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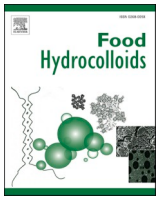

Transforming *Phaeodactylum tricornutum* by-product biomass: from industrial residue to high-value protein extracts

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ABSTRACT

Due to their rapid growth rate and high-value metabolites, microalgae are a good source for extracting products of biological interest, such as polyunsaturated fatty acids, pigments, polysaccharides or proteins. Industrially, the microalgae *Phaeodactylum tricornutum* is often produced to extract dietary essential omega-3 fatty acids, such as eicosapentanoic acid (EPA) or docosahexenoic acid (DHA) by using supercritical CO_2 as extraction solvent. This extraction produces a spent biomass by-product which can constitute more than 90 % of the initial algae biomass and contains many other interesting compounds that can be extracted. The by-product residual biomass of *P. tricornutum* was evaluated for its potential as a protein source. Initial compositional analysis revealed a high protein content. Protein-rich concentrates were extracted via a pH-shift method coupled with ultrasound treatment. The process involved alkaline solubilisation, ultrasonic disruption, and acid precipitation to isolate proteins, which were then purified via dialysis, achieving an extraction yield and protein content up to 26 % and 65.9 % respectively. Functional assessment revealed excellent foaming, emulsifying, and water and oil retention properties. This work underscores the potential of marine biomass residues as sustainable sources of high-quality proteins for food, feed, and cosmetic applications, advancing the circular bioeconomy.

1. Introduction

Due to their rapid growth and ability to store metabolites of high biological interest, microalgae are an excellent source for the extraction of a wide range of compounds which can be influenced by differences in the cultivation conditions to which they are subjected (Belachqer-El Attar et al., 2023).

Phaeodactylum tricornutum is a diatom, industrially cultured for its accumulation of polyunsaturated fatty acids (PUFAs), especially high-value eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Cui et al., 2021a). It also contains high concentrations of fucoxanthin: a carotenoid pigment, which presents high antioxidant activity (Fung et al., 2013). In the industry, *P. tricornutum* is used to extract its lipids for different applications, including biodiesel production (Mubarak et al., 2015), or as pharmaceutical and nutritional supplements (Barkia et al., 2019). Lipids can be extracted from microalgae biomass using different extractions techniques. Organic solvents have been commonly used for this purpose. However, new strategies combining supercritical or deep eutectic solvents with different cell disrupting methods are already performed at industrial scale to increase lipid extraction yields in a more efficient manner (Kumar et al., 2017). After lipid extraction, a substantial amount of biomass is managed as by-product with a largely unexploited number of bioactive compounds remaining in this biomass.

One of those interesting compounds left in the residual biomass are proteins. It is well-known that microalgae are a good source of protein, accumulating from 30 % to 55 % in dry weight (López et al., 2010). In this context, microalgae, owing to their rich protein content, emerge as a promising alternative to animal-derived proteins in vegan diets. These proteins exhibit a significant abundance of essential amino acids, complementary to plant vegan sources, which foster their valorisation as nutritional ingredients in both food and feed applications (Kusmayadi et al., 2021). Furthermore, a consensus among diverse studies is that proteins obtained from microalgae possess favourable technological properties, rendering them suitable for incorporation into food formulations as ingredients (Chen et al., 2019, Lupatini Menegotto et al., 2019).

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In order to recover protein from the biomass, an alkaline treatment followed by physical cell wall disruption is usually needed. Different cell disruption methods have been employed for protein extraction, including bead milling, ultrasound, microwave treatment, enzymatic digestion and high pressure, among others (Safi et al., 2014). Ultrasound is an efficient and environmentally-friendly technique that is widely used to extract proteins from marine biomass (Zheng et al., 2024), as it is a non-invasive technique that uses sound shock waves to create cavities in the biomass, facilitating the solvent entry and extraction of target compounds (Soria & Villamiel, 2010). Once the cell wall has been disrupted and the protein dissolved, an acidic pH change approaching the isoelectric point of the protein (Ursu et al., 2014) is necessary in order to precipitate the proteins and purify this fraction of interest.

Therefore, the main objective of this work was to obtain protein-rich concentrates from a *P. tricornutum* by-product after the industrial lipid extraction carried out by means of supercritical CO_2. To achieve this, a simple pH-shifting process assisted by an ultrasound treatment has been proposed. The extracts were characterized in terms of protein yield, composition, antioxidant and technological properties to evaluate their possible uses in the food industry.

2. Methods and materials

2.1. Raw material

P.tricornutum biomass (PTB) and its residual biomass generated after industrial lipid extraction using supercritical CO_2 (PTRB) were kindly supplied by IGV Planttech (Nuthetal, Germany). *P.tricornutum* (CCAP 1052/1B) was grown photoautotrophically into 30 L tubular bioreactors under controlled conditions. The biomass was collected by disc separation and then subjected to a lipid extraction using supercritical CO_2 as extracting solvent. PTRB and PTB were freeze-dried to remove all the water content and stored under dry conditions at room temperature.

2.2. Production of protein extracts

2.2.1. Treated samples

Prior to applying the protein extraction protocol, remaining lipids and fucoxanthin were removed from the *P.tricornutum* by-product residual biomass (PTRB) following the procedure previously described by Sun et al. (2022) with slight modifications. Briefly, 1 g of freeze-dried biomass was mixed with 40 mL of a 70 % ethanol:water (v/v) solution. This mixture was shaken in the dark for 2 h at room temperature, centrifuged at 33.700×*g* for 30 min and the treated pellet was dried in an oven at 50 °C for 48 h. The residual biomass resulting from the aforementioned procedure was denoted *Phaeodactylum tricornutum* treated by-product biomass (PTRTB).

2.2.2. Protein extraction from P. tricornutum

For comparative purposes, protein extraction was performed in the PTRTB and the PTRB (as supplied). Protein extraction was performed using a pH-shifting extraction protocol assisted by ultrasounds, following the procedure proposed by Gerde et al. (2013) with some modifications. First, the optimum pH for protein solubilisation was determined within a range of pH 9 to 12 by suspending 1g of each biomass in 30 mL of deionised water and adjusting the pH of the suspension using 1M NaOH solution. The suspension was subsequently centrifuged at 33,700×*g* for 30 min and the supernatant was then transferred to a pre-weighted tube and freeze-dried. The solubilisation yield and nitrogen content of the dry solubilized fraction were evaluated.

After identifying the optimum pH for protein solubilisation, the optimum pH for protein precipitation was determined in the range of pH 2 to 5. To this end, the proteins were solubilized using the previously described methodology in the optimum conditions and, the supernatant, containing the solubilized proteins, was adjusted to the selected acidic pH, using 1M HCl solution to precipitate the proteins present in the sample, followed by overnight incubation at −20 °C. Finally, after thawing, the sample was centrifuged at 3,700×*g* for 20 min to separate the supernatant from the solid pellet, which constituted the protein-rich concentrate.

To enhance the extraction yield and the protein content in the protein-rich concentrates, an ultrasonic treatment (US) was also performed after the alkaline solubilisation using an ultrasonic probe (UP400S, Hielscher), with a maximum power of 400W and 100 % amplitude (24 kHz). Two different times (2.5 and 5 min) were applied. To prevent overheating, the US treatment was performed in an ice bath. Finally, the precipitated protein extracts were dialyzed for 5 day at 4 °C using a molecular weight cut-off of 100–500 Da (SpectrumLabs).

2.3. Compositional characterization of the raw biomasses and the protein extracts

2.3.1. Protein content

The protein content of the initial biomass and the obtained concentrates were calculated, in triplicate, by measuring the nitrogen content through the Dumas method (Wiles et al., 1998), using an Elemental Analyzer Rapid N Exceed (Paralab S.L., Spain). The nitrogen content was multiplied by a nitrogen-to-protein conversion factor of 5.08, as previously reported for *P.tricornutum* biomass (Templeton & Laurens, 2015), and of 6.25 for the obtained protein concentrates.

