Title: Proteomic profiling of New Zealand endemic *Porphyra/Pyropia* (karengo) seaweeds: a predictive framework for functional food applications

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Highlights

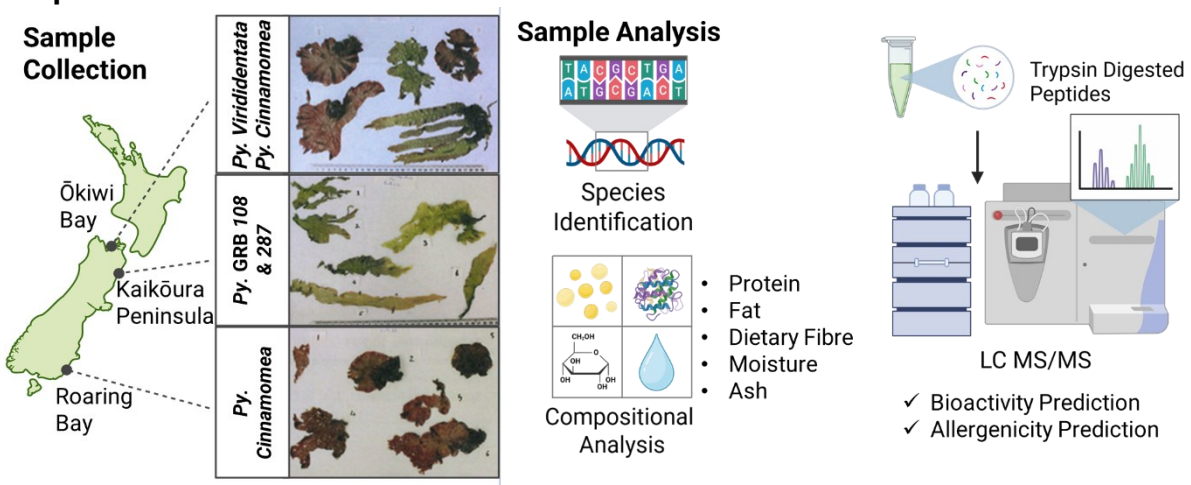
- New Zealand (NZ) *Porphyra/Pyropia* are protein- and fibre-rich with low fat and free sugars, comparable to commercial *Pyropia*.
- NZ *Porphyra/Pyropia* are enriched in sulphur-containing and umami-associated amino acids.
- *Porphyra* GRB contain higher level of phycocyanin, suggesting distinct functional potential.
- In silico analysis predicts phycobiliproteins to have low allergenicity risk and may release ACE and DPP-IV inhibitory peptides during gastrointestinal digestion

Abstract

Porphyra/Pyropia seaweeds are promising sources for functional foods development, offering a rich macro- and micronutrient profiles. In New Zealand (NZ), endemic *Porphyra/Pyropia* species (karengo), exhibit considerable variability driven by geography, seasonality, and climate, which may influence their nutritional quality. Despite their use as traditional foods, the NZ *Porphyra/Pyropia* remain underutilized commercially, in part due to the lack of biomolecular characterisation, particularly their bioactive protein components, hindering evidence-based species selection for

seaweed farming commercialisation and functional food development. This study presents the first proteomic characterization of three NZ *Porphyra/Pyropia* species: *Pyropia viridentata*, *Pyropia cinnamomea*, and *Porphyra* GRB complex. Mass spectrometry-based proteomics analysis identified differences in the phycobiliprotein composition among the species, with the *Porphyra* GRB complex containing higher levels of phycocyanin. Using the protein sequence information, *in silico* gastrointestinal digestion analysis predicted that phycobiliproteins from NZ *Porphyra/Pyropia* seaweeds can potentially release bioactive peptides capable of inhibiting angiotensin-converting enzyme (ACE) and dipeptidyl peptidase-IV (DPP-IV) activities. Sequence-based allergenicity prediction indicated possible cross-reactivity between NZ *Porphyra/Pyropia* β-phycoerythrin and β-phycocyanin against the β-phycocyanin allergen from spirulina, which is associated with a low incidence of allergy. Proximate analysis revealed that NZ *Porphyra/Pyropia* seaweeds have high protein (26-30.2%) and carbohydrate (48.3-50.9%) contents, and low fat and free sugar levels. Amino acid profiling further showed that NZ *Porphyra/Pyropia* seaweeds are relatively rich in sulphur-containing amino acids and umami-associated amino acids. Overall, these findings highlight the potential of NZ *Porphyra/Pyropia* seaweeds as a novel plant-based protein source for functional food applications.

Graphic Abstract



Keywords

New Zealand *Porphyra/Pyropia* species; Functional food; Phycobiliproteins; MS-based proteomics; Bioactive potential; Allergenicity prediction

1. Introduction

The *Porphyra/Pyropia* seaweeds are members of the Bangiaceae family of red algae and comprise many species which are found in the inter-tidal zone of high energy rocky coastlines throughout the temperate latitudes of both northern and southern hemispheres (Chen, 2009). The species within these two genera are regionally endemic (Kim, S.-M. et al., 2018; López-Vivas et al., 2015; Nelson, Wendy Alison, 2013). They are rich in macronutrients such as proteins, which provide a source of essential amino acids, as well as micronutrients, such as iron, calcium, potassium, iodine, and vitamins A, B6, B12 and C (MacArtain et al., 2007; Wang, H. et al., 2023). Their excellent nutritional profile may be linked to their long-standing history of human

consumption across various cultures (Mouritsen et al., 2013; Wang et al., 2020), with archaeological evidence of human consumption dating back to 14,000 years ago (Dillehay et al., 2008). In Asia, members of these two genera are commonly known as nori in Japan, gim in Korea, and zicai in China, where they are cultivated on a commercial scale. Global production of cultivated algae output has experienced remarkable growth, tripling from ~10.6 million wet tonnes in 2000 to ~34.7 million wet tonnes in 2019 (Cai et al., 2021). Notably, the *Porphyra/Pyropia* seaweeds contributed to 8.6% of the total cultivated seaweed production in 2019. This is largely driven by the increasing acceptance of sushi throughout the world as an everyday convenience food, but also an increasing appreciation of these seaweeds as a superfood, based on a deeper understanding on their nutraceutical effect (Carpena et al., 2022; Rawiwan et al., 2022; Wells et al., 2017; Wu et al., 2023). Marine algae are known to contain a repertoire of bioactive molecules, including protein-derived bioactive peptides (Cian et al., 2015; Cian et al., 2012; Lee et al., 2015), and the composition of these bioactive components can vary greatly due to differences in species, geographical location and seasonality (Guihéneuf et al., 2018; Rawiwan et al., 2022; Sanz et al., 2023). According to the World Bank Group, one future growth area for the algae industry is projected to be in the nutraceutical and alternative protein sector, which is expected to contribute another USD ~4.4 billion to the industry by 2030 (Bank, 2023). Currently, the global *Porphyra/Pyropia* cultivation market is dominated by Asian countries, with China accounting for ~71% of the global *Porphyra/Pyropia* cultivation production output (Cai et al., 2021). However, there are opportunities for other countries, especially those with indigenous algae species, to participate in this economy and offer diversified algae product with unique functional properties.

New Zealand's (NZ) possesses a rich marine ecosystem that is home to at least 47 taxa of Bangiales, all but six of which are endemic to the region (Nelson, W.A., 2013; Nelson and Zuccarello, 2025). These endemic NZ *Porphyra/Pyropia* species, collectively known as karengo, are prized by the Māori for their distinctive flavour and exceptional nutritional qualities, and have traditionally been consumed as whole food (Nelson and Zuccarello, 2024). On the other hand, the commercialization of NZ *Porphyra/Pyropia* seaweeds remain limited despite them being within the same genus as the commercially successful Japanese nori. Most commercially available NZ *Porphyra/Pyropia* products are wild harvested and has low and variable annual landings (White and White, 2020). With the NZ government identifying seaweed aquaculture as an emerging sector within its blue-economy development framework (Biancacci et al., 2026), efforts are made to advance the understanding of biodiversity (Nelson and Zuccarello, 2024; Nelson, Wendy Alison, 2013; Nelson et al., 2001; Schweikert et al., 2012) and cultivation techniques (Nelson and Knight, 1996) of NZ *Porphyra/Pyropia* seaweeds to support aquaculture development. Concurrently, nutritional studies on species such as Py*. cinnamomea*, *Py. viridiidentata*, *Py. plicata*, and the *P.* GRB complex, have demonstrated that these NZ red algae are rich in protein (30-35%), dietary fibre (50%), and essential micronutrients such as Omega-3 fatty acid and vitamin B12 (Wheeler, 2022).

