



# Iodine reduction and retention of nutrients and flavour-active compounds upon warm seawater treatment of the kelps *Alaria esculenta* and *Saccharina latissima*

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## ABSTRACT

Commercially cultivated kelp (seaweed) species represent a potential dietary source of iodine but may also put consumers at risk of excessive intakes upon frequent consumption. This study investigated warm seawater (W-SW) treatment as a simple method for reducing the iodine content of *Alaria esculenta* and *Saccharina latissima*. Iodine concentrations decreased in both kelps upon W-SW exposure at 45 °C, i.e. by 38 % and 78 %, respectively, in *A. esculenta* and *S. latissima* after 1 min and 51 % and 88 % after 2 min. Longer treatments resulted in further decrease in *A. esculenta* whereas only marginal further reduction in iodine concentrations were achieved in *S. latissima*. No reduction in iodine concentration was measured in *S. latissima* following treatment at 35 °C (not tested for *A. esculenta*). W-SW treatment at 45 °C induced loss of biomass in both kelps although the total retention was notably higher in *A. esculenta* compared to *S. latissima*. Among the analysed macronutrients, potassium and mannitol were associated with the lowest retentions. Losses of micronutrients (incl. vitamin B1 and B9) and trace elements were also measured in both kelps. The retention of free glutamate was high in both species suggesting that W-SW exposure does not negatively affect the umami potential of the final ingredients for food. Based on portions contributing 600 µg iodine, W-SW-treated *A. esculenta* was more nutritious than a comparable ingredient from *S. latissima*, with nutritionally relevant contribution (> 15 % of dietary reference intake (DRI)) per portion (4.6 g dry *A. esculenta* vs. 1.2 g *S. latissima*) of sodium, and notable contributions (> 5 % of DRI) of other minerals (calcium, magnesium and potassium) and vitamin B9. Short W-SW treatment is a simple approach that could be implemented in commercial kelp production and contribute to reducing the risk of excessive dietary iodine exposure from seaweed consumption.

## 1. Introduction

Strategies to meet the increasing demand for producing safe and nutritious food in a sustainable way point towards value chains favouring renewable resources and reducing environmental impacts including carbon emissions, freshwater use and decreased biodiversity. The environmental footprint of animal-based food products typically exceeds that of vegetable substitutes, highlighting the importance of a dietary change, particularly in developed countries [1]. In this context, the aquaculture of low-trophic primary biomass of seaweed (i.e.

macroalgae) is considered a part of the solution for the sustainable production of food and animal feed [2,3]. Aquaculture of seaweeds, primarily of the kelp species *Alaria esculenta* and *Saccharina latissima*, is an emerging industry in Europe, aiming to supply biomass to multiple commercial applications [2,4,5].

Seaweeds are traditional food items in Asia but have been largely overlooked in Western societies. Their use in industrial applications including health-promoting and flavour ingredients in the food industry has gained increasing interest over the past decades. Seaweeds are recognized as a source of bioactive compounds with applications in

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human nutrition such as functional ingredients (e.g. for salt replacement) and dietary supplements [6,7]. Besides, a wide range of edible species are recognized for their flavours (e.g. umami) and the potential to enhance the palatability of food dishes to which they are added [8,9]. Although kelp biomass from commercial cultivation is becoming increasingly available, the European food industry has not yet incorporated this resource in large-scale food applications. One major bottleneck to a broader use of kelps as food ingredients is related to food safety concerns to potentially toxic elements (PTEs).

Seaweeds may accumulate PTEs with negative effects on human health. Both non-essential (e.g. cadmium and inorganic arsenic) and essential elements (e.g. iodine), if occurring in high amounts, may cause problems when using seaweeds in large-scale food applications. In the case of edible kelps such as *S. latissima* and *A. esculenta*, high levels of iodine are identified as a potential food safety hazard which can limit their inclusion into commercial food formulations, due to the risk of excessive iodine exposure [10–12]. Optimal iodine intake is important for foetal development, development of brain and motoric skills in young children and for metabolic regulation. Iodine deficiency is a recognized problem globally and particularly among the European population [13,14]. Thus, seaweeds, especially edible kelps, could represent a dietary source of iodine which is relevant in a public health perspective. However, excessive iodine intakes, through e.g. the daily consumption of dried kelp, may affect the thyroid function, potentially resulting in hypo- or hyperthyroidism [15,16]. Currently, there are no regulatory limits in Europe for several of the relevant PTEs in seaweeds used as food, but the European Food Safety Authority (EFSA) has recently assessed the dietary exposure to iodine and heavy metals from seaweeds in EU to support the European Commission in establishing adapted future regulations [17]. The French Food Safety Agency has recommended a maximum concentration in the seaweed biomass of 2000 mg iodine per kg dry weight (DW) [18] that national food authorities use as guideline. Regarding the daily recommended intake of iodine, EFSA specified upper intake level (UL) of 600  $\mu\text{g day}^{-1}$  [19].

