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Seaweed as an alternative protein source: Prospective protein extraction technologies

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ABSTRACT

Demand for food production has increased due to population growth. The negative environmental impact of animal agriculture necessitates the search for alternatives to animal protein-based products. Potential health benefits from micronutrient-rich seaweeds have attracted significant attention for further research. This encourages the use of seaweed as an alternative protein-rich source. However, traditional seaweed protein extraction presents drawbacks because of energy and water-intensive treatments and poor extraction yield, which limit their application. There is an urge for cost-effective, novel, and sustainable technologies for seaweed protein extraction at the right techno-economics. Thus, this review article discusses the economic potential of seaweed proteins and identifies the traditional technologies applied to extract seaweed protein and their limitations. A detailed analysis of novel methods that could potentially be utilized to extract and enrich seaweed protein is presented. Various protein quantification/qualification approaches reported in the literature have been thoroughly assessed to compare their advantages and disadvantages. Technologies like the pulsed electric field, ultrasound- and microwave-assisted extraction, high-pressure processing, and subcritical-water extraction have huge potential to extract protein from seaweed sustainably yet are relatively under-explored. More research is required to develop further insights on the process-quality inter-relationship of these technologies for improved seaweed protein extraction. Essential amino acid index, protein digestibility corrected amino acid score, and digestible indispensable amino acid score are the best approaches to evaluate seaweed protein quality for human consumption. However, reported studies have barely covered these aspects, including seaweed protein's sensorial quality.

1. Introduction

The global food sector's greenhouse gas (GHG) emissions are ~13.7 billion tons of CO_2-equivalent: the livestock sector contributes 14.5% of global GHG emissions and 30% of biodiversity loss (Garnett, Smith, Nicholson, & Finch, 2016). Population growth and increased food production during the past decades have put enormous pressure on the planet and its resources. From 2010 to 2050, global consumption patterns of meat and dairy products are projected to increase by 173% and 158%, respectively (Fasolin et al., 2019; McLeod, 2011). The global demand for protein is expected to escalate, exacerbating the need for more sustainable production systems to reduce the carbon footprint (Ritala, Häkkinen, Toivari, & Wiebe, 2017). Economic advantages and social and environmental considerations will drive decisionmakers and the future market (Ritala et al., 2017; Tubb & Seba, 2021). These trends encourage the search for alternative protein sources, such as soy, wheat, legumes, rapeseeds, seaweed, etc., to replace livestock proteins-based diets (Onwezen, Bouwman, Reinders, & Dagevos, 2021).

Unconventional protein sources are pinned as the next disrupter of the existent industrial animal farming, as they will be cheaper, healthier (Wells et al., 2017), and more environment friendly (Tubb and Seba, 2021). Seaweeds contain nutritional compounds, such as vitamins, soluble dietary fibers, and flavonoids, which are associated with health benefits, for example, reduced risk of cardiovascular diseases (Hata, Nakajima, Uchida, Hidaka, & Nakano, 2001) and antihypertensive activity (H. A. Jung, Hyun, Kim, & Choi, 2006). Lu et al. (2020)

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demonstrated the anti-obesity effect of *Plocamium telfairiae* (Rhodophyta) in mice. This is also shown by Murakami et al. (2021), who associated ameliorating diet-induced metabolic diseases with the suppression of intestinal fat absorption after supplementing *Sargassum horneri* (Phaeophyta) to mice. M.-C. Kang et al. (2013) were able to attenuate type-II diabetes in mice by administrating a phlorotannin derivative isolated from *Ecklonia cava* (Phaeophyta). Seaweed has been consumed worldwide, especially in Asian countries, as a fresh product (Sanjeewa, Lee, & Jeon, 2018); however, through fermentation, other bioactive metabolites can be obtained and incorporated as food supplements or pharmaceuticals, such as flavor-enhancing compounds (glutamic and aspartic acids and alanine), bioactive amino acids (threonine, leucine, and proline), and bioactive dipeptides (seryl-tyrosine and glycyl-tyrosine) (Reboleira, Silva, Chatzifragkou, Niranjan, & Lemos, 2021).

As seaweed cultivation does not compete with food crops for land and natural resources, adopting seaweed proteins could play a role in enabling a sustainable dietary shift. Seaweed contains up to 47% protein on a dry weight basis; this is higher than traditional protein sources, such as cow's milk (3.4%) (Brien, Hayes, Sheldrake, Tiwari, & Walsh, 2022; Gellrich, Meyer, & Wiedemann, 2014). With the growing awareness of food production's impact on climate change, seaweed can be considered a major aquatic resource with the potential to play a vital role as a net zero feedstock for our food system. There are >10,000 species of seaweed, but only 145 species are harvested for human consumption (Baweja, Kumar, Sahoo, & Levine, 2016; Fleurence & Levine, 2016). Seaweeds are classified into three groups based on their distinct pigments: (1) Rhodophyta or red seaweeds (phycobilin (Rawiwan, Peng, Paramayuda, & Quek, 2022)); (2) Phaeophyta or brown seaweeds (fucoxanthin (H. Zhang et al., 2015)); and (3) Chlorophyta or green seaweeds (chlorophylls (Chen & Roca, 2018)). The growing trend in seaweed production is shown in Fig. 1, with red seaweed comprising 52.6% of the production in 2019.

The protein content of different seaweeds varies substantially depending on species, seasonality, harvest maturity, and environmental factors (Thiviya, Gamage, Gama-Arachchige, Merah, & Madhujith, 2022). Red, followed by green and brown seaweeds, usually present the highest protein content (Table 1), reaching up to 47% of their dry weight (Garcia-Vaquero & Hayes, 2016). Currently, several protein-rich seaweeds, such as *Ulva lactuca* (Chlorophyta), *Undaria pinnatifida, Fucus serratus* (Phaeophyceae), *Neopyropia tenera* (formerly *Porphyra tenera*), *Chondrus crispus*, and *Palmaria palmata* (Rhodophyta) are approved by the European Food Safety Authority for human consumption (Geada et al., 2021). Seaweeds are also a good source of EAAs – for example, green seaweed *Caulerpa acemose* var. *Peltata* possesses up to 40% of EAAs (Černá, 2011; Praiboon, Palakas, Noiraksa, & Miyashita, 2018).

Table 1
Protein content in seaweeds.

Group	Seaweed	Protein content (% DW)	Protein analysis method	References
Brown	*Ascophyllum nodosum*	7.1 ± 0.02	Dumas (N × 6.25)	(Shekhar U. Kadam, Álvarez, Tiwari, & O'Donnell, 2017)
	Durvillaea antarctica	9.7	Kejhdal (N × 6.25)	(Mateluna, Figueroa, Ortiz, & Aguilera, 2020)
Green	*Codium fragile*	11.7 ± 0.3	Bicinchinonic acid colorimetric	(Kulshreshtha et al., 2015)
	Ulva fenestrata	18.0 ± 0.7	Dumas (N × 5)	(Juul et al., 2022)
	Ulva rigida	11.2 ± 5.8	Kejhdal (N × 6.25)	(Fleurence, Le Coeur, Mabeau, Maurice, & Landrein, 1995)
	Ulva rotundata	10.0 ± 4.9	Kejhdal (N × 6.25)	(Fleurence, Le Coeur, et al., 1995)
	Ulva sp.	6.9 ± 0.3	Dumas (N × 5)	(Polikovsky et al., 2020)
	Ulva sp.	8.4 ± 0.1	Modified Lowry	(Prabhu, Levkov, Livney, Israel, & Golberg, 2019)
	Ulva sp.	20.2 ± 1.3	Modified Lowry	(Robin et al., 2018)
Red	*Chondrus crispus*	27.2 ± 1.4	Bicinchinonic acid colorimetric	(Kulshreshtha et al., 2015)
	Mastocarpus stellatus	16.9 ± 0.1	Kjeldahl (N × 4.59)	(Barral-Martinez, Florez-Fernandez, Dominguez, & Torres, 2020)
	Mastocarpus stellatus	18	Proximal composition	(Cid, Rodriguez-Seoane, Diaz-Reinoso, & Dominguez, 2021)
	Palmaria palmata	15.2	Dumas (N × 5)	(Naseri, Marinho, Holdt, Bartela, & Jacobsen, 2020)

Seaweed biomass comprises different types of proteins, such as glycoproteins (proteins covalently linked to glycans (Zheng et al., 2021)), phycobiliproteins (covalently attached open-chain tetrapyrroles known as phycobilins present in red algae (Li et al., 2019), lectins (proteins or glycoproteins containing at least one non-catalytic domain that binds

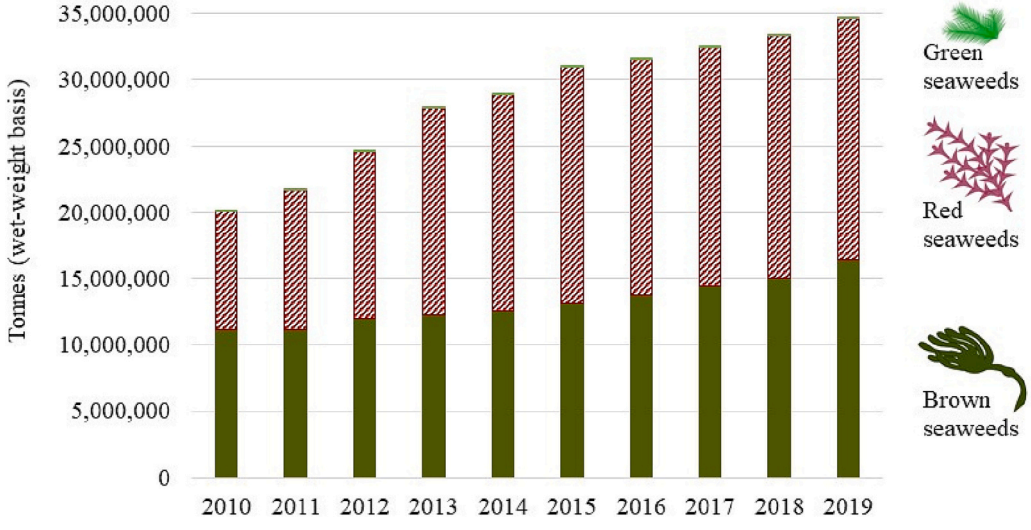

Fig. 1. Global production of seaweeds (2010–2019) [Data source: FAO, 2021].

reversibly to specific mono- or oligo-saccharides), peptides (protein fragments containing from 3 to 40 AAs), and cell wall-attached proteins, such as arabinogalactan proteins (highly glycosylated proteins present in some seaweeds) (Pliego-Cortés, Wijesekara, Lang, Bourgougnon, & Bedoux, 2020). Given the variability of protein types and their properties, extraction efficiency varies among species.

