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## **Future Foods**



## A step towards closing the food-waste gap in novel protein sources: Post-harvest protein boost of the seaweed crop *Ulva* by herring production tub water

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## ABSTRACT

Sea lettuce (*Ulva*) species have been identified as a future protein source, and post-harvest techniques, including the sustainable integration of liquid food side streams, have been developed to further increase their protein content and commercial market value. This study explores the post-harvest treatment of commercially produced seaweed biomass from large-scale, sea-based cultivations of *U. fenestrata* with residual water streams emerging from industrial storage of herring – so called herring production tub water (TUB). Growth rates of *U. fenestrata* were significantly higher in TUB treated seaweeds compared to controls. Further, the crude protein content was 71.26 % higher when cultivated in TUB, compared to controls, reaching a crude protein content of  $37.37 \pm 1.83$ % dry weight. Notably, there were no limiting amino acids, nor fish-related allergenic activity in the seaweed biomass. Our study demonstrates a new nutrient loop turning food waste into protein-rich biomass by applying sustainable seaweed cultivation.

#### 1. Introduction

Future predictions show that terrestrial crop vields will reach insufficient production by 2050 to cover global food demands (Ray and Foley, 2013). At the same time, it is predicted that relieving the pressure from agricultural expansion through methods such as mariculture could be a feasible and suitable option for increasing food production on a larger scale (Duarte et al., 2009). Especially, foodstuffs based on seaweeds are currently being discussed as a sustainable protein resource (Kazir et al., 2019; Pliego-Cortés et al., 2020; Juul et al., 2021; Steinhagen et al., 2021; Trigo et al., 2021; Stedt et al., 2022d; Steinhagen et al., 2022a, 2022b) that does not compete with existing terrestrial crops and could meet the needed agricultural expansion by reducing land-use pressures (Steinhagen et al., 2021; Spillias et al., 2023). Seaweeds can also fill an important niche in the current protein shift from red meat to vegetarian/vegan protein sources based on their unique sensory properties (e.g., umami-rich taste and marine flavor) and their content of macro- and micronutrients often lacking, or being low, in a vegetarian diet, e.g. long chain n-3 polyunsaturated fatty acids (LC n-3 PUFA) and vitamin B12 (Holdt and Kraan, 2011; Fleurence et al., 2012;

Mæhre et al., 2014; Machado et al., 2020). The huge growth within the area of vegan, plant-based proteins has recently stagnated to some extent (Neuhofer and Lusk, 2023), due to e.g. sensorial and nutritional challenges (Mayer Labba et al., 2022a, 2022b). This calls for new tasty vegan food alternatives to create a needed diversification within this food category.

Seaweeds contain all essential amino acids together with minerals, antioxidants, vitamins, dietary fibers, and fatty acids (Holdt and Kraan, 2011; Abdollahi et al., 2019; Stedt et al., 2022c), which make them ideal for the food industry. However, the average total protein content of most seaweeds (9–22 % dry weight (dw)) is - with the exception of a selection of some red seaweed species that exhibit higher average protein contents (30–47 % dw) (Rawiwan et al., 2022) – in several cases not competitive with terrestrial plant protein sources such as pea (20–30 % dw) or soybean (33–45 % dw) (Grieshop and Fahey, 2001; Holdt and Kraan, 2011; Meng and Cloutier, 2014; Trigo et al., 2021). Currently, green seaweeds of the genus *Ulva*, widely known as sea lettuce, are receiving considerable attention in aquaculture. *Ulva* representatives are used by the aquaculture sector due to their many beneficial traits such as high productivity, environmental tolerance (Bolton et al., 2016; Nardelli

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et al., 2019; Steinhagen et al., 2019, 2021, 2023), and their efficient bioremediation of nutrients (Sode et al., 2013; Al-Hafedh et al., 2015; Neveux et al., 2018). However, the biochemical composition of *Ulva* changes depending on the prevailing abiotic factors and cultivation conditions (Toth et al., 2020; Stedt et al., 2022b; Steinhagen et al., 2022a, 2022b), which also strongly affect the protein content. Therefore, the biomass value and its economic applicability in the food sector varies with the applied conditions during the cultivation period (Steinhagen et al., 2022a, 2022b) and seedling nursery (Steinhagen et al., 2021, 2022b).

