



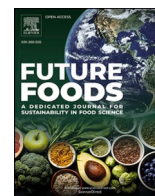
## **A step towards closing the food-waste gap in novel protein sources: Post-harvest protein boost of the seaweed crop *Ulva* by herring production**

Downloaded from: <https://research.chalmers.se>, 2024-06-30 13:00 UTC

Citation for the original published paper (version of record):

Steinhagen, S., Stedt, K., Trigo, J. et al (2024). 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. *Future Foods*, 9. <http://dx.doi.org/10.1016/j.fufo.2024.100347>

N.B. When citing this work, cite the original published paper.



# 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

Sophie Steinhagen<sup>a,1,\*</sup>, Kristoffer Stedt<sup>a,1</sup>, João P. Trigo<sup>b</sup>, Ingrid Undeland<sup>b</sup>, Henrik Pavia<sup>a</sup>

<sup>a</sup> Department of Marine Sciences, Tjärnö Marine Laboratory, University of Gothenburg, Laboratorievägen 10, Strömstad SE-452 96, Sweden

<sup>b</sup> Department of Biology and Biological Engineering-Food and Nutrition Science, Chalmers University of Technology, Gothenburg SE-412 96, Sweden

## ARTICLE INFO

### Keywords:

Allergens  
Essential amino acids  
Blue economy  
Blue protein  
Circularity  
Wastewater treatment

## 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 yields 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

\* Corresponding author.

E-mail address: [sophie.steinhagen@gu.se](mailto:sophie.steinhagen@gu.se) (S. Steinhagen).

<sup>1</sup> These authors contributed equally to this work.

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 (Vilarinho 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 Tjämnö 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 ( $>300$   $\mu\text{m}$ ), the TUB was filtered and subsequently stored at  $-60$  °C.

The total ammonium ( $\text{NH}_4^+$ ) 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  $\mu\text{m}$ +UV-light treated) deep-sea (40 m) seawater to 25  $\mu\text{M}$   $\text{NH}_4^+$ .

### 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  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . The starting biomass at the beginning of the experiment was set to 500 g fresh weight (fw) per tank (12 g fw  $\text{L}^{-1}$ ). 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 ( $32.8 \pm 0.9$  PSU 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  $\mu\text{M}$   $\text{NH}_4^+$ .

Herring Production Process water	Ammonium ( $\mu\text{M}$ $\text{NH}_4^+$ )	Inorganic phosphorus ( $\mu\text{M}$ P)	Dilution factor 25 $\mu\text{M}$ $\text{NH}_4^+$
TUB	2027.8 $\pm$ 218.1	8745.2 $\pm$ 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  $-60\text{ }^{\circ}\text{C}$  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 post-harvest 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).

**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, and (D) the total amino acid content after 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.

Source of Variance	A.Fresh weight (g)				B.Crude protein content (% dw)			
	DF	MS	F	<i>p</i>	DF	MS	F	<i>p</i>
Treatment	1	261.36	30.71	<0.005	2	189.00	108.44	<0.001
Residual	4	34.03			6	10.46		
Source of Variance	C.Total amino acids (% dw)				D.Total essential amino acids (% dw)			
	DF	MS	F	<i>p</i>	DF	MS	F	<i>p</i>
Treatment	2	47.95	70.64	<0.001	2	0.45	2.69	0.146
Residual	6	4.07			6	1.00		

