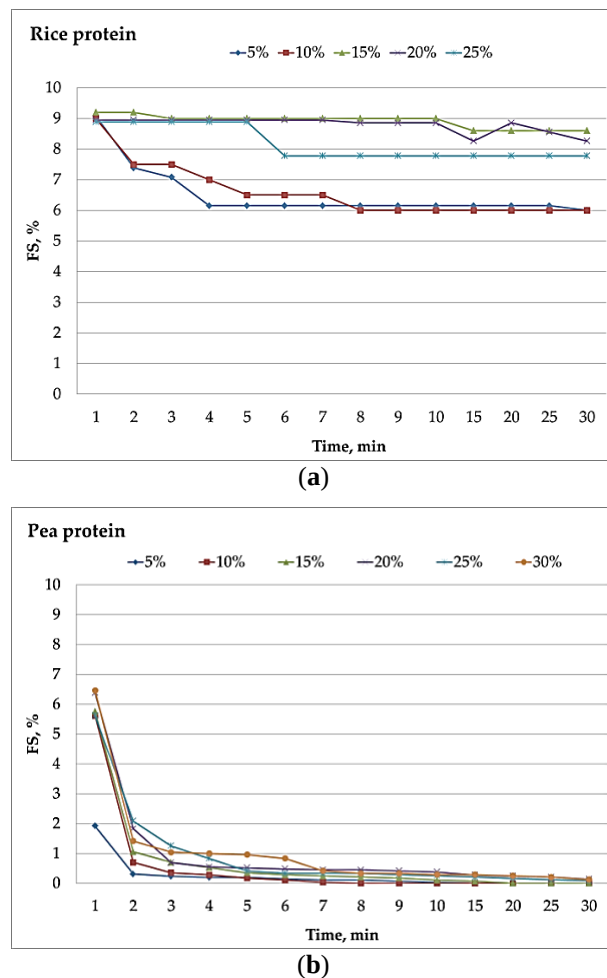


Figure 2. Foam stability (%) of aqueous protein solutions: (a) Rice protein; (b) Pea protein.

Rice protein formed foam at a maximum concentration of 25%. The reason was that as the concentration of RP increased, the viscosity of the aqueous solution increased, thus making difficult the introduction of air during aeration. The foam formed at concentration of 15, 20 and 25% was homogeneous and remained stable over time. At the

lower concentrations (5% and 10%), there was a sharp decline in the foam volume during the first 5 min. Once the small and similar sized air bubbles remained, the volume of foam remained stable after the 30-th min (Fig. 2a).

As seen from the results presented in Table 3, the pea protein is a good foaming agent, regardless of the solution concentration. However, the foam formed was unstable as it was completely destroyed after the 5-th min of its formation (Fig. 2b). Therefore, in order to improve the foam stability and to obtain stabile emulsion products, it is necessary to add stabilizers to the dispersion system.

Amino acid composition of proteins. The amino acid composition of pea and rice proteins is presented in Table 4.

Table 4. Amino acid composition of the proteins

Amino acids	Pea protein, mg.g ⁻¹	Rice protein, mg.g ⁻¹
Asparagine	60.27 ± 0.68	72.02 ± 3.08
Serine	48.91 ± 0.62	43.53 ± 0.97
Glutamic acid	79.64 ± 2.21	50.56 ± 1.51
Glycine	20.01 ± 0.69	16.96 ± 1.17
Histidine*	114.67 ± 1.68	95.93 ± 1.44
Tryptophan*	n.d.	n.d.
Arginine	155.90 ± 2.90	128.87 ± 2.04
Threonine*	52.10 ± 0.95	38.69 ± 0.63
Alanine	75.11 ± 1.50	88.29 ± 1.82
Proline	43.40 ± 1.20	48.15 ± 2.21
Cysteine	2.73 ± 0.21	2.59 ± 0.48
Tyrosine	53.61 ± 1.36	44.24 ± 1.91
Valin*	25.96 ± 0.12	25.08 ± 0.95
Methionine*	11.66 ± 0.16	19.80 ± 0.38
Lysine*	89.27 ± 0.73	73.90 ± 2.88
Isoleucine*	26.75 ± 0.35	24.87 ± 0.51
Leucine*	62.62 ± 0.43	47.58 ± 1.64
Phenylalanine*	33.56 ± 0.25	24.91 ± 1.34

*— essential amino acids; n.d. — not detected.

As seen from the results, the two proteins were characterized with the highest amount of arginine -

155.90 mg.g⁻¹ (PP) and 128.87 mg.g⁻¹ (RP), followed by the essential amino acids histidine (114.67 mg.g⁻¹ and 95.93 mg.g⁻¹, respectively), and lysine (89.27 mg.g⁻¹ and 73.90 mg.g⁻¹, respectively). Both proteins contained all essential amino acids, except tryptophan.