Food processing techniques have the potential to minimize food safety risks. Various studies have reported a drastic reduction of the iodine content of kelps (over 90 %) by heat treatment with fresh water [20–23]. However, this process is also associated with losses of nutrients (mainly minerals) [22,23] and flavour intensity [24]. Preliminary results suggest that heat treatment using seawater provide a better retention of nutrient and flavour-active compounds compared to similar treatments using fresh water, most likely due to the lower osmotic potential between the water and kelp tissue [24].

The objective of the present study is to further investigate the process parameters (i.e. time and temperature) of warm seawater (W-SW) treatment to lower the iodine concentration of *S. latissima* and *A. esculenta*. The characterization of the nutrient profile, levels of PTEs and free amino acids (FAAs, as major flavour-active compounds) in the kelps throughout the process served as a starting point to discuss the applicability of this processing method and resulting products to large-scale food applications.

## 2. Material and methods

### 2.1. Raw material

Wild *A. esculenta* was harvested from free diving at Øygarden, Norway (60°26'N, 4°56'E) on April 5, 2022, then immediately transported at Arctic Seaweed's facility and stored in ambient seawater in tanks. *Saccharina latissima* was cultivated on ropes at Tango Seaweed in Herøy, Norway (62°18'N, 5°44'E). The biomass was harvested on May 9, 2022, by cutting the ropes from the cultivation rig with kelp fronds attached to, transferred to an insulated tank and transported to Møreforsking's laboratory facilities within <2 h, where it was stored in running seawater at 8 °C.

Kelp fronds were sorted beforehand so that the pool of biomass used

in the experiment was relatively homogenous in size. Individual kelp frond size from subsamples is summarized in Table 1. Fronds covered with any visible epiphytes were discarded.

### 2.2. Warm seawater (W-SW) treatments

W-SW treatments were performed on *A. esculenta* and *S. latissima* at Arctic Seaweed AS and Møreforsking laboratory facilities respectively. The treatments were conducted in 40 L, temperature-regulated brewing tanks (DigiBoil 65, KegLand, Noble Park, Australia). For each replicate treatment, the tank was filled with 40 L of ambient seawater which was then heated to either 35 or 45 °C. Five nets were filled with 0.5 kg of whole kelp fronds (including stipes) and subsequently immersed in the tank at the beginning of the treatment. The total wet weight (WW) of the biomass in each net was registered before immersion. Unprocessed samples served as control. The nets were gently rotated in the tanks during treatments. One and one nets were sampled after 10 s and 1-, 2-, 4- and 8-min exposure to W-SW. After sampling, kelp fronds hung to drip for 5 min and the total WW in each net was registered again. The samples were then vacuum-packed and stored frozen at -20 °C until freeze-drying or cryo-milling for chemical analysis. The DW of the samples was determined gravimetrically as the residue remaining after freeze-drying. Water samples were also taken before adding kelp and after 2 and 8 min. The seawater used in this experiment (i.e. from both sites) was analysed for minerals and trace elements before treatment.

*Alaria esculenta* was only exposed to seawater at 45 °C ( $\pm 1$  °C) while *S. latissima* was treated at 35 and 45 °C. Each treatment was performed in 3 replicates. The experimental design including W-SW treatments and sampling is illustrated in Fig. 1.

