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# Ultrasound and enzymatic processing of *Palmaria palmata* – effects on biochemical components

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ABSTRACT

*Palmaria palmata* is gaining commercial interest in the northern hemisphere as a source of nutrients and bioactive compounds. However, the complex cell wall limits digestibility and extraction efficiency. This study investigated the use of ultrasound (US) and enzyme-assisted extraction (EAE), individually and in combination, to enhance the extraction of valuable components from *P. palmata* and to reduce iodine content. The US treatment was tested at two energy inputs (2.0 W/g and 9.1 W/g) alongside EAE using the multifunctional carbohydrase mixture Depol 793 EAE effectively degraded the cell wall components of *P. palmata*, resulting in a solid fraction rich in proteins. In contrast, the liquid fraction was enriched with sugars (xylose, glucose and galactose) and ash. In contrast, US alone did not affect extraction, and no additive or synergistic effects were observed when combining US with EAE. No significant reduction in iodine was obtained for any of the treatments, ranging from 10 % to 18 %. This study confirms that EAE is a promising approach for nutrient extraction from *P. palmata*, and future studies should explore additional enzymes and optimize US conditions to further improve extraction efficiency, in addition to including analysis of toxic elements to identify potential challenges for safe consumption.

## 1. Introduction

Macroalgae, or seaweed, has a long tradition as a source of food and traditional medicine in Asian countries. Seaweeds are gaining increased attention in Western countries as a source of bioactive components and as a food and feed ingredient (Stengel & Connan, 2015). They are a diverse taxonomic group with high chemical diversity (El Gamal, 2010), with a long tradition as a food source rich in polysaccharides, minerals, vitamins, proteins and lipids (Kadam et al., 2015). In addition, they produce various bioactive substances, such as polyphenols and polysaccharides, with various bioactivities, including antibacterial and antiviral properties (Holdt & Kraan, 2011). *Palmaria palmata* (commonly known as dulse) is increasingly gaining commercial interest due to its high protein content and nutritional value, making it a promising candidate for food applications (Stévant et al., 2023).

It is a widely used seaweed species for human consumption and is recognised for its strong umami flavour (Delaney et al., 2016; Mouritsen et al., 2013). The protein content in *P. palmata* can reach up to 35 % of its dry weight (DW) (Fleurence et al., 2018), and it has a high carbohydrate content, reaching up to 74 % of its DW. In addition, it is rich in micronutrients such as minerals and vitamins (Stévant et al., 2023). The characteristic red colour of *P. palmata* is caused by the pigments phycobiliproteins, specifically R-phycoerythrin and phycocyanin (Dumay & Morançais, 2016).

Seaweed generally has highly complex and structurally diverse cell walls that vary between species. It often comprises mixtures of sulphated and branched polysaccharides in strong associations with proteins and minerals (Fuller & Gibor, 1987; Jeon et al., 2011). The cell wall of *P. palmata* is primarily composed of β-(1,3)/β-(1,4)-D-xylans, accounting for up to 35 % of the total carbohydrate content (Deniaud et al., 2003; Kraan, 2012). This structural complexity poses a challenge for digestion and efficient extraction of valuable components, necessitating innovative approaches to overcome these barriers (Deniaud et al., 2003; O' Connor et al., 2020). Existing methods for disrupting the complex cell wall structure of seaweeds are insufficient for optimal extraction of valuable compounds. This study addresses the need for more effective techniques to enhance extraction efficiency. There is a growing interest in finding new, greener, more efficient extraction methods for valuable

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components from seaweeds (Carreira-Casais et al., 2021). To address these extraction challenges, various physical and chemical processing techniques are being investigated, including ultrasound (US), pulsed electric field, microwaves, and enzyme-assisted extraction (EAE) (Maribu et al., 2024; World Bank, 2023).

US uses high-frequency sound waves (20–100 kHz) that propagate through the biomass. This generates vapour bubbles within the structure that collide, causing perturbation within the biomass, also called cavitation. This can affect the extraction efficiency from the biomass and improve mass transfer. US is a simple and cost-effective alternative to conventional extraction methods (Kadam et al., 2013). EAE has gained increased interest due to its ability to weaken or disrupt the cell wall structure of seaweed and increase the extraction efficiency of intracellular compounds (Gil-Chávez et al., 2013; Wang et al., 2010). Several studies have shown the potential of US treatment on seaweeds. García-Oms et al. (2024) demonstrated that US treatment increased the antioxidant activity of polyphenols and pigments extracted from *P. palmata*. Inguanez et al. (2023) showed that US treatment under different conditions increased the extraction yields of nutrients from the brown seaweed *Alaria esculenta*. Therefore, the prospect of combining US and EAE to increase extraction efficiency is promising.

Further, processing of seaweeds has been suggested as a potential way to control and reduce potentially toxic elements, such as iodine, in seaweeds (Blikra, Aakre, & Rigutto-Farebrother, 2024). Several methods have been and are being investigated, including blanching (Nielsen et al., 2020), soaking (Blikra, Aakre, & Rigutto-Farebrother, 2024; Jordbrekk Blikra et al., 2021; Stévant et al., 2018), pulsed electric field (Blikra et al., 2022, 2024) and US (Noriega-Fernández et al., 2021). There are, however, limited studies showing this effect on *P. palmata*.

This study aimed to investigate whether the use of US at two different energy inputs and hydrolysis with a carbohydrate-degrading enzyme, alone or in combination, would be suitable strategies for extracting valuable components from *P. palmata* while simultaneously reducing the iodine content. The novelty of this study is its examination of US and enzyme-assisted extraction, both individually and in combination, on *P. palmata* biomass, a largely unexplored area in current research.