### 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 %.

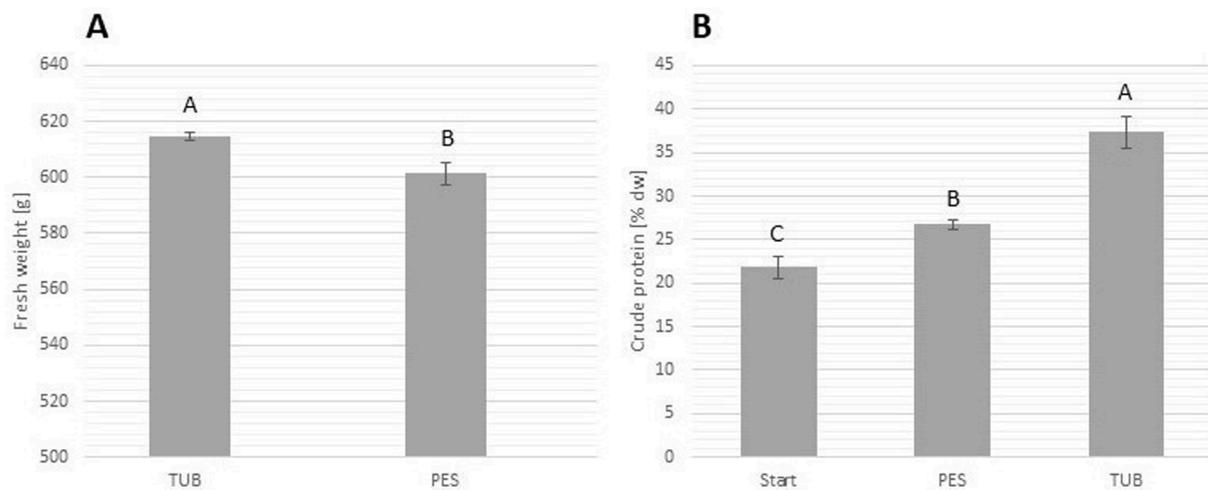
#### 3.2. Amino acid composition

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.



**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 high-value 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 post-harvest 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<sup>-1</sup> 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 ( $37.37 \pm 1.83$  % 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 ± 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>Δ</sup>	Egg white <sup>Δ</sup>	Soy protein <sup>Δ</sup>	Bean <sup>▲</sup>	Pea <sup>▲</sup>	Chickpea <sup>▲</sup>
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							
<b>Valine</b>	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
<b>Threonine</b>	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
<b>Isoleucine</b>	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
<b>Leucine</b>	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							
<b>Lysine</b>	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							
<b>Methionine</b>	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
<b>Histidine</b>	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
<b>Phenylalanine</b>	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 <sup>c</sup>	24.73±0.97 <sup>b</sup>	29.48±0.61 <sup>a</sup>							
TEAA (% of TAA)	36.91±0.14 <sup>a</sup>	37.93±0.51 <sup>a</sup>	37.16±0.25 <sup>a</sup>		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>Δ</sup>Friedman (1996)

<sup>▲</sup>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 post-harvest 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 short-term, 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

### Funding

This study was supported by the Swedish Government Research Council FORMAS ('A manual for the use of sustainable marine resources' grant no. 2022–00331; BlueGreen grant no. 2021–02340; and CirkAlg grant no. 2018–01839).

### 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

### Acknowledgments

We would like to thank Sweden Pelagic Ellös AB for providing herring production process waters for the experiment. The authors furthermore thank the FORMAS-funded projects 'A manual for the use of sustainable marine resources' (grant no. 2022–00331), BlueGreen (grant no. 2021–02340), and CirkAlg (grant no. 2018–01839) for financial support.

### Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.fufo.2024.100347](https://doi.org/10.1016/j.fufo.2024.100347).