Nonetheless, further characterization of the NZ *Porphyra/Pyropia* species is required to identify optimal candidates for commercial aquaculture development (Wheeler et al., 2021). One of the key knowledge gaps lies in the limited biomolecular characterization of these species, particularly regarding the identification of bioactive protein components that may contribute towards health benefits. Addressing this gap is essential for generating evidence-based insights to identify the right NZ Porphyra/Pyropia species for seaweed farming and the development of high-value functional food products. Therefore, this study aimed to characterise the proteome composition of three populations of NZ *Porphyra/Pyropia* species collected from the east coast of the South Island, namely *Py. viridiidentata*, *Py. cinnamomea*, and *P.* GRB complex and to compare their protein composition with that of a commercially sourced *Pyropia* seaweed (*Py. seriata*). In particular, phycobiliprotein content was evaluated, as these light harvesting proteins are prized for their bioactive properties in other red algae (Kim, E.Y. et al., 2018; Mune Mune et al., 2023; Wu et al., 2017). Based on the proteomics data, the bioactivity and allergenicity potential of these phycobiliproteins were predicted using *in silico* analysis. In addition, the amino acid profiles and carbohydrate composition were analysed to provide a more in-depth assessment of the suitability of NZ *Porphyra/Pyropia* seaweeds a source for functional food development.

2. Material and methods

2.1. Collection and processing of *Porphyra/Pyropia* biomass

A bulk sample of approximately 600 g wet weight of *Porphyra/Pyropia* seaweed was collected in September 2021 from three locations along the east coast of the South Island of New Zealand. Each bulk sample was transported to the laboratory within 12 h, rinsed in tap water, excess water removed using a salad spinner, and the morphology of five randomly selected thalli recorded. A 3 x 3 mm piece of each blade was excised. The remainder of the bulk sample was stored at -20ºC until further processing. The processing comprised freeze-drying and then subjecting to ball milling using an orbital shaker at 400 rpm for 20 min in a series of sealed 500 ml plastic containers with twenty 9 mm diameter stainless steel ball bearings. The resulting homogeneous fine powder was stored in sealed containers at room temperature until further analysis. A bulk sample of dried raw Korean-farmed *Py. seriata* was obtained from Geohae Co Ltd, Republic of Korea, through Pacific Harvest Ltd.

2.2. Species identification

DNA was extracted from a small sample of each dried powdered seaweed using a previously described method based on the use of Chelex 100 resin (Schweikert et al., 2012). A 1/10 dilution of each DNA extract was used to amplify a ~500 bp segment of the V9 region of the 18S ribosomal RNA gene using primers that are universal for *Porphyra*/*Pyropia* species (Broom et al., 1999; Nelson et al., 2001; Saunders and Kraft, 2011). Amplification of 2 µl of DNA was performed in PerfeCTa SYBR Green Fast mix (Quantabio) using a Mic Real Time PCR cycler (Bio Molecular Systems) for 45 cycles. The annealing and extension temperatures were 56ºC and 72ºC, respectively. The amplified DNA was purified using the GenepHlow Gel/PCR kit (Geneaid Biotech Ltd) and sequencing was performed by Genetic Analysis Services (University of Otago,

New Zealand). The sequences were used as query for similarity to known sequences in GenBank using BLAST. Identity as a particular species was confirmed based on greater than 99% similarity over the length of the sequence.

2.3. Compositional analysis

The proximate composition was determined from subsamples of each powdered sample as follows. Crude protein was determined by Kjeldahl assay using a conversion factor of 6.25, total fat was determined by extraction with ether and diethyl ether, ash was determined by combustion at 550ºC, and moisture was determined by weighing before and after heating to 105ºC for 16 hours. The total dietary fibre (TDF) and insoluble dietary fibre (IDF) were determined using a TDF100A-1KT enzymatic digestion kit (Sigma), following the manufacturer's instructions. The residual carbohydrate was estimated by difference from all the above components. These assays were carried out at/by the Cawthron Commercial Testing Laboratories, operating under ISO17025 compliant quality standards.

Additional analyses on total digestible carbohydrates and dietary fibre were performed by Eurofins Food Testing Netherlands (Heerenveen, Netherlands). Briefly, the analysis of digestible carbohydrates consists of both the dietary starch and free sugar quantification. The dietary starch analysis is based on AOAC 2017.16 method (Schmidt and Bunzel, 2025). First, dried and milled seaweed samples were treated with heat-stable α-amylase by incubation at 100°C for 1 h to gelatinize and partially hydrolyse the starch. After cooling, amyloglucosidase was added, and the mixture was incubated at 50°C for 2 h. The digested mixture was clarified, diluted as needed, and the glucose concentration was measured using a colorimetric glucose oxidase-peroxidase (GOPOD) assay at 505 nm. Dietary starch content is determined as 0.9 times the difference of glucose in the digested portion minus free glucose in an undigested portion of the sample (Weurding et al., 2001). For free sugar analysis, the ISO method 22184/IDF 244 (Brunt et al., 2020) was applied. In this method, free sugars, including fructose, galactose, glucose, lactose, maltose, and sucrose, were isolated from the algae samples by aqueous ethanol extraction and quantified using High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection (HPAEC-PAD).

As part of the carbohydrate analysis, a second total dietary fibre (TDF), which consist of high molecular weight dietary fibre (HMWDF) and low molecular weight dietary fibre (LMWDF), were determined based on Eurofins in-house method. Briefly, seaweed samples were enzymatically treated with heat-stable α-amylase and amyloglucosidase at 37°C for 4 h. The reaction was terminated by adjusting the pH to 8.2, followed by heat inactivation. Proteins in the sample were removed through protease digestion. The resulting reaction mixture was then used to quantify both HMWDF and LMWDF. HMWDF consist of water-insoluble dietary fibre (IDF) and ethanol-precipitable water-soluble dietary fibre (SDFP), while LMWDF consist of soluble dietary fibre that remain soluble in water and ethanol (SDFS). To isolate IDF, the aqueous reaction mixture was filtered, and the residue was washed, dried, and weighed gravimetrically. The filtrate containing SDFP and SDFS was then adjusted to

78% ethanol to precipitate SDFP, which was then recovered via filtration, washed, dried and measured gravimetrically. The remaining SDFS in the ethanol supernatant was quantified by high-performance liquid chromatography (HPLC). Total dietary fibre was calculated as the sum of IDF, SDFP, and SDFS.

Amino acid profiles were determined using a method based on EC Directive 98/64/EC Annex part A (EC, 1998) and EC Directive 2000/45/EC Annex Part C (EC, 2000). In brief, replicate samples of seaweed biomass were hydrolysed in the presence of either acid, acid with a pretreatment with performic acid, base, or base using barium hydroxide. The hydrolysed samples were then subjected to reverse phase HPLC spiked with individual amino acid standards and each amino acid peak was quantified from the elution profile.

