



Research Article

Characterization of protein extracted from the Omani seaweed- *Hypnea bryoides*

Hala Hilal Al-Mawali^a, Ahmed Ali Musalem Al-Alawi^{b,*}

^aDepartment of Food Science and Nutrition, Sultan Qaboos University/College of Agricultural and Marine Sciences, Sultan Qaboos University, Al-Khoud, 123, Oman

^bDepartment of Center of Excellence in MarBio, Sultan Qaboos University, Sultan Qaboos University, Al-Khoud, 123, Oman

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ABSTRACT

Hypnea bryoides (*H. bryoides*), a species of red seaweed (Rhodophyta), was previously found to contain a significant amount of protein. This study aimed to extract protein from *H. bryoides* and examine its functional and chemical properties, thereby facilitating its use. The protein was extracted in an alkaline solution (0.3 M NaOH, pH 12). The extraction yielded 31% from the original existing quantity, and the purity was 88.50 ± 0.71%. The protein demonstrated a higher oil-holding capacity (13.56 ± 0.26 g oil/g protein) compared to its water-holding capacity (9.61 ± 0.15 g water/g protein). The highest foaming capacity, solubility, emulsifying capacity, and stability were observed at pH 8 and 10 ($p \leq 0.05$), while the most outstanding foaming stability occurred at pH 4. The *in vitro* digestibility was 62.62 ± 3.29%. Furthermore, the molecular analysis revealed that β -sheet structures constitute the primary secondary structural component of *H. bryoides* proteins. A broad range of protein molecules with varying molecular weights (0.4–125.2 kDa) was identified. The amino acid profile indicated that essential amino acids accounted for 35.08% of the total, with methionine + cysteine as the limiting amino acid. Overall, this seaweed protein demonstrated good functional and chemical properties, making it suitable for many food applications.

1. Introduction

Global protein demand is anticipated to rise by 70% by 2050, driven by population growth reaching 9.7 billion people and rising living standards in developing nations (FAO, 2017; UN, 2019). This escalating global demand for protein has positioned seaweed as a compelling alternative to terrestrial crops, offering high protein content, exceptional biomass productivity, and minimal environmental impact. Several seaweeds have been identified as promising protein sources, including the brown seaweed *Undaria pinnatifida* (approximately 24% protein, dry basis) and the green seaweed *Ulva ohnoi* (up to 22% protein, dry basis) (Magnusson *et al.*, 2019; Twigg *et al.*, 2024). These seaweeds yield 26.4 and 70 tonnes of dry biomass per hectare annually, respectively, producing 6.34 and 15.4 tonnes of protein, respectively, which far surpasses that of terrestrial crops (Kraan, 2017). In contrast, soybean yields only 3–4 tons of dry seed per hectare annually (1.1–1.5 tons of protein), alfalfa provides 16.8–25.9 tons of dry biomass (4.1–5.4 tons of protein), and legumes yield 1–4 tons of seed with 22–26% protein (0.2–1 ton of protein) (Orloff & Putnam, 2007; Hartman *et al.*, 2011; Siddique *et al.*, 2012). These productivity advantages are particularly critical as arable land availability decreases by 0.3% annually while protein demand continues to rise (FAO, 2021). Unlike terrestrial crops, seaweeds require no synthetic fertilizers, freshwater, or arable land, reducing competition with traditional agriculture and minimizing ecological impacts (Angell *et al.*, 2016). Seaweed protein production generates > 90% fewer greenhouse gases than beef protein and requires zero freshwater irrigation, unlike soybean cultivation, which consumes 5,379–5,853 L of water per kilogram of protein produced (Mekonnen

& Hoekstra, 2011; Gephart *et al.*, 2021; Costa *et al.*, 2021; Breewood & Garnett, 2023).

Seaweed proteins have versatile applications in food, nutraceuticals, cosmetics, and biotechnology thanks to their unique bioactive and functional characteristics. Red seaweeds like *Porphyra* spp. are valued for their high protein content and functional roles in plant-based meat and dairy analogs (Cerná, 2011; Gamero-Vega *et al.*, 2020), while *Gracilaria* spp. produce R-phycoerythrin used as a fluorescent marker in biotechnology and for its antioxidant potential in nutraceuticals (Sekar & Chandramohan, 2008). Green seaweeds such as *Ulva* spp. yield ulvan-associated proteins like lectins, used for immune modulation and skin hydration in functional foods and cosmetics (Hardouin *et al.*, 2014). Spirulina produces phycocyanin, a blue pigment employed in beverages and nutraceuticals for its antioxidant effects (Sekar & Chandramohan, 2008). Additionally, seaweed proteins are being explored for antimicrobial and therapeutic peptides with potential antihypertensive and anticancer properties (Pina-Pérez *et al.*, 2017; Fitzgerald *et al.*, 2011).

The effectiveness of seaweed proteins in such applications depends on their structural characteristics, molecular weight, and amino acid composition. Red seaweeds, such as *Gracilaria* spp. and *Porphyra* spp., are rich in essential amino acids, including methionine and cysteine, which enhance their nutritional quality and digestibility for food and nutraceutical uses (Angell *et al.*, 2016; Tanna *et al.*, 2019). For instance, *Gracilaria corticata* (Rhodophyta) contains elevated sulfur-containing amino acids, contributing to its high protein quality (Tanna *et al.*, 2019). Molecular weight significantly influences functionality: low-molecular-weight peptides (<10 kDa) from *Kappaphycus alvarezii* (Rhodophyta) exhibit high solubility and bioactivity, including

***Corresponding author:**

E-mail address: ahmed543@sq.u.edu.om (A Al-Alawi)

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antioxidant, antihypertensive, and antidiabetic properties, making them ideal for nutraceutical supplements (Rawiwan *et al.*, 2022). In contrast, high molecular weight proteins (>50 kDa) from *Porphyra* spp. exhibit excellent emulsifying activity due to their hydrophobic amino acid residues, which stabilize emulsions in sauces and dressings, and form strong, firm gels for vegan desserts (Rawiwan *et al.*, 2022). Proteins extracted from *Gracilaria* spp. exhibit a relatively high water-holding capacity (4–6 g water per g protein), which can be utilized to enhance the texture in meat-analog formulations. They also show a moderate oil-holding capacity (2–3 g oil per g protein), which is useful in enhancing emulsion stability (Rawiwan *et al.*, 2022). Additionally, *Porphyra* spp. proteins exhibit better digestibility (>80%) compared to soy and balanced amino acid profiles, making them suitable for nutritional products (Rawiwan *et al.*, 2022). Secondary structure further governs performance: *Kappaphycus alvarezii* proteins, rich in β -sheets, contribute to gel strength, while *Gracilaria* spp. proteins with α -helices enhance emulsification, though processing conditions like temperature can disrupt these structures, impacting functionality (Rawiwan *et al.*, 2022; Chen *et al.*, 2015).

Among red seaweeds, *Hypnea bryoides* (*H. bryoides*), which is abundant in Omani seas, contains approximately 17% protein by dry weight, presenting significant potential for protein-based applications (Al-Alawi *et al.*, 2011, 2018). The worldwide seaweed industry, worth \$16.6 billion in 2020, is anticipated to reach \$25.5 billion by 2028 (Webb *et al.*, 2023). The second fastest-growing area within this market is protein applications, and it is the most strategically important segment because it supports several rapidly expanding downstream industries (World Bank, 2023). Despite its occurrence in regions such as Oman, Mauritius, and the Red Sea, the extraction and characteristics of *H. bryoides* proteins remain under-researched, limiting insights into their commercial value (Silva *et al.*, 1998; Jupp, 2002; Ibraheem *et al.*, 2014). Furthermore, *H. bryoides* is a recognized source of carrageenan, enabling it to produce two distinct commercial products: high-quality proteins and carrageenan (Al-Alawi *et al.*, 2011). This dual-product potential significantly enhances the commercialization prospects of *H. bryoides*. Characterizing these proteins is essential to determine their suitability for food, nutraceutical, and other applications.

2. Materials and Methods

2.1 Raw materials

2.1.1 Sample collection

H. bryoides samples were collected by Salalah Diving Services Company (Salalah, Sultanate of Oman) from the Sadh region (Dhofar Province, Sultanate of Oman). The coordinates of the geographical sampling locations were: 1782.96'04"N 5565.3'07"E, 1652.18'94"N 5447.89'80"E, 1615.19'95"N 5485.18'81"E, and 1607.90'96"N 5460.32'75"E. The samples were collected during the period 20 September - 20 November 2021. The seaweed specimens were identified in the field based on their morphological characteristics.