2.3.2. Total amino acid content of the protein-rich concentrates

The amino acid profiles of the samples were determined using microwave-assisted acid hydrolysis, adapted from the method of Moore and Stein (1963). Amino acid quantification was conducted via ion exchange chromatography with post-column ninhydrin derivatization for detection. Prior to hydrolysis, proteins were oxidized with performic acid containing phenol (a 9:1 mixture of formic acid and 30 % hydrogen peroxide with phenol), converting cysteine to cysteic acid and methionine to methionine sulfone. The samples were then hydrolysed in 6 M HCl at 160 °C for 15 min using a Discover 2.0 microwave synthesizer (CEM, North Carolina, USA). After hydrolysis, the samples were cooled on ice and adjusted to pH 2.2 using 7.5 N NaOH, then diluted to 20 mL with 0.2 M sodium citrate buffer (pH 2.2). A 1:2 dilution of the samples was prepared with the internal standard L-norleucine, yielding a final norleucine concentration of 125 nmol/mL. Amino acids were quantified using a Jeol JLC-500/V amino acid analyser (Jeol (UK) Ltd., Garden City, Herts, UK) equipped with a Jeol Na + high-performance cation exchange column.

2.3.3. Carbohydrate composition

Total carbohydrate profiles of all the samples were determined, in triplicate, by acid methanolysis using the method described by Martínez-Abad et al., (2018). Briefly, all the samples were hydrolysed using methanol (Panreac, Barcelona) acidified with acetyl chloride (Sigma, Germany), generating a concentration of 2M HCl in the anhydrous methanol (Panreac, Barcelona). Samples were heated at 100 °C for 5 h and then neutralized with pyridine. The samples were then treated with 2M trifluoracetic acid (Sigma, Germany) to remove methoxyl groups in the reducing end. All the samples were analysed using high performance anion exchange chromatography coupled to a pulsed amperometric detector (HPAEC-PAD) with a ICS-6000 system. The column used in these analyses was a CarboPac PA1 (4 × 250 mm). Elution conditions for uronic and neutral monosaccharides are described by Cebrián-Lloret et al., (2022). The neutral (fucose, arabinose, galactose, glucose and xylose) and uronic monosaccharide standards (mannuronic acid) were purchased from Sigma (Germany) and Carbosynth (UK).

2.3.4. Lipid content

The total lipid content was estimated using the Folch method (Löfgren et al., 2016), with some modifications. Briefly, lipids were

extracted with a mixture of methanol and dichloromethane in a volume ratio 1:2. Samples were weighed before and after the extraction, and the total lipid content was expressed as weight percentage. All experiments were performed in triplicate.

2.3.5. Ash content

The ash content (inorganic content) was determined, in triplicate, according the TAPPI T211 om-O7 standard method. Approximately 0.5 g of dried samples was added to a pre-weighed ceramic crucible and weighed. Calcination took place at 525 °C for 24 h in a muffle furnace and the ash content was gravimetrically calculated.

2.3.6. Total phenolic compounds (TPC)

The total phenolic content of the samples was determined in triplicate according to Singleton et al. (1999). Firstly, 5 mg/mL aqueous solutions of each biomass and the obtained extracts were prepared. 200 μL of each solution was mixed with 1000 μL of Folin-Ciocalteau reagent diluted 1:10 with water. After 5 min of incubation at room temperature, 800 μL of a 75 g/L sodium carbonate solution was added. The solution was incubated at 40 °C for 30 min. Then, the absorbance was measured at 760 nm. Following the same protocol, a standard curve with gallic acid was prepared in a concentration range of 0–70 μg/mL. The results of the samples were expressed as equivalents of gallic acid based on this calibration curve.

2.4. Antioxidant activity

The antioxidant activity of the raw materials and the obtained protein extracts was measured in triplicate according to Re et al. (1999). First, 0.192 g of 2, 2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) was diluted in 50 mL of phosphate buffered saline (PBS) 0.1M, then 0.033 g of potassium persulfate was added. The resulting solution was stirred overnight in the dark to allow the formation of the ABTS* radical. The absorbance of the solution was adjusted to 0.700 ± 0.02 by diluting the solution with 0.1M PBS. A 5 mg/mL solution of the sample was prepared. As a standard, 6-hydroxy-2, 5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) was used in a range from 0.02 to 0. 3 μmol/mL. The antioxidant activity was measured mixing 20 μL of the sample or standard solution with 230 μL of absorbance corrected ABTS* solution. 250 μL of PBS 0.1M was used as blank and 250 μL of ABTS* solution was used as control. The antioxidant activity of the sample was expressed as mg of Trolox equivalents (TEAC)/g extract.

2.5. Molecular weight of the protein-rich concentrates

The molecular weight distribution of protein-rich concentrates were measured by HPSEC, following the method described by Méndez et al. (2021), using a Waters ACQ Arc Sys core 1–30 cm CH (Waters,USA). The HPLC system was equipped with a Waters 2998 PAD module, a Waters 2414 refractive index detector (Waters, USA) and 2475 FLT module (Waters, USA). The columns used in the analysis were in series (PolySep-GEC-P 4000 and PolySep-GEC-P 2000; 300 mm × 7.8 mm; Phenomenex Inc, CA, USA). The mobile phase was a 0.01 M NaOH solution adjusted to pH 12. 3 mg of the samples were dissolved using 1 mL of the mobile phase at room temperature and agitated using a magnetic stirrer. The samples were filtered through 0.45 μm pore syringe filters and 10 μL of sample were injected into the system. The flow rate was adjusted to 0.5 mL/min. The calibration was made using pullulan standards (polymer standards service GmbH, Mainz, Germany).

2.6. Evaluation of technological and functional properties of the protein-rich concentrates

2.6.1. Water and oil-holding capacity of the protein-rich concentrates (WHC and OHC)

The water and oil-holding capacity were determined, in triplicate, according to the method described by Bencini (1986) with slight modifications. Briefly, 0.5 g of each protein-rich concentrate was mixed with 10 mL of distilled water or sunflower oil, for measuring WHC or OHC, respectively. The mixtures were shaken in an orbital shaker, LBX Orb-Pro (LBX instruments, Spain) for 5 min and then centrifuged at 4, 540×*g* or 30 min and the supernatant was separated from the pellet. Wet pellets were weighted and WHC and OHC were expressed as g water or oil/g protein (gram of water retained per gram of protein).

2.6.2. Foaming capacity and foam stability

Foam capacity (FC) and foam stability (FS) were determined using the method proposed by Nath and Narasinga Rao (1981), with some modifications. A suspension of the protein-rich concentrates was prepared by mixing 0.2 g of protein in 10 mL of deionised water. The pH of the suspensions was adjusted at 4, 7 and 10 using 0.1 M NaOH and 0.1 M HCl. For foam capacity, the suspensions were agitated with a homogeniser x1000D (CAT) at 10000 rpm for 2 min. After homogenisation, the increase in volume was measured and was expressed as foaming capacity (%).

$$FC = \frac{V_f - V_0}{V_0} \cdot 100 \quad (1)$$

Where V_f is the final volume of the solution with the foam after 2 min of homogenisation and V_0 is the initial volume of the suspension before homogenisation.

To determine foam stability, the volume loss of the suspension after homogenisation was measured at 30, 60, 90 and 120 min. Foam stability was expressed as (%).

$$FC = \frac{V_r}{V_f} \cdot 100 \quad (2)$$

Where V_r is the volume of the solution with the foam after resting and V_f is the volume of the foam and the solution after homogenisation.