2.4. Protein extraction and filter-assisted sample preparation

Protein extraction was independently performed on three subsamples of each seaweed biomass (dried and milled). Briefly, the seaweed powder was resuspended in 8M Urea, 1% SDS in 100mM Tris pH8 (1:100 w/v) and subjected to ultrasonication using the Q700 Sonicator (Qsonica, Newtown, CT, USA), followed by 30 min of vortexing. The sample solution was clarified through centrifugation at 16,000 x g for 10 min and protein quantification was performed using the DC protein assay kit (Bio-Rad, Hercules, CA, USA). 100µg of protein solution was reduced with 66.67 mM DTT for 1 h at room temperature. The reduced protein solution was loaded and trapped onto a 30K MWCO Microcon centrifugal filter (MilliporeSigma, Burlington, MA, USA) through centrifugation at 14,000 x g for 15 min. The protein was then alkylated with 100mM iodoacetamide (IAA) for 50 min at room temperature in the dark. On-filter protein digestion was performed using sequencing grade trypsin (1:50), (Promega, Madison, WI, USA) overnight at 37 °C. After incubation, 0.5 M NaCl was added to the column, and the digested peptides were eluted through centrifugation at 14,000 x g for 10 min. The digestion was quenched through acidification with trifluoroacetic acid. The digested peptides were desalted using ZipTip C18 resin prior to mass spectrometry analysis.

2.5. LC-MS/MS analysis and database search

LC-MS/MS analysis was performed based on Bi *et al* (Bi et al., 2018), with some modification. The tryptic peptides were reconstituted in 1% FA with 2% ACN and analysed using an LTQ-Orbitrap Elite MS (ThermoFisher Scientific, Waltham, MA, USA) coupled to a Waters nanoAcquity HPLC (Waters, Milford, USA). The peptide sample was first concentrated with an ACQUITY UPLC M-Class Symmetry C18 Trap Column (180 µm × 20 mm, 5 µm, 100Å), and then, separated using a Waters ACQUITY UPLC M-Class Peptide BEH C18 column (1.7 µm, 75 µm × 200 mm, 130 Å). The mobile phase buffers consisted of 0.1% formic acid (FA) in water (Buffer A) and 0.1% FA in acetonitrile (ACN) (Buffer B). A gradient of 5–40% B was applied for 40 min, followed by an increase from 40-70% B over 5 min to facilitate peptide separation, at a flow rate of 0.3 µL/min. Samples were injected into the LTQ Orbitrap Elite via an Nanospray Flex™ ion source (ThermoFisher Scientific, Waltham, MA, USA), operating in positive ionization mode at 1.8kV. Full MS spectra

were acquired in a top 15 peak data dependent mode at a resolution of 60,000 over an m/z range of 30 to 1600, followed by CID MS/MS with a normalized collision energy of 35.

Raw data from the LC-MS/MS analysis were analysed using the Maxquant 64G software package. Protein identification was performed using the Bangiaceae proteome retrieved from the NCBI Proteome Database (accessed in December 2023) with the Andromeda search engine. Carbamidomethylation was set as a fixed modification; acetylation (protein N-term) deamidation (NQ) and oxidation (M) were set as variable modifications. The maximum missed cleavage was set at 2. The parent mass error tolerance and precursor mass tolerance were set at 15 ppm and 0.5 Da. A false-detection rate (FDR) of < 1% and a minimum of two unique peptides were used for protein acceptance.

2.6. Bioinformatics analysis of proteomics data

Both *in silico* bioactivity and allergenicity prediction were performed based on Tan *et al* (Tan et al., 2022), with some modification. The protein sequences of the phycobiliproteins were retrieved to evaluate the bioactive potential of the peptides released upon *in silico* gastrointestinal (GI) digestion. Bioactive peptide prediction was carried out using the BIOPEP-UWM database (Minkiewicz et al., 2019), simulating enzymatic hydrolysis with the following digestive enzymes: pepsin (pH 1.3, EC 3.4.23.1), chymotrypsin (A) (EC 3.4.21.1), and trypsin (EC 3.4.21.4). Allergenicity predictions were performed using the AllerCatPro 2.0 web server (Nguyen et al., 2022), enabling the identification of potential allergenic risks based on protein sequence/structure cross-reactivity.

3. Results

3.1. Species identification of endemic NZ *Porphyra/Pyropia* biomass

Three bulk NZ *Porphyra/Pyropia* algae biomasses were collected along the east coast of the South Island, New Zealand, specifically at Ōkiwi Bay, north of Kaikōura, Kaikōura Peninsula, and Roaring Bay near Nugget Point, Otago. These locations were selected based on prior studies indicating the presence of extensive patches of NZ *Porphyra/Pyropia* being present, with each location harbouring a distinct species as the predominant *Porphyra/Pyropia* species in that location (Wheeler et al., 2021). DNA sequence analysis was performed to verify the species of NZ *Porphyra/Pyropia* algae biomass that were present at each site (Figure 1). At Ōkiwi Bay, the dominant species present were a mixture of *Py. viridiidentata* and *Py. cinnamomea* (Figure 1A). Each of these species has a distinct morphotype; *Py. viridiidentata* has broad green blades with yellow edges, while *Py. cinnamomea* has broad bronze-coloured blades with yellow edges. The Kaikōura Peninsula sample consisted solely of *P.* GRB complex, comprises of several very closely related taxa, with *P.* GRB *108* and *P.* GRB *287* being the most abundant. The *P.* GRB complex have yellowish-green elongated thalli (Figure 1B). The *Porphyra/Pyropia* sample from Roaring Bay contained only a single species, *Py. cinnamomea* (Figure 1C). The geography location of these NZ *Porphyra/Pyropia* agrees with previous collection data. *Py viridiidentata* were predominantly found in the lower part of North Island and the eastern coast of South Island in New Zealand, with

collection mainly fall in the late winter to early spring period (Belbin et al., 2021). *Py.cinnamomea* were predominantly found in the southern tip of the South Island, with collection mainly in the early spring period (Belbin et al., 2021). On the other hand, collection record of *P.* GRB are minimal, with one previous reported collection at Brighton Beach, South Island, New Zealand, during the autumn to winter period (Schweikert et al., 2012).

Figure 1: Species identification of NZ endemic *Porphyra/Pyropia* seaweeds collected from three coastal locations in New Zealand: *Py. viridiidentata & Py. cinnamomea* from Ōkiwi South (A); *P.* GRB *108 & 287* from north of Kaikōura Peninsula, New Zealand (B); *Py. cinnamomea* from Roaring Bay (C).

3.2. Proximate composition analysis and amino acid profiling of *Porphyra/Pyropia* biomass

Red algae are recognized for their nutritional quality, particularly due to their high levels of protein and carbohydrates content (Jiménez-González et al., 2023). Therefore, to better understand the nutritional value of the NZ *Porphyra/Pyropia* seaweed samples collected from the shores of NZ's South Island, proximate composition analysis was conducted (Table 1). A commercially sourced nori (*Py. seriata*) was also included for comparison. The analysis indicated that all samples contain relatively high levels of protein and carbohydrate, as expected for these taxa. The total protein content of *Py. viridiidentata* & *Py. cinnamomea, P.* GRB species complex, and *Py. cinnamomea* seaweed samples were 30.2%, 26.0%, and 28.1%, respectively, while the corresponding carbohydrate contents were 48.3%, 50.2%, and 50.9%.

The NZ *Porphyra/Pyropia* seaweed samples are also high in total dietary fibre, as showed by two independent analyses. HMWDF, which includes insoluble dietary fibre (IDF) and ethanol-precipitable water-soluble dietary fibre (SPFP), accounts for 43.5% in *Py. viridiidentata & Py. cinnamomea* sample, 39.9% in *P.* GRB complex sample, and 39.1% in the *Py. cinnamomea* sample. These values are similar to that obtained from the commercial *Py. seriata* sample, which has a HMWDF content of 39.8%. The LMWDF fraction, which consists of the water and ethanol soluble dietary fibre (SDFS), were below 1% across all samples. The NZ *Porphyra/Pyropia* seaweeds are all low in fat (≤4.0%) and starch (≤3.5%), with no detectable free sugar. This, combined with their high total dietary fibre content (37-43.9%) and other compositional attributes, makes them attractive as a source of biomass for food development.