2.1.2 Sample handling

After collection, seaweed samples were cleaned of any foreign materials (e.g., other seaweed plants, sand, etc.) and thoroughly washed with seawater. The samples were then placed in a cool box filled with clean seawater and transported to the facility of Salalah Diving Services Company. At the facility, the samples were thoroughly rinsed with fresh water, spread on a clean plastic sheet, and left to dry under the sun for 3 to 5 days, with daily flipping. The sun-dried seaweeds were then packed in a polyethylene bag and shipped to the Food Chemistry Lab (College of Agricultural and Marine Sciences, Sultan Qaboos University, Oman).

2.2 Extraction and determination of protein yield and purity

2.2.1 Extraction method

Prior to protein extraction, the sun-dried sample was depigmented following the procedure reported by Al-Alawi *et al.* (2011). Forty grams (40 g) of milled seaweed sample were mixed with 600 mL of an

acetone-methanol solution (1:1) in a 1000 mL bottle. The mixture was homogenized for 5 min using an ultra-mixer (Ultra TURRAX T25 Digital Dispenser, IKA, Germany) at 10,000 rpm. Next, the mixture was stirred for 1 h at room temperature, then centrifuged for 10 min at 4000 g. The supernatant was decanted, and the sediment was re-treated with a fresh methanol-acetone solution. The mixture was then stirred and centrifuged using the same procedure described above. The extraction was repeated three times, after which the sediment was used for protein extraction.

The protein extraction process was performed as described by Mæhre *et al.* (2016), with slight modifications. The depigmented sample was dispersed in 0.3 M NaOH (1:10, w/v), stirred at room temperature for 1 h, and then centrifuged at 5000 g for 10 min at 4 °C. After centrifugation, the supernatant was collected, and the precipitate was re-dispersed and treated as previously described. The supernatants from both steps were combined and dialyzed using dialysis membranes (MWCO 14,000 Da, Sigma-Aldrich, USA) against deionized water for three days at room temperature, with daily water replacement. The dialyzed samples were lyophilized using a Labconco Triad freeze dryer (Labconco Corporation, USA), packed into plastic containers, and stored at 4°C until further analysis.

2.2.2 Protein concentration and purity

The crude protein concentration and purity were determined using the Bicinchoninic Acid (BCA) assay Kit method (Sigma-Aldrich, USA) according to the manufacturer's instructions. A calibration curve was constructed using bovine serum albumin at concentrations from 0 to 1 mg/mL.

2.3 Chemical characterization of the extracted protein

2.3.1 Fourier-transform infrared (FTIR) analysis

The analysis was performed following the procedure described by Al-Alawi *et al.* (2011). A FTIR spectrometer (Agilent Cary 670, Malaysia), equipped with a single-bounce diamond attenuated total reflectance (ATR) cell (GladiATR, PIKE Technologies, USA), was used to analyze the extracted protein samples. Spectra were recorded at a resolution of 4 cm⁻¹, averaging 32 scans per sample.

2.3.2 Molecular weight measurement and protein profiling

The molecular weight of the protein extract was determined using size exclusion chromatography, following the method described by Sun *et al.* (2010), with slight modifications. The analysis was performed on a Nexera 2X system (Shimadzu, Japan), operated by LabSolutions software (version 5.82 SP1), and equipped with an autosampler (SIL-30AC), quaternary pump (LC-30AD), a diode array detector (SPD-M30A), and a column compartment (CTO-20AC). Separation was achieved using an Ultrahydrogel linear column (300 mm × 7.8 mm, Waters, USA) maintained at 25 °C.

The mobile phase consisted of a mixture of 0.05 M phosphate buffer (prepared from 1 M potassium phosphate dibasic and 1 M potassium phosphate monobasic) and 0.15 M NaCl solution. The flow rate was set to 0.5 mL/min, and the injection volume was 15 μ L. Prior to injection, samples were filtered through a 0.22 μ m nylon syringe filter. Molecular weight standards included amyloglucosidase (97 kDa) from *Aspergillus niger* (Fungi), carbonic anhydrase II (30 kDa) from bovine erythrocytes, trypsin inhibitor (20 kDa) from soybean, and vitamin B12 (cyanocobalamin, 1.355 kDa).

2.3.3 Amino acid profile and protein chemical score

The amino acid profile of *H. bryoides*'s protein was determined using a Nexera 2X system equipped with an LCMS-2020 Single Quadrupole LC/MS detector (Shimadzu, Japan), following the hydrolysis procedure described by Al-Saidi *et al.* (2011). 50 mg of the dried seaweed was placed in a hydrolysis tube with 5 mL of 6 M HCl containing 1% (v/v) phenol. The tube was flushed with nitrogen gas for 30 sec and then placed in a heating block (DRI-block DB-3D, TECHNE, UK) at 110°C for 24 h; 1 mL of the resulting hydrolysate was then mixed with 9 mL of

deionized water. Subsequently, 5 μL of the sample was injected into the LC-MS system. Prior to injection, the diluted hydrolysate was filtered using a 0.22 μm nylon syringe filter.

Amino acid separation was performed using gradient elution on an Intrada Amino Acid column (50 \times 3 mm, 3 μm , Intakt, Japan) maintained at 35°C, following the manufacturer's instructions. The mobile phases consisted of acetonitrile/formic acid (100/0.1) as solvent A, and 100 mM ammonium formate solution as solvent B. The flow rate was set at 0.6 mL/min. Elution began at 14% solvent B for 3 min, followed by a gradient increase from 14% to 100% B over 7 min, then returned to 14% B within 2 min.

The protein chemical score (CS) was calculated using the following equation, according to the method adopted by [FAO/WHO \(1991\)](#):

$$\text{Chemical score} = \frac{\text{mg of essential amino acid (EAA)/g protein}}{\text{mg of EAA/g FAO protein}} \times 100 \quad (1)$$

2.4 Functional properties of protein

2.4.1 Oil holding capacity (OHC) and water holding capacity (WHC)

The oil and water holding capacities (O/WHC) were determined according to [Garcia-Vaquero \(2017\)](#). The extracted protein (i.e., 0.3 g) was weighed and transferred to an empty centrifuge tube. Then, three milliliters (3 mL) of distilled water (for WHC) or sunflower oil (for OHC) was added, mixed for one minute using a vortex mixer, and centrifuged at 2200 g for 30 min. The tube containing the sediment was weighed, and the final weight was recorded after decanting the supernatant. The formula below was used to calculate W/OHC:

$$\text{W/OHC} = \frac{(\text{Wt of tube with the sediment} - \text{Wt of the empty tube})/\text{Wt of dry sample (g)} \times 100 \quad (2)$$

2.5 Solubility

The solubility of the extracted protein was determined following the method described by [Bleakley and Hayes \(2021\)](#). The protein was prepared in deionized water at a concentration of 1% (w/v), and the pH was adjusted using 1 M HCl and 1 M NaOH to produce samples with varying pH values ranging from 2 to 10. The samples were mixed for 45 min at room temperature using an orbital rotary shaker (Orbital Shaker HS 501 D, IKA, Germany), followed by centrifugation at 4000 g for 30 min at 4°C. The amount of soluble protein in the supernatant was quantified using the BCA method. The formula below was used to calculate protein solubility:

$$\text{Solubility (\%)} = \frac{\text{Concentration of protein in the supernatant/}}{\text{Concentration of protein in the total fraction}} \times 100 \quad (3)$$

2.6 Emulsifying properties

Emulsion stability (ES) and emulsion capacity (EC) were measured according to the method described by [Phongthai et al. \(2016\)](#), with slight modifications; 8 mL of oil was mixed with 24 mL of protein solutions (0.1%, w/v) prepared at different pH levels (2, 4, 6, 8, and 10). The mixture was homogenized at 10,000 rpm for one minute using an ultra-mixer to produce an emulsion. Subsequently, 50 μL of the emulsion was taken from the bottom of the container and mixed with five milliliters (5 mL) of 0.1% Sodium Dodecyl Sulfate (SDS) solution. The absorbance of the solution was measured at 500 nm at time 0 (A_0) and after 10 min (A_{10}). The following equations were used to calculate EC and ES:

$$\text{EC (mL/g)} = (2 \times 2.303 \times A_0) / (F \times \text{Protein weight (g)} \times \text{DF}) \quad (4)$$

$$\text{ES (min)} = (A_0 \times 10) / (A_0 - A_{10}) \quad (5)$$

Where A_0 and A_{10} are the absorbance values measured at 0 and 10 minutes, respectively, F is the oil volume fraction, and DF is the dilution factor.