## 2. Materials and methods

### 2.1. Raw material

The wild-harvested *P. palmata* was a mixture of gametophytes and tetrasporophytes of different sizes, harvested in Kårvik, Norway, in September 2022 (N69°52′05.8, E18°55′34.6). As described in Maribu et al. (2024), the seaweeds were shipped on ice overnight and stored in tanks with a constant flowthrough of fresh seawater at 8.4 ± 0.2 L/min and kept at 8.0 ± 0.1 °C to mimic the seawater temperature at the time of harvest at NORCE's facilities (Randaberg, Norway), to ensure fresh material throughout the study. The samples were washed in tap water and all epiphytes were removed. The seaweed was divided into batches of 10 g wet weight (WW) in 100 mL tap water (1:11), with three processing parallels for each treatment (n = 3). Water was chosen as the medium during ultrasound processing as it is a good medium for energy distribution. The samples were then homogenised using an IKA® T25 digital ULTRA TURRAX® (IKA, Staufen, Germany) at 3600 ± 200 rpm for 15 min.

### 2.2. Ultrasound treatment

The US treatment used a BT 130H benchtop ultrasonic water bath (35.6 cm W x 50.8 cm L x 35.6 cm H) (UPCORP, Freeport, IL, USA). Two different treatments with energy inputs of 500 W and 1000 W, with a nominal specific power of 2.0 W/g and 9.1 W/g, respectively, were applied to the homogenised seaweed batches (n = 3), operating at a frequency of 68/170 kHz. The ultrasound treatment employed frequencies of 68/170 kHz to evaluate their efficacy in disrupting cell structures and enhancing extraction yields. The US treatment was conducted for 30 min at room temperature (±20 °C). The temperature was controlled by having a constant flow of fresh water into the water bath. The temperature in the water bath increased during treatments, and the maximum temperature measured after treatment was 32.9 °C. The seaweed batches were placed in small steel bowls where the whole sample was under water during the US treatment.

### 2.3. Enzyme-assisted extraction

The EAE was conducted using the method previously described in Maribu et al. (2024). The extraction was performed on both US-treated and control samples in triplicates (n = 3), as seen in Fig. 1. EAE was conducted at 50 rpm (New Brunswick Incubator Shaker, Innova 40, Fisher Scientific, Hampton, NH, USA) for 1 h at 40 °C, where the enzyme, Depol 793 ((Batch number 27060) (Biocatalysts, London, United Kingdom) were added to a concentration of 1 % (V). After extraction, the samples were centrifuged at 10,000 × *g* for 20 min (Multifuge X3 FR, Thermo Fisher Scientific, Waltham, MA, USA). The liquid and solid fractions were freeze-dried before analysis (Gamma 2–16 LSCplus, CHRIST, Osterode am Harz, Germany).

### 2.4. Thermogravimetric analysis

Thermogravimetric analysis was used to determine the dry matter (DM) and ash content in triplicates (N = 3) using a TGA/DSC 3+. The results were analysed using the STARe evaluation software 16.40 (Mettler Toledo, Columbus, OH, USA). The samples (~30 mg WW) were subjected to a temperature gradient starting at 30 °C and up to 600 °C, with 10 K/min. When 105 °C and 600 °C were reached, the samples were held at these temperatures for 10 min to determine the DM and ash content. These temperatures were used as standard temperatures for dry matter and ash determination (Liu, 2019).

### 2.5. Protein analysis

The protein content was analysed using a Kjeltec™ 8400 (FOSS analytics, Hilleroed, Denmark) in triplicates (N = 3), with a specific conversion factor of 4.39 calculated for *P. palmata* harvested at the same latitude (Maribu, Elvevold, Eilertsen, & Blikra, 2025)). The samples (~10 mg) were hydrolysed in a heating block at 420 °C with 15 mL $H_2SO_4$ (Merck KGaA, Darmstadt, Germany) and 2 copper catalyst tablets (Kjeltabs Cu/3.5, Nerliens Meszansky, Oslo, Norway). After hydrolysis, the samples were cooled down, and 30 mL of distilled water was added before analysis, as previously described in Maribu et al. (2024).

### 2.6. Amino acid analysis

The amino acids in the liquid fractions were measured (N = 1) using a Biochrom 30+ amino acid analyser (Biochrom Co., Cambridge, UK) and analysed with Chromeleon software (Dionex, Sunnyvale, CA, USA), with A6407 and A6282 amino acid standards for identification. A lithium citrate equilibrated column with post-column derivatisation with ninhydrin was used. The samples (~40 mg) were hydrolysed for 26 h at 105 °C with 0.7 mL distilled water, 1.2 mL HCl (37 %) and 0.05 mL 20 mM DL-norleucine (Sigma Aldrich, Saint Louis, MO, USA). Prior to hydrolysis, the samples were flushed using nitrogen for approximately 10 s. The samples were centrifuged at 18,000 × *g* for 5 min (Eppendorf 5424 R centrifuge, Hamburg, Germany) and flushed with nitrogen until dry. Before analysis, the samples were dissolved in 1 mL lithium buffer (pH 2.2).

### 2.7. Sugar analysis

The sugar content was analysed in duplicates (N = 2) using GC-FID (Agilent Technologies, Santa Clara, CA, USA), as previously described

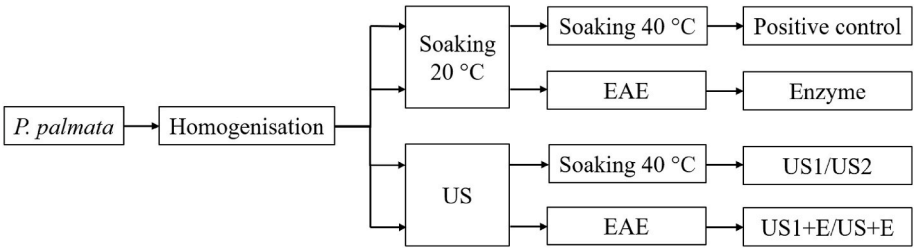


**Fig. 1.** Experimental workflow.

in Maribu et al. (2024), building on methodology from Englyst et al. (1994). Arabinose, fucose, galactose, glucose, mannose, rhamnose (all from Sigma Aldrich, Saint Louis, MO, USA) and xylose (Merck KGaA, Darmstadt, Germany) diluted in 50 % benzoic acid (Merck KGaA, Darmstadt, Germany) were used as standards.

