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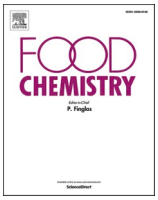

Short communication

Sea lettuce (*Ulva fenestrata*) as a rich source of cobalamin (vitamin B12) – both as processed whole biomass and as an extracted protein ingredient

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ABSTRACT

The seaweeds *Ulva fenestrata* and *Palmaria palmata* are promising food items; however, it remains unclear whether both contain true vitamin B12 and how post-harvest processing, storage, and protein extraction affect this vitamin. UHPLC-UV-MS/MS analysis revealed that untreated *Ulva* contained 681 ± 37 ng B12/g dry weight (dw) - 22 times more than *Palmaria*. Biomass soaking (16 °C, 3 min) in freshwater did not affect B12 content, but blanching (60 °C, 3 min) reduced its content in *Ulva* by 45 % (dw basis) and 61 % (protein content basis). Oven-drying and freeze-drying equally preserved B12; both techniques maintained B12 content during room-temperature dark storage for 4.8 months. Protein extraction via a new method (Trigo et al., 2025) resulted in a dried ingredient containing 60 % more B12 than untreated *Ulva*, and 29 times more than red meat on a moisture-equivalent basis. All *Ulva* samples – including the protein ingredient – qualified for the EU nutritional claim "High in Vitamin B12".

1. Introduction

Shifting from animal-based foods to plant-based alternatives has been linked to more environmentally friendly diets, resulting in reductions of up to 52 % in greenhouse gas emissions and up to 45 % in land use (Bunge et al., 2024). However, this dietary shift also carries a risk of lowering vitamin B12 intake (Bunge et al., 2024; Pawlak et al., 2014), which is concerning given the prevalence of low and marginal serum B12 in the e.g., United States population, ranging from 8 % to 29 % (Green et al., 2017). Vitamin B12 is essential for the e.g., normal function of the nervous and immune systems, erythropoiesis, and synthesis of DNA. Hence there are 8 authorized European Union (EU) health claims for food products containing this vitamin, provided that its content qualifies for a nutritional claim of "Source of Vitamin B12" (European-Comission, 2024).

Seaweed (like higher plants and animals) is unable to synthesize vitamin B12; yet, some species do contain the vitamin due to symbiotic interactions with B12-producing bacteria (Smith et al., 2007). Thus, integrating seaweed into plant-based diets – whether as a whole food or a protein ingredient – can help maintain a positive environmental impact of these diets and, in parallel, contribute to the intake of vitamin B12 (Gephart et al., 2021; Jacobsen et al., 2023). Quantification of vitamin B12 in seaweed via microbiological assays indicates that an amount of 4.7 g dry weight (dw) of *Ulva* sp. and 3.0–7.7 g dw of *Porphyra* sp. fulfills the adequate intake of 4 μg B12/day defined by EFSA (Brito et al., 2023; EFSA, 2015). While these seaweed genera appear to be good sources of vitamin B12 it is also known that microbiological assays tend to overestimate the biologically active vitamin B12, since the commonly used assay microorganisms (e.g., *Lactobacillus delbrueckii* ATCC 7830152) can give a response to analogs of vitamin B12, active in many microorganisms but inactive in animals (Edelmann et al., 2019). Therefore, highly selective and sensitive liquid chromatography (LC)-based techniques have been suggested for the quantification of biologically active vitamin B12 (Chamlagain et al., 2015a). As far as we know, vitamin B12 content quantified via LC-based techniques in seaweed has only been reported for *Ulva intestinalis* (Luhila et al., 2022). Thus, studies on other commercially relevant species within the European/Northern European context are lacking (Brito et al., 2023). Among these species,

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Ulva fenestrata and *Palmaria palmata* are of particular interest due to e.g., a large-scale off-shore cultivation potential has already been demonstrated for *U. fenestrata* (Steinhagen et al., 2021).

