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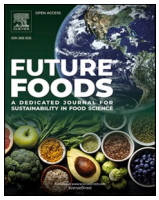

Harnessing microalgae for protein production: advances, functional properties, and industrial potential

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ABSTRACT

The escalating global population and rising demand for sustainable protein sources necessitate alternative solutions beyond traditional livestock and crop-based proteins. Microalgae have emerged as a promising protein source due to their high protein content (30–55 % dry weight), superior amino acid composition, and environmental benefits. Unlike conventional protein sources, microalgae can be cultivated on non-arable land using seawater, minimizing resource competition and reducing carbon emissions. Additionally, microalgal proteins exhibit promising functional properties, such as emulsification, foaming, and gelation, which have been demonstrated in selected species and highlight their potential in diverse food applications. Advances in microalgal cultivation strategies, such as mixotrophic and heterotrophic modes, have significantly improved protein yield. Moreover, biotechnological enhancements, including metabolic engineering and bioreactor optimization, have further increased their commercial viability. The sensory limitations can be alleviated through depigmentation, deodorization, and formulation strategies, thereby improving the food applicability of microalgal proteins. However, challenges such as high production costs and scalability barriers hinder large-scale adoption. Unlocking the microalgal potential as an alternative protein source will require advancements in cultivation efficiency, extraction methods, and cost reduction strategies. This review explores recent progress and emerging approaches to harnessing microalgal proteins for sustainable food and biotechnology applications.

1. Introduction

The global population is rapidly expanding and is projected to reach approximately 9.8 billion by 2050 (United Nations, 2017). To meet the escalating demand for food driven by this population surge, global food production must increase by an estimated 70 % (Falcon et al., 2022). According to data from the Food and Agriculture Organization (FAO), over 828 million people worldwide suffer from malnutrition due to insufficient access to protein-rich diets (Food and Agriculture Organization of the United Nations, 2022). This rising demand for dietary protein has already reached 2.02 million tons and is expected to increase significantly, ranging from an additional 3.6 to 12.5 million tons by 2050 (Safdar et al., 2023). The growing protein gap highlights an urgent need for sustainable, high-quality protein sources to ensure global food security and combat malnutrition on a large scale. Traditional protein sources, such as livestock and crops, require substantial amounts of land, water, and energy, making them increasingly unsustainable in the face of environmental concerns and food security issues (Noya et al., 2018). Microalgae offer a highly nutritious and sustainable protein source, rich in essential amino acids, vitamins, and bioactive compounds. With a short production cycle and significantly higher protein yield than animal and plant sources, they thrive on non-arable land and seawater, minimizing resource competition while reducing carbon emissions (Williamson et al., 2024). Unlike traditional protein sources, microalgae

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cultivation requires no fertilizers or pesticides and generates minimal waste (Li et al., 2024). Although initial production costs are high, technological advancements are expected to lower expenses. Additionally, their excellent functional properties make them versatile for food applications, with bioengineering enabling customized nutritional solutions (Table 1).

The protein content of microalgae typically ranges from 30 % to 55 % of their dry weight, and certain species, such as *Chlorella* and *Spirulina*, can reach even higher levels under optimized conditions (Lai et al., 2019; Thevarajah et al., 2022). Remarkably, studies have shown that microalgal protein yields can reach 4–15 t per hectare per year (MT ha^{-1} yr^{-1}), which is significantly higher than that of traditional protein sources such as soybeans (0.6–1.2 MT ha^{-1} yr^{-1}) and wheat (1.1 MT ha^{-1} yr^{-1}) (Koyande et al., 2019). This outstanding productivity highlights the potential of microalgae as a key resource for high-efficiency protein production, offering a scalable and environmentally friendly solution to global protein shortages. Beyond their impressive protein yield, microalgae possess an extraordinary ability to sequester carbon dioxide (CO_2) through photosynthesis, playing a crucial role in mitigating climate change (Onyeaka et al., 2021). They can also utilize industrial flue gases and wastewater as nutrient sources, converting waste into valuable biomass while simultaneously reducing greenhouse gas emissions (Wang et al., 2023b). This capability makes microalgae an essential technology for achieving carbon neutrality and promoting circular bioeconomy principles. Furthermore, microalgae can be cultivated in controlled environments, enabling year-round production with minimal environmental impact compared to conventional agriculture (Diankristanti et al., 2024).

Table 1
An analysis of the advantages of microalgae as an alternative protein source compared with traditional protein sources.

Comparison Items	Microalgae	Animal Protein	Plant Protein
Nutritional Components	High protein content (40–70 % DW), balanced essential amino acids, enriched with vitamins (B-complex, E), minerals, polyunsaturated fatty acids, and bioactive compounds; essential amino acid (EAA) index comparable to meat and soy.	Moderate protein content (15–25 % wet weight); complete EAA profile; rich in vitamin B12, heme-iron, zinc; but typically, high in total fat and saturated fatty acids.	Moderate protein content (20–40 % DW); abundant dietary fiber, vitamins, and minerals; often limited in lysine and/or methionine, requiring complementation.
Production Cycle	Rapid growth; doubling time <24 h under optimal conditions; scalable in bioreactors or open ponds year-round.	Longer, as the growth and development of animals require a certain amount of time	Longer, and plant growth is affected by factors such as seasons and climate
Production efficiency	High: protein yields of 4–15 t ha^{-1} yr^{-1} (e.g., Spirulina in raceway ponds >900 kg dry powder per mu annually).	Low: beef protein yields ~0.2–0.5 t ha^{-1} yr^{-1}; poultry and fish higher but still below microalgae.	Low to moderate: soybeans ~0.6–1.2 t ha^{-1} yr^{-1}; cereals typically <1 t ha^{-1} yr^{-1}.
Environmental Impact	Minimal land competition; can utilize non-arable land and seawater; mixotrophic cultivation can reduce or offset CO_2 emissions; high potential for circular production (wastewater, nutrient recycling).	High land and water demand; responsible for ~14.5 % of global GHG emissions; manure and runoff contribute to water and soil pollution.	Moderate: fertilizer and pesticide use cause eutrophication and soil degradation; significant land and water requirements.
Cost	High initial investment in industrial production equipment, but with the development of technology and large - scale production, the cost is expected to decrease	The breeding cost includes feed, breeding sites, epidemic prevention, etc., and the cost is relatively high	The planting cost includes seeds, chemical fertilizers, pesticides, labor, etc., and the cost is affected by market fluctuations
Processing and Application	Favorable functional properties (solubility, emulsification, foaming, gelation) comparable to whey and soy; amenable to protein engineering and formulation technologies (e.g., nanoencapsulation, 3D printing) for tailored applications.	Widely used in diverse food matrices; processing can enhance digestibility but may increase saturated fat intake or cause nutrient loss.	Established processing techniques (isolation, texturization); some plant proteins have off-flavors that reduce sensory acceptance.

In addition to their environmental and economic benefits, microalgal proteins offer superior nutritional quality. Microalgal protein, rich in essential amino acids, serves as an excellent alternative to both animal and plant proteins, offering a sustainable and nutritionally balanced solution for optimizing human dietary structures and enhancing food health benefits (Fig. 1). Among various microalgae species, *Chlorella pyrenoidosa* is primarily composed of approximately 60 % protein, 20 % carbohydrates, and 10 % lipids (Vani et al., 2022). Its protein content and essential amino acid profile are comparable to that of fishmeal, with notably high levels of l-lysine and polyunsaturated fatty acids. According to a FAO/WHO report, its amino acid scoring pattern is equivalent to that of egg protein (Cheng et al., 2020). This makes microalgae an outstanding candidate for addressing global protein demands while providing superior nutritional value. Microalgal-derived proteins can undergo extensive processing similar to conventional protein sources, allowing them to be transformed into protein powders, animal feed, food additives, functional foods, and dietary supplements (Yang et al., 2024b).

Beyond their use as a direct protein source, microalgal proteins can be further hydrolyzed via microbial fermentation or enzymatic digestion, either *in vitro* or *in vivo*, to produce bioactive peptides (Ashraf et al., 2025). These peptides, once purified to remove insoluble components, have demonstrated a range of significant biological activities, including anticancer, antihypertensive, anticoagulant, antioxidant, antitumor, lipid-lowering, hepatoprotective, and immune-modulating properties (Cunha and Pintado, 2022). Due to these remarkable biofunctional characteristics, microalgal-derived peptides hold great promise for applications in pharmaceutical research, functional food innovation, and the nutraceutical industry (Gong et al., 2025). As global interest in sustainable and health-promoting protein sources continues to rise, microalgal protein is poised to become a vital component in next-generation food and health products, contributing to both human well-being and environmental sustainability.

With increasing global demand for sustainable protein alternatives, microalgae represent a revolutionary approach to protein production. Their ability to thrive in non-arable environments, contribute to carbon sequestration, and provide high-quality protein makes them a key player in future food systems. Harnessing the potential of microalgae for large-scale protein production will require advancements in bioprocess engineering, genetic optimization, and cost-effective cultivation technologies. Nevertheless, ongoing research and industrial developments indicate that microalgae will play an essential role in addressing the world's protein needs while supporting global sustainability and environmental conservation efforts (Yu et al., 2024a).

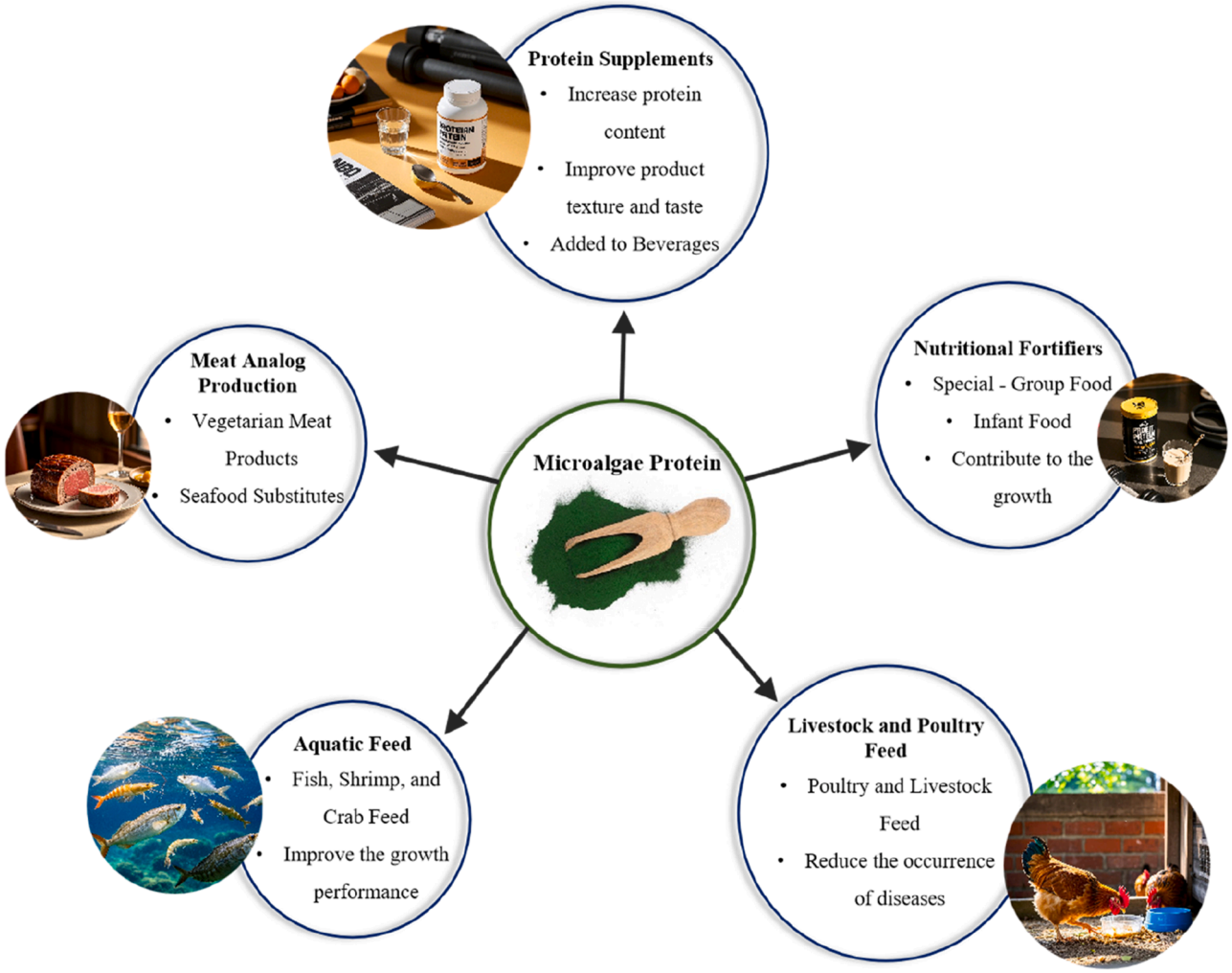

Fig. 1. The various application of microalgal protein in food and feed industries.

2. Technological and functional characteristics microalgal proteins

The techno-functionality of proteins refers to their unique physicochemical properties that influence their roles in food processing, beyond their nutritional value. These properties significantly impact food texture, viscosity, palatability, and mouthfeel. Techno-functional attributes are generally classified into surface properties (e.g., solubility, emulsification and foaming) and hydrodynamic properties (e.g., gelation, viscosity, thickening, and texturization) (Pan-utai and Iamtham, 2023). As the demand for sustainable functional ingredients grows, microalgal proteins have emerged as promising alternatives to traditional animal- and plant-based proteins. Studies, particularly on *Spirulina* and *Tetraselmis*, highlight their valuable techno-functional properties (Table 2). Additionally, specific microalgal species display promising techno-functional attributes: for instance, *Tetraselmis* protein exhibits emulsifying activity comparable to whey protein (Suarez Garcia et al., 2018), while *Spirulina* shows foaming performance similar to that of egg white (Lupatini Menegotto et al., 2019). When incorporating microalgal proteins into food formulations, factors such as processing methods, food matrices (e.g., emulsions, gels, foams), and interactions with other components must be carefully considered.

2.1. Emulsifying characteristics

The emulsifying properties of proteins refer to their ability to facilitate the formation and stabilization of emulsions between oil and water phases, making them valuable in various industries, including food, cosmetics, and pharmaceuticals (Lam and Nickerson, 2013). The underlying mechanism of emulsification lies in the unique structural characteristics of protein molecules. Their hydrophilic regions orient toward the aqueous phase, while the hydrophobic regions interact with the oil phase, effectively reducing surface tension at the oil-water interface and forming a stabilizing interfacial film. Additionally, proteins contribute to emulsion stability by generating steric hindrance and electrostatic repulsion, which help prevent the aggregation and coalescence of oil droplets (Lee et al., 2013). Several factors influence the emulsifying functionality of proteins, including their structural and physicochemical properties, as well as environmental conditions such as pH, temperature, and ionic strength (Binks and Rocher, 2009; Dai et al., 2020a; Othmeni et al., 2025; Perez et al., 2022).

