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Mild blanching prior to pH-shift processing of *Saccharina latissima* retains protein extraction yields and amino acid levels of extracts while minimizing iodine content

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ABSTRACT

The seaweed *Saccharina latissima* is often blanched to lower iodine levels, however, it is not known how blanching affects protein extraction. We assessed the effect of blanching or soaking (80/45/12 °C, 2 min) on protein yield and protein extract characteristics after pH-shift processing of *S. latissima.* Average protein yields and extract amino acid levels ranked treatments as follows: blanching-45 °C ~ control > soaking ~ blanching-80 °C. Although blanching-45 °C decreased protein solubilization yield at pH 12, it increased isoelectric protein precipitation yield at pH 2 ($p < 0.05$). The former could be explained by a higher ratio of large peptides/proteins in the blanched biomass as shown by HP-*SEC*, whereas the latter by blanching-induced lowering of ionic strength, as verified by a dialysis model. Moreover, blanching-45 °C yielded a protein extract with 49 % less iodine compared with the control extract. We recommend blanching-45 °C since it is effective at removing iodine and does not compromise total protein extraction yield.

1. Introduction

Consumption of plant-based proteins is on the rise as a result of current trends towards healthier and more sustainable foods. In parallel, EU policies aiming to restore European oceans by 2030 are expected to foster the development of an expanded seaweed aquaculture industry. This is because seaweed biomass can be a source of numerous products, including zero-carbon and low environmental impact protein (Lamy et al., 2020).

In Europe, seaweed aquaculture primarily focuses on ubiquitous brown species, especially the kelp *Saccharina latissima,* due to its potential for high biomass productivity (Commission, 2021; Stévant, Rebours, & Chapman, 2017). Over 15 companies in the EU and many startups already cultivate *S. latissima*, thereby making it a promising candidate to be used as a future protein source (Commission, 2021). Nevertheless, protein levels in *S. latissima* are relatively low – between 3 and 14 % on a dry weight (DW) basis (Holdt & Kraan, 2011) – thus calling for extraction processes to concentrate its protein. Another drawback of *S. latissima* is that it often contains high levels of the micronutrient iodine and non-essential elements, such as cadmium, lead, mercury, and inorganic arsenic. A partial reduction of iodine and some non-essential elements can be achieved by blanching *S. latissima*, as already studied by Jordbrekk Blikra, Wang, James, and Skipnes (2021), Nielsen et al. (2020), and Stévant et al. (2018). However, to date, it is not known how thermal treatments - particularly blanching - influence the extraction of seaweed proteins.

Industrial and food-grade processes to extract seaweed proteins are not well-established as current low extraction yields, usually from 1 to 29 %, hinder their economic sustainability (Harrysson et al., 2018, 2019). The following physiological and biochemical features of seaweed cells explain why protein extraction of seaweed remains a challenge: (i) scattered distribution of proteins in terms of their subcellular location, thus creating different and several physical barriers between the extraction solvent and a specific pool of proteins; (ii) protein heterogeneity in terms of charge, hydrophobicity, and solubility; (iii) complexation of proteins with polysaccharides, lipids, and phenolics; (iv) high

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levels of hydrocolloids such as alginate that increases the viscosity of the extraction system, thus possibly reducing mass transfer phenomena (Gregersen et al., 2021; Mendez & Kwon, 2021; Tamayo Tenorio, Kyriakopoulou, Suarez-Garcia, van den Berg, & van der Goot, 2018). Also, unpublished observations revealed that the relatively high salt content of seaweed slurry during alkaline protein extraction (74–83 mM NaCl-eq.) makes difficult their recovery with isoelectric precipitation. Therefore, novel methods or refinements of existing ones are warranted to allow the commercialization of seaweed protein ingredients. Among the extraction processes recently reported for seaweed, pH-shift processing stands out since it is relatively easy to scale up as confirmed by its industrial use in soy protein isolation (Keerati-u-rai & Corredig, 2011). From a nutritional standpoint, we recently found it to increase the digestibility of seaweed proteins after *in vitro* gastrointestinal digestion (Trigo et al., 2021). Regarding protein extraction yields with this process, a refined version - with protein solubilization at pH 12 and protein precipitation at pH 2 aided by a freeze-thawing cycle - yielded the highest protein yields for *S. latissima* (25 %) when compared to methods such as sonication in water followed by ammonium sulfate-induced protein precipitation and accelerated solvent extraction (protein yields of 3 and 1 %, respectively) (Harrysson et al., 2018). In this cited study, the pH-shift process also resulted in the most concentrated protein extracts (~40 % DW) (Harrysson et al., 2018). In other aquatic biomasses, such as the microalgae *Arthrospira* sp., thermal treatments conducted in a water bath (35–75 °C) have been reported to reduce protein solubility (Wang, Zhang, Liu, & Liu, 2019). In the pH-shift process, lower protein solubility would translate into reduced amount of protein being available for isoelectric precipitation, hence lower protein extraction yields. A similar negative relationship between thermal treatment and protein solubility was observed for vegetarian protein isolates produced at 70–90 °C from terrestrial sources, such as amaranth and is, as expected, primarily attributed to the formation of protein aggregates along with protein denaturation (Avanza & Añón, 2007). Others have also found that elevated temperature promoted interaction between phenolic acids and pea protein (Tsai & She, 2006). Based on this, we hypothesized that blanching of *S. latissima* would reduce protein extraction yields due to changes in protein conformation and complexation, thus yielding biomasses less desirable for protein extraction purposes. Moreover, the effect of protein extraction processes, such as the pH-shift-based process, on the co-extraction of iodine and heavy metals remains to be addressed. On this matter, we hypothesize that each metal will behave differently as their affinity for the same amino acid residue can differ (Jacobson & Turner, 1980).

The main goal of this study was to assess the effect of different blanching temperatures and soaking in tap water on the recovery of proteins during pH-shift processing of *S. latissima* biomass. A sub-goal was to evaluate the effects of blanching and pH-shift processing on levels of dry matter, ash, total monosaccharides, total amino acids, and essential and non-essential elements in biomasses and protein extracts made thereof. In order to better explain our findings, we also investigated the link between ionic strength (IS) and isoelectric protein precipitation as well as the influence of blanching on the potential activity of endogenous proteases during pH-shift processing.

2. Materials and methods

2.1. Seaweed biomass harvesting

Sugar kelp biomass (*S. latissima*) was outplanted in the autumn of 2019 at a cultivation site located in the Koster archipelago on the Swedish west coast within the Skagerrak region of the North Sea (58°51′34.0″N, 11°04′06.2″E). The *S. latissima* blades were harvested in May 2020, and on the same day transported in an icebox to Chalmers University of Technology for the blanching and soaking treatments.

2.2. Blanching and soaking treatments

The unprocessed *S. latissima* was blanched in 30 volumes (volume/weight) of tap water for 2 min at 45 or 80 °C in a water bath (SW22, Julabo). The choice of blanching at 45 °C for 120 s was based on the work by Nielsen et al. (2020), which tested different temperatures (30–80 °C) and time (2–300 s) bionomies. Overall, iodine content reached a constant level at 120 s and above 45 °C no further reduction in iodine was detected. Therefore, we selected 45 °C, 120 s as one of the blanching conditions. The selection of blanching at 80 °C for 120 s was supported by its industrial relevance since this condition is the main adopted one at the industrial scale. Finally, soaking in cold tap water served as a blanching control; thus, its processing time was set at 120 s. Right after, the biomass was drained using a kitchen drainer and then placed in polystyrene bags to cool down in ice water for 10 min. A parallel soaking treatment was done in tap water at 12 °C using the same processing time and seaweed-to-tap water ratio. The soaking temperature was dependent on the temperature of fresh tap water on that given day (which in this case was 12 °C) as we consider this would be the industrial-scale scenario. The control biomass was untreated and is here referred to as *unprocessed*. After that, all four biomasses were minced in a refrigerated room at 4 °C using a meat grinder (Model C-E22N, la Minerva) with a 2.0 mm hole plate. The biomasses were then stored at −80 °C in polystyrene bags until further use.