2. Market trends

The global seaweed industry is projected to grow from $15.01 billion in 2021 to $24.92 billion in 2028 (Insight, 2021). A recent FAO report (FAO, 2021) estimated that 34.7 million MT of aquatic plants, primarily marine macroalgae, were produced globally in 2019: 1.08 million MT (3.1%) by wild capture and 33.6 million MT (about 96.9%) by aquaculture (FAO, 2021). According to the report of Oilgae (2023), the total value of the European algae industry (in 2015) was estimated at € 6.3 billion, with ~78% belonging to the seaweed industry. Most global seaweed cultivation involves ~50 countries with a major share in East and Southeast Asia, and the wild harvest primarily occurs in Chile, China, and Norway (FAO, 2021). The major 28 producers are shown in Fig. 2. Seaweed for human consumption shares 85% of the world market, including seaweed-based food products (e.g., kombu, wakame, and Nori) and hydrocolloids as food ingredients. Various non-food uses of seaweeds, such as nutraceutical, pharmaceutical, animal feed, biofertilizers/bio-stimulants, bio-packaging, and biofuels, are also projected to drive market growth in the future. Japanese Wakame (*Undaria pinnatifida*), Nori (*Porphyra* sp.), and Japanese kelp (*Saccharina japonica*) were the most cultivated species for food applications, whereas, for the wild harvest, Chilean kelp (*Lessonia nigrescens*) accounts for approximately 22% of the total harvest (FAO, 2021).

According to Businesswire report (Markets, 2022), the seaweed protein market is projected to reach $1.131 billion by 2027. The increasing sustainability and health awareness among consumers drive the shift to livestock-free alternative plant protein consumption, accelerating the market growth of plant-based food. Seaweed protein, rich in nutrients, provides excellent innovation opportunities to formulate plant-based meat, seafood, and dairy alternatives. The US-based start-up UMARO (formerly known as Trophic; http://wwww.umarofoods.com) is focused on developing farmed red seaweed-based protein for making meat alternatives more meat-like. Israeli-based Genesea (http://www.genesea.net) is a spin-off from the Tel Aviv University that aims to commercialize the patented platform for making protein isolate from common seaweed varieties suitable for product formulation at low pH. Another California -based start-up called Triton (https://www.tritonai.com) aims to disrupt the food system with sustainable plant protein solutions from red and green seaweeds.

The high cost of seaweed farming slows down the market revenue growth of seaweed protein; therefore, improved protein extraction with higher yield can drive alternative protein industries. The relatively shorter shelf-life of freshly harvested seaweed requires immediate post-harvest treatment to stabilize the biomass for downstream processing and exportation. Typically fresh seaweeds become inedible due to the development of off-flavor and slime on the surface within ~4 days after harvest (Raja, Kadirvel, & Subramaniyan, 2022).

Besides proteins, seaweed offers a wide range of relevant bioproducts that can be co-obtained during protein extraction through multistage cascading processes, improving the economic aspect of seaweed protein commercialization (Baghel et al., 2020). These coproducts can show functionalities and bioactivities useful to produce bioactive ingredients, chemicals, and biofuels. Biorefineries have been proposed to promote an economically-viable "zero-waste" cascading process (Torres, Kraan, & Domínguez, 2019). Bikker et al. (2016) proposed a multistage process to combine extract protein and sugars (glucose, rhamnose, and xylose) from *U. lactuca* (Chlorophyta). The protein extraction was used for animal feed, whereas the coproduced sugars were successfully used to produce acetone, butanol, ethanol and 1,2-propanediol by clostridial fermentation. González-López, Moure, and Domínguez (2012) combined alkaline extraction, ultrafiltration, and autohydrolysis to recover alginate from *Sargassum muticum* and coextract antioxidants and other insolubles that could be destined for agriculture and energetic purposes. Protein accounted for 25% of the retentate.

3. Seaweed protein extraction

The major challenges for seaweed protein are the lack of eco-innovative extraction methods to overcome the limitations of the

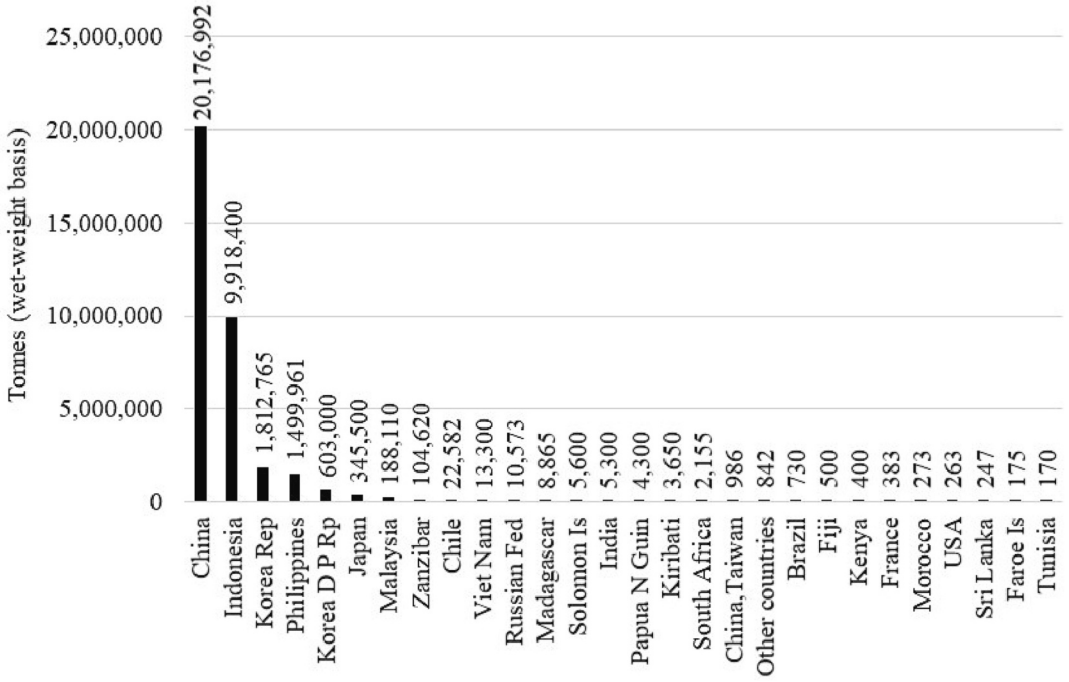

Fig. 2. Major producers of aquatic plants (annual production in 2019) [Data source: FAO, 2021].

conventional extraction processes, which incur the high cost associated due to low yield: for instance, Kulshreshtha et al. (2015) could recover only 2.9% and 1.4% of protein from *Codium fragile* using enzyme-assisted extraction (Neutrase) and osmotic shock, respectively; Fleurence, Le Coeur, et al. (1995) recovered <1% of protein from *Ulva rigida* and *Ulva lactuca* (as *Ulva rotundata*) using enzyme-assisted extraction (Cellulase). Seaweed proteins are cross-linked to polysaccharides via disulfide bridges within the cell wall assembly (Cermeño, Kleekayai, Amigo-Benavent, Harnedy-Rothwell, & FitzGerald, 2020). Due to high dispersion viscosity, the anionic or neutral polysaccharides and polyphenol-rich seaweed cell walls hinder protein release during the extraction. Moreover, the seaweed species, like brown algal kelp, are the most efficient iodine accumulators of all living systems. Species like *Laminaria digitata* (Phaeophyceae) contain an average iodine content of 1.0% dry weight, representing ~30,000-fold accumulation from the seawater. Such high iodine content in the seaweed protein somewhat limits its consumption due to related health issues. Thus, controlling iodine content during and/or before the protein extraction should address this food safety issue.

Additionally, seaweed protein extraction requires separating non-protein nitrogenous compounds to obtain purity. Different methods for seaweed protein extraction are described, and different efficiencies are reported depending on the species (Supplementary 1). Ideally, the protein extraction should reach high protein recovery, be cost-effective, non-destructive, and not time-consuming (Jeon, Wijesinghe, & Kim, 2011) to guarantee an economically viable production from seaweed. As per the literature, various enzymatic and chemical methods have been tested for seaweed protein extraction with little success in improving extraction efficacy (Fleurence, Le Coeur, et al., 1995; Shekhar U. Kadam et al., 2017).

Although there are extensive review articles on specific techniques for cell disruption of different plant biomasses for various purposes (Kant & Kumar, 2022; Lim, Chua, & Mustaffa, 2022; Oh, Kim, Ilhamsyah, Lee, & Kim, 2022; Sengar et al., 2022), there is a lack of recent reviews on the conventional and novel methods for protein extraction from seaweeds. Thus, this review aims to discuss the known methods to extract protein from seaweed and the novel techniques that still have not been broadly applied to seaweed but have demonstrated positive outcomes for other protein-rich biomass sources.

4. Methods for cell disruption and protein extraction

The typical protein extraction process can be divided into four categories: chemical, physical, biological and hybrid. There is no proper method to apply to all seaweeds. The extraction approach must be assessed for each species, and the pros and cons must be evaluated regarding the biomass composition to ensure the optimal protocol for obtaining protein-rich fractions (Table 2). Additionally, food regulations must be followed to ensure human safety, especially regarding using solvents (FAO; UK Goverment, 2020). Solvents are commonly required to aid mass and energy transfer, but besides the economic drawback of their application for protein extraction, they are environmentally and safety- concerning (Kerton & Marriott, 2013). Some greener alternatives have been proposed for conventional solvents, for example, natural deep eutectic solvents (NADES), which dissolve chemicals of low water solubility (Obluchinskaya et al., 2021), and instantaneous controlled pressure drop, which relies on heating the biomass followed by an abrupt pressure drop towards a vacuum to extract the intracellular material before condensation (Chemat, Fabiano-Tixier, Vian, Allaf, & Vorobiev, 2015). However, the application for seaweed protein extraction is still limited (Obluchinskaya et al., 2021). Nie, Chen, and Lu (2020) combined ultrasound- assisted extraction with NADES (choline chloride, 1,2-propanediol, and water) to extract polysaccharides from *S. horneri* (Phaeophyta): the extraction yield reached only 11.3% through optimal extraction conditions.

Despite the method used, protein recovery from seaweed follows the same overall steps: (1) Cell disruption and protein extraction – which can be further subclassified into (a) liquid conditions, where chemical (e.g., acid/alkaline and solvent extraction), biochemical (e.g., enzyme-assisted extraction), and physical (e.g., osmotic shock, ultrasound-assisted, microwave-assisted extraction) treatments can be applied individually or in combination to break down the cell wall and solubilize the protein content, and (b) dry conditions, where milling and sieving, air classification, and electrostatic separation can be employed; followed by (2) protein concentration, which can be achieved by acid, solvent, electrolyzed water and isoelectric precipitation, filtration, hydrolysis, and chromatography (Fig. 3) (Contreras et al., 2019).

4.1. Cell disruption

4.1.1. Enzymatic hydrolysis

Enzyme-assisted extraction has been extensively applied to treat lignocellulosic fraction and proteinaceous material from agri-food residues, such as oilseeds (Fetzer, Herfellner, Stäbler, Menner, & Eisner, 2018), olive (Vergara-Barberán, Lerma-García, Herrero-Martínez, & Simó-Alfonso, 2015), and pomegranate seeds (Talekar, Patti, Singh, Vijayraghavan, & Arora, 2018). This extraction process commonly combines carbohydrate-hydrolyzing enzymes and peptidases with neutral or mild acidic/alkaline conditions to digest specific polymer bonds and solubilize the protein content (Contreras et al., 2019). Carbohydrases degrade carbohydrate bonds and are important enzymes in releasing proteins linked to the lignocellulosic fraction (Sari, Mulder, Sanders, & Bruins, 2015). Peptidases cleave the carbon-nitrogen bond between two AAs in a protein (Neil David Rawlings, Barrett, & Bateman, 2011; Neil D. Rawlings & Bateman, 2019), although the combination of peptidases and carbohydrases does not improve protein extraction compared to peptidases only (Sari et al., 2015). Phospholipase, which breaks down phospholipid bonds, has also been applied to optimize protein extraction from olives (Vergara-Barberán, Lerma-García, Herrero-Martínez, & Simó-Alfonso, 2014). Despite showing better protein recovery by phospholipase compared to other enzymes (lipase, cellulase, and the enzymatic mixture Celluclast®) (Vergara-Barberán et al., 2014), its application has not been further explored (Contreras et al., 2019).