2.7 Foaming properties

The foaming properties of the protein extract were examined following the method reported by [Bleakley and Hayes \(2021\)](#). A protein

solution (1.5% w/v) was prepared at different pH values (2–10) by adjusting with 1 M HCl and 1 M NaOH. The protein solutions were homogenized in a graduated cylinder for 1 min using an ultra-mixer at a speed of 10,000 rpm. The total volume was recorded before and after homogenization. Foamability was calculated using the following equation:

$$\text{Foamability \%} = (V_2 - V_1) / (V_1) \times 100 \quad (6)$$

Where V_1 is the volume before homogenization, and V_2 is the volume after homogenization.

To evaluate foam stability, the volume of foam was recorded at 15, 30, 60, 90, and 120 min after homogenization. The reduction in foam volume over time was used to calculate foam stability (FS), expressed as the percentage of foam retained relative to the initial foam volume.

2.8 In vitro digestibility

The digestibility of the plant protein was determined following the procedure reported by [Phongthai et al. \(2016\)](#); 1 g of the milled, dried plant material was mixed with 99 mL of distilled water (1%, w/v), and the pH was adjusted to 1.5 using 1.0 M HCl. Pepsin enzyme was added to the mixture at a ratio of 1:100 (pepsin: protein, w/w) to initiate the digestion process. The mixture was then incubated in a shaking incubator (Gallenkamp, UK) at 37°C for 120 min at a speed of 100 rpm. After incubation, the digestion was stopped by neutralizing the mixture with 1.0 M NaOH.

Subsequently, trypsin enzyme was added to the neutralized pepsin-digested mixture at the same ratio (1:100, w/w), and the sample was incubated again at 37°C for 120 min under the same shaking conditions. After 30 min of trypsin digestion, the mixture was heated at 95°C for 10 min to inactivate the enzyme. Then, 10% trichloroacetic acid (TCA) was added, and the sample was centrifuged at 5500 g for 10 min. The resulting precipitate was collected and lyophilized.

Finally, the undigested protein content was measured using the Kjeldahl method, and protein digestibility (%) was calculated using the following formula:

$$\text{Protein digestibility (\%)} = \frac{\text{Total protein in the sample} - \text{Undigested protein}}{\text{Total protein in the sample}} \times 100 \quad (7)$$

2.9 Statistical analysis

Statistical analysis of the data was performed using SPSS software, version 18 for Windows. Significant differences between means were evaluated using one-way analysis of variance (ANOVA). Differences were considered statistically significant at the 5% level ($p < 0.05$). When significant differences were detected, Tukey's multiple comparison test was applied. All measurements were conducted in triplicate unless otherwise indicated, and the results were expressed as mean \pm standard deviation.

3. Results and Discussion

3.1 Protein extraction

3.1.1 Protein yield and purity

The crude protein yield obtained from *H. bryoides* was 6 \pm 0.35% (db) of the total seaweed biomass ([Fig. 1](#)), representing approximately 31% of the total protein initially present in the seaweed. The extracted protein exhibited a purity of 88.5 \pm 0.71%. Although other *Hypnea* species such as *H. charoides* (83.1 \pm 0.94%) and *H. japonica* (85.0 \pm 0.38%) showed comparable protein purity, their recovery yields relative to the existing protein content were markedly higher than that of *H. bryoides* (*H. charoides*: 46.3 \pm 0.61%; *H. japonica*: 45.4 \pm 0.23%) ([Wong & Cheung, 2000](#)). In contrast, the red seaweed *Kappaphycus alvarezii* (*K. alvarezii*) yielded 43% of its total protein with a purity of 62.3 \pm 1.62%, which was notably lower than that achieved in this study ([Kumar et al., 2017](#)).

The extraction method plays a critical role in determining both the quality and yield of the resulting protein extract. Alkaline conditions

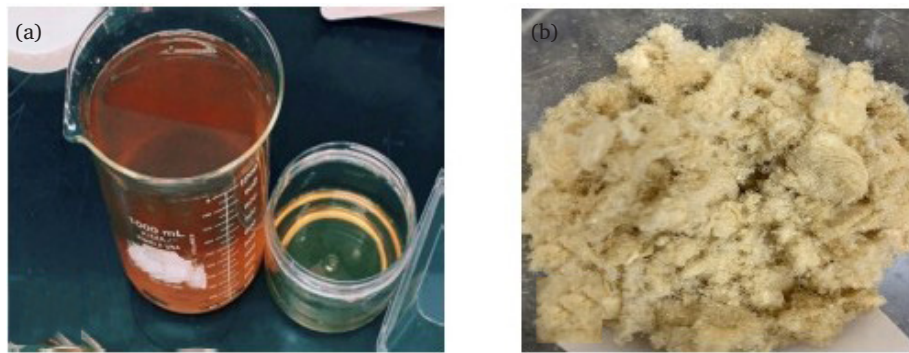


Fig. 1. The extracted protein from *H. bryoides* (a) before and (b) after freeze drying.

are generally known to enhance protein yield, and numerous studies support this. This increase is attributed to the improved solubilization of hydrophobic (water-insoluble) proteins from seaweed matrices through enhanced ionization under alkaline conditions (Kadam *et al.*, 2015). However, the relatively low protein yield observed in this study, despite the use of alkaline extraction conditions, can be attributed to several factors.

Proteins are classified into four distinct solubility classes based on their extraction needs: glutelins (alkali-soluble), prolamins (alcohol-soluble), globulins (salt-soluble), and albumins (water-soluble) (Osborne, 1907; Schalk *et al.*, 2017). Since this study employed only alkaline extraction conditions, it primarily targeted glutelin-type proteins while potentially leaving substantial amounts of globulins, prolamins, and albumins (Duranti *et al.*, 2008). Most proteins in *H. bryoides* may belong to solubility classes other than glutelins, which would explain the modest yield despite the use of alkaline conditions that are typically effective for protein extraction. This selectivity of the extraction method suggests that a sequential extraction approach using multiple solvents could potentially recover a more comprehensive protein fraction and achieve higher overall yields (Rasheed *et al.*, 2020).

Additionally, protein loss during the dialysis step, which used a 14,000 MW cut-off membrane, likely allowed low-molecular-weight proteins and peptides to pass through, further contributing to the reduced yield observed in this study.

Furthermore, specific proteins in macroalgal cells form complexes with non-protein compounds, like polyphenols and polysaccharides, which can complicate their extraction and purification (Harnedy & FitzGerald, 2013).

3.2 Chemical analysis

3.2.1 FTIR analysis

Fig. 2 presents the FTIR-ATR spectra of proteins extracted from *H. bryoides*. FTIR spectroscopy is a valuable analytical technique for identifying the secondary structure of proteins by examining characteristic vibrational bands associated with the peptide backbone (Barth, 2007; Kong & Yu, 2007).

In the spectrum, the prominent broad peak at approximately 3278 cm^{-1} corresponds to N-H stretching vibrations of polypeptide chains, indicating the presence of hydrogen-bonded amide groups (Barth, 2007). The peaks observed at 2961 cm^{-1} and 2928 cm^{-1} are attributed to asymmetric and symmetric C-H stretching vibrations associated with CH_2 and CH_3 functional groups from amino acid side chains, respectively (Goormaghtigh *et al.*, 2006). The spectral region ranging from 1700 to 1100 cm^{-1} encompasses the amide bands (I, II, and III), which are directly related to the protein's secondary structure and provide the most diagnostic information for conformational analysis.

The amide I band, which mainly indicates C=O stretching vibrations of the peptide bond. (70-85%) with minor contributions from C-N stretching (10-20%), appears as a sharp, intense peak near 1638 cm^{-1} (Kong & Yu, 2007). This frequency is characteristic of β -sheet secondary structures, which typically absorb in the 1630-1640 cm^{-1} range, due to intermolecular hydrogen bonding between peptide chains (Miller *et al.*, 2013; Usoltsev *et al.*, 2020). The medium-intensity peak at 1538

cm^{-1} corresponds to the amide II band, which arises from N-H bending vibrations (40-60%) coupled with C-N stretching vibrations (18-40%) and is generally less sensitive to secondary structure than amide I (Barth, 2007). Additionally, a smaller peak at 1241 cm^{-1} is attributed to the amide III band, which involves a combination of C-N stretching and N-H bending vibrations, providing supplementary structural information (Goormaghtigh *et al.*, 2006).