Table 1: Proximate composition of NZ *Porphyra/Pyropia* and commercial *Py. seriata* seaweed samples

Composition (g/100g)	*Py viridentata* & *Py. cinnamomea*	*P.* GRB complex	*Py. cinnamomea*	*Py. seriata*
Protein	30.2	26.0	28.1	37.6
Total fat	3.8	4.0	3.2	5.5
Ash	9.5	14.4	14.8	10.6
Moisture	8.2	5.4	3.0	2.6
Carbohydrates (by difference)	48.3	50.2	50.9	43.7
Total dietary fibre	42.0	37.0	42.0	37.0
Soluble dietary fibre	9.8	12.0	8.9	16.0
Dietary fibre analysis				
HMWDF (IDF +SDFP)	43.5	39.9	39.1	39.8
LMWDF (SDFS)	0.4	0.4	0.4	0.6
Total dietary fibre	43.9	40.3	39.5	40.4
Digestible carbohydrates analysis				
Starch*	1.4	3.5	1.8	0.8
Fructose	<0.1	<0.1	<0.1	<0.1
Galactose	<0.1	<0.1	<0.1	<0.1
Glucose	<0.1	<0.1	<0.1	<0.1
Lactose	<0.1	<0.1	<0.1	<0.1
Maltose	<0.1	<0.1	<0.1	<0.1
Sucrose	<0.1	<0.1	<0.1	<0.1

Legend: **HMWDF**: high molecular weight dietary fibre; **LMWDF**: low molecular weight dietary fibre; **IDF**: insoluble dietary fibre; **SDFP**: ethanol-precipitable water-soluble dietary fibre; **SDFS**: ethanol- and water-soluble dietary fibre.
***** Including maltodextrin (exclude glucose)

The amino acid composition analysis is presented in Table 2. All nine essential amino acids (EAAs) are present in the four samples, with leucine, valine, threonine, and lysine being the most abundant. The total EAAs content, expressed as a fraction of total protein was 271.5 mg/g, 323.1 mg/g and 298.9mg/g*, for* Py. *viridentata & Py. cinnamomea, P.* GRB complex, and *Py. cinnamomea* samples, respectively, and was comparable to that of commercial *Py. seriata* (300.5 mg/g). Further analysis of the individual EAA composition highlight that the threonine, valine, tryptophan, methionine + cysteine, and phenyalanine + tyrosine content in the NZ seaweeds meet the

respective daily essential amino acid (EAA) intake requirements for adults, assuming a protein consumption of 0.66 g protein/kg body weight/day as recommended by the World Health Organization (FAO/WHO, 2007).

Threonine is essential for the synthesis of structural proteins such as collagen and elastin and plays a role in maintaining immune function (Habte-Tsion et al., 2016; Tang et al., 2021; Wen et al., 2023). The threonine content in *Py. viridiidentata* & *Py. cinnamomea* (36.4 mg/g), *P.* GRB complex (46.2 mg/g), *Py. cinnamomea* (39.1 mg/g)*, and Py. seriata* (42.6 mg/g) all met the recommended daily threonine requirement of 23 mg/g. Valine, which support muscle metabolism, tissue repair, and energy production (Wang, C. et al., 2023; Wolfe, 2017), was also present in sufficient amounts in the four algae samples. Valine content in the *Py. viridiidentata* & *Py. cinnamomea* was 39.7 mg/g, *Py.* GRB complex was 46.2mg/g, *Py. cinnamomea* was 42.7 mg/g*, and Py. seriata* was 45.2 mg/g, meeting the recommended daily valine requirement of 39.2 mg/g. Lastly, tryptophan, a precursor to serotonin and niacin that is vital for mood regulation and metabolic processes (Richard et al., 2009) was present at an adequate level. The tryptophan content in *Py. viridiidentata* & *Py. cinnamomea* (6.6 mg/g), *Py.* GRB complex (11.5 mg/g), *Py. cinnamomea* (7.1 mg/g)*, and Py. seriata* (10.6 mg/g) all met the recommended daily tryptophan requirement of 6.0 mg/g. In addition, the NZ *Porphyra/Pyropia* seaweeds were relatively rich in sulphur-containing amino acids including methionine and cysteine (29.8-35.6 mg/g) which are commonly limiting in other plant-based proteins (Zhang et al., 2024). Nonetheless, further studies evaluating protein digestibility and amino acid bioavailability of the EAAs are required to assess the protein quality of these NZ *Porphyra/Pyropia* seaweeds.

The three NZ *Porphyra/Pyropia* seaweed samples also exhibited high level of flavour-enhancing amino acids, including alanine (115.4-128.1 mg/g), glutamic acid and glutamine (92.5-109.3 mg/g), aspartic acid and asparagine (85.4-92.7 mg/g), and glycine (43.0-53.8 mg/g) (Table 2). These concentrations are comparable to the levels observed in the *Py. seriata* sample and also those reported in other *Porphyra*/*Pyropia* species such as *P. dioica*, *P. umbilicalis*, *Py. yezoensis*, and *Py. tenera* (Fleurence, 1999; Jung et al., 2016; Machado et al., 2020). Alanine, asparagine, glutamine, and glycine are known to impart mild sweetness, while glutamic acid, glutamate, and aspartic acids contribute to the umami taste perception (Vilgis, 2023). As a result, the predicted taste profiles of the three NZ *Porphyra/Pyropia* seaweed samples (Figure S1) are comparable to the commercial *Py. seriata,* which is recognized for its favourable palatability (Wu et al., 2024).

Table 2: Total amino acid composition, expressed in mg/g of protein, of the three NZ *Porphyra/Pyropia* and commercial *Py. seriata* seaweed samples. The recommended adult daily essential amino acid requirement as determined by the FAO/WHO guideline are provided for comparison (FAO/WHO, 2007).

Amino Acids	**Py viridiidentata**	***P.* GRB complex**	***Py. cinnamomea***	***Py. seriata***	**FAO/WHO Guideline**

(mg/g protein)	& Py. cinnamomea				
Essential amino acids (EAA)					
Histidine	9.9	11.5	10.7	10.6	15.0
Threonine	36.4	46.2	39.1	42.6	23.0
Lysine	36.4	38.5	39.1	37.2	45.0
Methionine + Cysteine	29.8	30.8	35.6	26.6	22.0
Valine	39.7	46.2	42.7	45.2	39.0
Isoleucine	19.9	26.9	24.9	26.6	30.0
Leucine	49.7	57.7	53.4	55.9	59.0
Phenylalanine + Tyrosine	43.0	53.8	46.3	45.2	38.0
Tryptophan	6.6	11.5	7.1	10.6	6.6
ΣEAA	271.5	323.1	298.9	300.5	277.0
Non-essential amino acids (NEAA)					
Serine	39.7	42.3	46.3	39.9	-
Arginine	46.4	50.0	42.7	42.6	-
Aspartic acid & Asparagine	92.7	88.5	85.4	77.1	-
Glutamic acid & Glutamine	109.3	103.8	92.5	90.4	-
Alanine	125.8	115.4	128.1	109.0	-
Proline	29.8	38.5	32.0	34.6	-
Glycine	43.0	53.8	53.4	47.9	-
ΣNEAA	486.8	492.3	480.4	441.5	-
Total reported amino acids	758.3	815.4	779.4	742.0	-

3.3. LC-MS/MS-based proteomics analysis of *Porphyra/Pyropia* samples

Label-free LC-MS/MS quantitative analysis was conducted to evaluate and compare the proteome distribution of the three NZ *Porphyra/Pyropia* samples and a commercially sourced *Py. seriata* seaweed sample. After applying the filtering criteria of protein with at least two unique peptides and a peptide false-discovery rate (FDR) at <1%, 508 proteins were identified and quantified in the Py*. viridentata* & *Py. cinnamomea* sample*. P.* GRB complex contained 646 proteins, *Py. cinnamomea* contained 492 proteins, and *Py. seriata* contained 561 proteins. Principal component analysis (PCA) was performed on these identified proteins, revealing distinct clustering patterns between the NZ *Porphyra/Pyropia* seaweeds and with the *Py. seriata* seaweed as well (Figure 2A). The first principal component (PC1) accounted for 50.8% of the total variance, while the second principal component (PC2) explained 18.8%,

collectively capturing 69.6% of the total variation in the dataset (Figure 2A). Furthermore, the tight clustering of the three replicates from each sample underscores the robustness of the analysis, demonstrating high reproducibility in the LC-MS/MS analysis.