Microalgal proteins generally exhibit strong emulsifying capacity due to their amphiphilic nature and high solubility when removed from intact cell walls. For example, a water-soluble extract of *Chlorella protothecoides* stabilized oil-in-water emulsions for over a week, outperforming whey protein, with emulsions remaining stable even at 0.5 M NaCl and across pH 2–9 (Ebert et al., 2019; Grossmann et al., 2019a). Optimal emulsification typically occurs away from the protein isoelectric point: *Tetraselmis* sp. protein showed its highest emulsifying ability at neutral pH (5–7), whereas around pH 3–5 (isoelectric point below pH 3) solubility and emulsifying activity dropped sharply (Schwenzfeier et al., 2013a). Similarly, *H. pluvialis* proteins had better emulsifying

Table 2
Techno-functional properties of the crude protein extracts derived from microalgae.

Microalgae	Property	Remarks	References
Tetraselmis sp.	Foam formation	Facilitates the generation of stable foams through the selective adsorption of the protein fraction onto the air-water interface.	(Schwenzfeier et al., 2013b)
Tetraselmis sp.	Gelation, Foam formation, Emulsification	Exhibited superior surface activity and gelation characteristics compared with whey protein isolate.	(Suarez Garcia et al., 2018)
Tetraselmis sp.	Foam formation, Emulsification	The fractionation process resulted in a notable improvement in both the emulsion stability and the foaming properties.	(Schwenzfeier et al., 2014)
Arthrospira platensis	Foam formation	Exhibits more excellent foaming properties compared with whey protein isolate.	(Buchmann et al., 2019b)
Nannochloropsis gaditana	Protein solubility, Emulsification, Foaming	High protein solubility shows a positive correlation with the emulsifying and foaming properties.	(Valero-Vizcaino et al., 2024)
Arthrospira platensis	Emulsification, Oil-binding, Foam, formation Emulsification	The functional properties are significantly influenced by the pH value; the emulsifying and foaming capabilities are positively correlated with the protein solubility.	(Benelhadj et al., 2016)
Chlorella sp.	Foam stabilization, Porous material templating	Forms stable capillary foams via oil bridges, enabling porous material construction with high adsorption and regeneration efficiency.	(Yu et al., 2024b)
Chlamydomonas reinhardtii	Solubility, Foaming stability	Demonstrates the lowest foaming capacity but better foaming stability than *Spirulina platensis* and *Spirulina maxima* due to higher polysaccharide content.	(Hong et al., 2024)
Euglena gracilis	Water/oil binding, Emulsification, Foaming capacity	Exhibits the highest water/oil binding capacity, emulsifying activity, and foaming stability due to high surface hydrophobicity and β-sheet content.	(Hong et al., 2024)

performance when extracted at neutral pH than at pH 5.7 (Ba et al., 2016). Notably, several microalgae match or exceed conventional proteins in emulsification metrics: spray-dried *Spirulina platensis* achieved higher emulsion stability than egg white protein (Nirmala et al., 1992), and the red algae (*Porphyridium cruentum*) and a diatom (*Phaeodactylum tricornutum*) showed greater emulsifying capacity than soy protein isolate (Guil-Guerrero et al., 2004). Likewise, soluble protein fractions from *Chlorella* and *Haematococcus* were found to emulsify oil comparably to soy protein or sodium caseinate (Ba et al., 2016; Ursu et al., 2014). These findings underscore that, with proper extraction, microalgal proteins can form interfacial films as effectively as traditional animal or plant emulsifiers.

2.2. Foaming properties

The foaming capacity of a protein refers to its ability to generate a large interfacial area, while foam stability reflects its capability to maintain the air bubbles formed. Studies have shown that microalgal proteins exhibit excellent foaming properties. Several elements impact protein foaming performance, including source, composition, concentration, extraction and processing methods, solubility, pH, temperature, and the presence of carbohydrates or lipids (Ding et al., 2024; Yu et al., 2023; Zhang et al., 2023, 2022). For instance, proteins extracted from *Spirulina platensis* display increasing foaming capacity at pH levels above 3.0, reaching over 250 % in alkaline conditions (pH $\geq$10) (Benelhadj et al., 2016). Similar studies have shown that the foaming capacity and foam stability of *Spirulina* protein are strongly pH-dependent, reaching a minimum near its isoelectric point (~pH 3.5) and peaking at pH $\geq$ 7, where the proteins are more soluble and flexible (Devi and Venkataraman, 1984). This behavior parallels other proteins (e.g. egg albumen foams best at pH >6). Purity and concentration also influence foaming: a crude *Spirulina*-containing powder yielded less stable foams than a purified *Spirulina* protein isolate, and in general a concentrated protein isolate produces finer, more stable foams (Buchmann et al., 2019a). Enzymatic or thermal pretreatments can further enhance foamability; for instance, hydrolysis of insoluble *C. protothecoides* proteins increased their foaming capacity by exposing hidden hydrophobic site (Dai et al., 2020b). Ultrasound-assisted extraction (UAE) markedly enhanced the techno-functional performance of *Spirulina* protein, with foaming capacity from 40.56 % to 90.00 %, and stability from 35.85 % to 79.17 % (Purdi et al., 2023). Species-specific results highlight both strengths and limitations. *Tetraselmis chui* protein isolates formed foams more stable (at pH 5–7) than those stabilized by whey protein isolate or egg white albumin (Schwenzfeier et al., 2013b). *Haematococcus* protein likewise demonstrated higher foaming capacity than several plant proteins (barley, mung bean, chickpea, lupin), suggesting microalgal proteins can outperform many legume proteins in foam formation (Zhu et al., 2019). On the other hand, *Scenedesmus obliquus* protein concentrates show more moderate foaming performance: one report found a foaming capacity of ~20 % volume increase, which is only half that of egg white (~40 % under the same conditions) but far above soy protein isolate's negligible foam (~1 %) (Garcia-Encinas et al., 2025). The *Scenedesmus* foam was also less stable (about 25 % stability after a set time, vs. ~100 % for egg white) (Garcia-Encinas et al., 2025), indicating that while microalgal proteins can create foams, they may not match the long-term stability of egg white foams without formulation aids.

2.3. Gelling properties

The gelling property of proteins refers to their ability to form a structured three-dimensional network under specific conditions, facilitated by intermolecular interactions such as hydrogen bonding, hydrophobic interactions, electrostatic forces, and disulfide linkages (Nicolai, 2019). This network effectively traps water or other solvents, transforming the system from a liquid into a semi-solid or solid gel-like state. Several factors influence this process, including protein type and

concentration, pH, ionic strength, and temperature (Lv et al., 2022).

Microalgal proteins can form heat-induced gels, with required concentrations (minimum gelling concentrations) ranging from very low (similar to egg albumen) to moderate (similar to soy or pea proteins), depending on the species and preparation. Protein-rich extracts from *Chlorella, Spirulina, Tetraselmis*, etc., undergo unfolding and network aggregation upon heating to yield viscoelastic gels, much like conventional globular proteins (Chronakis, 2001). *Chlorella sorokiniana* protein isolate, for instance, began gelling at ~61 °C and formed a non-pourable gel after heating to ~65–80 °C; a minimum of ~9.9 % (w/v) protein was needed to achieve a self-supporting gel, a value comparable to the gelling thresholds of whey, pea, and soy protein isolates (Grossmann et al., 2019b). The gel formed by *Chlorella* protein was somewhat soft (yogurt-like in consistency), and its firmness was sensitive to conditions–higher temperature (≥80 °C) improved rigidity, whereas high ionic strength or pH away from neutral reduced gel elasticity and firmness (Grossmann et al., 2019b). In contrast, *S. platensis* proteins can gel at remarkably low concentrations under favorable conditions. Previous studies found that a *S. platensis* protein isolate could form a gel at only ~1.5 % protein (in 0.1 M Tris buffer, pH 7) and ~2.5 % in the presence of 0.02 M $CaCl_2$ (Chronakis, 2001). It should be noted that such low gelling concentrations for Spirulina were achieved under optimized conditions (neutral pH with buffering salts); in plain distilled water, the same isolate required a much higher ~12 % protein to gel, reflecting the importance of ionic environment and protein–protein interactions in network formation (Benelhadj et al., 2016). *Tetraselmis suecica* is another example: soluble protein extracts from *Tetraselmis* formed stronger gels at 10 % protein than did whey protein at the same concentration (Grossmann et al., 2019b). The *Tetraselmis* gel network gained rigidity upon cooling (after heating to 90 °C), implying that both covalent and non-covalent interactions (hydrophobic interactions, hydrogen bonding) developed to a greater extent than in the whey protein system (Grossmann et al., 2019b).

Mechanistically, microalgal proteins bring certain advantages to gelation. Many species produce high-molecular-weight, multimeric proteins that present multiple binding sites for network formation. For example, *Chlorella* and other green algae are rich in RuBisCO (ribulose-1,5-bisphosphate carboxylase/oxygenase), a large enzyme complex (~560 kDa holoenzyme, with ~55 kDa large subunits) that can contribute to gel structure (Prates, 2025). *Spirulina* and other cyanobacteria contain phycobiliprotein complexes such as C-phycocyanin (~210 kDa as a trimer of α/β subunits) which might be expected to aid gel formation by virtue of size and multimeric structure (Yu et al., 2024c). However, studies indicate that not all abundant proteins actually integrate into the gel network. In *S. platensis* gels, the pigmented phycobiliproteins were found *not* to be involved in gel cross-linking; instead, gelation was dominated by other *Spirulina* proteins through hydrophobic aggregation, hydrogen bonding, and intermolecular disulfide bond formation (Benelhadj et al., 2016). The exposure of hydrophobic residues (e.g. via unfolding at high temperature or by high-pressure treatment) appears to drive the initial aggregation and network assembly, while disulfide bridges (if cysteine content is sufficient) help stabilize the gel's elastic structure (Lozober et al., 2021). Microalgal proteins generally form opaque, irreversible heat-set gels (analogous to soy or egg gels) rather than transparent cold-set gels, given the need for unfolding and denaturation to enable intermolecular binding (Pipliya et al., 2025). As with other proteins, external factors can modify gelation behavior: for example, high-pressure homogenization pre-treatments have been shown to improve *Spirulina* protein gelation, yielding stiffer gels (higher storage modulus G') and lowering the gelation onset temperature by inducing partial unfolding of the proteins prior to heating (Pipliya et al., 2025). Conversely, proteolytic hydrolysis tends to impair gel formation–pepsin-treated *Spirulina* concentrate failed to gel, presumably because extensive hydrolysis disrupted the macromolecular network needed for gelation (Lozober et al., 2021).

2.4. Water-holding capacity

The water-holding capacity of proteins plays a crucial role in various biological and industrial applications. It refers to a protein's ability to bind and retain water, which significantly impacts food texture, taste, and shelf life (Zayas, 1997). In food systems, this property helps maintain moisture in products like meat and bread, preventing dryness and deterioration while preserving tenderness and juiciness (Schopf and Scherf, 2021; Szmańko et al., 2021). Beyond food applications, water retention is essential for maintaining cellular integrity and physiological function in living organisms. It ensures that biochemical reactions occur in an optimal aqueous environment and contributes to tissue elasticity and flexibility. For instance, collagen in skin and tendons relies on its water-binding properties to sustain tissue strength and resilience, playing a vital role in structural maintenance (Balasubramanian et al., 2012). Additionally, the water-holding ability of proteins is highly valuable in the cosmetics and pharmaceutical industries. In skincare formulations, it enhances moisture retention, helping to maintain skin hydration and improve product efficacy (Husein el Hadmed and Castillo, 2016).

Microalgal protein ingredients often demonstrate high water-holding capacity, which is beneficial for maintaining moisture in food products. *S. platensis* protein isolates, for instance, can hold substantial water – up to ~428.8 g water per 100 g protein (≈4.3 g H_2O per g) at pH 10 (Benelhadj et al., 2016). *H. pluvialis* protein concentrate exhibited water-holding capacity around 4.06 g water per g, which is comparable to the water-holding of yellow pea or green lentil protein concentrates and higher than that of certain other legume proteins like chickpea (Bertsch et al., 2021). *S. obliquus* proteins are likewise noted to have good water-holding ability (Prates, 2025), aligning with their high proportion of hydrophilic amino acids and the presence of high-molecular-weight fractions that can entrap water. Despite repeated demonstrations of emulsification and gelation, microalgal proteins still face key bottlenecks such as poor solubility near neutral pH, limited heat stability, and inconsistent performance in complex food matrices. These issues currently limit their competitiveness with plant proteins like soy or pea, highlighting the need for structural modification, enzymatic treatment, and advanced delivery systems to enable broader food applications.

3. Microalgal cultivation for protein improvement

The production of microalgal protein feed products involves multiple critical steps, with a primary focus on optimizing cultivation methods, strategic approaches, and reactor configurations. Furthermore, the development of protein cell factories through synthetic biology techniques is essential for improving amino acid profiles. Microalgae can be cultivated using various systems, including open raceway ponds, airlift bioreactors, photobioreactors, and fermenters (Sun et al., 2021).

3.1. Cultivation conditions

3.1.1. Light

Light plays a crucial role in the growth and development of microalgae, exerting its influence through two primary mechanisms. The first is an indirect effect via photosynthesis, a high-energy reaction, while the second is a direct effect through photomorphogenesis, a low-energy response. Both processes involve the perception of light signals through pigments and photoreceptors, which in turn regulate downstream gene pathways, ultimately influencing cell growth, metabolism, and functional changes (Maltsev et al., 2021; Straka and Rittmann, 2017).

Microalgae absorb light energy (photons) and convert it into chemical energy in the form of ATP and NADP. These reactions occur within the photosynthetic system, where light-harvesting complex (LHC) pigments capture photons and transfer the energy as an electron flow to the

reaction centers of photosystems (PS) (Mirkovic et al., 2016). Since different microalgal species possess distinct pigment compositions within their LHCs, they can absorb photons of varying wavelengths and efficiently convert them into chemical energy through photosynthesis (Lehmuskero et al., 2018). Typically, the effective wavelength range for microalgal photosynthesis falls between 400 nm and 700 nm, with blue light (420–490 nm) and red light (610–700 nm) being particularly beneficial (Esteves et al., 2025). It is well established that PSI is primarily driven by blue light, while PSII is more responsive to red light, meaning that specific wavelengths can influence microalgal growth and biochemical composition in a species-dependent manner.

