LWT - Food Science and Technology 203 (2024) 116402

Contents lists available at ScienceDirect

LWT

journal homepage: www.elsevier.com/locate/lwt

Processing of sugar kelp: Effects on mass balance, nutrient composition, and color

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A B S T R A C T

The European production of seaweed, including kelp species, is increasing, but efficient processing schemes for complete biomass valorization are still lacking. In this study, we investigated the effect of combining pulsed electric field (PEF) processing and soaking/blanching treatments (10, 45, and 60 °C) on the chemical composition of sugar kelp. Furthermore, we suggest applications for the various fractions resulting from the different pre-processing steps.

More dry matter was extracted from the biomass with increasing degree of processing. Combining PEF and blanching gave the highest extraction yield (60 %) compared to only using one of the steps (40 %). The extracted compounds contained ash (including iodine), polyphenols, and mannitol. Proteins, heavy metals, other sugars, and (presumably) fiber were concentrated in the solid fractions. Based on the composition, applications within food, feed, agriculture, nutraceuticals, and cosmeceuticals are suggested for the fractions and extracts.

These results can be relevant to researchers investigating novel technologies, processing of seaweed, and food safety, as well as to the seaweed industry, for the selection of processing technologies and product applications.

1. Introduction

From 1990 to 2020, global algae production has seen an average annual growth of 7.3%, reaching 35.1 million tons in 2020. Asia, with China and Indonesia, is the main contributing continent, with roughly 95% of seaweed production (FAO, 2022). In Europe, the seaweed harvest currently accounts for approx. 1% of the global industry by volume; essentially based on wild biomass. There is, however, a strong belief that Europe can increase production capacity, and recent projections estimate production volumes exceeding 8 million wet tons by 2030 (Vincent et al., 2020). Also in Norway, the annual harvest is increasing, and according to the Norwegian Seaweed Association, the aim is to reach 300, 000 tons of cultivated biomass by 2030. The most popular species for cultivation in Norway and Western Europe has until now been kelp species, with *Saccharina latissima* (sugar kelp) as the dominant species.

Seaweed is used for many purposes, including medicines, chemicals, building materials, packaging materials, and energy sources (Zhang et al., 2022), but cultivated kelp is primarily used as ingredients in food and feed. However, some issues need to be tackled to allow for more widespread utilization of the envisaged future production for these purposes, like improving food safety and thus enabling safe consumption of more significant amounts of seaweed products. Specifically, the iodine content of popular species, including sugar kelp, is high, limiting the recommended daily consumption. A mere portion of 0.04 g of unprocessed, dried sugar kelp provides the daily recommended amount of iodine for adults (150 μg; EFSA Panel on Dietetic Products, Nutrition and Allergies, 2014; Blikra et al., 2021). Iodine is required to maintain a normal thyroid function, but both too low and too high consumption correlate with adverse effects (Laurberg et al., 2009). Another potential concern related to sugar kelp is other potentially toxic elements (PTEs) such as cadmium and arsenic. However, the amount of these compounds has previously been found to be too low to pose a concern if consumption is kept below the advised upper limit for iodine per day (600 μg iodine; EFSA, 2018), which is equivalent to roughly 1 g boiled, dried sugar kelp (Blikra et al., 2021).

Post-harvest kelp must be stabilized or preserved quickly to avoid deterioration. All processing steps should add value to the product, resulting in optimal raw material utilization or improving later processing steps. A pre-processing strategy for macroalgae consisting of a combination of mechanical grinding, pulsed electric field (PEF)

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https://doi.org/10.1016/j.lwt.2024.116402
Received 21 December 2023; Received in revised form 25 May 2024; Accepted 23 June 2024
Available online 24 June 2024

processing, and water extraction was described by (Zollmann et al., 2019). By increasing temperature, pre-processing could include blanching, for which the effects are well documented (Krook et al., 2023; Nielsen et al., 2020; Stévant et al., 2021; Wirenfeldt et al., 2022). Pulsed electric field processing is a novel, energy-efficient, green processing step that can be run in tandem with other processes and has shown promising results for sugar kelp (Blikra et al., 2022).

Processing routes for extraction of high-value compounds while simultaneously increasing food safety by reducing the content of potentially toxic elements in a manner and scale suitable for seaweed production is, therefore, an exciting prospect. The aim of this work is to investigate the effects of PEF processing combined with soaking/blanching prior to drying on the mass balance and the release of specific compounds into the processing water. This was conducted to gain an overview of the effect of the various processing steps on the raw material and the prospects of both the liquid and solid fractions for further utilization. The data and insights from the present work are relevant for researchers investigating novel technologies, processing of seaweed, and food safety, and for the seaweed industry for deciding which technologies to invest in and which applications to pursue for their products. Hence, the implications of the results will depend on aims and contexts, and detailed interpretations and discussions of these are beyond the scope of this work.

2. Materials and methods

2.1. Sampling

Cultivated sugar kelp was harvested in June 2023 from Kraknes, Tromsø. The sporophytes were packaged in styrofoam boxes with ice in plastic bags and absorbent, stored in a cold room overnight, and shipped the following morning. Upon arrival at the lab, the lids were removed, and the boxes were covered in plastic film before overnight storage in a cold room (0.5 °C). The experiments were performed the following day.

2.2. Preparation of raw material

The kelp was fresh, dry, and crisp on the day of the experiments and had a dry matter content of 9.0 ± 0.8 %. The stipe and holdfasts were removed, as were any parts with fouling. The specimens were cut to pieces using a grinder (T. Myhrvold AS, Oslo, Norway) with a hole disc with openings of 10*20 mm to minimize initial variations in raw material and to facilitate mass transfer from the kelp during processing. Following grinding, the specimens were weighed into batches of 300 g, covered in plastic film, and put in a cold storage room (4 °C). The samples were then treated in triplicates by PEF and/or soaking/blanching as described below, except for unprocessed control samples (Fig. 1).

2.3. Processing

2.3.1. Pulsed electric field

Three batches of 300 g kelp were pooled (total 900 g) and processed with 1800 g tap water (1:2 ratio w/w). The PEF treatments were conducted using a PEF Pilot Dual (Elea GmbH, Quakenbrück, DE), equipped with a 10 L batch treatment chamber (electrode distance 24 cm). For the treatments, the following conditions were applied: electrode voltage of 24 kV, frequency of 30 Hz; and pulse width of 6 us. The set number of pulses was around 300, and the energy output was estimated to be 12.8 ± 5.0 J/g. The water had a start temperature of 11.8 ± 1.3 °C and a final temperature of 14.8 ± 0.9 °C after processing.

The PEF-treated samples were sieved for 1 min, followed by manual rotation of the sample and sieving for another minute. The sample was then weighed and split into three samples of approximately similar weight. The precise sample weight was recorded and used for the data processing, as was the weight of the water.