### 2.8. Iodine analysis

The iodine content in the liquid fractions (N = 1) and the raw material (N = 2) was performed and analysed by Mikroanalytisches Labor Kolbe (Oberhausen, Germany), which is an independent laboratory specialising in elemental analyses. As described in Jordbrekk Blikra et al. (2021), the samples were digested in a combustion unit from A1-Envirosciences (AQF-2100) with a manual sampler at 1100 °C and burned in an argon/oxygen stream. A Metrohm Model Plus ion chromatograph (IC) measured the gases produced during digestion. The detection limit was determined based on the sample mass, and the detection limit for iodine in the IC was 1 ppm. A control sample with a predetermined amount of iodine was added, and the significance level was set to $P < 0.05$.

### 2.9. Statistical analysis

One-way ANOVA was performed using Minitab® version 21.4.1. with a confidence interval of 95 % to test for significance between the sample groups. When more than two sample groups were present, a Tukey post hoc test was applied to test for variance ($P < 0.05$). All processing has been performed in triplicates (n = 3). In addition, all analyses have been performed in triplicates for each processing parallel unless otherwise specified. The results are presented as the mean with the standard deviation of the processing parallels (n = 3) (with each processing parallel having technical replication (N = 3)).

## 3. Results

### 3.1. Dry matter and ash

The DM in the raw material was 15.1 %. Following processing and centrifugation, the samples were separated into liquid and solid fractions. When the *P. palmata* was soaked in room-tempered water for 30 min, followed by warm-water soaking at 40 °C for 1 h (positive control), 44.6 % of the DM was recovered in the liquid fraction, as seen in Table 1. There was no significant increase in DM content extracted into the liquid fraction when the samples were treated with US at either low (US1) or high (US2) energy inputs (40.6–49.5 %) compared to the positive control. However, when the samples were treated with enzyme, the DM content in the liquid fraction increased significantly (82.5 %) compared to the positive control. A combination of US1/US2 and enzyme had no significant effect on the DM content in the liquid fraction compared to the sample treated with enzyme alone. As the DM content increased in the liquid fraction, it decreased in the solid fraction, resulting in significantly lower DM content in the samples treated with enzyme. As seen in Table 1, significant amounts of ash were extracted into the liquid fraction in the various treatments where the positive control, US1, and US2, had the highest ash content (33.1–35.7 % DM). The ash content in the solids was similar for most samples, where US2+E had the lowest ash content. The solid samples with higher DM content also had a higher ash content on a DM basis.

**Table 1**
Dry matter (DM) content in the different fractions as mean percent ± standard deviation (SD) and the ash content as percent DM ± SD (n = 3, with technical replication (N = 3), for each processing parallel) obtained from 10 g wet weight *P. palmata*. The DM content in the raw material was 15 %. The samples: Positive control: soaking; Enzyme: Enzyme assisted extraction (EAE); US1 and US2: treated with ultrasound (US) at low or high energy inputs; US1+E and US2+E: treated with US at low or high energy inputs combined with EAE.

| | Dry matter | | Ash | |
|---|---|---|---|---|
| | Liquid | Solid | Liquid | Solid |
| Positive Control | $44.6 \pm 4.5^{B}$ | $55.4 \pm 4.5^{A}$ | $33.1 \pm 3.3^{AB}$ | $28.9 \pm 1.0^{AB}$ |
| Enzyme | $82.5 \pm 2.9^{A}$ | $17.5 \pm 2.9^{B}$ | $29.0 \pm 6.9^{AB}$ | $22.9 \pm 1.4^{ABC}$ |
| US1 | $49.5 \pm 14.9^{B}$ | $50.5 \pm 14.9^{A}$ | $35.7 \pm 8.6^{A}$ | $24.2 \pm 1.4^{A}$ |
| US1+E | $71.3 \pm 10.0^{A}$ | $28.7 \pm 10.0^{B}$ | $30.4 \pm 2.5^{AB}$ | $22.4 \pm 1.3^{BC}$ |
| US2 | $40.6 \pm 2.3^{B}$ | $59.4 \pm 2.3^{A}$ | $36.4 \pm 4.6^{A}$ | $24.2 \pm 0.8^{A}$ |
| US2+E | $82.2 \pm 3.0^{A}$ | $17.8 \pm 3.0^{B}$ | $27.7 \pm 4.7^{B}$ | $21.8 \pm 0.7^{C}$ |

Capital letters indicate significant differences within groups.

### 3.2. Protein and amino acids

The extractable protein content varied between 6 and 7 % DW (of the liquid phase) across treatments (Fig. 2A). Significant differences were found in the protein content remaining in the solids after processing. The samples treated with enzyme alone or combined with US1 or US2 had a higher protein content in the solids (25.1–30.3 %) on a DW basis. The lower energy input treatment, US1, had a significantly higher protein content than the sample treated with the higher energy input, US2, which was similar to the positive control (~15.2 %).

