

Proteins Obtained from *Dunaliella Salina* Algae and Their Application in Gelation Processes and Metal Binding Compared to Legume Proteins

Rafał Szewczyk

Wroclaw University of Economics and Business

e-mail: rafalpraca5@interia.pl

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Abstract

Aim: Identification of research gap regarding the functional properties of microalgal proteins, specifically *Dunaliella salina*. The analysis aimed to determine whether these microalgal proteins exhibit similar properties to proteins from the following legumes: soybeans, lentils, chickpeas, and peas. The focus was on two key functional characteristics, i.e. gelation properties and metal binding properties.

Methodology: The analysis was based on a review of the scientific literature using the Mendeley database. The search included English-language keywords related to legume proteins and *Dunaliella salina*, particularly their functional properties. The selected publications were divided into two main thematic groups, namely protein composition and fractions, and their gelling and metal-binding capacity. Additionally, a quantitative analysis of the obtained results was performed to assess the research intensity for each species.

Results: A significant disparity in data availability was demonstrated between legumes and *D. salina*. While proteins from soybeans, peas, lentils, and chickpeas have been thoroughly characterised in terms of their function, the literature on *Dunaliella salina* is limited to general information on protein content and some studies on bioactive peptides. However, studies on the ability of these proteins to form gels and bind heavy metals are lacking, hence the results indicate a knowledge gap in this area.

Implications and recommendations: The identified research gap highlights the need for in-depth experimental studies on the functionality of *D. salina* proteins. It is recommended to evaluate their gelling properties and metal-binding capacity, and the findings of such studies could have applications

in the food industry. The potential of microalgae as an alternative protein source warrants further research.

Originality/value: This work contrasts the functional properties of legume proteins with the limited knowledge of *Dunaliella salina* proteins. It identifies a research gap in the area of microalgae as a potential source of functional proteins. It provides a basis for planning further studies on *D. salina* proteins in the context of their suitability for food applications.

Keywords: plant protein; *Dunaliella salina*, microalgae, protein gelation

1. Introduction

The basic macronutrients necessary for life are proteins, carbohydrates and fats, responsible for the proper functioning of the body and its growth. Proteins are the main building material for cells and tissues, and they are consumed in unprocessed or processed form and extracts or isolates are produced from them which are necessary for the design of functional food. Nowadays, high-protein plants, including legumes or oil plants, are considered a source of plant protein. Due to the increase in the world's population, sources of protein with high production potential and unique functional properties are constantly being sought. For years, microalgae have been of great interest to researchers due to the practically unlimited possibilities of their production and their insufficiently described properties. In analysing the current state of knowledge on plant protein, it was observed that in the case of soy (*Glycine max*), chickpeas (*Cicer arietinum*), lentils (*Lens culinaris*) and peas (*Pisum sativum*), there are many scientific reports on their properties in the gelation and metal binding processes. Research aimed at systematising current knowledge was undertaken in order to indicate the possibilities of research on microalgae, using *D. salina* as an example.

The research aim was to compare the level of knowledge on the properties of microalgae proteins with the existing knowledge on the properties of legume proteins. The findings could be helpful in indicating the directions of scientific research on microalgae, using *D. salina* as an example.

2. The State of Knowledge about Proteins Contained in Legumes and the Formulation of the Research Problem

2.1. Protein Composition

There is a lot of information in the scientific literature concerning the proteins contained in legumes, in which their composition and functional properties are described in detail. For example, soy is classified as a high-protein plant, with approximately 50% protein contained in the seeds. The dominant fraction is storage proteins, which constitute up to 80% of the total protein composition, the most important of which are 7S and 11S globulins, but 2S, 9S and 15S globulins can also be found. In addition, soy contains enzymes, protease inhibitors and lectins. (Garcia et al., 1997). An important aspect of assessing the properties of soy proteins is to take into account the influence of the food matrix. Reynaud et al. (2020) compared the digestion process of tofu, homemade soybean oil emulsion, soy juice and their corresponding protein concentrates, applying the INFOGEST protocol, a statistical simulation of food digestion in vitro. It was found that the food matrix had a significant effect on the degree of protein hydrolysis – soy juice was hydrolysed to a greater extent than fresh tofu (51.1% vs. 33.1%), whilst tofu was superior to soy protein isolate (33.1% vs. 17.9%). Soy contains all nine essential amino acids – histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine – as well as non-essential ones including arginine, alanine, aspartic acid, glutamic acid, glycine, proline, serine, tyrosine, and cysteine (Li et al., 2023).

