Modification and Glycation Microalgae Proteins by Non-Thermal Assisted Process

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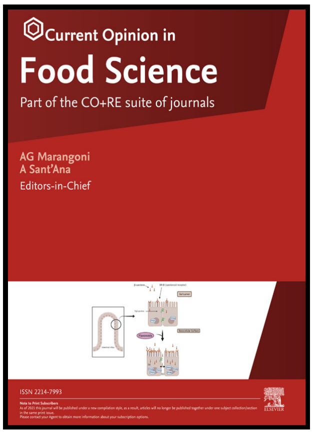

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Modification and Glycation Microalgae Proteins by Non-Thermal Assisted Process

Abstract

Plant proteins represent a promising solution worldwide due to their low production costs and easy accessibility. Additionally, plant proteins are more environmentally sustainable. Therefore, the potential of plant proteins to meet the protein requirements as an alternative source is increasingly gaining importance. Parallel to these developments, there is a growing interest and demand in food technologies for microalgae. The main reasons for this trend include their sustainable sources not only for proteins but also for various bioactive compounds and ingredients in food technology, as well as their positive impact on carbon emissions through carbon dioxide utilization. However, despite the significant demand for algae proteins from producers and consumers, their low solubility, viscosity, texture, and aroma defects limit their usage in foods. Various modification methods and glycation applications are needed to reduce these issues. However, it is crucial that these processes result in only the targeted changes in protein structures with minimal heat and chemical usage. Non-thermal processes such as sonication, high pressure, and cold plasma can be utilized for this purpose. Additionally, two main approaches can be used for microalgae protein modification: (i) modification of algae biomass followed by protein isolation or direct use/consumption of biomass, and (ii) isolation of protein from biomass followed by subsequent modification processes. This review offers a broad

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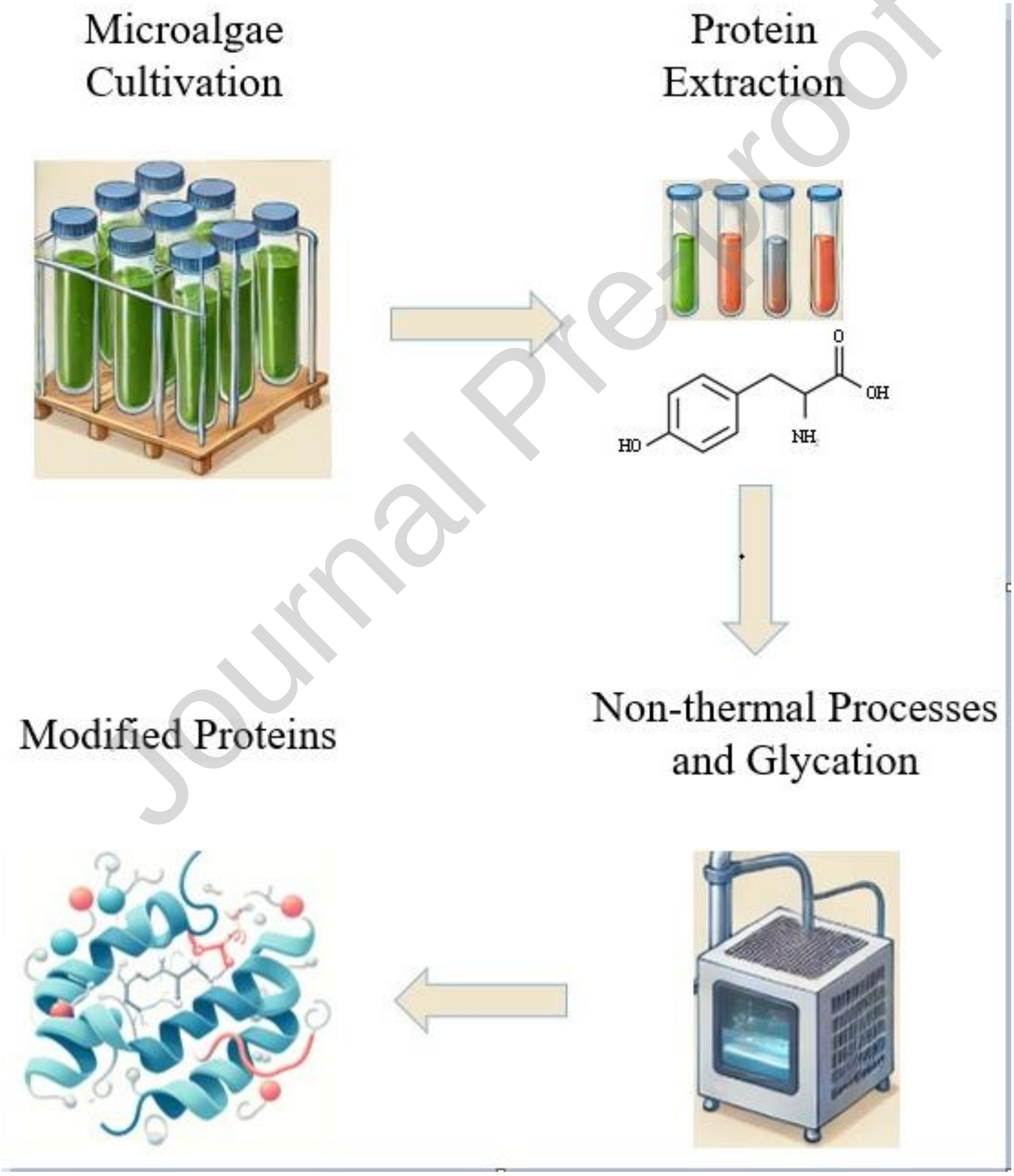
perspective regarding algal proteins. Modification techniques applied to plant proteins could be beneficial in enlightening the challenges linked to the digestibility, techno-functional, and sensory attributes of algae proteins. Much more research and innovation are required to enhance the potential of algae proteins.

