



Approaches for reducing the iodine content of the brown seaweed *Saccharina latissima*—effects on sensory properties

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Abstract

The effects of steam- or warm water treatments of the brown seaweed *Saccharina latissima* on iodine content and nutrient and sensory profiles were investigated. Warm freshwater or seawater treatments reduced the dry weight iodine content by 73% and 59%, respectively. Steam treatment was less efficient and only reduced the iodine content by 26%. Freshwater treatment resulted in a greater reduction in total solids and loss of water-soluble components, mainly reducing the content of ash and carbohydrates. Seawater treatment had a small effect on total solid content but altered the mineral profile leading to an increase in sodium and decrease in potassium content. The sensory profiles reflected the observed differences in composition as it revealed a significantly higher saltiness of the seawater-treated sample compared to other treatments, as well as a higher umami intensity compared to the freshwater-treated sample. The latter was characterized by low scores across all sensory attributes, indicating greater loss of flavour-active compounds. Further, including seawater-treated *S. latissima* at relatively low levels (0.5% and 1%) significantly altered the flavour of a commercial spinach soup compared to the same preparation without seaweed. Therefore, warm water treatment using seawater could be an alternative method for lowering the iodine content in brown seaweed while limiting nutrient loss and maintaining its flavour potential for applications in the food industry.

Keywords Sugar kelp · Phaeophyceae · Nutrient retention · Sensory properties · Food applications

Introduction

The seaweed aquaculture industry in Europe is growing rapidly. Seaweed is rich in valuable nutrients and can contribute to meet the increased demand for a more sustainable food production (Rotter et al. 2020; Blikra et al. 2021). Hence, it is gaining interest within the food industry. Seaweed production requires no input factors such as freshwater and chemicals and does not compete with terrestrial agriculture for land areas. With the right regulations, it can contribute to ecosystem services, support

biodiversity and counteract eutrophication through uptake of dissolved nutrients (Hasselström et al. 2018). Norway's long coastline and pristine waters offers favourable conditions for seaweed farming, and commercial biomass production is expanding (Stévant et al. 2017). The Norwegian industry is mainly focusing on the two brown seaweed species *Saccharina latissima* (sugar kelp) and *Alaria esculenta* (winged kelp), both due to their applicability as food for taste and nutritional profiles and high biomass yields in cultivation (Kraan 2013).

While seaweed is a traditional food and an integral part of the Asian cuisine, highly appreciated for its unique flavour and nutritional properties, it is unfamiliar to the majority of European consumers and the main use has been limited to the production of hydrocolloids entering the composition of food products. Along with an increasing focus on nutrition, health and sustainability, there is a positive attitude towards including seaweed in the diet (Mouritsen et al. 2013; Wendin and Undeland 2020). *Saccharina latissima* and other brown seaweeds are a rich source of dietary fibres, minerals, trace elements, certain

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vitamins and other bioactive compounds with potential health benefits (Holdt and Kraan 2011; Wells et al. 2017). Its content of the hydrocolloid alginate and capacity to bind water and oil may improve the texture of food, and seaweed antioxidants have a positive effect on shelf-life (Roohinejad et al. 2017). Together with the content of flavour-active compounds that elicit e.g., umami taste, with the ability to boost other flavours, brown seaweeds have a large potential as a sustainable ingredient in multiple food products. However, seaweed is yet to be incorporated as an ingredient in everyday food products in Europe. In order to enable for large scale usage of ingredients, there is a need to fill knowledge gaps regarding efficient post-harvest methods suitable to provide safe, nutritious and flavourful ingredients in sufficient volumes.

The high iodine content in some species, including *S. latissima*, and the potential to accumulate other potentially toxic elements (PTEs, i.e., inorganic arsenic and cadmium) are identified as major food safety hazards in the European seaweed chain (Banach et al. 2020; Blikra et al. 2022). There are currently no EU regulations for levels of PTEs in seaweed used as food and there is great variation in levels found in seaweed raw material (Duinker et al. 2020; Blikra et al. 2022). This raises concerns within the food industry and limits the amount that can be added to food products without risking excessive intake among consumers.

Iodine is an essential element for the production of thyroid hormones, which are involved in cell metabolism, growth and reproduction, and in the development of the central nervous system (Andersson et al. 2007; de Benoist et al. 2008). Mild to moderate iodine deficiency is a global problem affecting one third of the global population, particularly widespread in Europe including Norway (Andersson et al. 2007; Lazarus 2014; Brantsæter et al. 2018) and is the most common cause of preventable mental impairment worldwide (Zimmermann et al. 2008). In Norway, dairy products and fish are the main dietary sources of iodine but surveys show that consumption of these food items has decreased during the recent years (Helsedirektoratet 2019). In addition, there is an increasing number of people transitioning into a plant-based diet. Together this constitute a basis for assuming a higher probability of inadequate iodine intake among the population (Brantsæter et al. 2018). Although insufficient iodine intake is coupled to adverse, sometimes severe, health effects, also excessive intake can have negative consequences. Consequent exposure to high iodine levels is associated with the suppression of thyroid function, leading to hyperthyroidism or hypothyroidism, with similar consequences to those caused by iodine deficiency (Laurberg et al. 2009; Leung and Braverman 2014). Increased prevalence of thyroid dysfunction has been documented in association with seaweed consumption, due to excessive iodine intake (Miyai et al. 2008; Chung et al. 2009; Emdner and Jack 2011).

Based on the results from a large epidemiological study performed in European school children, EFSA has provided dietary reference values for adequate iodine intake levels (AI) of $150 \mu\text{g day}^{-1}$ for adults, $200 \mu\text{g day}^{-1}$ for pregnant and lactating women, $70 \mu\text{g day}^{-1}$ for infants and $90\text{--}130 \mu\text{g day}^{-1}$ for children and adolescents. The tolerable upper intake level for adults (UL) of $600 \mu\text{g day}^{-1}$ is based on dose–response studies with intakes up to $1800 \mu\text{g day}^{-1}$ showing no adverse clinical effects using an uncertainty factor of 3 (SCF 2002). Today, there are few seaweed-containing products available on the Norwegian market (excluding products containing seaweed-derived ingredients such as alginate and carrageenan) and in general they are not consumed frequently. Therefore, the current risk of excessive iodine intake is limited. However, to allow for a wider distribution and use in staple food products, recommended intake levels must be taken into consideration.