In the case of lentils, their main proteins are globulins, typical of legumes. Albumins, which are storage proteins, can also be found in them. The albumin fraction of lentils is characterised by a better amino acid profile in relation to the globulin fraction, with the level of methionine, tryptophan, lysine, and threonine being higher (Sharma et al., 2022). Lectins are also present, which have the ability to bind sugars. The functional properties of lentil protein isolate were also confirmed, including water and oil absorption capacity, solubility, emulsifying and foaming properties. The lowest protein solubility was found at pH 4 (20%), whereas the highest at pH 12 (85%). Lentil protein isolate showed better textural, visual and taste properties compared to lentil flour (Robert & Linn Ko, 2017). Choukri et al. (2020) studied the effect of heat stress and drought on the yield and nutritional value of lentils. 100 genotypes were analysed for iron, zinc and crude protein content under different environmental conditions. The concentration of micronutrients (iron and zinc) and crude protein decreased significantly under heat stress conditions, especially when heat and drought occurred simultaneously. Lentils contain essential amino acids, such as leucine, isoleucine, valine, threonine, methionine, phenylalanine, tryptophan, histidine, and lysine, whereas non-essential ones like arginine, aspartic acid, glutamic acid, alanine, serine, glycine or tyrosine (Osemwota et al. 2021).

The next legume – chickpeas – contain mainly globulins, albumins, prolamins and residual proteins, with a relatively high content of gluten in relation to other legumes. Important proteins in chickpeas are also 11S (legumins) and 7S (vicilins), whereby vicilins do not contain cysteines or disulfide bonds. Albumins, rich in sulphur amino acids, have a higher nutritional value than globulins (Boukid, 2021). Chickpeas contain essential amino acids such as leucine, isoleucine, valine, lysine, threonine, phenylalanine, methionine, histidine, and tryptophan, along with non-essential ones including glutamic acid, aspartic acid, arginine, alanine, serine, glycine, tyrosine, proline, and cysteine (Sumeyra et al., 2022).

Peas are one of the best-studied sources of vegetable protein. Its main fractions are globulins (70-80% of total protein) and albumins (10-20%). Albumins, soluble in water, are rich in lysine, methionine, tryptophan and threonine. In turn, globulins, soluble in salt solutions, act as storage proteins, and there are also legumins, vicilins and convicillins (Farshi et al., 2024). Peas contain essential amino acids, such as leucine, isoleucine, valine, lysine, threonine, phenylalanine, methionine, histidine, and tryptophan, along with non-essential ones including glutamic acid, aspartic acid, arginine, alanine, serine, glycine, tyrosine, proline, and cysteine (Plocina & Beitane, 2024).

In the case of the microalga *Dunaliella salina*, research revealed the presence of bioactive peptides. Xia et al. (2019) obtained antioxidant peptides <3000 Da using ultrasonic extraction and simulated in vitro hydrolysis, and the fraction of 500–1000 Da showed the highest antioxidant activity. Four novel peptides were identified containing 56% hydrophobic residues, indicating their high antioxidant potential. In another study, Wang et al. (2010) identified a novel MAR-binding protein (DMBP-1) in *Dunaliella salina* that is specifically bound to A/T-rich DNA sequences, and its presence in the cell nucleus was confirmed by green fluorescent protein. In another study, Tavallaie (2015) analysed the production of carotenoids and proteins by *Dunaliella salina* isolated from Lake Hoze-Soltan in Iran. The highest concentrations of carotenoids (14.95 g/ml) and protein (186 g/ml) were obtained at 10% NaCl and pH 8.5 after 42 days of culture, while the growth of the organism was significantly inhibited under 30% NaCl conditions.

Dunaliella salina contains essential amino acids, such as isoleucine, leucine, valine, histidine, lysine, methionine, phenylalanine, and threonine, and endogenous amino acids like glutamic acid, aspartic acid and proline (Sui et al., 2021).

To sum up, the research results suggest that *D. salina* has significant potential as a source of bioactive proteins. Further studies on the detailed characterisation of the proteins of this organism could contribute to its effective use in food products and dietary supplements.