Evidence suggests that blue LED light effectively promotes cell division and leads to the formation of larger cell sizes. Furthermore, blue light has been observed to regulate RuBisCO activity, enhancing the efficiency of the photosynthetic electron transport chain and increasing carbon fixation rates. It also plays a role in lipid metabolism by influencing the activity of carbonic anhydrase and ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO), thereby facilitating triglyceride accumulation and increasing lipid content. Additionally, blue light can improve lipid composition and stimulate the expression of fatty acid biosynthesis genes in *Chlorella* sp., leading to higher lipid yields (Baidya et al., 2021). Some studies have further demonstrated that *Chlamydomonas reinhardtii* cultivated under blue light contains higher protein levels, likely due to the inhibition of cell division, which in turn allows for greater protein synthesis and accumulation (Li et al., 2023). In contrast, red light is generally considered the optimal wavelength for biomass accumulation in microalgae. Red light enhances LHC function, providing energy to chlorophyll *a* and promoting electron flux, which supports organic biosynthesis and chlorophyll development (Esteves et al., 2025). For example, *S. obliquus* and *Nannochloropsis gaditana* achieved maximum algal biomass under red LED illumination, primarily due to the increased absorption of photons by chlorophyll, which enhances photosynthetic energy capture and accelerates metabolic activity (He et al., 2021; Kim et al., 2014). Regarding central carbon metabolism, the tricarboxylic acid (TCA) cycle, Embden-Meyerhof-Parnas (EMP) pathway, and pentose phosphate pathway (PPP) are all stimulated under red light, providing more energy and metabolic precursors to support rapid cell growth (Patelou et al., 2020).

3.1.2. Temperature

Temperature is a critical factor influencing microalgal growth, as it directly impacts nutrient uptake and CO_2 fixation, ultimately affecting biomass accumulation and lipid composition. Tan et al. (Tan et al., 2014) found that when *C. pyrenoidosa* was cultivated under varying temperature conditions, the proportion of unsaturated fatty acids increased at lower temperatures, helping to maintain membrane fluidity. In contrast, some studies have reported that certain microalgae reduce their unsaturated fatty acid content in response to cold stress (Venkata Subhash et al., 2014). These differences arise from the distinct optimal growth temperatures among microalgal species. When exposed to suboptimal temperatures, microalgae undergo adaptive metabolic adjustments to enhance their survival under stress. Consequently, maintaining an optimal temperature often requires cooling or heating systems, increasing operational costs—particularly in long-term continuous cultivation. To improve productivity and stimulate the accumulation of specific high-value biomolecules, temperature regulation is typically integrated with other cultivation strategies and system optimizations (Chen et al., 2019; Giossi et al., 2025; Hemker et al., 2024; Menegol et al., 2017).

3.2. Cultivation strategy

Microalgal cultivation modes can be classified into four categories based on carbon source, energy source, and light conditions: photoautotrophic, heterotrophic, mixotrophic, and photoheterotrophic. Photoautotrophic cultivation relies exclusively on CO_2 as the carbon source and converts light energy into chemical energy through photosynthesis. This is the most widely applied mode for large-scale production (Razzak, 2024). However, high-density photoautotrophic cultures face light penetration limitations, which can lead to reduced protein synthesis efficiency (Table 3). For instance, the highest reported productivity of *S. platensis* under photoautotrophic conditions is 0.62 g L^{-1} d^{-1} (Chaiklahan et al., 2022).

In contrast, heterotrophic cultivation enhances metabolism by utilizing organic carbon sources (e.g., glucose), achieving biomass productivities typically 5–10 times higher than those of photoautotrophic cultivation, depending on species and culture conditions (Table 3). In fed-batch cultures, *Chlorella* species have been shown to reach biomass concentrations of 200–300 g L^{-1} (Jin et al., 2021). However, heterotrophic metabolism tends to direct carbon flux toward starch or lipid accumulation rather than protein synthesis, often resulting in lower protein content. Recent research has explored precise nitrogen regulation strategies to overcome this limitation, particularly through luxury nitrogen assimilation (constant nitrogen excess) and phased nitrogen concentration shifts. The latter induces starch accumulation during the early phase under nitrogen limitation (up to 55 % of biomass), followed by high nitrogen supplementation in the later phase to stimulate protein accumulation, ultimately increasing protein content to 60 % in *Chlorella* sp. MBFJNU-17 (Xiao et al., 2022). At the molecular level, these shifts are likely mediated by nitrogen-responsive transcription factors and metabolic reprogramming, though further studies are needed to elucidate specific regulatory mechanisms.

Given the limitations of heterotrophic cultivation in maintaining high protein content, recent efforts have explored integrated cultivation approaches to leverage the benefits of both modes. For example, transferring heterotrophically cultivated cells into a photoautotrophic environment after dilution can trigger protein synthesis via light signaling, as evidenced by an increase in protein content from 38.55 % to 50.87 % in *Chlorella vulgaris* (Fan et al., 2012). Alternatively, optimizing carbon supply during the heterotrophic phase before shifting to photoautotrophic conditions can activate specific metabolic pathways, improving both biomass productivity and biochemical composition (Wang et al., 2020). The central challenge of mixotrophic cultivation is to balance the use of external carbon sources with light-driven photosynthesis. Excess glucose often inhibits the photosynthetic machinery. To address this, a fed-batch strategy that dynamically controlled glucose availability was implemented, leading to a 90.6 % increase in biomass concentration, reaching 1.62 g L^{-1} in a photobioreactor (Yang et al., 2021).

Mixotrophic and heterotrophic cultivation strategies have been widely studied, yet critical bottlenecks persist. Heterotrophic growth achieves high biomass yields but depends on carbohydrate supplementation, driving up costs and competing with food resources. Mixotrophic systems often show unstable productivity under fluctuating light and nutrient conditions, while dense cultures are further constrained by oxygen accumulation and light attenuation. Emerging approaches involve coupling photobioreactors with waste valorization (Wang et al., 2024) and applying metabolic engineering to prioritize protein over lipid biosynthesis (Wang et al., 2023a), thereby aligning cultivation with application-oriented goals.

3.3. Bioreactor design

The design of bioreactors is central to enhancing protein content in microalgae by optimizing growth conditions and metabolic regulation (Wang et al., 2025). Innovative designs are shifting from mere performance enhancement to deeper integration with the circular economy. For instance, a conical spiral photobioreactor can be freely suspended from the ceiling of a chicken house, capturing CO_2 and NH_3 exhaust gases while continuously cultivating *Spirulina* (productivity > 0.3 g L^{-1} d^{-1}). This system achieves both emission sequestration and biomass valorization, meeting dual objectives (Glockow et al., 2023). In addition, coupling microalgal cultivation with agricultural and industrial

Table 3
Microalgal cultivation strategies for protein production under varying conditions.

Microalgae	Biomass concentration (g L^{-1})	Protein content (% DW)	Protein concentration (g L^{-1})	Culture conditions	Reference
Bottleneck 1: Difficult to achieve heterotrophic high-density growth (<10 g L^{-1})					
Nannochloropsis oceanica	4.2	55	2.31	The light intensity is 100 μmol/m²/s.	(Verma et al., 2020)
Scenedesmus acutus	10.2	44	4.49	The light intensity is 120 μmol/m²/s, and the concentration of glucose is 15 g L^{-1}.	(Ma et al., 2017)
Spirulina maxima	0.61	77.3	0.47	Sugarcane vinasse as a carbon source in the two-step photoautotrophic-heterotrophic growth process	(dos Santos et al., 2016)
Spirulina sp.	1.1	56.8	0.62	Glucose concentration at 0.5 g L^{-1}	(Cruz et al., 2023)
Chlorella pyrenoidosa	4.5	61.8	2.79	Using enzymatic hydrolysis of wheat straw as substrate. Nitrogen at 1080 mg L^{-1}.	(Zhang et al., 2017)
Chlorella vulgaris	6.3	61.6	3.88	C/N ratio of 12:1 Supplementary ratio of 15 % for BRH.	(Cai et al., 2022)
Chlorella vulgaris	2.1	58	1.22	The light intensity is 150 μmol/m²/s, the concentration of CO_2 is 2 %, and the culture medium is BG11.	(Cao et al., 2023)
Chlorella pyrenoidosa	9.94	59.9	5.96	The mutant strain K05, with a 20 % substitution of sweet sorghum juice for glucose.	(Song et al., 2018)
Bottleneck 2: Decreased intracellular protein content (<40 % DW) after heterotrophic growth.					
Galdieria sulphuraria	80–116	<0.5 phycocyanin	0.25–0.4 phycocyanin	Light induction after heterotrophic growth	(Schmidt et al., 2005)
Galdieria sulphuraria	102.4	2.7 phycocyanin	2.94 phycocyanin	Fed-batch culture	(Graverholt and Eriksen, 2007)
Spirulina platensis	10.2	5.4 phycocyanin	0.55 phycocyanin	Two-step photoautotrophic-heterotrophic growth process.	(Chen and Zhang, 1997)
Galdieria sulphuraria	250.5	13.2	33.1	Heterotrophic-dilution-light induction	(Wan et al., 2016)
Chlorella sorokiniana	247	21.9	54.1	Glucose pulse flow with a C/N ratio of 16:1 (potassium nitrate)	(Jin et al., 2021)
Chlorella protothecoides	91.4	22.3	20.38	Nitrogen limitation two-step process	(Wang et al., 2016)
Galdieria sulphuraria	18	24.7	4.45	Heterotrophic-dilution-light induction	(Wan et al., 2016)
Chlorella regularis	90	<28	<25.2	Heterotrophic synchronous cultivation method	(Sansawa and Endo, 2004)
Chlorella protothecoides	64	34.5	22.0	Glycerol pulse feeding	(Ceron-Garcia et al., 2013)
Chlorella vulgaris	18.5	35	6.48	Glucose concentration at 30 g L^{-1}, urea 5 g L^{-1}, under dark conditions, pH 6.5	(Cao et al., 2023)
Scenedesmus obliquus	22.3	38	8.47	The concentration of glucose is 40 g L^{-1}, the concentration of sodium nitrate is 8 g L^{-1}, it is cultured by fed-batch operation, and the C/N ratio is 20	(Thiansathit et al., 2015)
Chlorella pyrenoidosa	25.6	32	8.19	The concentration of glucose is 50 g L^{-1}, the concentration of urea is 10 g L^{-1}, pH is equal to 7.0, and the dissolved oxygen (DO) is 40 %	(Chen et al., 2024)
Chlorella pyrenoidosa	21.8	41	8.94	The concentration of glucose is 50 g L^{-1}, the concentration of ammonium nitrate is 8 g L^{-1}, pH is equal to 7.0, and the dissolved oxygen (DO) is 40 %	(Chen et al., 2024)
Nannochloropsis oceanica	15.8	34	5.37	C/N ratio (Carbon from glucose and nitrate from $NaNO_3$)	(Verma et al., 2020)
Chlorella sorokiniana	52.1	33	17.19	Two-stage cultivation: In the first stage, there is a high carbon content (glucose 60 g L^{-1}), and in the second stage, nitrogen is supplemented (ammonium nitrate 10 g L^{-1})	(Xie et al., 2022)
Chlorella vulgaris	81.6	36.5	29.8	Glucose 80 g L^{-1} KNO_3 16.2 g L^{-1}	(Doucha and Lívanský, 2012)

side-streams has proven highly effective. Dairy wastewater supported mixotrophic cultivation of *Monoraphidium* sp. SVMIICT6 in flat-panel photobioreactors, enabling biomass productivities of 50 mg L^{-1} d^{-1} with COD, nitrate and phosphate removal efficiencies of 75 %, 85 % and 60 %, respectively, thereby coupling effective effluent remediation with biomass generation for downstream applications (Divya Kuravi and Venkata Mohan, 2022). Similarly, coupling microalgae cultivation with anaerobic–aerobic or sequencing batch reactors treating dairy wastewater promoted nutrient assimilation and biomass generation, achieving protein content of ~55 % while enhancing removal of COD, nitrogen, and phosphorus, thereby demonstrating the potential for integrated waste-to-biomass systems (Zkeri et al., 2021).

Conventional tubular and flat-panel reactors depend on stringent control of light and temperature to enhance productivity. By contrast, advanced configurations such as thin-layer cascade reactors employ ultra-thin suspension layers (0.5–1 cm), markedly increasing biomass density (Schädler et al., 2020). Photovoltaic-integrated systems have also attracted attention, achieving up to 75 % energy self-sufficiency while sustaining stable *Spirulina* production, with phycocyanin yields of 16.3 mg g^{-1} d^{-1} (Nwoba et al., 2020).

Additionally, photobioreactor systems used for wastewater treatment can simultaneously purify wastewater and enhance microalgal protein content, reaching up to 54.56 % (Solmaz and Işık, 2019). These cases highlight the core logic of bioreactor design: precision control of environmental factors (e.g., light intensity, mixing efficiency), coupled with low-carbon energy (e.g., photovoltaics) and waste inputs (e.g., exhaust gases or wastewater). Modular adaptations further reduce scaling-up costs. Future breakthroughs will rely on technological and contextual synergies to advance microalgal protein production from the laboratory to the circular economy network.

4. Amino acid metabolism and improvement in microalgae

4.1. Major amino acid metabolic pathways and biological functions

In recent decades, extensive research has been conducted on the role of amino acids in plant development and their responses to biotic and abiotic stresses. During photosynthesis, microalgae assimilate CO_2 and H_2O to synthesize carbohydrates, primarily glucose. The intracellular biosynthesis of amino acids occurs through three major pathways (Gorunmek et al., 2024). The pentose phosphate pathway (PPP) involves the direct oxidation of glucose, which is used for the synthesis of the histidine (His) family. The glycolysis pathway (EMP) facilitates the conversion of glucose in the cytoplasm to produce amino acids such as alanine (Ala), aromatic amino acids, and the serine (Ser) family. Meanwhile, the tricarboxylic acid (TCA) cycle generates pyruvate from mitochondrial glucose oxidation, which contributes to the synthesis of the glutamic acid (Glu) family. These pathways form an interconnected metabolic network with EMP, the TCA cycle, and PPP playing crucial roles in amino acid biosynthesis.

The amino groups required for amino acid synthesis primarily come from nitrate or ammonia absorbed by the cells, which are assimilated into glutamic acid and further converted into glutamine. Additionally, the carbon skeletons of amino acids frequently serve as precursors or intermediates in the TCA cycle. Studies have shown that amino acid metabolism significantly influences the overall metabolic state of microalgae. For instance, Liang et al. (Liang et al., 2009) reported that in *C. reinhardtii*, branched-chain amino acid catabolism enhances acetyl-CoA (AcCoA) levels, promoting lipid accumulation. Specifically, ketogenic amino acids, including leucine (Leu), lysine (Lys), tryptophan (Trp), phenylalanine (Phe), and tyrosine (Tyr), are metabolized into AcCoA. In contrast, glucogenic amino acids, such as glutamic acid (Glu), glutamine (Gln), aspartic acid (Asp), arginine (Arg), histidine (His), proline (Pro), methionine (Met), and threonine (Thr), are converted into glucose precursors and intermediates involved in glucose metabolism. Through the EMP pathway, glucose is further transformed into AcCoA, which is a key precursor for ATP generation and the biosynthesis of other amino acids under high-energy conditions.