The *P.* GRB complex exhibited a clear separation from the other two NZ samples along the PC1 axis, indicating a distinct proteome profile. This distinct separation may be attributed to two factors. First, the *P.* GRB complex typically inhabits at the high- to mid- intertidal zone whereas *Py. viridiidentata* and *Py. cinnamomea* are more commonly found in the mid- to low- intertidal zone (Nelson and Zuccarello, 2024). Differences in light quality across intertidal zone can influence phycobiliprotein composition (López-Figueroa and Niell, 1990), which represents a major protein component in these samples. Second, the thalli of the P. GRB complex are thought to be dioicous or temporal dioicous whereas the thalli of *Py. viridiidentata* and *Py. cinnamomea* are monoicous (Nelson and Zuccarello, 2024). This can potentially result in distinct protein repertoire associated with reproductive strategy. In contrast, the *Py. viridiidentata & Py. cinnamomea* sample clustered closely with the *Py. cinnamomea* sample, suggesting a high degree of similarity in their protein profiles, likely due to the presence of *Py. cinnamomea* in both samples. The commercially sourced *Py. seriata* sample was clearly separated from the *NZ Porphyra/Pyropia* samples, clustering independently along the negative PC2 axis, highlighting the differences in the protein composition of *Porphyra/Pyropia species* from different hemispheres. This observation is consistent with previous phylogenetic analysis of the red algal order Bangiales, in which the Pyropia clades are broadly resolved according to geographic regions (Sutherland et al., 2011).

The NZ *Porphyra/Pyropia* species remain poorly characterized at the biomolecular level, resulting in a limited species-specific proteome database for proteomics analyses. Therefore, to assess the proteome distribution of the four samples, a homology-driven approach (Junqueira et al., 2008) was adopted and a Bangiaceae-based proteome database was used for protein identification and quantification. Although this approach limits the quantification of individual protein and their isoforms, reliable protein identification and quantification were still achieved in this study using MaxQuant (Cox and Mann, 2008), which applies a parsimony-based protein inference strategy that enable robust protein-group level quantitative comparison across the samples. The identified proteins were broadly classified into four protein groups: “phycobiliproteins”, “other photosynthetic proteins”, “general proteins”, and “hypothetical proteins” (Figure 2B). Phycobiliproteins represented a significant proportion of the total protein content in the four samples. In the NZ *Porphyra/Pyropia* seaweed samples, phycobiliproteins constitute 36.1% of the proteome in *Py. cinnamomea*, 32.6% in *Py. viridiidentata* and *Py. cinnamomea*, and 30.7% in *P.* GRB complex samples. These proportions are comparable to that observed in *Py. seriata*, in which the phycobiliproteins constitute 32.7% of its proteome. The “other photosynthetic proteins” category was the second most abundant protein group across all samples. Their relative abundance in *Py. viridiidentata* and *Py. cinnamomea, P.* GRB complex, *Py. cinnamomea,* and *Py. seriata* samples was 24.6%, 16.3%, 25.8%,

and 20.7%, respectively. As autotrophic macroalgae, it is unsurprisingly that these species have proteomes that are highly enriched in phycobiliproteins as well as other photosynthetic proteins. Overall, these two protein groups accounted for approximately 47% to 61.9% of the total proteome across the studied samples. This reflects the central role of these protein groups in the light harvesting and energy conversion processes essential for algal photoautotrophic metabolism. The "general proteins" category represents the lower abundance fraction of the proteome, comprising between 8.4% and 13.5% of the total protein content across the four samples. The "hypothetical proteins" are proteins predicted to be expressed in these species based on matches to genome-derived proteome databases, but for which there is as-yet no experimental evidence for their expression or function. These hypothetical proteins comprise 31.0% of the total protein content in *Py. virididen*tata and *Py. cinnamomea,* 44.7% in *P.* GRB complex, 28.6% in *Py. cinnamomea,* and 33.1% in *Py. seriata*. This suggests that the proteome of *Porphyra/Pyropia* seaweeds remains fairly uncharacterized and highlights the need for further annotation and characterisation of the *Porphyra*/*Pyropia* proteomes.

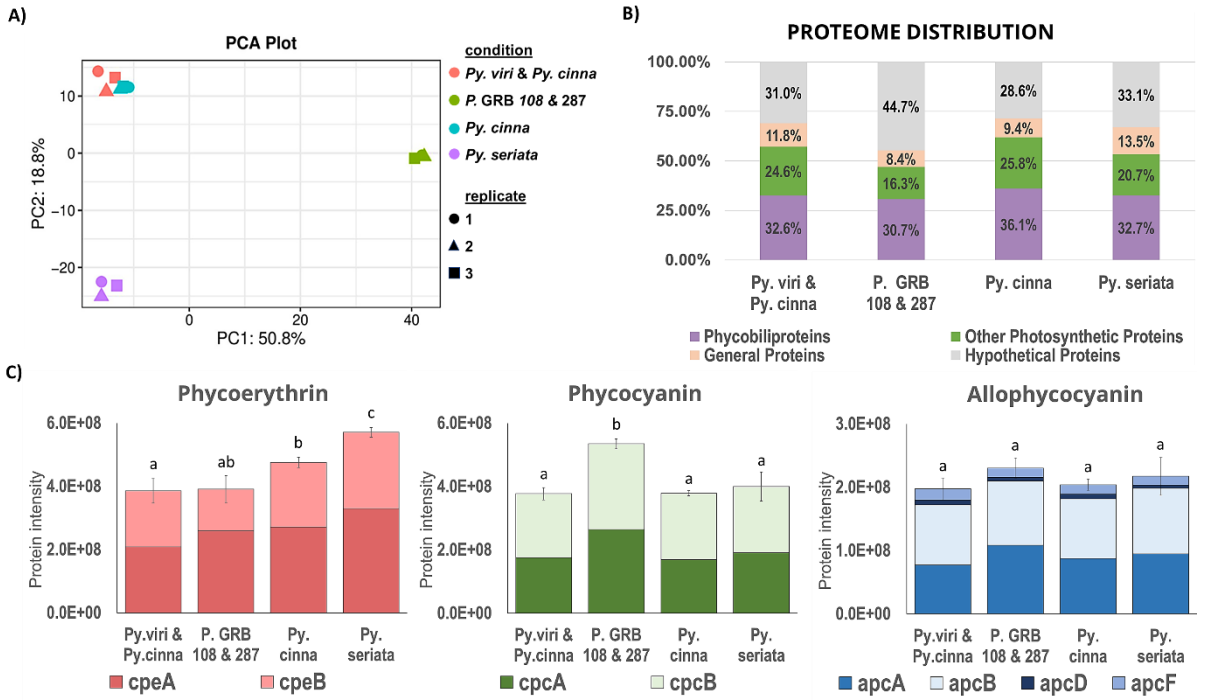


Figure 2: Mass spectrometry-based proteomics analysis of the *Porphyra*/*Pyropia* samples. Principal component analysis (PCA) plot illustrating the overall proteomic relationship among the four algae samples (A). Proteome abundance distribution in each algae sample. Identified proteins are classified into four functional categories and the protein abundance was calculated based on the intensity-based absolute quantification (iBAQ) values (B). Comparative analysis of phycoerythrin, phycocyanin, and allophycocyanin abundances highlights species-level differences in phycobiliprotein composition between the NZ *Porphyra/Pyropia* seaweeds and commercially sourced *Py. seriata* sample (C).
Legend: ***Py.viri:*** *Py. virididen*tata; ***Py.cinna*****:** *Py. cinnamomea*

The distribution of the three major phycobiliproteins: phycoerythrin, phycocyanin, and allophycocyanin, across the samples were characterized. Their relative abundance varied independently from one another across samples (Figure 2C). *Py. seriata* exhibited significantly higher levels of phycoerythrin compared to the NZ *Porphyra/Pyropia* seaweed samples. Among the three NZ seaweeds, the *Py. cinnamomea* sample had a greater phycoerythrin content than the mixed *Py. viridiidentata* and *Py. cinnamomea* sample. The alpha and beta subunits of phycocyanin were found to be significantly more abundant in the *P.* GRB complex sample compared to the three other samples, which may account for its distinct colouration. In contrast to the other phycobiliproteins, the four allophycocyanin subunits were similar across the four samples.