The use of enzymes is one of the most common techniques to assist protein extraction from red and green seaweeds (Echave et al., 2021). The brown seaweeds are the least studied regarding enzyme-assisted extraction due to their more complex cell wall composition (majorly composed of alginic acid (Draget, Smidsrød, & Skjåk-Bræk, 2005), see Table 3) (Cermeño et al., 2020), with only a few studies available (e.g., *L. digitata* (Costa et al., 2021)). Enzymes can be applied individually (Costa et al., 2021; Fleurence, Le Coeur, et al., 1995; Fleurence, Massiani, Guyader, & Mabeau, 1995; Kulshreshtha et al., 2015) or as a cocktail mixture (Fleurence, Le Coeur, et al., 1995; Fleurence, Massiani, et al., 1995; Harnedy and FitzGerald, 2013; Joubert & Fleurence, 2008; Mæhre, Jensen, & Eilertsen, 2016; Postma et al., 2018) (Table 4), presenting different efficiencies among seaweeds. However, enzymes do not always lead to improved protein extraction (Postma et al., 2018) because the effectiveness of enzymes in breaking down the seaweed cell wall depends on the cell wall composition, which varies across the species. For instance, cellulase, which efficiently aids in the recovery of protein from *P. palmata* (Joubert and Fleurence, 2008), is ineffective in recovering protein from *U. rigida* and *Ulva lactuca* (as *Ulva rotundata*), therefore requiring the aid of other enzymes to break the cell wall (Fleurence, Le Coeur, et al., 1995).

Kulshreshtha et al. (2015) demonstrated a significantly higher protein hydrolyzation by cellulase (7.1% ± 0.3% DW) in *C. crispus* than ultraflo (xylanase and beta-glucanase; 5.8% ± 0.5% DW) and beta-glucanase (4.1% ± 0.4% DW). The same authors, however, reported no significant difference in the protein hydrolyzation of *C. fragile* by using proteases (2.9% ± 0.1% DW) or beta-glucanase (2.6% ± 0.2%). On the other hand, Nasero et al. achieved the highest protein extraction

Table 2
Advantages and disadvantages of each protein extraction treatment.

Method	Advantage	Disadvantage	Purpose*	References
Biochemical				
Enzymatic	• Applied to recover target proteins • Well-documented use for green and red seaweeds • Possibility of using as a cocktail mixture and simultaneously with MAE, UAE	• Species-dependent • Long extraction time • Can be expensive • Requires specific temperature and pH • Not recommended for brown seaweeds because of their cell wall complexity	CD	(Brien et al., 2022; Cermeño et al., 2020; Garcia-Vaquero, Rajauria, O'Doherty, & Sweeney, 2017; Harnedy & FitzGerald, 2013)
Chemical				
Acid/Alkaline	• It is simple and cost-effective • It can be applied stepwise (acid followed by alkaline or alkaline followed by acid extraction) • It can be simultaneously used with UAE	• Partial degradation of the proteins and bioactive components • It is time-consuming • Its efficiency is species-dependent	CD and PE	(Shekhar U. Kadam et al., 2017; O'Connor, Meaney, Williams, & Hayes, 2020)
Physical				
Freeze-thawing	• It obtains protein with high emulsion activity • Simple	• Low emulsion stability • High-time and energy demanding • Not scalable	PE	(Abdollahi et al., 2019; Dewi, Santoso, Setyaningsih, & Hardingtyas, 2020; Kulkarni & Nikolov, 2018)
Osmotic shock	• Simple and cheap • It does not produce extra contaminants • Possibility of using simultaneously with any mechanical method	• Long extraction time (hours) • Low efficiency • Limited to highly water-soluble proteins	PE	(Barbarino & Lourenço, 2005; Cermeño et al., 2020)
Pressing (screw press, extrusion)	• Short treatment duration • Scalable • Non-thermal and chemical-free process	• It can be high-energy demanding when compared to other alternatives	PE	(Bals & Dale, 2011; Ghosh, Gillis, Sheviryov, Levkov, & Golberg, 2019; Guo et al., 2022; Robin et al., 2018)
High shear	• Associated with the total protein recovery improvement • Improvement of protein heat-stability • Reduction of viscosity and particle size	• Lower water-soluble protein recovery compared to osmotic shock • Application for protein extraction is not commonly applied • Energy-intensive	PE	(Harnedy and FitzGerald, 2013; Postma et al., 2018)
High-Pressure Processing (HPP)	• Non-thermal process • Shorter extraction time (seconds to minutes)	• Limited use due to its costs • Rarely applied for seaweed protein extraction	PE	(Laguna, Picouet, Guàrdia, Renard, & Sarkar, 2017; Mulla, Subramanian, & Dar, 2022)
Microwave-assisted (MAE)	• It does not require solvents • Short extraction times (minutes) • Facilitates the penetration of solvents • It can recover proteins without changing their structures • Promotes the recovery of high-purity protein	• Not recommended to extract heat-sensitive bioactive compounds • Not commonly used for protein extraction • Application at a large scale demands a high investment cost	PE	(Barba, Grimi, & Vorobiev, 2015; Cermeño et al., 2020; Farhadpour et al., 2016; Ochoa-Rivas, Nava-Valdez, Serna-Saldívar, & Chuck-Hernández, 2017)
Pulsed electric field (PEF)	• It can be applied to thermal-sensible proteins • It is energy efficient • It is considered a green technology • It can rapidly aid in disrupting cell membranes • It diminishes the need for organic solvents • It can be applied sequentially with any other method	• Limitations on scaling up • Its use for protein extraction needs further research	CD	(Brien et al., 2022; Cermeño et al., 2020; Polikovsky et al., 2016, 2019; Postma et al., 2018)
Subcritical water	• Short extraction times • Higher quality of the extracts • Lower costs of the extracting agent • Green technology • It does not require biochemicals • It allows the recovery of peptides	• The use for seaweed protein extraction is rare • It might cause thermal degradation • Examples of applications on a large scale are limited	PE	(Fan, Hu, Wang, Yang, & Zhang, 2020; Herrero, Cifuentes, & Ibañez, 2006; Polikovsky et al., 2020; Sereewatthanawut et al., 2008; Ziero et al., 2020)
Supercritical fluid	• Short extraction times • Lower costs of the extracting agent • Green technology • It does not require biochemicals	• Deterioration of AAs • Limited examples of applications	PE	(Di Domenico Ziero et al., 2020; Herrero et al., 2006)
Ultrasound-assisted (UAE)	• Fast processing time • Low energy demand • Limited use of organic solvents • It can be scaled up	• Can change the structure of polysaccharides • There might be some protein degradation • The active zone is restricted to a zone near the ultrasonic emitter • The presence of a dispersed phase can attenuate the wave, limiting the cell disruption	CD	(Barba et al., 2015; Brien et al., 2022; Shekhar U. Kadam et al., 2017)

(*continued on next page*)

Table 2 (*continued*)

Method	Advantage	Disadvantage	Purpose*	References
		• Other compounds are solubilized with protein (for instance, carbohydrates and phenols)		
Reverse micelle	• It has already been applied to extract and encapsulate target compounds	• Not yet applied for seaweed	PE	(Arshad et al., 2014; Pojić, Mišan, & Tiwari, 2018; Sagbas, Butun, & Sahiner, 2012)
Two-phase separation	• High separation efficiency	• Not commonly applied for seaweed protein extraction	PE	(Fleurence, Le Coeur, et al., 1995)

* CD = Cell disruption; PE = Protein extraction.

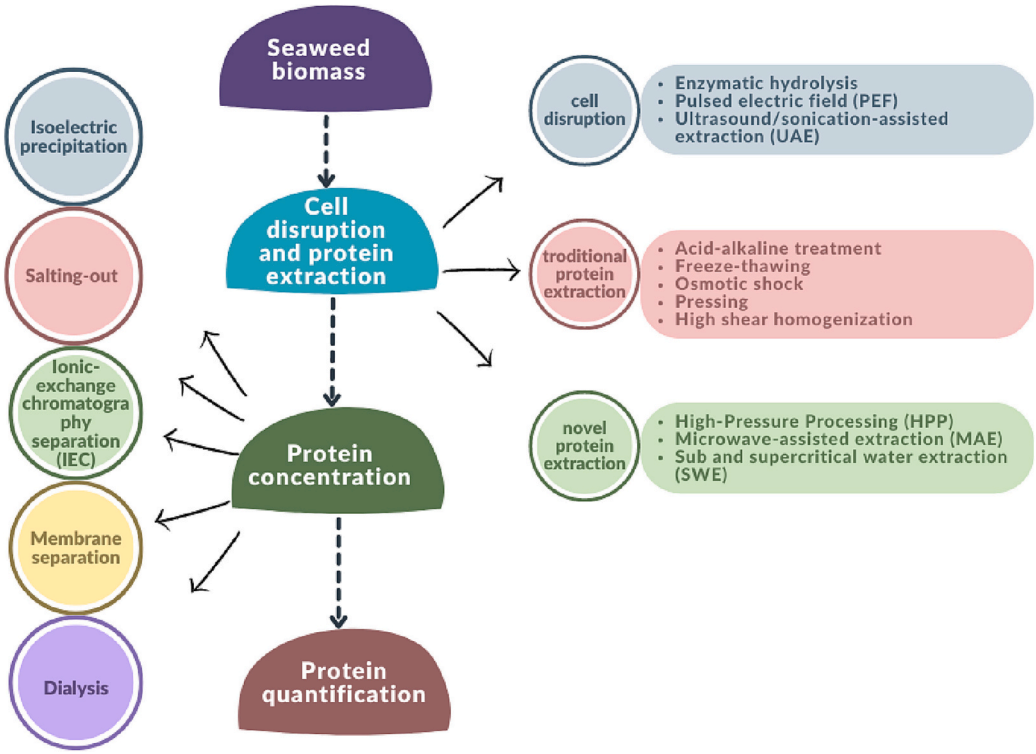

Fig. 3. A schematic for common cell disruption and protein extraction methods.

Table 3
Cell wall composition of seaweeds. Adapted from Goñi, Quille, and O'Connell (2020) and O'Connor et al. (2020).

Seaweed	Cell wall composition
Brown	Cellulose, glucan, sulfated xyloglucan, sulfated xylofucoglucuronan, alginate, fucoidan, laminarin, and mannitol
Green	Cellulose, glucan, mannans, xyloglucan, glucuronan, and ulvans
Red	Cellulose, sulfated mixed-linkage glucan, mannan, sulfated galactans, floridean starch, agars, carrageenans, glucomannan, and xylans

(> 85%) from *P. palmata* using protease combined with either cellulase or xylanase (Naseri et al., 2020). It has also been shown that a combination of cellulase and xylanase, as well as xylanase and Viscozyme® (cellulolytic enzyme cocktail), had a similar protein extraction efficiency compared to the control (without enzymes). This means that protein release from *P. palmata* requires more than only the breakdown of polysaccharides in the cell wall (Naseri et al., 2020).