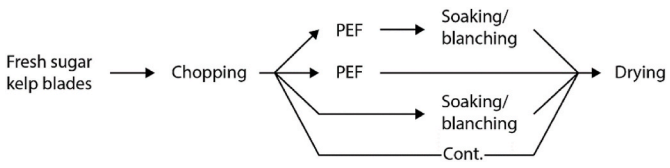

Fig. 1. Flow chart of the processing schemes. The soaking/blanching was performed at three temperatures, thus a total of eight groups were used. PEF: Pulsed electric field.

2.3.2. Blanching

Blanching was performed in a stainless-steel container with 600 g pre-tempered water, placed in a water bath to maintain the target temperatures (45 or 60 °C). A ratio of 1:2 w/w seaweed:water was applied. After adding the kelp, the sample was stirred (30 s), to obtain an even start temperature for the blanching process. The temperature was recorded in the center of the container. The measured temperatures were 37.3 ± 1.8 °C and 48.6 ± 2.1 °C for the 45 and 60 °C baths, respectively. After blanching for 2 min, the sample was sieved as described for the PEF treatment, and the weight of both the solid and liquid (water) fractions was recorded.

2.3.3. Soaking

Soaking at 10 °C was performed in the same manner as blanching in a stainless-steel container, with the same seaweed:water ratio, but no water bath was used. After 30 s the measured temperature in the container was 12.5 ± 1.7 °C. The liquid used for PEF, blanching and soaking was sampled in plastic tubes and stored at −30 °C until analysis.

2.3.4. Drying

All samples were dried until stable in a cooking and drying cabinet (Bastramat C1500, Bastra GmbH, Arnsberg, Germany) at 50 °C and 30 % relative humidity overnight (19 h). Two samples were weighed after 18h and again after 19h to check for weight stability. Since the weights of the samples remained unchanged, all samples were removed from the cabinet, and the final sample weight was recorded.

2.3.5. Analyses

Before analyses, the dried kelp samples were ground for 2 × 10 s using a Food Processor (R5, Robot coupe, France), followed by packaging in plastic bags. The liquid fractions from PEF, blanching, and soaking were thawed at 4 °C overnight before analyses.

2.3.6. Dry matter and ash

For dry matter analysis, samples were weighed (triplicate) in aluminum cups and dried at 105 °C for 18 h. The sample amounts used for analysis were 0.51 ± 0.01 g for the dried kelp, 2.16 ± 0.14 g for the fresh kelp (sampled after grinding), and 4.96 ± 0.09 g for the liquid fractions. The dry matter content was used to calculate the content per g dry weight. For analysis of ash content, 0.51 ± 0.01 g of dried kelp was weighed (duplicates), and the ash content was determined by drying the residue at 550 °C for 24 h in a Carbolite-Gero AAF 11 (Neuhausen, Germany) muffle furnace.

2.3.7. Polyphenols

Total phenolic content was analyzed using the Folin-Ciocalteu method (Nenadis et al., 2007; Singleton et al., 1999). Polyphenols were extracted from dried kelp according to Stévant et al. (2017). 250 mg dried sample was mixed with 10 ml of 80% (v/v) acetone and incubated for 1 h at room temperature in darkness. This was performed twice before the supernatants were pooled and filtered (0.45 μm). Liquid samples (after PEF or blanching) were thawed overnight at 4 °C before analysis. An aliquot of 0.5 ml sample was mixed thoroughly with 5 ml deionized water and 0.5 ml 2 M Folin-Ciocalteu phenol reagent. After 3

min, 1 ml of 20% sodium carbonate and 3 ml deionized water was added, mixed thoroughly, and incubated for 1 h in darkness at room temperature. The absorbance was measured in triplicate at 725 nm using a Synergy H1 multi-mode reader (BioTek Instruments, Winooski, VT, USA). The total phenolic content of the dried or liquid sample was expressed as milligrams of propyl gallate equivalents (PGE) per gram of dry weight sample, based on a calibration curve of propyl gallate in 80% methanol.

2.3.8. Total sugars and estimated fiber content

Before sugar analysis, the samples were hydrolyzed with a modified two-step sulphuric acid hydrolysis based on previously described methods (Nguyen et al., 2020; Sterner et al., 2017). For hydrolysis, 300 µl/5 ml of 72% (w/w) H_2SO_4 was added to 30 mg of dried sample or 0.5 ml of liquid sample. The samples were incubated at 30 °C for 1 h. The samples were then diluted to 4% (w/w) H_2SO_4 using deionized water and autoclaved (Panasonic MLS-3781L, Tottori, Japan) for 40 min at 121 °C The hydrolyzed material was centrifuged at 1246×*g* (Eppendorf Centrifuge 5810 R, Sigma-Aldrich, Saint-Louis, MO, USA) for 10 min and transferred to HPLC-tubes. The total sugar content was analyzed by ion-exchange high-performance liquid chromatography using a LC-20AT apparatus equipped with a CTO-20A column oven and a RID-20A refractive index detector (Shimadzu, Kyoto, Japan). The analysis was conducted at 65 °C with an injection volume of 10 µl. An isocratic flow rate of 0.6 ml/min for 30 min, with the mobile phase of 5 mM H_2SO_4 (Sharma et al., 2018) was used. Glucose, xylose, mannitol, and fucose were used as standards in concentrations of 2.5, 1.0, 0.5, 0.25 and 0.1 g/l.

The fiber content (Table 1) was estimated as the remaining fraction of the biomass according to the equation below. As some fiber compounds, e.g., laminarin (Devillé et al., 2004), are hydrolyzed and will be included in "the total sugars", the fiber content is underestimated.

$$Fiber\ (\%) = 100 - protein(\%) - total\ sugars(\%) - ash(\%) - polyphenols(\%)$$

2.3.9. Protein

The protein content in dried kelp was determined using the Kjeldahl method. Two copper catalyst tablets (Kjeltabs Cu/3.5, Nerliens Meszansky, Oslo, Norway) were added to approximately 0.5 g sample. The samples were then hydrolyzed with 15 ml of concentrated H_2SO_4 for 1 h in a heating block (Kjeltec system 2020 digestor, Tecator Inc, Herndon, VA, USA) at 420 °C. Then they were cooled down, and 30 ml of distilled water (ELGA Purelab Chorus 2^+, Veolia Water, High Wycombe, UK) was added. The total nitrogen in the samples was then measured using a Kjeltec™ 8400 (FOSS analytics, Hillerød, Denmark). The amount of total nitrogen was determined using the conversion factor 2.0, which has been proposed as an average conversion factor for sugar kelp (Bak et al., 2019). This factor was selected since the conventional conversion factor of 6.25 is known to overestimate the protein content in sugar kelp due to the high non-protein nitrogen content (Sharma et al., 2018).