Processing methods such as warm water treatment (WWT), boiling and fermentation has been reported to reduce the iodine content and to some extent the content of the other PTEs in *S. latissima* (Stévant et al. 2018b; Bruhn et al. 2019; Nielsen et al. 2020; Jordbrekk Blikra et al. 2021). Nielsen et al. (2020) reported a drastic reduction (over 90%) of the iodine content in cultivated biomass following WWT with freshwater (tap water). However, this was accompanied by a pronounced loss of other nutrients such as free amino acids, minerals, and phenolic compounds. Like other food items the flavour attributes of seaweeds are related to the content of flavour-active compounds such as free amino acids, volatile compounds, and minerals (Mouritsen et al. 2019; Stévant et al. 2020). These losses during processing would not only lead to a lower nutritional quality but also alterations in sensory properties. However, effects of processing of brown seaweeds on sensory properties is not well understood and studies on this topic are limited (Le Pape et al. 2002; Stévant et al. 2018a; Sánchez-García et al. 2019; Stévant et al. 2020; Akomea-Frempong et al. 2021).

Flavour is a major factor determining the consumers acceptance of food. Although, current focus on local, sustainable, and plant-based food are in favour of seaweeds, flavour and taste must meet the consumers palatability. Besides studies revealing a larger openness and curiosity towards seaweeds among Western consumers, there are still negative associations and emotions among the general population (Birch et al. 2019; Moss and McSweeney 2021) limiting a broader distribution of seaweed-containing foods. It is therefore of utmost importance to find methods for minimizing food safety risks providing the food industry with nutritious and tasty ingredients that can be included in food products attractive for the consumer.

The aim of this study was to explore the potential of WWT using freshwater or seawater and steam treatment to reduce the iodine content and other PTEs in *S. latissima*.

The effect of the tested treatments on nutrient retention and sensory properties was investigated. Furthermore, one sample was chosen based on its final iodine content and sensory profile for evaluation of its performance as an ingredient in a commercial food product.

Material and methods

Seaweed material & treatments

Cultivated *Saccharina latissima* from Tango Seaweed (Herøy, Møre & Romsdal, Norway) was harvested on 4 June 2020 and transported in insulated container to the laboratory within a few hours for warm water and steaming treatments. Freshwater (tap water) treatment was conducted on 1 kg fresh cultivated biomass (whole blades including stipes; weight approximately 100 g) in 5 L water per batch at 45 °C for 120 s as reported suitable to effectively reduce the iodine content of *S. latissima* (Nielsen et al. 2020). Steam treatment was conducted by placing *S. latissima* blades in a bench-top steamer (Phillips HD 9140) with steam at a temperature between 90 and 95 °C for 20 min.

Seawater treatment was not initially a part of the study, but samples from a pilot experiment performed by the industry were included since this method appeared as a promising alternative to freshwater-treatment and steaming that had not given the desired effects. Pilot-scale seawater-treatment was performed on wild *S. latissima* since cultivated biomass was not available. Wild *S. latissima* was harvested on 2 February 2021 (Misje, Vestland, Norway) and stored for one day in a mesh bag submerged in free-flowing seawater prior to further processing. These seaweeds were somewhat younger and smaller than the cultivated seaweeds. On the following day, multiple batches of *S. latissima* biomass (5 kg, whole blades including stipes) were rinsed in seawater (20 L) at 45 °C for 120 s. The same treatment water was used repeatedly. For all experiments, seaweeds with adhering matter (e.g., epiphytes and epibionts) were discarded and not included.

Freshwater-treatment and steaming treatments were performed in triplicates, whilst seawater-treatment was performed in duplicate. All samples were left to drip to remove the surface water, then vacuum-packed and frozen until freeze-dried prior to analysis.

A list of the samples that are included in the study and corresponding treatments are shown in Table 1.

Chemical composition

The proximate composition of the samples was analysed to investigate the nutrient retention following treatments.

Determination of moisture and ash

The moisture content was determined by drying samples at 105 °C until constant weight. Ash was determined by combustion of the sample in a muffle furnace at 590 °C for 12 h and the combustion residues were weighed. For further chemical characterization, fresh samples were freeze-dried until constant weight.

Iodine analysis

Iodine concentration in *S. latissima* samples was quantified using inductively coupled plasma-mass spectrometry (ICP-MS) according to the method described by Dahl et al. (2020). Samples were added 1 mL tetramethylammonium hydroxide (TMAH) and 5 mL deionized water before extraction at $90 \pm 3^\circ \text{C}$ for 3 h. The samples were then diluted and centrifuged. Prior to quantification, the samples were filtered through a 0.45 µm single use syringe and disposal filter. Tellurium was used as an internal standard in order to correct for instrument drift.

Fatty acid composition

The fatty acid content was determined according to ISO 12966-2:2017. Gas chromatography with flame ionization detection (GC-FID) was used to identify and quantify saturated, monounsaturated, and polyunsaturated fatty acids

Table 1 Overview of samples and treatments

Sample name	Species	Treatment description	Number of replicates (<i>n</i>)
Untreated	<i>Saccharina latissima</i> , cultivated	No treatment	3
Freshwater-treated	<i>Saccharina latissima</i> , cultivated	Rinsed in freshwater at 45 °C for 120 s. 1 kg in 5 L water	3
Steam-treated	<i>Saccharina latissima</i> , cultivated	Steamed for 20 min at 90–95 °C	3
Untreated	<i>Saccharina latissima</i> , wild	No treatment	1
Seawater-treated	<i>Saccharina latissima</i> , wild	Rinsed in in seawater at 45 °C for 120 s. 5 kg in 20 L water	2

(SA, MUFA and PUFA). Fatty acids were converted to free fatty acids by saponification, and methanolic acetyl chloride was used to convert free fatty acids into their corresponding methyl esters prior to extraction with heptane.

Protein content and carbohydrate content

Total nitrogen was determined as total N₂ gas after complete combustion of the sample in the presence of oxygen using a TruMac N Analyzer (LECO, USA) or a Rapid Max N Exceed Analyzer (Elementar, Germany). The total protein content was determined using a nitrogen-to-protein conversion factor of 5.0, which previously has been reported suitable for the prediction of protein in seaweed (Angell et al. 2016).

The carbohydrate content, including fibre, was estimated by using the following equation.

$$\text{Carbohydrates (g (100 g)}^{-1}) = 100 - (\text{moisture} + \text{Ash} + \text{Fatty Acids} + \text{Protein})$$

Determination of macro minerals and heavy metals

Macro minerals (Na, Mg, K and Ca) and heavy metals (As, Cd, Hg, Pb and Cu) were determined at the Institute of Marine Research (IMR) by ISO accredited methods according to Reksten et al. (2020). The elements were determined by ICP-MS after acid wet digestion in a microwave oven according to the method described by Julshamn et al. (2007), using an external calibration curve.