2.3. Protein extraction using pH-shift processing

Before processing, the pH-shift extraction protocol was fine-tuned by determining the pHs yielding maximum protein solubility and maximum protein precipitation of each biomass.

2.3.1. Fine-tuning of the pH-shift process

The fine-tuning of solubilization and precipitation pHs followed the protocol established by Harrysson et al. (2018) with minor modifications. Each biomass was processed in duplicate; for each replica, approximately 15 g of minced biomass was mixed with distilled water in a 1:6 (w/v) ratio. To promote cell breakage, the mixture was homogenised (LM5, Silverson) for 2 min at 8000 rpm, followed by an incubation step for 1 h with stirring. Afterwards, the pH of the homogenate was stepwise adjusted (PHM 210, Meterlab, Hach) from the native pH (7.3, 7.4, 8.3, and 8.6 for unprocessed, soaked, blanched 45 °C, and blanched 80 °C, respectively) to a maximum value of pH 12.0 with NaOH (1 M). Aliquots from each pH point were centrifuged at 8500×*g*, 4 °C for 20 min (Heraus Fresco 17, Thermo Scientific) and the resulting supernatants, along with the initial homogenate, were analysed for total protein content according to the Lowry method later modified by Markwell, Haas, Bieber, and Tolbert (1978). To determine the maximum protein precipitation, the supernatant recovered at the pH yielding the highest solubility (found to be pH 12.0 for all biomasses) was stepwise adjusted to a minimum value of pH 2 with HCl (1 M). Next, the aliquots at each examined pH were frozen overnight at −20 °C and thawed in cold water, followed by centrifugation, as described above. The resulting supernatants were analysed for total protein content as earlier mentioned. All steps were carried out on ice water unless stated otherwise.

2.3.2. Determination of protein yields

The production of the protein extracts from each of the four biomasses followed the protocol described in **Section 2.3.1**, but only using the pH that yielded the highest solubility (pH 12.0) and precipitation (pH 2.0) yields. The two centrifugation steps at 8500×*g* (20 min at 4 °C) were carried out in a Sorvall LYNX 6000 centrifuge (Thermo Scientific) and the resulting pellet from the second centrifugation is here referred to as *protein extract*. Pictures of the obtained pH-shift protein extracts can be found in **Supplementary Material**: **Fig. S1**. The solubility and precipitation yields, as well as the total protein yield, were calculated by measuring the total protein content (Markwell et al., 1978) in aliquots

withdrawn from the homogenate and supernatants 1 and 2 (S1 and S2). The pH-shift process was done in duplicate for each biomass.

2.3.3. Determination of protein degree of hydrolysis during pH-shift processing to identify potential endogenous protease activity of biomasses

The protein degree of hydrolysis during the osmotic shock incubation step of the pH-shift processing was analysed by withdrawing aliquots before and after the osmotic shock step. Then, the aliquots were mixed with 1 % SDS in a 1:1 ratio, followed by homogenization (Ultra-turrax T18 basic, IKA) at 10 000 rpm for 10 s. Afterwards, primary amines were detected with the TNBS method as described by Trigo et al. (2021) and the results were expressed as degree of hydrolysis (%DH). Samples were analyzed in triplicate, and a standard curve was made with leucine.

2.3.4. Ionic strength

The ionic strength during the pH-shift process and dialysis (Section 2.3.5) was monitored using a conductivity meter (CDM210, MeterLab). Sodium chloride (NaCl) was used as a standard, and the results were expressed as mM of NaCl equivalents. All measurements were conducted at 20 ± 1 °C.

2.3.5. Relationship between ionic strength (IS) and protein precipitation during pH-shift processing

To examine the link between IS and protein precipitation at acidic pH values, pH-shift processing was conducted as described in Section 2.3.2 with the following modification: the supernatant 1 (S1) was dialyzed (MWCO 1000 Da) against a citric acid-disodium phosphate buffer (pH 2.5, 45 mM NaCl eq.) at 4 °C to acidify S1 and to remove low molecular weight (LMW) compounds such as minerals contributing to the relatively high IS of seaweed biomass. The buffer was set to pH 2.5 - the lowest pH value for adequate buffering capacity. Then, the dialysate was subjected to a freeze-thawing cycle, followed by a centrifugation step to recover the protein extract. The control experiment was prepared as follows: the pH of S1 was adjusted to pH 2.5 with HCl (1 M), followed by an incubation step at 4 °C for the same length as the dialysis. Then, the acidified S1 was freeze-thawed and centrifugated. The effect of dialysis without the freeze-thawing cycle was also evaluated in order to know if the freeze-thawing step could be replaced in a hypothetical upscaling of the pH-shift process. All experiments were done in duplicate.

2.4. Sample characterization

2.4.1. Chemical composition

The dry matter of biomass and protein extracts was estimated by drying the samples at 105 °C in a furnace until constant weight. The ash content was determined by combusting 20 and 50 mg of dried initial biomasses and dried protein extracts, respectively, in the following steps: (1) ramp to 550 °C at a rate of 200 °C/h; (2) 550 °C for 6 h; (3) cooldown to 200 °C. After the last step, combusted samples were placed in a desiccator for further colling and then the weight was noted.

Total amino acids (TAA) were analysed, in triplicate, according to the protocol reported by Trigo et al. (2021). Briefly, initial biomasses and protein extracts were freeze-dried and grounded to fine powders. Next, 100 mg of these powders were weighed in screw cap glass tubes, followed by adding 4 mL HCl (6 M) to each tube. The air inside the tubes was replaced by nitrogen and hydrolysis took place in a heating block at 110 °C for 24 h. Hydrolysed samples were diluted with 0.2 M acetic acid and filtered (0.22 μm; Fisher Scientific). Then, two microliters of each sample were analysed by LC-MS (Agilent 1100 HPLC) with a Phenomenex column (C18 (2) 250 μm × 4.6 μm × 3 μm) preconditioned at 50 °C and coupled to an Agilent 6120 quadrupole in the SIM positive mode (Agilent Technologies). The separation was conducted at 0.7 mL/min for 40 min using different ratios of mobile phase A (3 % methanol, 0.2 % formic acid, and 0.01 % acetic acid) and mobile phase B (50 % methanol, 0.2 % formic acid and 0.01 % acetic acid). Collected data was compared against 17 different amino acids (Thermo Scientific) standards.

Carbohydrate analysis was performed following SCAN-CM 71:09 with modifications according to Sterner and Edlund (2016). Approximately 20 mg of freeze-dried powders (n = 2) of all samples were subjected to acid hydrolysis with 72 % (w/w) sulfuric acid at room temperature for 1.5 h. The resulting mixture was diluted with deionized water to obtain a 4 % sulfuric acid solution and then heated to 120 °C in an autoclave for 1.5 h. The monosaccharide content of the hydrolysates was identified using high-performance anion-exchange chromatography (HPAEC, Dionex, Sunnyvale, CA, USA) equipped with a pulsed amperometric detector (PAD, Dionex ICS-3000) and a CarboPac PA1 column (4 × 250 mm). The mobile gradient phase comprised (i) ultrapure water and (ii) 260 mM NaOH and 170 mM sodium acetate. All obtained data were processed using the Chromeleon 7.1 software. The carbohydrate standards (Sigma Alrich) were mannitol, fucose, arabinose, galactose, xylose, mannose, and alginic acid from *Macrocystis pyrifera*. Alginic acid underwent hydrolysis in the same manner as the samples. The uronic acid composition (guluronic acid/mannuronic acid ratio) of alginic acid was determined by ^{1}H NMR. NMR spectra were obtained with a Bruker DMX-500 NMR spectrometer; MestReNova software was used for data acquisition.