In previous studies, we showed that the quality and quantity of the Northern Hemisphere crop Ulva fenestrata Postels and Ruprecht cultivated in an offshore seafarm depend on the harvest season (Steinhagen et al., 2022a). Furthermore, we found that a circular approach of short-term post-harvest treatment with food production process waters from herring industries significantly increased the protein and total amino acid content in the seaweed biomass (Stedt et al., 2022c), thereby increasing the commercial value of the biomass for the food market. Such post-harvest treatments increased the protein and amino acid content three to five times, while heavy metal contents did not exceed health-based reference points of official food standards (Stedt et al., 2022a, 2022c). Furthermore, Stedt et al. (2022c) found no sensory attributes regarded as negative after cultivation in the food production process waters. This makes the cultivation technique valuable for supporting a circular food production system as it optimizes the output of sustainable protein resources while closing important production cycles to mitigate food waste.

Circular food production and low ecological footprints of protein sources are the core of future food systems that aim to eliminate food loss and waste and associated waste side streams, such as food production waters (Vilariño et al., 2017). Integrating food side streams into food production - which for long have been considered waste products with expensive disposal fees - therefore becomes a central part of sustainable, circular food production systems (Schieber, 2017). However, even though the economic relevance of side streams as a source of valuable compounds has been highlighted in several studies, their industrial utilization is only realized in a few examples (Schieber, 2017). Similar to the optimization of circular food production processes, the downstream processing e.g. the extraction of protein from green seaweeds can foster the sustainable growth of the future protein industry (Juul et al., 2021; Trigo et al., 2021). However, it is important to assess the allergen activity in biomass treated with food production waters to manage allergen risks, ensure consumer safety, and inform labelling practices when aiming for commercial application of the process. Nevertheless, there are only a few studies that evaluate the presence and potency of, for instance, fish allergens in seaweed biomass, highlighting the urgency of conducting such assessments to enhance allergen management and regulatory compliance in subsequent food production stages.

The aim of the present study was to determine if herring production tub water (TUB) can be applied as a short-term, post-harvest treatment to further boost the protein content of the commercially cultivated crop *U. fenestrata*, harvested from sea-based cultivations during its natural peak protein content (March/April) (see also Steinhagen et al., 2022a). Through this, we expect to contribute higher protein yields in *U. fenestrata* for subsequent downstream application of the biomass in the food value chain and aim to integrate previously regarded food waste as a viable circular resource. During a 14 day post-harvest experiment with TUB, we measured growth, crude protein content, amino acid profile, and tested the fish allergen activity of the *U. fenestrata* biomass.

#### 2. Materials and methods

#### 2.1. Seaweed source material and taxonomic identification

Seaweed material was harvested from a commercial sea-based seaweed farm of the company Nordic Seafarm, located at the Bohuslän coastline (Skagerrak), Sweden (N 58.64271, E 11.216433). Seedlings of *U. fenestrata* were transplanted to the seafarm in September 2022. The area of the cultivation site is characterized by rocky shores which are typical for the Scandinavian west coast of Sweden and Norway, and it is one of the most biodiverse marine areas in Sweden. For mean salinity of the sea surface and mean temperatures of the region, see Steinhagen et al. (2021). For a detailed schematic representation of an *Ulva* seafarm and detailed handling of processes during cultivation see also Steinhagen et al. (2021). Individuals of *U. fenestrata* were collected from the seaweed farm in early spring conditions on the 27th of February 2023 and had a mean crude protein content of  $21.83 \pm 1.25$  % dw (mean  $\pm$  SD).

Molecular identification of the seaweed source material – which originated from a previous long-term cultivation of the identical gametophytic clonal strain at the Tjärnö Marine Laboratory – has been described in detail by Toth et al. (2020) (GenBank accession numbers: MN240309, MN240310, MN240311).