### References

- Abdollahi, M., Axelsson, J., Carlsson, N.G., Nylund, G.M., Albers, E., Undeland, I., 2019. Effect of stabilization method and freeze/thaw-aided precipitation on structural and functional properties of proteins recovered from brown seaweed (*Saccharina latissima*). *Food Hydrocoll.* 96, 140–150. <https://doi.org/10.1016/j.foodhyd.2019.05.007>.
- Al-Hafedh, Y.S., Alam, A., Buschmann, A.H., 2015. Bioremediation potential, growth and biomass yield of the green seaweed, *Ulva lactuca* in an integrated marine aquaculture system at the Red Sea coast of Saudi Arabia at different stocking densities and effluent flow rates. *Rev. Aquac.* 7 (3), 161–171. <https://doi.org/10.1111/raq.12060>.
- Angell, A.R., Mata, L., de Nys, R., Paul, N.A., 2016. The protein content of seaweeds: a universal nitrogen-to-protein conversion factor of five. *J. Appl. Phycol.* 28 (1), 511–524. <https://doi.org/10.1007/s10811-015-0650-1>.
- Bolton, J.J., Cyrus, M.D., Brand, M.J., Joubert, M., Macey, B.M., 2016. Why grow *Ulva*? Its potential role in the future of aquaculture. *Perspect. Phycol.* 3 (3), 113–120. <https://doi.org/10.1127/pip/2016/0058>.
- Califano, G., Kwantes, M., Abreu, M.H., Costa, R., Wichard, T., 2020. Cultivating the macroalgal holobiont: effects of integrated multi-trophic aquaculture on the microbiome of *Ulva rigida* (Chlorophyta). *Front. Mar. Sci.* 7 <https://doi.org/10.3389/fmars.2020.00052>.
- del Olmo, A., Picon, A., Nuñez, M., 2020. Preservation of five edible seaweeds by high pressure processing: effect on microbiota, shelf life, colour, texture and antioxidant capacity. *Algal. Res.* 49, 101938 <https://doi.org/10.1016/j.algal.2020.101938>.
- Duarte, C.M., Holmer, M., Olsen, Y., Soto, D., Marbà, N., Guiu, J., Black, K., Karakassis, I., 2009. Will the oceans help feed humanity? *Bioscience* 59 (11), 967–976. <https://doi.org/10.1525/bio.2009.59.11.8>.
- EFSA, 2017. Dietary reference values for nutrients summary report. EFSA Support. Publ. 14 (12), e15121E. <https://doi.org/10.2903/sp.efsa.2017.e15121>.
- Faber, I., Henn, K., Brugarolas, M., Perez-Cueto, F.J., 2022. Relevant characteristics of food products based on alternative proteins according to European consumers. *J. Sci. Food Agric.* 102 (12), 5034–5043. <https://doi.org/10.1002/jsfa.11178>.
- Figuerola, V., Bunger, A., Ortiz, J., Aguilera, J.M., 2022. Sensory descriptors for three edible Chilean seaweeds and their relations to umami components and instrumental texture. *J. Appl. Phycol.* <https://doi.org/10.1007/s10811-022-02848-2>.
- Fleurence, J., Morancais, M., Dumay, J., Decottignies, P., Turpin, V., Munier, M., Garcia-Bueno, N., Jaouen, P., 2012. What are the prospects for using seaweed in human nutrition and for marine animals raised through aquaculture? *Trends. Food Sci. Technol.* 27 (1), 57–61. <https://doi.org/10.1016/j.tifs.2012.03.004>.
- Friedman, M., 1996. Nutritional value of proteins from different food sources. *A Rev. J. Agric. Food Chem.* 44 (1), 6–29. <https://doi.org/10.1021/jf9400167>.