Besides the limited data on vitamin B12 in seaweed, it remains unclear how its content is affected by industrial practices, such as seaweed post-harvest and preservation treatments as well as storage. Gaining insights into such effects would help the seaweed industry to ensure optimal preservation of B12 in the produced biomasses. Regarding post-harvest treatments of seaweed, soaking is commonly used to remove sand and epiphytes. Meanwhile, blanching is primarily applied in kelp species to lower iodine content to levels safer for consumption (Banach et al., 2024; Trigo, Palmnäs-Bédard, et al., 2023). Applying blanching to non-kelp species may serve similar purposes as it does for vegetables – namely, reducing microbial load and inactivating enzymes that would otherwise induce unwanted sensory changes, such as bitterness or rancidity. Earlier studies reported that blanching of insects does not affect vitamin B12 content (Khatun et al., 2021; Lenaerts et al., 2018), thus we hypothesize the same might occur in seaweed. For long-term storage, oven-drying is the most common technique to reduce the water activity of seaweed in cold climates (e.g., Northern Europe). To our knowledge, the effect of oven-drying on vitamin B12 content has only been reported for the microalgae *Chlorella vulgaris*. In this case, drying *C. vulgaris* at 60 °C for 6 h significantly lowered vitamin B12 content in comparison to the freeze-dried biomass (Madhubalaji et al., 2021).

U. fenestrata is a potential contributor to the ongoing dietary shift to more sustainable alternative protein sources (Vega-Gómez et al., 2024), and we recently reported on cultivation regimes that raised protein content to 30 % dw (Steinhagen et al., 2024). This finding is important because protein concentration from the raw material helps to match the protein content of meat and to improve protein digestibility and amino acid accessibility (Trigo et al., 2021). An additional advantage, yet to be confirmed, is the co-concentration of vitamin B12 since it is typically bound to protein (Rakuša et al., 2023).

This short communication aimed to evaluate the effects of post-harvest treatments, drying techniques, and storage conditions on the content of true vitamin B12 in *U. fenestrata* and *P. palmata* biomasses. It also aimed to assess the effect of downstream protein extraction on B12 levels in *U. fenestrata*.

2. Materials and methods

2.1. Seaweed biomass harvesting

U. fenestrata and *P. palmata* were harvested from different cultivation tanks at Tjärnö Marine Laboratory (Strömstad, Sweden) in October 2022. Gametophytic algal material of *U. fenestrata* was taken after a long-term indoor tank cultivation; details on cultivation conditions and molecular identification are described in Steinhagen et al. (2021). Cultivation of *P. palmata* followed the seasonal variation in terms of water temperature, salinity, and light. Also, the tank was supplied with continuous flow-through deep-water filtered to 5 µm, along with aeration to ensure water motion.

2.2. Post-harvest treatments

Around 700 g of fresh weight of each biomass species was blanched in tap water at 60 °C for 3 min in a biomass:water ratio of 1:30 (*w/v*). A temperature of 60 °C was selected as it has been shown to inhibit the activity of LOX in *Ulva* sp. by >90 % (Kuo et al., 1997). After blanching, the biomasses were drained with a colander and immediately placed in zip-lock polystyrene bags, which were then cooled down in an ice-water bath. The soaking treatment shared the same processing conditions, except for the water temperature, which was the tap water temperature (16 °C) on that specific day. The control biomass was untreated and is hereafter referred to as *untreated*. A portion of all treated and untreated biomasses was freeze-dried, while the remainder biomass was dried at 40 °C for 24 h in an oven-dryer (MKASZ 25 drying cabinet) and then milled (Severin KM 3868). The storage test involved placing a portion of the oven-dried samples in closed zip-lock polystyrene bags, which were then stored at room temperature in darkness for 144 days (4.8 months). Storage in the darkness prevents (i) conversion of B12-cofactors to the aquo/hydroxocobalamin (HO-Cbl), the latter form tends to be less stable in complex biological mixtures, and (ii) lipid oxidation (Harrysson et al., 2021; Pratt, 1972). All samples, including sampling points from the storage test, were stored at −80 °C under vacuum until analysis.

2.3. Protein extraction from U. fenestrata

The protein extraction method used is reported in Trigo et al. (2025). Briefly, the method consisted of processing *U. fenestrata* with 0.1 % aqueous Triton X-114 and reprocessing the seaweed pellet with water later adjusted to pH 12. Then, the solubilized proteins were precipitated at pH 2. The tested *U. fenestrata* and protein extract were the same samples as in the cited study (Trigo et al., 2025).

2.4. Moisture content

The moisture content of all powders was analyzed gravimetrically after oven-drying at 105 °C for 24 h (Termaks TS4057).