2.6.3. Emulsifying activity and emulsion stability

Emulsifying activity (EA) and emulsion stability were determined according to the method proposed by Naczk et al. (1986) with slight modifications. Protein concentrates were suspended in distilled water at a concentration of 1 % (w/v). The pH of the solutions was adjusted to 4, 7 and 10 using 0.1 M NaOH and 0.1M HCl. Sunflower oil was added to produce an emulsion in an oil/water ratio of 3:2. The total height of the mixture was measured. The mixture was homogenised for 1 min at 9000 rpm using an *homogenizer Unidrive X 1000D (*CAT Ingenieurbüro, Germany).After homogenisation, the emulsion was left and after 30 min of resting the height of the emulsion layer was measured. The emulsifying activity was calculated using equation (3).

$$EA = \frac{V_e}{V_t} \cdot 100 \quad (3)$$

Where V_e is the volume of the emulsion layer after homogenisation and V_0 is the total volume of the solution before homogenisation.

To determine emulsion stability (ES), the height loss in the emulsion layer was measured at 0, 30, 60, 90 and 120 min after homogenisation. Emulsion stability was calculated using equation (4).

$$ES = \frac{V_r}{V_e} \cdot 100 \quad (4)$$

Where V_e is the volume of the emulsion layer after homogenisation and

V_r is the volume of the emulsion layer after resting.

2.7. Confocal laser scanning microscopy of the emulsions

Emulsions were prepared with treated and non-treated protein-rich extracts using the method described by Tang and Ghosh (2021). Briefly, the oil phase was stained by adding 50 μL of 0.1 % (w/v) Nile red (Sigma, Germany) solution in acetone, whereas 0.1 % (w/v) Fast Green FCF (Sigma, Germany), acidified with a 1 % of acetic acid, was added to the final emulsion samples. Fluorescence images were obtained with a ZEISS LCS 980 inverted microscope (Carl Zeiss Microscopy GmbH, Jena, Germany), a fully motorised confocal laser scanning system. The dry objective lens employed was a Plan-Neofluar 10x (Carl Zeiss Microscopy GmbH, 420340-9901-000, Jena, Germany). Two different tracks were utilised. For the Nile red fluorescence experiment, a pinhole size of 40 μm (1.00 Airy Units) was employed, and the laser wavelength was set to 488 nm, with a power output of 0.20 %. The detection wavelength was selected to be 495–620 nm, and a detector gain of 620V was employed, using a GaAsP-PMT detector. For the Fast Green fluorescence experiment, the pinhole size was set to 40 μm (0.81 Airy Units), the laser wavelength to 639 nm, and the power to 0.20 %. The detection wavelength was selected to be 643–735 nm, with a detector gain of 650V, once again using a GaAsP-PMT detector. It is also noteworthy that the scanning conditions for both tracks were set to frame mode, operating in a bidirectional manner without the application of averaging. All microscopic images were captured as a Z-stack containing 21 to 25 optical slices with a 4.8 μm z-step. The properties of each individual optical slice are outlined below: 8 bits, frame size of 1024x1024 pixels, image dimensions of field of view 848.53 × 848.53 μm (0.829 μm/pixel).

2.8. Statistical analysis

The data is represented by the average ± standard deviation. Letters indicate the significant differences in both graphs and tables with a confidence level of 95 %. Statgraphics Centurion 18 was used, employing an analysis of variance (ANOVA) and a Tukey test.

3. Results and discussion

3.1. Compositional characterization of Phaeodactylum tricornutum biomass

The composition of the starting *P. tricornutum* raw biomass (PTB), the residual biomass (PTRB) generated after industrial lipid extraction using supercritical CO_2 and the treated one (PTRTB) was determined and the results are summarized in Table 1. Protein, carbohydrates and lipids, representing 36 wt%, 9 wt% and 23 wt% of the total biomass (dry weight), respectively, were the major components of the raw biomass. Similar values have been reported in literature (Branco-Vieira et al., 2018; Cui, Thomas-Hall, Chua, & Schenk, 2021a, 2021b). On the other hand, as expected, the lipid content was significantly decreased in the PTRB and it decreased three-times in PTRTB sample. Some of the most abundant fatty acids presents in the *P. tricornutum* biomass that has been previously reported are hexadecanoic acid, palmitoleic acid or eicosapentaenoic acid (Fajardo et al., 2007; Yang et al., 2017).

The high protein content remaining in the PTRB and PTRTB is of potential interest for cascade valorisation schemes which would increase the eco-sustainability of the currently used process. The total phenolic content (TPC) of the raw biomass was estimated as 0.94 %, being in the range of values previously reported for this biomass (German-Báez et al., 2017) and, interestingly, it did not significantly change, neither after the lipid extraction industrial process, nor after the pretreatment carried out with ethanol:water. Regarding the antioxidant capacity, samples showed lower antioxidant capacity values than those previously reported for *P. tricornutum* and other species (Conde et al., 2021). Additionally, a total ash content of approximately 15 % was estimated in all the samples.

Table 1
Gross composition, polyphenol content and antioxidant activity of the *Phaeodactylum tricornutum* raw biomass (PTB), the original untreated residual biomass (PTRB) and the treated by-product biomass (PTRTB).

	PTB	PTRB	PTRTB
Proteins (%)	36.2 ± 0.2^a	35.6 ± 0.0^a	36.9 ± 0.3^a
Lipids (%)	22.9 ± 1.4^a	10.0 ± 2.0^b	3.2 ± 1.2^c
Ashes (%)	15.1 ± 0.1^a	15.5 ± 1.6^a	15.8 ± 0.2^a
TPC (%)	0.9 ± 0.1^a	0.9 ± 0.1^a	1.1 ± 0.2^a
AC (mgTE/g sample)	29.3 ± 0.1^a	30.0 ± 0.1^a	30.2 ± 0.1^a
Carbohydrates (%)[a]	9.8 ± 0.5^a	13.1 ± 0.8^b	7.9 ± 0.3^a
Fucose (%)	0.19 ± 0.01^a	0.36 ± 0.02^b	0.27 ± 0.01^c
Arabinose (%)	0.65 ± 0.05^a	0.98 ± 0.07^b	0.66 ± 0.06^a
Galactose (%)	2.50 ± 0.30^a	2.71 ± 0.12^a	0.39 ± 0.02^b
Glucose (%)	1.67 ± 0.05^a	2.37 ± 0.18^b	0.21 ± 0.01^c
Xylose (%)	2.90 ± 0.20^a	4.33 ± 0.18^b	3.86 ± 0.15^c
Mannuronic acid (%)	1.60 ± 0.20^a	2.11 ± 0.01^b	2.55 ± 0.08^b

TPC = Total Phenolic Compounds; AC = Antioxidant capacity; TE = Trolox equivalents.
Values with different letters within the same row are significantly different ($p \leq 0.05$).
[a] Total carbohydrates calculated as the adding of all the monosaccharides (fucose, arabinose, galactose, glucose and xylose).

The carbohydrate analysis, revealed significant differences among samples. PTB and PTRB were richer in carbohydrates, the difference being a higher contribution of galactose and glucose, compared to PTRTB. PTRB has a total carbohydrate content of 13.14 %, which was higher than that of the raw biomass. Finally, PTRTB has the lowest carbohydrate content of the biomasses (7.92 %), where the most abundant monosaccharides are mannuronic acid and xylose. Cui et al. (2021a, 2021b), Branco-Vieira et al. (2020) and Niccolai et al. (2019) reported a total carbohydrate content of 9 %, 7.85 % and of 11 % in the *P. tricornutum* biomass respectively, being very similar to the values obtained after the analysis of the different samples in this work.

3.2. Protein extraction

Once the industrial by-product and its treated counterpart sample were characterized, the process to obtain rich-protein extracts was studied and the effect of an ultrasound treatment was also evaluated with the aim of increasing the extraction yield.