Table 1
Nutritional content in the differently pre-processed dried kelp samples (% dry weight).

Sample	Protein	Total sugars	Ash	Fiber[1]	Polyphenols
Cont.	3.3 ± 0.2^{D}	23.1 ± 3.4^{A}	44.3 ± 2.5^{A}	25.4 ± 5.0^{D}	4.5 ± 0.5^{A}
S10	4.0 ± 0.2^{C}	20.7 ± 6.6^{A}	35.7 ± 3.1^{B}	35.3 ± 5.8^{C}	3.9 ± 1.1^{AB}
B45	4.5 ± 0.2^{B}	13.9 ± 2.3^{B}	29.4 ± 0.9^{C}	49.7 ± 2.6^{B}	2.5 ± 0.4^{BC}
B60	4.6 ± 0.3^{B}	14.0 ± 3.2^{B}	30.0 ± 1.9^{C}	48.7 ± 2.3^{B}	2.5 ± 0.6^{BC}
PEF	4.8 ± 0.1^{B}	13.7 ± 0.7^{B}	34.2 ± 0.8^{B}	45.1 ± 1.3^{B}	2.8 ± 0.1^{BC}
PEF + S10	5.6 ± 0.1^{A}	9.9 ± 1.3^{B}	23.4 ± 0.8^{D}	58.9 ± 1.4^{A}	1.7 ± 0.5^{C}
PEF + B45	5.5 ± 0.1^{A}	9.1 ± 1.6^{B}	21.9 ± 0.6^{D}	61.0 ± 0.3^{A}	1.9 ± 0.1^{C}
PEF + B60	5.6 ± 0.2^{A}	9.2 ± 0.5^{B}	21.9 ± 0.4^{D}	60.4 ± 1.3^{A}	1.3 ± 0.3^{C}

Capital letters indicate significant differences within columns.
1: The fiber content was estimated from the unidentified dry matter content.

2.3.10. Iodine

Elemental iodine analysis was performed by Mikroanalytisches Labor Kolbe (Oberhausen, Germany), as previously described (Blikra et al., 2021), but with some modifications. In brief, ground kelp samples (n = 3) were crushed and sieved to 0.5 mm. The subsequent digestion was performed in a special combustion unit at 1100 °C and burned in an argon/oxygen stream. Initial analytical attempts gave a fast clogging of the filter and an inhibition of gas flow, likely due to elevated silicon levels in the samples due to later harvesting time than our previous experiments (Sharma et al., 2018). To compensate for this, the filter was changed more often than usual, and water was constantly added during combustion to prevent the buildup of the resulting lane on the combustion tube and promote flow through the filter. The combustion time was also significantly longer to ensure that all the sample gas exited the chamber during the analysis. The resulting gases were collected in an aqueous solution and measured on a Metrohm Model 883 Plus ion chromatograph. The lower limit of detection was 1 ppm. One analytical replicate was taken from each parallel (=3 replicates per treatment).

2.3.11. Metals

Transition and heavy metal analysis was performed by ALS Scandinavia AB (Luleå, Sweden), following SE-SOP-0128 (SS-EN 13805:2014) and using accredited methodology. Briefly, the samples were dissolved in nitric acid/hydrogen peroxide with traces of hydroflouric acid in a microwave oven following B-PF51HF-MW or B-PF51-MW. The analysis was performed using ICP-SFMS/ICP-AES. The method has been described elsewhere (Blikra et al., 2021).

2.3.12. Color

Approx. 5.0 g dried sample was transferred to a white container (diameter 6.5 cm) and photographed. A light chamber (VeriVide's DigiEye, VeriVide ltd., Leicester, UK) with day-light lamps (6500K) coupled to a digital camera (Nikon D90, Tokyo, Japan) and DigiEye software (Version 2.9) was used. The entire surface of the sample was marked for data collection, and the color was measured using CIE *L**, *a**, *b**.

2.4. Statistical analysis

Analysis of variance (ANOVA) was performed to test for significant differences between sample groups, using Minitab® version 19.2020.1 and a 95% confidence interval. When more than two sample groups were present, a Tukey post hoc test was applied. The results are given as the average ± sample standard deviation.

3. Results and discussion

3.1. Mass transfer during pre-treatments and drying

Dry matter components were extracted and released to the liquid phase from the kelp biomass during all immersion steps, including soaking, blanching, and PEF processing. The degree of dry matter extraction depended on the amount of processing the kelp was exposed to, and increasing dry matter was extracted from the kelp for each pre-processing step (Fig. 2). Around 20% dry matter was extracted from samples that were only soaked (10 °C), whereas roughly 40% dry matter was extracted from samples processed by PEF or blanching (45 or 60 °C). Similarly, a previous study investigating blanching of whole thallus

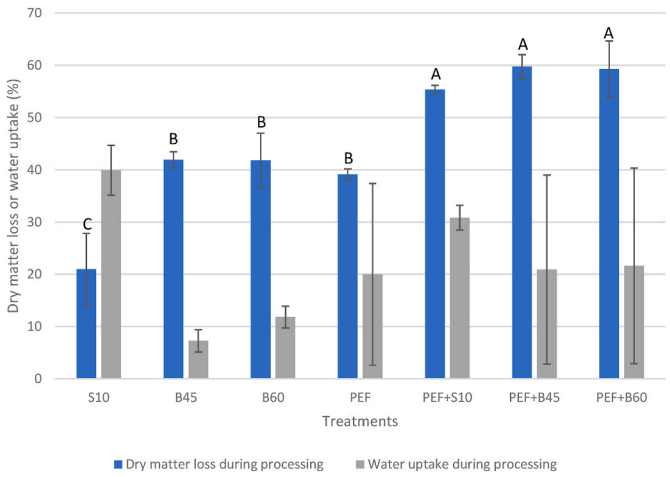

Fig. 2. The loss of dry matter and uptake of water for solid kelp fractions during processing treatments. Significant differences are indicated by different capital letters (for dry matter loss – no significant differences were found for water uptake). The samples: S10, B45, B60: soaked/blanched at 10, 45, and 60 °C, respectively; PEF: only PEF treated before drying; PEF + S10, PEF + B45, PEF + B60: PEF treated followed by soaking/blanching at 10, 45 and 60 °C, respectively.

sugar kelp found extractions of 29–54% dry matter, with higher extraction rates for longer blanching times and at higher temperatures (Nielsen et al., 2020). In our study, between 55 and 60% dry matter was extracted from samples which were treated by both PEF and blanching. Thus, pre-processing before drying extracted 20–60% of the dry matter, and the dry weight of the kelp was between 4 and 9% of the raw materials' wet mass, depending on pre-processing (S1). This implies that when adding pre-processing steps, utilization of the liquid fractions is advantageous and necessary for full utilization of the biomass.