Graphical abstract



Keywords: Algal protein; Glycation; Physical modification; Non-thermal technology; Alternative proteins

1. Introduction

Plant proteins are classified according to their sources, including legumes, grains, nuts, etc. Both plant and animal proteins have their advantages, and increased demand for vegan products, tendency towards healthy eating, religious beliefs, socio-economic concerns, environmental safety, cost, and sustainability have led to an increase in the use of plant protein sources in the food industry [1]. As seen in Figure 1, the supply of plant and animal-based proteins has continually increased over the years and has rarely shown any decline [2]. The plant-based food market has grown worldwide, and this increase is expected to continue in the coming years. This expansion can be attributed to manufacturing goods that mimic the taste, feel, and functionality of conventional animal products to appeal to general customers. Companies of all sizes, from start-ups to large food manufacturers, achieved this expansion by significantly investing in and developing their plant-based product lines [3][4]. These trends highlight the increasing importance of plant protein sources and the growing demand for sustainable alternative protein sources (Figure 1). Among alternative protein sources, algal proteins are also considered promising alternatives.

In general, algae can be referred to as plant-like organisms that are usually photosynthetic and aquatic but do not have true roots, stems, leaves, and vascular tissue. Microalgae are rich in proteins, lipids and functional protein sources [5]. The main reasons for this trend include their sustainable sources not only for various nutrients but also for bioactive compounds and various ingredients in food technology [6]. Approximately 40 different species of microalgae are used to produce these products. Among them, *Spirulina*, *Chlorella sp.*, *Dunaliella sp.*, *Haematococcus sp.*, and *Nannochloropsis sp.* are species with high-value component productivity and commercial value, and they constitute more than 95% of the total mass of microalgae used in the food, feed, cosmetic, and health sectors. For example, there is a high market demand for various bio-products derived from *Chlorella sp.* (food ingredient, food supplement, and animal feed), with global sales of approximately 38 billion USD/year [7] [8]. Additionally, providing optimum controlled conditions for microalgae cultivation is advantageous, considering the effects of stress conditions on components for specific purposes. There are various risks associated with microalgae cultivation in open ponds and other systems, such as high CO_2 consumption at low efficiency, inadequate control of some environmental factors, contamination risks, and high demand for salt and water. Therefore, the use of photobioreactors (PBR) is emphasized. Designing and scaling up PBR for high efficiency and use with different species are essential parts of microalgae studies [9]. Moreover, it is necessary to conduct microalgae cultivation studies to control environmental conditions and for different aimed uses.