For precise secondary structure analysis, the amide I band was quantitatively evaluated by second-derivative spectroscopy, followed by deconvolution using curve-fitting (Kong & Yu, 2007). The deconvolution analysis revealed that the predominant peak at 1625 cm^{-1} accounts for approximately 56.1% of the total amide I band area, indicating that β -sheet structures constitute the major secondary structural component of *H. bryoides* proteins. Additional minor components were identified at 1654.9 cm^{-1} (α -helix, ~18.2%) and 1681.7 cm^{-1} (β -turns or antiparallel β -sheet, ~25.7%), providing a comprehensive secondary structure profile (Usoltsev *et al.*, 2020).

β -sheet-rich proteins demonstrate good gel-forming capabilities due to their capacity to form intermolecular networks through hydrophobic interactions and hydrogen bonding (Dickinson, 2003). This structural arrangement enhances the water-holding capacity and mechanical strength of protein gels, making them suitable for modifying food products. The extended conformation of β -sheets also provides excellent emulsification properties, as the hydrophobic regions can interact with lipid phases while hydrophilic regions remain in the aqueous phase (Foegeding *et al.*, 2002).

In food processing applications, β -sheet proteins show enhanced thermal stability compared to α -helical proteins, maintaining their functional properties under moderate heating conditions commonly used in food preparation (Damodaran, 2017). This thermal resilience makes *H. bryoides* proteins particularly suitable for processed food applications that require heat treatment. Furthermore, the β -sheet structure facilitates the formation of protein films and coatings with good barrier properties, which could be valuable for food packaging applications (Gennadios *et al.*, 1994).

The predominant β -sheet structure of *H. bryoides* proteins also suggests potential applications in plant-based protein products, where β -sheet-rich proteins can contribute to the fibrous texture characteristic of meat analogs through controlled aggregation and alignment during processing (Dekkers *et al.*, 2018). β -sheet proteins' capacity to form ordered and extended structures makes them especially suitable for creating anisotropic textures that imitate muscle fiber architecture.

Comparative analysis with other seaweed proteins reveals that the β -sheet predominance in *H. bryoides* is relatively uncommon, as many marine algae proteins exhibit mixed secondary structures with significant α -helical content (Fleurence, 1999). This unique structural feature may enhance the distinctive functional qualities of *H. bryoides* proteins and their potential benefits in certain food uses.

3.3 Molecular weight (MW) profile

Fig. 3 presents the chromatogram of molecular weight separation of proteins from *H. bryoides*, providing detailed insights into the complex protein profile of this red seaweed species. This analytical representation reveals multiple distinct peaks corresponding to

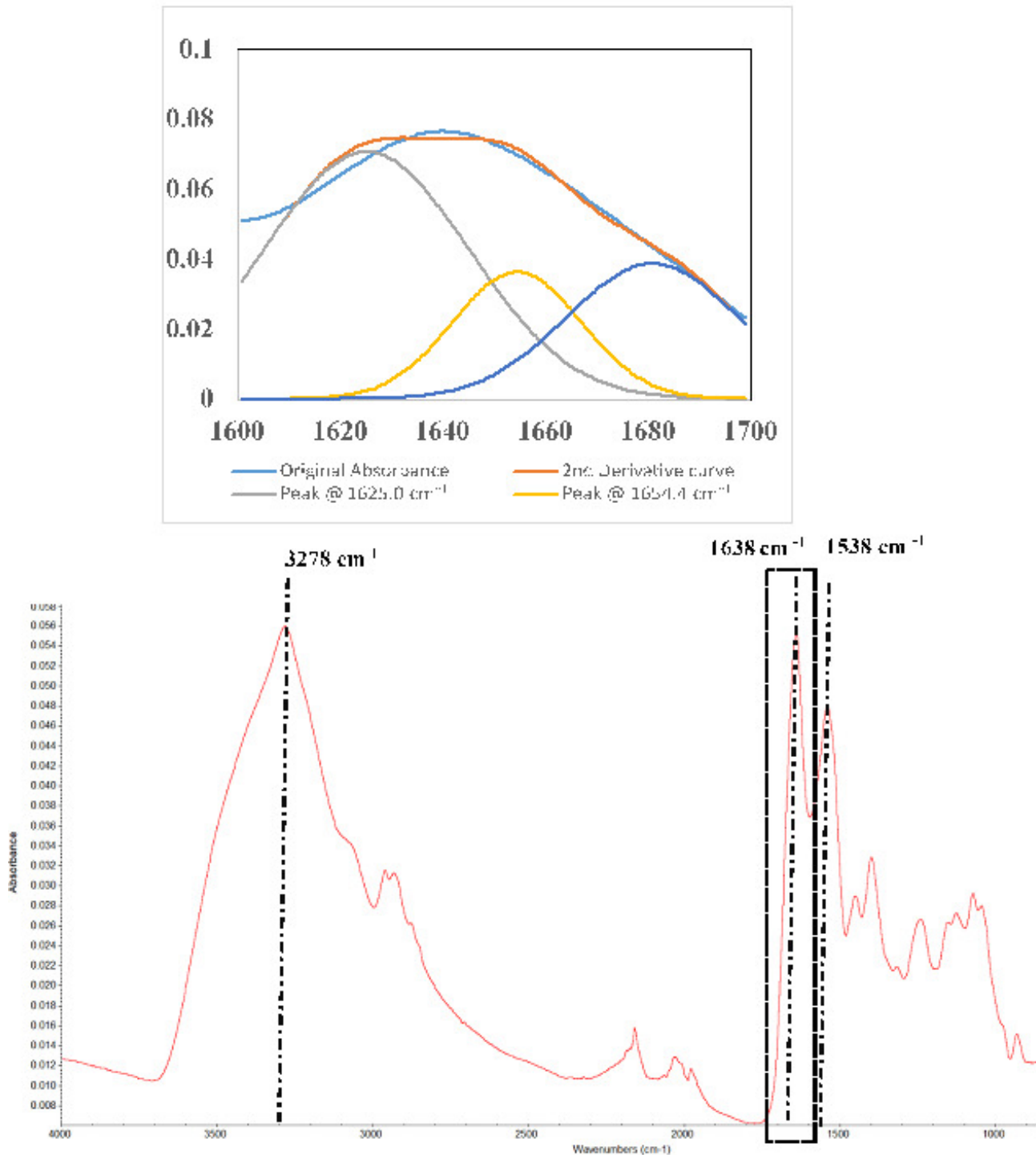


Fig. 2. FTIR-ATR spectra of an *H. bryoides* protein and the second derivative and curve-fitting deconvolution of the amide I band.

various molecular weights, which were accurately determined from a calibration curve correlating molecular weight with retention time using standard protein markers (Hong *et al.*, 2012; Burgess, 2018). The chromatographic analysis demonstrates a heterogeneous protein composition characteristic of marine algae, reflecting the diverse functional roles these proteins serve in cellular metabolism and structural organization (Garcia-Vaquero *et al.*, 2017).

The chromatogram shows three major peaks of highest intensity, corresponding to the predominant protein fractions in the *H. bryoides* extract. These major peaks appear at molecular weights of 15.6 kDa (retention time: 17.9 min), 11.9 kDa (retention time: 18.7 min), and 9.7 kDa (retention time: 19.3 min), collectively accounting for the most

significant proportion of the total protein content. The prominence of these low-to-medium molecular weight proteins suggests their abundance in the cellular protein machinery of *H. bryoides*, likely representing enzymes involved in photosynthetic processes, metabolic pathways, or structural parts of the thylakoid membrane system (Thiviya *et al.*, 2022). The retention time pattern follows the expected inverse relationship between molecular weight and elution time, confirming the reliability of the size exclusion chromatographic separation (Irvine, 2001).