2.5. Analysis of true vitamin B12

The vitamin B12 content was determined according to Chamlagain et al. (2015a). Before quantification, *P. palmata* samples were subjected to a second milling step with dry ice using a Sorvall Omni-mixer 17,220. This ensured a similar particle size to the *U. fenestrata* samples (between 0.25 and 0.88 mm). The natural vitamin B12 forms were extracted from the powdered biomasses as cyanocobalamin (CNCbl) using an extraction buffer (at pH 4.5) containing sodium cyanide; this was followed by boiling in a water bath for 30 min Chamlagain et al. (2015b). Then, B12 (CNCbl) was purified with the immunoaffinity column “Easi-Extract” (R-Biopharma) and quantified on a Waters Acquity UPLC coupled to a PDA detector (Fig. 1a). The confirmation of the vitamin form is shown with an example mass spectra in Fig. 1b and was based on the UHPLC-MS/MS method reported by Chamlagain et al. (2018). It is important to note that during the cyanide treatment, the upper ligand is replaced by a cyano group regardless of the B12 form (i.e. true or pseudo) and that the treatment does not affect the lower ligand 5,6-dimethylbenzidimazole only present in true vitamin B12. These aspects enable cyano true B12 to (i) elute at a different time than cyano pseudo B12 and (ii) be identified via a unique mass spectrum (Fig. 1b) (Chamlagain et al., 2015b; Chamlagain et al., 2018). No pseudo-B12 forms were identified in all tested samples.

2.6. Compositional analysis

Total amino acids as a measure of protein, ash, and fatty acids were measured via LC-MS, gravimetry, and GC–MS, respectively, according to Trigo et al. (2025).

2.7. Statistical analysis

The statistical tests one-way ANOVA followed by Tukey’s post hoc or *t*-test were used. For non-normally distributed data, the non-parametric Kruskal-Wallis test was chosen. All tests were conducted using SPSS Statistics software and differences were considered statistically significant at $p < 0.05$.

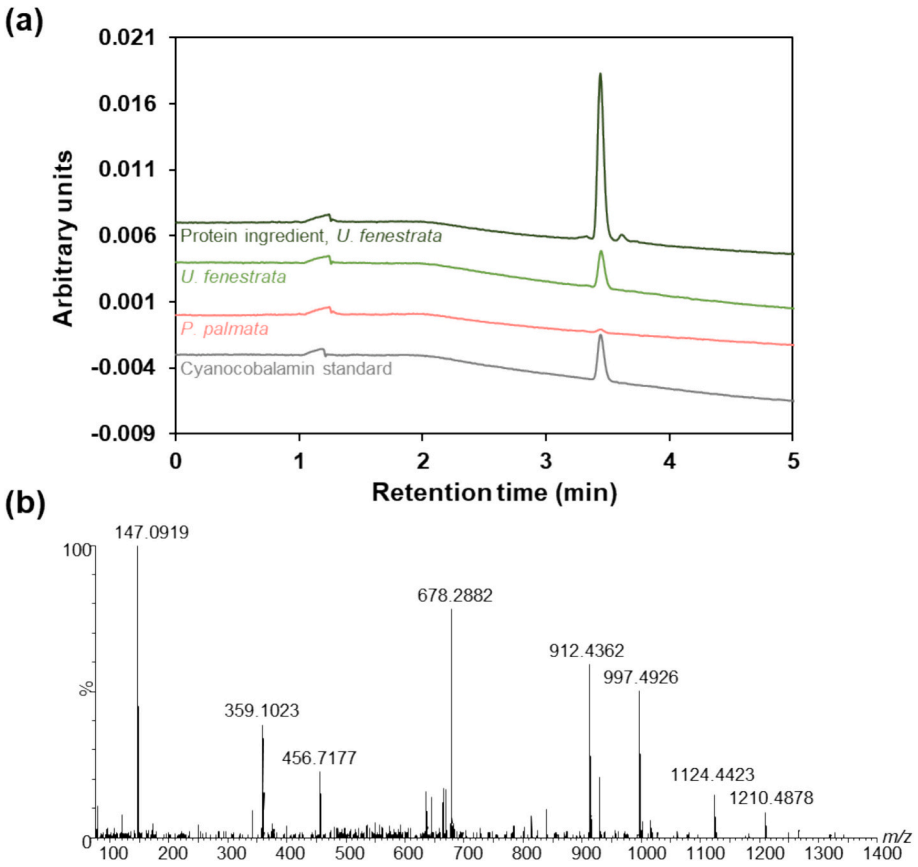

Fig. 1. - (a) Example chromatograms of a cyanocobalamin standard and the immunoaffinity-purified extract of the seaweed biomasses and *U. fenestrata* protein ingredient showing the retention of cyanocobalamin at 3.45 min; (b) LC-MS/MS analysis of the peak eluting at the retention time of cyanocobalamin in the *U. fenestrata* extract. Examples of mass spectra of the cyanocobalamin peak from the other three samples are provided in Supplementary Material: Figure S1.