The amino acid composition (AA) of the liquid fractions from the different treatments is shown in Table 2. The extracted AA ranged from 18.5 to 31.1 % of the total amino acid (TAA) content, where the positive control had the lowest content and enzyme had the highest. The liquid fractions' essential amino acids (EAA) content ranged from 26.0 % to 32.5 %. Some of the AA were extracted to a large degree (>80 %). This includes the EAA threonine and the non-essential AA glutamic acid, aspartic acid and proline. Tryptophan was not detected due to complete degradation during acid hydrolysis before the analysis.

### 3.3. Sugars

All treatments readily extracted sugars, and no significant differences (Table S1) in total sugar content were found between the treatments. However, the results indicate that the various sugars were extractable to varying degrees in the different treatments (Fig. 3). The positive control, US1 and US2 samples exhibited a higher galactose content than those subjected to EAE. However, on a DW basis, galactose was efficiently extracted across all treatments, including the positive control, with similar amounts on a WW basis. EAE enhanced the

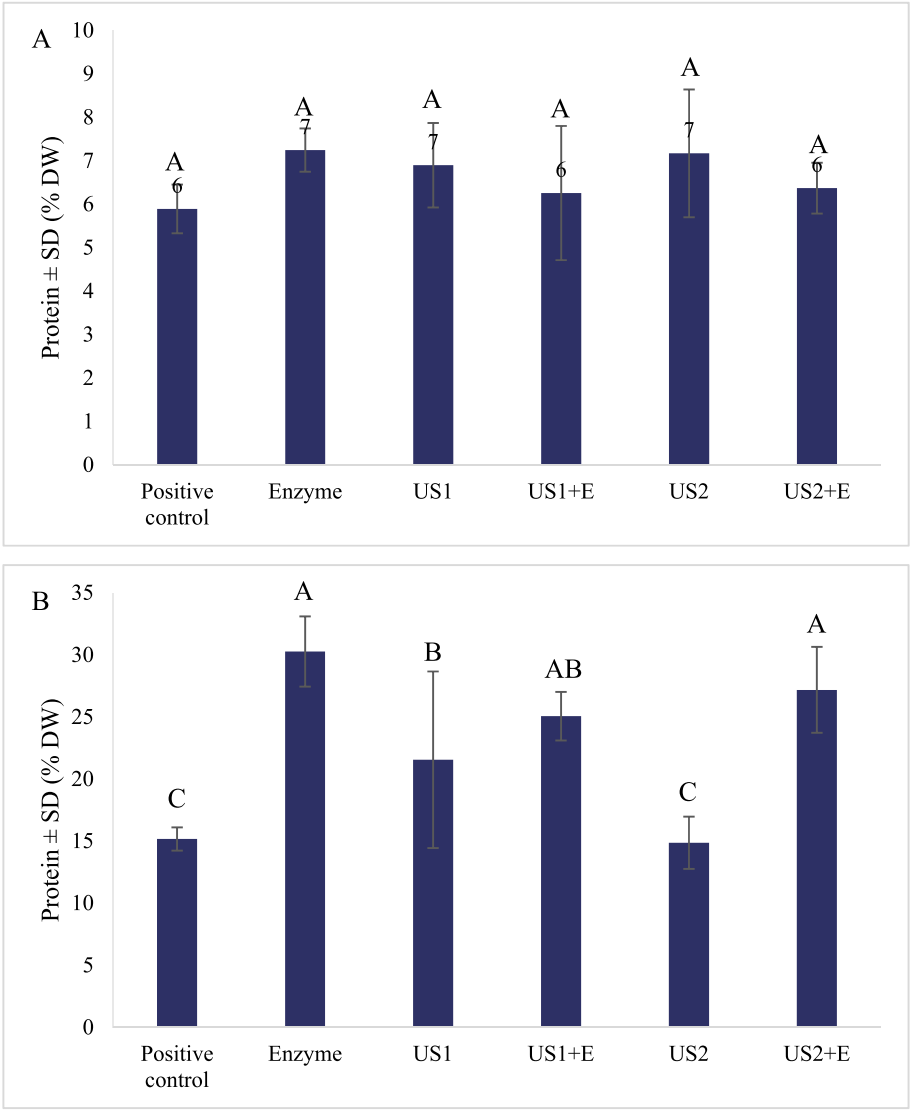


**Fig. 2.** Protein content in the liquid (A) and solid (B) fraction after treatment with ultrasound (US) and enzyme (EAE) given as mean percent dry weight ± standard deviation (n = 3, with technical replication (N = 3), for each processing parallel). Capital letters indicate significant differences within groups. The samples: Positive control: soaking; Enzyme: EAE; US1 and US2: treated with US at low or high energy inputs; US1+E and US2+E: treated with US at low or high energy inputs combined with EAE.

extraction of xylose, mannose and glucose compared to US alone and the positive control. Glucose and xylose levels were increased in enzyme-treated samples relative to the positive control, US1, and US2, constituting 11.3 % and 20.7 % DW, respectively. Mannose and arabinose were detected in minimal amounts varying from 0.1 to 1.3 % DW and 0.1–0.3 % DW, respectively, for all treatments. Fucose and rhamnose were not detected in any samples.

### 3.4. Iodine

The iodine content in the liquid fractions varied between 11.7 and 21.3 mg/kg DW, with no significant differences between the various treatments (Table 3). This resulted in a 10.1–18.4 % reduction of iodine in the solid fractions. The estimated iodine content in the solid fractions varied between 94.7 and 103.3 mg/kg DW (81.6–89.9 % of the iodine content).

## 4. Discussion

Degradation of the complex cell wall structure of seaweeds is essential for releasing valuable compounds. The complex and diverse polysaccharide composition of these cell walls might hinder extraction efficiency, particularly when using conventional extraction methods (Wijesinghe & Jeon, 2012). US and EAE are green technologies that have proven cost-efficient and promising for extracting valuable components from seaweed (Kadam et al., 2013; Quitério et al., 2022).