Beyond their fundamental role as protein building blocks, amino acids are integral to multiple physiological processes. They support cell growth and development, regulate intracellular pH balance, contribute to energy production and redox homeostasis, and enhance stress resistance by helping microalgae adapt to fluctuating environmental conditions. Additionally, amino acids participate in cellular signaling and gene expression regulation, affecting key metabolic pathways. Research indicates that microalgae alter their amino acid profiles in response to environmental stresses, leading to significant metabolic adaptations (Navarova et al., 2012). Certain amino acids, such as Lys, Trp, His, Leu, and Ser, are particularly crucial as they actively contribute to microalgal growth, metabolism, and productivity through their involvement in cell signaling and metabolic regulation (Wu et al., 2014).

The importance of free amino acids extends beyond their role in protein biosynthesis. They serve as essential intermediates in diverse metabolic pathways and contribute to microalgal adaptation under stress conditions. Despite the rapid advancement of microalgal biotechnology, the specific roles of amino acids in stress responses remain incompletely understood due to variations in species diversity and differences in stress type, duration, and intensity.

A notable challenge in traditional plant-based proteins is the deficiency of essential amino acids, particularly lysine and methionine. In contrast, microalgae are rich in lysine, which not only enhances nutrient absorption through non-competitive inhibition mechanisms but also accelerates growth and improves overall biomass quality (Table 4). As lysine is a key limiting amino acid in *C. pyrenoidosa*, understanding its role in stress adaptation and optimizing its metabolic pathway within microalgal cells is crucial for improving its biotechnological applications.

4.2. Lysine metabolism and biological functions

Lysine biosynthesis in plants occurs through a specific branch of the aspartate family pathway. As illustrated in Fig. 2, the first enzyme in this pathway is aspartate kinase (AK), which catalyzes the phosphorylation of aspartate to form aspartyl-phosphate. This enzyme is subject to feedback inhibition by lysine and threonine. The next step involves the NADPH-dependent reduction of aspartyl-phosphate into aspartate semialdehyde, a reaction catalyzed by aspartate-semialdehyde dehydrogenase (ASADH). Subsequently, homoserine dehydrogenase (HSDH) utilizes NADH to convert aspartate semialdehyde into homoserine (Stepansky et al., 2006).

At this stage, lysine biosynthesis proceeds via two possible pathways: the α-amino adipic acid (AAA) pathway and the diaminopimelic acid (DAP) pathway, the latter of which is predominantly found in algae, fungi, plants, and bacteria. In the DAP pathway, tetrahydropyridine dicarboxylate is first formed through the aspartate pathway, and lysine is ultimately synthesized via the action of diaminopimelate decarboxylase. A crucial enzyme unique to lysine biosynthesis is

Table 4
Comparison of the amino acid composition between foods and microalgae.

Amino acids (%)	Soybean meal (Winkler et al., 2011)	Dried yeast (Winkler et al., 2011)	Fish meal (Øverland et al., 2013)	*Chlorella vulgaris* (Becker, 2007)	*Nannochloropsis* (Gerde et al., 2013)
Essential amino acid					
Arginine	8.30	5.54	6.68	6.60	6.70
Histidine	3.04	3.30	2.51	2.06	2.00
Isoleucine	5.22	5.54	4.28	3.92	4.77
Leucine	8.30	8.75	7.98	9.07	9.57
Lysine	6.95	9.70	8.67	8.66	7.23
Methionine	1.97	2.98	3.19	2.27	2.00
Phenylalanine	4.88	4.34	4.16	5.15	5.27
Threonine	4.09	5.74	5.07	4.95	4.78
Valine	3.85	9.00	5.02	5.67	6.44
Tryptophan	–	–	1.07	2.16	2.47
Non-essential amino acid					
Alanine	4.61	6.27	6.38	8.14	6.77
Aspartic acid	11.91	9.73	10.52	9.28	9.56
Glycine	3.99	4.09	5.62	5.98	5.49
Glutamic acid	18.07	10.39	15.22	11.96	12.19
Cysteine	2.20	1.93	1.07	1.44	1.00
Tyrosine	3.22	3.49	3.37	3.51	4.01
Proline	4.37	3.99	4.16	4.95	5.99
Serine	5.04	5.23	5.04	4.23	3.77

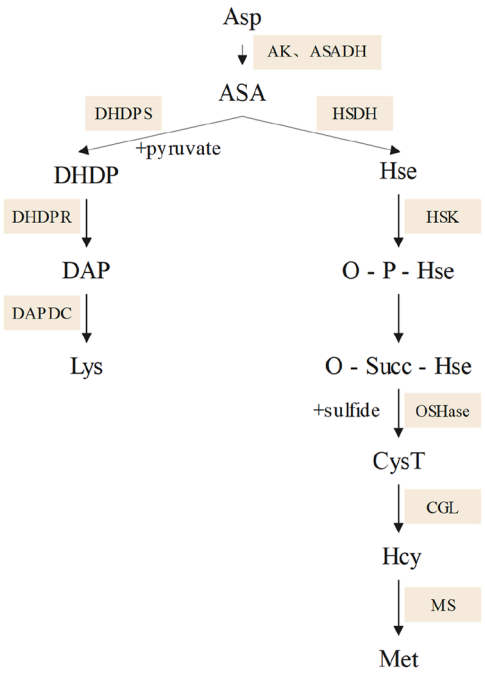

Fig. 2. The biosynthetic pathways of lysine and methionine.

dihydrodipicolinate synthase (DHDPS), which facilitates the condensation of pyruvate and aspartate semialdehyde to form dihydrodipicolinate. This reaction is a key regulatory step in lysine biosynthesis, as it is highly specific to the lysine branch and subject only to lysine inhibition (Stepansky et al., 2006).

Lysine degradation is also triggered by biotic and abiotic stresses. While the complete catabolic pathway of lysine remains poorly defined, its initial steps are well documented, linking lysine degradation to the synthesis of N-hydroxy-pipecolic acid (NHP) and AAA. Lysine is recognized as one of the most energy-rich amino acids, capable of providing electrons to the mitochondrial electron transport chain (ETC) either indirectly, through conversion into intermediates of the TCA cycle, or directly as an electron donor (Hildebrandt et al., 2015). Notably, in response to carbon starvation induced by darkness, lysine levels increase in *Arabidopsis thaliana* to support cellular survival (Hirota et al., 2018). These findings suggest that lysine biosynthesis and degradation play essential roles in cellular stress responses. For example, lysine accumulation has been shown to protect yeast cells from freezing, dehydration, and oxidative stress (Olin-Sandoval et al., 2019) and to aid plant cells in coping with drought-induced environmental stress (Demirel et al., 2020). Experimental evidence further supports that enhanced lysine accumulation improves the heat tolerance of *Escherichia coli* under high-temperature conditions (Isogai and Takagi, 2021). In plants, blue light stimuli have been found to upregulate lysine biosynthesis pathway genes, highlighting its potential role in photoregulatory mechanisms. Wang et al. (2024a) demonstrated that red light-emitting diode (LED) treatment of *C. pyrenoidosa* markedly increased protein content and lysine accumulation, elevating lysine levels from ~6.2 % to 9.8 %—comparable to those of fish meal.

These studies underscore the strong correlation between lysine metabolism and environmental stress adaptation. In microalgae, stress resistance mechanisms involve metabolic adjustments that sustain growth under harsh conditions. However, the precise molecular mechanisms underlying lysine-mediated stress tolerance in microalgae remain largely unexplored. To fully understand the role of lysine in stress response, further research into lysine gene regulatory networks is necessary.

Despite growing interest in leveraging lysine biosynthesis for stress adaptation, the extent to which lysine accumulation enhances stress tolerance remains poorly understood. The ability of lysine to support the development of robust microalgal strains is particularly relevant for optimizing microalgal production of valuable compounds under challenging environmental conditions. Additionally, lysine plays a crucial role in regulating the expression of essential intracellular enzymes, thereby ensuring normal cellular growth and high metabolic productivity.

A deeper understanding of how lysine contributes to stress adaptation could pave the way for the rational design and optimization of microalgal strains, enhancing both their resilience and productivity. Accurate sensing and regulation of amino acid levels are of particular importance, as they influence protein synthesis, amino acid metabolism, and cellular energy balance. In recent years, advancements in molecular biology have enabled the development of new analytical tools to explore the physiological and biochemical roles of amino acids in cellular growth. However, the molecular-level regulation of plant amino acid metabolism remains a largely uncharted territory, posing a major challenge and a promising area for future research. Understanding this complex regulatory network will be key to unlocking the full potential of lysine metabolism in microalgal biotechnology.

5. Downstream process for protein recovery

Microalgal biomass is a rich source of protein, containing all essential amino acids required by humans, making it a promising raw material for food production. However, most of these proteins are intracellular, and their extraction is hindered by the complex, multilayered structure of the cell walls (Geada et al., 2021). Therefore, an effective method to break down the cell wall barrier is necessary to extract the desired proteins. (Fig. 3).

5.1. Cell lysis and protein recovery

The microalgal protein extraction process typically follows the well-established protocols used for plant protein processing, involving critical stages such as cell wall disruption, protein release, solid-liquid separation, flocculation, and subsequent purification (Amorim et al., 2021). Several techniques are employed for protein extraction, including acid extraction, alkali extraction, organic solvent extraction, salt solution extraction, and enzymatic hydrolysis. To improve the efficiency of protein extraction and maximize protein release from microalgal cells, various physical techniques, such as bead milling, ultrasonication, pulsed electric field (PEF), and microwave-assisted extraction, have been applied.

Among these methods, bead milling combined with centrifugation has proven to be effective in protein yield and cost efficiency. For instance, in the case of *C. vulgaris*, a protein recovery rate of approximately 96 % can be achieved after 60 min of bead milling (Kulkarni and Nikolov, 2018). Ultrasonic-assisted extraction disrupts cell walls through high-frequency sound waves, significantly improving protein diffusion and enhancing the solvent's mass transfer properties. This method offers advantages such as energy efficiency, minimal chemical usage, and compatibility with other technologies, such as enzymatic or ionic liquid coupling (Hildebrand et al., 2020). For instance, combining proton ionic liquids with ultrasonication has increased phycocyanin recovery to 14.85 mg g^{-1} (Rodrigues et al., 2015). Liu et al. (2022) reported that ultrasonic disruption enables efficient protein recovery from *C. vulgaris*, achieving a yield of 92 %—25 % higher than conventional alkaline extraction—while simultaneously reducing energy consumption by 40 %. However, optimizing parameters such as ultrasound power, treatment time, and strain specificity is still crucial for further improvements (Carreira-Casais et al., 2021).

Pulsed electric fields (PEF) apply short-duration electric pulses that induce electroporation of cell membranes, increasing membrane permeability and facilitating protein release. This technique offers advantages such as selective membrane permeabilization, the use of water

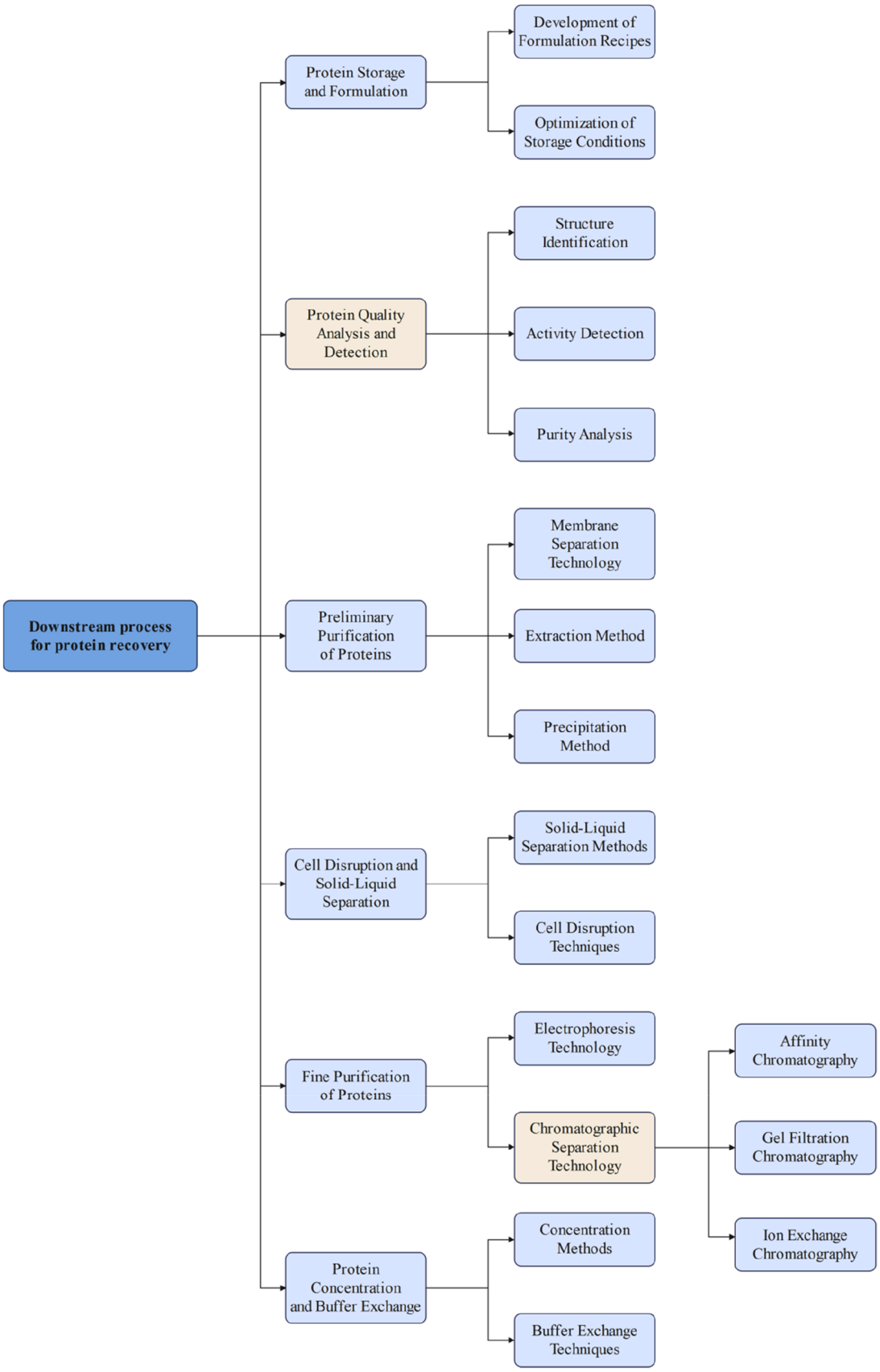

Fig. 3. Downstream process for protein recovery from microalgae.

as a medium (avoiding harmful solvents), and good compatibility with downstream processes (Gateau et al., 2021; Machado et al., 2022). However, scaling up this process requires optimizing conductivity and electrode spacing to ensure uniform electric field distribution. Microwave-assisted extraction utilizes dielectric heating, pressure buildup within cells, and enhanced mass transfer, which significantly reduces extraction times. For example, protein extraction from *Spirulina* can achieve a recovery rate of 78 % in just 3 min of microwave treatment (Mahali and G, 2019). The integration of multiple extraction techniques can greatly improve both protein yield and extraction efficiency.