Table 1
– "True" vitamin B12 content (n = 3) and moisture content ($n \leq 2$) of *U. fenestrata* and *P. palmata* subjected to different post-harvest treatments, followed by drying and subsequent storage at room temperature and in darkness for 144 days. Data were compared to the adequate intake (AI) of 4 μg B12 per day, recommended by EFSA (2015).

Species	Post-harvest treatment	Drying technique	Storage time	Moisture (%)	Vitamin B12	
					ng/g sample dw	Amount to meet B12 AI (g dw)
U. fenestrata	Untreated	Freeze-dried	Day 0	3.6 ± 0.2	681 ± 37^{a}	5.9
		Oven-dried	Day 0	12.4 ± 0.2	894 ± 45^{b}	4.5
		Oven-dried	Day 144	11.4 ± 0.1	755 ± 32^{ab}	5.3
	Soaked	Freeze-dried	Day 0	4.1*	767 ± 47^{ab}	5.2
		Oven-dried	Day 0	10.2 ± <0.01	868 ± 89^{b}	4.6
		Oven-dried	Day 144	10.7 ± <0.01	760 ± 81^{ab}	5.3
	Blanched	Freeze-dried	Day 0	4.8*	378 ± 25^{c}	10.6
		Oven-dried	Day 0	11.1 ± <0.01	368 ± 33^{c}	10.9
		Oven-dried	Day 144	11.8 ± <0.01	326 ± 47^{c}	12.3
P. palmata	Untreated	Freeze-dried	Day 0	6.7 ± 0.3	30.7 ± 4.5 ^{A}	130
		Oven-dried	Day 0	12.8 ± 1.1	37.6 ± 3.3 ^{A}	106
		Oven-dried	Day 144	8.1 ± 0.6	28.8 ± 4.2^{A}	139
	Soaked	Freeze-dried	Day 0	5.7*	35.7 ± 2.5 ^{A}	112
		Oven-dried	Day 0	10.2 ± 0.6	36.5 ± 3.0 ^{A}	110
		Oven-dried	Day 144	6.6 ± 0.1	31.1 ± 4.0 ^{A}	129
	Blanched	Freeze-dried	Day 0	5.7*	26.9 ± 5.2^{A}	149
		Oven-dried	Day 0	12.5 ± 0.6	32.3 ± 1.7 ^{A}	124
		Oven-dried	Day 144	8.9 ± 0.4	32.0 ± 1.6 ^{A}	125

Values with different lowercase letters for *U. fenestrata* and uppercase letters for *P. palmata* mean statistically different B12 content (Tukey's post-hoc test, $p < 0.05$); *$n = 1$, due to limitations on sample amount; n.a. *not analyzed*; AI *adequate intake*; dw *dry weight.*

3. Results and discussion

3.1. Effect of species

Untreated *U. fenestrata* contained 22 times more vitamin B12 than untreated *P. palmata* (Table 1). To our knowledge, this is the first study to report the B12 content of both species using LC-UV-MS/MS. Studies using the same technique reported a B12 content of 49–276 ng/g dw for *U. intestinalis* (Luhila et al., 2022) and 30–560 ng/g dw for the microalgae *Spirulina*, though in the latter case, the material also contained large quantities of inactive pseudovitamin B12 (Edelmann et al., 2019). For *P. palmata*, the reported range was 100–734 ng/g dw, as quantified by microbiological methods (Martínez Hernández et al., 2018; Watanabe et al., 2002). Altogether, the vitamin B12 content of *U. fenestrata* in this study was ≥1.6 times higher than that of *U. intestinalis* and *Spirulina*, while the B12 values for *P. palmata* were lower than the given range. The latter comparison might indicate either an overestimation of true B12 by the microbiological assays (reactive to several vitamin B12 analogs) or compositional differences caused by a variation in the harvest season. For instance, Luhila et al. (2022) found that the B12 content of the red seaweed *Furcellaria lumbricalis* was the highest during August–September, compared to the entire harvest period from April to September. Another factor, yet to be explored, concerns whether microbiome differences between wild seaweed used in the cited studies and the tank-cultivated seaweed of the present study influence the B12 content. For example, a recent study conducted by van der Loos et al. (2024) reported microbiome variations between wild vs. tank-cultivated seaweed.