As mentioned above, PP and RP were characterized with a high protein content of 81.88% and 89.71%, respectively. PP is known as easy to digest and does not cause stomach irritation and intolerance, with a good amino acid profile and very high digestibility and solubility. RP is a quality vegetable protein, and a suitable supplement to the daily menu. It is often compared to the whey protein, that possesses high digestibility, good tolerance, is lactose-free, gluten-free, without causing irritations and allergies (Silva et al., 2020).

Microbiological analyses of proteins. The microbial contamination of rice protein (RP) and pea protein (PP) - was determined. The results presented in Table 6 show that the TPC (mesophilic aerobic and facultatively anaerobic microorganisms) in PP and RP was in the permissible limits. The yeasts and fungal counts in PP and RP were below the permissible values and met the standard requirements. In all plant proteins *Salmonella* sp. was not detected, whereas the presence of coliforms, *E. coli*, coagulase-positive staphylococci (*S. aureus*) as well as spore-forming species (*B. cereus* and *Clostridium* spp.) was below the permissible limits.

Physicochemical characterization of emulsion systems

Emulsifying activity and emulsion stability. The stability changes of the food emulsion used as a mayonnaise base was evaluated immediately after its preparation and on the 20-th day of the storage at 4°C. The emulsion stability was assessed by spectrophotometric determination of the emulsifying activity index (EAI) and the emulsion stability index (ESI) presented in Table 5.

As seen from the results at the end of the storage period (20th day), the emulsion base retained stable despite the decrease in the ESI value.

Table 5. Emulsifying activity index (EAI) and emulsion stability index (ESI) of the emulsion base (the control)

day	A ₀	A ₅	EAI, m ² .g ⁻¹	ESI, min
0	0.910	0.853	69.9	79.8
20	0.919	0.836	70.5	55.4

The preparation of stable emulsion products is essential for the food, pharmaceutical and cosmetic industries. Food emulsions are thermodynamically unstable systems that tend to separate back into their individual oil and water phases over time. EAI is a measure of the ability of emulsifiers to form an emulsion, while ESI evaluates the capacity of the emulsifier to stabilize the emulsion within a given time period (Lima et al. 2024). Changes in emulsion properties may be a result of physical processes that cause changes in the spatial distribution of its components (creaming, sedimentation, flocculation, coalescence, Ostwald ripening and phase inversion) or they may be a result of chemical processes that cause alterations in the type of molecules present (oxidation, hydrolysis, polymerization). Over time, during the storage of emulsions, the two phases move toward a more stable state due to the difference in density between the oil and water phases. Thus, measurements of emulsion stability are often followed by changes in the emulsified phase as a function of time elapsed after preparation by visual observation and using the transmitted light method and the backscattered light technique (McClements, 2015).

Microbiological analyses of emulsion systems

The microbial contamination of the four experimental groups (the control, sample T, sample PP and sample RP) was determined. The results presented in Table 7 demonstrate that during the storage at 4°C for 20 days, the values of TPC parameter reached 10² cfu.g⁻¹. Until the end of the storage period, yeasts were not detected in any of the food emulsions, while fungi were detected on the 5-th day (sample T), on the 10-th day (the control) and on the 15-th day (samples PP and RP). Considering the microbiological quality standards, according to which the number of yeasts and fungi should not exceed 500 cfu.g⁻¹ (5×10² cfu.g⁻¹), the obtained values ranged in the permissible limits.

In all emulsions *Salmonella* sp. was not detected, whereas the coliforms, *E. coli*, coagulase-positive staphylococci (*S. aureus*) as well as spore-forming species (*B. cereus* and *Clostridium* spp.) were below the permissible limits.

Sensory analysis of emulsion systems

The good overall appearance of food products is a major sensory parameter. In most cases, the consumer's expectations are related to a product that looks homogeneous; therefore, the separation of emulsion phases is an undesirable process. Another important characteristic of food emulsions that is perceived by the human senses is the aroma. Flavour is important to manufacturers and marketers because it contributes to good consumer acceptance of the food product (Hu et al. 2017).

The results from the organoleptic analysis of the control used as a base food emulsion (a) and the three food emulsions with the addition of encapsulated thyme (b), pea protein (c), rice protein (d), are presented in Fig. 3.