#### 2.2. Herring production tub water

The herring production tub water used in the experiment was collected in a food-grade state in October 2022. The water originated from in-house storage of whole herring in tubs from a primary industrial processor (TUB). A detailed explanation of TUB can be found in Stedt et al. (2022d). To remove coarse particles (>300  $\mu$ m), the TUB was filtrated and subsequently stored at -60 °C.

The total ammonium (NH<sup>+</sup><sub>4</sub>) and inorganic phosphorus (P) contents of TUB were analyzed using commercial enzymatic kits, as outlined in Stedt et al. (2022d), while the inorganic phosphorus/orthophosphate content was analyzed with a standard curve made with monopotassium phosphate, as reported by Qvirist et al. (2015) (Table 1). When used in the experiment, the TUB was diluted with filtered (0.2  $\mu$ m+UV-light treated) deep-sea (40 m) seawater to 25  $\mu$ M NH<sup>+</sup><sub>4</sub>.

#### 2.3. Experimental setup

The experiments included the TUB, as well as a control of 1:100 Provasoli Enriched Seawater (PES), known to be an effective growth media for seaweeds (Provasoli, 1968). The treatments were set up in triplicates. The *U. fenestrata* collected from the commercial seaweed farm were placed in 45 L tanks at 12 °C, under 16:8 h (L:D) light cycle, and at an irradiance of 90–110  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>. The starting biomass at the beginning of the experiment was set to 500 g fresh weight (fw) per tank (12 g fw L<sup>-1</sup>). The experiment was conducted for 14 days, starting on the 28th of February 2023. The respective nutrient solution was added every second day to avoid nutrient depletion. No water exchange was needed during the experimental period, as no detrimental microbial degradation was observed. Salinity and pH (WTW MultiLine 3420, Xylem Analytics) remained stable throughout the experiment in all treatments (32.8  $\pm$  0.9PSU and 8.0  $\pm$  0.4, respectively, mean  $\pm$  SD), and no

#### Table 1

Characterization of undiluted TUB (mean  $\pm$  SEM, n = 3) collected in October 2022, and its corresponding dilution factor to reach 25  $\mu$ M NH<sup>+</sup><sub>4</sub>.

Herring Production Process water	Ammonium (µM NH <sub>4</sub> )	Inorganic phosphorus (µM P)	Dilution factor 25 µM NH4+
TUB	$2027.8 \pm 218.1$	$\textbf{8745.2} \pm \textbf{141.8}$	81

additional adjustments were necessary. At the start of the experiment, random tissue samples were collected to analyze the crude protein content. At the end of the experiment, the total fw, crude protein content, amino acid profile, and fish allergen content of the seaweeds were measured.

#### 2.4. Growth and crude protein content

The fresh weight (fw) in each tank was determined on an analytical balance (Sartorius TE1502S, Göttingen, Germany) after excess water had been removed using a salad spinner, following a standardized protocol. Random tissue samples were collected from each tank, frozen, lyophilized (24 h), homogenized to a fine powder, and stored at -60 °C before further analysis.

The total nitrogen content was determined by combustion using a GSL elemental analyzer coupled to an isotope-ratio mass spectrometer (EA-IRMS, 20 - 22, Sercon Ltd., Crewe UK). Subsequently, the crude protein content was estimated based on the nitrogen-to-protein conversion factor of 5 for seaweeds (Angell et al., 2016).

## 2.5. Amino acid composition and allergen activity

Analyses of amino acid profiles and fish allergen content of *U. fenestrata* were performed by the commercial provider Eurofins Food & Feed Testing Sweden AB (ISO 13,903:2005 /IC-UV & ISO/IEC 17,025:2018). The method for analysis of amino acids cannot recover tryptophan, while glutamine and asparagine are co-determined with glutamic and aspartic acid, respectively. Amino acid profiles were generated in triplicates on both PES controls and TUB treatments, whereas fish allergen activity was determined on pooled samples of the respective treatments by the application of standardized molecular DNA markers.

#### 2.6. Statistical analysis

Data on growth, protein, and amino acid content of input biomass and post-harvest treated *U. fenestrata* were statistically analyzed in JMP (JMP®, Version 15, SAS Institute Inc., Cary, NC, USA) using analysis of variance (ANOVA). The effect of post-harvest treatment was analyzed for each variable using a one-way ANOVA with the respective postharvest treatment (PES, TUB) and the initial starting point (Start) as a two or three level, fixed factor. Significant differences among means were compared using Tukey's HSD test. All data were visually checked for homogeneity and normality with diagnostic plots (density-, normality- and QQ-plots).