The samples absorbed water during each processing step involving immersion in water. Soaking and PEF processing resulted in higher uptakes of roughly 40 and 30% water, respectively, while blanching resulted in an uptake of 9.5% water on average, with no significant differences between the two temperatures (Fig. 2). For blanching following PEF-treatments, the additional water uptake ranged from negative to 12%. Further studies are needed to assess whether this uptake has consequences for energy consumption during water removal steps such as drying.

3.2. Chemical composition

3.2.1. Protein

The relative protein content in the dried kelp samples increased significantly after pre-processing compared to the untreated control sample (Table 1). Samples treated with PEF and subsequently blanched had the highest protein content, roughly 5.6 ± 0.2% protein, compared to control samples with 3.3 ± 0.2%. The samples treated with either PEF or blanching contained 4.5–4.8% protein, significantly lower than those treated with both PEF and blanching. This indicates a concentration of protein in the dried kelp samples with an increasing degree of processing, which was also found in a recent study with seawater blanching (Lafeuille et al., 2023). Previous studies have found that although the protein content was unchanged or increased after blanching, amino acids leached into the blanching liquid (Nielsen et al., 2020). In the latter study, *S. latissima* was blanched at 45–80 °C for 2–120 s. Here they observed a significant decrease in the content of two specific amino acids, alanine and glutamic acid, with a decrease from 178 to 128 mg/g protein and 173 to 146 mg/g protein, respectively. Thus, it can be expected that the blanching liquid contains some amino acids, but that the majority of amino acids are retained within the *S. latissima* specimen. Extractions of certain amino acids (incl. glutamic acid) have implications for taste.

Traditionally, the general nitrogen-to-protein conversion factor is 6.25. Bak et al. (2019) reported an average conversion factor of 2.0 for *S. latissima* from the Faroe Islands, ranging from 1.2 in June to 2.7 in April, thus indicating a large seasonal variation in the protein content of *S. latissima*. According to Manns et al. (2017), the conversion factor of S. latissima in Danish waters varied from 2.1 to 5.9 with season and location. However, several other studies conducted on brown seaweeds have reported higher conversion factors, e.g., Lourenço et al. (2002) suggested a general conversion factor of 5.38, and Angell et al. (2016) applied 5.00 for brown seaweeds. To limit uncertainties regarding species and geographical variations, it was decided to use the average conversion factor found by Bak et al. (2019). Regardless of conversion factor choice, the results indicate the same, namely a concentration of protein with an increasing degree of processing.

3.2.2. Sugars

Table 2 shows the sugar content in the dried kelp samples after the various treatments. The glucose content varied between 27 and 47 g/kg dry weight for the pre-processed samples, and there were no significant differences compared to the control sample. The xylose content decreased with increasing processing, but the content was only significantly lower than the control for samples treated with both PEF and soaking/blanching. Both PEF and blanching resulted in a significantly lower fucose content compared to controls, where the combination of the two had the lowest fucose content of 18 ± 2 g/kg dry weight. Even though the glucose, xylose, and fucose decreased due to processing, the sugars, are not detected in the liquid phases after PEF and blanching, i.e., the content in the liquid phases were below the detection limit of the analysis (LOD >0.16 g/L).

The mannitol content in the dried kelp samples decreased significantly when the kelp was pre-processed with PEF and blanching, where the combination of the two resulted in the highest extraction yield of mannitol (Table 3). High amounts of mannitol (147–221 g/kg dry weight) were detected in the liquid samples after PEF and blanching. Wirenfeldt et al. (2022) found that blanching (2 min, 76 °C) in tap water (50 g sugar kelp in 1 L water) drastically reduced the content of mannitol in sugar kelp samples from 16.6 to 0.7%. Mannitol is a highly soluble carbohydrate used to store carbon and as an osmoprotectant in the algae (Zhang & Thomsen, 2019). In this study, more mannitol was extracted when applying PEF and/or blanching than present in the control (Table 3). A possible reason could be that the hydrolysis was incomplete in the dried samples. However, a two-step hydrolysis using sulphuric acid is proposed to be the best method for quantifying sugars from brown algae (Manns et al., 2014). Further investigations are needed to validate the result.

The sugar content in this study was analyzed using ion-exchange

Table 2
Sugar content (g/kg dry weight) of differently pre-processed dried kelp samples. The samples: Cont.: negative control sample: dried only; PEF: only PEF treated before drying; PEF + S10, PEF + B45, PEF + B60: PEF treated followed by soaking/blanching at 10, 45, and 60 °C, respectively; S10, B45, B60: soaked/blanched at 10, 45, and 60 °C, respectively. The mannitol content is reported in Table 3.

Sample	Glucose	Xylose	Fucose
Cont.	43 ± 12^{A}	33 ± 1^{A}	30 ± 2^{A}
S10	47 ± 23^{A}	33 ± 4^{A}	28 ± 2^{B}
B45	37 ± 14^{A}	30 ± 3^{ABC}	23 ± 1^{CD}
B60	43 ± 14^{A}	31 ± 3^{AB}	22 ± 1^{CD}
PEF	39 ± 9^{A}	29 ± 2^{ABCD}	25 ± 2^{C}
PEF + S10	30 ± 13^{A}	26 ± 2^{BCD}	20 ± 1^{DE}
PEF + B45	27 ± 2^{A}	25 ± 2^{D}	18 ± 1^{E}
PEF + B60	30 ± 19^{A}	25 ± 4^{CD}	18 ± 2^{E}

Capital letters indicate significant differences within columns.

Table 3
Mannitol content (g/kg dry weight) of differently pre-processed dried kelp and liquid samples after PEF and blanching. The samples: Cont.: negative control sample: dried only; PEF: only PEF treated before drying; PEF + S10, PEF + B45, PEF + B60: PEF treated followed by soaking/blanching at 10, 45, and 60 °C, respectively; S10, B45, B60: soaked/blanched at 10, 45, and 60 °C, respectively.

Sample	Dried kelp	Liquid samples	
		PEF water	Blanching water
Cont.	125 ± 24^{A}	–	–
S10	99 ± 37^{A}	–	147 ± 10^{D}
B45	50 ± 10^{B}	–	217 ± 2^{A}
B60	43 ± 14^{B}	–	221 ± 31^{A}
PEF	44 ± 6^{B}	172 ± 7^{C}	–
PEF + S10	24 ± 3^{B}	172 ± 7^{C}	180 ± 5^{BC}
PEF + B45	20 ± 1^{B}	172 ± 7^{C}	195 ± 7^{B}
PEF + B60	19 ± 2^{B}	172 ± 7^{C}	189 ± 9^{B}

Capital letters indicate significant differences within the dried kelp and the liquid samples.

chromatography with an RI detector. RI detectors are often used in the quantitative estimation of carbohydrates. However, RI detectors are highly sensitive and small changes in temperature, pressure, and mobile phase can influence the baseline. Previous studies using HPLC-RID for detection of sugars have reported a limit of detection between 0.067 and 0.16 g/L (Galant et al., 2015).