Following microalgal cell disruption, centrifugation is commonly employed to separate cell debris from the protein-rich supernatant, facilitating solid-liquid clarification (Yang et al., 2024a). To enhance protein extraction efficiency, cell disruption or lysate incubation is often conducted under alkaline conditions (pH $>$ 10), which increases the surface charge of protein molecules—shifting them away from their isoelectric point (pI $\approx$ 6)—thereby improving solubility (Momen et al., 2021). Under alkaline conditions, protein extraction efficiency can increase from approximately 70 % to nearly 95 %, primarily due to enhanced solubility driven by electrostatic repulsion (Ursu et al., 2014). Protein hydration is strongly influenced by the ionic strength of the solvent: at low salt concentrations, a reduced dielectric constant and weakened salt-bridge interactions between proteins promote dispersion, whereas at high salt concentrations, competition for water molecules diminishes protein-water interactions, leading to aggregation and precipitation. pH adjustment serves as a critical strategy for modulating salt effects and optimizing protein solubility (Martins de Oliveira et al., 2018).

The application of strong acids or bases in protein extraction presents considerable environmental and process-related drawbacks. These chemicals generate non-recoverable waste, contribute to environmental pollution, and may cause protein degradation through deamination, decarboxylation, and peptide bond hydrolysis. Such degradation not only compromises protein integrity but may also lead to the formation of potentially cytotoxic byproducts, thereby reducing the functional and nutritional quality of the extracted proteins (El Achkar et al., 2021). Furthermore, while increasing the liquid-to-solid ratio can improve extraction efficiency, excessive solvent usage may destabilize the protein hydration shell, leading to incomplete precipitation and lower recovery yields (Vo et al., 2024). At both industrial and laboratory scales, optimizing solvent selection is crucial to achieve high protein recovery while maintaining process sustainability. Alkaline extraction remains a widely used method due to its effectiveness in disrupting rigid cell walls and solubilizing protein aggregates (Hadidi et al., 2023). However, the growing emphasis on green processing technologies has led to the development of alternative extraction strategies. These include osmotic shock-assisted biphasic systems (Krishna Koyande et al., 2020), ultrasound-assisted ionic liquid extraction (Lee et al., 2017), and autolysis/enzyme-assisted aqueous extraction (Sierra et al., 2017). These emerging techniques, especially when applied to robust strains such as *C. vulgaris*, hold significant promise for enhancing protein yield while maintaining a balance between cost-effectiveness, scalability, and environmental sustainability.

5.2. Protein enrichment and purification

After protein extraction, it is necessary to concentrate the target proteins. Dialysis, a widely used technique, facilitates solute exchange through semi-permeable membranes. Although simple and requiring no specialized equipment, it is time-consuming and may lead to protein denaturation (Soto-Sierra et al., 2018). Ultrafiltration (UF) presents a more efficient alternative, utilizing membranes with specific molecular weight cutoffs (MWCO) to selectively retain larger molecules (Naz and Mukherjee, 2025). Ursu et al. reported that a 300 kDa UF membrane achieved a protein recovery rate of 95 %, significantly higher than the 76 % recovery observed using acid precipitation (Ursu et al., 2014). However, challenges in achieving high protein purity remain. For instance, Safi et al. found that UF products contained $<$20 % protein by dry weight (DW), which is insufficient for food-grade applications. Additionally, increasing the MWCO to 1000 kDa did not improve flux and resulted in further protein loss (Safi et al., 2017).

To enhance purity, precipitation or ion-exchange chromatography can be employed to remove impurities such as polysaccharides and pigments (Tang et al., 2020). Three-phase extraction techniques have demonstrated significant potential in overcoming the limitations of traditional membrane filtration. In a previous study, a liquid triphasic flotation (LTF) system, optimized from three-phase partitioning (TPP), was used to extract proteins from *C. vulgaris*. This method achieved a protein recovery rate of 87.23 % and a separation efficiency of 56.72 % (Chia et al., 2019). However, the high cost of solvents remains a barrier to the scalability of this technique. Various treatment techniques can be integrated in the application process to optimize performance efficiency, tailored to the specific characteristics of algal cell walls and the selection of appropriate extraction and concentration methods.

6. Sensory limitations and improvement strategies of microalgal proteins

The sensory limitations of microalgal proteins are largely attributed to chlorophyll and volatile metabolites, which impart undesirable colors and off-flavors that constrain their incorporation into food products. To mitigate these drawbacks, several refinement strategies have been developed. For instance, in *C. pyrenoidosa*, supercritical fluid extraction was shown to effectively eliminate chlorophyll a, which fell below the detection limit as confirmed by HPLC–UV analysis, while the protein fraction retained its solubility and functional integrity (Miyazawa et al., 2021). In *A. platensis*, solvent extraction was also shown to mitigate undesirable odors while preserving nutritional quality. Hexane achieved the strongest deodorizing effect, and key odor-active compounds—including 1,4:3,6-anhydro-α-d-glucopyranose, palmitic acid methyl ester, and hexadecanamide—were identified as major contributors to the fishy profile. Notably, the treatments did not substantially alter the amino acid composition (Cuellar-Bermudez et al., 2017). Mechanistically, enzymatic hydrolysis disrupts the rigid cell wall, facilitating the release of volatile compounds, which are subsequently removed through solvent extraction. This strategy not only achieved a protein retention rate above 90 % but also markedly enhanced overall sensory acceptability (Verni et al., 2023).

The practical relevance of these strategies has been demonstrated in food reformulation. For instance, pasta fortified with *A. platensis* showed marked increases in phenolic compounds, chlorophylls, and carotenoids, while sensory evaluations indicated that consumer preference was maximized at a supplementation level of 2 % (Fradinho et al., 2020). Beyond processing, complementary formulation approaches—such as combining microalgal proteins with herbs, spices, or natural flavor maskers—offer further potential to mitigate residual off-notes while simultaneously enhancing nutritional and functional profiles. Taken together, these studies highlight that depigmentation, deodorization, and formulation adjustments are effective strategies to mitigate the sensory limitations of microalgal proteins and enhance their suitability for food applications.

7. Future perspectives and translational pathways for microalgal proteins

With the full awakening of consumers' health awareness, microalgal protein has continuously attracted market attention due to its high-quality nutritional density, which is rich in essential amino acids, natural vitamin groups, and carotenoids with high bioavailability. As a result, more companies have entered the microalgal protein market, which has increased competition and driven down prices. Such competition has further spurred efforts to cut costs for maintaining

profitability (Table 5).

Microalgal proteins have significant potential in precision nutrition. Synthetic biology enables the customization of amino acid composition, and when combined with Artificial intelligence (AI)-driven peptide design, has facilitated the development of a microalgae-derived ACE-inhibitory peptide targeting hypertension (Ashraf et al., 2025). Furthermore, in 3D-printed foods, microalgal protein gels can be engineered to achieve controllable textures through pH adjustment, thereby meeting personalized nutritional and sensory requirements (Mirzapour-Kouhdasht et al., 2024). Building on these advances, cross-disciplinary innovations are expected to further accelerate application-oriented development. AI offers new opportunities in protein structure prediction and *de novo* bioactive peptide discovery, while protein engineering provides strategies to improve solubility, stability, and interfacial functionality—long-standing challenges that limit incorporation into complex food matrices (Wu et al., 2025). In parallel, advances in formulation science, such as nanoencapsulation and next-generation food printing, open avenues for producing functional products with tailored texture, targeted delivery, and enhanced consumer acceptance (Rasia et al., 2024). Collectively, these developments position microalgal proteins as a versatile platform for personalized, application-driven food solutions rather than mere nutritional supplements.

Despite the promising potential of microalgae in protein production, their application remains largely confined to laboratory settings (Fig. 4). The primary barrier lies in the high costs of large-scale production. For instance, considering protein feed as an example, soybean meal, a widely used plant-based protein source, is priced at approximately $0.55 per kilogram, with an annual global demand of 200 million tons. Similarly, fishmeal, a key component in aquaculture feed, costs less than $2.18 per kilogram, with an annual demand of 7 million tons. In contrast, the current market demand for microalgae as a feed ingredient is only 25,000 tons per year, with prices ranging from $32.69 to $326.94 per kilogram. To render microalgal biomass economically viable as a feed protein without driving up meat prices, its production costs would need to be reduced to €1.10–5.45 per kilogram (Acien Fernandez et al., 2021).

Production costs arise from multiple stages of the value chain. Cultivation media alone account for 30–40 % of total input (Guldhe et al., 2017), with nitrogen source fluctuations directly impacting cost stability. Downstream cell disruption is similarly burdensome: the bead milling method consumes ~500 kWh per ton of biomass, while low biomass concentrations (<10 g L^{-1}) reduce centrifugation efficiency by ~40 % (Vázquez-Romero et al., 2022a).

Emerging solutions target these barriers across biological, engineering, and systems levels. CRISPRa–VP64 elevated protein levels in *C. sorokiniana* UTEX 1602 to 60 % of dry cell weight (570 mg L^{-1}), whereas CRISPRi–KRAB with ASGARD further increased protein to 65 % and lipid yields to 150–250 mg L^{-1} (WT: 150 mg L^{-1}), illustrating the capacity of programmable gene regulation to optimize biomass composition (Lin et al., 2022). Photovoltaic-integrated reactors offer substantial potential for energy self-sufficiency, lowering cultivation costs, and enabling the utilization of industrial exhaust gases as carbon sources (Chakraborty and Peter, 2025; Tawfik et al., 2021). Systems-level assessments show that wastewater-coupled cultivation can lower biomass production costs to $0.39–0.92 per kg and wastewater treatment expenses to $0.18–1.69 per m^3, thereby reducing medium inputs while providing concurrent benefits in biomass generation and effluent remediation (Chaudry, 2021). These strategies collectively represent viable pathways to reduce input costs while sustaining productivity.

Another fundamental constraint is the inverse relationship between protein content and biomass accumulation. Under autotrophic conditions, microalgae typically exhibit high protein levels but relatively low biomass productivity. Conversely, heterotrophic and mixotrophic cultivation substantially enhance biomass yields but often reduce protein content to 15–30 %. Overcoming this trade-off will require optimization of cultivation conditions, refinement of culture strategies, and application of metabolic engineering to generate strains capable of balancing high protein yields with robust biomass accumulation.

Scaling up cultivation from laboratory to industrial reactors introduces additional challenges. Many studies demonstrating high protein productivity have been conducted in shake flasks (<0.50 L) (Table 3). However, industrial-scale systems are constrained by light attenuation, nutrient diffusion limitations, and contamination risks. As microalgae transition into large-scale photobioreactors, efficient management of light penetration, gas exchange, and nutrient delivery becomes increasingly essential. Accordingly, further research into cost-efficient cultivation platforms that sustain high productivity while ensuring stability is indispensable.

Table 5
Production cost and selling price of microalgae and their products.

Category	Production price (Cost) in USD kg^{-1}	Sales price in USD/kg	Reference
Microalgae biomass (Dry powder)			
Photoautotrophic mode (Open pond)	2.5 - 5	4 - 8	(Vázquez-Romero et al., 2022a; Yadav et al., 2020)
Photoautotrophic mode (photobioreactor)	8 - 15	15 - 30	(Vázquez-Romero et al., 2022b)
Heterotrophic mode (Using glucose as carbon source)	3 - 5	6 - 10	(Vázquez-Romero et al., 2022a)
Microalgae protein concentrate			
Feed grade protein (60 % purity)	5 - 8	10 - 12	(Vázquez-Romero et al., 2022b)
Food grade protein (80 % purity)	15 - 25	50 - 150	(Vázquez-Romero et al., 2022b)
High-value added products			
Algal omega-3 extract	80 - 150	200 - 500	(Vázquez-Romero et al., 2022b)
Phycocyanin (Pharmaceutical grade)	500 - 1000	2000 - 5000	(Sekar and Chandramohan, 2008)

Declaration of generative AI and AI-assisted technologies in the writing process

While preparing this manuscript, the authors employed ChatGPT solely to enhance the manuscript's language and readability. After, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Ethical statement

This article is a review and does not involve any studies with human participants or animals performed by any of the authors.

CRediT authorship contribution statement

Jie Zheng: Writing – original draft, Investigation, Conceptualization. **Jiaman Geng:** Writing – original draft, Investigation. **Jiaxin Li:** Writing – original draft, Investigation. **Dongyu Li:** Writing – original draft. **Shufang Yang:** Writing – original draft. **Xue Lu:** Writing – original draft. **Jin Liu:** Writing – review & editing. **Chengrong Zhang:** Writing – review & editing, Conceptualization. **Han Sun:** Writing – review & editing, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial

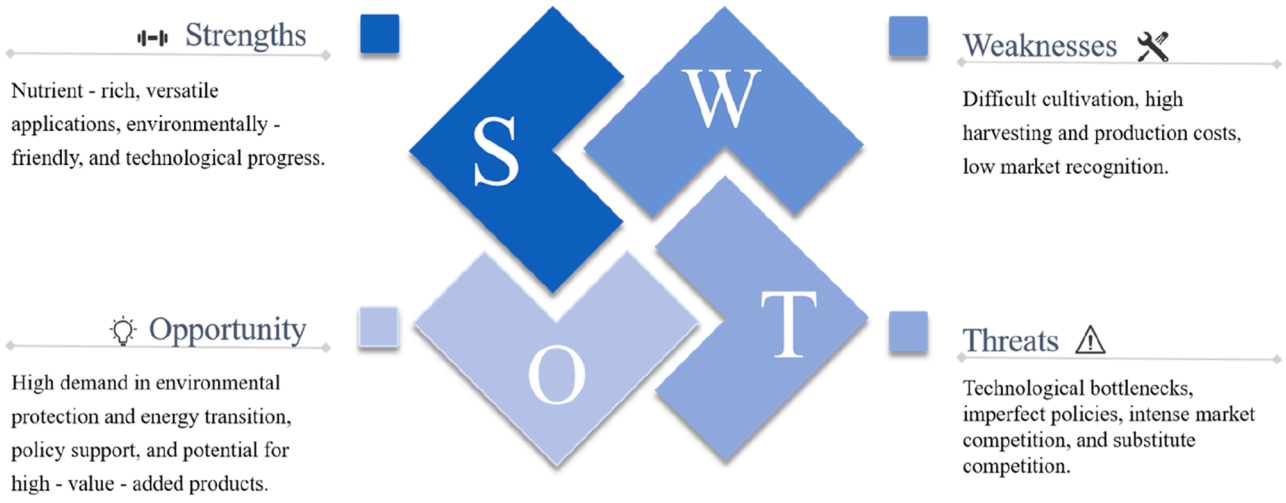

Fig. 4. Microalgae biotechnology is facing the challenges, transmutation and innovation opportunities of sustainable development.

interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

References

Acien Fernandez, F.G., Reis, A., Wijffels, R.H., Barbosa, M., Verdelho, V., Llamas, B., 2021. The role of microalgae in the bioeconomy. New Biotechnol. 61, 99–107.
Amorim, M.L., Soares, J., dos Reis Coimbra, J.S., Leite, M.d.O., Teixeira Albino, L.F., Martins, M.A., 2021. Microalgae proteins: production, separation, isolation, quantification, and application in food and feed. Crit. Rev. Food Sci. Nutr. 61 (12), 1976–2002.
Ashraf, A., Guo, Y., Yang, T., Din, A.S.U., Ahmad, K., Li, W., Hou, H., 2025. Microalgae-derived peptides: exploring bioactivities and functional food innovations. J. Agric. Food Chem. 73 (2), 1000–1013.
Ba, F., Ursu, A.V., Laroche, C., Djelveh, G., 2016. *Haematococcus pluvialis* soluble proteins: extraction, characterization, concentration/fractionation and emulsifying properties. Bioresour. Technol. 200, 147–152.
Baidya, A., Akter, T., Islam, M.R., Shah, A.K.M.A., Hossain, M.A., Salam, M.A., Paul, S.I., 2021. Effect of different wavelengths of LED light on the growth, chlorophyll, β-carotene content and proximate composition of chlorella ellipsoidea. Heliyon 7 (12).
Balasubramanian, P., Prabhakaran, M.P., Sireesha, M., Ramakrishna, S., 2012. Collagen in human tissues: structure, function, and biomedical implications from a tissue engineering perspective. Polymer Composites – Polyolefin Fractionation – Polymeric Peptidomimetics – Collagens, pp. 173–206.
Becker, E.W., 2007. Micro-algae as a source of protein. Biotechnol. Adv. 25 (2), 207–210.
Benelhadj, S., Gharsallaoui, A., Degraeve, P., Attia, H., Ghorbel, D., 2016. Effect of pH on the functional properties of Arthrospira (Spirulina) platensis protein isolate. Food Chem. 194, 1056–1063.
Bertsch, P., Boecker, L., Mathys, A., Fischer, P., 2021. Proteins from microalgae for the stabilization of fluid interfaces, emulsions, and foams. Trends Food Sci. Tech. 108, 326–342.
Binks, B.P., Rocher, A., 2009. Effects of temperature on water-in-oil emulsions stabilised solely by wax microparticles. J. Colloid Interface Sci. 335 (1), 94–104.
Buchmann, L., Bertsch, P., Bocker, L., Krahenmann, U., Fischer, P., Mathys, A., 2019a. Adsorption kinetics and foaming properties of soluble microalgae fractions at the air/water interface. Food Hydrocoll. 97.
Buchmann, L., Brändle, I., Haberkorn, I., Hiestand, M., Mathys, A., 2019b. Pulsed electric field based cyclic protein extraction of microalgae towards closed-loop biorefinery concepts. Bioresour. Technol. 291, 121870.
Cai, Y.H., Zhai, L.G., Fang, X.M., Wu, K.P., Liu, Y.H., Cui, X., Wang, Y.P., Yu, Z.G., Ruan, R.G., Liu, T.Y., Zhang, Q., 2022. Effects of C/N ratio on the growth and protein accumulation of heterotrophic chlorella in broken rice hydrolysate. Biotechnol. Biofuels Bioprod. 15 (1), 11.
Cao, Y., Yang, S., Wang, J., Kong, W., Guo, B., Xi, Y., Zhang, A., Yue, B., 2023. Metabolomic exploration of the physiological regulatory mechanism of the growth and metabolism characteristics of Chlorella vulgaris under photoautotrophic, mixotrophic, and heterotrophic cultivation conditions. Biomass Bioenergy 173, 106775.
Carreira-Casais, A., Otero, P., Garcia-Perez, P., Garcia-Oliveira, P., Pereira, A.G., Carpena, M., Soria-Lopez, A., Simal-Gandara, J., Prieto, M.A., 2021. Benefits and drawbacks of ultrasound-assisted extraction for the recovery of bioactive compounds from marine algae. Int. J. Environ. Res. Public Health 18 (17).
Ceron-Garcia, M.C., Macias-Sanchez, M.D., Sanchez-Miron, A., Garcia-Camacho, F., Molina-Grima, E., 2013. A process for biodiesel production involving the heterotrophic fermentation of Chlorella protothecoides with glycerol as the carbon source. Appl. Energy 103, 341–349.
Chaiklahan, R., Chirasuwan, N., Srinorasing, T., Attasat, S., Nopharatana, A., Bunnag, B., 2022. Enhanced biomass and phycocyanin production of Arthrospira (Spirulina) platensis by a cultivation management strategy: light intensity and cell concentration. Bioresour. Technol. 343, 126077.
Chakraborty, S., Peter, S.C., 2025. Solar-fuel production by photodriven CO2 reduction: facts, challenges, and recommendations. ACS Energy Lett. 10 (5), 2359–2371.
Chaudry, S., 2021. Integrating microalgae cultivation with wastewater treatment: a peek into economics. Appl. Biochem. Biotechnol. 193 (10), 3395–3406.
Chen, B., Gong, R., Qiao, Z., Men, J., Tan, L., Tian, S., Jin, H., 2024. Improving Chlorella protein production under heterotrophic high cell density fed-batch cultivation with a two-stage nitrogen nutrient supply strategy. Green Carbon.
Chen, F., Zhang, Y.M., 1997. High cell density mixotrophic culture of Spirulina platensis on glucose for phycocyanin production using a fed-batch system. Enzyme Microb. Technol. 20 (3), 221–224.
Chen, M., Bi, R., Chen, X., Ding, Y., Zhang, H., Li, L., Zhao, M., 2019. Stoichiometric and sterol responses of dinoflagellates to changes in temperature, nutrient supply and growth phase. Algal Res. 42.
Cheng, P., Chu, R., Zhang, X., Song, L., Chen, D., Zhou, C., Yan, X., Cheng, J.J., Ruan, R., 2020. Screening of the dominant chlorella pyrenoidosa for biofilm attached culture and feed production while treating swine wastewater. Bioresour. Technol. 318, 124054.
Chia, S.R., Mak, K.Y., Khaw, Y.J., Suhaidi, N., Chew, K.W., Show, P.L., 2019. An efficient and rapid method to extract and purify protein - Liquid Triphasic flotation system. Bioresour. Technol. 294, 122158.
Chronakis, I.S., 2001. Gelation of edible blue-green algae protein isolate (*Spirulina platensis* strain Pacifica): thermal transitions, rheological properties, and molecular forces involved. J. Agric. Food Chem. 49 (2), 888–898.
Cruz, C.G., da Rosa, A.P.C., Costa, J.A.V., 2023. Identification of the phytohormones indole-3-acetic acid and trans-zeatin in microalgae. J. Chem. Technol. Biotechnol. 98 (4), 1048–1056.
Cuellar-Bermudez, S.P., Barba-Davila, B., Serna-Saldivar, S.O., Parra-Saldivar, R., Rodriguez-Rodriguez, J., Morales-Davila, S., Goiris, K., Muylaert, K., Chuck-Hernandez, C., 2017. Deodorization of arthrospira platensis biomass for further scale-up food applications. J. Sci. Food Agric. 97 (15), 5123–5130.
Cunha, S.A., Pintado, M.E., 2022. Bioactive peptides derived from marine sources: biological and functional properties. Trends Food Sci. Technol. 119, 348–370.
Dai, L., Hinrichs, J., Weiss, J., 2020a. Ionic strength and pH stability of oil-in-water emulsions prepared with acid-hydrolyzed insoluble proteins from Chlorella protothecoides. J. Sci. Food Agric. 100 (11), 4237–4244.
Dai, L., Shivananda, R., Hinrichs, J., Weiss, J., 2020b. Foaming of acid-hydrolyzed insoluble microalgae proteins from *Chlorella protothecoides*. Food Biophys. 15 (3), 368–375.

Demirel, U., Morris, W.L., Ducreux, L.J.M., Yavuz, C., Asim, A., Tindas, I., Campbell, R., Morris, J.A., Verrall, S.R., Hedley, P.E., Gokce, Z.N.O., Caliskan, S., Aksoy, E., Caliskan, M.E., Taylor, M.A., Hancock, R.D., 2020. Physiological, biochemical, and transcriptional responses to single and combined abiotic stress in stress-tolerant and stress-sensitive potato genotypes. Front. Plant Sci. 11.

Devi, M.A., Venkataraman, L.V., 1984. Functional-properties of protein products of mass cultivated blue-green-alga Spirulina-platensis. J. Food Sci. 49 (1), 24–27.

Diankristanti, P.A., Hei, E., Ho, N., Chen, J.-H., Nagarajan, D., Chen, C.-Y., Hsieh, Y.-M., Ng, I.S., Chang, J.-S., 2024. Unlocking the potential of microalgae as sustainable bioresources from up to downstream processing: a critical review. Chem. Eng. J. 488.

Ding, Y., Xiao, N., Guo, S., Lin, J., Chen, L., Mou, X., Ai, M., 2024. Impact of NaCl perturbation on physicochemical and structural properties of preheat-treated egg white protein modulating foaming property. Food Chem. 459, 140377.

Divya Kuravi, S., Venkata Mohan, S., 2022. Mixotrophic cultivation of monoraphidium sp. in dairy wastewater using flat-panel photobioreactor and photosynthetic performance. Bioresour. Technol. 348, 126671.

dos Santos, R.R., Araujo, O.D.F., de Medeiros, J.L., Chaloub, R.M., 2016. Cultivation of Spirulina maxima in medium supplemented with sugarcane vinasse. Bioresour. Technol. 204, 38–48.

Doucha, J., Lívanský, K., 2012. Production of high-density chlorella culture grown in fermenters. J. Appl. Phycol. 24 (1), 35–43.

Ebert, S., Grossmann, L., Hinrichs, J., Weiss, J., 2019. Emulsifying properties of water-soluble proteins extracted from the microalgae *chlorella sorokiniana* and *phaeodactylum tricornutum*. Food Funct. 10 (2), 754–764.

El Achkar, T., Greige-Gerges, H., Fourmentin, S., 2021. Basics and properties of deep eutectic solvents: a review. Environ. Chem. Lett. 19, 3397–3408.

Esteves, A.F., Pardilhó, S., Gonçalves, A.L., Vilar, V.J.P., Pires, J.C.M., 2025. Unravelling the impact of light spectra on microalgal growth and biochemical composition using principal component analysis and artificial neural network models. Algal Res. 85.

Falcon, W.P., Naylor, R.L., Shankar, N.D., 2022. Rethinking global food demand for 2050. Popul. Dev. Rev. 48, 921–957.

Fan, J., Huang, J., Li, Y., Han, F., Wang, J., Li, X., Wang, W., Li, S., 2012. Sequential heterotrophy-dilution-photoinduction cultivation for efficient microalgal biomass and lipid production. Bioresour. Technol. 112, 206–211.

Food and Agriculture Organization of the United Nations, 2022. New UN Report Finds 828 million People Going Hungry in a world of Plenty.

Fradinho, P., Niccolai, A., Soares, R., Rodolfi, L., Biondi, N., Tredici, M.R., Sousa, I., Raymundo, A., 2020. Effect of *arthrospira platensis* (spirulina) incorporation on the rheological and bioactive properties of gluten-free fresh pasta. Algal Res. 45, 101743.

Garcia-Encinas, J.P., Ruiz-Cruz, S., Juarez, J., Ornelas-Paz, J.d.J., Del Toro-Sanchez, C.L., Marquez-Rios, E., 2025. Proteins from Microalgae: nutritional, functional and bioactive properties. Foods 14 (6).

Gateau, H., Blanckaert, V., Veidl, B., Burlet-Schiltz, O., Pichereaux, C., Gargaros, A., Marchand, J., Schoefs, B., 2021. Application of pulsed electric fields for the biocompatible extraction of proteins from the microalga *haematococcus pluvialis*. Bioelectrochemistry 137, 107588.

Geada, P., Moreira, C., Silva, M., Nunes, R., Madureira, L., Rocha, C.M.R., Pereira, R.N., Vicente, A.A., Teixeira, J.A., 2021. Algal proteins: production strategies and nutritional and functional properties. Bioresour. Technol. 332, 125125.

Gerde, J.A., Wang, T., Yao, L., Jung, S., Johnson, L.A., Lamsal, B., 2013. Optimizing protein isolation from defatted and non-defatted Nannochloropsis microalgae biomass. Algal Res. 2, 145–153.

Giossi, C.E., Bitnel, D.B., Wunsch, M.A., Kroth, P.G., Lepetit, B., 2025. Synergistic effects of temperature and light on photoprotection in the model diatom phaeodactylum tricornutum. Physiol. Plant. 177, e70039.

Glockow, T., Velaz Martín, M., Meisch, L., Kapieske, D., Meissner, K., Correa Cassal, M., Kaster, A.K., Rabe, K.S., Niemeyer, C.M., 2023. A photobioreactor for production of algae biomass from gaseous emissions of an animal house. Appl. Microbiol. Biotechnol. 107, 7673–7684.

Gong, Q., Wang, J., Zhao, W., Liu, J., Yang, S., Gao, Y., Mou, H., Zhu, C., Sun, H., 2025. Development of fast-growing chlorophyll-deficient chlorella pyrenoidosa mutant using atmospheric and room temperature plasma mutagenesis. Bioresour. Technol. 422, 132245.

Gorunmek, M., Ballik, B., Cakmak, Z.E., Cakmak, T., 2024. Mycosporine-like amino acids in microalgae and cyanobacteria: biosynthesis, diversity, and applications in biotechnology. Algal Res. 80, 103507.

Graverholt, O.S., Eriksen, N.T., 2007. Heterotrophic high-cell-density fed-batch and continuous-flow cultures of galdieria sulphuraria and production of phycocyanin. Appl. Microbiol. Biotechnol. 77, 69–75.

Grossmann, L., Ebert, S., Hinrichs, J., Weiss, J., 2019a. Formation and stability of emulsions prepared with a water-soluble extract from the microalga Chlorella protothecoides. J. Agric. Food Chem. 67 (23), 6551–6558.

Grossmann, L., Hinrichs, J., Goff, H.D., Weiss, J., 2019b. Heat-induced gel formation of a protein-rich extract from the microalga *Chlorella sorokiniana*. Innov. Food Sci. Emerg. 56, 102176.