Interestingly, the untreated *Saccharina latissima* biomass tested in Trigo, Stedt, et al. (2023) was also analyzed in the present study, and its B12 content was assessed as 71.1 ± 0.6 ng/g dw. This content is higher than the result for *P. palmata* but almost ten times lower than that for *U. fenestrata*.

3.2. Effect of post-harvest treatment

The effect of post-harvest treatment was only significant for *U. fenestrata* (one-way ANOVA, $F(2) = 78$, $p < 0.001$). Specifically, blanched samples contained 44 % to 64 % less B12 than the untreated and soaked biomasses (Tukey's post-hoc test; $p < 0.05$), which is contrary to the initial hypothesis based on studies with insects (Khatun et al., 2021; Lenaerts et al., 2018). To understand if such reductions were due to a loss of protein-bound vitamin B12, results were normalized to protein content (Fig. 2a). After blanching, the ratio of B12-to-protein was reduced by 61 % for *U. fenestrata* and 45 % for *P. palmata*,

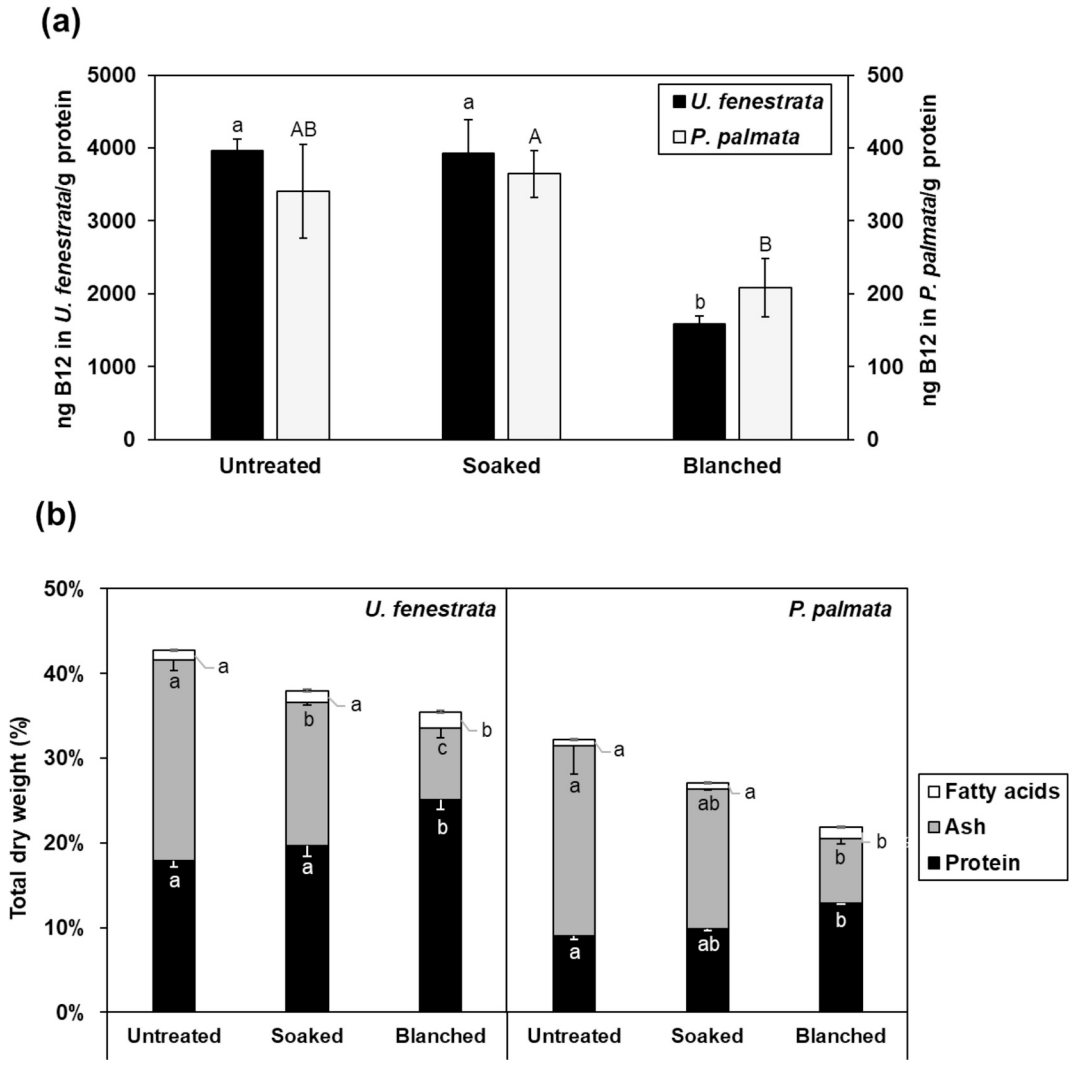

Fig. 2. – (a) Vitamin B12 content normalized to protein content of freeze-dried biomasses previously subjected to soaking or blanching. Within each species, different lowercase letters mean statistical differences at $p < 0.05$ (One-way ANOVA or Kruskal-Wallis 1-way ANOVA, the latter for *U. fenestrata*); (b) composition of the biomasses in (a) in terms of protein (measured as total amino acids), ash, and total fatty acids on a dry weight basis. Within each species and category, values with different lowercase letters mean statistical differences at $p < 0.05$ (One-way ANOVA or Kruskal-Wallis 1-way ANOVA, the latter for ash and protein of *P. palmata*).

whereas after soaking, this ratio was similar to the untreated biomasses (Fig. 2a). Therefore, a higher water temperature (60 °C vs 16 °C) likely denatures B12-binding proteins and/or promotes the leaching of free vitamin B12 into the blanching water. As B12 is known to be heat-resistant (Ball, 2005), this loss is unlikely to originate from its thermal breakdown. Both free and protein-bound B12 in seaweed have been confirmed in the red species *Porphyra tenera* (Yamada et al., 1996), though the ratio of free-to-bound B12 in seaweed has yet to be reported. The free form can be especially relevant for individuals with protein-bound vitamin B12 malabsorption (Bito et al., 2018).