The research goal was to determine whether *Dunaliella salina* proteins have functional properties comparable to those of legume proteins. To address this issue, literature sources were analysed for gelling and metal-binding properties of the proteins from the studied legumes and microalgae.

2.2. Gelling Properties and Binding of Metals

The gelling properties of proteins include their ability to form a three-dimensional network that allows them to retain water and impart a gel-like consistency to food products. This process is the result of protein denaturation due to factors, such as temperature, pH, and salt.

Regarding soy, Cabodevila et al. (1994) compared gels obtained from soy protein isolate using xylose and glucono- δ -lactone (GDL). Gels obtained by the Maillard reaction showed less syneresis, higher breaking strength, and higher elasticity than gels obtained using GDL, which was attributed to the presence of additional covalent cross-links. In another study, Comfort et al. (2002) analysed the gelation of soy and whey protein isolates, both separately and in blends (ratios from 17:1 to 1:17). Rheological results showed phase separation, especially at the 5:1 (soy/whey) ratio, which was associated with reduced shear modulus and increased syneresis, indicating instability of the systems.

In the case of lentils, Joshi et al. (2011) showed that the drying method of the protein isolate significantly affects its gelling properties. Spray-dried and freeze-dried proteins showed better gel formation and strength compared to vacuum-dried proteins. Jo & Chen (2023) obtained a cold-setting gel from lentil protein using fibrillar aggregates and a combination of glucono- δ -lactone and transglutaminase. This process resulted in the formation of thick-linked networks stabilised by covalent and hydrophobic interactions. Gelation of lentil protein isolate was also studied in relation to pH, protein concentration, and heating time (Jo et al., 2020). Gels at pH 2 were translucent, homogeneous and showed good mechanical properties such as compressive strength (2.37 kPa) and high water retention capacity (80.62%). Mung beans showed the lowest critical protein concentration (14% w/v) and lower gelation temperature (77 °C), forming stronger gels. In the study by Guidi et al. (2022), it was found that pea and lentil protein mixtures showed linear mixing behaviour during gelation, while the addition of hemp caused nonlinearity and decreased gel strength.

Zhang et al. (2007) showed that chickpea proteins formed more elastic gels under high ionic strength and at a specific pH. The strength of these gels was up to 26.4 g for CaCl_2 and 9.3 g for NaCl, respectively. Papalamprou et al. (2009) stated that isolates obtained by ultrafiltration contained both globulins and albumins, which translates into better gelling properties than isolates prepared by the isoelectric method.

Moreno et al. (2020) showed that the gelling properties of pea proteins can be improved by the addition of microbial transglutaminase, which increases the gel strength and stability. Chen et al. (2022) found that resistive heating improves the mechanical properties of gels by developing protein structures and increasing the number of disulfide bonds. Kornet et al. (2021) compared the gelling properties of pea protein concentrate and its protein fractions with whey protein isolate. The albumin fraction showed the best gelling properties after diafiltration. De Berardinis et al. (2023) developed a map of the gelling conditions of pea and soy proteins, indicating that soy protein gels efficiently at 15% concentration and high ionic strength, while pea protein requires higher concentration and forms weaker gels. Sun & Arntfield (2012) evaluated mixtures of myofibrillar protein and pea as ingredients of additives to meat products. The addition of transglutaminase improved gel strength, but the effect was smaller than in mixtures with soy protein. Başığit et al. (2024) studied the production of pea protein hydrogels using cryogenic technology, obtaining samples with high porosity and water retention capacity (up to 83.70%). Rodriguez & Beyrer (2023) emphasised the importance of pea soluble protein extracts, which improve gelling properties and may reduce the need for deep refining of the isolate.

Proteins capable of binding metals, known as metalloproteins, enable plants to regulate the level of metal ions in cells and prevent their excessive accumulation. Dionisio et al. (2018) showed that soy protein fractions bind Zn^{2+} ions, mainly through 7S globulins, which was confirmed by ion chromatography and mass spectrometry. Reinecke et al. (1989) identified a protein fraction (18,100 g/mol) from soy that binds Cu^{2+} with high stability ($K = 1.046 \cdot 10^3$), analysing the effect of different chelating agents. Capraro