Microalgae's positive impact on carbon emissions due to their consumption of CO_2 also contributes to sustainability, which is one of the most important issues in the world today. Their biomass being rich in various macromolecules and containing bioactive and minor components is an important advantage. [10,11]. Among the macromolecules, there has been a significant acceleration in interest in proteins and academic research in this field. Three main motivating factors emerge in protein chemistry and studies related to proteins: (i) identifying alternative and sustainable plant protein sources, (ii) characterizing the technological and functional properties of alternative plant proteins using innovative methods, and (iii) improving these properties and "protein quality" with new approaches.

There is a general and common consensus on microalgae being a sustainable and alternative protein source among the motivating factors of this study. Additionally, methods are needed to characterize algae proteins and their modifications with or without glycation. Determining the usage possibilities of modified algae proteins in different model foods can answer current questions and needs in food science and technology. This study uses non-thermal and green technology techniques to discuss the potential outcomes and requirements of glycation and/or modifications of microalgae proteins.

2. Algae as Protein Sources

Proteins are significant and crucial fractions of microalgae biomass. The total protein content of microalgae biomass varies depending on the species. The protein content of *C. vulgaris* biomass is >50.0 g/100 g [12], whereas for other species like *D. salina*, it is >30.0 g/100 g [13]. Microalgae are nutritionally comparable to specific animal proteins, such as eggs, and have an extremely favorable amino acid profile [14]. Bioactive peptides in algae have many beneficial effects, including antithrombotic, anti-oxidative, anti-hypertensive, opioid, hypocholesterolemic, appetite suppression, mineral binding, immunomodulatory, anti-microbial, and cytomodulatory properties [15].

Thus, microalgae are among the rich, nutritious protein sources that obtain protein isolates for modification and/or glycation purposes. These processes can be applied to protein isolates or directly to biomass. Due to the large number of microalgae species and their high biochemical variation in biomass, selecting suitable microalgae for specific purposes is essential. For example, *D. salina* has high-quality protein content, and the amount and composition of essential amino acids vary depending on the cultivation conditions [13]. Cultivation techniques and stress conditions significantly affect the composition of microalgae biomass. These stress conditions include the type and concentration of carbon source, temperature, light intensity, salinity, nutrient deficiencies, salinity, and residence time in the bioreactor [10,13,16]. UVB light exposure increases D3 vitamin production in *D. salina* while decreasing protein accumulation [10]. Protein digestibility-corrected amino acid score (PDCAAS) index of *C. vulgaris*, *C. sorokini* and *Acutodesmus obliquus* was determined as 0.63, 0.64, and 0.29, respectively [17]. The protein content and PDCAAS values of some food products and microalgae biomasses are presented in Table 1. These values are lower than animal proteins and close to plant proteins. The cell wall restricts the extraction of target substances and/or limits the bioavailability and bioaccessibility properties. As a result, various methods may be required to break down cell walls in microalgae processing (such as thermal, physical (sonication and mechanical) and/or chemical methods) [8]. Microalgae rigid cell walls contain insoluble carbohydrates, other polysaccharides, proteins, lipids, and inorganic salts [18]. Therefore, cell disruption processes are performed using different techniques. For this purpose, various methods can be utilized, such as enzymatic as a chemical method and physical methods providing mechanical effects, such as ball-milling and high-pressure homogenization [19]. Consequently, the mechanical properties of cell walls are among the criteria considered for non-thermal processes.