Current seaweed intake in Japan is estimated at 2.6 g dw or 25.5 g fw per day (Trigo, Palmnäs-Bédard, et al., 2023). Assuming a similar intake would be achieved in Europe, then 2.6 g dw of dried *U. fenestrata* from the untreated, soaked, and blanched biomasses would respectively provide 58 %, 56 %, and 25 % of the adequate intake (AI) for vitamin B12 (4 μg/day; EFSA, 2015). For fresh *U. fenestrata* subjected to the aforementioned treatments, a portion of 25.5 g fw would respectively correspond to 94 %, 81 %, and 34 % of the AI; the moisture content of these biomasses was estimated as 78 %, 83 %, and 85 %. Therefore, despite some loss of B12 due to blanching, the resulting dried *U. fenestrata* remained rich in this vitamin. For dried *P. palmata,* the percentages relative to the AI were below or equal to 2 %, whereas a fresh portion would contribute to 3–4 % of the AI; moisture contents of 77 %, 83 %, and 86 %, respectively.

According to European Regulations No 1169/2011 and 1924/2006, all tested *U. fenestrata* samples qualify for an EU nutritional claim of "High in Vitamin B12". This also permits the use of the 8 authorized EU health B12-claims, including e.g., contribution to normal neurological and psychological functions, normal homocysteine metabolism, and reduction of tiredness and fatigue. It is important to note that neither regulations specifically require demonstrating nutrient bioavailability for such claims.

Regarding proximate composition, blanching led to higher protein and total fatty acid content compared to the untreated biomass when normalized to dw (Fig. 2b). This relative increase is mainly due to the release of minerals into the blanching water, as described in other seaweed species (Trigo, Stedt, et al., 2023). Amino acid and fatty acid analyses also revealed that due to blanching, the essential amino acid content increased from 6.9 ± 0.3 % to 9.4 ± 0.5 % ($t(4) = -5.6$, $p = 0.005$) and the polyunsaturated fatty acids (PUFA) from 0.6 ± 0.1 % to 1.1 ± <0.1 % of the total dw ($t(4) = -8.2$, $p < 0.001$); the increase in PUFA content was retained after oven-drying (0.5 ± <0.1 % vs. 0.8 ± 0.1 %, $p = 0.006$).