2.4.2. Elemental composition

Total content of essential and non-essential elements was determined by ALS Scandinavia AB (Luleå, Sweden), following a method based on their accredited routine. In order to achieve the lowest possible detection limits and to avoid contamination risks associated with additional homogenization of samples, sample amount was increased to > 1 g per digestion. Each sample was weighted into acid-washed 50 mL propylene vessels. After adding concentrated nitric acid (10:1, v/m), the mixture was left to react overnight, followed by digestion at 105 °C for 2 h in a heating block. Then, digests were diluted with MQ-water in two steps to provide a total dilution factor of 100 and a nitric acid concentration of 1.4 M. The content of all elements was measured by HR-ICP-MS (ELEMENT XR, Thermo Scientific) operated with methane gas addition to the plasma. The matrix effect was corrected by adding internal standards (Indium and Lutetium) to all solutions at 1 μg/L concentration. Quantification was done using external calibration with standards matching the acid concentration of the sample digests. A set of preparation blanks and reference materials was prepared together with the samples. Samples for the iodine determination were prepared by microwave-assisted, closed vessel nitric acid digestion, followed by sample dilution with a mixture of Ammonia (0.5 %) – Triton X-100 (0.01 %) – EDTA (0.01 %). The detection limit of all analysed elements can be found in **Supplementary Material: Table S1**. Initial biomasses were analysed in triplicate and protein extracts in duplicate.

The determination of inorganic arsenic (iAs) was performed at the Swedish Food Agency (Uppsala, Sweden) according to the European standard EN 16802:2016. The samples (0.2–0.5 g) were accurately weighed into polypropylene tubes with a screwcap, followed by the addition of 10 g of extraction solution (composed of 0.1 M nitric acid and 3 % hydrogen peroxide). After mixing, the samples were placed in water bath at 90 °C for 2 h followed by centrifugation (10 min, 308*g*). The supernatant was carefully sampled and filtered (Minisart® RC25 Syringe Filter, 0.45 μm) directly into new sample tubes for further dilution. The iAs was quantified isocratically by HPLC-ICP-MS (Agilent 1260 Infinity Quaternary LC and Agilent 7700x ICP-MS) using a strong anion exchange column (Dionex Ionpac AS7 with guard column Dionex Ionpac AG7) and ammonium carbonate as the mobile phase (50 mM ammonium carbonate and 3 % methanol, pH 10.3). The method performance is described in Kollander et al. (2019). Due to a shortage of samples, only one replicate was performed per sample. The Swedish Food Agency participates annually in proficiency tests arranged by the European Reference Laboratory for metals and nitrogenous compounds in order to determine iAs in different matrices. In addition, certified and in-house reference materials are routinely analysed and evaluated

together with the samples for careful control of the quality of the analyses.

2.4.3. Protein/peptide relative size distribution

Qualitative analysis of the protein/peptide molecular weight from initial biomasses and protein extracts thereof was analysed as previously explained by Trigo et al. (2021) with minor modifications. Shortly, freeze-dried powders of all samples were dissolved in a mobile phase made with phosphate buffer (0.1 M; pH 7.50) to a final protein content of 1 mg/mL. Subsequently, the mixture was homogenized (Ultra-turrax T18 basic, IKA) at 18 000 rpm for 30 s and centrifuged to remove coarse particles. The supernatants were analysed in a high-performance size-exclusion chromatograph (HP-*SEC*) (Dionex HPLC, Dionex GmbH) equipped with two serially connected Agilent columns - Agilent Bio *sEC*-5 (5 μm particle size, 150 Å pore size) and Agilent Bio *sEC*-5 (5 μm particle size and 300 Å pore size) - and a UV-detector. A commercial protein standard mix ranging from 1 to 670 kDa was used to create a calibration curve of known molecular weights as a function of retention time. The average area of each peak from three independent injections (n = 3) was calculated and presented in relation to the total peak area.

2.5. Statistical analysis

Significant differences between the different treatments were tested using the one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for pairwise comparison. The differences were considered statistically significant at $p < 0.05$. All statistical tests were conducted using SPSS Statistics software (version 26.0.0.0).

3. Results and discussion

3.1. Characterization of initial biomasses

3.1.1. Chemical composition

The effects of soaking and blanching on the dry matter, ash, total amino acids, and total monosaccharides of *S. latissima* are presented in Table 1. Blanching and soaking treatments yielded biomasses with 49 and 25 % lower ash content, respectively, compared to the unprocessed control ($p < 0.05$). The lower ash content was also reflected in a reduced IS of biomasses after blanching or soaking – 9.8 and 157 mM, compared to 371 mM of untreated biomass. Moreover, the large ash removal during blanching at either 45 and 80 °C resulted in biomasses with higher TAA content when compared to the soaked and unprocessed biomasses ($p < 0.05$). Nielsen et al. (2020) also reported significant leaching of ash (>90 %) after blanching *S. latissima* for 2 min at 45 and 80 °C with a consequent increase in TAA. The most abundant fraction in all biomasses was carbohydrates, with the total monosaccharide content ranging from 65 to 78 % of the total DW. Soaking was the only treatment that significantly affected total monosaccharide content ($p < 0.05$) since it increased the levels of glucose (the main monomer of cellulose and laminarin) and mannitol (**Supplementary Material: Table S2**).

Table 2 shows the elemental composition of all tested biomasses. Soaking maintained the levels of all non-essential elements (e.g. cadmium), albeit a non-significant 46-percentage points reduction for lead was observed. For blanching, the 45 and 80 °C temperatures reduced total arsenic by 19 and 33 percentage points, respectively ($p < 0.05$). Other works on blanched *S. latissima*, found slight up-concentrations in cadmium and lead, although the increase was not statistically significant (Bruhn et al., 2019; Jordbrekk Blikra et al., 2021). It is well-documented that the bioabsorption of heavy metals by brown seaweed can be related to the alginate content. This is because alginate contains an abundance of carboxylic groups capable of binding light metals (e.g., sodium) and exchanging them for harmful elements if present in the cultivation media/surrounding water (Santos, Ungureanu, Volf, Boaventura, & Botelho, 2018). To confirm that blanching of *S. latissima* likely concentrated most non-essential elements due to an increased proportion of alginate, we quantified the primary alginate residues i.e., mannuronic and guluronic acids. The results revealed that the alginate fraction increased from 21.2 % to 38.7 and 45.3 % total DW after blanching at 45 and 80 °C, respectively, while soaking resulted in a similar alginate content to the unprocessed biomass (24.4 % total DW) (**Supplementary Material: Table S2**). These data support the hypothesis of alginate in non-essential elements binding.

Most analyzed essential elements were concentrated during

Table 1
Chemical composition of unprocessed, soaked, and blanched *S. latissima* and pH-shift protein extracts thereof. Values are shown as average ± SD, n = 3 for initial biomasses and n = 2 for protein extracts.

		Dry matter (% of WW)	Ash (%DW)	Total amino acids (%DW)	Total monosaccharides (%DW)
Initial biomasses	Unprocessed	**12.0** ± 0.3^{a}	**29.9** ± 0.9^{a}	**4.9** ± 0.3^{a}	**64.9** ± 0.8^{b}
	Soaked	**8.6** ± 0.2^{b}	**22.4** ± 1.8^{bc}	**5.4** ± 0.2^{a}	**77.6** ± 2.1^{a}
	Blanched 45 °C	▼ **5.6** ± 0.2^{c}	▼ **15.3** ± 1.4^{e}	▲ **10.1** ± 0.1^{b}	▲ **66.2** ± 0.1^{b}
	Blanched 80 °C	▼ **5.7** ± 0.2^{c}	▼ **15.4** ± 2.5^{e}	▲ **11.1** ± 0.4^{b}	**71.1** ± 4.5^{ab}
Protein extracts	PE-unprocessed	▼ **3.8** ± 0.2^{d}	▼ **18.3** ± 0.3 ^{cd}	▲ **20.2** ± 0.2^{c}	**34.4** ± 0.8^{e}
	PE-soaked	▼ **1.8** ± <0.1^{e}	▼ **22.7** ± 0.5^{b}	▲ **14.3** ± 0.7^{d}	▼ **49.5** ± 4.1 ^{cd}
	PE-blanched 45 °C	▼ **2.0** ± <0.1^{e}	**10.4** ± 0.9^{f}	▲ **21.3** ± 0.6^{c}	▼ **52.1** ± 0.1^{c}
	PE-blanched 80 °C	▼ **2.6** ± <0.1^{f}	▼ **7.4** ± 0.6^{f}	▲ **13.8** ± 0.1^{d}	▼ **44.7** ± 1.2^{d}
		▼	▼	▲	▼

Different letters (a-f) denote significant differences ($p < 0.05$) within each column; For initial biomasses, ▲ and ▼ indicate a significant increase and decrease relative to the unprocessed biomass; For protein extracts, ▲ and ▼ indicate a significant increase and decrease relative to the respective biomass;
WW wet weight; DW dry weight.