Costa et al. (2021) identified that alginate lyase could effectively disrupt the cell wall of L. *digitata* to release the trapped fatty acids but not the protein. This could be associated with limited access to protein due to binding with other compounds or poor protein separation due to the increased viscosity of the medium. For example, the extracellular phenolic compounds cross-linked to alginates have protein binding properties, whereas hydrocolloidal anionic polysaccharides increase viscosity (Costa et al., 2021). Additionally, Naseri et al. (2020) reported that protease had the highest essential amino acid:amino acid (EAA:AA) ratio (0.44) in the liquid fraction among the enzymes assessed for *P. palmata*; the EAA:AA ratio in the residual biomass (after extraction) ranged from 0.45 to 0.55. Despite the higher EAA:AA ratio in the residual biomass, after protein precipitation from the liquid fraction, the AA content was higher in the precipitate than in the residual biomass, except for lysine and valine. Moreover, the high content of glutamic and aspartic acids imparts extracts with a strong umami flavor, an attractive quality for food flavoring (Mouritsen, 2012). The AA recovery could potentially be higher by optimizing the protein precipitation, considering that up to 70% of the extracted protein remained in the liquid fraction after conventional isoelectric precipitation (Naseri et al., 2020).

The concentration of enzymes also influences protein extraction to a certain extent (Mendez and Kwon, 2021). Joubert and Fleurence (2008) evaluated the impact of using different concentrations (expressed as units, U) of cellulose and xylanase on the protein extraction from *P. palmata*. They showed that a 24 U (i.e., 24 U cellulose +24 U xylanase) resulted in an improvement in the protein extraction compared to the control (0 U) and 12 U. However, increasing the concentration to 48 U and 72 U did not enhance the overall protein extraction, meaning that all the polysaccharide bonds could be cleaved with 24 U mixture. This study demonstrated a varied protein extraction efficiency by cellulolytic

Table 4
Enzymes used to disrupt seaweed's cell wall.

Enzyme	Seaweed group	Species	References
Carbohydrase			
–	Brown	*Laminaria digitata*	(Costa et al., 2021)
Beta-glucanase	Red	*Chondrus crispus*	(Kulshreshtha et al., 2015)
Cellulase	Green	*Codium fragile*	(Kulshreshtha et al., 2015)
	Red	*Chondrus crispus*	(Kulshreshtha et al., 2015)
	Red	*Gracilaria fisheri*	(AMIN, LEE, & SHARMIN, Amin, Lee, & Sharmin, 2020)
	Red	*Devaleraea mollis*	(Mendez & Kwon, 2021)
Cellulase + agarase	Red	*Gracilariopsis longissima* (formely *Gracilaria verrucosa*)	(Fleurence, Massiani, et al., 1995)
Cellulase + beta-glucanase + hemicellulose	Green	*Ulva lactuca* (as *Ulva rotundada*)	(Fleurence, Le Coeur, et al., 1995)
	Green	*Ulva rigida*	(Fleurence, Le Coeur, et al., 1995)
	Red	*Devaleraea mollis*	(Mendez and Kwon, 2021)
Cellulase + carrageenase	Red	*Chondrus crispus*	(Fleurence, Massiani, et al., 1995)
Cellulase + xylanase	Red	*Palmaria palmata*	(Mæhre et al., 2016)
	Red	*Palmaria palmata*	(Harnedy and FitzGerald, 2013)
	Red	*Palmaria palmata*	(Joubert and Fleurence, 2008)
Pectinase	Green	*Ulva lactuca* (as *Ulva rotundada*)	(Postma et al., 2018)
Xylanase	Red	*Palmaria palmata*	(Fleurence, Massiani, et al., 1995)
Xylanase + beta-glucanase	Red	*Chondrus crispus*	(Kulshreshtha et al., 2015)
Peptidase			
Protease	Red	*Palmaria palmata*	(Naseri et al., 2020)
	Red	*Gracilaria fisheri*	(AMIN et al., Amin et al., 2020)
	Green	*Codium fragile*	(Kulshreshtha et al., 2015)
	Red	*Chondrus crispus*	(Kulshreshtha et al., 2015)
Sulfatase			
Bacterial enzymes	Green	*Ulva* sp.	(Reisky et al., 2019)

enzymes depending on the cultivation months (April and July) of seaweed, suggesting the seasonal variation of the polysaccharides content or polysaccharides-protein organization in seaweeds. Similarly, Postma et al. (2018) could not identify a proportional increase in protein extraction efficiency upon increasing enzymes concentration (cellulase, pectinase, cellulase and pectinase, β-glucuronidase, and abalone powder) from 0.5 to 2% DW.

The enzyme activity is optimum at a certain pH and temperature, meaning that significant variations of these parameters should influence the efficacy. Naseri et al. (2020) studied the protein extraction from *P. palmata* using protease (Alcalase®) at different temperatures (30–60 °C) and pHs (7–9). In their studies, the lowest and highest extraction efficiency were achieved at 30 °C (<76.4%) and 60 °C (>83.8%), respectively. pH did not affect the Alcalase activity on the protein recovery in the soluble/liquid fraction, although the protein recovered in the insoluble/pellet fraction was higher at pH 7 (12.6%) compared to pH 8 (10.4%) at 60 °C. Mendez and Kwon (2021) reported considerable recoveries (>3- and 2-fold for water and salt-soluble proteins, respectively) at 37 °C and 7 °C using cellulases via a sequential treatment approach (aqueous + saline + alkaline + ethanol extraction).

Enzyme-assisted protein extraction must be assessed for each seaweed, even for the same species, due to geographical distribution and seasonality differences, which can affect the cell wall polysaccharide content; hence the enzyme performance to solubilize the protein. Parameters such as enzyme and substrate ratio, temperature, and pH must be carefully optimized to ensure the highest protein extraction (Brien et al., 2022; Garcia-Vaquero et al., 2017). Optimizing incubation time is also important because treatment longer than 4 h does not seem to improve further the protein extraction (Postma et al., 2018).

4.1.2. Pulsed electric field (PEF)

PEF induces cell stress through transient permeabilization and electrophoretic movement of charged species between cellular compartments by generating high-voltage electric currents (Barba et al., 2015; Robin et al., 2018). It is a food-grade process and a green technology that can rapidly and effectively aid cell membrane disruption (Cermeño et al., 2020) with limited use of organic solvents (Brien et al., 2022). PEF is commonly used as a pretreatment to facilitate biocompound recovery (Prabhu et al., 2019; Robin et al., 2018). It also induces the permeability of other compounds, such as phenolics and carbohydrates (Einarsdóttir et al., 2022; Postma et al., 2018) and is used to extract lipids for biofuel (Brien et al., 2022). Robin et al. (2018) reported an approximately 7-fold increase in the total extracted protein concentration from *Ulva* sp. after PEF treatment (50 pulses, 50 kV) followed by mechanical press compared to osmotic shock followed by the mechanical press (control). By increasing the charging voltage from 20 to 50 kV, they achieved around 40% more protein extraction, demonstrating the positive impact of charging voltage on electroporation and concomitant protein extraction from *Ulva* sp. Interestingly, increasing the energy input per kg of biomass does not seem to enhance protein extraction. Postma et al. (2018), however, only achieved 15.1% protein yield from *U. lactuca* using PEF (2 pulses, 3 kV) compared to 20% using osmotic shock. This study reported that pulse duration did not have a significant effect on protein yield at electric field strengths of 3 and 5 kV cm^{-1} while a shorter pulse duration (0.05 ms) resulted in higher protein extraction than longer pulse duration (0.5 and 5 ms) at an electric strength field of 7.5 kV cm^{-1}. PEF can potentially help seaweed protein extraction, yet different operational parameters must be adjusted to facilitate optimized cell disruption and improve extraction yield.

4.1.3. Ultrasound/sonication-assisted extraction (UAE)

The sound wave in UAE is transformed into mechanical energy that disrupts the cell wall through the microturbulence resulting from the implosion of air bubbles (acoustic cavitation phenomena) (Fan et al., 2020; Fleurence and Levine, 2016), thereby promoting the depolymerization (Barrio et al., 2022) and the liquefaction of cell compounds (Shekhar U. Kadam et al., 2017). Ochoa-Rivas et al. (2017) demonstrated that UAE could promote changes in protein's secondary and tertiary structure. Two different types of equipment may be applied for this purpose: ultrasonic water bath (more common and cheaper) and ultrasonic probe (more powerful, requiring a shorter processing time) (Shekhar U. Kadam et al., 2017). This technology is cheaper (compared to PEF, for example) and can be scaled up (Brien et al., 2022). Nevertheless, there might be other issues regarding the use of UAE: (a) possible protein degradation, (b) restriction of the active zone near the ultrasonic emitter, (c) wave attenuation due to the presence of a dispersed phase (Barba et al., 2015), and (d) the solubilization of other compounds (Shekhar U. Kadam et al., 2017). Consequently, the process must be optimized, and further purification may be required to guarantee a good-quality protein yield.

Time and power are key factors that affect the Fan et al. (2020) reported an increase in the protein extraction rate from *Arthrospira platensis* (formerly *Spirulina platensis*) (Cyanobacteria) with a longer sonication time, reaching equilibrium after 60 min, while the extraction rate increased with power to 200 W and decreased afterward. Barrio et al. (2022) found that extraction time significantly affected agar extraction from *Gelidium corneum* (formerly *Gelidium sesquipedale*) (Rhodophyta), but regarding protein extraction and AA profile (rich in

aspartic acid), neither extraction time (30 to 60 min) nor power (50 to 100%) affected them. Similarly, Ochoa-Rivas et al. (2017) showed that extraction time was essential for the protein extraction from peanut protein only at a lower amplitude (20 μm), while at a higher amplitude (100 μm), the extraction for 15 or 40 min impacted neither the protein extraction yield nor its purity Lafarga, Álvarez, Bobo, and Aguiló-Aguayo (2018) reported a significantly higher protein extraction from "Ganxet" beans using 0.4 M NaOH, followed by UAE for 60 min, which can be attributed to the facilitation of deeper penetration of solvent into the cell. Mateluna et al. (2020) showed that the alginate in *Durvillaea antarctica* (Phaeophyceae) produced a damping and insulating effect on the ultrasound waves in the first minutes. Although these authors compared relatively short times (5 and 15 min), it can be expected that this effect depends on the biomass composition. It has been demonstrated that extended US processing on brown seaweed *Ascophyllum nodosum* could compensate for the lower power (Shekhar U. Kadam et al., 2017). This study coupled acid, alkali or combined acid-alkali extraction with and without US pre-treatment, which clearly showed that the US processing step facilitated the reduction in the acid/alkali concentration (from 0.4 M to 0.1 M) and time required (from 60 to 10 min) to extract protein, although compromised the purity due to the solubilization of other compounds even at a shorter time.

Despite the limited use for seaweed protein extraction, UAE seems a promising technology to be combined with other conventional extraction methods, such as pH-shift. However, the effect of time and power must be individually assessed, and the protein yield must counterbalance the costs associated with the UAE implementation.