3.3. Effect of drying technique

As presented in Table 1, the B12 content in freeze-dried biomass was similar to the B12 content in oven-dried biomass for both *U. fenestrata* ($t(25) = -0.58$, $p = 0.57$) and *P. palmata* ($t(25) = -0.97$; $p = 0.34$). These results contrast with those from the microalgae *C. vulgaris*, where the B12 content was lower after oven-drying compared to freeze-drying (Madhubalaji et al., 2021). The lower oven-drying temperature in our study (40 °C vs 60 °C) could explain why B12 was better preserved, despite the longer drying time (24 h vs 6 h). In our study, the overall effect of the drying technique was non-significant; though, oven-dried untreated and soaked *U. fenestrata* contained 31 % and 13 % more vitamin B12, respectively, than the corresponding freeze-dried samples (Tukey's; $p < 0.05$). Similar findings were reported for the insect *Gryllus assimilis*, although no explanation was provided (Khatun et al., 2021). In another work that aimed at optimizing vitamin B12 extraction from *Ulva lactuca*, the boiling extraction method at pH 5 without cyanide addition yielded 2.7 times more vitamin B12 from the oven-dried material compared to freeze-dried material (Susanti et al., 2022); yet, the reason for this difference was not discussed. We hypothesize that the observed difference is due to a smaller particle size of the freeze-dried material, despite our efforts to control this parameter (refer to section 2.5). A smaller particle size probably caused a higher degradation of B12 right after the material resuspension (to allow subsequent B12 extraction in the presence of cyanide), where natural B12 forms such as adenosylcobalamin and methylcobalamin likely converted to HO-Cbl due to exposure to light and oxygen (Pratt, 1972). The latter B12-form is known to be unstable in the presence of ascorbate and thiols like DMS (Frost et al., 1952; Pratt, 1972), both of which are naturally present in *Ulva* sp. (Harrysson et al., 2021; Li et al. 2023.

3.4. Effect of storage time

The B12 content of the oven-dried biomasses was measured at the both the beginning and end of a 144-day storage period in darkness at room temperature, as indicated in Table 1. Generally, vitamin B12 was preserved during storage for both *U. fenestrata* ($t(16) = 0.83$, $p = 0.42$) and *P. palmata* ($t(16) = 0.83$; $p = 0.42$). Very few studies have monitored the changes in B12 in low-moisture foods during storage. Of those available, Hemery et al. (2020) found that CNCbl – the most stable form of vitamin B12 normally used in food fortification – was preserved for 6 months in B12-fortified wheat flour under storage conditions similar to those in the present study.

3.5. Effect of downstream protein extraction

Fig. 3 presents the vitamin B12 and protein content of a protein ingredient from *U. fenestrata* and the untreated *U. fenestrata* biomass. As initially hypothesized, protein extraction up-concentrated B12 with the final dried ingredient containing 60 % more of this vitamin ($t(4) = -7.69$, $p = 0.002$) compared to the untreated biomass, when normalized to their dw. Since protein up-concentration was around 120 %, the B12-to-protein ratio was higher for seaweed biomass than the protein ingredient (3.7 ± 0.2 vs. 2.6 ± 0.2 μg B12/g protein). The most plausible explanation is that the extreme pH conditions during the extraction process may have denatured B12-binding proteins, thereby liberating the ligand from its protein complexes. Another possible explanation is the partial degradation of B12, likely first to OH-Cbl and subsequently to forms that could not be recovered as cyanocobalamin and, therefore, were not detected in the analysis (Figs. 1b and S1). This degradation is likely attributable to B12's sensitivity to both light and extreme pH (Lie et al., 2020), as the extraction process included steps at pH 12 and pH 2. According to mass balances, the relative percentage of lost/degraded B12 relative to the raw material was 83.7 %, whereas the relative

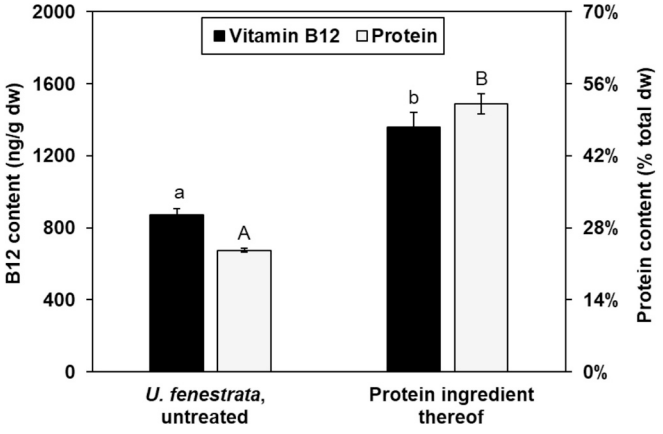

Fig. 3. – Vitamin B12 content ($n = 3$) and protein content (measured as total amino acids, n = 3) of untreated *U. fenestrata* and protein ingredient thereof on a dry weight basis. Protein extraction followed the method 0.1Trit + Alk described in Trigo et al. (2025). Different lowercase letters mean differences in B12 content, whereas uppercase letters mean differences in total amino acid content (*t*-test, $p < 0.01$). Amino acid data was retrieved from the cited work since the same samples were used.

amount of non-recovered protein was 77.4 %.