Beyond these dominant fractions, the chromatogram reveals additional protein populations of varying abundances. Two medium-intensity peaks are observed at molecular weights of 125.2 kDa (retention

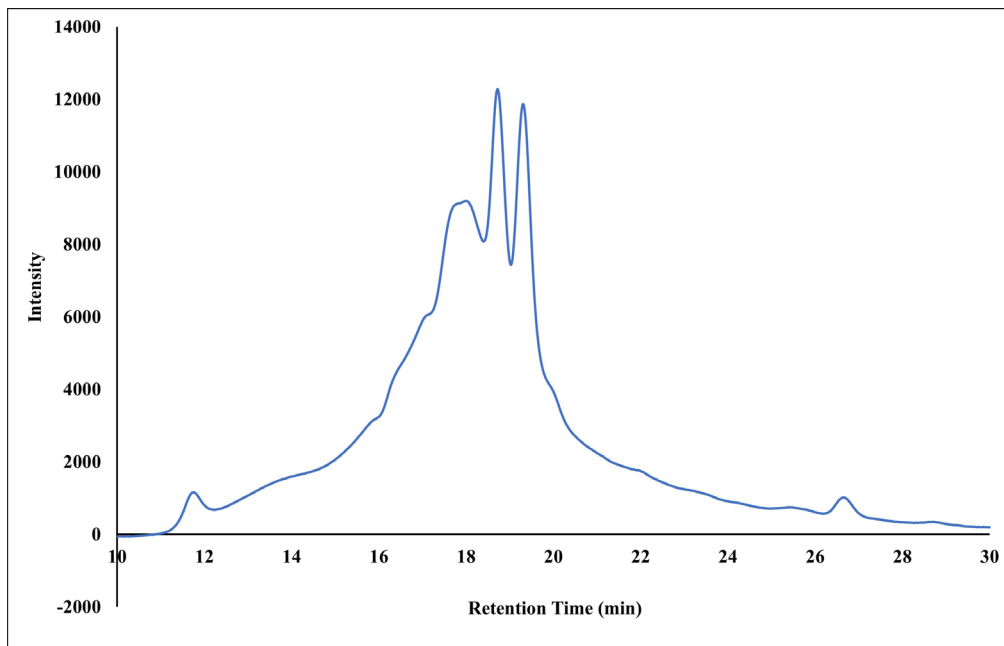


Fig. 3. Chromatogram of molecular weight profile of *H. bryoides* protein.

time: 11.8 min) and 0.7 kDa (retention time: 26.7 min), representing the extremes of the molecular weight distribution. The high molecular weight peak at 125.2 kDa likely corresponds to large enzyme complexes or structural proteins (Echave *et al.*, 2021). Conversely, the 0.7 kDa peak represents small peptides or amino acid clusters that may result from natural protein turnover or partial hydrolysis during extraction.

The chromatogram further displays several smaller peaks distributed across the molecular weight spectrum, indicating the presence of minor protein fractions at molecular weights of 71.1 kDa (retention time: 13.4 min), 32.0 kDa (retention time: 15.8 min), 26.9 kDa (retention time: 16.3 min), 20.5 kDa (retention time: 17.1 min), 7.6 kDa (retention time: 20.0 min), 3.9 kDa (retention time: 21.9 min), 1.2 kDa (retention time: 25.4 min), and 0.4 kDa (retention time: 28.6 min). This diverse molecular weight distribution suggests different biological functions, including structural support, regulatory processes, transport mechanisms, and enzymatic catalysis (Thiviya *et al.*, 2022). The time-based separation pattern shows that the initially detected peaks correspond to protein molecules with higher molecular weights (eluting earlier due to size-exclusion principles). In comparison, proteins with progressively lower molecular weights were detected after 18 min, as systematically illustrated in Fig. 3.

The comprehensive molecular weight analysis revealed that *H. bryoides* proteins span an exceptionally broad range from 0.4 to 125.2 kDa, demonstrating remarkable protein diversity that significantly exceeds the molecular weight range previously reported by Harnedy & FitzGerald (2013) for *Palmaria palmata* (Rhodophyta) protein extract, which was limited to 14.8-55 kDa. This expanded molecular weight distribution in *H. bryoides* suggests either a more diverse protein complement or different extraction efficiency compared to other red seaweed species (Zhang *et al.*, 2024). The broader range may indicate the presence of both intact high-molecular-weight protein complexes and smaller bioactive peptides, potentially offering enhanced functional versatility for commercial applications (Pereira, 2024).

The observed variation in molecular weights of proteins can be attributed to multiple interconnected factors related to extraction methodology and the inherent characteristics of the protein source. Critical factors influencing the molecular weight profile include the nature of the extraction media (aqueous, alkaline, or acidic solutions), pH conditions, temperature parameters, and extraction duration, all of which collectively determine both the quality and quantity of the resulting protein extract (Al-Alawi *et al.*, 2011). The alkaline extraction conditions employed in this study likely contributed to the broad molecular weight distribution by effectively solubilizing proteins

of varying sizes while maintaining the integrity of larger protein complexes. Generally, the utilization of acid or alkali in extraction protocols can significantly enhance protein solubility by altering protein charge distribution and disrupting protein-matrix interactions; however, excessively harsh conditions may induce protein hydrolysis through peptide bond cleavage, resulting in the formation of shorter peptides and potentially explaining the presence of very low molecular weight fractions observed in the chromatogram (Kadam *et al.*, 2015).

The predominance of the two major peaks with lower molecular weights (11.9 kDa and 9.7 kDa) provides a mechanistic explanation for the exceptional emulsification and foaming properties exhibited by *H. bryoides* protein extracts. Small-to-medium molecular weight proteins possess optimal surface-active properties due to their enhanced molecular flexibility and rapid diffusion to oil-water or air-water interfaces (Damodaran, 2005; Sánchez *et al.*, 2005). These proteins can undergo conformational changes more readily than larger proteins, allowing for better adsorption at interfaces and more effective reduction of surface tension (Hettiarachchy *et al.*, 1996). The 15.6 kDa, 11.9 kDa, and 9.7 kDa fractions likely contain amphiphilic proteins with balanced hydrophobic and hydrophilic regions, enabling them to stabilize emulsions and foams through interfacial film formation (Chao *et al.*, 2018). This molecular weight profile positions *H. bryoides* proteins as particularly suitable for food applications requiring emulsification and foaming functionality, such as plant-based meat alternatives, dairy analogs, and bakery products where texture and stability are critical performance parameters (Mune *et al.*, 2016).

3.4 Amino acid profile

Table 1 reveals the comprehensive amino acid profile of *H. bryoides*, providing detailed insights into the nutritional composition and protein quality of this red seaweed species. Amino acids comprised $79.6 \pm 4.5\%$ of the protein extract, which is in close agreement with the value indicated by the purity test (88.5%), demonstrating good agreement between the analytical methods (Machado *et al.*, 2020). The missing content represents the amino acids lost during the hydrolysis step, a common occurrence in acid hydrolysis procedures used for amino acid analysis (Černá, 2011).

As indicated in Table 1, glutamic acid exhibited the highest percentage ($12.9 \pm 0.5\%$), followed by aspartic acid ($9.7 \pm 0.3\%$), reflecting the characteristic amino acid distribution pattern observed in marine macroalgae. Similar trends have been reported in other seaweeds (Wong and Cheung 2000; Cian *et al.*, 2014; Bleakley & Hayes,

Table 1.
Amino acids profiling of *H. bryoides* protein.

Amino acids	Concentration (%)
Phenylalanine	5.8 ± 0.5
Tryptophan	ND
Leucine	6.6 ± 0.1
Isoleucine	2.8 ± 0.3
Methionine	0.9 ± 0.1
Histidine	2.1 ± 0.4
Lysine	6.0 ± 0.2
Valine	5.6 ± 0.3
Threonine	6.4 ± 0.4
Alanine	5.5 ± 0.1
Glutamic acid	12.9 ± 0.5
Aspartic acid	9.7 ± 0.3
Glycine	7.2 ± 0.3
Serine	6.8 ± 0.2
Glutamine	5.1 ± 0.1
Asparagine	1.7 ± 0.0
Cysteine	ND
Tyrosine	4.7 ± 0.1
Arginine	6.4 ± 0.2
Proline	6.3 ± 0.5
Total Essential amino acids	35.1 ± 2.4
Total Non-essential amino acids	64.9 ± 2.2
Total Polar Amino Acids	61.7 ± 2.3
Total Non-polar amino acids	40.6 ± 2.2

*ND; not detected

2021), with aspartic and glutamic acids consistently being the most common amino acids across various seaweed species (Thiviya *et al.*, 2022; Raja *et al.*, 2022). The high proportion of acidic amino acids is typical in red seaweed. Their elevated amounts contribute to the unique taste and flavor of these species (Cian *et al.*, 2014), with aspartic and glutamic acids being particularly responsible for the characteristic umami taste that makes seaweeds valuable as natural flavor enhancers (Kumar *et al.*, 2017).