### 2.3. Chemical analyses

#### 2.3.1. Iodine analysis

The iodine content of the samples was quantified at the Institute of Marine Research using inductively coupled plasma-mass spectrometry (ICP-MS) according to the method described by Dahl et al. [25]. Freeze-dried samples were added 1 mL tetramethylammonium hydroxide (TMAH) and 5 mL deionized water before extraction at  $90 \pm 3$  °C for 3 h. The samples were then diluted and centrifuged. Prior to quantification, the samples were filtered through a 0.45  $\mu\text{m}$  single use syringe and disposal filter. Tellurium was used as an internal standard to correct instrument drift.

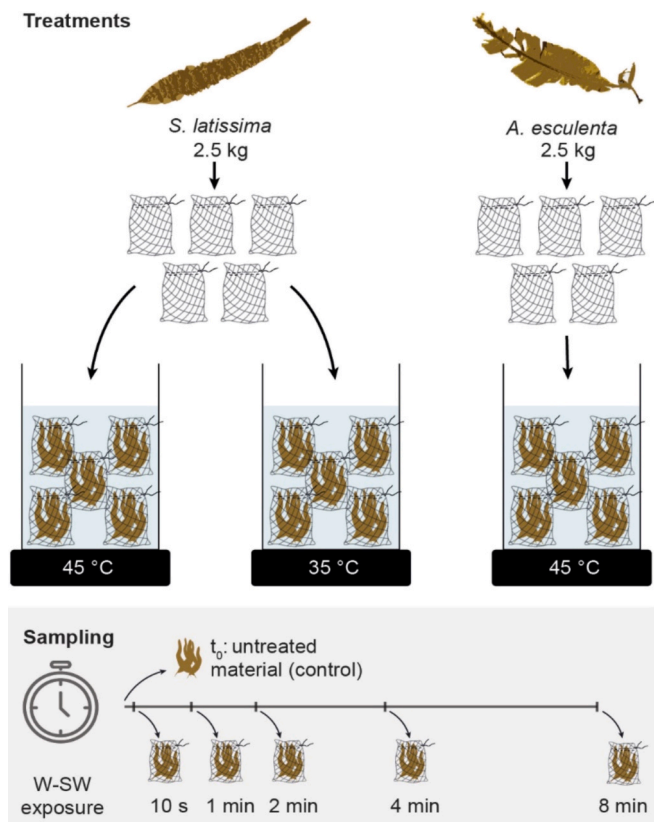
#### 2.3.2. Determination of macro-, micro- and trace elements

Macrominerals (sodium (Na), potassium (K), magnesium (Mg), calcium (Ca), phosphorus (P)), microminerals (arsenic (As), copper (Cu), iron (Fe), manganese (Mn), zinc (Zn) and trace elements (silver (Ag), cadmium (Cd), chromium (Cr), mercury (Hg), nickel (Ni), lead (Pb), selenium (Se), vanadium (V)) were determined at the IMR in freeze-dried samples by ISO accredited methods according to Moxness Reksten et al. [26]. The elements were determined by ICP-MS after acid wet digestion in a microwave oven according to the method principles described by Julshamn et al. [27] using an external calibration curve

**Table 1**

Frond size of a representative pool of *Alaria esculenta* and *Saccharina latissima* individuals used in warm seawater (W-SW) treatments.

Species	Length (cm)	Width (cm)
<i>A. esculenta</i> (n = 30)	min = 105.0	min = 4.5
	mean = 162.6	mean = 7.1
	st.dev. = 42.2	st.dev. = 1.3
	max = 285.0	max = 10.6
<i>S. latissima</i> (n = 27)	min = 54.0	min = 6.0
	mean = 86.8	mean = 11.37
	st.dev. = 24.8	st.dev. = 3.8
	max = 139.0	max = 20.0



**Fig. 1.** Experimental design to study the iodine reduction and retention of nutrients and free amino acids upon warm seawater (W-SW) exposure of the kelps *Saccharina latissima* and *Alaria esculenta*. Treatments were conducted in 40 L temperature-regulated tanks. Each treatment was repeated thrice, providing replication for exposure at different durations and temperature.

with internal standardisation.