3. Modification Approaches for Algae Proteins

Proteins play a crucial role in product composition due to their ability to form emulsions, foams, films, and gels, significantly impacting foods' rheological, sensory, and textural properties [20]. Protein modification alters amino acid residues and polypeptide chains using various external factors. These changes in protein structures and physicochemical properties result in alterations in functional and nutritional properties. Modification applications can be performed using different physical, chemical, and biological techniques. To increase the bioactive qualities of proteins, biological modification techniques such as fermentation and germination are used to break down or build new protein structures; however, they are unstable and expensive. Moreover, chemical solvents or chemical processes are used in chemical modification to create proteins with improved and/or changed characteristics. Therefore, appropriate methods should be adopted to modify the properties of algae proteins, improve their functionality, eliminate allergenic substances, remove unpleasant tastes, reduce anti-nutritional factors, and meet the specific needs of applications.

4. Algae Protein Glycation

Unlike acetylation, deamidation, and succinylation, the Maillard reaction is spontaneous and naturally occurring. It is greatly accelerated by heat and no external chemicals are required. It is a chemical modification technique, is considered a mild process, and can be classified as green technology [21] [22]. Foods exposed to the Maillard reaction may include heterocyclic aromatic amines (HAAs) and advanced glycation end products (AGEs) [23]. Optimization of reaction conditions comes to the forefront of controlling Maillard reactions. These reactions occur naturally without adding chemical compounds and can alter important food properties such as stability, flavor, and color. This contributes to the potential solution to off-flavor problems, which are important issues in microalgae consumption and food technology [8]. Furthermore, glycation reactions can enhance the functional properties and antioxidant activity of proteins, peptides, and amino acids. Using these conjugates to successfully encapsulate other bioactive compounds from algae biomass or algae proteins will increase possibilities [24]. Additionally, this situation may lead to the obtainment of novel encapsulation agents. Also, improving and developing the technological properties of algae proteins with these processes is important because foods in which microalgae compositions are used include those found in emulsions and various beverage forms.

Proteins typically exhibit good solubility properties depending on pH conditions. However, their solubility decreases significantly under acidic conditions. The increase in solubility is due to an increase in electrostatic repulsion between protein molecules and the hydration of charged residues. *Spirulina* sp. protein extract showed the minimum solubility at pH 4 as 4.99%, but it rose to 32.44% at pH 10. *Isochrysis* sp. protein extract showed a minimum solubility at pH 2 14.12% and maximum solubility at pH 12 19.25%. Both protein extracts were lower than whey and flax seed proteins [25]. Extraction techniques of algae proteins cause differences in solubility values [26]. This is associated with the ability of polysaccharides to alter protein structures, reduce sulfhydryl groups, decrease protein-protein interactions, and prevent protein aggregation. The conjugation process may increase the solubility of algae proteins. Solubility of the rice protein conjugated with dextran increased from 32% to 46% with reaction time. Increasing dextran concentration also increased solubility to 52.02%, which was obtained in the conjugate having the mass ratio of protein to dextran 5:1 [27]. Previous studies [27,28] suggested that the reduced solubility during prolonged heating periods might be due to the progression of the Maillard reaction to its final stage and the formation of insoluble compounds. Dry and wet heating are the two traditional methods for the glycation of proteins. Dry heating requires drying of the material before the reaction and needs to be controlled by humidity and temperature. Due to the costly process and long preparation time, it is unsuitable for large-scale and industrial applications [29]. In the wet heating technique, forming the Maillard reaction in aqueous solutions fastens the reaction and reduces the process time. Higher temperature conditions result in denaturation and aggregations of proteins and result in weak techno-functional properties. Furthermore, in food technology and industry, thermal stability should be considered to determine the resistance of algae proteins against high temperatures that can potentially cause damage to proteins and lead to precipitation and structural loss, which can reduce nutritional value. Maillard glycation has been successfully used to improve the thermal stability of proteins such as rice protein isolate [30] and canola protein isolate [31], particularly under acidic conditions, by preventing intermolecular protein-protein interactions (e.g., hydrophobic-hydrophobic) and preserving the natural structure of proteins during thermal processing.