Table 2
Elemental composition of unprocessed, soaked, and blanched *S. latissima* and pH-shift protein extracts thereof. Values are shown as average ± SD, n = 3 for initial biomasses and n = 2 for protein extracts.

		Non-essential elements (mg/kg DW)							Essential elements (mg/kg DW)									
		tAs	iAs[1]	Cd	Hg	Pb	Ni	Ag	Cr	Co	Cu	I	Fe	Mn	Mo	Se	Zn	Na
Initial biomasses	Unprocessed	**60.0** ± 2.9 ^{cd}	**0.119**	**0.360** ± 0.006^{c}	**0.021** ± 0.004^{a}	**0.147** ± 0.006^{a}	**0.73** ± 0.13^{a}	**0.007** ±<0.001^{a}	**2.53** ± 0.39^{a}	**0.104** ± 0.013^{a}	**1.35** ± 0.07^{a}	**2648** ± 120^{ab}	**124** ± 13^{ab}	**7.92** ± 0.44^{ab}	**0.24** ± 0.02^{a}	**0.597** ± 0.517^{a}	**27.4** ± 1.5^{ab}	**46 148** ± 825^{f}
	Soaked	**58.3** ± 2.4 ^{cd}	**0.079**	**0.335** ± 0.003^{bc}	**0.023** ± 0.002^{a}	**0.075** ± 0.001^{a}	**0.70** ± 0.12^{a}	**0.005** ± 0.001^{a}	**2.86** ± 0.40^{a}	**0.083** ± 0.010^{a}	**1.50** ± 0.08^{a}	**2749** ± 168^{ab}	**66** ± 6^{a}	**6.18** ± 0.33^{a}	**0.21** ± 0.02^{a}	**0.445** ± 0.430^{a}	**28.0** ± 1.3^{b}	**19 364** ± 259^{c}
	Blanched 45 °C	**48.6** ± 2.2^{ab}	**0.115**	**0.608** ± 0.016^{d}	**0.034** ± 0.001^{a}	**0.214** ± 0.027^{ab}	**1.45** ± 0.23^{a}	**0.010** ± 0.001^{a}	**7.28** ± 1.15^{ab}	**0.111** ± 0.013^{a}	**3.41** ± 0.13^{ab}	**461** ±<1^{a}	**124** ± 13^{ab}	**10.44** ± 0.59 ^{cd}	**0.27** ± 0.02^{a}	**0.391** ± 0.249^{a}	**45.7** ± 1.7^{d}	**11 780** ± 64^{b}
	Blanched 80 °C	**40.0** ± 2.6^{a}	**0.082**	**0.695** ± 0.025^{d}	**0.035** ± 0.002^{a}	**0.584** ± 0.038^{bc}	**4.31** ± 1.47^{b}	**0.023** ± 0.003^{a}	**15.30** ± 4.14^{bc}	**0.184** ± 0.039^{ab}	**5.07** ± 0.29^{b}	**373** ± 2^{a}	**186** ± 21^{bc}	**12.25** ± 0.67^{d}	**0.28** ± 0.04^{a}	**0.410** ± 0.243^{a}	**48.7** ± 1.8^{de}	**12 471** ± 42^{b}
Protein extracts	PE-unprocessed	**64.2** ± 3.0^{cde}	**0.089**	**0.233** ± 0.055^{ab}	**0.126** ± 0.005^{a}	**1.11** ± 0.18^{d}	**3.00** ± 0.82^{ab}	**0.041** ± 0.001^{a}	**21.80** ± 2.77^{c}	**0.536** ± 0.057^{d}	**12.62** ± 0.43^{c}	**2439** ± 444^{ab}	**458** ± 43^{e}	**6.53** ± 0.77^{a}	**2.42** ± 0.01^{c}	**1.29** ± 0.06^{a}	**19.9** ± 2.8^{a}	**28 169** ± 21^{e}
	PE-soaked	**71.9** ± 1.0^{e}	**0.135**	**0.216** ± 0.018^{ab}	**0.189** ± 0.054^{a}	**1.01** ± 0.06^{d}	**2.42** ± 0.47^{ab}	**0.027** ± 0.007^{a}	**13.27** ± 1.44^{abc}	**0.438** ± 0.014 ^{cd}	**11.02** ± 1.23^{c}	**4622** ± 1511^{b}	**378** ± 16de	**7.24** ± 0.17^{ab}	**1.51** ± 0.19^{d}	**1.46** ± 0.51^{a}	**27.2** ± 1.4^{ab}	**23 631** ± 233^{d}
	PE-blanched 45 °C	**56.2** ± 0.8^{bc}	**0.115**	**0.416** ± 0.002^{c}	**0.198** ± 0.061^{a}	**0.80** ± 0.04 ^{cd}	**1.91** ± 0.13^{ab}	**0.015** ± 0.007^{a}	**8.71** ± 0.52^{ab}	**0.297** ± 0.009^{b}	**10.29** ± 0.42^{c}	**1205** ± 131^{a}	**262** ± 3 ^{cd}	**9.24** ± 0.17^{bc}	**0.77** ± 0.01^{b}	**0.70** ± 0.26^{a}	**54.6** ± 1.0^{e}	**9488** ± 95^{a}
	PE-blanched 80 °C	**67.2** ± 0.6^{de}	**0.078**	**0.182** ± 0.006^{a}	**0.139** ± 0.039^{a}	**1.03** ±<0.01^{d}	**1.46** ± 0.06^{a}	**0.037** ± 0.018^{a}	**7.34** ± 0.27^{ab}	**0.265** ± 0.010^{bc}	**12.71** ± 0.29^{c}	**1181** ± 214^{a}	**427** ± 33^{e}	**6.23** ± 0.49^{a}	**0.94** ± 0.02^{b}	**1.19** ± 0.45^{a}	**35.8** ± 0.5^{c}	**8543** ± 242^{a}

Different letters (a-f) denote significant differences ($p < 0.05$) within each column; For initial biomasses, ▲ and ▼ indicate a significant increase and decrease relative to the unprocessed biomass; For protein extracts, ▲ and ▼ indicate a significant increase and decrease relative to the respective biomass; [1](n = 1, accredited method measurement uncertainty ± 19 %).
DW dry weight; *tAs* total arsenic; *iAs* inorganic arsenic.

blanching, with significant increases only detected for chromium, copper, manganese, and zinc after blanching at 80 °C ($p < 0.05$). Regarding soaking, the levels of all essential elements remained unaffected, except for sodium which decreased ($p < 0.05$). In the literature, Nielsen et al. (2020) studied the removal of iodine from *S. latissima* as a function of different blanching conditions by varying its temperature (30–80 °C) and duration (2–300 s). Comparing their study to ours revealed that for identical time/temperature combinations (i.e., 45 and 80 °C for 2 min), similar iodine reductions were achieved – the percentage reductions and final iodine levels being 85–93 % and 293–460 mg/kg DW, respectively, in both studies. Another evaluation reported a significant decrease in iodine, sodium, and total As after boiling re-hydrated *S. latissima* for 1, 2, 5, and 20 min; in contrast to our data, selenium was partially removed regardless of the processing time (Correia et al., 2021). Other recent studies on *S. latissima* assessing the impact of soaking (Jordbrekk Blikra et al., 2021) and boiling (Bruhn et al., 2019; Jordbrekk Blikra et al., 2021) found similar trends for iodine and total As.