Firstly, the protein solubilisation conditions were evaluated in both biomasses and the optimal pH was determined to maximize protein solubilisation. Table 2 compiles the solubilisation yields and protein concentration. As observed, both the extraction yield and the protein content increased at stronger alkaline conditions (pH = 12.0), reaching yields of 51 wt% and 39 wt% for PTRB and PTRTB, respectively. This indicates that other components (i.e. polysaccharides, as it will be detailed below) were also solubilized together with proteins. Phusunti and Cheirsilp (2020) reported same pH value (pH 12.0) as the optimum for the solubilisation of proteins from *Chlorella vulgaris* raw biomass, reaching an extraction yield of 46.6 %.

Interestingly, it was noted that the protein extraction yield was higher in PTRB samples than in their PTRTB counterparts. Probably, the presence in high concentration of polar lipids such as glycolipids or phospholipids in the microalgae biomass can improve the solubilisation yield due to their surfactant activity (Markwell et al., 1978).

Grossmann et al., (2019) also obtained isolated proteins from *P. tricornutum* and studied their solubility at different pH. The concentrates yielded a protein content of 50.3 %, which is comparable to the protein content observed in the present work for PTRTB and they also found pH 12 as the optimal point for protein solubilisation.

After solubilisation, proteins were precipitated at different pH values within the range 2–5, and the extraction yields and protein

Table 2
Extraction yield and protein content of the untreated and treated by-product residual biomass *Phaeodactylum tricornutum* in the solubilisation and precipitation steps.

	Protein solubilisation test			
	PTRB		PTRTB	
pH	Extraction yield (%)	Protein content (%)	Extraction yield (%)	Protein content (%)
9	45.6 ± 0.7^{a}	35.1 ± 0.4^{a}	17.9 ± 0.9^{a}	34.9 ± 0.3^{a}
10	47.1 ± 1.5^{ab}	35.4 ± 0.1^{a}	19.3 ± 1.6^{a}	37.4 ± 0.1^{b}
11	49.9 ± 0.8^{bc}	36.4 ± 0.1^{b}	21.1 ± 0,1^{a}	42.9 ± 0.7^{c}
12	51.51 ± 0.13^{c}	39.1 ± 0.3^{c}	31.9 ± 0.3^{b}	51.3 ± 0.1^{d}
	Protein precipitation test			
	PTRB		PTRTB	
pH	Extraction yield (%)	Protein content (%)	Extraction yield (%)	Protein content (%)
5	1.8 ± 1.1^{a}	58.2 ± 0,1^{a}	4.7 ± 1.0^{a}	66.6 ± 0.1^{a}
4	11.1 ± 0.5^{b}	63.8 ± 0.2^{c}	9.2 ± 0.2^{b}	72.8 ± 0.5^{b}
3	14.1 ± 0.5^{bc}	63.8 ± 0.1^{c}	10.7 ± 0.1^{c}	70.6 ± 1.4^{b}
2	15.3 ± 0.1^{c}	59.1 ± 0.5^{b}	12.6 ± 0.1^{d}	65.2 ± 1.0^{a}

Values with different letters within the same columns are significantly different ($p \leq 0.05$). PTRB= *P. tricornutum* by-product residual biomass. PTRTP= *P. tricornutum* residual treated by-product biomass.

concentration are also summarized in Table 2. As shown, more acidic conditions promoted the precipitation of a greater amount of a protein-rich concentrate while the protein purity was not affected to a great extent, the highest being at pH 4. Therefore, at pH 2, for PTRB and PTRTB, protein recovery was the highest.

Cavonius et al. (2015) reported for *Nannochloropsis oculata* a protein extraction using an alkali treatment at pH 10, followed by an acid precipitation at pH 3. Those conditions were milder than the ones used in this work, but the maximum protein content obtained was 24 %, which differs from the 65.2 ± 0.1 % obtained with the PTRTB. Grossmann et al. (2018) reported that the minimum solubility of the proteins from *Chlorella protothecoides* was at pH 3.0, which also differs from the ones obtained for the PTRB. This suggests that this species of microalgae requires harsher conditions to extract proteins than other species of microalgae, but provides a very rich protein concentrate extract.

Thus, pH 12.0 and pH 2.0 were selected as the optimum pH for protein solubilisation and precipitation conditions, respectively. However, the extraction yield and protein content (−12.6 and 65.2 %, respectively) were still quite low, indicating that protein was still remaining in the residue obtained after the solubilisation step.

3.3. Ultrasound treatment

In order to increase the protein extraction yield, an ultrasound treatment was applied on the non-treated and the original (untreated) by-product biomass to disrupt or permeabilize the cell walls during the solubilisation step (pH 12.0). Two different times 2.5 and 5 min were tested for the US treatment, followed by acidic precipitation at pH 2. The extraction yields, as well as the protein content in the protein-rich concentrates were determined. Table 3 shows the results obtained after the ultrasound treatment coupled to the previous optimized conditions.

The results, shown in Table 3, evidenced that the extraction yield increased with the application of ultrasound and the values obtained after 2.5 and 5 min were similar for both PTRB and PTRTB. For PTRB, the extraction yield increased from 15.3 ± 3 % to 27 ± 3 % after 2.5 min of ultrasonication. For PTRTB, the extraction yield increased from 12.1 ± 1.4 % to 25.0 ± 0.7 %. There were no significant differences in extraction yield between PTRB and PTRTB concentrates after 2.5 and 5 min of ultrasound treatment. However, the protein content in the PTRB protein concentrates is lower than that in the treated ones. The results

Table 3
Extraction yield and protein content of the protein-rich extracts from treated and non-treated *Phaeodactylum tricornutum* industrial by-product residual biomass.

	PTRB		PTRTB	
US time	Extraction yield (%)	Protein content (%)	Extraction yield (%)	Protein content (%)
0 min	15.3 ± 0.1^{a}	59.1 ± 0.5^{a}	12.6 ± 0.1^{a}	65.2 ± 1.0^{a}
2.5 min	27.0 ± 3.0^{b}	65.2 ± 0.1^{b}	25.0 ± 0.7^{b}	67.2 ± 0.7^{a}
5 min	26.0 ± 3.0^{b}	64.4 ± 0.3^{b}	24.9 ± 0.1^{b}	72.1 ± 0.1^{b}

Values with different letters within the same columnsare significantly different ($p \leq 0.05$). PTRB= *P. tricornutum* by-product residual biomass. PTRTP= *P. tricornutum* residual treated by-product biomass.

demonstrate that the application of 2.5 min of ultrasound results in doubling the protein extraction yield. Given the similarity in extraction yields at 2.5 and 5 min and the minor differences in protein content between these durations, a treatment time of 2.5 min was selected as an optimal compromise between sample processing time, extraction efficiency and protein content.

Different conditions have been reported in the literature for protein extraction from *P. tricornutum*. For instance, Al Khawli et al., (2021) applied an alkaline treatment at pH 8.5 combined with 30 min of ultrasound, producing a protein precipitate containing 6.10 % protein. Conversely, Cui et al. (2021a, 2021b) achieved a protein extract with 31.2 % protein by applying the ultrasound treatment for only 3 min. Interestingly, in this work shorter ultrasound extraction times were applied under alkaline conditions, promoting much higher protein extraction yields which ranged between 65.2 % and 67.2 %. The difference between the work of these other authors and this work is that they used raw biomass of *P. tricornutum* as starting material, whereas this work used the by-product that had been pre-treated with supercritical CO_2. This improvement can be attributed to the combination of the pre-treatment of the sample, which is likely to have increased cell wall disruption, facilitating the release of intracellular compounds and the harsher solubilisation conditions (pH 12.0), improving overall efficiency.

Therefore, protein concentrates can be obtained from both PTRB and PTRTB *P. tricornutum* residual biomass through an ultrasound treatment under alkali conditions, followed by a simple pH-shifting process.