3.2.3. Polyphenols

The level of polyphenols in dried kelp decreased during processing, and the results ranged from ~45 in control samples to ~13 mg PGE/g dry weight in samples treated with both PEF and blanching at 60 °C (Fig. 3A). The control samples had significantly higher levels of polyphenols than all pre-processed samples except those that were only soaked and subjected to the mildest treatment. The samples that were treated with a combination of PEF and blanching had lower polyphenol content. However, they were not significantly different from samples treated with only PEF or soaking/blanching.

The content of polyphenols extracted to the liquid fraction during pre-processing is illustrated in Fig. 3B. Samples were pooled during PEF treatment. Hence, an average of all PEF-treated samples, including those PEF treated prior to soaking/blanching, is shown in the figure with identical values (n = 12). The results indicate that PEF-treatment is a better extraction method that gives significantly higher levels of polyphenols in liquid fractions than the soaking/blanching at the temperatures used in this experiment. Combining the two treatments further increased the total content of extracted polyphenols. Extraction under higher temperatures can result in the degradation of polyphenols, as they are shown to be temperature sensitive (Generalić Mekinić et al., 2019). This problem can be avoided by using PEF, as the electrical fields will cause electroporation of the cell wall and result in the release of bioactive compounds without the use of heat (Matos et al., 2021). A comparison of the extraction yield during PEF versus the soaking treatment illustrates that the electroporation effect was needed for efficient extraction (Fig. 3B).

3.2.4. Ash

The results of the ash content analysis for sugar kelp after the various treatments are given in Table 1. The results showed a statistically significant decrease in ash during processing compared to the untreated control. Previous studies also found a reduction in ash content during the blanching of Saccharina latissima (Lafeuille et al., 2023; Nielsen et al., 2020) and during PEF processing of green algae (Prabhu et al., 2019; Robin et al., 2018). In our study, the ash content was almost 50% reduced in the samples treated with both PEF and soaking/blanching compared to the controls. Soaking and PEF-treatment alone resulted in higher residual ash content than the other pre-treatments (35% ash). However, combining these treatments, first PEF and then soaking,

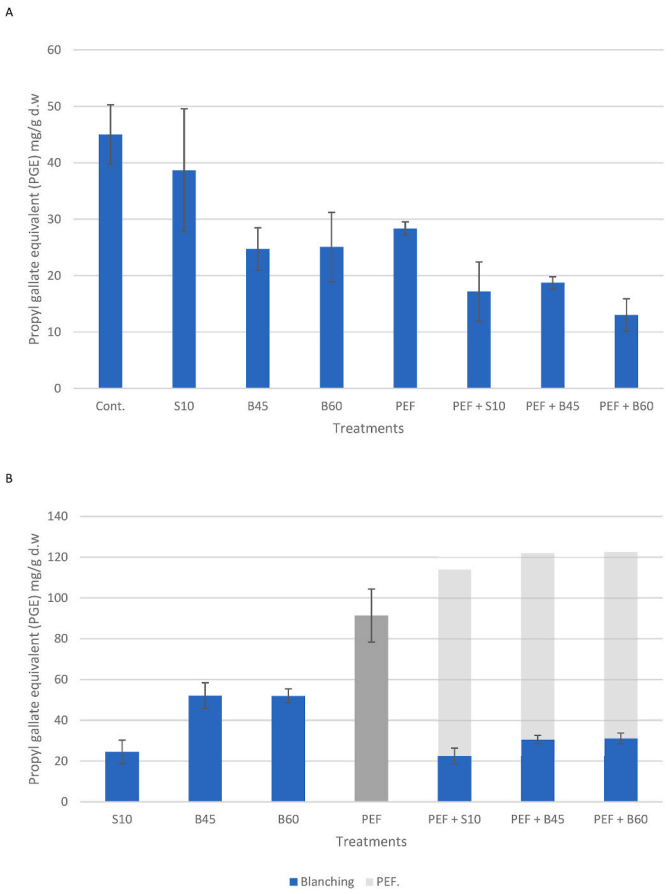

Fig. 3. Total phenolic content (mg PGE/g dry weight) of dried kelp (A) and liquid fractions (B) after pre-processing. The samples: Cont.: negative control sample: dried only; PEF: only PEF treated before drying; PEF + S10, PEF + B45, PEF + B60: PEF treated followed by soaking/blanching at 10, 45 and 60 °C, respectively; S10, B45, B60: soaked/blanched at 10, 45, and 60 °C, respectively. Grey color represents liquid fractions after PEF treatment. Blue color represents liquid fraction after blanching. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

significantly reduced the ash content (23.4% ash) compared with the two treatments separately. It is also interesting that PEF alone gave a significantly higher ash content (about 10% higher) than PEF combined with soaking/blanching regardless of the soaking and blanching temperature. Comparing the ash results with the dry matter loss (Fig. 2), we see that similar amounts of dry matter were extracted by PEF and blanching (~40 %), but the composition of the dry matter differed in that more ash remained in the biomass after PEF (~35 %) compared to after blanching (~30 %).

3.2.5. Iodine

The iodine content in the raw material was 5200 ± 210 mg/kg dm, which is within range of previously reported values for cultivated sugar kelp (2630–7977 mg/kg dm; Afonso et al., 2021; Bruhn et al., 2019; Krook et al., 2023). In this study, all the investigated processing steps reduced the iodine content in the kelp (Table 4). The most common method of iodine reduction in kelp sp. is blanching, and results ranging from 59 to 95 % reductions are previously reported for sugar kelp (Krook et al., 2023; Lafeuille et al., 2023; Nielsen et al., 2020; Wirenfeldt et al., 2022). In our study, blanching reduced the iodine content by 90%, which is in the upper range of previously reported results. Our highest iodine reduction was achieved for samples treated with PEF processing prior to blanching (94 %; 300 ± 100 mg/kg dw), but the difference was not significant, compared to blanching only. This content is lower than

Table 4
Concentration (mg/kg dry weight) and reduction (%) of potentially toxic elements iodine, arsenic, cadmium, mercury, and lead. Values are shown as mean ± standard deviation (n = 3).