In the present study, US treatment has been combined with EAE to enhance extraction efficiency. It has been reported that treating the biomass with US before EAE enhanced the interaction between the enzyme and the biomass and promoted extraction by generating and exposing more target sites for further enzymatic breakdown of the biomass (Dabbour et al., 2019). However, combining US at 500 W or 1000 W with Depol 793 did not enhance the extraction, and EAE alone had the highest impact on extraction. Further, the US treatment was conducted using a frequency of 68/170 kHz, resulting in nominal specific powers of 2.0 W/g (500 W) and 9.1 W/g (1000 W). This coupling of frequencies was chosen as lower frequencies (68 kHz) will only release the molecules on the surface of the biomass (Noriega-Fernández et al., 2021). Several studies show that increasing powers increase the force of the cavitation bubbles generated, leading to increased extraction

**Table 2**
Amino acid composition in the liquid fraction from the control, ultrasound (US) and enzyme-assisted extraction (EAE) treated *P. palmata*. The data is given as mean mg AA/g dry weight ± standard deviation (n = 3, with technical replication (N = 1) of each processing parallel). The samples: Positive control: soaking; Enzyme: EAE; US1 and US2: treated with US at low and high energy inputs; US1+E and US2+E: treated with US at low or high energy inputs combined with EAE.

| | Positive control | Enzyme | US1 | US1+E | US2 | US2+E |
|---|---|---|---|---|---|---|
| Histidine | 0.0$^{B}$ | 0.7 ± 0.6$^{B}$ | 0.1 ± 0.2$^{B}$ | 0.7 ± 0.6$^{B}$ | 0.3 ± 0.4$^{B}$ | 0.9 ± 0.9$^{AB}$ |
| Isoleucine | 1.8 ± 0.3$^{B}$ | 3.7 ± 0.4$^{A}$ | 2.2 ± 0.3$^{B}$ | 2.7 ± 0.6$^{B}$ | 2.0 ± 0.3$^{B}$ | 3.5 ± 0.6$^{B}$ |
| Leucine | 3.2 ± 0.8$^{B}$ | 6.2 ± 0.5$^{AB}$ | 4.7 ± 1.3$^{B}$ | 4.8 ± 1.0$^{B}$ | 3.9 ± 0.4$^{B}$ | 6.0 ± 1.4$^{AB}$ |
| Phenylalanine | 1.9 ± 0.3$^{B}$ | 3.8 ± 0.3$^{AB}$ | 2.5 ± 0.6$^{B}$ | 2.8 ± 0.6$^{B}$ | 2.2 ± 0.3$^{B}$ | 4.0 ± 1.3$^{AB}$ |
| Methionine | 1.1 ± 0.3$^{B}$ | 1.8 ± 0.4$^{B}$ | 1.6 ± 0.5$^{B}$ | 1.4 ± 0.3$^{B}$ | 1.1 ± 0.1$^{B}$ | 1.8 ± 0.2$^{B}$ |
| Lysine | 2.5 ± 0.7$^{B}$ | 2.8 ± 0.2$^{B}$ | 3.3 ± 0.8$^{B}$ | 1.9 ± 1.2$^{B}$ | 2.8 ± 0.1$^{B}$ | 2.3 ± 0.9$^{B}$ |
| Threonine | 5.8 ± 2.0$^{A}$ | 7.1 ± 0.3$^{A}$ | 6.6 ± 2.1$^{A}$ | 5.9 ± 1.3$^{A}$ | 5.7 ± 1.2$^{A}$ | 7.1 ± 1.3$^{A}$ |
| Tryptophan | n. d | n. d | n. d | n. d | n. d | n. d |
| Valine | 2.3 ± 0.4$^{B}$ | 5.1 ± 0.3$^{B}$ | 3.2 ± 0.7$^{B}$ | 3.8 ± 0.9$^{B}$ | 2.9 ± 0.3$^{B}$ | 4.7 ± 1.3$^{B}$ |
| Alanine | 4.6 ± 1.3$^{B}$ | 7.2 ± 0.3$^{AB}$ | 6.1 ± 1.4$^{B}$ | 5.6 ± 1.5$^{B}$ | 5.3 ± 0.3$^{B}$ | 6.8 ± 1.5$^{B}$ |
| Arginine | 2.1 ± 0.3$^{B}$ | 4.7 ± 1.4$^{B}$ | 3.1 ± 0.9$^{B}$ | 2.8 ± 1.1$^{B}$ | 3.2 ± 1.5$^{B}$ | 6.7 ± 3.0$^{B}$ |
| Asparagine | n. d | n. d | n. d | n. d | n. d | n. d |
| Aspartic acid | 8.0 ± 2.0$^{A}$ | 9.3 ± 0.8$^{A}$ | 12.8 ± 6.7$^{A}$ | 7.4 ± 2.4$^{A}$ | 9.1 ± 1.4$^{A}$ | 9.1 ± 2.1$^{A}$ |
| Cysteine | 2.2 ± 0.5$^{A}$ | 2.7 ± 0.7$^{A}$ | 2.7 ± 0.4$^{A}$ | 2.2 ± 0.4$^{A}$ | 3.0 ± 0.6$^{A}$ | 2.5 ± 1.3$^{A}$ |
| Glutamine | n. d | n. d | n. d | n. d | n. d | n. d |
| Glutamic acid | 20.6 ± 0.2$^{AB}$ | 16.5 ± 1.2$^{AB}$ | 27.4 ± 8.4$^{A}$ | 13.2 ± 4.2$^{B}$ | 23.3 ± 2.7$^{AB}$ | 15.9 ± 4.4$^{AB}$ |
| Glycine | 3.8 ± 1.1$^{B}$ | 6.7 ± 0.3$^{B}$ | 5.0 ± 1.2$^{B}$ | 5.0 ± 0.9$^{B}$ | 4.3 ± 0.2$^{B}$ | 6.5 ± 1.2$^{B}$ |
| Proline | 5.8 ± 1.5$^{A}$ | 7.0 ± 0.4$^{A}$ | 6.4 ± 1.4$^{A}$ | 4.8 ± 0.8$^{A}$ | 6.4 ± 1.1$^{A}$ | 7.0 ± 1.2$^{A}$ |
| Serine | 2.8 ± 0.3$^{B}$ | 6.6 ± 0.2$^{AB}$ | 3.6 ± 0.8$^{B}$ | 5.2 ± 1.2$^{AB}$ | 3.3 ± 0.5$^{B}$ | 6.7 ± 1.4$^{AB}$ |
| Tyrosine | 1.6 ± 0.1$^{B}$ | 4.0 ± 0.5$^{AB}$ | 2.0 ± 0.3$^{B}$ | 3.1 ± 0.6$^{B}$ | 1.6 ± 0.2$^{B}$ | 4.2 ± 1.1$^{AB}$ |
| TAA | 69.8 ± 12.0$^{B}$ | 95.8 ± 6.1$^{AB}$ | 93.0 ± 25.4$^{AB}$ | 73.2 ± 18.5$^{B}$ | 80.2 ± 5.2$^{B}$ | 95.7 ± 23.5$^{AB}$ |
| % Protein | 6.0 ± 1.0$^{B}$ | 8.2 ± 0.5$^{AB}$ | 8.0 ± 2.2$^{AB}$ | 6.2 ± 1.6$^{B}$ | 6.9 ± 0.5$^{B}$ | 8.2 ± 2.0$^{AB}$ |
| % Kjeldahl protein (4.39) | 6.3 ± 0.6$^{B}$ | 7.8 ± 0.5$^{B}$ | 7.4 ± 1.0$^{B}$ | 6.7 ± 1.6$^{B}$ | 7.7 ± 1.7$^{B}$ | 6.9 ± 0.6$^{B}$ |