The protein extraction method used in the present study achieved extraction yields 3.4 times higher than a conventional pH-shift method (Trigo et al., 2025). The yield (~25 %), measured as total amino acids, is among the highest reported for *U. fenestrata* (Juul et al., 2024). Therefore, the current method avoids time- and/or energy-consuming steps like freeze-thawing, heating, dialysis, and ultrasonication as well as toxic reagents (e.g., β-mercaptoetanol and ammonium sulfate), relatively high seaweed-to-water ratios, or expensive enzymes so far available for research purposes (e.g., ulvan lyase). While a yield of ~25 % is lower than those typically achieved for e.g., soy protein (~50 %; Verfaillie et al., 2023), we consider that focusing solely on yields overlooks other key aspects of ingredient value, such as nutritional multifunctionality due to the presence of e.g., omega-3 fatty acids (Trigo et al., 2025) and vitamin B12, the latter not naturally present in soybeans. Further, we envision this protein extraction method as a part of an integrated seaweed biorefinery concept, where the protein recovery step is followed by subsequent extractions of e.g. polysaccharides; an approach we previously proposed in a study on the red seaweed *Porphyra umbilicalis* (Wahlström et al., 2018).

About 2.9 g dw of the *Ulva* protein ingredient would meet the adequate daily intake of vitamin B12 (EFSA, 2015). The ingredient also qualifies for an EU nutritional claim on "High in Vitamin B12" along with the EU health claims exemplified in **Section 3.2**. Moreover, compared to cooked beef, which contains 1.9–2.4 μg B12/100 g fw (Gille & Schmid, 2015; USDA, 2011), the protein ingredient contains 23 to 29 times more vitamin B12 when both products are normalized to a typical moisture content of cooked meat, here beef (~60 %; USDA, 2011). Moisture normalization allows for a fair comparison, as the native moisture content of the *Ulva* protein ingredient was about 92 %. Even assuming a one-third loss of B12 during extrusion for a hypothetical meat analog containing the *Ulva* protein ingredient, the B12 content would be 15 to 19 times greater than that of cooked beef.

4. Conclusion

This short communication aimed to investigate the effect of species, post-harvest treatment, drying technique, storage time, and downstream protein extraction on the vitamin B12 content in seaweed biomass. The study showed that the B12 content of *U. fenestrata* was one order of magnitude higher than that of *P. palmata* and *S. latissima*. Moreover, biomass soaking did not affect the B12 content on a dw basis, whereas blanching significantly reduced it, namely in *U. fenestrata*. Despite this reduction, the blanched *U. fenestrata* remained a rich source of this vitamin due to the high B12 content in the untreated biomass. Overall, oven-drying and freeze-drying equally preserved B12; dark storage of up to 4.8 months preserved the vitamin, regardless of the drying technique. The protein ingredient obtained from *U. fenestrata* contained more B12 than the original biomass and red meat, when normalizing the B12 contents to dry weight and equal moisture, respectively. However, considerable losses of B12 were observed during the preparation of the protein ingredient.

Altogether, food products developed from whole *U. fenestrata* and its protein ingredients show strong potential as dietary sources of vitamin B12. Nevertheless, bioavailability trials are necessary to determine the extent to which vitamin B12 from these products is absorbed and reaches systemic circulation.

CRediT authorship contribution statement

João P. Trigo: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Bhawani Chamlagain:** Writing – review & editing, Validation, Investigation, Formal analysis. **Jonatan Thorén:** Validation, Methodology, Investigation. **Rebecca Strand:** Writing – review & editing, Validation, Methodology, Investigation. **Mar Vall-llosera Juanola:** Writing – review & editing, Validation, Methodology, Investigation. **Sophie Steinhagen:** Writing – review & editing, Resources, Funding acquisition. **Alexandra Kinnby:** Writing – review & editing, Resources, Funding acquisition. **Gunilla Toth:** Writing – review & editing, Supervision, Resources, Funding acquisition. **Susanna Kariluoto:** Writing – review & editing, Resources, Funding acquisition. **Ingrid Undeland:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: João P. Trigo and Ingrid Undeland have a patent pending to Chalmers Ventures AB. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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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.2025.144302.

Data availability

Data will be made available on request.

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