- Gaudry, A., Zeroual, S., Gaie-Levrel, F., Moskura, M., Boujrial, F.Z., El Moursli, R.C., Guessous, A., Mouradi, A., Givernaud, T., Delmas, R., 2007. Heavy metals pollution of the atlantic marine environment by the Moroccan phosphate industry, as observed through their bioaccumulation in *Ulva lactuca*. *Water. Air. Soil. Pollut.* 178 (1), 267–285. <https://doi.org/10.1007/s11270-006-9196-9>.
- Grieshop, C.M., Fahey, G.C., 2001. Comparison of quality characteristics of soybeans from Brazil, China, and the United States. *J. Agric. Food Chem.* 49 (5), 2669–2673. <https://doi.org/10.1021/jf0014009>.
- Harrysson, H., Konasani, V.R., Toth, G.B., Pavia, H., Albers, E., Undeland, I., 2019. Strategies for improving the protein yield in pH-shift processing of *Ulva lactuca* Linnaeus: effects of Ulvan lyases, pH-exposure time, and temperature. *ACS Sustain. Chem. Eng.* 7 (15), 12688–12691. <https://doi.org/10.1021/acscuschemeng.9b02781>.
- Holdt, S.L., Kraan, S., 2011. Bioactive compounds in seaweed: functional food applications and legislation. *J. Appl. Phycol.* 23 (3), 543–597. <https://doi.org/10.1007/s10811-010-9632-5>.
- Ismail, B.P., Senaratne-Lenagala, L., Stube, A., Brackenridge, A., 2020. Protein demand: review of plant and animal proteins used in alternative protein product development and production. *Anim. Front.* 10 (4), 53–63. <https://doi.org/10.1093/af/vfaa040>.
- Jarvis, T.A., Bielmeyer-Fraser, G.K., 2015. Accumulation and effects of metal mixtures in two seaweed species. *Comp. Biochem. Physiol. C. Toxicol. Pharmacol.* 171, 28–33. <https://doi.org/10.1016/j.cbpc.2015.03.005>.
- Juul, L., Danielsen, M., Nebel, C., Steinhagen, S., Bruhn, A., Jensen, S.K., Undeland, I., Dalsgaard, T.K., 2021. *Ulva fenestrata* protein – Comparison of three extraction methods with respect to protein yield and protein quality. *Algal. Res.* 60, 102496 <https://doi.org/10.1016/j.algal.2021.102496>.
- Kazir, M., Abuhassira, Y., Robin, A., Nahor, O., Luo, J., Israel, A., Golberg, A., Livney, Y. D., 2019. Extraction of proteins from two marine macroalgae, *Ulva* sp. and *Gracilaria* sp., for food application, and evaluating digestibility, amino acid composition and antioxidant properties of the protein concentrates. *Food Hydrocoll.* 87, 194–203. <https://doi.org/10.1016/j.foodhyd.2018.07.047>.
- Kibler, K.M., Reinhart, D., Hawkins, C., Motlagh, A.M., Wright, J., 2018. Food waste and the food-energy-water nexus: a review of food waste management alternatives. *Waste Manag.* 74, 52–62. <https://doi.org/10.1016/j.wasman.2018.01.014>.
- Machado, M., Machado, S., Pimentel, F.B., Freitas, V., Alves, R.C., Oliveira, M., 2020. Amino acid profile and protein quality assessment of macroalgae produced in an integrated multi-trophic aquaculture system. *Foods* 9 (10), 1382. <https://doi.org/10.3390/foods9101382>.
- Mæhre, H.K., Malde, M.K., Eilertsen, K.E., Elvevoll, E.O., 2014. Characterization of protein, lipid and mineral contents in common Norwegian seaweeds and evaluation of their potential as food and feed. *J. Sci. Food Agric.* 94 (15), 3281–3290. <https://doi.org/10.1002/jsfa.6681>.
- Mayer Labba, I.C., Hoppe, M., Gramatkovski, E., Hjellström, M., Abdollahi, M., Undeland, I., Hulthén, L., Sandberg, A.S., 2022a. Lower non-heme iron absorption in healthy females from single meals with texturized fava bean protein compared to