3.4 Bioactivity and allergenicity prediction of phycobiliproteins

Bioactive peptides derived from phycobiliproteins have demonstrated health-promoting properties, when liberated through enzymatic hydrolysis (Kim, E.Y. et al., 2018; Mune Mune et al., 2023; Wu et al., 2017). Given the high abundance of phycobiliproteins in the NZ *Porphyra/Pyropia* seaweed samples, a sequence-based approach was utilized to predict possible bioactivities in these proteins. Although a species-specific proteome database for the LC-MS/MS analysis is not available, evaluation of the potential bioactivity of phycobiliproteins remains feasible due to the high sequence conservation of these proteins within the Bangiaceae family (Figure S2). *In silico* bioactivity prediction was conducted using the BIOPEP-UWM database to evaluate the potential bioactivity of phycobiliproteins following simulated gastrointestinal (GI) digestion. The theoretical degree of hydrolysis (DH) of the phycobiliproteins following the sequential digestion with pepsin (pH1.3, EC3.4.23.1), chymotrypsin (A)(EC3.4.21.1) and trypsin (EC3.4.21.4) are as follows: 33.74% and 31.25% for the phycoerythrin α-subunit (*cpeA*) and β-subunit (*cpeB*), respectively; 34.78% and 32.75% for the phycocyanin α-subunit (*cpcA*) and β-subunit (*cpcB*), respectively; and 33.13%, 35.00%, 35.00%, and 37.50% for the allophycocyanin α-subunit (*apcA*), β-subunit (*apcB*), γ-subunit (*apcD*), and β18-subunit (*apcF*), respectively (Table 3). The simulated digestion process generated a diverse array of predicted peptides and amino acids, predominantly within the molecular weight range of 150 to 300 Da (Figure S3). This suggests that most of these peptides produced are di- and tri- peptides which have higher propensity to confer bioactivity due to their efficient absorption and interaction with biological targets (Kim, S.-M. et al., 2018; Korhonen and Pihlanto, 2006).

The potential bioactivity of these peptides was then predicted by mapping the *in silico* digested protein sequences against the BIOPEP bioactive peptide database. The analysis revealed that all phycobiliprotein subunits contained a range of short-chain peptides with diverse bioactivities, predominately with angiotensin-converting enzyme (ACE) and dipeptidyl peptidase IV (DPP-IV) inhibitory activities. Among these proteins, *apcF*, *cpcA*, and c*peB* were identified to have the top 3 highest frequency of peptides released that conferred DPP-IV inhibition activity, while *apcA, apcB,* and *cpcB* have the highest release frequency of peptides with ACE inhibitory activity. Peptides such as EL, VY, and GSH from *cpcA* and IR and GW from *apcD* are predicted to possess

antioxidative properties, while the VL and IL peptide sequences from *apcF* were also predicted to stimulate glucose uptake.

Additionally, several peptide sequences identified in this analysis have been reported in other food materials to exhibit bioactivities verified by in-vitro assays or animal studies. For example, the dipeptide sequences, SF (found in *cpcA*, *apcA*) and QY (*cpcA*), derived from *Moringa oleifera* seeds, were demonstrated to possess anti-oxidative properties by reducing hydrogen peroxide-induced oxidative damage in Chang liver cells (Liang et al., 2020). The SF peptide was also identified in *Phaseolus vulgaris* and shown to possess ACE-inhibitory activity based on a TNBS assay (Tagliazucchi et al., 2015). Protease N-hydroylsed royal jelly protein has been reported to release antioxidative peptides such as AL (*cpeB, cpcB, apcF*) and IR (*apcD*), with strong hydroxyl radical scavenging activity (Guo et al., 2009). Nakahara et al. identified several peptides, including GW (*apcD*), GY (*apcB*), AF (c*peA*), SY (*cpeA, apcF*), with ACE inhibitory properties in *Aspergillus sojae*-fermented soybean using in-vitro ACE inhibition assay, and further demonstrated that GY and SY peptides reduced systolic blood pressure in spontaneously hypertensive rats following a single dose administration (Nakahara et al., 2010; Nakahara et al., 2011). The AF peptide was also identified in *Phaseolus vulgaris* (Tagliazucchi et al., 2015) and hydrolysed corn gluten meal (Yang et al., 2007), where ACE inhibitory activity was observed. Lastly, hydrolysed milk proteins have been shown to yield peptides with DPP IV inhibition and/or antioxidative properties. Milk-derived dipeptides such as AY (*cpcB, apcA*), VY (*cpcA*), SL (*cpeA, cpcA, apcA*), EK (*apcD*), GL(*cpeB, cpcA, cpcB, apcB, apcF*), were reported to exhibit both proton radical scavenging and DPP-IV inhibition activities (Nongonierma and FitzGerald, 2013).

Taken together, these reported bioactivities suggest that the phycobiliproteins from NZ *Porphyra*/*Pyropia* algae may release peptide sequences with health-promoting properties upon gastrointestinal digestion. Nonetheless, further empirical validation is required to confirm the release, stability, intestinal absorption, and bioactivity of these peptides.

Table 3: *In silico* prediction of bioactivity and allergenicity risk of phycobiliproteins based on protein sequence information.

Protein Name	Gene Name	Theoretical DH	Predicted Bioactivity	Identified Peptide Sequence	A_E	Allergenic Potential
Phycoerythrin	*cpeA*	33.74%	DPP IV inhibition	**AF**, DR, **SL**, **SY**, TL, VN	0.043	Weak
			ACE inhibition	**AF**, AR, DM, DY, DR, **SY**	0.037	
	cpeB	31.25%	DPP IV inhibition	**AL**, **GL**, TN	0.051	Strong
			ACE inhibition	**GL**, PGL	0.028	
Phycocyanin	*cpcA*	34.78%	DPP IV inhibition	**GL**, PY, **QY**, **SF**, SL, TK, TL, TN, **VY**	0.056	Weak

			ACE inhibition	DY, **GL**, GR, GSH, **SF**, **VY**	0.037	
			antioxidative	EL, GSH, **VY**	0.019	
	cpcB	32.75%	DPP IV inhibition	**AY**, DL, DM, DR, GEF, **GL**	0.047	Strong
			ACE inhibition	**AL**, **AY**, DR, **GL**, IN	0.047	
Allo-phycocyanin	*apcA*	33.13%	ACE inhibition	**AY**, DL, DR, DY, ER, QK, **SF**	0.05	Weak
			DPP IV inhibition	**AY**, DR, **SF**, **SL**, TL, VL	0.037	
	apcB	35.00%	ACE inhibition	DL, DY, **GL**, GM, GU, VR	0.05	Weak
			DPP IV inhibition	**GL**, **GY**, **SL**, VL, VR	0.044	
	apcD	35.00%	DPP IV inhibition	**EK**, **GW**, **IR**, QL, SL	0.044	Weak
			ACE inhibition	DY, **EK**, GM, **GW**	0.037	
			antioxidative	**IR**, **GW**	0.019	
	apcF	37.50%	DPP IV inhibition	**AL**, **SL**, **GL**, DR, IL, IN, QH, **SY**, VL	0.065	Weak
			ACE inhibition	DR, DL, **GL**, IL, **SY**	0.041	
			Stimulating (Glucose Uptake)	IL, VL	0	

Note: The peptides highlighted in **bold** have been experimentally confirmed in other food materials to exhibit bioactivity, as supported by in-vitro assays or animal studies.