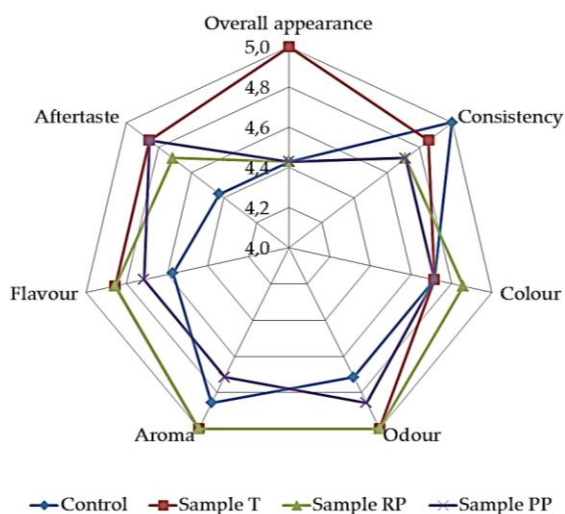


Figure 3. Organoleptic properties of the food emulsions.

The data from the sensory evaluation showed that sample T exhibited the best organoleptic profile (total score 32.14 ± 0.11), followed by sample RP (31.43 ± 0.20). The scores were close to that of the control sample (31.29 ± 0.21), while sample PP received the lowest evaluation (30.14 ± 0.14), out of a maximum possible score of 35.

The graphical dependence between the seven sensory parameters is presented in Fig. 3. As seen

from the diagram, the reason for the most preferred emulsion product was the overall appearance and aftertaste due to the addition of calcium alginate capsules with thyme to the emulsion. Thyme gives additional colour, taste and pleasant aroma to the final product. The dark green colour of the capsules with thyme stands out among the creamy-white colour of the food emulsion that contributes to improving the appearance of the final product and better acceptance from the testers.

Table 6. Microbial contamination of the proteins used in the study

Parameter, cfu.g ⁻¹	Rice protein	Pea protein
TPC*	70	20
<i>Bacillus cereus</i>	<100	<100
Sulfite-reducing bacteria	<10	<10
Yeasts	<10	<10
Fungi	<10	<10
<i>Escherichia coli</i>	<10	<10
Coliforms	<100	<100
<i>Staphylococcus aureus</i>	<100	<100
<i>Salmonella</i> spp.	—**	—**

* TPC – Total plate count (mesophilic aerobic and facultative anaerobic microorganisms); ** – not detected

The alginate capsules containing PP were colourless, tasteless and remained unnoticeable in the emulsion. A part of the testers considered the presence of these transparent spheres in the emulsion product to be an undesirable effect, and on the other hand, this led to a decrease in the smoothness and homogeneity of the final emulsion product. For some testers, the colour of sample RP was rated as preferable, whose alginate capsules were darker and more noticeable compared to PP capsules. On the other hand, samples with encapsulated thyme flour were distinguished by the best appearance, color standing out from the emulsion base, taste and aroma.

Based on the results of the sensory evaluation, we can conclude that food emulsions with thyme alginate pearls have the highest sensory score

(32.14 ± 0.11), which is distinguished by a good overall appearance, texture, taste and stability, followed by sample RP (31.43 ± 0.20), which is close to the control (31.29 ± 0.21). Capsules with encapsulated rice protein worsen the quality of the food emulsion. Their average organoleptic score has values lower than those of the base - 30.14 ± 0.14 .

The use of plant proteins as sources of essential nutrients is of great importance to the food sector. In addition, the application of plant proteins as alternatives to chemical stabilizers is an effective technological approach in obtaining stable emulsion products. Due to its rich physicochemical composition and high nutritional value (high protein content, carbohydrates, vitamins, minerals, dietary fibers) pea can serve as an ingredient in different functional food products (Tulbek et al. 2016; Lu et al. 2020). Pea protein is a plant protein rich in amino acids, such as lysine, leucine, aspartic acid, glutamic acid and arginine (Boye et al. 2020). Rice and pea proteins have significant potential when they are used in combination. Pea protein is characterized by higher levels of lysine, but lower levels of methionine, while rice protein is rich in methionine, but has lower amounts of lysine. This synergistic effect significantly improves the functionality of food products. Vélez-Erazo et al. (2021) investigated the effect of chia oil and the addition of pea protein content on the stability of emulsions. The authors demonstrated that a formulation with 13.5% (w/w) chia oil and 3.87% (w/w) pea protein showed the best stability as the phases remained stable for 7 days. Tong et al. (2024) studied the synergistic effects of the interaction between pea protein and rice bran protein for stabilizing a palm kernel oil-in-water emulsion, using high-pressure assisted homogenization. The results demonstrated that the combination of these two proteins improved the functionality and led to the formation of a stable emulsion. Olsmats and Rennie (2024) found that a minimum of 5% (w/v) pea protein and 30-50% (v/v) rapeseed oil resulted in the formation of a thick, dense protein layer as well as hydrated protein dispersed in the continuous phase. The resulting emulsion was stable against creaming and coalescence.