Assuming iodine is 100 % bioavailable to humans, the daily amount of dry *S. latissima* biomass that can safely be consumed increased 5.5-fold from 0.2 g in the unprocessed state to 1.3 g DW when blanched at 45 °C (TDI of 600 μg iodine/day/adult according to EFSA, 2006). The increase by blanching at 80 °C was marginally higher when compared to blanching at 45 °C - only 6.8-fold vs 5.5-fold (**Supplementary Material: Table S3**). Additionally, comparing both blanching treatments, revealed that milder temperatures limited the up-concentration of harmful elements such as lead, nickel, or silver. Lastly, from an industrial standpoint, blanching at 45 °C saves costs and time related to heating of water and cooling of the biomass.

3.1.2. Protein/peptide relative size distribution

HP-*SEC* was carried out to assess the molecular weight of protein/peptides of the initial biomasses. The chromatograms and the relative peak size distribution compared to the total peak area are shown in Fig. 1. Blanching increased the relative proportion of the > 670 kDa fraction compared to the unprocessed and soaked biomasses ($p < 0.01$). This increase appeared to be temperature dependent as the relative

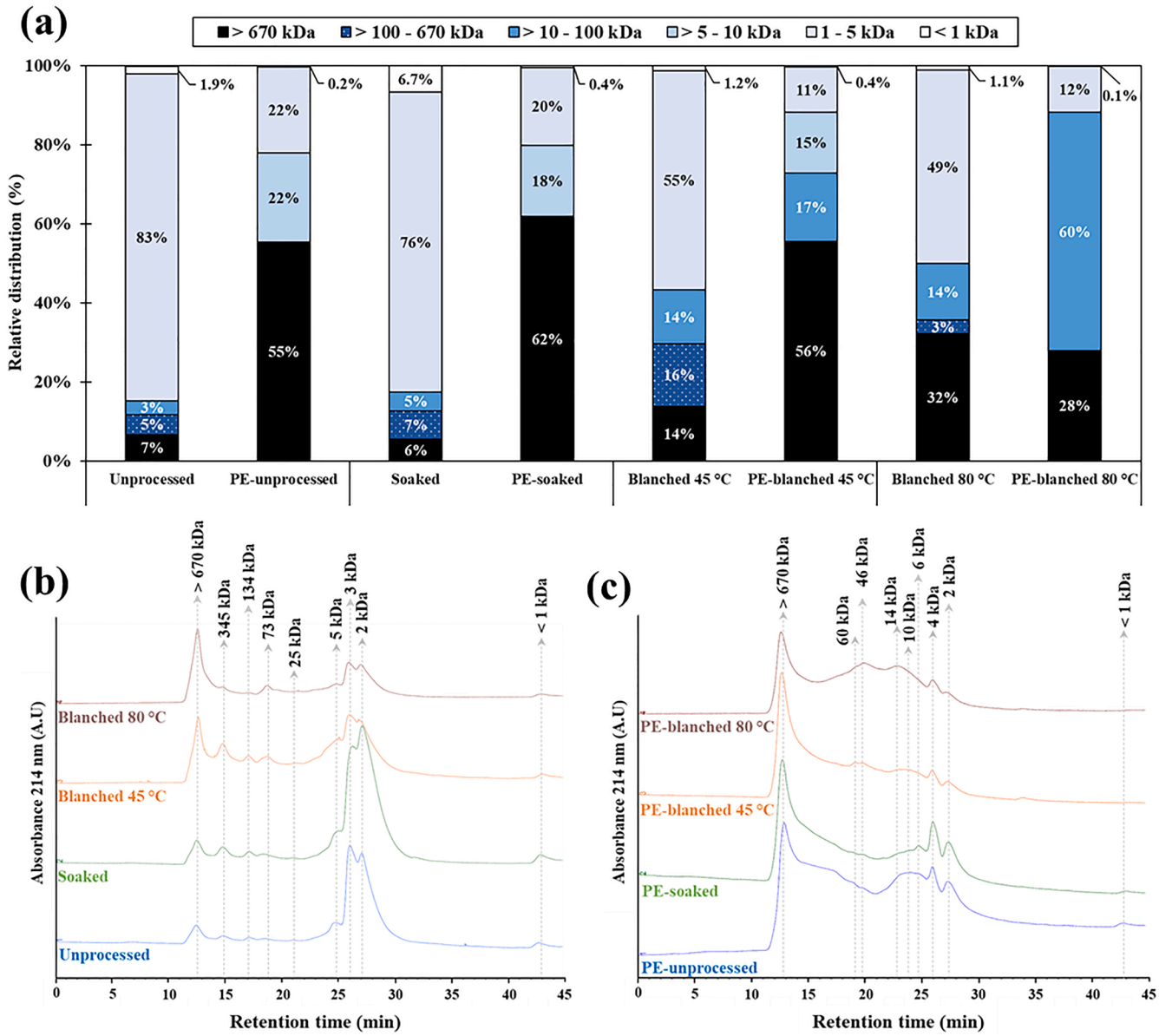

Fig. 1. Apparent molecular weight of protein/peptides from unprocessed, soaked, and blanched *S. latissima* and pH-shift protein extracts thereof. Results are shown as relative percentage of total peak area (a), and HP-*SEC* chromatograms of initial biomasses (b) and protein extracts thereof (c). Arrows point at the molecular weight of main peaks. PE: protein extract.

distribution of > 670 kDa fraction was ranked in the following order: blanched 80 °C > blanched 45 °C > soaked. It is well-documented that temperature contributes to the formation of protein aggregates mainly due to the promotion of hydrophobic interactions between proteins (W. Wang, Nema, & Teagarden, 2010). Thus, the > 670 kDa fraction likely represents soluble protein aggregates. In another work, when thermally processing a pea protein isolate for 1 h at 90 °C, the fraction > 650 kDa increased 14-fold and accounted for 61 % of the relative peak area (Mession, Sok, Assifaoui, & Saurel, 2013). We also found that both blanched biomasses had a higher relative proportion of the 73 kDa fraction ($p < 0.01$). Indeed, after blanching, the size distribution was characterized by a predominance of proteins/polypeptides > 10 kDa, making up 44 to 49 % of the total relative peak area. This value was 15 and 18 % for unprocessed and soaked biomasses, respectively. Blanching also reduced the proportion of protein/peptides ~ 2 kDa compared to unprocessed and soaked *S. latissima* ($p < 0.01$), suggesting these were highly water soluble.

3.1.3. Amino acid profile

Fig. 2**a** presents the initial biomasses' amino acid (AA) profile. Both blanching treatments resulted in higher relative content of glycine, histidine, proline, and arginine ($p < 0.05$). Moreover, blanching at 80 °C increased the relative content of aspartate, lysine, and leucine ($p < 0.05$), whereas blanching at 45 °C kept similar values of these AAs. Generally, blanched biomasses, regardless of blanching temperature, showed a lower relative content of the remaining amino acids i.e., alanine, serine, valine, threonine, isoleucine, glutamate, methionine, phenylalanine, and tyrosine. Regarding the soaking treatment, most amino acids were present at similar levels as in the unprocessed *S. latissima*, except for alanine and glutamate (higher levels, $p < 0.05$) and threonine, lysine, phenylalanine, and tyrosine (lower levels, $p < 0.05$).

The relative amount of essential amino acids (TEAA) ranked the biomasses in the following order: unprocessed (44.3 ± 0.5 %) < blanched 80 °C (42.4 ± 0.7 %) < soaked (41.4 ± 1.1 %) < blanched 45 °C (41.0 ± 0.3 %). The ratios differed significantly between the two highest and the two lowest values ($p < 0.05$). Previous studies have also found similar TEAA ratios for unprocessed *S. latissima*, which ranged from 42 to 47 % (Abdollahi et al., 2019; Harrysson et al., 2018; Nielsen et al., 2020). However, under identical processing conditions as in our study, i.e., the same time, temperature, and seaweed-to-processing water ratio, Nielsen et al. (2020) reported a significant increase in the TEAA ratio from 45.9 to 49.3 % after blanching at 80 °C and no statistical difference after blanching at 45 °C; the effect of soaking was not included in their study design. Our biomass was harvested in May on the Swedish west coast, whereas theirs was harvested in April on the Norwegian north coast. Therefore, different harvest location and time might explain why the TEAA ratio behaved differently between the studies.