NZ *Porphyra/Pyropia* seaweeds are not widely consumed globally and as a result, there is limited information on the potential allergenicity risks associated with their consumption. However, instances of algae-induced food allergies have been documented in other populations (James et al., 2023). This underscores the importance of evaluating the allergenic potential of NZ *Porphyra/Pyropia* seaweeds to enable a quick assessment on their suitability as a novel food source and to better understand possible allergy risk to consumers. To address this concern, *in silico* allergenicity assessment based on protein sequences were conducted to evaluate the phycobiliproteins present in the NZ *Porphyra/Pyropia* samples, using the AllerCatPro 2.0 allergenicity prediction algorithm (Nguyen et al., 2022). The results suggest that both *cpeB* and *cpcB* proteins may possess allergenicity risk due to their high structural similarity (>93% similarity) to the epitope of a known allergen, β-phycocyanin, derived from *Arthrospira platensis* (commonly known as spirulina) (Figure S4). The *cpeB* protein and spirulina β-phycocyanin protein share a common epitope region spanning amino acid residues 101 to 115 and 167 to 170. Whereas, the *cpcB* protein showed sequence similarity to spirulina β-phycocyanin protein between position 59 to 76. Spirulina is one of the most consumed microalgae and the first documented case of

spirulina allergy with anaphylaxis was reported in 2010, in which the phycocyanin protein was found to be the causative allergen (Petrus et al., 2010). Since then, four more cases of spirulina-related allergies with unknown aetiology have been reported (Gromek et al., 2024), three of which involved anaphylactic reaction. However, food allergies from consuming nori product remain rare, and the allergy are generally associated with either the amphipods that can reside within them (Motoyama et al., 2007) or due to porphyran, a major sulphated polysaccharide in *Porphyra/Pyropia* seaweeds (Thomas et al., 2019). Overall, considering the low incidence of both spirulina- and nori-based food allergies, consuming NZ *Porphyra/Pyropia* seaweeds is not likely to pose a significant allergenicity risk.

4. Discussion

4.1. Nutritional quality and proteomics insight of NZ *Porphyra/Pyropia* seaweeds

This study reports the proximate composition and proteomics analysis of three NZ *Porphyra/Pyropia* seaweeds, *Py. viridiidentata*, *Py. cinnamomea*, and *P.* GRB complex collected from the South Island of New Zealand. These native NZ *Porphyra/Pyropia* seaweeds have a high protein and carbohydrate content, similar to other commercial Porphyra/*Pyropia* seaweed varieties from East Asia such as *Py. yezoensis* (Cho and Rhee, 2019; Jung et al., 2016), *Py. haitanensis (Hwang et al., 2013)*, and *Py. tenera* (Hwang et al., 2013; Rawiwan et al., 2022). These Asian species have a reported protein content that ranges between 32.16% to 47% and levels of carbohydrate from 48.14% to 57.9%. The findings are also in line with the proximate composition of NZ *Porphyra* spp. reported previously (Smith et al., 2010), while having a higher protein to carbohydrate ratio than the NZ *Py. plicata* (Battershill, 2024). Moreover, when benchmarked against conventional plant-based protein source, the three studied NZ Porphyra*/Pyropia* seaweeds have a protein content that is comparable to pea (20%-25%) (Shen et al., 2022), higher than wheat (10%-15%) (Alomari et al., 2023), but lower than soybean (36%-40%) (Cabanos et al., 2021). The amino acid analysis revealed that the NZ *Porphyra/Pyropia* seaweeds to be a good source of valine, threonine and tryptophan, which is close to those reported in *Py. yezoensis* (Jung et al., 2016) but lower than *Py. tenera* (Rawiwan et al., 2022). The valine and threonine content in NZ *Porphyra/Pyropia* seaweeds are also comparable to pea (Valine: 4.5%; Threonine: 4.2%) (Ciurescu et al., 2018) and soybean (Valine: 4.7%; Threonine: 3.7%) (Kudełka et al., 2021) and higher than wheat (Valine: 3.9%; Threonine: 2.8%) (Jiang et al., 2008). Furthermore, the NZ *Porphyra/Pyropia* seaweeds were found to contain a high amount of dietary fibre (>30%) and have a fat content of <5%, which consists mainly of omega-3 fatty acids (Wheeler et al., 2021). Collectively, these suggest that the *Py. viridiidentata*, *Py. cinnamomea*, and *P.* GRB complex seaweeds have nutritional attributes that are desirable for further development into functional foods.

The proteomics evaluation revealed distinct PCA plot clustering patterns, with the NZ *Porphyra/Pyropia* seaweeds species being separated from *Py. seriata*. This suggests that *Porphyra/Pyropia* species from northern and southern hemispheres may have differing protein composition and also highlights the proteome diversity in the three NZ *Porphyra/Pyropia* seaweed species. In particular, the *P.* GRB complex has a different phycobiliprotein composition compared to the other samples, having more

phycocyanin, which may in part account for its distinct colouration. Colour variation in the *Porphyra/Pyropia* seaweeds can be attributed to the specific makeup of their phycobilisomes (Adir et al., 2020; Dominguez-Martin et al., 2022), which are specialised light-harvesting pigmented protein complexes, found adhered to the thylakoid membrane of the chloroplasts. The phycobilisome contains several types of phycobiliproteins, which are responsible not only for capturing light energy for photosynthesis but also for the distinctive hue of these species.

Phycobiliproteins from red algae, which include the pinkish-red phycoerythrin, the blue phycocyanin, and the cyan allophycocyanin, have been extensively studied and have been shown to contain specific amino acid sequences which, when enzymatically cleaved, release peptides with health promoting effects (Dagnino-Leone et al., 2022; Mysliwa-Kurdziel and Solymosi, 2017). In our study, the simulated GI digestion analysis indicated that phycobiliproteins from the NZ *Porphyra/Pyropia* seaweed samples could potentially release bioactive peptides with anti-hypertensive (ACE inhibition) and blood glucose lowering (DPP-IV inhibition) effects upon digestion. ACE inhibitory properties from phycobiliprotein-derived peptides have been demonstrated in other red algae species in previous studies. It was demonstrated that thermolysin-derived *Gracilariopsis chorda* hydrolysate exhibited higher angiotensin-converting enzyme (ACE) inhibitory activity compared to its non-hydrolysed protein counterpart, as confirmed through an in-vitro ACE inhibitory assay (Mune Mune et al., 2023). Moreover, their study identified two key peptide sequences, IDHY from the phycoerythrin α-subunit and LVVER from phycoerythrin β-subunit, that had strong interaction with the human ACE, based on *in silico* molecular docking analysis. Similarly, the sequential digestion of R-phycoerythrin from *Bangia fusco-purpurea* with pepsin and trypsin generated ACE inhibitory peptides (Wu et al., 2017). Notably, the synthesized versions of these peptides retained their ACE inhibitory activity even after exposure to pancreatin digestion, suggesting their stability and potential efficacy in physiological condition.

4.2 Commercial potential of NZ *Porphyra/Pyropia* seaweeds as functional food

Commercialization of NZ *Porphyra/Pyropia* seaweeds as high value functional food products must address nutritional efficacy, sensory appeal and regulatory approval, in order to enhance consumer acceptance and market penetration (Rombach and Dean, 2024). While our present finding suggests that the native NZ *Porphyra/Pyropia* seaweed samples have potential usage as a biomass for functional food manufacturing, clinical outcomes from consumption of other *Porphyra/Pyropia* seaweed species have shown mixed results. Previous intervention studies indicated that *Porphyra/Pyropia* seaweeds exert an anti-hypertensive effect only in certain demographics. In a 10-week randomized, open-label, parallel-group study, the consumption of 1.76gm of nori (*Pyropia* spp.) daily significantly reduces the diastolic blood pressure but not systolic blood pressure in young healthy Japanese preschool boys (Wada et al., 2021). However, nori consumption did not improve both the diastolic and systolic blood pressure in the female participants. While another clinical intervention study showed that *P. yezoensis*-derived peptides reduce the blood pressure of hypertensive patients that followed a 35-days regimen involving the daily

intake of 1.8 gm of *P. yezoensis* peptides and this effect was not observed in normotensive participants (Saito et al., 2002). On the other hand, in an acute, randomised, controlled, crossover trial, the consumption of Korean nori (*Py. seriata*) biomass or protein extract did not significantly affect glucose, insulin, ghrelin, and glucagon-like peptide-1 (GLP-1) levels in healthy Chinese male participants when compared to a soy protein-based meal over a 3-hour period post prandial sampling (Wu et al., 2024). To the best of our knowledge, clinical studies evaluating the health benefit of NZ *Porphyra/Pyropia* seaweeds are lacking; hence, future clinical assessments of health outcomes associated with their consumption are needed to guide their application as functional food ingredient.