3.4. Compositional characterization of the obtained protein-rich concentrates

The composition of the protein-rich concentrates from PTRB and PTRTB residual biomasses obtained with the US (2.5 min) treatment and optimum solubilisation pH (12.0) and precipitation at pH 2.0 was studied and the results are summarized in Table 4. As observed, protein was the main component in both concentrates, having ca. 65–66 %. It should be noted that the protein content was significantly higher than in other *P. tricornutum* concentrates reported in the literature (Ebert et al., 2019) in which protein and carbohydrates were the major components (37 and 35 % for protein and carbohydrate content, respectively).

Furthermore, the lipid fraction was similar to that reported for the PTRB and PTRTB, the PTRB concentrates showing the highest content.

The carbohydrate content of the concentrates was around 6–9 %, suggesting that some of them co-precipitate with the protein probably as glycoproteins (Waghmare et al., 2016). Differences in the carbohydrate composition of the two concentrates are evident. The total carbohydrate content in the PC-PTRB is 6.7 %, whereas it is 8.7 % in PC-PTRTB. In PC-PTRB, the predominant monosaccharides were xylose, galactose, and mannuronic acid, while in the PC-PTRTB, xylose, glucose, and arabinose were most abundant. Compared with other microalgae protein concentrates, for the soluble concentrate protein from *Tetraselmis* spp., Schwenzfeier et al. (2014) reported a total carbohydrate content of 7.3

Table 4
Gross composition of protein-rich concentrates from *Phaeodactylum tricornutum* original by-product (PC-PTRB) and treated (PC-PTRTB) by-product residual biomass.

	PC-PTRB	PC-PTRTB
Proteins (%)	64.4 ± 0.3[a]	65.9 ± 0.5[b]
Lipids (%)	10.0 ± 1.5[a]	3.1 ± 0.1[b]
Ashes (%)	2.4 ± 0.9[a]	3.6 ± 0.1[b]
Carbohydrates (%)[a]	6.7 ± 0.2[a]	8.7 ± 0.8[b]
Fucose (%)	0.54 ± 0.01[a]	0.76 ± 0.06[b]
Arabinose (%)	–	1.60 ± 0.40
Galactose (%)	1.19 ± 0.02[a]	1.07 ± 0.06[b]
Glucose (%)	1.09 ± 0.07[a]	1.22 ± 0.11[a]
Xylose (%)	1.97 ± 0.02[a]	2.70 ± 0.20[b]
Mannuronic acid (%)	0.89 ± 0.09[a]	1.23 ± 0.01[b]
TPC	2.2 ± 0.1[a]	1.4 ± 0.2[b]
AC (mg TE/g simple)	135.7 ± 0.1[a]	35.9 ± 0.1[b]

TPC = Total Phenolic Compounds; AC = Antioxidant capacity; TE = Trolox equivalents.
Values with different letters within the same row are significantly different ($p \leq 0.05$).
[a] Total carbohydrates calculated as the adding of all the monosaccharides (fucose, arabinose, galactose, glucose and xylose).

%, with galactose and glucuronic acid being the most abundant monosaccharides.

Concerning the ash content in the obtained protein-rich concentrates, the values were 5-fold lower than the original biomass, ranging between 2.5 and 3.5 %, thus evidencing that most of the salts were removed during the extraction and dialysis processes.

The amino acid profile of the obtained concentrates was also characterized and the results are shown in Fig. 1.

Tryptophan, which is one of the essential amino acids, could not be determined by the method employed, and thus no results were available for this compound in the protein concentrates. The essential amino acids represented a total of 43.9 % of the protein fraction in the PC-PTRB and 43.1 % in the PC-PTRTB. This ratio was higher than that observed in some animal protein sources, including milk (39 %), casein (34 %), or egg (32 %) (Gorissen et al., 2018), suggesting the potential use of these protein concentrates as ingredients in food formulations. In comparison with other types of marine biomasses, Cebrián-Lloret et al. (2024) reported that the ratio of essential amino acids in proteins present in red algae such as *Gelidium corneum* or *Gracilaria longuissima* was 35.5 % and 34.7 %, respectively. These values were lower than the ratio obtained with the microalgae *P.tricornutum* in this work.

In both protein concentrates, leucine was the essential amino acid with the highest concentration, accounting for 9.45 % in the PC-PTRTB and 9.16 % in the PC-PTRB. Conversely, histidine was the limiting essential amino acid, with levels of 2.45 % in the PC-PTRB and 1.99 % in the PC-PTRTB. This trend has been previously documented in protein concentrates from other microalgae and cyanobacteria species. For instance, Andreeva et al. (2021) analysed the amino acid composition of *Chlorella vulgaris*, *Arthrospira platensis, Nostoc* sp., *Dunaliella salina* and *Pleurochrysis carterae*, observing a similar pattern. Both of the protein-rich concentrates analysed in this study demonstrated amino acid profiles that consistently exceeded the levels recommended by the FAO for all essential amino acids analysed (FAO, 2013).This finding indicates that this protein extract offers a highly favourable amino acid composition, rendering a potentially valuable source of protein for nutritional applications. The high concentration of essential amino acids further highlights their suitability for addressing dietary protein requirements, particularly in contexts where meeting recommended intake levels is critical.

Regarding the non-essential amino acids, it can be observed that the most abundant in the samples were aspartic acid and glutamic acid. It is important to highlight that proteins derived from marine biomass exhibit elevated concentrations of these two amino acids, which are responsible for contributing to the umami taste profile of the algae (Ahmed et al., 2024; Pootthachaya et al., 2023). It can be observed that the non-essential amino acid with the lowest concentration in both *P. tricornutum* protein concentrates was cysteine. These results are consistent with those obtained in different marine biomass species (León-Vaz et al., 2023).

The amphiphilic nature of the proteins is conferred by their hydrophilic and hydrophobic amino acid composition, which gives them surfactant properties that allow them to form emulsions and foams. The hydrophilic-hydrophobic balance of both protein concentrates was also evaluated, yielding 51 % hydrophilic amino acids and 49 % hydrophobic amino acids. A balanced hydrophobic-hydrophilic ratio in proteins improves emulsion stability by facilitating interactions with both aqueous and oil phases. However, if the balance is overly neutral, the emulsion may lack long-term stability, as stronger protein-interface interactions are often necessary for maintaining structural integrity (Zhang et al., 2022).

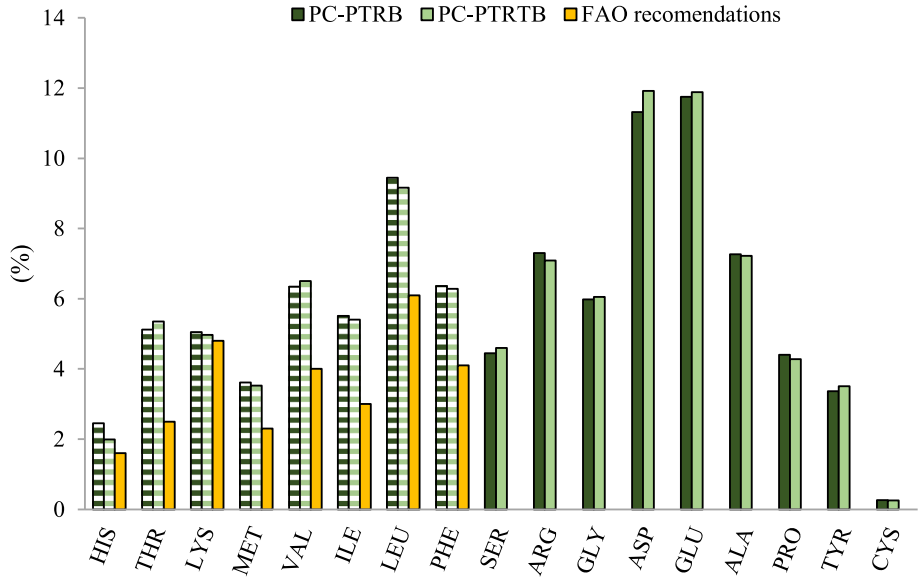

Fig. 1. Total amino acid profile, expressed as % (g AA/100g protein) of the protein-rich concentrates from the original (PC-PTRB) and treated (PC-PTRTB) *Phaeodactylum tricornutum* by-product biomass. The horizontal line fill indicates the essential amino acids.