In the literature, some studies emphasize that the viscosity of proteins increases with conjugation. After the conjugation of pea protein with inulin, the apparent viscosity at 1 s^{-1} of the emulsion prepared with pea protein isolate was found to be about 0.06 Pa.s and it increased to about 0.3 Pa.s depending on the preparation method of the conjugates. The preparation method of the conjugates remarkably affected the rheological properties of the emulsion. Authors attributed this situation to the fact that inulin may have significantly increased the viscosity of the continuous phase due to its relatively large effective volume in water [32]. Among the foods where microalgae are commonly used are beverages. In this case, the type of saccharide used for conjugation should be considered when developing a method. Food products containing Maillard-type algae protein-sugar conjugates can maintain their rheological properties during processing, transportation, and storage. These conjugates are anticipated to increase viscosity, prevent phase separation in food emulsions, and prevent foam collapse in foam-based products, making this property particularly important in emulsion and foam-based food products. This allows algae protein conjugates to be used as alternative stabilizers in commonly consumed foods such as ice cream.

Algae proteins' potential uses can be further expanded by enhancing their emulsification properties, thereby increasing their potential use as surfactants in the food industry. The interaction between algae proteins and polysaccharides can alter the protein surface charge distribution and improve the thickness of the interfacial layer. Additionally, it has been determined that polysaccharides incorporated into the protein structure can generate specific steric hindrance between oil droplets, promote protein adsorption on the surface of oil droplets to form a dense multilayer structure, prevent aggregation and flocculation between emulsion droplets, and thus increase emulsion stability [33,34]. Emulsions stabilized by algae protein-saccharide conjugates may exhibit higher stability under acidic conditions, high ionic strength, and high temperatures. Therefore, compared to denaturation-sensitive other emulsifiers at high salt concentrations and acidic pH values, glycosylated proteins may be more effective agents in food systems. Additionally, protein structure or conformation changes through glycation with saccharides increase solubility behavior and adsorption rate at air-water interfaces. Therefore, the foaming effect of algae protein can be enhanced by conjugation with different saccharides, and the stability of these foams can be improved. However, parameters such as protein type, temperature, duration, and protein: saccharide ratios are important in applying glycation in protein modification [21]. For this reason, optimization studies should be conducted by considering parameters such as solubility, gel formation capacity, emulsion stability, degree of glycation, and HMF content, considering different microalgae species.

5. Non-Thermal Modification of Algae Proteins

Non-thermal technologies have been focused on improving post-harvest processes in microalgae, particularly in enhancing extraction processes of specific components. Among these, methods such as cold atmospheric plasma [35], sonication [36], high-pressure and pulsed electric fields [37,38] have been utilized. Strong hydrodynamic shear forces and elevated heat fluxes are released at the bubble collapse zone under cavitation conditions due to gas bubbles' quick formation and collapse caused by localized pressure differences that occur in a few microseconds. The protein extraction yields were 87.8 % and 76.6 % for *Arthrospira platensis* and *Chlorella vulgaris*, respectively [39]. More flexible and mobile protein aggregates are formed as a result of pressure and heat generated during ultrasonication treatment [40].

By applying high-energy cold plasma, covalent bonds can be broken, the spatial structure can alter, and macro clusters can be opened, leading to a decrease in particle size [35]. In addition, reactive species generated by plasma can promote the addition of some hydrophilic groups, increasing the interaction and bonding of water molecules to the protein surface. Cold plasma treatment can alter protein structures and properties, affecting solubility, particle size, hydrophilicity, and functional characteristics such as emulsifying and foaming properties. The use of cold plasma technologies as an alternative non-thermal method for foods has recently been approved. The Food and Drug Administration (FDA), the Environmental Protection Agency (EPA), and the United States Department of Agriculture (USDA) have approved the use of this technology for the processing of food products and food packaging [41].

High hydrostatic pressure applies hydrostatic pressures in the 100–800 MPa range for a few minutes. Dynamic high-pressure is a technology in which a liquid is forced through a narrow valve gap by high pressure, resulting in an extensive mechanical force on the liquid. High-pressure treatments significantly affect the secondary structure of proteins, which significantly improves the holding capacity, foaming, and emulsifying properties [42]. Applying high-pressure treatment, solubility, surface hydrophobicity, and gel stiffness increased, and the gelation point decreased [43].