4.2. Traditional protein extraction

4.2.1. Acid-alkaline treatment

Different acidic and alkaline conditions have been applied to extract protein from seaweed, resulting in shorter peptides and lower molecular weight of proteins due to hydrolysis (Shekhar U. Kadam et al., 2017). Acidic and alkaline extraction can be used individually or combined sequentially (acidic-alkaline or alkaline-acidic extraction) (Supplementary 1). The pH influences protein extraction through two mechanisms: (1) rupturing the cell wall either by acidic or alkaline conditions and (2) protein solubilization at alkaline pH (Shekhar U. Kadam et al., 2017; Sari et al., 2015). Other compounds may also be solubilized depending on the pH, affecting the protein purity (Shekhar U. Kadam et al., 2017).

Shekhar U. Kadam et al. (2017) found an increase in the protein extraction yield from *A. nodosum* with increasing acid concentration (>2 times higher recovery at 0.4 M HCl compared to 0.1 M HCl). The extraction in the alkaline condition showed 3.2 to 6.5 times higher protein recovery than in the acidic condition. The acidic condition facilitated the release of protein bound to the cell wall polysaccharide but did not promote protein solubilization (Sari et al., 2015). In contrast, the alkaline condition favored the liberation of structural and intercellular proteins and protein solubilization (Naseri et al., 2020). The alkaline extraction also increased phenylalanine, serine, glycine, and valine retention. However, threonine was degraded only under alkaline conditions, while arginine, isoleucine, leucine, and tyrosine were entirely degraded regardless of the extraction method employed. Vilg and Undeland (2017) reached 100% protein solubilization from *Saccharina latissima* (Phaeophyceae) biomass at pH 12 and isolation of 16.01% using isoelectric precipitation.

Overall, alkaline seems more reliable than acid extraction (Contreras et al., 2019), yielding a higher protein purity (Shekhar U. Kadam et al., 2017; Vilg and Undeland, 2017). The ionic strength might be one factor that compromises the solubility of seaweed proteins at low pH due to the interaction between anions and positively-charged groups of proteins (Vilg and Undeland, 2017). Pretreatment with osmotic shock could overcome this problem by lowering the ionic strength of the system; however, it depends on the shape of the salting-in/salting-out curve (Vilg and Undeland, 2017). Fleurence, Le Coeur, et al. (1995) demonstrated that osmotic shock coupled with alkaline extraction showed better protein recovery from *Ulva rigida* and *U. lactuca* (as *Ulva rotundata*) (Chlorophyta) than enzymatic extraction (cellulase and polysaccharidase mixture), and the extracted protein was rich in aspartic and glutamic acids, alanine, and glycine. On the other hand, a lower yield of protein extraction (45%) from *P. palmata* using alkaline treatment was reported compared to enzyme-assisted extraction (>90%) (Naseri et al., 2020). *D. mollis* is a red seaweed containing 50% water-soluble protein with a cellulose-rich cell wall (Mendez and Kwon, 2021), which means that only alkaline extraction might not be the best approach. Mendez and Kwon (2021) reported 44–80% more protein recovery from *D. mollis* by combining enzymatic extraction using cellulase followed by alkaline treatment to recover both the water-soluble and salt-soluble proteins fractions.

Like enzyme-assisted extraction, time, temperature, and solvent-seaweed ratio must be optimized (O'Connor et al., 2020; Sari et al., 2015). Temperature and extraction time can compensate for each other; longer extraction times allow the extraction at lower temperatures (Sari et al., 2015). This can help extract high thermal-sensitive proteins. However, longer treatments and high pH contribute to lysinoalanine formation, which is of safety concern (Mendez and Kwon, 2021). Additionally, higher temperatures could result in lower protein solubility, possibly due to protein denaturation (Vilg and Undeland, 2017). The little temperature effect on protein extraction can be promising since it points to lower energy requirements during industrial extraction (Vilg and Undeland, 2017). The biomass:solvent ratio should be low enough to guarantee the highest protein extraction but not so much that it would compromise the process scalability (Sari et al., 2015). A 1:15 (*w/v*) biomass:solvent ratio seems to be enough to extract protein from *P. palmata*, using 0.12 M NaOH and 0.1 g/100 mL of the food-grade reducing agent *N*-acetyl-L-cysteine (NAC) (Harnedy and FitzGerald, 2013). The same ratio has been applied to brown seaweed *A. nodosum* (Shekhar U. Kadam et al., 2017). Vilg and Undeland (2017)reported a lower protein solubility for *S. latissima* at 1:60 compared to higher ratios (≥1:20) using alkaline extraction. The required concentration of the alkaline can be lowered in cases where there is no interference by other components that reacts to the alkaline, for example, lignin (Sari et al., 2015) (although it is found in low concentrations in seaweed (Wi, Kim, Mahadevan, Yang, & Bae, 2009)).

Despite the positive effect on protein extractability, some measures must be taken when applying alkaline treatment, such as checkpoints throughout the process to monitor the formation of lysinoalanine (Mendez and Kwon, 2021). However, the formation of this compound should not be an issue when extracting protein from cysteine, serine, and lysine-absent seaweeds, especially at low temperatures (Harnedy and FitzGerald, 2013). Additionally, depending on the expected extracted protein quality, it might not be suitable, as alkaline and acid extraction can lead to the degradation of EAAs (Shekhar U. Kadam et al., 2017).

4.2.2. Freeze-thawing

Freeze-thawing involves one or more cycles of freezing at very low temperatures (normally < −20 °C (Abdollahi et al., 2019)) and thawing the biomass to obtain the desired bioproduct (Abdollahi et al., 2019; Hu & Xie, 2021)such as thermosensitive bio-compounds (Dewi et al., 2020). It induces protein denaturation (Abdollahi et al., 2019) and subsequent phase separation (Abdollahi & Undeland, 2020). For its simplicity, the freeze-thawing method can be applied as a pretreatment to aid biomass solubilization and dispersion for downstream processes like high-pressure homogenization, ultra-sonication, and bead milling (Kulkarni and Nikolov, 2018). Abdollahi et al. (2019) compared various pretreatments to obtain protein from *S. latissima*. They reported different protein extraction yields due to freeze-thawing at −20 °C (79.9%) and −80 °C (65.7%), compared to that from freeze-drying (90.9%) and ensiling (25.4%). One of the explanations for better solubilization of freeze-thawed protein at −20 °C than −80 °C might be the formation of larger ice crystals at slow freezing (−20 °C), causing more damage to the

cell wall structure and therefore providing higher protein yield (Abdollahi et al., 2019). However, the freeze-thawing process can be time- and energy-consuming (Kulkarni et al., 2018). It can also cause protein oxidation, decrease its molecular weight (Abdollahi et al., 2019), and change its physical characteristic (color, hardness, and springiness), especially after multiple freeze-thawing cycles (Hu and Xie, 2021). As freeze-thawing is difficult to scale-up and not a robust method for permeabilization, its application on a large scale is practically undoable; therefore, other technologies like pulsed electric field (PEF) or high-pressure homogenization to permeabilize the cell should be favored. A study by Kulkarni and Nikolov (2018) concluded that freeze-thawing did not significantly impact protein extraction from the green microalga *Chlorella vulgaris*.

4.2.3. Osmotic shock

Osmotic shock is considered one of the simplest and greenest methods to recover protein from seaweed, as it only requires distilled water to break the cell wall. Under hypotonic conditions, the influx of water into the cell can lead to swelling and rupture of the cytoplasmic membranes (Kot, Gientka, Bzducha-Wróbel, Błażejak, & Kurcz, 2020; Mæhre et al., 2016). Water volume positively impacts the extraction of water-soluble proteins, as demonstrated by Vilg and Undeland (2017), who obtained a 58% increase in protein solubility for *S. latissima* at a ratio of 1:60 (dry biomass:water) compared to 1:20. Similarly, higher protein solubility can be reached at higher temperatures (Vilg and Undeland, 2017). Although it is expected that longer extraction times would result in higher protein solubilization, Mendez and Kwon (2021) demonstrated that treatments longer than 4 h did not improve the extraction of proteins from *D. mollis* and increased the phenolic coextraction, which interferes with protein extraction (Vilg and Undeland, 2017). Vilg and Undeland (2017) reported a decrease in the protein yield from the brown seaweed *S. latissima* at longer (16 h) compared to shorter (1 or 2 h) treatments. These results are contradictory to the one reported by Postma et al. (2018), where protein extraction increased linearly with time from <5% protein yield (1 h) to approximately 20% (24 h). However, extensive holding times may hinder protein extraction for large volumes at industrial scales (Postma et al., 2018). The same authors found a similar positive effect on protein yield regarding temperature, although it was not linearly: increasing the temperature from 4 to 22 °C did not lead to more protein extraction but increasing further to 30 °C did (19.5% at 30 °C 4.3% at 22 °C, and roughly 7% at 4 °C after 24 h).

Usually, the protein recovery is low by osmotic shock, considering that only water-soluble proteins will be extracted from the seaweed biomass (Cermeño et al., 2020). For this reason, osmotic shock is commonly applied as a pretreatment to aid the cell wall disruption and ease the extraction (O'Connor et al., 2020) by the following treatments: for example, alkaline extraction (Harnedy and FitzGerald, 2013), mechanical press (Polikovsky et al., 2019), and ultrasound (Galland-Irmouli et al., 1999). Other examples are listed in Supplementary 1.

4.2.4. Pressing

Pressing processes such as screw press and extrusion are convenient for protein extraction due to their short treatment duration and scalability (Robin et al., 2018), commonly applied to extract protein from plant leaves (Tamayo Tenorio, Gieteling, de Jong, Boom, & van der Goot, 2016) but also applied to recover other bioproducts, such as oils (Hojilla-Evangelista & Evangelista, 2017). The efficiency of cell disruption by pressing depends on the biomass source, fiber (cellulose/lignin) content, presence of mucilage, acids, tanning agents, enzymes, and the mechanical disruption technique such as heat generation during pressing (Guo et al., 2022). Labuckas, Maestri, and Lamarque (2014) did not find a significant difference in the protein content in walnut flour obtained using screw-pressing at different temperatures (25 °C, 50 °C, and 70 °C). However, protein solubility was the highest at 50 °C, which could result from protein denaturation caused by mechanical stress and/or temperature. Additionally, the intensity of bands between 15 and 40 kDa decreased, whereas low molecular weight peptides (<10 kDa) increased at 70 °C. This demonstrates that even though the temperature might not affect protein extraction, it impacts protein quality.

Intact cells might be found in the waste cake after pressing, and some protein fractions might be retained by its fibrous material (Tamayo Tenorio et al., 2016). So, it is recommended that the extracted juice goes through some solid-liquid separation, for instance, filtering, to separate the protein-rich juice from the fibers (Guo et al., 2022). Additionally, pressing can follow cell disruption by other technologies, such as (PEF) (Robin et al., 2018) and enzymatic treatment (Moure, Domínguez, Zúñiga, Soto, & Chamy, 2002). Although few papers reported the use of pressing for seaweed protein extraction, the scalability of this technology is unquestionable. The simplicity and fast operation enable its combination with other technologies.

4.2.5. High shear homogenization

High shear force is a physical process where large forces are transmitted to the solid by hydraulic shear, fluid layer friction, centrifugal extrusion, and collision, causing granulation (Tardos, Hapgood, Ipadeola, & Michaels, 2004) and fibrillation (J. Zhao et al., 2013). The high shear homogenization is typically performed on a laboratory scale using, for example, the IKA Ultra-Turrax unit, where the liquid medium enters the dispersion head axially at high rotation speeds and is drawn radially out the stator slots (IKA, 2023). Such high shear force has been shown to promote the release of water-soluble proteins from *U. lactuca* (Postma et al., 2018).