Sensory profile of processed samples of *S. latissima*

A sensory descriptive analysis was performed to evaluate how the selected treatments affected the flavour, texture, and mouthfeel of *S. latissima* samples and to evaluate which sample had the largest potential as a flavourful ingredient in a food product.

A generic descriptive analysis (GDA) was used for the sensory profiling of three samples of *S. latissima* (freshwater-treated, seawater-treated, and steam-treated samples). The sensory panel consisted of 9 judges selected and trained according to ISO:8586:1 (2012). The samples were evaluated using 10 sensory attributes (Table 2) as described by Stévant et al. (2018a). A scale from 0 to 9 (lowest to highest intensity) was used and the panellists were trained using two characteristic samples. All samples were coded with three-digit numbers and evaluated in dry powder form (milled to > 1 mm) in two duplicate sessions. The sensory program RedJade (Tragon Corporation, Palo Alto, CA, USA) was used to collect the sensory data which was processed and stored according to the General Data Protection Regulation (GDPR).

Sensory evaluation of seawater-treated *S. latissima* as an ingredient in a commercial product

Dried powder of seawater-treated *S. latissima* was added to a dehydrated spinach soup (TORO, Orkla Foods Norway) to evaluate its flavour contribution to a food formulation. Each portion consisted of 19.75 g dehydrated soup powder where 2.0 dL water and 0.5 dL milk were added to a final weight of approximately 270 g. Dried and milled seawater-treated *S. latissima* was added to the soup at approximately 0.5% and 1% inclusion level, equivalent to 0.13 g and 0.26 g per portion (equivalent to 1.3 and 2.6 g of rehydrated ingredient). The levels of inclusion were determined based on the dietary iodine contribution of the seawater-treated sample, covering 50 and 100% respectively of the UL of 600 µg iodine day⁻¹ for adults (EFSA 2014). The soups were prepared by combining all ingredients in a pan and heating under agitation until boiling and was then left to boil for 5 min. All soup samples were served hot. The sensory evaluation of the product was performed by 16 untrained panellists in two tetrad tests, one for each inclusion level (0.5 and 1%), to detect perceptible difference between seaweed-containing soup and the same standard

Table 2 Sensory attributes and their definition used by the panellists in the general descriptive analysis. Scale anchors define the endpoints of the 9-point scale used in the analysis

Sensory attribute	Scale anchors	Description
<i>Aroma & Flavour</i>		
Fresh sea	none much	Fresh sea odour and flavour
Fermented	none much	Fermented odour and yeast flavour, matured cheese, cured ham
Hay	none much	Dry hay odour and flavour, green tea
Salt	none much	Salty taste
Umami	none much	Umami taste e.g., meat stock, brown crabmeat
Bitter	none much	Bitter taste
<i>Texture</i>		
Crispy	none much	During first bites, how crispy is the samples
Tough	tender tough	When chewing, how difficult is it to break up
Dissolves	none much	When chewing, how the samples dissolves or melts
Viscous	thin viscous	Viscous, slimy, porridge-like

soup without kelp. Panellists were given four samples, i.e., two with seaweed and two control (without seaweed) per test and instructed to sort the samples into two groups based on their similarity. All samples were coded with three-digit numbers and the evaluation took place in a sensory test facility equipped with individual booths. The sensory software EyeQuestion (Logic8 BV, Elst, Netherlands) was used to collect data.

Data analysis

Statistical analysis was performed using R (version 4.1.0, R Development Core Team (2021)). The results from the analysis of replicate samples were described as mean \pm standard deviation. A one-way analysis of variance (ANOVA, R function aov) was used to detect significant differences ($p < 0.05$) among cultivated untreated, freshwater and steam treated *S. latissima* samples regarding the iodine content. The data from the sensory profiling was analysed using a mixed model ANOVA (R function lmer (Bates et al. 2014)). In this model, individual panellists were treated as random factors to detect significant differences in sensory profile (scores for each attribute) among samples. The Benjamini–Hochberg procedure was applied to control the false discovery rate under multiple testing. Tukey's honest significant differences (HSD) were computed for the pairwise comparison of samples (R function glht). A principal component analysis (PCA) based on covariance matrix (no scaling) was applied to visualize sensory profiles of samples (R function prcomp). The p -values from the tetrad tests were determined using binomial distribution (R function bionom.test) The critical number of correct answers to conclude that samples were perceptibly different were fixed at 10 ($\alpha = 0.05$).

Results

Iodine content

The iodine content of untreated samples and after steaming and warm water treatments are shown in Fig. 1. Cultivated and wild untreated *S. latissima* contained 7977 ± 2317 and 5580 mg iodine kg^{-1} DW, respectively. Treatment with freshwater resulted in an iodine content reduction of 73% relative to the cultivated untreated sample, to a final content of approximately 2189 ± 209 mg kg^{-1} DW. Treatment with seawater resulted in a similar final content of about 2310 ± 227 mg kg^{-1} DW, which was equal to a reduction of 59% compared to the wild untreated sample. Steam exposure for 20 min did not significantly reduce the iodine content of cultivated *S. latissima*.

Mass balance and retention of nutrients

Proximate composition

To account for the loss of total solids (TS), the content of macro constituents was estimated relatively to the wet weight (WW) as reported previously by Nielsen et al. (2020). In contrast, expressing the content relative to the DW would not correctly reflect the nutrient losses or water uptake occurring during the treatments. The content of ash, carbohydrates, fatty acids, and proteins as well as total solids can be seen in Fig. 2. The DW of the wild *S. latissima* (untreated) (Fig. 2b), harvested in February 2021, was considerably lower compared to the cultivated untreated sample (Fig. 2a), harvested in June 2020. The DW content

Fig. 1 Iodine content in **a)** cultivated *Saccharina latissima* samples untreated and subjected to freshwater or steam treatment and **b)** wild *Saccharina latissima* samples untreated and subjected to seawater treatment. Results are given as mean ($n = 3$ in (a), $n = 2$ in (b)). Error bars in (a) represent standard deviation. Different subscript letters in (a) indicate significant differences among samples (Tukey HSD, $p < 0.05$)