- beef and cod protein meals: two single-blinded randomized trials. *Nutrients* 14 (15). <https://doi.org/10.3390/nu14153162>.
- Mayer Labba, I.C., Steinhagen, H., Almius, L., Bach Knudsen, K.E., Sandberg, A.S., 2022b. Nutritional composition and estimated iron and zinc bioavailability of meat substitutes available on the Swedish market. *Nutrients* 14 (19), 3903. <https://doi.org/10.3390/nu14193903>.
- Meng, Y., Cloutier, S., 2014. Chapter 20 - gelatin and other proteins for microencapsulation. In: Gaonkar, A.G., Vasisht, N., Khare, A.R., Sobel, R. (Eds.), *Microencapsulation in the Food Industry*. Academic Press, San Diego, pp. 227–239.
- Nardelli, A.E., Chiozzini, V.G., Braga, E.S., Chow, F., 2019. Integrated multi-trophic farming system between the green seaweed *Ulva lactuca*, mussel, and fish: a production and bioremediation solution. *J. Appl. Phycol.* 31 (2), 847–856. <https://doi.org/10.1007/s10811-018-1581-4>.
- Neuhofer, Z.T., Lusk, J.L., 2023. "Impacts of variety-seeking, substitution, and demographics on demand for plant-based meat alternatives", in: 2023 Annual Meeting, July 23-25, Washington D.C, Agricultural and Applied Economics Association).
- Neveux, N., Bolton, J.J., Bruhn, A., Roberts, D.A., Ras, M., 2018. The bioremediation potential of seaweeds: recycling nitrogen, phosphorus, and other waste products. *Blue Biotechnol.* 1, 217–239 production and use of marine molecules.
- Nghiem, L.D., Koch, K., Bolzonella, D., Drewes, J.E., 2017. Full scale co-digestion of wastewater sludge and food waste: bottlenecks and possibilities. *Renew. Sustain. Energy Rev.* 72, 354–362. <https://doi.org/10.1016/j.rser.2017.01.062>.
- Nielsen, M.M., Bruhn, A., Rasmussen, M.B., Olesen, B., Larsen, M.M., Moller, H.B., 2012. Cultivation of *Ulva lactuca* with manure for simultaneous bioremediation and biomass production. *J. Appl. Phycol.* 24 (3), 449–458. <https://doi.org/10.1007/s10811-011-9767-z>.
- Paull, R.E., Chen, N.J., 2008. Postharvest handling and storage of the edible red seaweed *Gracilaria*. *Postharvest Biol. Technol.* 48 (2), 302–308. <https://doi.org/10.1016/j.postharvbio.2007.12.001>.
- Pliego-Cortés, H., Wijesekara, I., Lang, M., Bedoux, G., Bourgoignon, N., 2020. Chapter Nine - Current knowledge and challenges in extraction, characterization and bioactivity of seaweed protein and seaweed-derived proteins. In: Bourgoignon, N. (Ed.), *Advances in Botanical Research*. Academic Press, pp. 289–326.
- Provasoli, L., 1968. "Media and prospects for the cultivation of marine algae", in: *cultures and Collections of Algae*. In: *Proceedings of the US-Japan Conference*. Hakone. September 1966. (Japan Society of Plant Physiology).
- Qvirist, L., Carlsson, N.G., Andlid, T., 2015. Assessing phytase activity—methods, definitions and pitfalls. *J. Biol. Methods* 2 (1), e16. <https://doi.org/10.14440/jbm.2015.58>.
- Rawiwan, P., Peng, Y., Paramayuda, I.G.P.B., Quek, S.Y., 2022. Red seaweed: a promising alternative protein source for global food sustainability. *Trends. Food Sci. Technol.* 123, 37–56. <https://doi.org/10.1016/j.tifs.2022.03.003>.
- Ray, D.K., Foley, J.A., 2013. Increasing global crop harvest frequency: recent trends and future directions. *Environ. Res. Lett.* 8 (4) <https://doi.org/10.1088/1748-9326/8/4/044041>.
- Sá, A.G.A., Moreno, Y.M.F., Carciofi, B.A.M., 2020. Plant proteins as high-quality nutritional source for human diet. *Trends. Food Sci. Technol.* 97, 170–184. <https://doi.org/10.1016/j.tifs.2020.01.011>.
- Schieber, A., 2017. Side streams of plant food processing as a source of valuable compounds: selected examples. *Annu Rev. Food Sci. Technol.* 8 (1), 97–112. <https://doi.org/10.1146/annurev-food-030216-030135>.
- Shuuluka, D., Bolton, J.J., Anderson, R.J., 2013. Protein content, amino acid composition and nitrogen-to-protein conversion factors of *Ulva rigida* and *Ulva capensis* from natural populations and *Ulva lactuca* from an aquaculture system, in South Africa. *J. Appl. Phycol.* 25 (2), 677–685. <https://doi.org/10.1007/s10811-012-9902-5>.
- Sode, S., Bruhn, A., Balsby, T.J.S., Larsen, M.M., Gottfredsen, A., Rasmussen, M.B., 2013. Bioremediation of reject water from anaerobically digested waste water sludge with macroalgae (*Ulva lactuca*, Chlorophyta). *Bioresour. Technol.* 146, 426–435. <https://doi.org/10.1016/j.biortech.2013.06.062>.
- Sosulski, F.W., Imafidon, G.I., 1990. Amino acid composition and nitrogen-to-protein conversion factors for animal and plant foods. *J. Agric. Food Chem.* 38 (6), 1351–1356. <https://doi.org/10.1021/jf00096a011>.