The disruption of the cell wall of *P. palmata* via high-shear homogenization resulted in alkaline soluble protein recovery (Harnedy and FitzGerald, 2013). Postma et al. (2018) found a better protein yield from *U. lactuca* (approximately 39%) by using high shear homogenization compared to the enzymatic extraction (30%), osmotic shock (19.5%), and PEF (15.1%). By varying biomass conditions (pre-cut and cut into pieces with different sizes), biomass concentration, and shear force (via different rotor tip speeds), the study showed that increased rotor speeds and low biomass concentration resulted in the highest yield of protein extraction for the uncut biomass. In contrast, for the pre-cut biomass, the highest protein yield was obtained by using a low to moderate biomass concentration and rotor speed.

High shear could be a promising pretreatment to disrupt seaweed cells for protein extraction, but its efficiency and operational setting depend on how the seaweed samples were prepared before proceeding. Moreover, further experimental evaluation is required to assess its applicability for seaweed protein extraction on a large scale.

4.3. Novel methods for protein extraction

4.3.1. High-pressure processing (HPP)

The application of non-thermal pressure-assisted protein extraction methods can potentially shorten the extraction time and decrease the organic solvent consumption. It has been shown that HPP destabilizes hydrogen bonds and disrupts electrostatic interactions between proteins, which imparts denaturation, conformation, aggregation, or gelation (Laguna et al., 2017; S. Zhao et al., 2022) and influences the protein secondary (Velazquez, Méndez-Montealvo, Welti-Chanes, Ramírez, & Martínez-Maldonado, 2021), tertiary, and quaternary structure (Z. Zhang, Yang, Zhou, Zhang, & Wang, 2017). Such structural changes expose the hydrophobic groups previously buried in interior regions of the protein, thereby increasing the surface hydrophobicity of proteins (Z. Zhang et al., 2017) and protein digestibility (Mulla et al., 2022).

The applied pressure in HPP varies (100–600 MPa) (Laguna et al., 2017; Mateluna et al., 2020; Velazquez et al., 2021; Z. Zhang et al., 2017; S. Zhao et al., 2022): 100 MPa can lead to partial protein denaturation (Velazquez et al., 2021), whereas higher pressures can result in protein degradation and collapse of the gel network structure (>300 MPa) (Z. Zhang et al., 2017), reduction of protein hydrophobicity

(>400 MPa) (Mulla et al., 2022), and formation of aggregates and protein precipitation (500 MPa) (S. Jung & Mahfuz, 2009). However, there are various reports about the effect of pressure on protein solubilization. For instance, He et al. (2019) demonstrated that high pressures (>400 MPa) increase the solubility of the protein in chicken breast in the presence of *Eucheuma denticulatum* (formerly *Eucheuma spinosum*) (Rhodophyta) polysaccharides, while Z. Zhang et al. (2017) reached the highest protein solubility at 200 MPa from broiler meat. Jung and Mahfuz (2009) reported a decrease in yield for the protein extraction from soybean flakes due to protein insolubility with increasing pressure in HPP (from 200 MPa to 500 MPa) followed by either enzyme-assisted or aqueous extraction. Overall, protein denaturation and extraction may happen at a wide range of pressure, which could be associated to the decreasing denaturation enthalpy (Jung and Mahfuz, 2009).

HPP has been applied to improve the water-holding capacity and gelation properties of meat products (Velazquez et al., 2021; Z. Zhang et al., 2017; S. Zhao et al., 2022) and legumes (Mulla et al., 2022) and extend products' shelf-life (del Olmo, Picon, & Nuñez, 2019; Marcal et al., 2021). The application of HPP in seaweed is rare. del Olmo et al. (2019) reported HPP application to extend the shelf-life of *Laminaria ochroleuca* (kombu, Phaeophyceae); Mateluna et al. (2020) analyzed the effect on the texture and microstructure of the brown seaweed *D. antarctica*, and Martelli et al. (2020) evaluated the antimicrobial activity of HPP-treated *Himanthalia elongata* (although their results demonstrated that HPP prejudicated the antimicrobial activity). To the best of the authors' knowledge, only one article has reported the application of HPP for seaweed protein extraction. O'Connor et al. (2020) showed protein recovery from *Fucus vesiculosus* (23.7%), *Alaria esculenta* (15.0%), *P. palmata* (14.9%), and *C. crispus* (16.1%) using HPP pretreatment (600 MPa, 4 min). Their results demonstrated that HPP could not improve protein extraction compared to other conventional methods (autoclave or osmotic shock + sonication + freeze-thawing + salting). However, HPP improved the extraction of glutamic acid-rich fraction for all the seaweeds compared to the conventional method. The inferiority of HPP compared to the conventional method in the seaweed protein extraction could be attributed to the latter comprising more steps that facilitated better cell wall degradation. The difference in protein extraction among the species could result from different cell wall compositions and protein solubility. This demonstrates that the use of HPP to extract protein from seaweed requires further in-depth assessment.

Moreover, the fast treatment (minutes) and lower operational cost enable HPP to treat large volumes of material (O'Connor et al., 2020). Optimal pressure and combination with other treatments must be assessed. HPP seems to be a novel yet under-explored method to extract protein on a large scale.

4.3.2. Microwave-assisted extraction (MAE)

MAE is a novel technique applied mainly to extract seaweed carbohydrate or phenolic compounds rather than proteins. Microwaves induce the water molecule vibration in a solution, disrupting hydrogen bonds and making dissolved ions migrate. As a result, the penetration of solvents is facilitated (O'Connor et al., 2020), allowing the use of a much less solvent volume than conventional methods, as demonstrated by Yuan and Macquarrie (2015). They reported using 3 times less solvent than acid-assisted extraction to extract fucoidan from *A. nodosum*. MAE has been considered a low-energy extraction approach that reduces extraction time and solvent usage and improves extraction yield (Barba et al., 2015) and protein purity (Ochoa-Rivas et al., 2017). Nevertheless, its application to extract heat-sensitive compounds may not be recommended (Cermeño et al., 2020; Shekhar U Kadam, Tiwari, & O'Donnell, 2013).

Barral-Martinez et al. (2020) demonstrated that the protein content from *M. stellatus* in the solid and liquid fractions increased after using microwave at different irradiation power (100–500 W). This can be associated with cell wall activation and breakdown. Abeln et al. (2019) demonstrated that MAE could depolymerize mono and polysaccharides in seaweed, which could explain the improvement in the protein extraction with increasing irradiation up to 300 W reported by Barral-Martinez et al. (2020). Cid et al. (2021) combined a sequential supercritical extraction and microwave-assisted subcritical water extraction (190 °C, 3 min) to extract protein, fatty acids, and phenolics from *M. stellatus*. The reported protein yield (1.7%) was similar to their previous work (1.8%) using only microwave-assisted subcritical water extraction (190 °C, 3 min) (Ponthier, Domínguez, & Torres, 2020). Chew, Chia, Lee, Zhu, and Show (2019) reported that microalgal protein extraction was higher at 100 W than at 180 and 300 W. These results are expected since microwaves increase the intracellular pressure to a point where it exceeds the expansion capacity of the matrix, bursting the cell and allowing the intracellular content to migrate to the surrounding medium (Rodriguez-Jasso, Mussatto, Pastrana, Aguilar, & Teixeira, 2011). However, at higher irradiations, it denaturalizes and insolubilizes the protein.

Temperature is another crucial factor regarding MAE. Yuan and Macquarrie (2015) evidenced that higher temperatures decrease the molecular weight of extracted fucoidan. Similarly, Farhadpour et al. (2016) documented a positive temperature effect on cyclotide extraction from *Viola ignobilis* (flowering plant) resulting from the augment of solvents to solubilize analytes at higher temperatures by increasing the surface tension and solvent viscosity. However, using some buffers can negatively affect the recovery of some proteins at higher temperatures, which can be attributed to the denaturation following irreversible aggregation and formation of covalent complexes, decreasing protein solubility and extractability (Amponsah & Nayak, 2016). Sagu, Huschek, Homann, and Rawel (2021) identified better enzymatic protein recovery from nuts at 37 °C than at 50 °C. The same behavior can be expected when using longer treatments. Farhadpour et al. (2016) found that cyclotides extraction increased with time ≤ 20 min, followed by a decrease due to the decrease in cyclotide solubility, while Chew et al. (2019) found an increase in protein extraction from *C. vulgaris* with time (≤ 2 min) followed by a decrease afterward. Their experiments also demonstrated that longer MAE treatments could be portioned into shorter cycles to avoid protein degradation. Ponthier et al. (2020) highlighted that extraction time (3 or 6 min) did not influence protein extraction from *M. stellatus*, although it improved the solubilization of carrageenan within the temperatures 70–150 °C (at higher temperatures, extraction time was not significant).

Despite less use of MAE for protein extraction from seaweed, many previous papers have demonstrated that it is a valuable tool for recovering other biomolecules like polysaccharides (e.g., fucoidan (Rodriguez-Jasso et al., 2011; Yuan and Macquarrie, 2015), cellulose (Singh, Gaikwad, Park, & Lee, 2017)), carrageenan (Ponthier et al., 2020), hydrocarbons (Punín Crespo, Cam, Gagni, Lombardi, & Lage Yusty, 2006), organic acids (Tedesco, Hurst, Randviir, & Francavilla, 2021), and iodine (Romarís-Hortas, Bermejo-Barrera, & Moreda-Piñeiro, 2013). MAE causes dipole polarization and ionic conduction, solubilization-desorption and inside-cell diffusion, and changes in cell structure walls (Rodriguez-Jasso et al., 2011), which could allow the extraction of internal compounds (López-Hortas, Gely, Falqué, Domínguez, & Torres, 2019), such as proteins, and short times favor polysaccharides extraction (Yuan and Macquarrie, 2015). Since efficient protein/peptide extraction with MAE has been documented for several protein sources (e.g., (Amponsah and Nayak, 2016; Behere, Patil, & Rathod, 2021; Chew et al., 2019; Farhadpour et al., 2016; Ochoa-Rivas et al., 2017; Sagu et al., 2021)), it is wise to notice the promising application for seaweed.

4.3.3. Sub (SWE) and supercritical water extraction

SWE uses water at temperatures from 100 °C to 374 °C (critical point) under high pressure (usually from 10 to 60 bar) (Herrero et al., 2006) to break the cell wall (Tasaki, 2021), whereas supercritical water extraction happens at higher temperatures (>374 °C). At 250 °C, the relative dielectric constant of water decreases from approximately 80 (polar solvent) at room temperature to around 27, which is similar to

ethanol's dielectric constant (Mohsen-Nia, Amiri, & Jazi, 2010), allowing the dissolution of hydrophobic material (Amagliani, O'Regan, Kelly, & O'Mahony, 2017). Supercritical water extraction involves two thermodynamic steps: firstly, the extraction happens driven by the subcritical water process's thermodynamic condition during the system's heating; secondly, the system is heated towards temperatures above 374 °C. However, temperatures above 250 °C seem uncommonly applied (Di Domenico Ziero et al., 2022; Fan et al., 2020; Tasaki, 2021; Yilmaz-Turan et al., 2020), which could be an approach to avoid AA deterioration due to thermos sensitivity (K. Kang et al., 2001), explaining the limited study on protein extraction using supercritical systems.