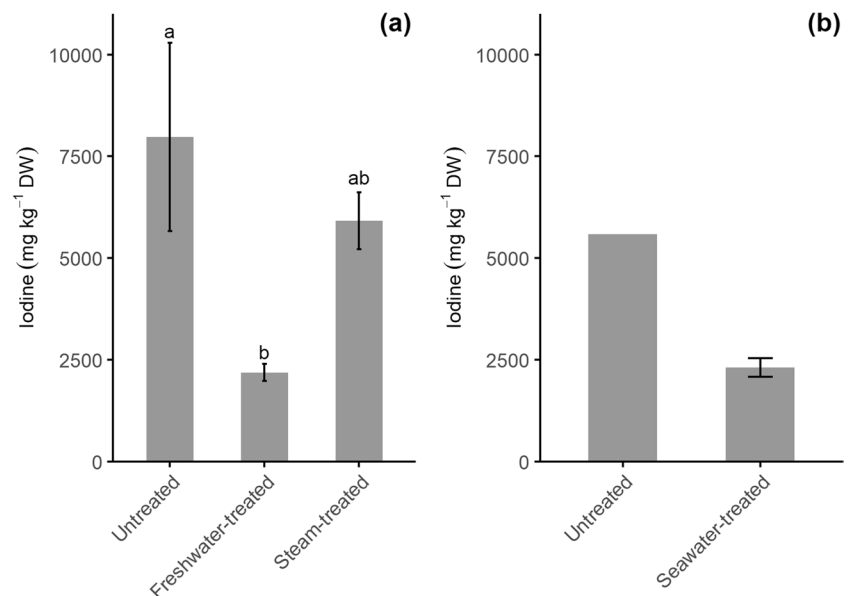
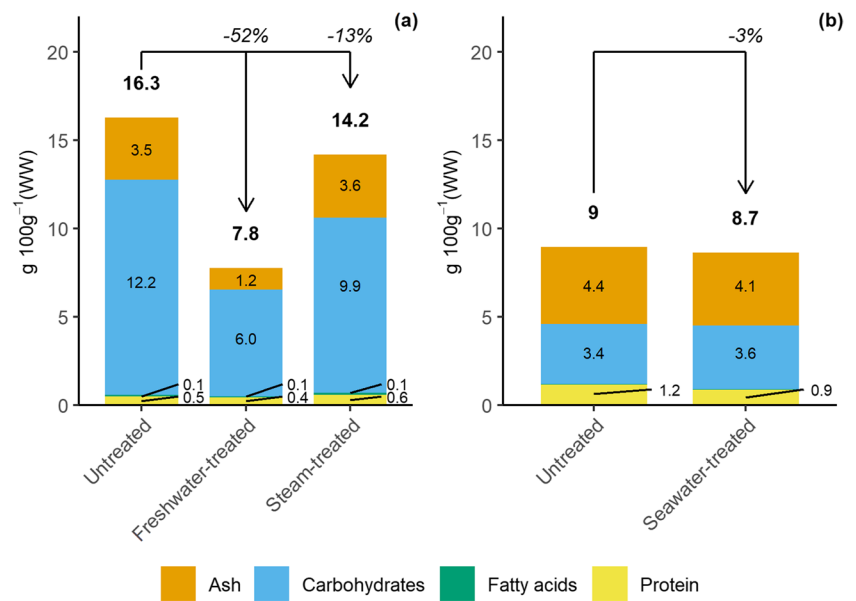


Fig. 2 Composition of (a) cultivated *Saccharina latissima* samples, untreated and subjected to freshwater or steam treatment ($n=3$) and (b) wild *Saccharina latissima* samples, untreated ($n=1$) and subjected to seawater treatment ($n=2$). The results are presented relative to the WW (mean values) and the values in bold indicate total total solid content, i.e. the sum of ash, protein, fatty acids and carbohydrates. The percentages in connection to the arrows represent the loss of total solids



in the cultivated untreated sample ($16.3 \text{ g } (100 \text{ g})^{-1}$) was about twice as high compared to the wild untreated sample ($9.0 \text{ g } (100 \text{ g})^{-1}$). The main difference in proximate composition was the notably higher carbohydrate content of $12.2 \text{ g } (100 \text{ g})^{-1}$ (WW) in the cultivated seaweed, compared to $3.4 \text{ g } (100 \text{ g})^{-1}$ (WW) in the wild untreated sample. Freshwater treatment increased the moisture content, resulting in a decrease in total solids of 52% compared to the untreated. The steam treatment had less effect on the moisture content, resulting in a total solid content of 14.2% corresponding to a reduction of 13% when compared to the cultivated untreated sample. Freshwater treatment mainly led to a reduction in ash and carbohydrate content, while

the protein content remained stable. Seawater treatment on the other hand did not seem to affect the seaweed composition with only a minor reduction in total solids (3%). All samples had a low content of fatty acids which was minimally affected by the treatments.

Minerals and trace metals

In Fig. 3, the content of the macro minerals Ca, K, Mg and Na (also including I) following different treatments is shown. Relative to the WW of the sample treatment with freshwater gave a clear reduction (70%) in the total content of analysed minerals and resulted in lower concentrations

Fig. 3 Content of selected macro minerals Ca, K, Mg, Na, and I relative to the WW (mean values) in (a) cultivated *Saccharina latissima* samples, untreated and subjected to freshwater treatment and steam treatment ($n=3$) and (b) wild *Saccharina latissima* samples, untreated ($n=1$) and subjected to seawater treatment ($n=2$). The values in bold above the bars indicate the total amount of the five elements and the percentages in connection to the arrows represents the total loss of elements following treatments

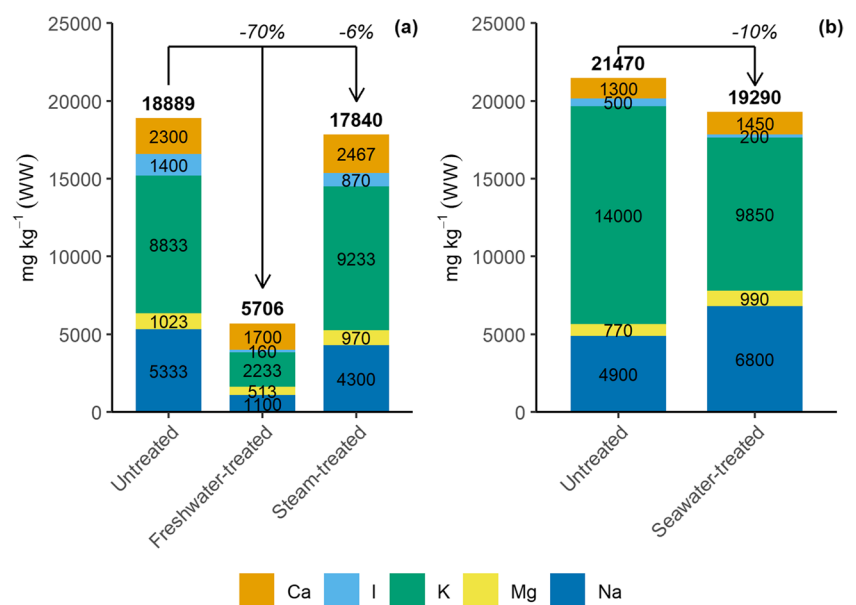


Table 3 Iodine and mineral profile of freeze dried (mg kg^{-1} DW) *Saccharina latissima* samples. Values are given as mean of replicate measurements \pm standard deviation or as single measurement. Number of replicates are stated for each sample