Comparing the EAA profile of the initial biomasses with the FAO/WHO recommendation for adults revealed that only the blanched biomasses met the recommendation (WHO/FAO/UNU, 2007). This is because the histidine requirement (≥1.5 g/100 g protein) was only fulfilled in these biomasses, as opposed to the unprocessed and soaked *S. latissima* that showed a histidine content below 1.0 g/100 g protein. Other works have also reported histidine as a limiting amino acid in unprocessed *S. latissima* (Abdollahi et al., 2019; Nielsen et al., 2020).

3.2. Protein solubilities and yields during pH-shift processing

The maximum protein solubility and precipitability of all biomasses were at pH 12 and 2, respectively (**Supplementary Material: Fig. S2**), which agreed with the pH values previously reported for unprocessed *S. latissima* (Abdollahi et al., 2019; Harrysson et al., 2018). Interestingly, we were expecting an increase in the pI after blanching/soaking as both treatments resulted in biomasses with lower IS (**Section 3.1.1**) (Belitz, Grosch, & Schieberle, 2004). The expected shift in the pI was likely offset by the relative increase of alginate and fucoidan after blanching/soaking (**Supplementary Material: Table S2**) since anionic polysaccharides can push the pI downwards (Schwenzfeier, Wierenga, Eppink, & Gruppen, 2014).

Fig. 3 summarizes the protein solubilization yield, protein precipitation yield, and total protein yield obtained during pH-shift processing of unprocessed, soaked, and blanched *S. latissima* when using solubilization/precipitation pH at 12 and 2, respectively. Both soaking and blanching treatments negatively affected the protein solubilization yield ($p < 0.05$), with the blanched 80 °C biomass showing the lowest value, followed by the blanched 45 °C and soaked biomasses. Since blanching reduced the proportion of small molecular weight protein/peptides in the biomass (Fig. 1**a**), the lower protein solubilization yield could be explained by two hypotheses. The first one being the leaching of these soluble proteins/peptides into the blanching waters. This hypothesis could be confirmed by analysing the protein/peptide relative size

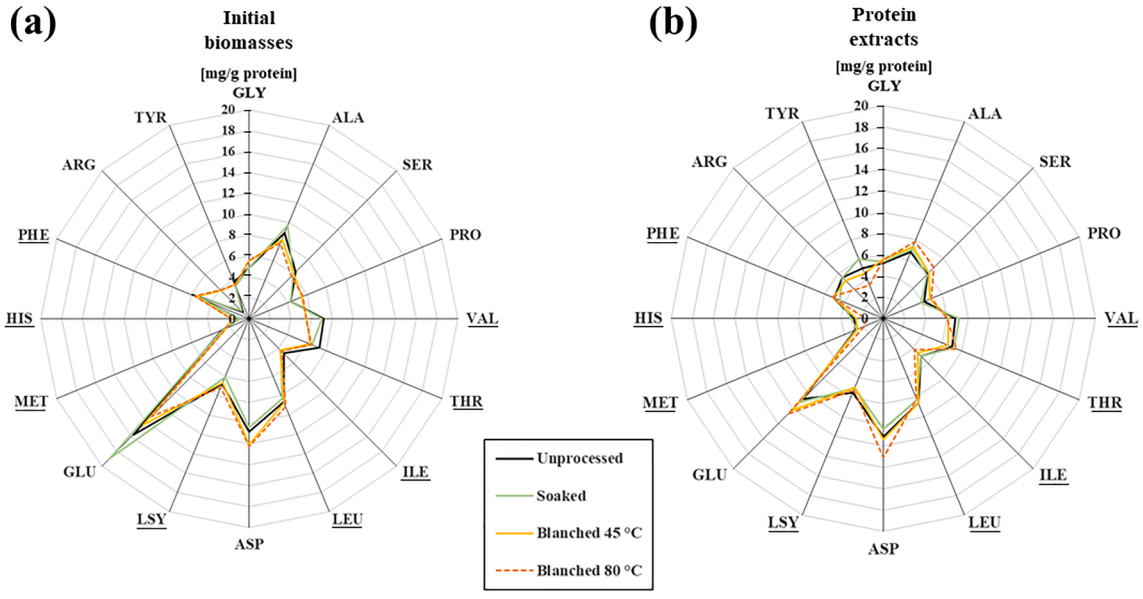

Fig. 2. Amino acid profile (mg/g protein) of unprocessed, soaked, and blanched *S. latissima* (a) and pH-shift protein extracts thereof (b). Essential amino acids are underlined.

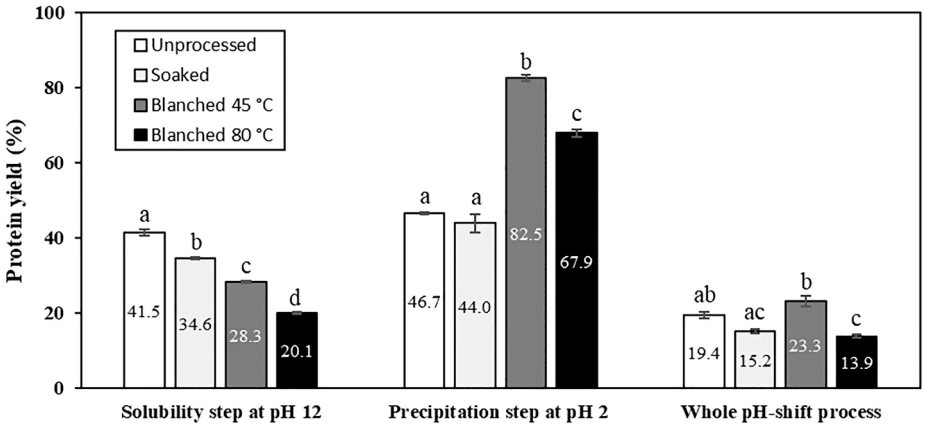

Fig. 3. Protein yields obtained during pH-shift processing of S. latissima biomasses. Values are shown as average ± SD and different letters (a-d) denote significant differences ($p < 0.05$) within each treatment.

distribution of these waters, albeit we would need to aliquot substantial volumes of sample as peptides would be highly diluted due to the 1:30 dilution (weight/volume) preceding the blanching. Secondly, it is also likely that blanching promoted the formation of water-insoluble protein aggregates. For example, Sashikala, Sreerama, Pratape, and Narasimha (2015) reported a significant increase in the relative proportion of water-insoluble protein aggregates after subjecting mung bean cultivars to boiling water.

Blanching the biomass at 45 °C significantly improved the protein precipitation yield during pH-shift processing by>35 percentage points, when compared to unprocessed and soaked biomasses ($p < 0.01$) (Fig. 3). The higher protein precipitation yield translated into a slightly higher total protein yield for this biomass compared to the unprocessed biomass ($p = 0.11$), and a significantly higher protein yield compared to the soaked and blanched 80 °C biomasses ($p < 0.05$). To better explain these results, we hypothesized that (i) blanching would inactivate endogenous proteases, thereby preventing the production of low-molecular-weight peptides that can be difficult to precipitate due to their high solubility and (ii) the dilution of salt during blanching at 45 °C reduced the chance of a "salting-in" scenario, thus facilitating protein precipitation. Regarding the first hypothesis, for *Saccharina* sp., the optimum pH for protease activity is between pH 7 and 9 (Ying, Xiao-Yu, Cun-Shuan, & Ji-Xun, 1998). Therefore, endogenous protease activity is unlikely to play a role in dictating the higher precipitation yields achieved with the blanched biomasses. However, no significant differences in the degree of hydrolysis (DH%) were detected before and after the osmotic shock step for all biomasses (**Supplementary Material: Fig. S3**); something that may be explained by the reduction of the native ionic strength when adding water in a 1:6 ratio (% w/v). Therefore, it is unlikely that endogenous protease activity played a role in dictating the higher precipitation yields achieved with the blanched biomasses. The second hypothesis is based on the assumption that in the IS range 0.3–100 mM NaCl eq., protein solubility declines with decreasing salt concentrations due to the promotion of electrostatic protein–protein interactions (Belitz et al., 2004; Stefansson & Hultin, 1994). Thus, we dialyzed the first supernatant (S1) obtained during pH-shift processing of unprocessed biomass using a buffer solution at pH 2.5 with a final IS adjusted to 44 mM. This was the same IS found for S1 from the biomass blanched at 45 °C. As shown in Table 3, dialysis significantly increased protein precipitation by 17.7 percentage points ($p < 0.05$) compared to direct acidification, which yielded an IS of 106 mM. As a result of this increase, total protein yield was also improved by 6.1 percentage points, though not statistically significant ($p = 0.082$). In an industrial setting, adopting a freeze-thawing cycle to improve protein precipitation can jeopardize the economic sustainability of the pH-shift process. Thus, we also evaluated the effect of dialysis without this step. Overall, dialysis per se reached similar ($p > 0.05$) precipitation yield and total yield as the pH-shift process with a freeze-thawing cycle (Table 3), which suggests a dialysis step can replace this cycle.