Sample	Iodine		Arsenic		Cadmium		Mercury		Lead	
	Conc.	Red.	Conc.	Red.	Conc.	Red.	Conc.	Red.	Conc.	Red.
Literature	2630–7977	–	28–120	–	0.2–4.6	–	0.01-0.06	–	0.1–1.1	–
Cont.	5200 ± 200^{A}	–	72.3 ± 6.3 ^{A}	–	1.60 ± 0.17 ^{C}	–	0.0185 ± 0.0018 ^{A}	–	0.151 ± 0.066 ^{A}	–
S10	4300 ± 300^{B}	17	63.4 ± 6.8 ^{AB}	12.4	1.83 ± 0.19 ^{C}	−14.3	0.0217 ± 0.0036 ^{A}	−17.5	0.114 ± 0.013 ^{A}	24.6
B45	500 ± 100^{E}	90	60.6 ± 2.9 ^{BC}	16.2	2.45 ± 0.15 ^{B}	−53.0	0.0235 ± 0.0026 ^{A}	−27.2	0.109 ± 0.010 ^{A}	27.9
B60	500 ± 100^{E}	90	53.9 ± 2.3 ^{BC}	25.4	2.30 ± 0.12 ^{B}	−43.3	0.0245 ± 0.0010 ^{A}	−32.6	0.135 ± 0.020 ^{A}	10.6
PEF	2600 ± 200^{C}	50	73.3 ± 2.2 ^{A}	−1.4	2.58 ± 0.02 ^{B}	−60.7	0.0289 ± 0.0047 ^{A}	−56.5	0.145 ± 0.013 ^{A}	3.8
PEF + S10	1600 ± 200^{D}	69	57.9 ± 1.7 ^{BC}	20.0	3.19 ± 0.09 ^{A}	−99.2	0.0316 ± 0.0013 ^{A}	−71.3	0.177 ± 0.004 ^{A}	−17.5
PEF + B45	300 ± 100^{E}	94	50.9 ± 2.2 ^{C}	29.6	3.15 ± 0.19 ^{A}	−96.3	0.0311 ± 0.0058 ^{A}	−68.4	0.156 ± 0.011 ^{A}	−3.3
PEF + B60	300 ± 100^{E}	94	51.7 ± 2.6 ^{C}	28.6	3.43 ± 0.21 ^{A}	−114	0.0419 ± 0.0217 ^{A}	−126.9	0.176 ± 0.017 ^{A}	−16.6
UL (iodine) and TWI (metals)	600 μg		Not establ.		2.5 μg/kg BW		1.6 μg/kg BW		Not establ.	
Kelp needed to reach TWI[1]	14 g		–		51 g		2600 g		–	

Capital letters indicate significant differences within columns. Literature values are from: Afonso et al. (2021), Bruhn et al. (2019), Krook et al. (2023) and Ometto et al. (2018). Abbreviations: UL: tolerable upper intake level (daily); TWI: tolerable weekly intake; BW: body weight.
1: Calculated based on content in kelp (dry weight) processed by PEF and blanching at 60 °C, which had the lowest iodine and highest heavy metal content.

the threshold proposed in France (2000 mg/kg dw; CEVA, 2019), but not below the threshold to enter the German food market (20 mg/kg dw; BfR, 2007). To the best of our knowledge, no other European countries have such regulations. Alternatively, in industrial food production, the content of added algae can be calculated based on the iodine content of interest to add per portion size. For instance, 100 μg iodine/portion would be roughly 0.3 g kelp if using kelp processed by both PEF and blanching and 0.2 g per portion if using kelp processed by blanching alone. Such an addition in a few, common food items could provide a healthy and natural iodine food source, potentially contributing to improving the iodine status, which is characterized as insufficient in ≥21 countries worldwide (Zimmermann & Andersson, 2021). Soaking in tap water at 10 °C resulted in a small but significant reduction in iodine (17%). This finding was within the range of previous studies reporting reductions between 0 and 35 % for soaking, washing, and rinsing of sugar kelp in water below 20 °C (Blikra et al., 2021; Stévant et al., 2018; Wirenfeldt et al., 2022). In contrast, the kelp samples processed by both PEF and soaking in tandem contained 70 % less iodine than control samples. This combination could be a promising option for extracting iodine from kelp at low temperatures. Processing with PEF without subsequent blanching or soaking led to a significant reduction in iodine content (50%) compared to soaking alone (17%), which was conducted at a similar temperature. This difference in extracted iodine highlights that the PEF-treatment made more iodine available for extraction, probably by tissue perforation.

3.2.6. Metals

The concentrations of the transition metal arsenic and the heavy metals cadmium, mercury, and lead for the untreated control and the processed samples are shown in Table 4. The values are in the range of those previously reported (Afonso et al., 2021; Bruhn et al., 2019; Krook et al., 2023; Ometto et al., 2018).

All treatments except for the soaking and PEF-treatment alone reduced the processed samples' arsenic content. The samples processed by both PEF and blanching showed the highest reduction in arsenic (30%). However, the differences between these samples and those only blanched were insignificant. Based on these results, it seems that the PEF-treatment had little effect on arsenic reduction in sugar kelp. Soaking at 10 °C did not give a significant reduction in arsenic content. This is in contrast to our previous study, where the arsenic content was significantly reduced both during rinsing in tap water and boiling of whole thallus *S. latissima* (Blikra et al., 2021).

The cadmium concentration was higher in the processed samples than in controls. The samples that were PEF-treated and blanched at 60 °C had an average of 114% higher cadmium concentration than the control samples. Hence, it seems that the cadmium was concentrated in the biomass during processing due to the extraction of other dry matter components. No significant differences were observed in the mercury and lead concentrations. However, the results indicate a mercury concentration in all samples and of lead for all samples that were PEF-treated and blanched.

The tolerable weekly intakes of cadmium and mercury (EFSA, 2012a; EFSA Panel on Contaminants in the Food Chain, 2012) and the recommended upper daily limit of iodine (EFSA, 2018) are indicated in Table 4, along with the minimum amount of kelp needed to reach the weekly limits. Since mercury speciation was not performed in the present study, the calculations were conservatively performed presuming the most toxic chemical species (i.e., methylmercury). There is no established level for safe lead consumption (EFSA, 2012b). Regarding arsenic, only total arsenic content was measured in this study. Inorganic arsenic compounds are carcinogenic, and there are no established limits for safe consumption (EFSA, 2014). However, previous studies have shown that arsenic content in seaweeds, including sugar kelp, is mainly in the organic form (Díaz et al., 2012; Krook et al., 2023). Despite the presence and concentration of heavy metals during processing, an iodine content of 300 μg/g dry weight in kelp processed by both PEF and blanching only allows for the consumption of 14 g dry weight per week (Dujardin et al., 2023). Thus, iodine is the limiting factor for the consumption of this kelp.