Sample	I	Ca	K	Mg	Na	
Cultivated <i>S. latissima</i>	Untreated ($n=3$)	7977 \pm 2317 ^a	14 421 \pm 935 ^a	55 348 \pm 2142 ^a	6 421 \pm 557 ^a	33 488 \pm 3 696 ^a
	Freshwater-treated ($n=3$)	2189 \pm 209 ^b	26 007 \pm 1 963 ^b	34 146 \pm 1 809 ^b	7 838 \pm 272 ^b	16 830 \pm 1 042 ^b
	Steam-treated ($n=3$)	5916 \pm 699 ^{ab}	15 764 \pm 1 133 ^a	59 018 \pm 5 502 ^a	6 198 \pm 429 ^a	27 513 \pm 2 570 ^a
Wild <i>S. latissima</i>	Untreated ($n=1$)	5580	14 509	156 250	8 594	54 688
	Seawater-treated ($n=2$)	2310 \pm 227	16 752 \pm 549	113 804 \pm 1 536	11 438 \pm 96	78 566 \pm 1 019

Different subscript letters in each column indicate significant differences between the cultivated untreated, freshwater-treated and steam-treated samples.

for all (Fig. 3a). Steam treatment on the other hand had little effect on the content of minerals. Treatment with seawater seemed to have little effect on the total content of analysed minerals compared to the untreated sample (wild), as shown in Fig. 3b. The total content decreased with 10% on a WW basis and there was a reduction in I and K content, while the Ca, Na and Mg content increased.

On a DW basis (Table 3) freshwater treatment led to a significant decrease in both K and Na with approximately 40 and 50%, respectively, while the Ca concentration increased by 80% compared to the cultivated untreated sample. This is in accordance with the mass balance, showing great losses of Na and K and only a slight reduction in Ca on a WW basis. The largest difference between the treatments were the final Na content, where the seawater-treated samples had concentration more than four times higher than the freshwater-treated sample ($78\,566 \pm 1\,019$ and $16\,830 \pm 1\,042$ mg kg^{-1} , respectively).

The ability of seaweed to accumulate heavy metals is identified as a potential food safety issue, thus the content of As, Cd, Cu, Hg and Pb were analysed. As can be seen in Table 4, the content of Cd, Cu, Pb and Hg was to varying extent concentrated in the freshwater-treated sample. Treatment with seawater had minimal effect on the content of analysed heavy metals. Inorganic As (iAs) was analysed in the cultivated untreated and freshwater-treated sample where low levels were found.

Sensory profile of processed *S. latissima* samples

Significant ($p < 0.05$) differences were found between samples for most aroma and flavour attributes, except for bitterness and fermented flavour and aroma, which can be seen in Table 5. Regarding the textural properties, the samples differed in terms of how crispy they appeared as well as how they dissolved in the mouth. The freshwater-treated sample was to some extent associated with hay flavour and aroma as well as a tough texture. There was also a significant difference in umami flavour of the freshwater-treated sample compared to the other samples. The largest difference between samples was the saltiness, where the freshwater-treated sample had the lowest intensity, and the seawater-treated the highest. The latter was otherwise characterized by an aroma and flavour of fresh sea and a dissolving mouthfeel. The steam-treated sample had higher saltiness and umami intensity than the freshwater-treated sample and was overall rated between the two other samples.

The average scores from the panellists were used to conduct a PCA to visualise the differences in sensory profiles between samples. The 1st and 2nd principal component (PC) together accounts for 92.2% of the total variation as revealed by the PCA biplot in Fig. 4 (85.5 and 6.7% by PC1 and PC2, respectively). The samples are mainly separated in terms of saltiness and hay flavour and aroma by PC1 (along the x-axis), with the seawater-treated samples being associated with a saltier taste and flavour and odour of fresh sea whilst the freshwater-treated sample was

Table 4 Heavy metals in freeze dried samples (mg kg^{-1} DW) *Saccharina latissima* samples. Values are given as mean of replicate measurements \pm standard deviation or as single measurement. Number of replicates are stated for each sample

Sample	As	iAs	Cd	Cu	Pb	Hg	
Cultivated <i>S. latissima</i>	Untreated ($n=3$)	46 \pm 2 ^a	0.067 \pm 0.001	0.21 \pm 0.02 ^a	0.55 \pm 0.04 ^a	0.05 \pm 0.005 ^a	0.01 \pm 0.0004 ^a
	Freshwater-treated ($n=3$)	34 \pm 2 ^b	0.063 \pm 0.004	0.42 \pm 0.06 ^b	7.67 \pm 0.81 ^b	0.12 \pm 0.01 ^b	0.03 \pm 0.002 ^b
	Steam-treated ($n=3$)	52 \pm 1 ^c	-	0.28 \pm 0.01 ^a	1.24 \pm 0.17 ^a	0.10 \pm 0.02 ^a	0.02 \pm 0.001 ^c
Wild <i>S. latissima</i>	Untreated ($n=1$)	71	-	0.47	0.66	0.41	0.06
	Seawater-treated ($n=2$)	56 \pm 2	-	0.49 \pm 0.005	0.67 \pm 0.01	0.28 \pm 0.08	0.05 \pm 0.01

Different subscript letters in each column indicate significant differences between the cultivated untreated, freshwater-treated and steam-treated samples.