3.5. Evaluation of technological and functional properties of the protein-rich concentrates

Following the characterisation of the protein-rich concentrates, the technological and functional properties of the protein-rich extracts were evaluated. The extracts were obtained using a 2.5-min ultrasound pre-treatment at pH 12.0 for protein solubilisation and pH 2.0 for precipitation. The water- and oil-holding capacities (WHC and OHC), in addition to their foaming and emulsifying abilities, were analysed with the objective of establishing structure–function relationships.

3.5.1. Water (WHC) and oil-holding capacity (OHC)

The water-holding capacity (WHC) and the oil-holding capacity (OHC) of the protein-rich concentrates are gathered in Fig. 2.

As observed, the WHC was significantly affected by the starting biomass, being higher in protein concentrates obtained from the treated by-product biomass. This effect can be ascribed to different factors, including the protein's conformation, the balance of hydrophobic and hydrophilic amino acids and other intrinsic characteristics (Chavan et al., 2001). Another possible cause is the composition effect, as the PC-PTRTB protein-rich concentrate has a higher carbohydrate content, and carbohydrates are known to increase water absorption capacity (Bojorges et al., 2025). Aletor et al. (2002) proposed that protein-rich ingredients with WHC values similar to the ones found in this study, could be used in viscous foods such as soups and gravy. Chen et al. (2019) reported WHC values of 2.02 ± 0.05, 2.81 ± 0.04 and 2.87 ± 0.04 $g_{water}/g_{protein}$, for protein-rich extracts from *Chlorella pyrenoidosa, Arthrospira platensis* and *Nannochloropsis oceanica*, respectively. Those results are lower than the ones obtained with the protein concentrates from *P. tricornutum* by-product biomass. When compared with other traditional plant protein sources, Chen et al. (2019) reported a WHC of 6.13 ± 0.18 $g_{water}/g_{protein}$ for soy bean protein concentrates, which is slightly higher than the one obtained for the *P. tricornutum* residual treated biomass.

The oil-holding capacity of the PC-PTRB and PC-PTRTB protein-rich concentrates was 3.31 ± 0.07 and 3.24 ± 0.05 $g_{oil}/g_{protein}$, respectively. There were not significant differences between both of the concentrates OHC values. Higher values have been reported for protein-rich concentrates obtained from *Arthrospira platensis, Nannochloropsis oceanica and Chlorella pyrenoidosa* (8.37 ± 1.45, 8.25 ± 0.44 and 6.68 ± 0.41 g oil/g protein, respectively (Chen et al., 2019; Cui et al., 2020). In contrast, Kinsella (1979) reported values from 1.19 to 1.54 $g_{oil}/g_{protein}$ for soybean protein concentrates, being significantly lower than the ones obtained for *P. tricornutum* by-product residual biomass.

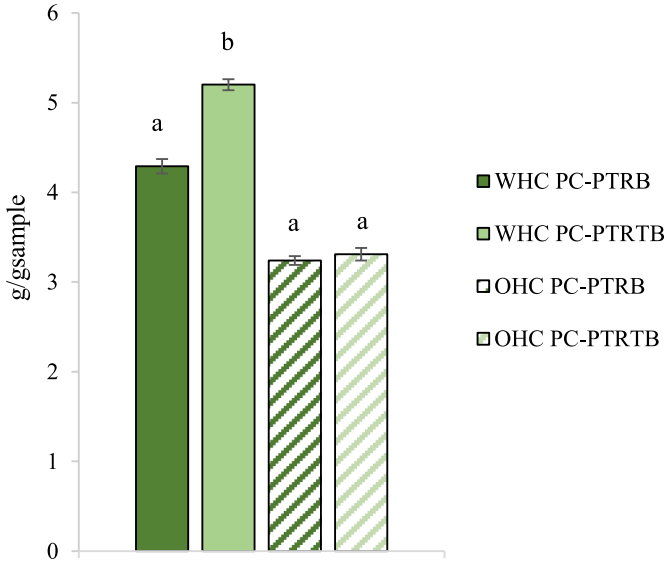

Fig. 2. WHC and OHC of the protein-rich concentrates obtained from original (PC-PTRB) and treated (PC-PTRTB) *Phaeodactylum tricornutum* by-product biomass.

3.5.2. Foaming capacity and foam stability

The foaming capacity (Fig. 3A) was carried out in both PC-PTRB and PC-PTRTB protein-rich concentrates at pH 4, 7 and 10, and the foam stability, shown in Fig. 3B, was evaluated over a 2-h period, with measurements taken every 30 min.

In general, a similar trend was observed in both types of protein-rich concentrates, showing higher foaming capacity at pH values of 7 and 10, which did not change significantly between samples.

Ragab et al. (2004) suggested that the foaming capacity is directly influenced by the solubility of proteins in the medium, evidencing that the protein-rich concentrates were better solubilized at pH 7 and 10. Conversely, at pH 4 (near the isoelectric point), protein concentrates could be partially solubilized, leading to lower foaming capacity compared to alkaline and neutral conditions. Interestingly, the foaming capacity of the obtained concentrates was significantly higher than that obtained for other plant proteins such as soybean protein concentrates, which has been reported to have a foaming capacity of approximately 55 % at pH 7 (Ijarotimi et al., 2018).

Foam stability of the PC-PTRB and PC-PTRTB protein-rich concentrates is shown in Fig. 3B. The first clear observation was that PC-PTRTB concentrates showed higher foam stability than their counterparts, being more evident at pH 4, probably ascribed to the higher lipid content (see Table 4) which has been reported to negatively affect the foam stabilization (Wilde et al., 2004). Regarding the effect of pH on the foam stability, it was significantly affected by the composition of the protein-rich concentrates. For PC-PTRTB protein concentrates, foams obtained at pH 4 and pH 10 exhibited slightly higher stability compared to those obtained at neutral pH, with similar behaviour between the extremes. In contrast, in the PC-PTRB protein-rich concentrates, foaming was observed at pH 4 in freshly prepared samples but it was suddenly destabilized within a few minutes (and thus, it is not shown in Fig. 3B), while at neutral and alkaline conditions (pH 7 and 10, respectively), a similar behaviour was observed over time. Amagliani et al. (2021) pointed out that the protein solubility is intimately related to foam stability. Therefore, at a pH close to the protein isoelectric point (pH 4), foam stability is generally low. However, Kim et al. (2021) highlighted that defatting processes could enhance foam stability, even when surface hydrophobicity and protein solubility decrease, which could explain the differences observed at pH 4.0 for PC-PTRTB and PC-PTRB protein-rich concentrates. As a result, PC-PTRTB concentrates provided better foaming capacity and stability.

As compared to other algae-derived protein concentrates, Lupatini Menegotto et al. (2019) reported higher foam stability values (63.63 %) for *Arthrospira platensis* protein-rich concentrates prepared at pH 7 and stored for 1h. Zhu et al. (2019) reported a foam stability of approximately 61 % at pH 6 after 30 min for protein-rich concentrates from *Haematococcus pluvialis*, which was slightly lower than the one obtained for PC-PTRTB protein concentrates from *P.tricornutum* and measured under similar conditions. Interestingly, our results evidenced higher foaming capacity and stability of PC-PTRB and PC-PTRTB protein-rich concentrates as compared to other plant-derived protein concentrates such as soybean protein concentrates (53 % after 30 min storage and pH 7.0).

The results show that the pre-treatment with ethanol: water carried out with the by-product residual biomass of *P. tricornutum* affects the composition of the protein concentrates and the behaviour of the foams formed with these proteins.