3.2.7. Color

The color was only measured in dry kelp specimens. The color is essential for two reasons: 1) The color could indicate the presence of carotenoids, many of which have bioactive properties (Pereira et al., 2021). 2) The color could affect consumer perception of sugar kelp as a food and food ingredient. The color of the differently pre-processed samples showed significant differences in blue/yellow (b*) and yellowness (Fig. 4), but not lightness (L*) and red/greenness (a*). The samples that were both PEF treated and soaked/blanched were less yellow than others, and significantly less so than control samples. The reduction in yellowness could be attributed to the reduction in polyphenols found in the present study but could also be related to the extraction or denaturation of other compounds. Sugar kelp contains several colored carotenoids, including the brown-colored fucoxanthin and also the orange-colored violaxanthin (Hallerud, 2014), for which a reduction in content could be associated with a reduction in yellow color. A reduction in total carotenoids has previously been found during the blanching of sugar kelp (Lafeuille et al., 2023). Since the color may affect consumer perception of food, the color of kelp ingredients is important when creating food recipes, e.g., for ready-to-eat food products. A blander color, which was achieved after processing with both PEF and blanching, may be a benefit in some foods where it is

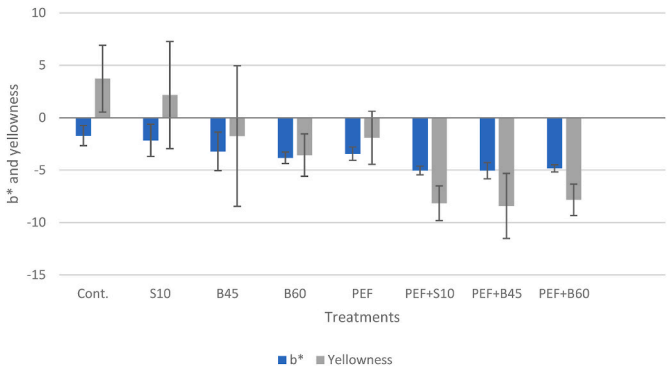

Fig. 4. Blue-yellow tone (b*) and yellowness of differently pre-processed dried kelp samples following CIELAB color space. The samples: S10, B45, B60: soaked/blanched at 10, 45, and 60 °C, respectively; PEF: only PEF treated before drying; PEF + S10, PEF + B45, PEF + B60: PEF treated followed by soaking/blanching at 10, 45 and 60 °C, respectively; Cont.: negative control sample: dried only. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

appropriate to "hide" the kelp. In contrast, a yellower/greener ingredient could be beneficial in other items where the kelp can be showcased. However, further studies assessing consumer perception of food items with processed kelp ingredients are needed to highlight such issues.

3.2.8. *The potential in pre-processing for valorization of kelp and seaweed in general*

The best combination of processing methods and order of processing steps to meet the market needs is not obvious. To utilize the solid fraction of the kelp for food, a promising combination found in this study was PEF processing followed by soaking, resulting in a product with low iodine (−70%) and ash content compared to the control, without elevating the temperature. Furthermore, PEF may facilitate quicker drying and reduce energy consumption, which has been documented for several vegetables (Liu et al., 2018; Liu et al., 2020; Yamada et al., 2020). For other raw materials, like potatoes, PEF is used to facilitate cutting operations, and it should be further investigated whether PEF processing before cutting/grinding could have favorable effects on kelp as well. However, the timing of the grinding step will also affect the chemical composition after processing. Regarding the cost-effectiveness of PEF, the major cost is investment in the equipment (ranging from $45,000 to $2,000,000), with low energy-cost during running compared to, e.g., blanching (Blikra et al., 2022). Thus, the cost-effectiveness depends largely on the amount of processed biomass per hour and intermediate use of the equipment for other raw materials in between the seaweed harvesting season. The PEF process is currently mainly commercially used on a full scale in the potato snack industry, and one equipment producer indicates a total PEF processing cost of 0.1 €/kg (Boevere, 2018). It should also be noted that sensory analysis of the PEF processed kelp has not been conducted, and this should be prioritized in later studies assessing the applicability of PEF processing of kelp for food. Alternative applications for the solid fraction are soil components, feed ingredients, and raw materials for bioplastic production (Table 5).

Due to the large reduction in mass, combinational processing of kelp using PEF and soaking/blanching should be combined with the utilization of the extracted components (Table 5). In the case of PEF-treatment followed by soaking/blanching, the mineral, polyphenol, and mannitol contents of the liquid fractions were high, thus giving opportunities for multi-extraction of single components. Polyphenols are a diverse group of bioactive components, many of which have health-promoting effects. Isolated polyphenols have shown diverse bioactivity, including anti-tumor, anti-cholesterol, antioxidant, and anti-inflammatory, and are, therefore, attractive as nutraceuticals and pharmaceuticals (Cotas et al., 2020). Mannitol has numerous applications in industries such as food, pharmaceutical, and cosmetic (McElroy et al., 2023; Zhang & Thomsen, 2019). It has a high relative sweetness and a low energy value compared to sucrose, making it suitable for low-calorie sweets. While it is cheaper to produce mannitol using industrial synthesis, consumer trends show a preference for natural products, which could lead to higher prices for natural extracts.

Alternatively, the extracted liquid could be used with few or no added processing steps, e.g., in health food applications, functional feed ingredients, or as plant biostimulant (Table 5). Plant biostimulants are products used to promote health and robustness, e.g., within agriculture (Calvo et al., 2014; Stirk et al., 2020). Such liquids are typically complex mixtures and have previously been extracted from several seaweed raw materials, including *Ascophyllum nodosum*, *Macrocystis pyrifera*, *Ecklonia maxima*, *Durvillea potatorum,* and *Durvillea antarctica* (Khan et al., 2009). They contain various chemical compounds, including sugars, polyphenols, amino acids, polysaccharides, macro-and micronutrients, and more (Deolu-Ajayi et al., 2022). The requirement for biostimulants is a measured effect on plant function, and further studies assessing the effect of extracts (including aqueous extracts) from sugar kelp are needed before commercial exploitation. Regarding utilization of the liquid in food applications, it is essential to ensure stability in the iodine content and not use too much liquid per portion size. If used cautiously, such an ingredient could provide a healthy iodine source (e.g., 25–100 μg per portion), bioactive polyphenols, and taste (incl. mannitol, glutamic acid).

An alternative to the processing scheme explored in the present work could be combining PEF with cascade biorefinery (e.g., McElroy et al. (2023)) for extraction of the lipid and acid-soluble fractions (Fig. 5).

3.2.9. *Methodological weaknesses*

During PEF processing, the set number of pulses was 322, achieved in only 2/4 samples. The reason this was not achieved in the remaining samples was the high conductivity in the extraction liquid during PEF processing, caused by the low water-to-kelp ratio (2:1). The estimated energy output (12.8 ± 5.0 J/g) had a high standard deviation, which could influence the results. However, the setup was randomized, which should have spread the deviations and limited the effect on the data.