Table 5 Mean sensory scores on a scale from 0 to 9 (\pm standard deviation) from the descriptive analysis of three ingredients from *Saccharina latissima* by panellists ($n=9$) during repeated sessions ($n=2$) and based on 10 sensory attributes

Sensory attribute	Freshwater treated	Seawater-treated	Steam-treated	<i>p</i> -value
<i>Aroma & flavour</i>				
Fresh sea	3.9 \pm 1.7 ^a	5.9 \pm 2.1 ^b	4.3 \pm 1.9 ^a	0.004
Fermented	3.4 \pm 1.7 ^a	3.3 \pm 1.5 ^a	3.9 \pm 1.9 ^a	0.460
Hay	5.4 \pm 1.9 ^b	3.6 \pm 2.0 ^a	4.7 \pm 2.1 ^{ab}	0.030
Salt	1.8 \pm 0.8 ^a	7.7 \pm 1.1 ^c	4.8 \pm 1.5 ^b	0.000
Umami	3.2 \pm 2.1 ^a	4.9 \pm 1.7 ^b	4.8 \pm 1.9 ^b	0.005
Bitter	1.9 \pm 1.4 ^a	2.4 \pm 1.5 ^a	2.3 \pm 1.2 ^a	0.460
<i>Texture</i>				
Crispy	3.0 \pm 1.7 ^{ab}	2.6 \pm 1.4 ^a	4.1 \pm 2.1 ^b	0.016
Tough	4.4 \pm 2.1 ^a	3.2 \pm 1.8 ^a	3.6 \pm 1.9 ^a	0.114
Dissolves	3.3 \pm 1.7 ^a	6.4 \pm 1.1 ^b	4.3 \pm 2.1 ^a	0.000
Viscous	3.5 \pm 2.0 ^a	4.3 \pm 2.4 ^a	3.3 \pm 2.1 ^a	0.312

Significant ANOVA results ($p < 0.05$) following the correction with the Benjamini–Hochberg procedure are in bold. Different superscript letters in the same row indicate significant differences among samples (Tukey HSD, $p < 0.05$).

more associated with dry hay and green notes as also seen in Table 5. The steam-treated sample are separated from the other samples by PC2 (along the y-axis), mainly by differences in textural attributes but also fermented flavour. However, as can be seen from the length of the vectors these attributes are characterized by a moderate intensity and the amount of variability accounted for by PC2 (6.7%), indicates that actual differences can be small and should be interpreted with care. Despite this, the main pattern seen in Table 5 is confirmed in the PCA.

Sensory analysis of spinach soup with and without seawater-treated *S. latissima*

Based on the sensory profile determined in the GDA, characterized by intense saltiness and umami taste, and

the final iodine content ($2\,310 \pm 227$ mg kg⁻¹), the seawater-treated *S. latissima* was selected for testing as an ingredient in a commercial dehydrated spinach soup to evaluate its flavour contribution to the product, following two inclusion levels based on iodine concentration in prepared product. The results can be seen in Table 6. At both inclusion levels, the majority of panellists were able to differentiate between the two samples, with 11 correct sorting of samples at the lower inclusion level (0.5%, test 1) and 13 at the higher inclusion level (1% test 2). The result from the binomial distribution reveals a significant difference ($p < 0.05$) in the sensory perception of the soups containing seawater-treated *S. latissima* (both at 0.5% and 1%) and the control soup without seaweed ingredient.

Fig. 4 Principal component analysis (PCA) biplot (1st and 2nd principal component) based on average scores from the panellists ($n=9$) obtained in the descriptive sensory analysis. Variation in intensity for attributes are represented by vectors of different length based on loadings

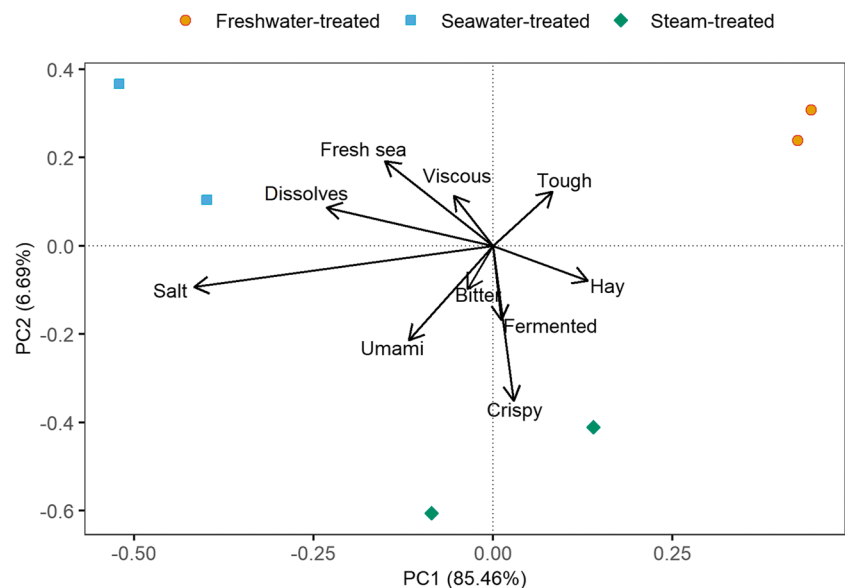


Table 6 Tetrad test to detect perceptible differences between kelp (seawater-treated *Saccharina latissima*) containing soup and standard soup without seaweed ingredient. The soups were assessed in one tetrad test per variant (level of inclusion of seaweed ingredient) by 16 panellists

Test	Sample (soup) tested	Inclusion level	Number of correct sorting	Number of incorrect sorting	<i>p</i> -value
1	Seawater-treated <i>Saccharina latissima</i>	0.5%	11	5	0.04
2	Seawater-treated <i>Saccharina latissima</i>	1%	13	3	<0.001

Discussion

Methods for iodine reduction content in *S. latissima*

The iodine content of kelps varies with season, harvest location and the physiological state of the plant (Ar Gall et al. 2004; Lüning and Mortensen 2015; Roleda et al. 2018) and levels ranging from 670 to 10 000 mg kg⁻¹ DW have been reported from *S. latissima* harvested along the Norwegian coast (Duinker et al. 2020). This constitutes an additional challenge for the predictability of the iodine level in the final product. Both the cultivated and wild untreated samples in this study had an iodine content within the range reported by Duinker et al. (2020). There are several studies investigating potential methods for reducing iodine content in *S. latissima*. Methods such as rehydration, boiling, blanching at temperatures between 30 and 80 °C as well as fermentation has all shown to reduce the iodine content (Lüning and Mortensen 2015; Stévant et al. 2018b; Bruhn et al. 2019; Correia et al. 2021; Jordbrekk Blikra et al. 2021). The WWT in the present study was adopted from Nielsen et al. (2020) who achieved a 90% reduction of the iodine content in *S. latissima* from WWT at 45 °C for 120 s, to a final content of 346 mg kg⁻¹ DW (starting content was 4 605 mg kg⁻¹ DW). In this study, freshwater- and seawater treatments resulted in an iodine loss of 73 and 59%, respectively, and a final iodine content above 2 000 mg kg⁻¹ DW for both samples, which is the limit recommended by French food authorities for seaweed products (ANSES 2018). Several factors impact the amount of iodine that are extracted during processing, such as iodine species present in the seaweed and its solubility, processing conditions including time, temperature, choice of solvent and solvent-to-substrate ratio (Blikra et al. 2022). The high initial iodine concentration in the cultivated *S. latissima* and higher biomass-to-water ratio compared to the experiment conducted by Nielsen et al. (2020), i.e., 1 kg (WW) in 5 L water versus 150 g (WW) in 5 L could explain the limited effect obtained during the freshwater treatment in the present study. Higher biomass-to-water ratio would most probably lead to higher iodine concentration in the treatment water which will limit the extraction of iodine. This may also explain the limited effect obtained from seawater treatment in this study as it was performed