n. d = not detected.
Capital letters indicate significant differences within groups.

(García-Oms et al., 2024; Kumar et al., 2021).

Our results demonstrate a significant increase in DM content in the liquid fraction from 44.6 % in the positive control to 82.5 % in the enzyme-treated samples. In contrast, US treatments resulted in DM content of 49.5 % (US1) and 40.6 % (US2). Lahaye and Vigouroux (1992) reported an increase of 79.8–81.4 % DM from fresh *P. palmata* when using the enzyme xylanase. The enzyme Depol 793, is a mixture of beta-glucanase, pectin lyase, and cellulase activity that efficiently targets and breaks down the complex polysaccharide matrix. The results of this present study show that the enzyme efficiently targets the polymer bonds present in *P. palmata,* and the liquid fraction consisted mainly of sugars and ash, specifically xylose, glucose and galactose. The extracted xylose is most likely obtained from the degradation of the structural xylans in the cell wall (Bajpai, 2014).

EAE resulted in a liquid fraction rich in sugars and ash, leaving a solid fraction rich in proteins. The protein content in the solid fraction made up 30.3 % DW, compared to 15.2 % DW in the positive control. Similar protein enrichment in the solid fraction of *P. palmata* has been reported previously by Aasen et al. (2022) using a different enzyme, xylanase, and by Maribu et al. (2024) when using Depol 793. Thus, these enzymes (xylanase and Depol 793) show similar effectiveness in degrading the cell wall structure in *P. palmata*. Depol 793 is a much more cost-efficient enzyme (approximately half the price of xylanase), making it a preferred option for EAE of *P. palmata*. The protein content in the liquid fraction after EAE treatment (alone or combined with US) varied between 6.3 and 7.2 % DW. Previous studies on *P. palmata* show that EAE (using xylanase) increased the extractability of water-soluble proteins, specifically their main pigment, R-phycoerythrin. This was confirmed in this present study, as the liquid fractions had the characteristic pink colour of the pigment (not shown) (Dumay et al., 2013).

It has previously been reported that deionized water alone extracted 52.7–58.8 % DM from fresh *P. palmata* after a 24-h incubation (Lahaye & Vigouroux, 1992). The results in the present study show that soaking in room-tempered water for 30 min followed by warm-water soaking at 40 °C for 1 h (positive control) resulted in a similar extraction of DM content. However, differences in incubation time and degree of homogenisation of *P. palmata* might be a reason for the differences seen in extraction yields. Similarly to EAE, the liquid phase after US consisted largely of sugars and ash. However, with US, galactose was the primary sugar extracted, in addition to small amounts of xylose and glucose. It has previously been shown that almost all the galactose present in *P. palmata* is in water-soluble form (Rødde et al., 2004) and that soaking and US treatment in water should be efficient in extracting galactose. The low content of xylose points to a low degradation of xylans and the cell wall structure using both US1 and US2, where the content is similar to the positive control. This show that US treatment at a nominal specific power of 2.0 W/g (500 W) and 9.1 W/g (1000 W) had little effect on degrading the structural xylans.