One key consideration for any clinical evaluation of the effects of consuming *Porphyra/Pyropia* seaweeds is the form in which these red algae are being consumed. Whether as whole seaweed, extracts, or isolated bioactive molecules, the nutrient profile, absorption, and bioactivity can vary significantly. In particular, the complex cell wall structures of red algae can reduce the bioavailability of these beneficial compounds, including proteins and peptides (Cebrián-Lloret et al., 2024). To overcome this, further research is required to optimise extraction and processing strategies to improve protein bioavailability, along with identifying strategies to effectively enrich protein content and reduce anti nutrient factors, such as phenolic compounds, and the high fibre content to improve protein digestibility. Food processing technologies such as extrusion (Cebrián-Lloret et al., 2024), fermentation (Luo et al., 2025), and heat treatment (Maehre et al., 2015) can help break down the complex cell wall structures of red algae, thereby improving protein release and facilitating digestion of protein into simpler peptides and amino acids for improved bioavailability. Additionally, fractionation techniques such as ultra/micro filtration (Manzanilla-Valdez et al., 2024) or electrostatic separation (Wang et al., 2024) can be explored to selectively concentrate the beneficial protein fraction while reducing fibre content and anti-nutrient factors that limits nutrient absorption.

Functional foods, while primarily valued for their health-promoting properties, also need to possess a sensory profile that appeals to consumers in order to achieve commercial success. In this regard, the present study found that the NZ *Porphyra/Pyropia* seaweeds are rich in umami amino acids, including alanine, asparagine, aspartic acid, glycine, glutamine, and glutamic acid that may provide a sweet and umami taste profile (Diepeveen et al., 2022). Moreover, previous studies have indicated a favourable palatability of other *Porphyra/Pyropia* seaweeds that may enable higher consumer acceptance. A previous clinical study had indicated that the visual appeal, smell, and taste tolerance of *Py. seriata* biomass and protein concentrate were comparable to each other and to soy protein, the most widely used plant-based protein on the market (Wu et al., 2024). Additionally, the *Py. seriata* biomass also scored significantly higher for aftertaste preference by the participants. In a separate stud*y,* 12 commonly consumed edible algae was analysed, and *Porphyra/Pyropia* species were found to have the highest equivalent umami concentration values while containing lower level of fishy smelling aldehydes compounds, such as (E)-2-octenal and nonanal (Chua et al., 2024). Taken together,

these studies, along with our *in-silico* taste prediction analysis (Figure S1) suggest that the NZ *Porphyra/Pyropia* seaweeds may possess a sensory profile favourable for consumer acceptance.

Currently, no dedicated Codex Alimentarius guidelines exist for seaweed food safety. Even though the Codex Alimentarius has established a regional standard for lavar (*Pyropia*) product (CXS 323R-2017) (CAO, 2017), this standard does not include seaweed-specific limits for chemical hazards or comprehensive food safety risk management. As such, various national and regional agencies have developed regulatory framework to govern seaweed food safety, but international harmonization of these regulations have yet to be achieved (Bomfeh et al., 2022). Nonetheless, these existing frameworks can be leveraged to support the commercial development of NZ *Porphyra/Pyropia* seaweeds. For example, seaweeds are known to accumulate chemical hazards such as arsenic, cadmium, lead and iodine. These hazards may be accumulated at different part of the farmed seaweed value chain, such as in the hatchery and cultivation stage (Banach et al., 2020; Bomfeh et al., 2022). In contrast, post harvesting processing steps, such as cleaning and washing, can reduce the arsenic and iodine, thereby providing defined points for food safety evaluation and risk management. Similarly, the New Zealand Food Safety authority published a technical assessment evaluating the food safety risk associated with seaweed and seaweed products (Cressey et al., 2023). A key finding of this assessment highlights the impact of food processing methodology on mitigating both chemical and microbiological hazards. While most processing methods reduce overall food safety risk, the report highlighted that thermal processing such as boiling, can convert organic arsenic into harmful inorganic arsenic in shellfish. Accordingly, these guidelines underscores the need for appropriate food safety evaluation during processing of NZ *Porphyra/Pyropia* seaweeds.

4.3 Limitation of study

The present study has several limitations that constrain a comprehensive evaluation of the three studied NZ *Porphyra/Pyropia* seaweeds as a source for functional food development. First, the *Porphyra/Pyropia* species lacks a well annotated protein database that result in limited proteome coverage, and this can limit our understanding on their biology and characterization of novel bioactive protein. Future work focusing on comprehensive annotation of the NZ *Porphyra/Pyropia* proteome will enable a deeper understanding of their biology. Such efforts will provide insight into studying seaweeds metabolic regulation and identifying key growth-promoting drivers. These drivers can then be targeted to optimize propagation conditions for aquaculture, to support sustainable production of NZ *Porphyra/Pyropia* seaweed as a future food source [56]. Next, this study focused mainly on the phycobiliprotein composition due to their potential bioactivity and commercial value (Dagnino-Leone et al., 2022; Mysliwa-Kurdziel and Solymosi, 2017). Nonetheless, the *Porphyra/Pyropia* species also contain other beneficial components such as sulphated polysaccharides (porphyran), mycosporine-like amino acids, vitamin B12 and eicosapentaenoic acid (EPA), all of which have health promoting effects (Cho and Rhee, 2019). Defining their composition in the NZ *Porphyra/Pyropia* seaweeds will provide a more holistic

understanding of their nutritional profile and, more importantly, guide protein extraction processes towards tailoring of optimal compositional profiles for further functional characterization to determine their potential food application.

5. Conclusion

In conclusion, this study provides a systematic characterisation of the nutritional composition of NZ *Porphyra/Pyropia* seaweeds. These seaweeds have a high protein and carbohydrate content while remaining low in fat and free sugars on a dry weight basis. Proteomics analyses revealed differences in the phycobiliprotein composition among the three NZ *Porphyra/Pyropia* seaweeds. Subsequent *in silico* analyses of the phycobiliprotein sequences suggested the potential release of ACE and DPP-IV inhibitory peptides after gastrointestinal digestion. Allergenicity prediction further indicated a low potential risk of food allergenicity associated with these NZ *Porphyra/Pyropia* seaweeds. Overall, the data presented here serve as a foundation for future validation studies, which can focus on the characterisation of non-protein functional components and assessment of the protein digestibility and peptide bioactivity. Such studies will support the optimization of extraction and processing strategies to enhance the bio-accessibility of proteins and other bioactive constituents in NZ *Porphyra/Pyropia* seaweeds for functional food applications, as well as the validation of health claims through clinical studies.

Supplementary Materials

Refer to Attachment

Author Contributions

Tan Chee Fan: Writing – original draft**,** Writing – review & editing, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Kuin Tian Pang:** Writing – original draft, Methodology, Investigation, Formal analysis. **Alok Tanala Patra:** Writing – original draft**,** Visualization, Data curation. **Felicia Ong:** Methodology, Investigation. **Rita Lee:** Methodology, Formal analysis, Investigation. **Ian Walsh:** Investigation, Data curation. **Siew Young Quek:** Writing – review & editing, Investigation **Tom Wheeler:** Writing – original draft, Writing – review & editing, Investigation, Data curation, Methodology, Conceptualization. **Bi Xuezhi:** Writing – review & editing, Supervision, Methodology, Conceptualization.

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Data Availability Statement

The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD069102 (Vizcaíno et al., 2014).

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Conflicts of Interest

The authors declare a conflict of interest in the relationship with the Future Food journal as one of the co-authors, Siew Young Quek, is one of the editor-in-chief of the journal. Given their role as editor-in-chief, Siew Young Quek had no involvement in the peer-review of this article and has no access to information regarding its peer-review. Full responsibility for the editorial process for this article was delegated to another journal editor.

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