at a higher biomass-to-water than the freshwater treatment. Further studies would be needed to confirm these observations. However, the potential for reduction of iodine content is probably higher also when using seawater, as seen from results from more recent studies which have obtained 90% reduction in iodine content from seawater-treatment of 2.5 kg (WW) *S. latissima* in 40 L seawater at 45 °C for 120 s (*unpublished results*). Steam treatment for 20 min at relatively high temperatures did not significantly reduce the iodine content, which is in accordance with results reported in an earlier study (Stévant 2019).

Nutrient and flavour retention

One of the aims of this study was to investigate how the tested treatments affected the nutrient content and composition of *S. latissima* in order to identify processing methods with minimal impact on desirable nutrients and flavour compounds. The treatments investigated in this study all influenced the nutrient content of *S. latissima* and resulted in samples which differed in sensory profile. The freshwater treatment had the greatest effect on the content of soluble nutrients and resulted in a loss primarily of Na, K, and carbohydrates, while minerals such as Mg and Ca, together with fatty acids and proteins were retained and concentrated. This is in accordance with results reported in previous studies (Stévant et al. 2018b; Nielsen et al. 2020). The difference in umami intensity of the freshwater-treated sample compared to the steam-treated samples suggests that water-soluble flavour compounds, such as amino acids glutamate and alanine, are partially released during the WWT as reported by Nielsen et al. (2020). The free glutamate level of dried *S. latissima* is reported low (0.5 – 1.5 mg g⁻¹ DW) (Mouritsen et al. 2013; Stévant et al. 2018b) compared to known umami sources like kombu (ca. 15 mg g⁻¹ DW) (Ninomiya 1998) although it may be increased following storage (up to 6.4 mg g⁻¹ DW) (Stévant 2019). The free glutamate content of the samples was not measured in the present study, but this compound is highly water-soluble, therefore the free glutamate levels in treated samples are most likely low. Hence the perception of umami from these samples may be caused by aroma compounds (e.g., volatile compounds) (Frøst et al. 2021) as well as other flavors influencing the overall taste experience

(Mouritsen et al. 2019). In this study the difference in saltiness, as shown to be significant between samples, could be coupled to the difference in mineral profile with Na and K being mostly affected by treatments. Freshwater treatment resulted in loss of Na and K, while the relative proportions of Ca, Mg, Cd and Cu remain stable or increased. It should be noted that seasonal variations will also influence the composition of the seaweed material.

The retention of some minerals is influenced by interactions with cell wall polysaccharides, e.g. the affinity of alginate for divalent cations, known to sequester minerals and metals (Davis et al. 2003). The affinity is determined by the ratio between monomers guluronic (G) and mannuronic (M) acid in the alginate as it is blocks of G monomers that bind divalent cations. Increasing proportions of G-blocks have been reported as the algae grows older along with variations across natural *S. latissima* populations (Indergaard et al. 1990; Manns et al. 2017). A higher G-content in the wild biomass used for seawater-treatment, could contribute to higher retention of certain minerals, i.e., Ca and Mg. Moreover, the content of soluble storage carbohydrates (i.e., mannitol and laminarin) varies with season and is lowest during the winter and highest during spring and early summer (Schiener et al. 2015; Manns et al. 2017; Sharma et al. 2018). This is in accordance with the observations in this study where the carbohydrate content in the cultivated *S. latissima*, harvested in June, was close to four times higher than in the wild, harvested in February. Although the carbohydrate composition was not analysed in this study, loss of mannitol was most likely the main reason for the decrease in carbohydrate content in the freshwater-treated sample. Whereas the main carbohydrates in the wild biomass was most likely structural cell-wall polysaccharides, such as alginate and cellulose, which are not as easily extracted and thus retained during treatment, resulting in only a minor variation in total carbohydrate content.

Seawater treatment resulted in higher nutrient retention, most likely due to lower osmotic difference between the water and seaweed tissue. However, the treatment seemed to alter the relative proportion of macro minerals and the data indicates that the relative content of K decreased, while the content of Na increased compared to the untreated sample. The seawater-treated sample had the highest content of both minerals, which was reflected in the sensory analysis where it had the highest score for saltiness. The steam-treated sample had a mean score of salty taste lower than the seawater-treated and higher than the freshwater-treated sample. This also corresponds to the mineral profiles and the content of Na, which was slightly lower than in the seawater-treated sample but higher than in the freshwater-treated sample. Furthermore, it is important to note that the perception of salty taste is dependent not solely on the Na-content but also on content of the aforementioned free amino acids, which were not analysed in this study, and other minerals such as

K (Linscott and Lim 2016; Figueroa et al. 2021) which was lowest in the freshwater-treated sample.

Nutritional benefits and hazards

The content of Na in food products is important from a public health perspective as it is closely associated to the prevalence of cardiovascular diseases as a consequence of high blood pressure (O'Halloran et al. 2016). Low sodium-to-potassium (Na/K) ratio of foods are beneficial and decrease risk of high blood pressure. The World Health Organisation (WHO) recommends a Na/K ratio below 1 to maintain cardiovascular health, which is interesting as foods like meat, bread, sauces, and condiments are often characterized by high ratios above 5 (WHO 2012). Therefore, measures to reduce intake of sodium and reduce the Na/K ratio of the diet are of high priority globally (Santos et al. 2021). Brown seaweeds, such as *S. latissima* are characterized by low Na/K ratios, often below 0.5 (Cofrades et al. 2011; Chapman et al. 2015; Stévant et al. 2018a). Although the mineral profile was affected during processing and the relative Na-content increased, the Na/K ratio of the seawater-treated sample remained low (0.7). One gram of ordinary table salt (NaCl) contains approximately 400 mg of Na, whilst 1 g of the sample with the highest final sodium content in this study, i.e., the seawater-treated sample, contains close to 80 mg Na. Thus, replacement of salt using such a seaweed ingredient has the potential to reduce the final Na-content of food products. Additionally, it would contribute with other beneficial nutrients and trace elements. Although the extent to which salt can be replaced is most likely dependent on type of application and has to be tested, this suggests that, if seaweed is processed in a manner that retains minerals and components that provides a salty taste, it can be used to obtain food products with maintained saltiness and reduced Na-content and thus a healthier mineral profile. However, it should be noted that the Na/K ratio resulting from seawater treatment in this study is low compared to those seen in more recent studies which obtained ratios up to 4 using the same time and temperature but lower biomass-to-water ratio (*unpublished results*). Despite being more efficient in reducing the iodine content, the final Na-content should be considered dependent on the main aim of inclusion. Additionally, these results imply that the processing conditions for WWT with seawater to some extent can be adapted to meet final quality requirements.