The pulsed-electric field (PEF) technology has been used as a non-thermal process in which the materials are subjected to short high-power electrical pulses between electrodes. Electrophoretic movement of charged species between cell compartments induces cell rupture, and PEF is beneficial to the extraction of algae protein. The PEF process generates electrical energy, which may cause the protein to unfold and interact more with the solute. Revealing hydrophobic amino acids and the generation of free radicals as a result of protein polarization can occur by applying PEF. Moreover, secondary and tertiary structures of the proteins are modified by PEF treatment [44].

These techniques prevent thermal degradation and loss of bioactive components, making them suitable for protein extraction from foods with heat-sensitive components. Studies conducted in the last five years suggest significant opportunities for protein modification in microalgae technology. Still, they also indicate that an adequate number and scope of studies have not yet been conducted. However, research conducted with other plant proteins provides important clues about the potential outcomes and changes that can be encountered due to the modification of algae proteins.

6. Sustainability and Energy Efficiency in Thermal and Non-Thermal Food Processing

Understanding the environmental and ecological impacts of food production and processing is becoming increasingly significant. Thermal processing methods, such as pasteurization, baking, drying and sterilization, are widely used in the food industry for microbial inactivation and extending shelf life. However, these methods are often energy-intensive and can degrade food's nutritional and sensory qualities. Studies have shown that the heat used in these processes can significantly reduce the levels of heat-sensitive nutrients such as vitamins, color, and flavor compounds. In contrast, non-thermal technologies like High-Pressure Processing (HPP), Pulsed Electric Field (PEF), US, Cold Plasma (CP), Ozone, and Electrolyzed Water offer significant energy savings while maintaining food quality. Table 1 compares thermal and non-thermal food processing technologies, highlighting their energy efficiency, advantages, and challenges. To further understand the environmental benefits of using modified algae proteins, Life Cycle Assessment (LCA) can be applied. LCA studies have shown that algae proteins when modified using non-thermal methods, present a lower environmental footprint than conventional protein sources. LCA data shows frozen algae have a climate footprint of 0.96 kg CO_2e/kg, with significant emissions from processing, packaging, and other sources. Incorporating non-thermal methods for protein modification can further reduce the environmental impact, aligning with sustainable production practices [40]. Algae have a relatively low land use and greenhouse gas emission footprint compared to other protein sources such as poultry and plant-based proteins [45].

7. Future Perspectives

The increase in the plant-based food market, which has been on the rise for many years, is expected to continue due to the growing world population and the demands of consumers. According to the Statista data, the plant-based food market in 2023 was 52.5 billion dollars; it will be 64.7 billion dollars in 2024, and it is expected to reach 161.9 billion dollars in 2030. This increase in the plant-based food market necessitates the widespread use of plant-based proteins, which are crucial for the nutritional and other quality characteristics of such products. According to data compiled by the World Economic Forum, by 2035, the alternative protein market is projected to grow seven times its current size, from 13 million metric tons per year to 97 million metric tons per year, with an 11% share of the overall protein market (with regulatory support and technological advances, this share could reach 22%). The nutritional properties of algal proteins appear to be lower than those of animal proteins. This situation can be overcome by making mixtures of different algal proteins by targeting nutritional properties. Physical or physical/chemical methods can also improve the techno-functional properties of algal proteins. For this aim, different physical and dual methods, including a combination of physical and chemical methods, can be employed to improve the algal proteins' nutritional, bioactive, and techno-functional properties. Moreover, fermentation technology can also be employed to improve algal proteins' techno-functional and nutritional properties. In the future, there is the potential to see many food products enriched with algal proteins or protein-rich biomasses on the market. In addition, preparations consisting of algal proteins or algal proteins and different plant origins proteins are also expected to be available in the food supplement market [46].