#### 3. Results

#### 3.1. Growth and crude protein content

The effect of TUB treatment on seaweed growth was significantly higher (p < 0.005) compared to the PES control treatment (Table 2). After 14 days of post-harvest treatment, the biomass had increased by  $20.3 \pm 0.8$  % in the control treatments supplemented with PES, whereas the TUB treatment yielded a 22.9  $\pm$  0.3 % (mean  $\pm$  SD) increase (Fig. 1A).

After 14 days the crude protein content of the seaweeds showed a statistically significant increase in both treatments, compared to the starting point at harvest, with a significantly stronger effect in the TUB treatment compared to PES (p < 0.001) (Table 2). The protein content increased from  $21.8 \pm 1.3$  % dw at harvest, to  $26.8 \pm 0.5$  % dw in the control PES treatment and to  $37.4 \pm 1.8$  % dw in the post-harvest TUB treatment (mean  $\pm$  SD) (Fig. 1B). To conclude, the TUB post-harvest treatments increased the crude protein content of the biomass by an average of 71.3 %, whereas the PES treatment increased the crude protein content by 22.7 %.

## 3.2. Amino acid composition

The amino acid profiles of the *U. fenestrata* biomass before the treatment and after 14 days of the respective post-harvest treatment (PES or TUB) are presented in Table 3. Generally, the total amino acid (TAA) content of the seaweed biomass after the post-harvest treatments (PES 24.7  $\pm$  1.0 and TUB 29.5  $\pm$  0.6 % dw) was significantly higher than compared to the biomass at the start of the experiment (21.5  $\pm$  0.2 % dw, mean  $\pm$  SD) (p < 0.001) (Tables 2, 3). Furthermore, the TAA were significantly higher in the TUB treatment than in the PES treatment (p < 0.001) (Table 2).

The total essential amino acid (TEAA) content of the input biomass at the start of the experiment was  $36.9 \pm 0.1$  % of TAA, whereas the biomass had a TEAA content of  $37.9 \pm 0.5$  % in the PES and  $37.2 \pm 0.3$  % of TAA in the TUB treatment (mean  $\pm$  SD, n = 3). No significant differences in TEAA contents between the treatments were detected (p = 0.146). Based on the WHO/FAO/UNU (2007) requirements, there were no limiting amino acids in the seaweed biomass used in this study, and critical values of TEAA in freshly harvested and post-harvest treated seaweed biomass exceeded critical reference values (Table 3).

#### 3.3. Allergens

Analyses to verify a potential allergenic effect of the post-harvest treatment with TUB and PES on the seaweed biomass were negative. Specific DNA from fish as a marker for potentially allergenic material has not been demonstrated in this study. The detection level corresponds to 0.001–0.005 g of fish allergen per 100 g of sample.

#### Table 2

One-way ANOVA of (A) fresh weight (g) of *Ulva fenestrata* after 14 days of post-harvest treatment with either the control PES or herring production process water TUB, and (B) the crude protein content at the start of the experiment and after 14 days of post-harvest treatment with either PES or TUB, (C) the total amino acid content at the start of the experiment and after 14 days of post-harvest treatment with either PES or TUB, in the experiment amount the PES or TUB treatments. Significant *p*-values are indicated with italics. Data are visualized in Fig. 1 and all data are available in the supplement.

	A.Fresh weight (g)				B.Crude protein content (% dw)			
Source of Variance	DF	MS	F	р	DF	MS	F	р
Treatment Residual	1 4	261.36 34.03	30.71	< 0.005	2 6	189.00 10.46	108.44	< 0.001
	C.Total amino acids (% dw)							
	C.Total an	nino acids (% dw)			D.Total e	essential amino acio	ds (% dw)	
Source of Variance	C.Total an DF	nino acids (% dw) MS	F	р	D.Total e	essential amino acio MS	ds (% dw) F	р



**Fig. 1.** (A) Mean fresh weight (g) per experimental tank of the seaweed biomass after 14 days of post-harvest treatment with either the control PES or herring production process water TUB (start input biomass per tank: 500 g), and (B) crude protein content (% dw) of *Ulva fenestrata* at the start point of the experiment and after 14 days post-harvest treatment in PES and TUB. Error bars show SD, n = 3, and capital letters above bars show significant differences between means based on Tukey's HSD test (p < 0.005).