3.5.3. Emulsifying activity and emulsion stability

The effects of pH on the emulsifying activity and emulsion stability of both protein-rich concentrates are presented in Fig. 4A and B, respectively, while the visual appearance of the developed emulsions is shown

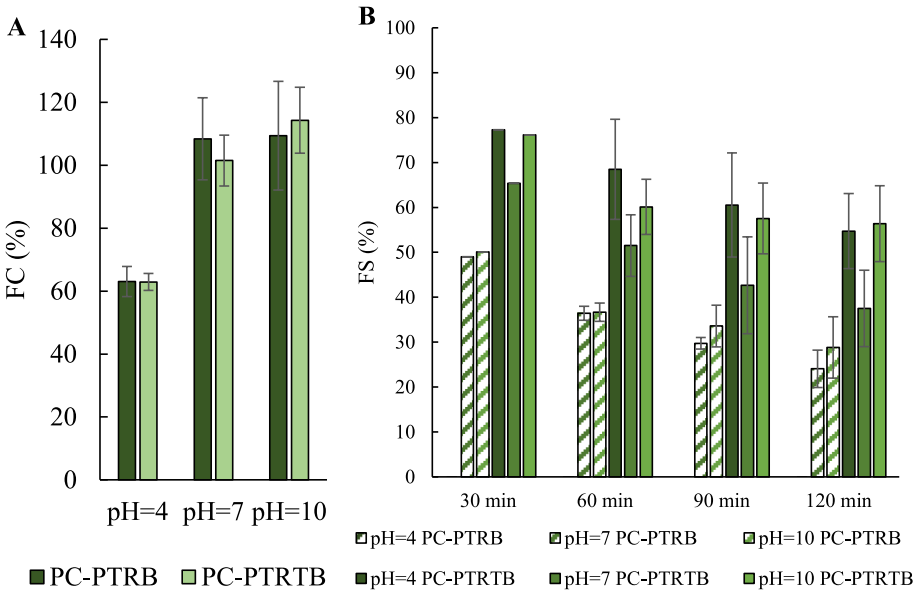

Fig. 3. Foaming capacity (A) and foam stability (B) of protein-rich concentrates from the original (PC-PTRB) and the treated (PC-PTRTB) *Phaeodactylum tricornutum* by-product biomass at different pH values.

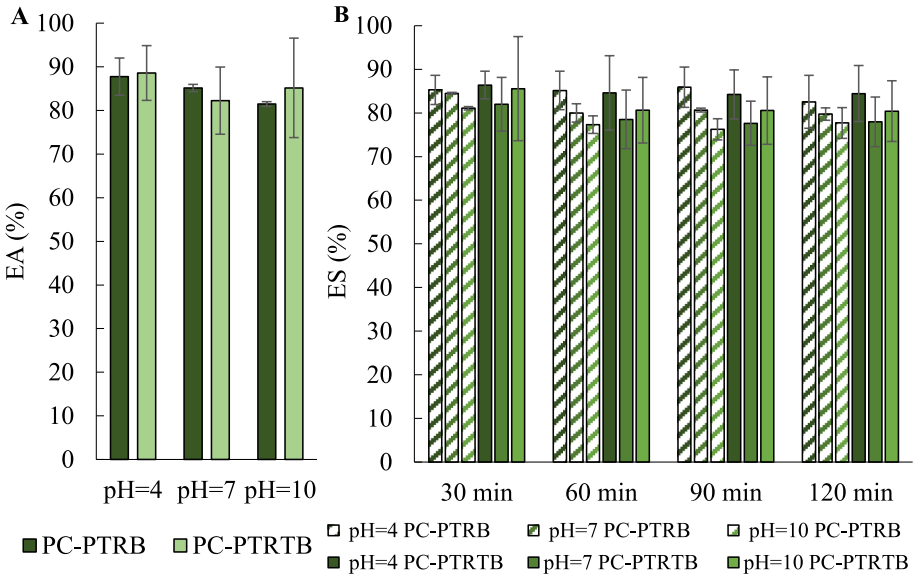

Fig. 4. Emulsifying activity (A) and emulsion stability (B) of the protein rich-concentrates form original (PC-PTRB) and treated (PC-PTRTB) *P.tricornutum* by-product residual biomass at different pH.

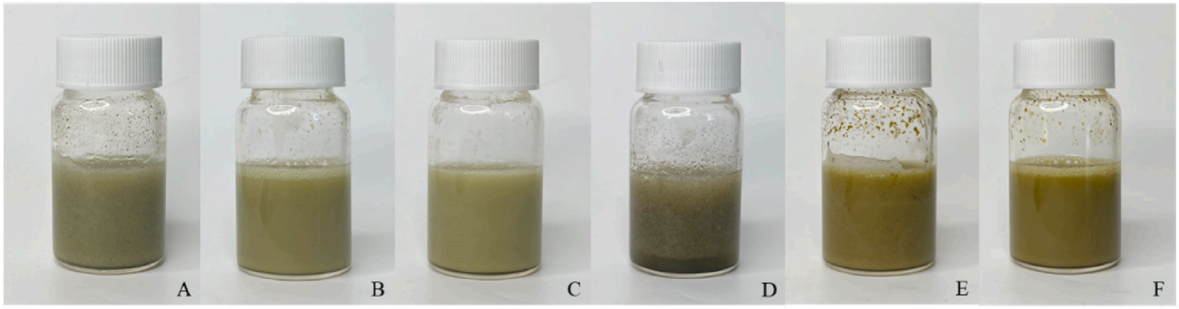

Fig. 5. Emulsions of PC-PTRTB protein-rich concentrate at pH 4 (A) 7 (B) and 10 (C) and emulsions of PC-PTRB protein-rich concentrate at pH 4 (D), 7 (E) and 10 (F).

in Fig. 5. As expected, both protein concentrates exhibited good emulsifying properties at different pH conditions, mainly ascribed to the well-known emulsifying properties of proteins (Ebert et al., 2019; Tang, 2017), and all the emulsions had a creamy appearance, showing a thicker consistency for the ones prepared at pH 4 (cf. Fig. 5). This was probably due to an increase in the oil droplet size and the presence of protein aggregates (see Fig. 6, confocal laser microscopy images) under acidic conditions since the protein and oil concentration in the concentrates were exactly the same. To better understand these differences, the microstructure of freshly-prepared emulsions was investigated by means of confocal laser scanning microscopy and representative images are shown in Fig. 6. The dispersed phase, in this case sunflower oil, was stained using Nile Red dye, which is highlighted in red in the images. The proteins present in the continuous phase, in this case water, were stained using the dye Fast Green, and can be observed in green in the images.

In general, emulsions prepared with PC-PTRTB protein-rich concentrates showed a more heterogenous particle size distribution than their counterparts prepared with PC-PTRB concentrates. This effect can be ascribed to the different composition of the concentrates, as shown in Table 4, which could also influence the consistency of the resulting emulsions.