The protein content in the liquid fractions of US1 and US2 varied between 6.8 and 7.1 % DW. Previous studies have shown that a sonication power of 200 W increased protein release from microalgae, while the protein release decreased with higher sonication powers (Fan et al., 2020; Zheng et al., 2024). This present study tested US powers of 500 W and 1000 W, resulting in protein contents in the solid fraction of 21.5 % and 14.9 %, respectively. The US was, therefore, efficient in terms of concentrating protein in the solids but not in terms of protein extraction. Despite a lack of efficiency with regard to extraction, seaweed processing has been shown to increase seaweed protein digestibility. In its native form, seaweeds have a low protein digestibility at around 50 % due to the complex cell wall (Cebrián-Lloret et al., 2024). Mæhre et al. (2016) reported that boiling *P. palmata* for 15 and 30 min increased the accessibility of the proteins and essensial amino acids in the solid samples, while Braspaiboon et al. (2022) showed that US treatment followed by alkaline extraction increased protein accessibility in microalgae. In both cases, the protein was left in the solid fraction, as observed in this present study. US treatments have also been shown to increase the

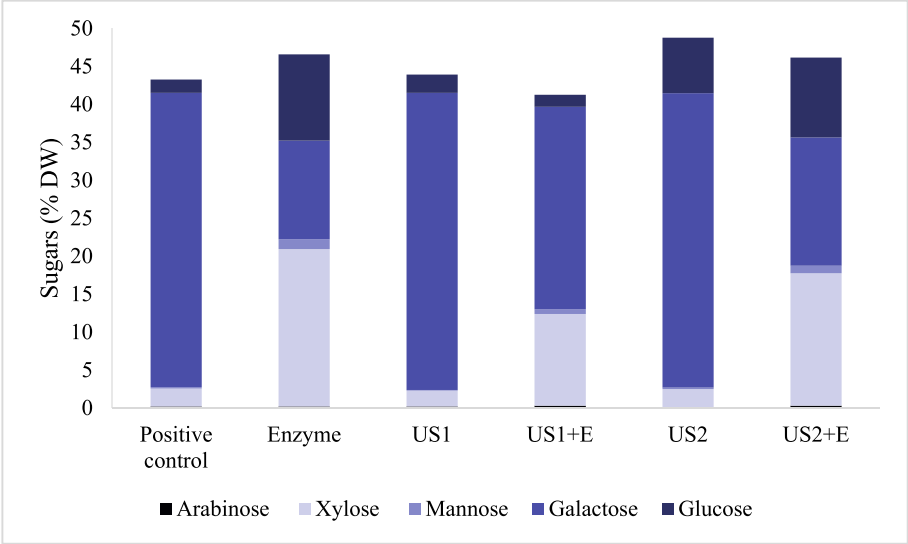


**Fig. 3.** Sugar content in the liquid fraction is given as percent dry weight (n = 3, with technical replication (N = 2) of each processing parallel). The samples: Positive control: soaking; Enzyme: Enzyme-assisted extraction (EAE); US1 and US2: treated with ultrasound (US) at low or high energy inputs; US1+E and US2+E: treated with low or high energy inputs combined with EAE. Table S1 in the supplementary material shows data, standard deviation, and statistical differences for the different sugars.

**Table 3**
Iodine content in mg/kg dry weight ± standard deviation (n = 3, with technical replication (N = 1) of each processing parallel) in the liquid fraction and the estimated content in the solid fractions. The estimated reduction of iodine in the solid is given as percent. The samples: Positive control: soaking; Enzyme: enzyme-assisted extraction (EAE); US1 and US2: treated with ultrasound (US) at low or high energy inputs; US1+E and US2+E: treated with US at low or high energy inputs combined with EAE.

| | Iodine content (mg/kg DW) in the liquid fraction | Estimated iodine content (mg/kg DW) in the solid fraction | Estimated iodine reduction in the solid fraction (%) |
|---|---|---|---|
| Positive control | $13.7 \pm 4.0^{A}$ | $102.3 \pm 4.0^{A}$ | 12.9 |
| Enzyme | $15.0 \pm 7.0^{A}$ | $101.0 \pm 7.0^{A}$ | 11.8 |
| US1 | $15.0 \pm 3.6^{A}$ | $101.0 \pm 3.6^{A}$ | 10.1 |
| US1+E | $11.7 \pm 2.1^{A}$ | $104.3 \pm 2.1^{A}$ | 10.9 |
| US2 | $21.3 \pm 4.5^{A}$ | $94.7 \pm 4.5^{A}$ | 12.9 |
| US2+E | $12.7 \pm 3.2^{A}$ | $103.3 \pm 3.2^{A}$ | 18.4 |
| Raw material | $116.0 \pm 22.6^{B}$ | $116.0 \pm 22.6^{A}$ | – |

Capital letters indicate significant differences within groups.

digestibility of plant protein through structural transformations (Aghababaei et al., 2024). It is not unlikely that US treatment can have a similar effect on seaweed protein. However, this needs to be confirmed through digestion experiments.

As mentioned in the introduction processing of seaweeds has been suggested for controlling and reducing potential toxic elements, such as iodine. In the present study, no significant reduction (10.1–18.4 %) in iodine content was seen for either of the treatments, including the positive control. This is in accordance with Nitschke and Stengel (2016) that observed a ~15 % iodine reduction in *P. palmata* after soaking for 1 h at 15 °C.

To date, there is limited data on the effects of US on the iodine content in seaweed, except from a few studies on different species of brown algae. When using US operating at 68 kHz and 500 W with a nominal specific power of 0.016 W/g, Noriega-Fernández et al. (2021) reported no significant reduction in iodine in the kelp *Laminaria hyperborea* compared to untreated control. This is a much lower nominal specific power than used in this study of 2.0 W/g (US1) and 9.1 W/g (US2), indicating that US at both low and high energy inputs was not efficient for iodine reduction. Also, Jönsson and Nordberg Karlsson (2024) reported a low iodine reduction (3 %) in *S. latissima* when using US, compared to 73 % and 70 % with soaking under high pressure and boiling, respectively.