The essential amino acid profile revealed that valine, leucine, threonine, lysine, and phenylalanine were present in relatively high amounts, indicating good nutritional potential for human consumption (De Bhowmick *et al.*, 2022). However, tryptophan, methionine, isoleucine, and histidine displayed low concentrations in the extracted protein, a pattern commonly observed in seaweed proteins where sulfur-containing amino acids and tryptophan often serve as limiting factors (Reynolds *et al.*, 2022). The total essential amino acids were approximately 35.1%, representing a substantial proportion that supports the nutritional value of *H. bryoides* protein. This percentage is similar to that reported in *H. charoides* (36.2%) (Wong & Cheung, 2000) but lower than the percentage found by Siddique *et al.* (2013) in *Gelidium pusillum* (Rhodophyta) (52.1%), indicating species-specific variations in essential amino acid content among red seaweeds (Bunda *et al.*, 2015).

On the other hand, the non-essential amino acids accounted for approximately 64.9% of the total amino acids, with an essential to non-essential amino acids ratio of 0.54, which is within the range typically observed for seaweed proteins (Krogdahl *et al.*, 2021).

The protein chemical score of the *H. bryoides* protein extract was 0.003, with methionine and cysteine being the limiting amino acids, excluding tryptophan, which highlights the typical limitation of sulfur-containing amino acids in seaweed proteins (Thiviya *et al.*, 2022). This value is significantly lower than that calculated for *Pyropia columbina* (formerly *Porphyra columbina*) (Rhodophyta) protein, which had

a chemical score of 0.57, with tryptophan being the limiting amino acid, demonstrating the variability in protein quality among different seaweed species (Machado *et al.*, 2020). Tryptophan plays a crucial role in determining protein quality (FAO, 2011), serving as an essential amino acid for protein synthesis and various metabolic processes. However, its concentration was very low or nearly undetectable in the *H. bryoides* extract, which is consistent with findings in other seaweed species where tryptophan often represents a limiting factor (Reynolds *et al.*, 2022).

Additionally, sulfur-containing amino acids (cysteine and methionine) were also present in low concentrations, even though red seaweed is confirmed to contain high amounts of sulfur-containing amino acids compared to green seaweed (Wong & Cheung, 2000). This apparent contradiction suggests that extraction and analysis conditions may significantly impact the recovery and detection of sensitive amino acids (Kumar *et al.*, 2017).

The reason for these low concentrations could be that some amino acids were destroyed by the HCl hydrolysis method employed for amino acid analysis. It has been demonstrated that exposing proteins to strong acids or alkalis can cause specific amino acids, such as tryptophan, to degrade completely, while other amino acids, like methionine and cysteine, may partially break down into different nitrogen-containing compounds (Kadam *et al.*, 2015). This degradation phenomenon is well-documented in protein analysis literature, where harsh hydrolysis conditions necessary for complete protein breakdown can simultaneously destroy acid-labile amino acids (Černá, 2011; Machado *et al.*, 2020). This methodological limitation also explains why the total amino acid percentage in the extracted protein did not reach 100%, as some amino acid residues are inevitably lost during the hydrolysis process, particularly those containing sulfur or indole groups, which are susceptible to acid-catalyzed degradation reactions (De Bhowmick *et al.*, 2022).

3.5 Functional properties

3.5.1 Water and oil holding capacities (W/OHC)

W/OHC are valuable to the food industry due to their influence on texture and flavor (Garcia-Vaquero *et al.*, 2017). These functional properties serve as critical indicators of protein performance in food systems, directly affecting product quality and consumer acceptance (Damodaran, 2005). The WHC describes the protein's capability to bind to water within the food matrix through several molecular interactions, such as electrostatic forces and hydrogen bonding (Bleakley & Hayes, 2021).

The WHC of the *H. bryoides* protein extract was 9.61 ± 0.15 g water/g protein, demonstrating a substantial water-binding capacity, positioning this seaweed protein as a functional ingredient for moisture-sensitive applications. This finding is slightly lower than the values of WHC found for *H. japonica* (11.8 ± 0.05 g water/g protein) and *H. charoides* (10.9 ± 0.30 g water/g protein), but comparably higher than the value found in the protein extracted from *K. alvarezii* (2.22 ± 0.04 g water/g protein) (Wong & Cheung, 2000; Kumar *et al.*, 2017). Furthermore, the *H. bryoides* WHC is close to the WHC of legume proteins (i.e., pea, chickpea, and lentil), which range between 10.5 and 13 g water/g sample, indicating comparable functionality to established plant protein sources (Ettoumi & Chibane, 2015).

The WHC of protein is important in various foods, especially viscous foods such as dough, soup, custard, and baked goods, as they are intended to absorb water, causing protein suspension, so improving the product's thickening and viscosity (Ettoumi & Chibane, 2015). Different factors contribute to a protein's capability to absorb water, including protein conformation, amino acid composition, surface polarity or hydrophobicity, and physical properties such as particle size and porosity (Wong & Cheung, 2000). The molecular structure of proteins creates specific hydration sites where water molecules can be bound through various mechanisms, with polar amino acid residues playing a vital role in water retention (Zayas, 1997).

OHC is an important characteristic of food ingredients that improves mouthfeel and preserves flavor, making it a key factor in lipid-rich food products. (Ettoumi & Chibane, 2015). In this study, the OHC of

the *H. bryoides* was found to be 13.56 ± 0.26 g oil/g protein, which is relatively higher than the OHC values reported for many other seaweeds, indicating exceptional lipid-binding capacity. For example, the OHC of *K. alvarezii* was 1.29 ± 0.20 g oil/g of protein (Kumar *et al.*, 2017). Moreover, other seaweeds from the genus *Hypnea* showed lower OHC values, such as *H. charoides* (0.95 ± 0.04 g oil/g protein) and *H. japonica* (0.82 ± 0.01 g oil/g protein) (Wong & Cheung, 2000).

The OHC is affected by capillary attraction, which physically entraps oil via both surface adsorption and internal absorption mechanisms (Sharoba *et al.*, 2013). Hydrophobic proteins also play a major role in oil absorption through non-polar interactions with lipid molecules (Chen *et al.*, 2019). Thus, the variation in OHC values among different seaweed species is linked to the protein content, the type of protein, amino acid composition, and the non-polar side of the amino acids that interact with fat molecules (Zielińska *et al.*, 2015). The current OHC value is high, even higher than those reported in legumes (i.e., peas, chickpeas, and lentils) (6 to 8 g oil/g protein) (Ettoumi & Chibane, 2015). Therefore, it is suggested that *H. bryoides* contains more nonpolar amino acids. High OHC is crucial for bakery products and other food applications, such as formulations containing ground meat and meat substitutes (Zielińska *et al.*, 2015). Therefore, due to its high water and oil-holding capacity, *H. bryoides* is a valuable source of functional protein that could have enormous applications in food manufacturing.

3.5.2 Protein solubility

Protein solubility is regarded as the most important property of proteins as a food ingredient because it affects several functional traits, such as emulsification and foaming, and is essential to other functional characteristics (Ettoumi & Chibane, 2015). The solubility behavior of proteins depends on the balance between protein-solvent and protein-protein interactions, which are strongly influenced by environmental factors like ionic strength and pH (Kinsella, 1979). The solubility of *H. bryoides* protein was tested at different pHs ranging from 2 to 10. The results indicated that protein solubility increased as pH rose (Fig. 4).

Furthermore, the protein exhibited minimal solubility at pH 2; therefore, pH 2 was identified as the protein's isoelectric point, the pH where its net charge is zero, and protein-protein interactions are maximized. Generally, in higher acidic or alkaline conditions, protein molecules acquire charges (positive or negative), leading to electrostatic repulsion and increasing protein solubility (Kumar *et al.*, 2017). *H. bryoides* protein showed a significant ($p < 0.05$) increase in solubility starting at pH 2 ($40.96 \pm 3.40\%$) to pH 6 ($93.24 \pm 13.80\%$), followed by a slight increase ($p > 0.05$) up to pH 10 ($97.92 \pm 0.90\%$).