The extraction of biomolecules can result from altering non-covalent bonds (e.g., hydrogen bonds), ionic bonds, and hydrophobic interactions, breaking large molecule structures, such as proteins, into soluble peptides (Contreras et al., 2019). The efficiency of these mechanisms depends on the holding time, the biomass:liquid ratio, temperature (Xi, 2017), and biomass properties (Ziero et al., 2020). Although important, the pressure does not impact protein extraction, as temperature and extraction time do; protein extraction seems to remain stable at pressures from 10 to 20 MPa (Fan et al., 2020). Temperature and extraction time are commonly associated with superior protein recovery. However, Fan et al. (2020) reached maximum protein/peptide extraction at 150 °C, which remained stable at higher temperatures (<180 °C), demonstrating that temperature improves protein extraction to a specific limit. Saravana, Cho, Woo, and Chun (2018) studied the influence of temperature (100–150 °C) and pressure (10–50 bar) combined with NADES to extract polysaccharides from *S. japonica* (Phaeophyceae). Higher temperature (150 °C) and lower pressure (19.85 bar) were the optimal conditions to recover alginate (28.12%) and fucoidan (14.93%).

High temperatures might cause thermal degradation (Sereewatthanawut et al., 2008), especially of high-thermal sensitive proteins. This can be overcome by heating the water separately from the biomass, shortening the biomass exposure to heat, as demonstrated by Trigueros et al. (2021), who used a looping system to heat and avoid the water entering the reactor before reaching the working temperature (185 °C), similarly to Fan et al. (2020), who preheated the water to 80–100 °C to extract peptides from *Arthrospira platensis*. However, a heating ramp can facilitate the extraction of target polysaccharides by exploiting the different solubilities at different temperatures (Trigueros et al., 2021), which can be helpful when combining protein and carbohydrate extraction.

SWE has been applied for different purposes and in different biomasses, such as extraction of proteins and feruloylated arabinoxylans from wheat bran (Yilmaz-Turan et al., 2020), protein from cow manure digestate solid (Tasaki, 2021), peptides from *A. platensis* (Fan et al., 2020), and AAs from poultry feathers (Di Domenico Ziero et al., 2022). Considering the variety of biomass objected to SWE, it is expected the possibility of using it for seaweeds. However, the application of SWE for seaweed seems uncommon. Its use for protein extraction is even rarer (Polikovsky et al., 2020): Polikovsky et al. (2020) extracted approximately 85% of the total protein in *Ulva* sp. by applying SWE; Trigueros et al. (2021) reached even higher protein extraction from *Gelidium corneum* (formerly *Gelidium sesquipedale*) residue (after agar extraction) using SWE (96.1%).

Since the SWE employs high temperatures, some AAs might undergo Maillard reactions with carbonyl groups or reducing carbohydrates. Trigueros et al. (2021) identified threonine, lysine, glutamic acid, and serine in the raw biomass of *G. corneum*, but not in the extract after SWE treatment (185 °C) or at least in much lower concentrations, even though nearly 100% of the protein was solubilized. However, by shortening the residence time (increasing the water flow from 4 to 8 mL min^{-1}), the degradation of peptides and AAs was reduced, and the diffusion of proteins was increased, obtaining higher hydrolysis yields. Interestingly, the diffusion of AAs was not as affected as peptides with the increase in flow rate due to the higher diffusivity coefficients of AAs (Trigueros et al., 2021). These results contrast Di Domenico Ziero et al. (2022), who demonstrated that aspartic acid and serine concentrations from SWE-treated poultry feathers were maximized at a flow rate of 10 mL min^{-1} at 210 °C, while isoleucine and methionine were maximized at 5 mL min^{-1} and 250 °C. This means that specific AAs can be obtained at the expense of others by optimizing the temperature and flow rate of SWE systems.

The impact of SWE on the AA profile must be evaluated to ensure protein extraction with desirable quality, mainly due to the degradation of heat-sensitive proteins. The economic assessment of scalability must also be the goal of future studies. Nevertheless, SWE has been demonstrated to be an alternative to extracting protein without demanding the use of solvents that can either be applied alone (Di Domenico Ziero et al., 2022), followed by filtration to increase the protein yield (Tasaki, 2021), or in combination with other protein extraction methods (e.g., following enzymatic treatment (Yilmaz-Turan et al., 2020), or ultrasound-assisted extraction (Fan et al., 2020).

5. Protein concentration processes

After protein extraction, separation and purification processes are usually applied to produce protein isolates and concentrates. Isoelectric precipitation, application of organic solvents, salting, membranes, chromatography, and adsorption are known approaches to concentrating protein (Contreras et al., 2019). For simplicity and objectivity, processes aiming at the removal of other biocompounds prior to protein concentration were not covered in the discussion; for example, Harrysson et al. (2018) recovered protein from *Porphyra umbilicalis*, *U. lactuca*, and *S. latissima* after accelerated-solvent extraction of lipids, phlorotannins, and carbohydrates. Nevertheless, we highlighted that such methods are relevant and can aid in protein concentration and, therefore, should be considered to improve protein yield.

5.1. Isoelectric precipitation

This method is widely applied to concentrate protein from plants (Contreras et al., 2019). The basic mechanism of isoelectric precipitation, also known as pH-shifting, is the reduction of protein solubility by decreasing the pH of the medium so that the protein reaches its isoelectric point and therefore precipitates due to the zero net charge (Türker, Selimoğlu, & Taşpınar-Demir, 2022). This is often combined with the solubilization of proteins under alkaline conditions, where proteins gain a negative charge, promoting repulsion between protein molecules (Abdollahi et al., 2019). After solubilization and centrifugation to remove the pellet containing non-protein fractions, the pH is dropped to precipitate the solubilized protein. It presents high efficiency

Table 5
Documented concentration factor for different seaweeds using isoelectric precipitation.

Seaweed	Pretreatment	Concentration factor*	References
Saccharina latissima	Oven-drying	5.1	(Abdollahi et al., 2019)
Porphyra umbilicalis	Sonication + milling	2.2	(Harrysson et al., 2018)
Ulva lactuca	Sonication + milling	2.6	(Harrysson et al., 2018)
Saccharina latissima	Sonication + milling	4.0	(Harrysson et al., 2018)
Ulva ohnoi	Acidic extraction	2.1	(Magnusson et al., 2019)
Ulva ohnoi	Osmotic shock	3–5	(Angell, Paul, & de Nys, 2017)

* Concentration factor is calculated by dividing the final protein concentration by the initial biomass protein concentration.

in recovering proteins devoid of lipids (Table 5).

Abdollahi et al. (2019) elaborated a novel method for protein precipitation from seaweed using pH-shifting combined with freeze-thawing of pre-treated (sun-dried, oven-dried, frozen at −20 °C or −80 °C, ensiled, and freeze-dried) *S. latissima* biomass. This study involved protein solubilization at pH 12 and the pH-shifting (from 12 to 2) combined with freeze-thawing, which has increased the protein extraction yield by 1.4–2.6 times compared to the classical pH-shifting without freeze-thawing. However, this method caused valine, isoleucine, and leucine degradation in some pre-treated biomasses, whereas alanine and glutamic acid were reduced regardless of the precipitation method. Harrysson et al. (2018) also reported that freeze-thawing-aided pH-shifting precipitation resulted in less glutamate (~7–9% AA) than accelerated-solvent extraction (16–26% AA). The acidic condition can irreversibly damage the protein, which could explain the results of Abdollahi et al. (2019). Additionally, it may cause the coprecipitation of undesired compounds, such as lignin (Rommi, Niemi, Kemppainen, & Kruus, 2018) and fatty acids (Harrysson et al., 2018). For instance, Harrysson et al. (2018) showed a fatty acid concentration of 2.2 and 1.6 times for *U. lactuca* and *S. latissima*, respectively, after pH-shift precipitation. However, the same behavior was not reported for *P. umbilicalis*, meaning the coprecipitation of fatty acids is species-dependent. The isoelectric precipitation is unquestionably an easy and fast way to concentrate protein which still requires optimization of parameters like pH based on the biomass type (Naseri et al., 2020) to increase the protein yield and make seaweed a competitive protein source.

5.2. Salting-out

The salting-out process involves the use of a high concentration of salt to increase the ionic strength, decreasing the protein solubility. Ammonium sulfate is the preferred choice as it is food-grade, cheap, highly soluble, and efficiently capable of stabilizing protein structures (Burgess, 2009; Duong-Ly & Gabelli, 2014). Although it has been demonstrated to positively affect protein concentration for some seaweeds, the salting-out process's efficiency seems less than other concentration approaches (Tables 5, 6, and 7), and it is species-dependent.

Precipitation with ammonium sulfate decreased the AA concentration compared to the crude biomass of *P. umbilicalis*, *U. lactuca*, and *S. latissima* (Harrysson et al., 2018), which is in accordance with the result reported for *P. palmata* by O'Connor et al. (2020). The later authors identified a decrease in some EAAs after sonication and salting out with ammonium sulfate, especially histidine, cysteic acid, taurine, and cysteine. Interestingly, the EAA concentration increased by almost 1.2 times in the final biomass, meaning the main contributor to the overall reduction in protein (1.5 times lower) was the decrease in the total AA content and/or the precipitation of fat, which roughly doubled in the pellet of *P. palmata*. It is important to track down the impact of previous steps on the final protein content. For instance, Harrysson et al. (2018) and O'Connor et al. (2020) applied sonication as a pretreatment, and sonication can degrade some proteins and solubilize other compounds (Barba et al., 2015; Brien et al., 2022; Shekhar U. Kadam et al., 2017), resulting in lower final protein concentration. Nevertheless, such an effect depends on the cell wall composition and susceptibility of the seaweeds to cell lysis (O'Connor et al., 2020).

Table 6
Documented concentration factor for different seaweeds using salting-out.

Seaweed	Pretreatment	Concentration factor*	References
Phorphyra umbilicalis	Sonication	<1	(Harrysson et al., 2018)
Ulva lactuca	Sonication	<1	(Harrysson et al., 2018)
Saccharina latissima	Sonication	<1	(Harrysson et al., 2018)
Fucus vesiculosus	Sonication	1.3	(O'Connor et al., 2020)
Alaria esculenta	Sonication	1.5	(O'Connor et al., 2020)
Palmaria palmata	Sonication	<1	(O'Connor et al., 2020)
Chondrus crispus	Sonication	1.6	(O'Connor et al., 2020)

* Concentration factor is calculated by dividing the final protein concentration by the initial biomass protein concentration.

Table 7
Documented concentration factor for different seaweeds using membrane separation.

Seaweed	Pretreatment	Concentration factor[a]	Molecular weight cut-off (kDa)	References
Ascophyllum nodosum	Ultrasound (22.8 μm) + alkaline extraction	4.1[b]	1	(Shekhar U. Kadam et al., 2017)
	Ultrasound (68.4 μm) + alkaline extraction	3.9[b]	1	(Shekhar U. Kadam et al., 2017)
Grateloupia turuturu	Osmotic shock	5	30[c]	(Denis et al., 2009)

[a] Concentration factor is calculated by dividing the final protein concentration by the initial biomass protein concentration.
[b] Concentration factor was calculated by comparing the concentration after the pretreatment and after filtration.
[c] The authors applied ultrafiltration to concentrate R-phycoerythrin selectively.