The present study reports an iodine content of 116 mg/kg DW in the raw material. Interestingly, this is substantially lower than previously found in *P. palmata* harvested at the same latitude as the material used in this study (750 mg/kg DW) (Maribu, Elvevold, Eilertsen, & Blikra, 2025). This difference in iodine can be a result of different harvested locations and, therefore, different ocean currents and areas prone to industry and anthropogenic activities. In the previous study, we reported that arsenic and lead could become potential bottlenecks for the safe consumption of *P. palmata* if the iodine content were lowered. No significant reduction was observed in the present study during processing; however, the much lower iodine content in this raw material compared to that observed in our previous study increases the amount of *P. palmata* needed to reach the reference daily intake of iodine (150 µg) (EFSA et al., 2023) from 0.2 g to 1.5 g (7.5 times increase).

The liquid and solid fractions of *P. palmata* have several potential uses, and some are listed in Table 4. The liquid fraction is rich in sugars, especially galactose, minerals/ash (including iodine), and a small amount of protein. Potential uses include food and feed ingredients as a source of minerals, bioactive compounds, and taste components (umami) (Holdt & Kraan, 2011; Mouritsen et al., 2013). Extracted galactose from the liquid fraction has the potential for pharmaceutical applications to improve drug delivery and absorption (Conte et al., 2021). The solid fraction is rich in protein, sugars/carbohydrates (in the

**Table 4**
Potential applications of the liquid and solid fractions.

| Fraction | Market | Applications | Effect | Compounds | References |
|---|---|---|---|---|---|
| Liquid | Food | Food ingredient | Taste enhancer, soluble dietary fibre | Bioactive compounds, molecules associated with taste, sugars | (Holdt & Kraan, 2011; Mouritsen et al., 2013) |
| | Feed | Feed ingredient | Source of minerals, bioactives, and soluble dietary fibre | Minerals, bioactive compounds, sugars | Holdt and Kraan (2011) |
| | Agriculture | Biostimulants | Increase crop yields, increase nutrient availability, improve soil conditions | Polyphenols, sugars, minerals | (Calvo et al., 2014; Khan et al., 2009; G. Kumar & Sahoo, 2011) |
| | Pharmaceutical | Drug delivery | Substrates for galactose-containing conjugates improving drug delivery and adsorption | Galactose | Conte et al. (2021) |
| Solid | Food | Food ingredient | Iodine fortification, source of fibre and protein | Minerals, carbohydrates, proteins | (Blikra, Aakre, & Rigutto-Farebrother, 2024; Holdt & Kraan, 2011) |
| | Feed | Feed ingredient | Iodine fortification, source of fibre and protein | Minerals, carbohydrates, proteins | (Blikra, Aakre, & Rigutto-Farebrother, 2024; Krogdahl et al., 2021) |
| | Agriculture | Biostimulants | Increase crop yields, increase nutrient availability, improve soil conditions | Polyphenols, sugars, carbohydrates, minerals | (Calvo et al., 2014; Khan et al., 2009; G. Kumar & Sahoo, 2011) |

positive control and US samples), and minerals/ash. This makes the solids promising for food and feed ingredients as a source of protein, fibre, and minerals (Blikra, Aakre, & Rigutto-Farebrother, 2024; Holdt & Kraan, 2011; Krogdahl et al., 2021). Both the liquid and solid fractions have the potential as plant biostimulants, which can increase the growth, yield, and stress tolerance of different crops (Calvo et al., 2014; Khan et al., 2009; G. Kumar & Sahoo, 2011).

## 5. Methodological considerations

The sample size used in the experiments was low, ranging between 10 g and 40 g WW *P. palmata*. There are some uncertainties regarding how the US waves are distributed in the water bath, resulting in different amounts of waves reaching the material. The sample size might also affect this. Due to the limited raw material available, several analyses have no negative control.

The samples were freeze-dried after processing with a combination of US and enzyme. Changes in the biochemical properties were observed 2 min after removal from the freeze-dryer, and the material became "caramelised," probably due to binding moisture from the air. This made the material challenging to handle and process. However, the calculations have accounted for the differences in sample size and differences in moisture content.

The choice of conversion factor when analysing protein content with the Kjeldahl method is an ongoing discussion. Traditionally, the general nitrogen-to-protein conversion factor is 6.25. This is known to overestimate protein content in seaweed, and a suggested conversion factor of 4.39 was calculated for raw material harvested at the same latitude as the material used in this present study (Maribu, Elvevold, Eilertsen, & Blikra, 2025).

## 6. Conclusion

This study aimed to investigate whether using ultrasound at two different energy inputs and extraction with a carbohydrate degrading enzyme, alone or in combination, would be suitable strategies for extracting valuable components from *P. palmata.* US treatment with a nominal specific power of 2.0 W/g (500 W) and 9.1 W/g (1000 W) was not suitable for extracting components, such as proteins and carbohydrates, from *P. palmata*. The liquid fractions consisted mainly of galactose and ash, indicating minimal degradation of the cell wall components. However, Depol 793 efficiently degraded the cell wall components, resulting in a liquid fraction rich in sugars (xylose, glucose and galactose) and ash, leaving a protein-rich pellet. Combining US and enzymatic extraction showed no synergistic or additive effects where the results point to a significant contribution from the enzyme. No significant reduction in iodine was observed for any of the treatments. Further research should investigate the combination of US and other enzymes and optimize the US treatment for seaweed extraction. In addition, analyses of the effect of processing on the PTE content should be included for potential bottlenecks for safe consumption of *P. palmata*.

## CRediT authorship contribution statement

**Ingrid Maribu:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Marthe Jordbrekk Blikra:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Karl-Erik Eilertsen:** Writing – review & editing, Conceptualization. **Kjetil Elvevold:** Writing – review & editing, Supervision, Conceptualization.

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## 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.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2025.117859.

## Data availability

Data will be made available on request.

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