- Spillias, S., Valin, H., Batka, M., Sperling, F., Havlík, P., Leclère, D., Cottrell, R.S., O'Brien, K.R., McDonald-Madden, E., 2023. Reducing global land-use pressures with seaweed farming. *Nat. Sustain.* <https://doi.org/10.1038/s41893-022-01043-y>.
- Stedt, K., Gustavsson, O., Kollander, B., Undeland, I., Toth, G.B., Pavia, H., 2022a. Cultivation of *Ulva fenestrata* using herring production process waters increases biomass yield and protein content. *Front. Mar. Sci.* 9 <https://doi.org/10.3389/fmars.2022.988523>.
- Stedt, K., Pavia, H., Toth, G.B., 2022b. Cultivation in wastewater increases growth and nitrogen content of seaweeds: a meta-analysis. *Algal. Res.* 61, 102573 <https://doi.org/10.1016/j.algal.2021.102573>.
- Stedt, K., Steinhagen, S., Trigo, J.P., Kollander, B., Undeland, I., Toth, G.B., Wendin, K., Pavia, H., 2022c. Post-harvest cultivation with seafood process waters improves protein levels of *Ulva fenestrata* while retaining important food sensory attributes. *Front. Mar. Sci.* 9 <https://doi.org/10.3389/fmars.2022.991359>.
- Stedt, K., Trigo, J.P., Steinhagen, S., Nylund, G.M., Forghani, B., Pavia, H., Undeland, I., 2022d. Cultivation of seaweeds in food production process waters: evaluation of growth and crude protein content. *Algal. Res.* 63, 102647 <https://doi.org/10.1016/j.algal.2022.102647>.
- Steinhagen, S., Enge, S., Cervin, G., Larsson, K., Edlund, U., Schmidt, A.E.M., Wahlström, N., Kollander, B., Pavia, H., Undeland, I., Toth, G.B., 2022. Harvest time can affect the optimal yield and quality of sea lettuce (*Ulva fenestrata*) in a sustainable sea-based cultivation. *Front. Mar. Sci.* 9 <https://doi.org/10.3389/fmars.2022.816890>.
- Steinhagen, S., Enge, S., Larsson, K., Olsson, J., Nylund, G.M., Albers, E., Pavia, H., Undeland, I., Toth, G.B., 2021. Sustainable large-scale aquaculture of the northern hemisphere sea lettuce, *Ulva fenestrata*, in an off-shore seafarm. *J. Mar. Sci. Eng.* 9 (6), 615. <https://doi.org/10.3390/jmse9060615>.
- Steinhagen, S., Hoffmann, S., Pavia, H., Toth, G.B., 2023. Molecular identification of the ubiquitous green algae *Ulva* reveals high biodiversity, crypticity, and invasive species in the Atlantic-Baltic Sea region. *Algal. Res.* 73, 103132 <https://doi.org/10.1016/j.algal.2023.103132>.
- Steinhagen, S., Karez, R., Weinberger, F., 2019. Cryptic, alien and lost species: molecular diversity of *Ulva sensu lato* along the German coasts of the North and Baltic Seas. *Eur. J. Phycol.* 54 (3), 466–483. <https://doi.org/10.1080/09670262.2019.1597925>.
- Steinhagen, S., Larsson, K., Olsson, J., Albers, E., Undeland, I., Pavia, H., Toth, G.B., 2022b. Closed life-cycle aquaculture of sea lettuce (*Ulva fenestrata*): performance and biochemical profile differ in early developmental stages. *Front. Mar. Sci.* 9 <https://doi.org/10.3389/fmars.2022.942679>.
- Toth, G.B., Harrysson, H., Wahlstrom, N., Olsson, J., Oerbekke, A., Steinhagen, S., Kinby, A., White, J., Albers, E., Edlund, U., Undeland, I., Pavia, H., 2020. Effects of irradiance, temperature, nutrients, and pCO<sub>2</sub> on the growth and biochemical composition of cultivated *Ulva fenestrata*. *J. Appl. Phycol.* 32, 3243–3254. <https://doi.org/10.1007/s10811-020-02155-8>.
- Trigo, J.P., Engström, N., Steinhagen, S., Juul, L., Harrysson, H., Toth, G.B., Pavia, H., Scheers, N., Undeland, I., 2021. In vitro digestibility and Caco-2 cell bioavailability of sea lettuce (*Ulva fenestrata*) proteins extracted using pH-shift processing. *Food Chem.* 356, 129683 <https://doi.org/10.1016/j.foodchem.2021.129683>.
- van den Burg, S.W.K., Dagevos, H., Helmes, R.J.K., 2019. Towards sustainable European seaweed value chains: a triple P perspective. *ICES J. Mar. Sci.* 78 (1), 443–450. <https://doi.org/10.1093/icesjms/fsz183>.
- Vilarino, M.V., Franco, C., Quarrington, C., 2017. Food loss and waste reduction as an integral part of a circular economy. *Front. Environ. Sci.* 5 <https://doi.org/10.3389/fenvs.2017.00021>.
- Wendin, K., Undeland, I., 2020. Seaweed as food – attitudes and preferences among Swedish consumers. A pilot study. *Int. J. Gastron. Food Sci.* 22, 100265 <https://doi.org/10.1016/j.ijgfs.2020.100265>.
- WHO/FAO/UNU, 2007. "Protein and amino acid requirements in human nutrition", in: WHO Technical Report Series 935. Report of Joint WHO/FAO/UNU Expert Consultation. (Geneva, Switzerland).