Compared to other studies that followed a similar protein extraction protocol on *S. latissima*, the total protein yield obtained with the blanched 45 °C biomass is among the highest reported and is comparable to the yields achieved when using unprocessed freeze-dried *S. latissima* as starting material (yields of 25–26 % vs 23 % in our study) (Abdollahi et al., 2019; Harrysson et al., 2018; Veide Vilg & Undeland, 2017). Overall, these results contradict with our initial hypothesis that blanching would yield less suitable biomasses for protein extraction purposes. We have also demonstrated that the choice of blanching temperature greatly influences the total protein yields. Therefore, we consider further studies are warranted aiming at finding

Table 3
Relationship between ionic strength (IS) and protein precipitation yield of unprocessed *S. latissima* biomass. The effect of freeze-thawing aided protein precipitation was also studied with or without dialysis. Values are shown as average ± SD.

	Yields of pH-shift processing		IS (mM NaCl eq.)		
	Precipitation yield at pH 2.5 (%)	Total yield (%)	Supernatant 1 at pH 12	Supernatant 1 after dialysis	Supernatant 1 after HCl addition
pH-shift					
No freeze-thawing	**21.0** ± 3.7^{a}	**7.7** ± 1.8^{a}	82	–	106
With freeze-thawing	**42.4** ± 0.6^{b}	**14.5** ± 0.2^{ab}	82	–	106
pH-shift with dialysis*					
No freeze-thawing	**40.3** ± 2.5^{b}	**14.7** ± 1.7^{ab}	82	43	–
With freeze-thawing	**60.1** ± 0.1^{c}	**20.6** ± <0.1^{b}	82	43	–

Letters (a-c) denote significant differences ($p < 0.05$) within each column; *Dialysis of supernatant 1 at pH 12 against citric acid/sodium phosphate buffer at pH 2.5 adjusted with 20 % NaCl to an IS of 44 mM (same IS as supernatant 1 of blanched 45 °C biomass).

the best time/temperature combination that simultaneously maximizes protein yield and minimizes levels of non-essential elements and iodine in the final protein extract. Moreover, further work is required to improve total protein yields when pH-shift processing seaweed since values are still lower compared to e.g., soy protein isolation. For instance, Mendez and Kwon (2021) improved protein solubility up to 3-fold when subjecting the red seaweed *Devaleraea mollis* to the Osborne protein fractionation approach. The authors also observed a significant increase in protein yield when combining this approach with polysaccharide-degrading enzymes (Mendez & Kwon, 2021).

3.3. Protein extract characterization

3.3.1. Chemical composition

Table 1 depicts the dry matter, ash, total amino acids, and total monosaccharides of protein extracts from unprocessed, soaked, and blanched *S. latissima*. Overall, pH-shift processing reduced ash levels in protein extracts from unprocessed and blanched biomasses ($p < 0.05$). Moreover, the two protein extracts from blanched biomass showed the lowest ash levels ($p < 0.05$), most likely due to these biomasses' already low ash levels. The highest TAA content was detected in protein extracts from unprocessed and blanched 45 °C biomasses, with around 20 % DW of each extract being amino acids. Additionally, the up-concentration of TAA after pH-shift-based extraction was 4.2-, 2.6-, 2.1-, and 1.2-fold for protein extracts from unprocessed, soaked, blanched 45 °C, and blanched 80 °C biomasses, respectively ($p < 0.05$). Other publications on pH-shift-based protein extraction from *S. latissima* have reported up to 5.0-fold protein up-concentration and a protein purity ranging from 19 to 41 % DW (Abdollahi et al., 2019; Harrysson et al., 2019). Although the purity of protein extracts from unprocessed and blanched 45 °C biomasses falls within this range, the values are still lower when compared to pH-shift protein extracts with > 90 % protein from other vegetarian sources, e.g., soybean (Keerati-u-rai & Corredig, 2011). The monosaccharide analysis revealed that pH-shift processing significantly reduced total monosaccharide content ($p < 0.05$). However, this fraction was still predominant in our protein extracts, with values ranging from 34 to 52 % of the total DW. Interestingly, we detected high fucose levels in all protein extracts (9–15 % of the total DW versus 2–3 % in the initial biomasses), thus suggesting co-extraction of fucoidan during pH-shift processing (**Supplementary Material: Table S2**). Fucoidan is a sulfated polysaccharide known to possess several bioactivities (Holdt & Kraan, 2011). Hence its presence in *S. latissima* protein extracts could provide a basis for novel and multifunctional protein ingredients.

Table 2 shows the elemental composition of all protein extracts produced. After pH-shift protein extraction, total arsenic levels varied depending on which type of initial biomass was processed. For instance, only in the blanched biomasses the total arsenic content significantly decreased ($p < 0.05$). Inorganic arsenic, a known arsenic toxic species, accounted for 0.1–0.2 % of the tAs. No evident changes in iAs levels appeared to occur after protein extraction. Regarding cadmium, all protein extracts except those from the soaked biomass had lower levels when compared to their respective biomass ($p < 0.05$). Lead was extensively concentrated after pH-shift processing regardless of the sample type ($p < 0.05$). No significant differences were found in the remaining non-essential elements.

Generally, pH-shift processing increased the contents of cobalt, copper, iron, and molybdenum ($p < 0.05$). For the remaining essential elements, no clear trend was detected after protein extraction since the effect of the different biomass treatments was more prominent. For instance, when compared to the corresponding biomass, protein extract from the blanched 45 °C biomass had slightly higher levels of iodine (2.6-fold) and zinc (1.2-fold, $p < 0.05$) and similar levels of selenium. Conversely, the unprocessed biomass resulted in a protein extract with similar content in selenium, iodine, and zinc.

Based on the guidelines established by EFSA for iAs, Cd, Pb, Cu, I, Se, and Zn, we calculated the amount of protein extracts that an adult can safely consume (EFSA, 2006, EFSA, 2009a, EFSA, 2009b, EFSA, 2013). The results from all calculations can be found in **Supplementary Material: Table S3**. In summary, iodine limited the consumption of protein extracts to amounts between 0.1 and 0.5 g DW, where the upper range corresponded to protein extracts from blanched biomasses. Lead was the second most-limiting element, although the amounts needed to attain its tolerable daily intake were substantially higher than those from iodine – between 29 and 40 g DW. Therefore, the relative high iodine levels in all protein extracts alongside low protein purities may compromise their use as food protein ingredients. Appropriate bioavailability trials need to be conducted on the iodine issue to assess how much of this element reaches the systemic circulation. Until now, human intervention studies reported an iodine bioavailability ranging from 57 to 71 % for *Saccharina* species (Blikra, Henjum, & Aakre, 2022).