4. Conclusion

In this study, we investigated the movement of chemical constituents in sugar kelp during processing. Sugar kelp biomass was processed by PEF, blanching, and soaking, and the resulting chemical composition was assessed in both the liquid and solid fractions. In general, increasing processing led to greater mass extraction to liquid fractions. Some components were readily extracted, such as minerals (ash) including iodine, polyphenols, and mannitol. In contrast, proteins, and heavy metals were concentrated in the solid fractions, as was (presumably) fiber.

The combination of PEF and soaking reduced iodine content in the biomass (−70%) without elevating the temperature and could thus be a more energy-efficient method than the currently used blanching treatments for this species. However, this combination led to greater extraction of dry matter as compared to blanched only.

Several applications of solid and liquid fractions were suggested based on the chemical composition, as well as the need for further processing and research. These should be followed up in ongoing and novel projects to unleash the potential of seaweed. Further studies should also investigate the effect of various processing schemes on the 3Ps of sustainability: people (who eat/use kelp), planet (i.e., energy and water usage, solvent use for extractions, etc.), and profit (i.e., kelp producers' bottom line, the effect on national and international economy).

Table 5
Suggested product applications for solid and liquid fractions.

Fraction		Market	Applications	Further processing needs	Further research needs	Estimated TRL
Solid		Food	Food ingredient	Drying, mixing, further food processing, and packaging	Optimization of energy efficient low temperature drying. Recipe formulation (could include co-creation or other consumer studies). Assessment of stability in chemical composition.	Medium to high
		Domestic plants and horticulture	Soil component for increasing water retention (alginates) – as replacement for turf	Evaluate the need for reduction in salt and heavy metals, possibly using chelating agents	Effect on plant growth and water retention. Sustainability assessment (CO_2 equivalents).	Medium to high
		Feed/Agriculture	Feed ingredient	Fermented product in use for ruminants. Evaluate the need for salt and heavy metals reduction, possibly using chelating agents.	Fermentation methods to be optimized. Bioavailability of protein and carbohydrates. Bioavailability of heavy metals. Effect on gut health and animal/fish welfare. Effect on composition of meat/egg/milk.	Medium to high
		Packaging materials	Bioplastic/packaging material	Milling of seaweed powder to low particle size	Improve mechanical strength	Low to medium
Liquid		Domestic plants & agriculture	Liquid fertilizer providing mannitol, nitrogen, and minerals, e.g., potassium	Salt reduction	Effect on plant growth and microbiome.	Medium
		Domestic plants & agriculture	Biostimulants (plant hormones)	Salt reduction, possibly up-concentration (water reduction), or extraction of relevant components	Optimize methods for salt reduction, could include soaking biomass in fresh water before processing or filtration.	Medium
		Feed/Agriculture	Feed components with bioactive ingredients for health-promoting effects	Salt reduction, possibly iodine reduction	Effect on gut health and animal/fish welfare, nutritional claims.	Low to medium
		Food/Health food	Ingredient in food or drinks to improve iodine status	Reduce iodine either by processing or mixing with other ingredients	Assessment of stability in iodine content.	High
		Health food/ Nutraceutical	Iodine supplement to provide optimal iodine status	Encapsulation and possibly mixing with other health-promoting ingredients (e.g., fruit extracts or omega 3 sources)	Assessment of stability in iodine content and resulting risk of consumption. The content should be stable and consistent (e.g., 75–125 μg).	Medium to high
		Health food/ Nutraceutical	Health-promoting shot or smoothie providing, e.g., 100 μg iodine and bioactive components (incl. polyphenols)	Mixing with other ingredients, packaging	Assessment of stability in iodine content and resulting risk of consumption. The content should be stable and consistent (e.g., 75–125 μg). Health claim investigation of bioactive compounds and health claim application.	Medium to high
Compounds extracted from the liquid	Mannitol	Food ingredients, cosmeceuticals, pharmaceuticals	Low-energy sweeteners, natural sweeteners	Separation, purification, drying	Method optimization.	Medium to high
	Polyphenols	Nutraceutical, pharmaceutical	Nutraceutical tablet or liquid extract with bioactive properties	Separation, possibly purification and low-temperature drying (e.g., freeze-drying)	Health claim investigation of bioactive properties (*in vitro* studies) and health claim application.	Medium
	Iodine	Nutraceutical, pharmaceutical	Iodine tablet or liquid extract	Separation, concentration (e.g., drying)	Assessment of stability in iodine content and resulting risk of consumption. The content should be stable and consistent and provide a safe dose (e.g., 100 μg).	Medium to high

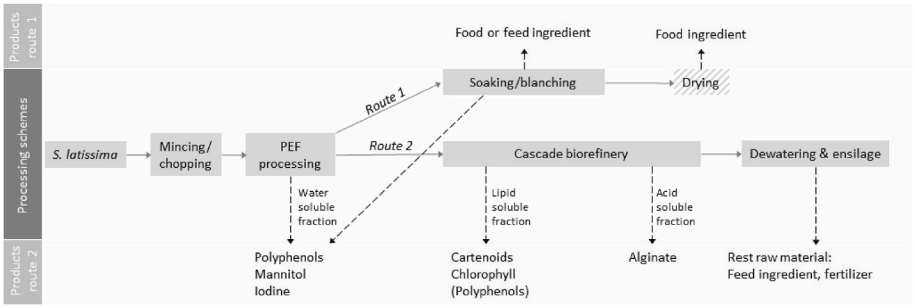

Fig. 5. Suggested processing schemes for full biomass utilization of sugar kelp.

CRediT authorship contribution statement

Marthe Jordbrekk Blikra: Writing – review & editing, Writing – original draft, Visualization, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Tone Mari Rode:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Torstein Skåra:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Conceptualization. **Ingrid Maribu:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis. **Randi Sund:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Methodology, Formal analysis. **Mette Risa Vaka:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis. **Dagbjørn Skipnes:** Writing – review & editing, Writing – original draft, Validation, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Dagbjørn Skipnes reports financial support was provided by Horizon Europe. Marthe J. Blikra reports financial support was provided by Research Council of Norway. Dagbjørn Skipnes reports financial support was provided by Research Council of Norway. The other authors were in part supported by the same grants disclosed above.

Data availability

Data will be made available on request.

Acknowledgments

This study was supported by the following projects: AlgaeProBANOS, which was co-funded by the European Union through the Horizon Europe program (project number 101112943); SUSKelpFood, funded by the Research Council of Norway (grant number 326803); BEIS, funded by Nofima and the Research Council of Norway (grant number 194959), and the Nofima-funded project “PepTek”.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2024.116402.

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