It should be noted that, at low pH, higher mean particle size was attained and some agglomerates were even observed in PC-PTRTB samples, while the average particle size decreased and size distribution remained almost unchanged at pH 7 and pH 10, except in PC-PTRTB samples prepared at pH 10. This suggests a different stabilization mechanism depending on the pH. At pH 4, it is hypothesised that the

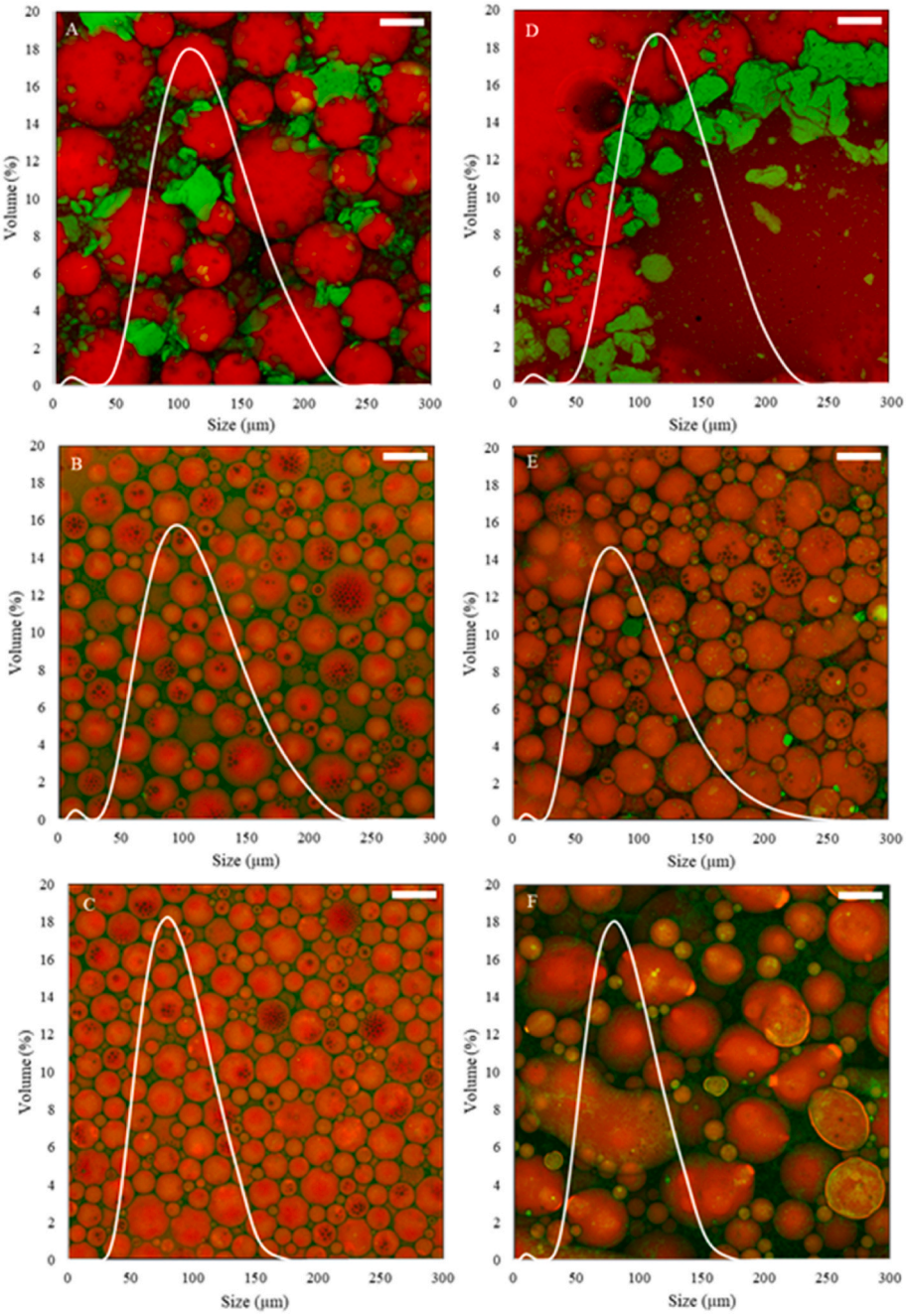

Fig. 6. Confocal microscopy images of emulsions prepared with PC-PTRB (A, B, C) and PC-PTRTB (E, F, G) protein-rich concentrates at different pH conditions: 4 (A, D), 7 (B,E) and 10 (C, F). Scale bar is 100 μm width.

stabilization mechanism in PC-PTRTB samples prepared under acidic conditions is based on the higher viscosity provided by the continuous aqueous phase (thicker consistency) and not to a direct absorption to the O/W interface. In previous works, Cui et al. (2022) and Gao et al. (2017) reported that oil droplets in protein emulsions at a pH value near to the isoelectric point of the protein are stabilised by a three-dimensional network comprising protein aggregates present at the oil-water interface and proteins in the continuous phase. In line with the observed effect of the particle size, at pH 4 (near the isoelectric point), an increase in protein-protein interactions resulted in a low surface hydrophobicity and decreased net charge and solubility of proteins, thus resulting in a higher oil droplet size and broader particle size distribution (Cui et al., 2022). In contrast, proteins were better solubilized at higher pH values (7.0 and 10.0), being mainly located at the O/W interface, as observed in the confocal images, thus stabilizing the oil droplets. Interestingly, emulsions prepared with the PC-PTRB protein-rich concentrate, contained residual lipids or pigments with probably surfactant properties that could also act at the O/W interface. Contrary to these findings, Bertsch et al. (2021) observed that defatting crude concentrates substantially enhanced their functionality by eliminating oil-soluble surfactants, which otherwise compete with protein adsorption. Furthermore, the presence of polyphenol compounds, found in both samples, has been reported to improve emulsifying properties (Lima et al., 2023; Yan et al., 2022).

Benelhadj et al. (2016), reported that proteins extracted from *Arthrospira platensis* exhibited an emulsifying activity of 46.0 % at pH 4, which increased to approximately 64.0 % at pH 10. Interestingly, both PC-PTRB and PC-PTRTB protein-rich concentrates obtained in the present work exhibited markedly higher emulsifying capacities at both pH values (4 and 10), reaching values up to 90 % at pH 4.

Emulsion stability has been quantified as the percentage (%) decrease in volume of the emulsion layer relative to its initial volume following homogenisation. After a 2-h interval, the creaming layer was measured. Interestingly, no significant differences were observed in the emulsion stability neither between PC-PTRB and PC-PTRTB protein-rich concentrates nor by pH, although it was slightly higher at pH 4. Specifically, emulsion stability of those prepared with PC-PTRTB protein-rich concentrates decreased to 84 ± 6 %, 78 ± 6 %, and 80 ± 8 % in emulsions formulated at pH 4, 7, and 10 respectively, after the 2-h of storage. In the case of PC-PTRB protein-rich concentrates, it decreased to 82 ± 6 %, 80 ± 1 %, and 77 ± 3 % at pH 4, 7, and 10, respectively, after the 2-h period.

4. Conclusions

Protein-rich concentrates have been effectively extracted from the residual biomass of *P. tricornutum* through a method that combines ultrasound-assisted alkaline solubilisation followed by precipitation via pH acidification. The protein-rich concentrates obtained were comprehensively analysed, revealing a physicochemical composition and amino acid profile that meet essential nutritional requirements, demonstrating their potential as a valuable protein source.

In addition to their nutritional properties, the protein-rich concentrates exhibited promising functional properties, including high oil and water-holding capacities. These functionalities, coupled with the ability to form foams and emulsions, underscore their suitability for incorporation into a wide range of formulations. From a technological perspective, these characteristics make the concentrates as promising ingredients for applications in food development, cosmetic formulations and animal feed. Given the excellent nutritional and tecno-functional properties of both PC-PTRB and PC-PTRTB protein-rich concentrates, the pretreatment process will ultimately depend on the intended applications, as this additional processing step increases production costs. Beyond these specific applications, this work emphasizes the wider potential of using microalgal residues to support a circular bioeconomy, thereby enhancing the value of industrial by-products. Overall, the findings highlight the dual advantage of promoting resource use while fostering the development of high-value products, offering innovative pathways for the valorisation of microalgal biomass.

CRediT authorship contribution statement

Josep Biosca-Micó: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis. **Antonio Martínez-Abad:** Writing – review & editing, Visualization, Funding acquisition, Conceptualization. **Laura G. Gómez-Mascaraque:** Writing – review & editing, Methodology, Formal analysis. **Amparo López-Rubio:** Writing – review & editing, Visualization, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **María José Fabra:** Writing – review & editing, Visualization, Supervision, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that there are no conflicts of interest.

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Data availability

Data will be made available on request.

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