3.3.2. Protein/peptide relative size distribution

The protein/peptide relative size distribution of the four protein extracts is represented in Fig. 1. pH-shift processing of the unprocessed, soaked, and blanched 45 °C biomasses increased the relative proportion of the > 670 kDa fraction ($p < 0.05$) up to 62 %. We have previously reported a similar finding for pH-shift protein extracts from unprocessed *Ulva fenestrata* (Juul et al., 2021; Trigo et al., 2021), supporting the hypothesis that pH-shift processing likely promotes cross-linking reactions between proteins and/or selectively recovers high molecular weight proteins. Interestingly, for the fraction > 670 kDa, protein extracts from blanched 80 °C showed a similar relative proportion as was found in its original biomass (Fig. 1). This can be attributed to partial sedimentation of soluble protein aggregates during the first centrifugation step. That specific polypeptides sediment in the first centrifugation step when subjecting seaweed to pH-shift processing was recently also demonstrated by Juul et al. (2021). The fraction 100–670 kDa was no longer visible in any of the protein extracts, possibly due to aggregation of 345 and 134 kDa proteins with the > 670 kDa fraction or because these fractions were not recovered using pH-shift processing (Fig. 1**b-c**). Similarly, the fraction 10–100 kDa was not detected in protein extracts from unprocessed and soaked biomasses, even though these biomasses exhibited peaks at 73 and 25 kDa. For protein extracts from blanched 45 °C biomass, the only peak in the fraction 10–100 kDa was found at 60 kDa, while its biomass showed peaks at 73 and 25 kDa. Likewise, protein extract from blanched 80 °C biomass showed two new peaks at 47 and 14 kDa and the total relative proportion of this fraction increased from 14.3 to 60.2 %. The proportion of low molecular weight fractions (i.e., 1–5 and < 1 kDa) decreased in all protein extracts, most likely due to the removal of soluble small peptides in the second centrifugation and/or tentative cross-linking reactions. Overall, our results suggest that the choice of soaking/blanching temperature (e.g., 12, 45, or 80 °C) profoundly affects the molecular weight profile of the final protein extracts. This finding can be particularly relevant when tailoring specific functional properties, such as the emulsification capacity of the protein extracts (Abdollahi et al., 2019).

3.3.3. Amino acid profile

Fig. 2**b** shows the AA profile of the pH-shift protein extracts produced from the different biomasses. The extract from blanched 45 °C biomass, which had the most concentrated AA, presented higher relative contents of proline and glutamate and lowered relative contents of valine, lysine, and tyrosine compared to protein extracts from the unprocessed biomass ($p < 0.05$). Furthermore, comparing the protein extracts from blanched 45 °C and blanched 80 °C biomasses, the former sample showed higher relative levels of methionine, histidine, arginine, tyrosine, and aspartate ($p < 0.05$). In contrast, the only AAs being most abundant in the blanched 80 °C extract were serine and threonine ($p < 0.05$). A comparison between extracts from blanched 45 °C and soaked biomasses revealed that proline, phenylalanine, tyrosine, and aspartate were present in relatively higher contents in the former ($p < 0.05$). On the other hand, protein extracts from soaked biomass had higher relative contents

of valine, threonine, and histidine ($p < 0.05$). The effect of the pH-shift processing on the AA profile has already been investigated for different seaweed species (Harrysson et al., 2018) and seaweed post-harvest stabilization methods (Abdollahi et al., 2019), but so far not for thermally treated and soaked seaweed. We found that the effect of pH-shift processing on the AA profile was identical for 13 out of the 16 quantified AAs, when comparing extracts from unprocessed and blanched 45 °C biomasses. The AAs that responded differently were proline, valine, and methionine. When comparing extracts from unprocessed and soaked biomasses, 11 out of 16 AAs followed similar trends. Conversely, comparing extracts from unprocessed and blanched 80 °C biomasses revealed that only 4 out of 16 AAs (glycine, valine, isoleucine, phenylalanine) responded similarly.

The TEAA ratios of protein extracts from the four biomasses followed the order unprocessed (45.5 ± 0.2 %) > soaked (45.0 ± 0.5 %) > blanched 45 °C (43.1 ± 0.4 %) > blanched 80 °C (42.0 ± 0.4 %); with significant differences between the highest and the two lowest values ($p < 0.05$). The above values fall between the TEAA ratios reported for soy (40 %) and egg (48 %) as well as between *S.latissima* derived pH-shift extracts reported elsewhere (43–49 %) (Abdollahi & Undeland, 2018; Abdollahi et al., 2019; Harrysson et al., 2018). Moreover, the pH-shift processing significantly increased the TEAA ratio on the soaked and blanched 45 °C samples ($p < 0.05$). All protein extracts complied with the WHO/FAO recommendations for adults. Moreover, if potential health-related problems due to harmful elements are disregarded, an adult with 63.3 kg body weight would need to consume 143 g, 209 g, 135 g or 279 g DW of dried protein extracts from unprocessed, soaked, blanched 45 °C, or blanched 80 °C biomasses, respectively, to cover the recommended daily intake of EAAs (WHO/FAO/UNU, 2007).

Summing up, the selectiveness of the pH-shift process for different AA depended on the temperature at which the *S. latissima* biomass was pre-treated. Despite the distinct AA selectiveness, all four protein extracts ultimately showed a complete EAA-profile. Moreover, to fulfill the WHO/FAO recommendations, one would need to consume relatively lower amounts of extracts derived from blanched 45 °C or unprocessed biomasses compared to those derived from soaked and blanched 80 °C biomasses.

4. Conclusion

In this study, we aimed to assess the effect of blanching (at 45 and 80 °C) and water soaking (12 °C) on the yield of proteins recovered after pH-shift processing of *S. latissima*. We also examined the influence of blanching, soaking, and pH-shift processing on the protein/peptide size distribution and chemical composition, including essential and non-essential elements, of biomasses and protein extracts thereof. Based on our findings, only blanching at 45 °C retained similar protein yields to the unprocessed biomass. Despite similar yields, blanching at 45 °C decreased protein solubilization yield at pH 12 but increased isoelectric protein precipitation yield. Analysis of the protein/peptide size distribution revealed a higher ratio of large peptides/proteins in the blanched biomass, which could explain the lower protein solubilization yield. Also, we confirmed a link between lower ionic strength after blanching and increased protein precipitation. Moreover, compared to the pH-shift protein extract from unprocessed *S. latissima*, blanching at 45 °C resulted in a protein extract with similar amino acid levels. In order to improve protein extraction yield and amino acid levels, we suggest further work should focus on aspects such as (i) finding the best binomial time–temperature for blanching (ii) interactions between polysaccharides and proteins, (iii) the pronounced protein heterogeneity in terms of solubility, and (iv) the intrinsic high ionic strength of seaweed, which creates an unwanted salting-in scenario during protein precipitation.

Regarding elemental composition, the two blanched biomasses and protein extracts thereof presented the lowest iodine content with around 49 % total removal after the pre-treatment and pH-shift processing. Nevertheless, iodine was still the element limiting consumption at higher amounts of all biomasses and respective proteins extracts. Non-essential elements such as cadmium, lead, and nickel were concentrated after blanching treatments. Analysis of the monosaccharide composition revealed that such up-concentrations were probably partly due to an increased proportion of alginate after blanching. A side finding from the monosaccharide analyses was that fucose was up-concentrated, indicating co-extraction of fucoidan.

Overall, we recommend mild blanching temperatures if *S. latissima* is used for protein extraction. This is because it retains the original protein yields and total amino acid content while minimizing iodine levels in the final protein extracts.

CRediT authorship contribution statement

João P. Trigo: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization. **Kristoffer Stedt:** Resources, Writing – review & editing. **Alina E.M. Schmidt:** Resources, Investigation, Visualization, Writing – review & editing. **Barbro Kollander:** Resources, Investigation, Visualization, Writing – review & editing, Funding acquisition. **Ulrica Edlund:** Resources, Supervision, Writing – review & editing. **Göran Nylund:** Resources, Supervision, Funding acquisition. **Henrik Pavia:** Resources, Supervision, Writing – review & editing, Supervision, Funding acquisition. **Mehdi Abdollahi:** Conceptualization, Methodology, Validation, Resources, Writing – review & editing, Supervision, Funding acquisition. **Ingrid Undeland:** Conceptualization, Methodology, Validation, Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2022.134576.

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