Conclusion

The assessment of the literature shows that there is an increasing need for sustainable protein sources in a variety of food applications. Innovation of novel processes for efficiently utilizing protein-rich agro-industrial products, promising many value-added products and significant contributions to increasing incomes and reducing waste management and environmental pollution. Non-thermal food processing methods offer significant advantages over thermal methods, including shorter processing times, lower temperatures, and reduced energy consumption while maintaining similar food quality. These benefits result in substantial reductions in carbon emissions. Additionally, the lower temperatures used in non-thermal processing help preserve foods' nutritional and aromatic properties. Non-thermal methods have proven to be more effective in terms of energy usage, sensory properties, and nutritional quality for the glycation of microalgae proteins. These findings highlight the potential of non-thermal technologies to enhance the sustainability and quality of food production, particularly for alternative protein sources like microalgae. Moreover, improving functional properties, enhancing bio-accessibility and successfully translating these advances into commercial opportunities have not reached the potential for algae proteins. Research and development efforts are necessary to discover the full potential of algal proteins in meeting the demands of various industries.

Data Availability

No data was used for the research described in the article.

Declaration of Competing Interest

The authors do not have any conflict of interest.

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Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

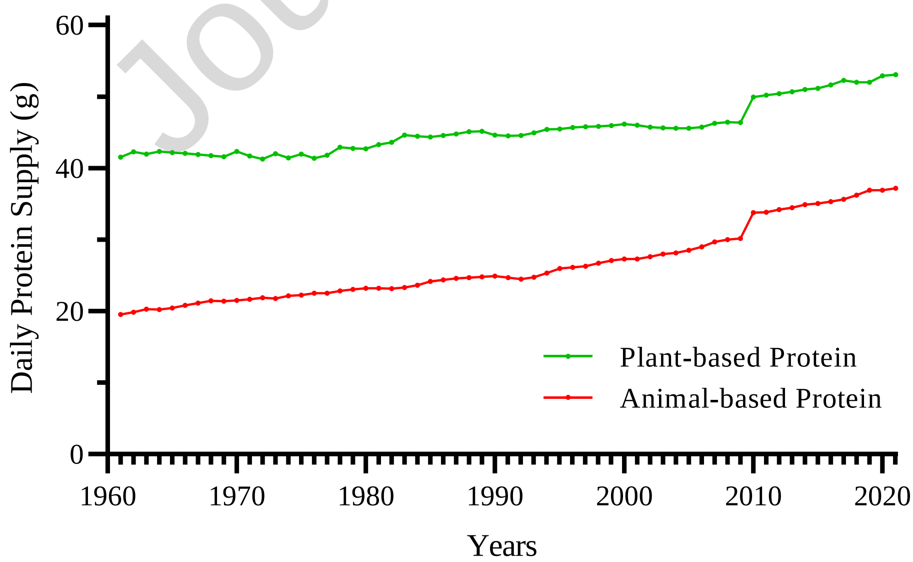

Fig 1. Daily protein supply from animal and plant-based foods, world, 1961 to 2021 (Protein of animal origin includes protein from all meat commodities, eggs and dairy products, and fish & seafood

)

Table 1. Protein content and Protein digestibility-corrected amino acid score (PDCAAS) index of some food prodcts and microalgae biomass [29-32]

Some widely consumed products			Microalgae biomass		
Product	Protein content (%)	PDCAAS	Microalgae	Protein content (% dry matter)	PDCAAS
Milk	3	1.00	Chlorella Sorokini	40	0.64
Egg	12-13	1.00	Chlorella vulgaris	53.50	0.63-0.77
Beef	18-23	0.92	*Chlorella sp.*	38-58	0.63-0.64
Chicken	17-24	1.00	*Acutodesmus obliquus*	38	0.29
Fish	16-24	1.00	*Scenedesmus obliquus*	40-56	0.29
Oat	17	0.57-0.64	*Arthrospira sp.*	46-71	0.84
Whole wheat	10-16	0.45-0.54	*Spirulina sp.*	59.16	0.41
Soy	39-43	0.91			

Highlights

- Novel technologies were discussed to alter protein structures, leading to functionality improvement.
- The sustainability and energy efficiency of these techniques were discussed to understand the environmental benefits.
- Potential usage areas of algae proteins and conjugates were emphasized.
- Novel Technologies offer opportunities to improve the protein digestibility of algae proteins
- Algae proteins can be applied in microencapsulation as wall materials.