#### 4. Discussion

The nutritional value of a foodstuff is strongly determining its market value. Seaweeds are currently increasing in the European food and feed markets and attract many commercial businesses by their various highvalue exploitable compounds (van den Burg et al., 2019). Previous studies have highlighted that seaweed biomass quality and quantity often show a trade-off effect during cultivation processes (Steinhagen et al., 2022a). The biochemical profiles of Ulva biomass for example show strong dependencies on environmental factors and hence seasonal conditions (Steinhagen et al., 2022a). Therefore, the harvest time of the biomass is strongly interdependent on its downstream application purpose. To optimize shelf-life, biomass conditions, and biochemical profiles of seaweeds, various post-harvest approaches have been highlighted in recent years (Paull and Chen, 2008; del Olmo et al., 2020; Stedt et al., 2022c). Innovative techniques involve the application of side streams from food production systems, e.g. processing waters, which after being used in the production of the primary food source is discarded as waste connected to significant costs (Nghiem et al., 2017). A recently developed technique for the value-adding post-harvest treatment of the commercially interesting green seaweed Ulva involves such food production process waters and provides a sustainable technique to help reduce food waste (Stedt et al., 2022a, 2022c).

Our work reveals that post-harvest treatment using herring production process water (TUB), which is typically treated as waste, can enhance the nutritional quality of the Northern Hemisphere crop U. fenestrata. Specifically, the treatment elevates the crude protein content and essential amino acids beyond the naturally occurring levels. The results demonstrate that the post-harvest treatment of the crop Ulva with TUB has a high potential to minimize the food waste gap. We confirm this with five lines of evidence including that (1) the growth rate of Ulva was significantly higher when treated with TUB compared to the PES control, (2) the crude protein content of Ulva cultivated in TUB was significantly higher compared to PES controls and increased by 71 % (from 21.8  $\pm$  1.3 % dw, to 37.4  $\pm$  1.8 %) in comparison to when harvested, (3) the total amino acids were significantly higher after postharvest treatment and highest in biomass treated with TUB, (4) the Ulva biomass contained all the essential amino acids in the required amounts recommended by WHO (tryptophan not measured), and (5) no fish-related allergenic effects could be detected in the biomass. Combined, our results reveal the increased market value of Ulva biomass which has been post-harvest treated by a currently wasted nutrient resource, paving the way for a new circular production route of vegan protein, contributing to closing the food waste gap.

Reducing food loss and waste can lead to more efficient use of resources, improved management of water resources, and fewer environmental consequences, which could positively impact climate change and livelihoods (Kibler et al., 2018). Developing novel food sources and integrating food process side streams into production systems to optimize quality and quantity may help pave the way toward more sustainable and circular food systems. Our study shows that Ulva biomass, which is rapidly developing in crop systems in Europe (Califano et al., 2020; Steinhagen et al., 2021, 2022a, 2022b), benefits from such food side stream integration. This not only provides a means to contribute to closing the food waste gap but also imparts higher market value to the biomass e.g. through added protein content. Presuming the consistency of the data generated and using a dw:fw ratio of 1:7, harvesting 1 hectare (ha) from a commercial sea-based seaweed farm that produces 4 t fw  $ha^{-1}$  in early spring (personal communication, Nordic Seafarm), and post-harvest cultivating it with TUB, could increase the biomass to 4.9 t fw in 14 days, while simultaneously binding 28 kg N from the water. If performed in a raceway pond that is 0.5 m deep, a total area of <0.1 ha is needed (assuming start density 12 g/ fw L<sup>-1</sup>). This concept could complement year-round land-based cultivation at the industries as discussed in Stedt et al. (2022a) by yielding biomass tailored for high protein content. However, on-site studies in industrial settings with a continuous supply of process water are needed to investigate and evaluate the bioremediation efficiency of the cultivation systems. Nevertheless, the results indicate that the concept can provide both economic and environmental benefits to industries.