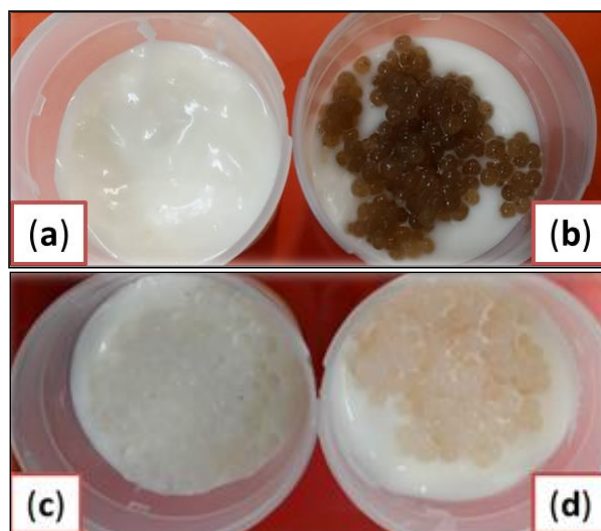


Figure 4. Overall appearance of the food emulsions: the control (a); with the addition of encapsulated thyme (b); encapsulated pea protein (c) and encapsulated rice protein (d).

The incorporation of pea protein into ice cream is a promising technological approach to reducing the saturated fatty acids in the final product. Pea protein was suitable for stabilizing oil/water emulsions due to its high emulsifying activity (EAI), high emulsifying stability index (ESI), flexible structure, and suitable particle sizes. It was demonstrated that a stabilized emulsion system (pH 7.0) with the addition of pea protein has the potential to replace 60% of cream in the production of ice cream with reduced saturated fat content. The resulting ice cream had a stable structure, similar properties and sensory characteristics, comparable to the control (without the addition of pea protein) (Qin et al. 2024).

Romero et al. (2012) investigated the emulsifying behaviour of rice protein concentrate for stabilizing an oil-in-water emulsion and found that the interfacial and emulsifying properties of protein concentrate had the best emulsifying potential at low pH levels. Gomes and Kurozawa (2023) found that rice protein concentrate (degree of hydrolysis 6% and 10%) showed better emulsion stabilizing properties at lower protein concentration (1%) compared to higher one (1.5%).

Table 7. Microbiological changes in the food emulsions during the storage at 4 °C for 20 days

Sample	Control					Sample T					Sample PP					Sample RP				
Parameter, cfu.g ⁻¹	Day																			
	1	5	10	15	20	1	5	10	15	20	1	5	10	15	20	1	5	10	15	20
TPC*	<10	30	30	40	4×10 ²	60	90	1×10 ²	3×10 ²	7×10 ²	40	50	50	1.10 ²	1.5×10 ²	<10	<10	30	60	1×10 ²
EBC**	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100
<i>Bacillus cereus</i>	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100
<i>Escherichia coli</i>	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
<i>Staphylococcus aureus</i>	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100
<i>Salmonella</i> sp.	—***	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Yeasts	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
Fungi	<10	<10	10	20	80	<10	60	1×10 ²	4.8×10 ²	9×10 ²	<10	<10	<10	1.2×10 ²	7.6×10 ²	<10	<10	<10	1.1×10 ²	4.3×10 ²

*TPC – total plate count; **EBC – *Enterobacteriaceae* *** – not detected

Krstonošić et al. (2020) characterized protein-stabilized oil-in-water emulsions by examining the emulsifying ability of different proteins. In pea protein isolate, the authors found a moisture content of 7.13%, and a protein content of 76.17%. The prevalent amino acids in the protein composition were glutamine (11.81%), asparagine (8.47%), arginine (6.00%), leucine (5.96%) and lysine (5.70%).

Conclusions

The application of plant proteins as a source of essential nutrients in the development of novel functional products is of growing interest to the food industry. Based on the present results, the obtained new food emulsions with enhanced nutritional value exhibited excellent texture, high stability during the storage period and improved organoleptic properties. The three types of mayonnaise were highly appreciated by the consumers and regarded as attractive. The future efforts of our work in this field are going to be focused on the additional extending the shelf life of the products.

Author Contributions

Conceptualization, A.P., T.Y., I.V. and Y.T.; methodology, A.P., I.V., V.Y. and Y.T.; formal analysis, A.P., I.V., V.Y. and Y.T.; investigation, A.P., I.V., V.Y. and Y.T.; resources, A.P., I.V., V.Y., I.D. and Y.T.; data curation, A.P., T.Y., I.V., V.Y. and Y.T.; writing - original draft preparation, T.Y., I.V., D.D., and Y.T.; writing - review and editing, A.P., T.Y. and Y.T.; visualization, I.V.; supervision, A.P. and Y.T.; project administration, A.P.; funding acquisition, A.P. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement

The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding authors.

Conflicts of Interest

The authors declare no conflicts of interest.

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