Guil-Guerrero, J.L., Navarro-Juárez, R., López-Martínez, J.C., Campra-Madrid, P., Rebolloso-Fuentes, M.M., 2004. Functional properties of the biomass of three microalgal species. J. Food Eng. 65 (4), 511–517.

Guldhe, A., Kumari, S., Ramanna, L., Ramsundar, P., Singh, P., Rawat, I., Bux, F., 2017. Prospects, recent advancements and challenges of different wastewater streams for microalgal cultivation. J. Environ. Manage. 203, 299–315.

Hadidi, M., Aghababaei, F., McClements, D.J., 2023. Enhanced alkaline extraction techniques for isolating and modifying plant-based proteins. Food Hydrocoll. 145, 109132.

He, Z., Han, W., Jin, W., Yang, J., Gao, S., Li, S.-f., Tu, R., Han, S., Chen, Y., Zhou, X., 2021. Cultivation of scenedesmus obliquus and chlorella pyrenoidosa in municipal wastewater using monochromatic and white LED as light sources. Waste Biomass Valor. 12, 4873–4883.

Hemker, F., Zielasek, F., Jahns, P., 2024. Combined high light and salt stress enhances accumulation of PsbS and zeaxanthin in Chlamydomonas reinhardtii. Physiol. Plant. 176, e14233.

Hildebrand, G., Poojary, M.M., O'Donnell, C., Lund, M.N., Garcia-Vaquero, M., Tiwari, B. K., 2020. Ultrasound-assisted processing of chlorella vulgaris for enhanced protein extraction. J. Appl. Phycol. 32, 1709–1718.

Hildebrandt, T.M., Nunes Nesi, A., Araújo, W.L., Braun, H.-P., 2015. Amino acid catabolism in plants. Mol. Plant 8, 1563–1579.

Hirota, T., Izumi, M., Wada, S., Makino, A., Ishida, H., 2018. Vacuolar protein degradation via autophagy provides substrates to amino acid catabolic pathways as an adaptive response to sugar starvation in Arabidopsis thaliana. Plant Cell Physiol. 59, 1363–1376.

Hong, X., Fan, L.P., Yan, X.W., Li, J.W., 2024. Structural and functional properties of proteins isolated from four species of microalgae and their application in air-water interface stabilization. Int. J. Biol. Macromol. 283.

Husein el Hadmed, H., Castillo, R.F., 2016. Cosmeceuticals: peptides, proteins, and growth factors. J. Cosmet. Dermatol. 15, 514–519.

Isogai, S., Takagi, H., 2021. Enhancement of lysine biosynthesis confers high-temperature stress tolerance to *Escherichia coli* cells. Appl. Microbiol. Biotechnol. 105, 6899–6908.

Jin, H., Chuai, W., Li, K., Hou, G., Wu, M., Chen, J., Wang, H., Jia, J., Han, D., Hu, Q., 2021. Ultrahigh-cell-density heterotrophic cultivation of the unicellular green alga Chlorella sorokiniana for biomass production. Biotechnol. Bioeng. 118, 4138–4151.

Kim, C.W., Sung, M.-G., Nam, K., Moon, M., Kwon, J.-H., Yang, J.-W., 2014. Effect of monochromatic illumination on lipid accumulation of nannochloropsis gaditana under continuous cultivation. Bioresour. Technol. 159, 30–35.

Koyande, A.K., Chew, K.W., Rambabu, K., Tao, Y., Chu, D.-T., Show, P.-L., 2019. Microalgae: a potential alternative to health supplementation for humans. Food Sci. Human Wellness 8, 16–24.

Krishna Koyande, A., Tanzil, V., Murraly Dharan, H., Subramaniam, M., Robert, R.N., Lau, P.-L., Khoiroh, I., Show, P.-L., 2020. Integration of osmotic shock assisted liquid biphasic system for protein extraction from microalgae Chlorella vulgaris. Biochem. Eng. J. 157.

Kulkarni, S., Nikolov, Z., 2018. Process for selective extraction of pigments and functional proteins from Chlorella vulgaris. Algal Res. 35, 185–193.

Lai, Y.C., Chang, C.H., Chen, C.Y., Chang, J.S., Ng, I.S., 2019. Towards protein production and application by using Chlorella species as circular economy. Bioresour. Technol. 289, 121625.

Lam, R.S., Nickerson, M.T., 2013. Food proteins: a review on their emulsifying properties using a structure-function approach. Food Chem. 141, 975–984.

Lee, L.L., Niknafs, N., Hancocks, R.D., Norton, I.T., 2013. Emulsification: mechanistic understanding. Trends Food Sci. Tech. 31 (1), 72–78.

Lee, S.Y., Show, P.L., Ling, T.C., Chang, J.-S., 2017. Single-step disruption and protein recovery from Chlorella vulgaris using ultrasonication and ionic liquid buffer aqueous solutions as extractive solvents. Biochem. Eng. J. 124, 26–35.

Lehmuskero, A., Skogen Chauton, M., Boström, T., 2018. Light and photosynthetic microalgae: a review of cellular- and molecular-scale optical processes. Prog. Oceanogr. 168, 43–56.

Li, P., Luo, Y., Tian, J., Cheng, Y., Wang, S., An, X., Zheng, J., Yan, H., Duan, H., Zhang, J., Pan, Z., Chen, Y., Wang, R., Zhou, H., Wang, Z., Tan, Z., Li, X., 2024. Outdoor tubular photobioreactor microalgae-microorganisms biofilm treatment of municipal wastewater: enhanced heterotrophic assimilation and synergistic aerobic denitrogenation. Bioresour. Technol. 408, 131151.

Li, X., Huff, J., Crunkleton, D.W., Johannes, T.W., 2023. Light intensity and spectral quality modulation for improved growth kinetics and biochemical composition of chlamydomonas reinhardtii. J. Biotechnol. 375, 28–39.

Liang, Y., Sarkany, N., Cui, Y., 2009. Biomass and lipid productivities of Chlorella vulgaris under autotrophic, heterotrophic and mixotrophic growth conditions. Biotechnol. Lett. 31, 1043–1049.

Lin, J.-Y., Lin, W.-R., Ng, I.S., 2022. CRISPRa/i with Adaptive Single Guide assisted regulation DNA (ASGARD) mediated control of chlorella sorokiniana to enhance lipid and protein production. Biotechnol. J. 17.

Liu, Y., Liu, X., Cui, Y., Yuan, W., 2022. Ultrasound for microalgal cell disruption and product extraction: A review. Ultrason. Sonochem. 87, 106054.

Lozober, H.S., Okun, Z., Shpigelman, A., 2021. The impact of high-pressure homogenization on thermal gelation of Arthrospira platensis (Spirulina) protein concentrate. Innov. Food Sci. Emerg. 74, 102857.

Lupatini Menegotto, A.L., Silva de Souza, L.E., Colla, L.M., Vieira Costa, J.A., Sehn, E., Stival Bittencourt, P.R., de Moraes Flores, E.L., Canan, C., Colla, E., 2019. Investigation of techno-functional and physicochemical properties of *Spirulina platensis* protein concentrate for food enrichment. LWT 114, 108267.

Lv, X., Huang, X., Ma, B., Chen, Y., Batool, Z., Fu, X., Jin, Y., 2022. Modification methods and applications of egg protein gel properties: a review. Compr. Rev. Food Sci. Food Saf. 21, 2233–2252.

Ma, C., Zhang, Y.B., Ho, S.H., Xing, D.F., Ren, N.Q., Liu, B.F., 2017. Cell growth and lipid accumulation of a microalgal mutant *scenedesmus* sp. Z-4 by combining light/dark cycle with temperature variation. Biotechnol. Biofuels 10, 260.

Machado, L., Carvalho, G., Pereira, R.N., 2022. Effects of innovative processing methods on microalgae cell wall: prospects towards digestibility of protein-rich biomass. Biomass 2 (2), 80–102.

Mahali, M., G, S., 2019. Extraction methods and functional properties of protein from *Arthospira platensis* for bioavailability of algal proteins. Int. J. Pharm. Chem. 5 (2).