Other seaweed proteins showed different patterns, reflecting species-specific variations in amino acid composition and protein structure. For example, the solubility of *K. alvarezii* protein was lowest at pH 4 ($33.72 \pm 1.23\%$) and highest at pH 12 ($58.72 \pm 1.68\%$) (Kumar *et al.*, 2017). These solubility data are lower than the solubility of *H. bryoides* protein

in our study. On the other hand, our results were close to the data reported by Garcia-Vaquero *et al.* (2017), where *H. elongata*'s protein showed maximum and minimum solubility at pH 12 ($96.15 \pm 0.15\%$) and pH 4 ($22.5 \pm 0.5\%$), respectively. It is worth mentioning that *H. elongata* is a brown seaweed, and we compared it to our study due to the limited number of studies on the functional properties of proteins extracted from red seaweeds specifically and from all seaweeds in general.

High protein solubility is crucial for protein extracts used in several food products like beverages, coffee whiteners, confections, dressings, and whipped toppings, as functional ingredients, where complete dissolution is essential for product clarity and stability (Garcia-Vaquero *et al.*, 2017).

3.5.3 Emulsifying activity (EA) and emulsifying stability (ES)

The EA of *H. bryoides* protein at five different pHs using sunflower oil has been shown in Fig. 5. Emulsification is a complex process that involves reducing the interfacial tension between oil and water phases through protein adsorption at the interface (McClements, 2004). The lowest EA was observed at pH 2 (5.16 mL/g), then increased as the pH increased to 46.4 mL/g at pH 8. After that, no significant increase ($p > 0.05$) at pH 10 (54.6 mL/g) was noticed. This pH-dependent behavior reflects the relationship between protein solubility and emulsifying capacity, as proteins need to first dissolve before moving to the oil-water interface.

EA was also analyzed at pH 2 to pH 10 (Fig. 6). Protein at pH 10 showed the best stability (107.76 min), followed by pH 8, 6, and 4 (97.77 min, 87.63 min, and 44.03 min, respectively) with no significant differences between pH 6 and 8 and between 8 and 10 ($p > 0.05$). The pH 2 had the lowest stability (18.27 min) with highly significant differences ($p < 0.05$) from the other pHs. Protein extracts from the brown seaweed *H. elongata* showed a somewhat similar trend, as the lowest activity and stability were noticed at pH 4, while the highest activity and stability were noted at pH 8 (Garcia-Vaquero *et al.*, 2017).

Along with the role of pH, the type of oil used in the analysis affects emulsion properties due to differences in viscosity and interfacial tension (Pearce & Kinsella, 1978). Moreover, the emulsion activity of some products might be affected by protein concentration and its nature. On the other hand, stability can be affected by several factors beyond pH, such as protein conformation, net charge, viscosity, interfacial tension, and droplet size (Bleakley & Hayes, 2021). The hydrophobic surface and the flexibility of protein molecules could be manipulated by enzymatic hydrolysis, leading to protein denaturation. It is also possible to improve the hydrophilic-lipophilic balance by uncovering the buried non-polar groups for better emulsification (Phongthai *et al.*, 2016).

However, the strength of the hydrophobic and hydrophilic interactions affects the EA more, whereas the amount and the distribution of non-polar amino acids are responsible for ES, and a

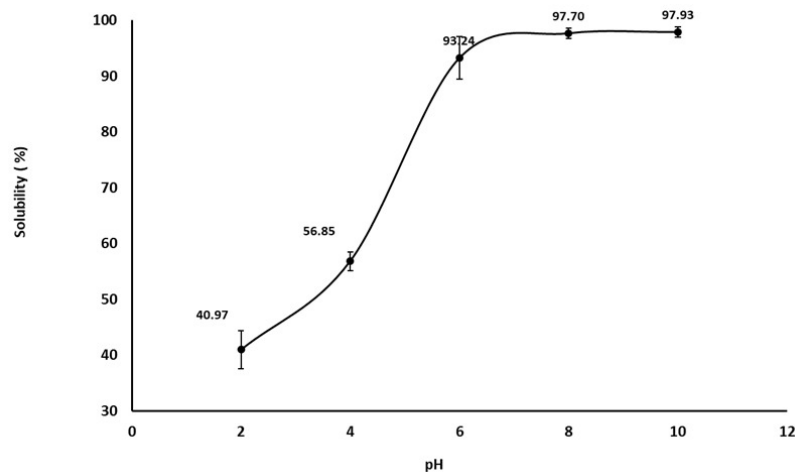


Fig. 4. Solubility (%) of *H. bryoides* protein at different pH values.

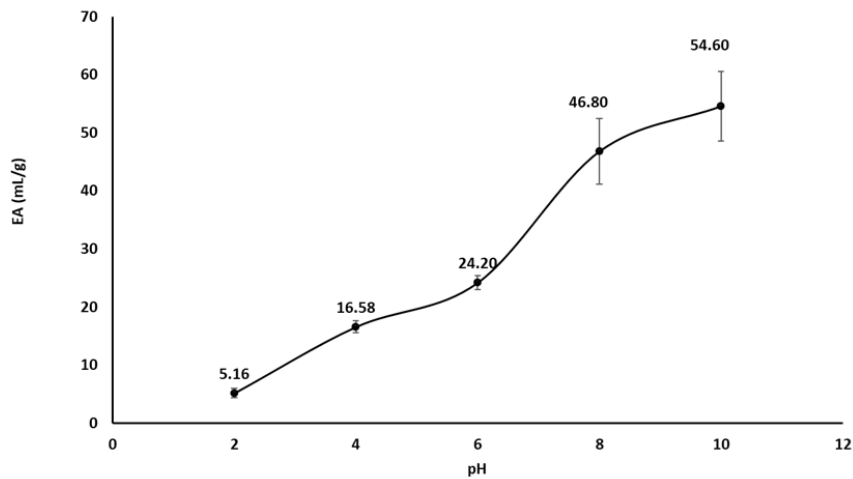


Fig. 5. Emulsifying activity % of *H. bryoides* protein at different pH values.

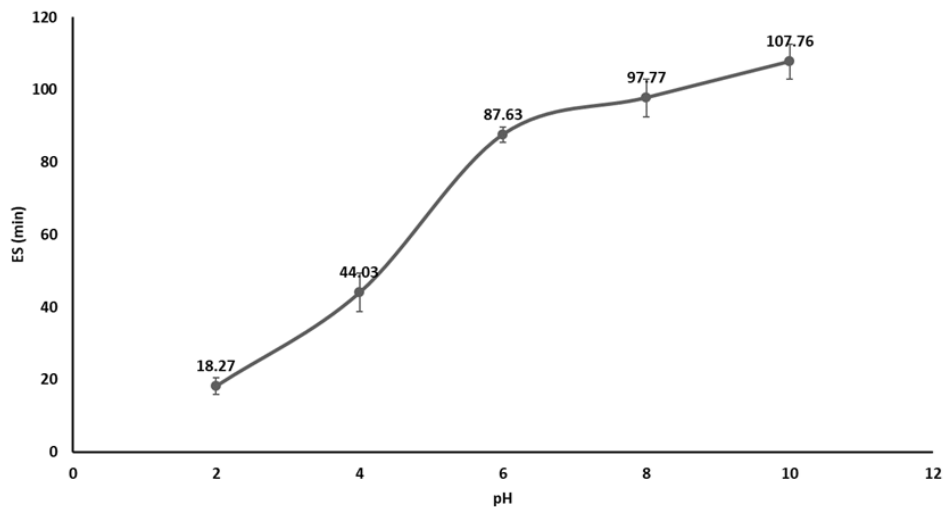


Fig. 6. Emulsifying stability (min) of *H. bryoides* protein at different pH values.

high proportion of non-polar amino acids in the protein fraction favors emulsification (Kumar *et al.*, 2017). Protein solubility is also crucial for the emulsion, as rapid migration to the water-oil interface is required (Phongthai *et al.*, 2016). Moreover, a protein with a lower molecular weight is attributed to increasing EA, thus, better interfacial properties of these molecules at the water-oil interface (Felix *et al.*, 2019). The higher ES observed in this study likely results from the fact that the extracted protein includes a substantial amount of nonpolar amino acids.

3.5.4 Foaming capacity (FC) and stability (FS)

FC of *H. bryoides* protein at various pH values has been displayed in Fig. 7. Foaming is a surface phenomenon that requires proteins to rapidly migrate to the air-water interface and form stable films that can entrap air bubbles (Damodaran, 1997). The FC of all tested samples increased with increasing pH. The lowest value of FC was observed at pH 2 ($233.33 \pm 28.86\%$) and increased at pH 4 ($406.66 \pm 11.54\%$), pH 6 ($500 \pm 0\%$), and pH 8 ($543 \pm 5.77\%$) until it reached its highest value at pH 10 ($576.66 \pm 5.77\%$). No significant differences appeared between pHs 8 and 10 ($p > 0.05$).