Considering the obtained iodine levels in this study, the iodine content in the cultivated untreated sample only allows for the consumption of 0.02 g (DW) to cover the AI level of 150 µg. Freshwater treatment increased this amount to 0.07 g. The same calculation for the wild untreated and seawater-treated sample results in an allowable intake level of 0.03 and 0.06 g, respectively. Considering the recommended UL of 600 µg, it would correspond to consumption of approximately

0.27 and 0.26 g of freshwater- and seawater-treated samples, respectively. For comparison, an iodine level of 346 mg kg⁻¹ DW as reported by Nielsen et al. (2020), would allow for consumption of 0.4 and 1.7 g to reach AI and UL, respectively. If added to the soup used in this study using the same basis for inclusion (i.e., contribution with 50 and 100% of UL) it would correspond to approximately 3 and 6% in the prepared product. Although the iodine content of *S. latissima* is a challenge for the food industry it is also an opportunity to provide a natural, plant-based source of iodine to population groups who are at risk of developing deficiency. Further research should focus on how seaweed ingredients safely can be used as iodine enrichment in food products or as supplements to help improve iodine status among those with insufficient dietary intake. The levels of analysed heavy metals in this study, i.e., As, Cd, Pb, Cu, and Hg, were low in all samples and below the content limits proposed for seaweed by the French Food Authorities (ANSES 2018). A tolerable weekly intake (TWI) of 2.5 µg Cd per kg bodyweight (bw) was established by EFSA (2012), corresponding to a maximum daily intake of 25 µg day⁻¹ (175 µg week⁻¹). At the highest inclusion level used in the soup in this study one portion would contribute with 0.5% of the maximal dose (0.13 µg per portion). The risk of overexposure of these elements upon consumption of these samples is therefore small.

Testing in a commercial food formulation

Including seaweed in commercial food formulations can provide several benefits e.g., iodine enrichment, salt replacement or flavour enhancement (Chapman et al. 2015). The test-product in this study aimed to investigate if there were any perceptible differences in flavour resulting from the addition of an iodine-reduced *S. latissima* ingredient to a typical commercial product (i.e., a spinach soup). The two inclusion levels (0.5 and 1%) were based on iodine intake recommendations and equal to 300 and 600 µg iodine per portion (portion size according to the manufacturer). The potential loss of iodine during cooking of the soup was not considered in this experiment. However, boiling experiments have shown that despite significant extraction of iodine from the raw material into the water, more than 90% of the initial content was accounted for in the water phase and cooked raw material (Chung et al. 2013). Thus, the prepared soup would contain most of the iodine from the seaweed, despite the heat treatment. The milk used in the preparation also contains iodine but at a relative low level (16 µg 100 g⁻¹ according to the nutrition declaration) and has a minor contribution to the total content in the final meal.

Exploring the relation between the content of iodine and salts (along with other nutrients) and the flavour contribution of *S. latissima* across treatments is the first step to optimize processing strategies. The results from the tetrad tests showed that at both 0.5 and 1% inclusion, the panellists were

able to distinguish between the soup with seawater-treated *S. latissima* and the control soup without seaweed ingredient. The panellists were given the opportunity to give comments after each test and in general the impressions of better/off-taste and more/less taste were equally distributed between the soup samples. There were no comments implying that any of the samples had a taste of sea or a marine flavour that could be coupled to the seaweed addition. However, 5 out of 16 judges reported that the soup with 1% kelp was saltier (data not shown). It should be noted that the salt content in this soup was not compensated for upon addition of kelp ingredient. However, it should be further investigated to which extent salt can be reduced and in which concentrations such an ingredient must be added to compensate without negative impact on sensory properties.

Conclusion

This study demonstrates that WWT in both freshwater and seawater significantly reduced the iodine content of *S. latissima* while steam treatment had little effect, which is compliant with results reported in earlier studies. While freshwater treatment led to a greater loss of soluble nutrients, mainly carbohydrates and minerals, seawater treatment seemed to result in a greater nutrient retention but with an alteration of the relative proportion of minerals. The difference in chemical composition was reflected in the sensory profile where the seawater-treated *S. latissima* was characterized with highest scores for saltiness and umami taste, which are properties beneficial for possible applications as a salt replacer and taste enhancer in food products. The addition of relatively small amounts of seawater-treated *S. latissima* (i.e., 0.5% and 1%) significantly altered the flavour of a commercial food product.

Despite loss of iodine during processing, the final levels obtained in this study were higher than current available recommendations. Intakes of 1 g would exceed the daily recommended upper tolerable level with close to 4 times for both freshwater- and seawater-treated samples. This is limiting a broader use of brown seaweed ingredients in the food industry, especially the addition to products likely to be consumed frequently. Further research is needed to identify processing strategies to reduce the iodine content in brown seaweed while maximizing nutrient and flavour retention. This is key for broadening the use of ingredients based on brown seaweeds in the food industry which may contribute to more sustainable food products.

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Authors' contributions Material preparation, data collection and analysis were performed by Johanna Liberg Krook, Pierrick Stévant, Arne Duinker, Wenche Emblem Larssen and Ingri Mjelde Birkeland. The first draft of the manuscript was written by Johanna Liberg Krook. Reviewing and editing of the manuscript was performed by Svein Jarle Horn, Siv Skeie, Pierrick Stévant and Arne Duinker. All authors read and approved the final manuscript.

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Data availability All data generated or analysed during this study are included in this published article.

Declarations

Competing interests Johanna Liberg Krook and Ingri Mjelde Birkeland are employed by Orkla Ocean AS, a supplier of seaweed containing products.

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