To our knowledge, this study provides the highest crude protein values  $(37.37 \pm 1.83\% \text{ dw})$  achieved in *Ulva* biomass when compared with previous studies (5 %-30 %) (Holdt and Kraan, 2011; Nielsen et al., 2012; Steinhagen et al., 2021; Stedt et al., 2022a; Steinhagen et al., 2022a). The recent trend for vegan and vegetarian diets has significantly increased the demand for alternative proteins (Ismail et al., 2020; Faber et al., 2022), making novel crops such as sea lettuce with high protein content especially valuable for the food market. Additionally, the *Ulva* biomass, when post-harvest treated with TUB, demonstrates promising commercial applicability due to its relatively high content of umami-enhancing components such as glutamic and aspartic acid (Figueroa et al., 2022). The build-up of proteins from cultivation in TUB also resulted in biomass with a higher content of total amino acids, compared to at the start of the experiment, or when cultivated in PES.

#### Table 3

Amino acid profiles (g 100 g protein<sup>-1</sup>) of dried biomass of *Ulva fenestrata* at the start of the experiment harvested from an industrial seaweed farm in early spring (27.02.2023) and after 14 days of post-harvest treatment in control (PES) and in herring production process water (TUB) (mean  $\pm$  SD, n = 3). For reference, recommended amino acid profiles of required essential amino acids by WHO/FAO/UNU (2007) are indicated. Further, EAA of protein-rich foodstuffs (light grey) for direct comparison of the seaweed biomass used in this study are listed. EAA are indicated by bold font. The references cited in this table are Friedman (1996) and Sá et al. (2020).

Amino acid	Amino acid profile (g 100g protein <sup>-1</sup> )									
-	Start	PES	TUB	WHO/FAO/UNU requirements	Beef <sup>∆</sup>	Egg white <sup>∆</sup>	Soy protein <sup>∆</sup>	Bean▲	Pea▲	Chickpea▲
Glycine	5.97±0.01	6.15±0.12	6.23±0.21							
Alanine	7.55±0.09	10.71±0.35	10.77±0.32							
Serine	4.81±0.05	6.93±0.31	6.79±0.18							
Proline	6.16±0.04	3.56±0.39	3.50±0.05							
Valine	5.87±0.04	6.99±0.27	6.44±0.07	3.90	4.54	6.78	4.91	4.3	4.72	5.7
Threonine	5.14±0.08	7.21±0.47	6.56±0.06	2.30	4.21	4.68	3.84	2.4	3.45	4.7
Isoleucine	3.91±0.05	4.26±0.15	4.24±0.09	3.00	4.18	5.28	4.71	4.0	4.23	6.0
Leucine	7.46±0.04	6.25±0.37	5.99±0.16	5.90	7.75	8.76	8.51	7.2	7.11	10.0
*Aspartic acid	10.44±0.07	12.38±0.07	12.13±0.20							
Lysine	5.31±0.12	4.53±0.18	5.03±0.08	4.50	7.94	6.98	6.34	6.2	6.93	8.5
*Glutamic acid	14.91±0.11	11.38±0.56	12.00±0.22							
Methionine	2.41±0.02	1.67±0.15	1.79±0.12	1.60	3.27	6.64	6.81	0.7	5.0	2.1
Histidine	1.85±0.08	1.75±0.05	2.01±0.06	1.50	3.20	2.25	2.54	3.0	2.22	3.3
Phenylalanine	4.96±0.04	5.27±0.09	5.11±0.04	3.80**	7.02	9.08	9.68	5.4	4.87	7.9
Arginine	7.43±0.24	5.48±0.08	5.65±0.45							
Tyrosine	3.35±0.14	2.53±0.01	2.90±0.17							
Hydroxyprolin	1.14±0.19	1.34±0.03	1.02±0.36							
Cystein & Cystine	1.71±0.02	2.51±0.09	2.19±0.15							
TAA (% dw)	21.53±0.21 °	24.73±0.97 <sup>b</sup>	29.48±0.61ª							
TEAA (% of TAA)	36.91±0.14 ª	37.93±0.51ª	37.16±0.25 °		42.11	50.45	47.34	33.3	38.53	43.78