et al. (2015) proved that metal binding by soy 11S globulin is associated with the presence of histidine residues in the C-terminal fragment of the chain. In the case of lentils, Loris et al. (1994) described the crystal structure of metal-binding lectins, identifying key amino acid residues responsible for stabilizing the complexes. Wan et al. (2009) described chickpea metallothionein 2, demonstrating its ability to bind five heavy metal ions and pH- and ionic strength-dependent complex stability. Mukhamedov et al. (2022) isolated a 3896 Da peptide from chickpea albumin fraction with Zn^{2+} binding ability. Zhang et al. (2023) identified Fe^{3+} -binding peptides in pea proteins, mainly from the vicilin family. Shoaib et al. (2022) used FTIR spectroscopy to analyse changes in the cell wall structure of pea grown in Cu(II)-enriched soil and inoculated with *Sclerotium rolfisii*, a plant pathogen causing fungal diseases. The results showed limited Cu(II) translocation from root to shoot and changes in carbohydrate and protein regions, indicating adaptive mechanisms under transition metal stress. Geiken et al. (1998) described the effect of cadmium (CdCl_2) on the activity of photosystem II in peas and broad beans. Cadmium caused increased electron transfer, followed by damage to the oxygen-evolving complex, D1 protein degradation, and granule reorganization. Broad bean demonstrated greater stress tolerance due to more efficient D1 protein exchange.

De Souza Celente et al. (2024) described the ability of the halophilic microalga *Dunaliella salina* to purify saline wastewater by removing heavy metals and nutrients while producing bioproducts, such as skin cosmetics, as its extracts have antioxidant properties.

Literature analysis showed that proteins from legumes, such as soybeans, lentils, chickpeas and peas, have well-documented functional properties – in particular the ability to form gels and emulsify, which makes them attractive raw materials in the food industry. The gelation of these proteins depends mainly on their fractional composition, environmental pH and technological processing. Moreover addition, they show a moderate ability to bind metals, which may be important in detoxification or biofortification. *Dunaliella salina*, despite limited data, should be the subject of research on the gelling and metal-binding properties of proteins. This may open new perspectives for industrial applications, especially in the context of wastewater treatment and food production.

3. Research Methods

To achieve the research aim, a comparative literature analysis of the properties of proteins from the selected legumes was conducted, using soy, lentils, chickpeas, peas and the microalgae *Dunaliella salina*. The focus was on important aspects such as the ability to gel and bind metals.

For this purpose the Mendeley database was used, containing a collection of scientific publications, the selection of which was made using appropriately selected keywords in English. Both general entries, e.g. soy protein, proteins from pea, and more specific phrases such pea protein gelation, *Dunaliella salina* metal binding, were employed

Selected publications from the period 1989-2024 were analysed for protein functionality and analytical methods used in the studies. The collected information was organised thematically, categorised by protein characteristics and their ability to gel and bind metals. The results allowed to formulate conclusions and recommendations regarding potential directions for further research on *D. salina* proteins.

4. Research Results

By analysing data from the Mendeley database, presented in Tables 1 to 3, it was possible to assess the level of knowledge related to proteins derived from selected legumes and the microalga *Dunaliella salina*. A large disproportion in the number of publications referring to the organisms discussed was observed.

The most search results concerned pea proteins (over 9000), soybeans (over 2000), lentils and chickpeas, whilst for *Dunaliella salina* the number of publications on proteins was much lower (279 items).

The situation was similar in terms of functional properties: for entries such as pea protein gelation, 92 publications were identified, while for lentil protein metal binding – 170. For *D. salina* there were no publications referring to protein gelation and only one article on metal binding.

The collected quantitative data indicate that research on the functional properties of microalgae proteins is much less developed than for legumes, despite the potential benefits resulting from knowledge of their composition and resistance to environmental conditions.

Based on the identified research gap regarding *Dunaliella salina* proteins, further studies can be undertaken in the following areas:

- fractionation of *Dunaliella salina* proteins and identification of their molecular structures,
- examination of their gelling properties in relation to pH or ionic strength and comparison with plant proteins,
- description of the ability to bind heavy metals using, e.g. FTIR spectroscopy,
- determination of plans for the use of this microalgae proteins in the food industry.

Table 1. Results of the search for publications on proteins from selected legumes and *Dunaliella salina*

Database	Keywords	Quantity
Mendeley	Proteins from soybeans	2256
	Proteins from lentils	1613
	Proteins from peas	9085
	Proteins from chickpeas	2184
	Proteins from <i>Dunaliella salina</i>	279

Source: own research.