Maltsev, Y., Maltseva, K., Kulikovskiy, M., Maltseva, S., 2021. Influence of light conditions on microalgae growth and content of lipids, carotenoids, and fatty acid composition. Biology 10 (10).
Martins de Oliveira, V., Godoi Contessoto, V., Bruno da Silva, F., Zago Caetano, D.L., Jurado de Carvalho, S., Pereira Leite, V.B., 2018. Effects of pH and salt concentration on stability of a protein G variant using coarse-grained models. Biophys. J. 114 (1), 65–75.
Menegol, T., Diprat, A.B., Rodrigues, E., Rech, R., 2017. Effect of temperature and nitrogen concentration on biomass composition of *heterochlorella luteoviridis*. Food Sci. Technol. 37 (spe), 28–37.
Mirkovic, T., Ostroumov, E.E., Anna, J.M., van Grondelle, R., Govindjee, Scholes, G.D., 2016. Light absorption and energy transfer in the antenna complexes of photosynthetic organisms. Chem. Rev. 117 (2), 249–293.
Mirzapour-Kouhdasht, A., Biparva, P., McClements, D.J., Garavand, F., Garcia-Vaquero, M., 2024. Formulation of inks for 3D printing of microalgae-based meat analogues and the role of modified starch: a review. Int. J. Food Sci. Technol. 59 (11), 8618–8629.
Miyazawa, T., Higuchi, O., Sasaki, M., Ota, M., Aida, T., Takekoshi, H., Inomata, H., Miyazawa, T., 2021. Removal of chlorophyll and pheophorbide from *Chlorella pyrenoidosa* by supercritical fluid extraction: potential of protein resource. Biosci. Biotechnol. Biochem. 85 (7), 1759–1762.
Momen, S., Alavi, F., Aider, M., 2021. Alkali-mediated treatments for extraction and functional modification of proteins: critical and application review. Trends Food Sci. Technol. 110, 778–797.
Navarova, H., Bernsdorff, F., Doering, A.-C., Zeier, J., 2012. Pipecolic acid, an endogenous mediator of defense amplification and priming, is a critical regulator of inducible plant immunity. Plant Cell 24 (12), 5123–5141.
Naz, S., Mukherjee, A., 2025. Extraction and purification of protein from algae (microalgae and seaweeds). Marine Molecules from Algae and Cyanobacteria, pp. 25–39.
Nicolai, T., 2019. Gelation of food protein-protein mixtures. Adv. Colloid Interface Sci. 270, 147–164.
Nirmala, C., Prakash, V., Venkataraman, L.V., 1992. Physicochemical and functional-properties of proteins from spray-dried algae (Spirulina-platensis). Nahrung-Food 36 (6), 569–577.
Noya, I., González-García, S., Bacenetti, J., Fiala, M., Moreira, M.T., 2018. Environmental impacts of the cultivation-phase associated with agricultural crops for feed production. J. Clean. Prod. 172, 3721–3733.
Nwoba, E.G., Parlevliet, D.A., Laird, D.W., Alameh, K., Moheimani, N.R., 2020. Outdoor phycocyanin production in a standalone thermally-insulated photobioreactor. Bioresour. Technol. 315, 123865.
Olin-Sandoval, V., Yu, J.S.L., Miller-Fleming, L., Alam, M.T., Kamrad, S., Correia-Melo, C., Haas, R., Segal, J., Peña Navarro, D.A., Herrera-Dominguez, L., Méndez-Lucio, O., Vowinckel, J., Mülleder, M., Ralser, M., 2019. Lysine harvesting is an antioxidant strategy and triggers underground polyamine metabolism. Nature 572 (7768), 249–253.
Onyeaka, H., Miri, T., Obileke, K., Hart, A., Anumudu, C., Al-Sharify, Z.T., 2021. Minimizing carbon footprint via microalgae as a biological capture. Carbon Capture Sci. Technol. 1.
Othmeni, I., Blecker, C., Karoui, R., 2025. pH-dependent emulsifying properties of pea protein isolate: investigation of the structure-function relationship. Int. J. Biol. Macromol. 290, 139105.
Øverland, M., Karlsson, A., Mydland, L.T., Romarheim, O.H., Skrede, A., 2013. Evaluation of *Candida utilis, kluyveromyces marxianus* and *saccharomyces cerevisiae* yeasts as protein sources in diets for Atlantic salmon (*Salmo salar*). Aquaculture 402-403, 1–7.
Pan-utai, W., Iamtham, S., 2023. Techno-functional properties of microalgae in food products. Handbook of Food and Feed from Microalgae, pp. 293–304.
Patelou, M., Infante, C., Dardelle, F., Randewig, D., Kouri, E.D., Udvardi, M.K., Tsiplakou, E., Mantecón, L., Flemetakis, E., 2020. Transcriptomic and metabolomic adaptation of *nannochloropsis gaditana* grown under different light regimes. Algal Res. 45.
Perez, D., Harte, F., Lopez-Pedemonte, T., 2022. Ionic strength and buffering capacity of emulsifying salts determine denaturation and gelation temperatures of whey proteins. J. Dairy Sci. 105 (9), 7230–7241.
Pipliya, S., Kumar, S., Gupta, R.K., Das, R.S., Meena, D., Srivastav, P.P., Tiwari, B.K., Garcia-Vaquero, M., 2025. The future of algal proteins: innovations in extraction and modifications, functional properties, and sustainable food applications. Fut. Foods 11, 100549.
Prates, J.A.M., 2025. Unlocking the functional and nutritional potential of microalgae proteins in food systems: a narrative review. Foods 14 (9), 1524.
Purdi, T.S., Setiowati, A.D., Ningrum, A.J., 2023. Ultrasound-assisted extraction of *Spirulina platensis* protein: physicochemical characteristics and techno-functional properties. J. Food Meas. Charact. 17 (5), 5474–5486.
Rasia, T.A., da Silva Vaz, B., Costa, J.A.V., Morais, M.G., 2024. Nanoencapsulation of *spirulina* sp. LEB 18 microalgae biomass using electrospray technique and application in chocolate milk. Food Process. Bioprod. 148, 491–506.
Razzak, S.A., 2024. Comprehensive overview of microalgae-derived carotenoids and their applications in diverse industries. Algal Res. 78.
Rodrigues, D.B., Menezes, C.R., Mercadante, A.Z., Jacob-Lopes, E., Zepka, L.Q., 2015. Bioactive pigments from microalgae *phormidium autumnale*. Food Res. Int. 77, 273–279.
Safdar, L.B., Foulkes, M.J., Kleiner, F.H., Searle, I.R., Bhosale, R.A., Fisk, I.D., Boden, S. A., 2023. Challenges facing sustainable protein production: opportunities for cereals. Plant Commun. 4 (6), 100716.
Safi, C., Olivieri, G., Campos, R.P., Engelen-Smit, N., Mulder, W.J., van den Broek, L.A. M., Sijtsma, L., 2017. Biorefinery of microalgal soluble proteins by sequential processing and membrane filtration. Bioresour. Technol. 225, 151–158.
Sansawa, H., Endo, H., 2004. Production of intracellular phytochemicals in *Chlorella* under heterotrophic conditions. J. Biosci. Bioeng. 98 (6), 437–444.
Schädler, T., Neumann-Cip, A.-C., Wieland, K., Glöckler, D., Haisch, C., Brück, T., Weuster-Botz, D., 2020. High-density microalgae cultivation in open thin-layer cascade photobioreactors with water recycling. Appl. Sci. 10 (11).
Schmidt, R.A., Wiebe, M.G., Eriksen, N.T., 2005. Heterotrophic high cell-density fed-batch cultures of the phycocyanin-producing red alga *Galdieria sulphuraria*. Biotechnol. Bioeng. 90 (1), 77–84.
Schopf, M., Scherf, K.A., 2021. Water absorption capacity determines the functionality of vital gluten related to specific bread volume. Foods 10 (2).
Schwenzfeier, A., Helbig, A., Wierenga, P.A., Gruppen, H., 2013a. Emulsion properties of algae soluble protein isolate from *tetraselmis* sp. Food Hydrocoll. 30 (1), 258–263.
Schwenzfeier, A., Lech, F., Wierenga, P.A., Eppink, M.H.M., Gruppen, H., 2013. Foam properties of algae soluble protein isolate: effect of pH and ionic strength. Food Hydrocoll. 33 (1), 111–117.
Schwenzfeier, A., Wierenga, P.A., Eppink, M.H.M., Gruppen, H., 2014. Effect of charged polysaccharides on the techno-functional properties of fractions obtained from algae soluble protein isolate. Food Hydrocoll. 35, 9–18.
Sekar, S., Chandramohan, M., 2008. Phycobiliproteins as a commodity: trends in applied research, patents and commercialization. J. Appl. Phycol. 20 (2), 113–136.
Sierra, L.S., Dixon, C.K., Wilken, L.R., 2017. Enzymatic cell disruption of the microalgae *chlamydomonas reinhardtii* for lipid and protein extraction. Algal Res. 25, 149–159.
Solmaz, A., Işık, M., 2019. Polishing the secondary effluent and biomass production by microalgae submerged membrane photobioreactor. J. Sustain. Environ. Technol. 34, 1–8.
Song, X.J., Wang, J., Wang, Y.C., Feng, Y.G., Cui, Q., Lu, Y.D., 2018. Artificial creation of *chlorella pyrenoidosa* mutants for economic sustainable food production. Bioresour. Technol. 268, 340–345.
Soto-Sierra, L., Stoykova, P., Nikolov, Z.L., 2018. Extraction and fractionation of microalgae-based protein products. Algal Res. 36, 175–192.
Stepansky, A., Less, H., Angelovici, R., Aharon, R., Zhu, X., Galili, G., 2006. Lysine catabolism, an effective versatile regulator of lysine level in plants. Amino Acids 30 (2), 121–125.
Straka, L., Rittmann, B.E., 2017. Effect of culture density on biomass production and light utilization efficiency of *Synechocystis* sp. PCC 6803. Biotechnol. Bioeng. 115 (2), 507–511.
Suarez Garcia, E., van Leeuwen, J., Safi, C., Sijtsma, L., Eppink, M.H.M., Wijffels, R.H., van den Berg, C., 2018. Selective and energy efficient extraction of functional proteins from microalgae for food applications. Bioresour. Technol. 268, 197–203.
Sun, H., Wu, T., Chen, S.H.Y., Ren, Y., Yang, S., Huang, J., Mou, H., Chen, F., 2021. Powerful tools for productivity improvements in microalgal production. Renew. Sustain. Energy Rev. 152.
Szmańko, T., Lesiów, T., Górecka, J., 2021. The water-holding capacity of meat: a reference analytical method. Food Chem. 357.
Tan, X., Chu, H., Zhang, Y., Yang, L., Zhao, F., Zhou, X., 2014. Chlorella pyrenoidosa cultivation using anaerobic digested starch processing wastewater in an airlift circulation photobioreactor. Bioresour. Technol. 170, 538–548.
Tang, W., Liu, D., Yin, J.-Y., Nie, S.-P., 2020. Consecutive and progressive purification of food-derived natural polysaccharide: based on material, extraction process and crude polysaccharide. Trends Food Sci. Technol. 99, 76–87.
Tawfik, A., Ni, S.-Q., Awad, H.M., Ismail, S., Tyagi, V.K., Khan, M.S., Qyyum, M.A., Lee, M., 2021. Recent approaches for the production of high value-added biofuels from gelatinous wastewater. Energies 14 (16).
Thevarajah, B., Nishshanka, G.K.S.H., Premaratne, M., Nimarshana, P.H.V., Nagarajan, D., Chang, J.-S., Ariyadasa, T.U., 2022. Large-scale production of Spirulina-based proteins and c-phycocyanin: a biorefinery approach. Biochem. Eng. J. 185.
Thiansathit, W., Keener, T.C., Khang, S.-J., Ratpukdi, T., Hovichitr, P., 2015. The kinetics of Scenedesmus obliquus microalgae growth utilizing carbon dioxide gas from biogas. Biomass Bioenergy 76, 79–85.
United Nations, 2017. World Population Projected to Reach 9.8 billion in 2050, and 11.2 billion in 2100.
Ursu, A.V., Marcati, A., Sayd, T., Sante-Lhoutellier, V., Djelveh, G., Michaud, P., 2014. Extraction, fractionation and functional properties of proteins from the microalgae Chlorella vulgaris. Bioresour. Technol. 157, 134–139.
Valero-Vizcaino, A., Villaró-Cos, S., Morillas-España, A., Cerdá-Moreno, C., Lafarga, T., 2024. Production of techno-functional proteins and plant biostimulants from Nannochloropsis gaditana. Food Biosci. 59, 104000.
Vani, M.V., Basha, P.O., Rajesh, N., Riazunnisa, K., 2022. Development of chlorella pyrenoidosa EMS mutants with enhanced biomass and lipid content for biofuel production. Syst. Microbiol. Biomanuf. 3 (4), 693–701.
Vázquez-Romero, B., Perales, J.A., de Vree, J.H., Böpple, H., Steinrücken, P., Barbosa, M. J., Kleinegris, D.M.M., Ruiz, J., 2022a. Techno-economic analysis of microalgae production for aquafeed in Norway. Algal Res. 64, 102679.
Vázquez-Romero, B., Perales, J.A., Pereira, H., Barbosa, M., Ruiz, J., 2022b. Techno-economic assessment of microalgae production, harvesting and drying for food, feed, cosmetics, and agriculture. Sci. Total Environ. 837, 155742.
Venkata Subhash, G., Rohit, M.V., Devi, M.P., Swamy, Y.V., Venkata Mohan, S., 2014. Temperature induced stress influence on biodiesel productivity during mixotrophic microalgae cultivation with wastewater. Bioresour. Technol. 169, 789–793.
Verma, R., Kumari, K.V.L.K., Srivastava, A., Kumar, A., 2020. Photoautotrophic, mixotrophic, and heterotrophic culture media optimization for enhanced microalgae production. J. Environ. Chem. Eng. 8 (5), 104149.

Verni, M., Demarinis, C., Rizzello, C.G., Pontonio, E., 2023. Bioprocessing to preserve and improve microalgae nutritional and functional potential: novel insight and perspectives. Foods 12 (5).
Vo, T.P., Nguyen, D.Q., Thi Ho, T.A., Nguyen, T.M., Huy Ha, N.M., Vo, P.H.N., 2024. Novel extraction of bioactive compounds from algae using green solvent: principles, applications, and future perspectives. J. Agric. Food Res. 18.
Wan, M., Wang, Z., Zhang, Z., Wang, J., Li, S., Yu, A., Li, Y., 2016. A novel paradigm for the high-efficient production of phycocyanin from Galdieria sulphuraria. Bioresour. Technol. 218, 272–278.
Wang, J., Wang, Y., Gu, Z., Mou, H., Sun, H., 2023a. Stimulating carbon and nitrogen metabolism of chlorella pyrenoidosa to treat aquaculture wastewater and produce high-quality protein in plate photobioreactors. Sci. Total Environ. 878, 163061.
Wang, J., Wang, Y., Xiao, M., Liang, Q., Yang, S., Liu, J., Zhang, Y., Mou, H., Sun, H., 2024. Upcycling food waste into biorefinery production by microalgae. Chem. Eng. J. 484.
Wang, Q., Yu, Z., Wei, D., 2020. High-yield production of biomass, protein and pigments by mixotrophic chlorella pyrenoidosa through the bioconversion of high ammonium in wastewater. Bioresour. Technol. 313, 123499.
Wang, T., Tian, X., Liu, T., Wang, Z., Guan, W., Guo, M., Chu, J., Zhuang, Y., 2016. Enhancement of lipid productivity with a novel two-stage heterotrophic fed-batch culture of chlorella protothecoides and a trial of CO2 recycling by coupling with autotrophic process. Biomass Bioenergy 95, 235–243.
Wang, Y., Yang, S., Liu, J., Wang, J., Xiao, M., Liang, Q., Ren, X., Wang, Y., Mou, H., Sun, H., 2023b. Realization process of microalgal biorefinery: the optional approach toward carbon net-zero emission. Sci. Total Environ. 901, 165546.
Wang, Y., Zhao, W., Wang, J., Yang, S., Liu, J., Zheng, J., Mou, H., Sun, H., 2025. Enhancing astaxanthin production from Chromochloris zofingiensis via blue light and exogenous inducers in plate photobioreactors. Bioresour. Technol. 420, 132106.
Williamson, E., Ross, I.L., Wall, B.T., Hankamer, B., 2024. Microalgae: potential novel protein for sustainable human nutrition. Trends Plant Sci. 29 (3), 370–382.
Winkler, B., Tosi, H., Webster, A.J.F., Resende, F.D., Oliveira, A.A.M.A., Villela, L.C.V., 2011. Dried yeast (Saccharomyces cerevisae) as a protein source for horses. Livest. Sci. 137 (1), 168–177.
Wu, G., Bazer, F.W., Dai, Z., Li, D., Wang, J., Wu, Z., 2014. Amino acid nutrition in animals: protein synthesis and beyond. Annu. Rev. Anim. Biosci. 2 (1), 387–417.
Wu, Y., Shan, L., Zhao, W., Lu, X., 2025. Harnessing artificial intelligence to revolutionize microalgae biotechnology: unlocking sustainable solutions for high-value compounds and carbon neutrality. Mar. Drugs 23 (5).
Xiao, X., Zhou, Y., Liang, Z., Lin, R., Zheng, M., Chen, B., He, Y., 2022. A novel two-stage heterotrophic cultivation for starch-to-protein switch to efficiently enhance protein content of chlorella sp. MBFJNU-17. Bioresour. Technol. 344 (Pt A), 126187.
Xie, Y., Zhang, Z., Ma, R., Liu, X., Miao, M., Ho, S.-H., Chen, J., Kit Leong, Y., Chang, J.-S., 2022. High-cell-density heterotrophic cultivation of microalga Chlorella sorokiniana FZU60 for achieving ultra-high lutein production efficiency. Bioresour. Technol. 365, 128130.
Yadav, G., Dubey, B.K., Sen, R., 2020. A comparative life cycle assessment of microalgae production by CO2 sequestration from flue gas in outdoor raceway ponds under batch and semi-continuous regime. J. Clean. Prod. 258, 120703.
Yang, S., Fan, Y., Cao, Y., Wang, Y., Mou, H., Sun, H., 2024a. Technological readiness of commercial microalgae species for foods. Crit. Rev. Food Sci. Nutr. 64 (22), 7993–8017.
Yang, S., Wang, Y., Wang, J., Cheng, K., Liu, J., He, Y., Zhang, Y., Mou, H., Sun, H., 2024b. Microalgal protein for sustainable and nutritious foods: a joint analysis of environmental impacts, health benefits and consumer's acceptance. Trends Food Sci. Technol. 143.
Yang, S., Zhao, W., Mou, H., Sun, H., 2021. Improving astaxanthin production of haematococcus pluvialis by an efficient fed-batch strategy in a photobioreactor. Algal Res. 60.
Yu, B.S., Pyo, S., Lee, J., Han, K., 2024a. Microalgae: a multifaceted catalyst for sustainable solutions in renewable energy, food security, and environmental management. Microb. Cell Fact. 23 (1), 308.
Yu, H., Zhu, Y., Duan, F., Hui, A., Wang, A., 2024b. Fabrication of porous adsorbents templated from capillary foam stabilized with Chlorella for highly efficient removal of cationic dyes. Chem. Asian J. 19 (19).
Yu, Z., Ma, L., Liu, B., Wang, W., Shang, Z., Dang, H., Liu, C., 2023. Improvement of foaming properties of ovalbumin: insights into the synergistic effect of preheating and high-intensity ultrasound on physicochemical properties and structure analysis. Ultrason. Sonochem. 101, 106672.
Yu, Z., Zhao, W., Sun, H., Mou, H., Liu, J., Yu, H., Dai, L., Kong, Q., Yang, S., 2024c. Phycocyanin from microalgae: a comprehensive review covering microalgal culture, phycocyanin sources and stability. Food. Res. Int. 186, 114362.
Zayas, J.F., 1997. Water holding capacity of proteins. In: Functionality of Proteins in Food, pp. 76–133.
Zhang, T., Chen, T., Jiang, H., Liu, J., Wang, E., Zhang, M., Liu, X., 2023. pH-induced egg white protein foaming properties enhancement: insight into protein structure and quantitative proteomic analysis at protein adsorption layer. Food Hydrocoll. 144.
Zhang, T., Gong, P., Wang, Y., Jiang, H., Zhang, M., Yang, M., Liu, J., Liu, X., 2022. Lipid oxidation induced egg white protein foaming properties enhancement: the mechanism study revealed by high resolution mass spectrometry. Food Res. Int. 152, 110713.
Zhang, T.Y., Wang, X.X., Wu, Y.H., Wang, J.H., Deantes-Espinosa, V.M., Zhuang, L.L., Hu, H.Y., Wu, G.X., 2017. Using straw hydrolysate to cultivate chlorella pyrenoidosa for high-value biomass production and the nitrogen regulation for biomass composition. Bioresour. Technol. 244, 1254–1260.
Zhu, Y., Zhao, X., Zhang, X., Liu, H., 2019. Extraction, structural and functional properties of *haematococcus pluvialis* protein after pigment removal. Int. J. Biol. Macromol 140, 1073–1083.
Zkeri, E., Iliopoulou, A., Katsara, A., Korda, A., Aloupi, M., Gatidou, G., Fountoulakis, M. S., Stasinakis, A.S., 2021. Comparing the use of a two-stage MBBR system with a methanogenic MBBR coupled with a microalgae reactor for medium-strength dairy wastewater treatment. Bioresour. Technol. 323, 124629.