Low foamability coincides with low protein solubility (Fig. 4), where the polypeptide chains cannot move rapidly to the interface due to limited molecular mobility. The lowest capacity value observed at pH 2 might refer to the behavior of protein molecules at their

isoelectric point (zero net charge), where protein aggregation is maximized. Whereas an increase of FC as the pH rises could be due to an increased net charge of the protein, which results in the weakening of the hydrophobic interaction, thus enhancing protein flexibility. This enhanced flexibility helps proteins diffuse and quickly move toward the air-water interface, facilitating air encapsulation and foam formation (Phongthai *et al.*, 2016; Garcia-Vaquero *et al.*, 2017).

In general, the current sample showed a high FC at all pH levels compared to that observed in the red seaweed *K. alvarezii* ($53.33 \pm 2.31\%$ at pH 4.0) and the brown seaweed *Himantalia elongata* (71.52% at pH 10) (Kumar *et al.*, 2017; Garcia-Vaquero *et al.*, 2017). The FS was measured at different times for the different pH levels (Fig. 8). The results demonstrated that foam stability decreased over time due to gravitational drainage and bubble coalescence (Murray, 2007).

The result showed a significant ($p \leq 0.05$) drop in FS after 15 min in pH 2 and 4 and after 30 min in pH 6, 8, and 10. No further statistical differences were observed over time ($p > 0.05$). Although the results showed that pH 4 had a lower FC than pH 6, 8, and 10, it exhibited the highest stability in foaming behavior, and after 30 min, there were no significant differences in FS ($p > 0.05$). Moreover, the findings demonstrated no significant difference between pH 8 and pH 10 in FS over time ($p > 0.05$). pH 4 was optimal for FS, where protein solubility/denaturation is moderate. Generally, the high FC relies on the dispersing ability of a protein, but the FS is mainly affected by the degree of protein denaturation (Kumar *et al.*, 2017).

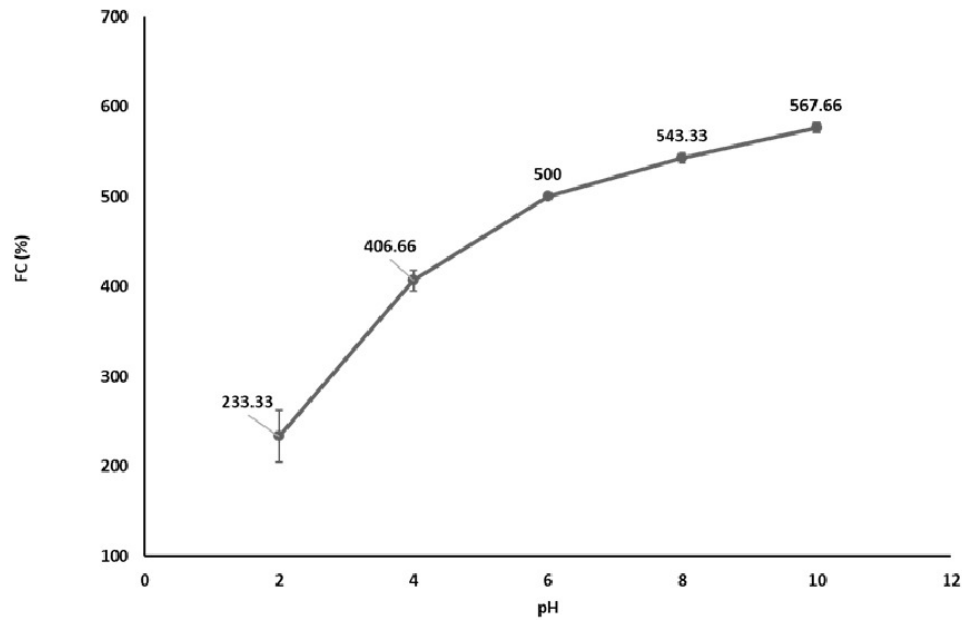


Fig. 7. Foaming capacity of *H. bryoides* protein at different pH values.

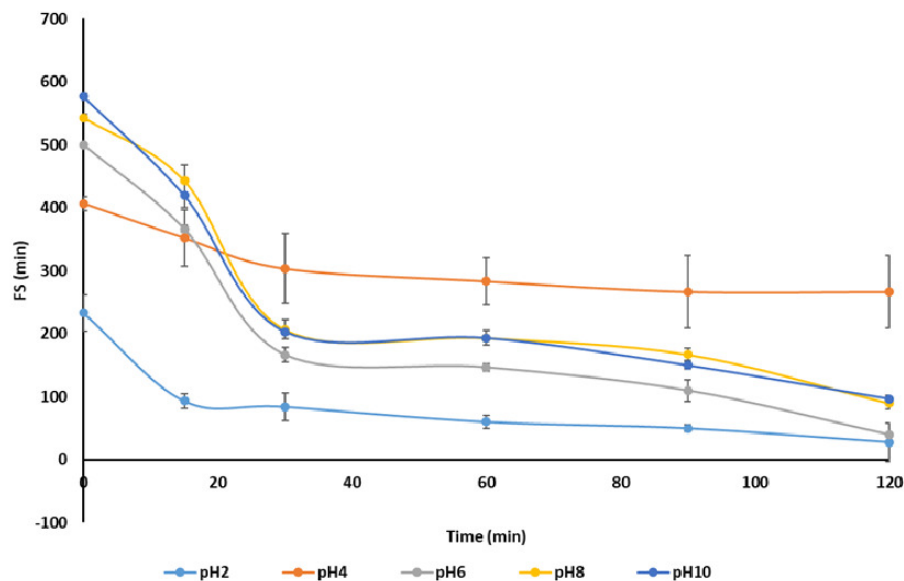


Fig. 8. Foaming stability (min) of *H. bryoides* protein at different pH values.

3.6 In vitro protein digestibility

The in-vitro digestibility of *H. bryoides* protein was determined to be $62.62 \pm 3.29\%$. This value is lower than that for *Pyropia columbina* protein ($74.33 \pm 3.0\%$) as reported by [Cian et al. \(2014\)](#). It is worth noting that both our study and the one by [Cian et al. \(2014\)](#) assessed protein digestibility using the entire plant. The variability in digestibility could be due to differences in seaweed species, which cause variations in composition. The factors that limit digestibility are either polysaccharide molecules (soluble or insoluble fibers) or phenolic compounds. The high levels of polysaccharides, particularly in seaweed species, limit access to digestive enzymes, leading to a decrease in proteolytic enzyme activity.

Previous work on *H. bryoides* has demonstrated the presence of a significant percentage of carrageenan ([Al-Alawi et al., 2011](#); [Al-Nahdi et al., 2019](#)). Carrageenan is a water-soluble polysaccharide used as a thickening agent. Polysaccharides that increase viscosity, like those

found in *H. bryoides*, further inhibit enzyme activity. Additionally, the presence of phenolic compounds reduces digestibility by reacting with amino acids and proteins, thereby inhibiting proteolytic enzyme activity ([Fleurence, 1999](#); [Wong & Cheung, 2000](#); [Cian et al., 2014](#)). The extraction of protein would improve protein digestibility, as shown in *H. charoides* ($88.7 \pm 0.70\%$) and *H. japonica* ($88.9 \pm 1.40\%$) ([Wong & Cheung, 2000](#)).

5. Conclusions

The red seaweed *H. bryoides* represents a promising source of protein in terms of both quality and quantity. Although the protein yield was moderate (6% of the original biomass, corresponding to 31% of the total existing protein), the extract exhibited high purity (88.5%). The protein demonstrated a greater oil-holding capacity than water-holding capacity, suggesting its potential utility in lipid-rich food formulations.

Other functional properties, namely foaming, emulsifying, and solubility, were strongly influenced by pH. Additionally, the protein showed moderate digestibility and contained a substantial proportion of EAA (35.08%). It exhibited a broad molecular weight distribution, dominated by relatively small proteins, and its secondary structure was primarily composed of β -sheet conformations. Future research should further investigate the bioactive potential of this protein, given its favorable functional and chemical properties.

CRedit authorship contribution statement

Hala Al-Mawali: Lab work, data analysis, writing – original draft.
Ahmed Al-Alawi: Conceptualization, supervision, writing—review, and editing

Declaration of competing interest

The authors declare that they have no competing financial interests or personal relationships that could have influenced the work presented in this paper.

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

The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript, and no images were manipulated using AI.

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