Table 2. Results of the search for publications on gelation of legume isolates and *Dunaliella salina*

Database	Keywords	Quantity
Mendeley	Soya isolate gelation	15
	Lentil isolate gelation	21
	Pea isolate gelation	92
	Chickpea isolate gelation	19
	<i>Dunaliella salina</i> isolate gelation	0

Source: own research.

Table 3. Results of the search for publications on metal binding by proteins from legumes and *Dunaliella salina*

Database	Keywords	Quantity
Mendeley	Soya metal binding proteins	5
	Lentil metal binding proteins	12
	Pea metal binding proteins	205
	Chickpea metal binding proteins	14
	<i>Dunaliella salina</i> proteins metal binding	1

Source: own research.

5. Conclusions

Legumes, such as peas, soybeans, lentils and chickpeas, are well-studied sources of protein, and their functional properties have been described in detail as results from the analysis of the literature. Despite their small number, selected studies indicate that *Dunaliella salina* has bioactive peptides. The lack of data on gelation and metal binding by *Dunaliella salina* proteins reveals a significant research gap. Filling this gap would help develop new, functional protein raw materials derived from algae. Therefore, it is recommended to conduct studies covering the fractionation of *D. salina* proteins, their structure and functionality, using techniques including spectroscopy, rheology and chelation tests. The results could serve as the basis for the commercial use of this microalgae in the food industry.

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Białka pozyskiwane z alg *Dunaliella salina* i ich zastosowanie w procesach żelowania i wiązaniu metali na tle białka z roślin strączkowych

Streszczenie

Cel: Rozpoznanie luki badawczej dotyczącej funkcjonalnych właściwości białek mikroalg, z uwzględnieniem *Dunaliella salina*. Analiza miała na celu określenie, czy białka tej mikroalgi wykazują podobne właściwości do białek pochodzących z wybranych roślin strączkowych: soi, soczewicy, ciecierzycy i grochu. Skupiono się na dwóch najważniejszych cechach funkcjonalnych: zdolności do żelowania oraz wiązania metali.

Metodologia: Analiza została przeprowadzona na podstawie przeglądu literatury naukowej z wykorzystaniem bazy danych Mendeley. Przeszukiwanie obejmowało anglojęzyczne frazy kluczowe związane z białkami roślin strączkowych i *Dunaliella salina*, zwłaszcza ich właściwościami funkcjonalnymi. Zebrane publikacje podzielono na dwie główne grupy tematyczne: skład i frakcje białkowe oraz ich zdolność do żelowania i wiązania metali. Dodatkowo przeprowadzono analizę ilościową uzyskanych wyników, aby ocenić intensywność badań dla każdego gatunku.

Wyniki: Wykazano znaczącą dysproporcję w dostępności danych między roślinami strączkowymi a *D. salina*. Podczas gdy białka soi, grochu, soczewicy i ciecierzycy zostały dokładnie opisane pod względem ich funkcji, literatura dotycząca *Dunaliella salina* ogranicza się do ogólnych informacji o zawartości białka oraz kilku prac dotyczących peptydów bioaktywnych. Brakuje natomiast badań poświęconych zdolności tych białek do tworzenia żeli oraz wiązania metali ciężkich. Wyniki wskazują na istnienie luki badawczej w tym obszarze.

Implikacje i rekomendacje: Zidentyfikowana luka badawcza wskazuje na potrzebę przeprowadzenia pogłębionych badań eksperymentalnych nad funkcjonalnością białek *D. salina*. Zaleca się ocenę ich właściwości żelujących, jak również zdolności do wiązania metali. Wyniki takich badań mogą mieć zastosowanie w przemyśle spożywczym. Potencjał mikroalg jako alternatywnego źródła białka uzasadnia dalsze badania.

Oryginalność/wartość: Praca zestawia właściwości funkcjonalne białek roślin strączkowych z ograniczoną wiedzą na temat białek *Dunaliella salina*. Wskazuje lukę badawczą w obszarze mikroalg jako potencjalnego źródła białek funkcjonalnych. Stanowi podstawę do planowania dalszych badań nad białkami *D. salina* w kontekście ich przydatności w zastosowaniach spożywczych.

Słowa kluczowe: białko roślinne, *Dunaliella salina*, mikroalgi, żelowanie białek