TAA total amino acids, TEAA total essential amino acids

Significant differences between treatments are denoted by superscript letters

\* Glutamine and asparagine were co-determined with glutamic and aspartic acid, respectively

\*\* sum of phenylalanine and tyrosine

<sup>△</sup>Friedman (1996)

▲Sá et al. (2020)

The differences between the total amino acid content and the crude protein content can possibly be attributed to the method chosen for analysis. The acidic hydrolysis prevents the recovery of tryptophan, and the hydrolysis time could degrade some amino acids (Angell et al., 2016). Moreover, some protein consists of more nitrogen-rich amino acids than others (Sosulski and Imafidon, 1990; Shuuluka et al., 2013). However, the biomass contained all the essential amino acids in the required amounts recommended by WHO/FAO/UNU (2007) (tryptophan not measured), making the biomass an attractive and high-quality biomass for human consumption. If an adult weighing 63.3 kg (EFSA, 2017) were to only consume U. fenestrata cultivated in TUB, at least 140 g dw needs to be consumed to reach the recommended daily intake of all EAA WHO/FAO/UNU (2007) (data not shown). This value is comparable to the required amount for soybean (170 g dw; Grieshop and Fahey, 2001). By concentrating the proteins with similar extraction methods as for soybeans (Harrysson et al., 2019; Juul et al., 2021; Trigo et al., 2021), the prospects of using *U. fenestrata* as a protein source for humans are further increased.

Given that seaweeds can accumulate heavy metals from their environment (Gaudry et al., 2007; Jarvis and Bielmyer-Fraser, 2015), we

examined the seaweed's accumulation from the TUB water used in this study in our previous research (Stedt et al., 2022a, 2022c). These results found no enriched or health concerning values of the tested heavy metals, indicating that seaweeds cultivated in TUB water are a safe food source (Stedt et al., 2022a, 2022c). Further emphasis in this study was put on evaluating potential fish-related allergenic activity of the TUB post-harvest treated seaweed biomass, and the results show that there was no allergenic activity, making the biomass a safe and versatile option in the food sector. Furthermore, our earlier studies found no negative effect of the TUB water on the seaweed biomass sensory profiles, which further underlines its commercial suitability for foodstuffs (Stedt et al., 2022c). The consistently rising positive consumer attitudes towards seaweeds as a food source (Wendin and Undeland, 2020) underscores the need to provide novel food and protein sources and strengthens the sustainable European Blue Economy and market value of alternative proteins.

## 5. Conclusion

This study contributes to the "farm to fork" strategy emphasized by

the EU's Blue Deal by providing details on the value-adding of postharvest treatment of seaweeds. Additionally, it introduces a methodology to help close the food-waste gap by integrating food side streams and seaweed cultivation. Our study reveals that the naturally optimal protein content of *U. fenestrata* biomass can be boosted through shortterm, post-harvest treatments with herring production process waters. After 14 days in the process water, the crude protein content of *U. fenestrata* was increased by 71.26 % and reached a maximum of 37.37  $\pm$  1.83 % dw, resulting in the highest crude protein values achieved in commercially produced *Ulva* biomass. Furthermore, we show that the biomass was not only free from traceable fish-related allergenic compounds but also enriched with all the essential amino acids in the required amounts recommended by WHO.

#### **Ethics declaration**

This work did not involve the use of human and/or animal subjects

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## CRediT authorship contribution statement

**Sophie Steinhagen:** Writing – review & editing, Writing – original draft, Visualization, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Kristoffer Stedt:** Conceptualization, Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **João P. Trigo:** Investigation, Writing – review & editing. **Ingrid Undeland:** Writing – review & editing, Supervision, Funding acquisition. **Henrik Pavia:** Writing – review & editing, Supervision, 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. All authors declare no conflicts of interest.

## Data availability

All data used for this study is included in the manuscript

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## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.fufo.2024.100347.

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