5.3. Ionic-exchange chromatography separation (IEC)

Different chromatography categories can be applied to purify extracts: gas-liquid, gas-solid, adsorption, partition, ion exchange, and gel filtration chromatography (Batool & Menaa, 2020). Ionic-exchange chromatography is widely applied to purify charged biomolecules, such as proteins, peptides, and AAs, presenting high efficiency as it can be used to separate different proteins aided by high-affinity and cost-effective buffers. In IEC systems, charged molecules in the mobile phase interact with charged groups of the stationary phase (column packing matrix). The degree of purification depends on how strongly the compound interacts with the stationary phase, holding on to the charged groups more firmly while the elements with weaker interaction pass through the system (Batool and Menaa, 2020). As different AAs possess different charges, which also depend on the pH, IEC separation can be performed by targeting specific AAs (Echave et al., 2021).

5.4. Membrane separation

Different filtration approaches (nano-, micro-, and ultrafiltration) have been used to concentrate and enrich proteins. The type of membrane used depends on the target molecule to concentrate. Ultrafiltration is commonly applied to separate peptides with molecular masses between 1 and 10 kDa, but membranes with higher molecular masses cut-off can be used for proteins (Denis, Massé, Fleurence, & Jaouen, 2009). In such systems, the liquid is pumped (usually under pressure) across a semi-permeable membrane that presents a specific molecular weight cut-off, diffusing undesirable compounds, for example, carbohydrates, salts, phenols, and other low molecular mass molecules (Cermeño et al., 2020; Shekhar U. Kadam et al., 2017) while retaining proteins (Shekhar U. Kadam et al., 2017).

As shown in Table 7, ultrafiltration is reliable for concentrating seaweed proteins. However, membrane-based systems often suffer from fouling due to the accumulation of particles on the membrane surface,

which decreases efficiency and increases operating costs and processing times. For brown seaweed extraction, alginates, which tend to form a gel, have been pointed out as the possible driver of fouling in ultrafiltration membranes (Madeira, Marçal, Cardoso, Gando-Ferreira, & Costa, 2022).

5.5. Dialysis

Another membrane-based technique is dialysis. This method is commonly used to remove small-molecule contaminants by selectively and passively diffusion through a semipermeable membrane, such as a dialysis tube. The difference between dialysis and other membrane techniques is that the first utilizes buffers on both sides of the membrane. In this system, proteins are retained inside the dialysis tube while low molecules, such as salts, reducing agents, or preservatives, diffuse through the semipermeable membrane until the buffers from both sides reach electric neutrality, according to the Donnan effect (Whitford, 2013). Although it is a simple technique, it can be time-demanding as it depends on the diffusion rate (Echave et al., 2021), making its upscaling difficult and unattractive for industries (Harrysson et al., 2018). This might explain why dialysis seems not commonly applied to purify seaweed protein, except for some target proteins, for example, phycoerythrin from *Kappaphycus alvarezii* (Dewi et al., 2020).

6. Seaweed protein quantification

Protein quantification is routinely done through different well-established methods, either by direct analysis, for example, AA quantification, or indirect protocols, such as nitrogen-based conversion (e.g., the Dumas combustion or the Kjeldahl methods). Direct quantification is usually applied in physiological and ecological studies, while indirect quantification is mostly used in biochemical profiling, cultivation/bioremediation, and feeding trial studies, where protein quality assessment is not the main goal (Angell, Mata, de Nys, & Paul, 2016).

The nitrogen-to-protein conversion is widely accepted as it can easily and accurately quantify the protein content of biomass. However, the non-protein nitrogenous substance fraction must be considered and used to calibrate the results, as they also contribute to the nitrogen content. The nitrogen-to-protein conversion factor for seaweed is 6.25, but the non-protein nitrogenous substance fraction is highly variable among seaweed species, and the level also depends on the extraction and purification processes. For instance, Angell et al. (2016) suggested using a nitrogen-to-protein factor of 5.1, 4.49, and 4.56 for red, green, and brown seaweed, respectively, based on a meta-analysis of 236 articles.

Generally, the Lowry method results in higher protein content than total AA analysis, and the degree of difference between these results depends on the type of biomass analyzed. Harrysson et al. (2018) demonstrated that the Lowry method and total AA analysis resulted in similar protein content for the crude biomass of *P. umbilicalis* and *U. lactuca*. In contrast, the Lowry method provided higher protein content than the total AA analysis for the extracted proteins. For *S. latissima*, the same authors reported higher protein content in the crude biomass and extract when the Lowry method was applied. This suggests that some components in the crude biomass or extract can interfere with the Lowry method, such as pigments and types of AAs (Harrysson et al., 2018; Legler, Müller-Platz, Mentges-Hettkamp, Pflieger, & Jülich, 1985). Although the total AA analysis seems the most accurate, this method can also suffer from limitations, for example, the inability to quantify/identify some specific AAs depending on how the samples were treated or the conducted method (Harrysson et al., 2018).

It is somewhat accepted that varied precision is inherent regardless of the protein quantification or estimation method. No single method is likely to be accurate and full-proof, as extrinsic factors, such as protein extraction techniques, protein quality, and the presence of the other compounds in the matrices, might interfere with the results (Zaguri, Kandel, Rinehart, Torsekar, & Hawlena, 2021). One important aspect regarding protein for human consumption is the quality of protein, which can be classified by the EAA index (EAAI), the protein digestibility-corrected AA score (PDCAAS), or the digestible indispensable AA score (DIAAS, recommended to replace the PDCAAS), both adopted by the FAO/WHO (Consultation, 2011). Both approaches use the EAA composition to classify the protein quality.

The EAAI compares the protein quality employing the geometric mean value of EAAs relative to a reference protein (Machado et al., 2020). Machado et al. (2020) reported an EAAI of 0.90–1.14, 0.9–1.16, 1.02, and 1.23 for *Porphyra dioica*, *P. umbilicalis*, *Gracilaria vermiculophylla*, and *U. rigida*, respectively. These results mean seaweeds can be a high-quality alternative protein (EAAI >1 (Kent, Welladsen, Mangott, & Li, 2015)) source. The PDCAAS is based on comparing the concentration of the first limiting EAA in the sample with the concentration in a reference (scoring) pattern (Schaafsma, 2000), giving a score up to 1.0 (excellent protein quality). This approach is helpful because higher crude protein content does not always mean higher digestibility (De Bhowmick & Hayes, 2022b). De Bhowmick and Hayes (2022b) determined the PDCAAS values of *P. palmata* (0.69), *F. serratus* (0.63), and *A. esculenta* (0.59), which are compared to chickpea (0.62–0.71) (Bai, Nosworthy, House, & Nickerson, 2018). Unfortunately, the PDCAAS value is not currently reported in the literature for many seaweed species (De Bhowmick & Hayes, 2022a). This also seems to be true for DIAAS.

Assessing the seaweed protein bioavailability, i.e., bioactivity and bioaccessibility (Carbonell-Capella, Buniowska, Barba, Esteve, & Frígola, 2014), is critical to assess the digestion and uptake of functional components. However, quantifying it can be tenuous as the analytical concentration determination of a component does not always reflect what actually is happening in the digestive system due to its complexity and interacting factors (Wells et al., 2017). Bacterial flora and the presence of other substances in the digestive tract and biomass composition and manipulation can either facilitate or inhibit metabolite uptake (Sensoy, 2014). Currently, some analytical methods provide a reliable and accurate approach to assess bioavailability, such as simulated gastrointestinal digestion (Ma et al., 2022), xenobiotic animal models, and genetic and molecular biological techniques; however, nutritional and functional quantification of seaweed protein remains barely encompassed (Wells et al., 2017).

7. Conclusion

The increased consumption of alternative protein drives the market demand, encouraging the further search for sustainable and diversified protein alternatives. Seaweed can offer a great source of good-quality protein to supply the global market. However, protein extraction from seaweed is still challenging despite the efforts, especially due to (1) cell wall complexity which impairs cell rupture, (2) co-solubilization of other compounds, reducing overall protein content, for example, polysaccharides and polyphenols have been reported to hinder protein release during extraction, (3) seasonal variation influencing protein and cell wall composition, (4) lack of consensus in extraction parameter optimization, which is species dependent, (5) the use of solvents is unsustainable and enzymes can be costly, (6) limited or inexistent data regarding extraction costs and energy consumption of the process as well as upscaling feasibility, (7) lack of systematic research to assess individual process step within protein extraction method for comparative yield and efficiency. Despite the health and environmental benefits of seaweed-derived proteins, some compounds, such as iodine and arsenic accumulated by some seaweed species, possess food safety and health concerns. Additionally, most studies are limited to quantifying solubilized protein, which does not always reflect the overall protein content in the final product. Hence, novel purification/concentration steps must be developed further, ensuring a competitive production of seaweed protein at an industrial scale. The high cost of farming combined with high-cost protein extraction and low efficiency are the main factors slowing down the market revenue growth for the seaweed

protein market.

Chemical and biochemical processes have been widely used to obtain high yields in plant protein extraction. Current research has clearly indicated that the efficiency of cell disruption in seaweed depends on the cell wall composition of seaweed which is, in turn, linked to the seaweed group (green, red, and brown) and species. However, traditional technologies that disrupt cells and extract proteins from seaweed present drawbacks, such as poor yield, long time, high-energy consumption, high costs, and organic solvent usage, limiting their sustainable proposition and scalable adaptation. Enzymatic cell disruption is not recommended for brown seaweed due to its cell complexity, and the same limitation is found for ultrafiltration to concentrate protein from brown seaweed. Nevertheless, an in-depth systematic study for enzymatic cell disruption of different seaweed species may provide further science-led insights on the relationship between cell wall composition and efficiency of the enzyme-based extraction technology. Applying extraction methods like acid/alkaline, microwave-assisted, subcritical water, supercritical fluid, and ultrasound-assisted extraction has been reported for seaweed protein extraction. However, their poor cost-effectiveness and long processing require seeking eco-innovative extraction processes that are efficient, sustainable, cost-effective, non-destructive, and faster. Technologies like HPP, MAE, SWE, UAE, and PEF are promising for seaweed protein extraction and present the advantage of requiring none or less solvents and shorter times to extract but require further studies, especially concerning the economic viability and extraction efficiency as well as quality, especially digestibility and sensorial quality. Other technologies, such as reverse micelle extraction, aqueous two-phase system extraction, and greener solvents, must be evaluated, given the limited information available for seaweed protein extraction. Nevertheless, different approaches can be applied comprising different sets of technologies to guarantee the yield of target proteins, and co-products from protein extraction with industrial applications can be obtained and improve the techno-economic aspect.

CRediT authorship contribution statement

Gleison de Souza Celente: Conceptualization, Writing – original draft, Methodology, Data curation, Visualization, Writing – review & editing, Validation, Investigation. **Yixing Sui:** Writing – original draft, Writing – review & editing, Validation, Investigation. **Parag Acharya:** Writing – review & editing, Funding acquisition, Resources, Supervision.

Declaration of Competing Interest

The authors declare no conflict of interest.

Data availability

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

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Appendix A. Supplementary data

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