### 2.3.3. Free amino acids (FAAs)

FAA analysis was performed using an ultra-performance liquid chromatography (UPLC) system (Waters China Ltd., Hong Kong, China) with the AccQTag kit from Waters. Freeze-dried sample (0.5 g) was transferred to a pyrex tube with a screw cap, together with 100  $\mu$ L internal standard (norvaline 2,5 mM in 0,1 M HCl), 900  $\mu$ L of 0,1 M HCl and 9 mL MQ water. The solution was vortex-homogenized for 1 min and flushed with nitrogen before the cap was screwed on. Samples were kept at 90 °C for 2 h. The solution was cooled and filtered through a 0.45  $\mu$ m syringe filter, before derivatization using the AccQTag kit. The following were added, in sequence, in UPLC sample vials: 70  $\mu$ L AccQTag Ultra borate buffer, 10  $\mu$ L sample extracts and 20  $\mu$ L AccQTag Ultra reagent. Vials were vortexed for 5 s and placed in a 55 °C heating block for 10 min, then cooled and analysed on a Waters AccQTag Ultra Column 2.1  $\times$  100 mm according to the manufacturer's UPLC program. Results are determined using a one-point standard curve for each of the amino acids.

### 2.3.4. Water-soluble carbohydrates (WSCs)

WSCs were determined by hydrolysis of freeze-dried solid samples (only untreated and 4 min W-SW exposed) at high temperature and strong acid to quantify the monomers. 0.2 g of sample was added in a glass bottle with 2 mL 72 % w/w sulfuric acid then incubated for one hour at room temperature followed by a dilution to 4 % w/w sulfuric acid. The diluted sample was then heated to 120 °C for 40 min in an autoclave. The solution was neutralised to a pH between 3.0 and 10.0 before filtration through a 0.22  $\mu$ m nylon filter. High-performance

anion-exchange chromatography with pulsed amperometric detection (HPAE-PAD) was performed on a Dionex ICS3000 (Thermo Scientific, USA) with a CarboPac MA10 column to quantify the monomers. A 10  $\mu$ L sample was injected in an isocratic mobile phase consisting of 0.5 M NaOH in milliQ water. Standards of fucose, mannitol and glucose were used to establish a calibration curve. Monosaccharides were corrected for the addition of water when glycosidic linkages were hydrolyzed, to express the result on a DW basis.

### 2.3.5. Vitamins

Vitamins were analysed after homogenising wet, frozen samples in a cryogenic grinder cooled with liquid nitrogen (CG-500 Freezer/Mill®, Cole-Parmer Ltd, USA), and freeze-dried DW percentages were used for calculations of concentrations relatively to the samples' DW. Thiamine (vitamin B1) was determined at the IMR using high performance liquid chromatography (HPLC) with fluorescence detector (FLD) following acid extraction of the samples, hydrolysis and enzyme treatment, as described by Moxness et al. [26]. Ascorbic acid (vitamin C) was determined at the IMR by HPLC with an electrochemical detector following the protocol described by Mæland and Waagbø [28]. Folic acid (vitamin B9) was measured at the IMR by a validated HPLC-tandem mass spectrometry (HPLC-MS/MS) method adapted from standard protocols [29–31]. Folate concentration was calculated as the sum of the six vitamers (folic acid equivalents): tetrahydrofolate (THF), 5-methyltetrahydrofolate (5-CH<sub>3</sub>-THF), 5,10-methenyltetrahydrofolate (5,10-CH = THF), 10-formylfolic acid (10-CHO-FA), 5-formyltetrahydrofolate (5-CHO-THF), and folic acid (FA).

### 2.4. Retention factors

The total retention (TR) of biomass was calculated using eq. 1 considering the variation in WW and DW during W-SW exposure (at treatment time  $t$ ) compared to before treatment ( $t_0$ ).

$$TR = \frac{WW(t) \times DW(t)}{WW(t_0) \times DW(t_0)} \quad (1)$$

Retention factors (RFs) of individual nutritional compounds were computed using eq. 2, where  $X$  is the concentration of a specific compound (expressed as part of the DW) in a sample at  $t_0$  and during treatment ( $t$ ).

$$RF = \frac{WW(t) \times X(t)}{WW(t_0) \times X(t_0)} \quad (2)$$

### 2.5. Comparison of iodine and PTEs data to toxicological guideline values and nutrient contribution

The obtained results for the iodine content of untreated and W-SW-exposed kelp were compared to dietary reference values for iodine intake. The value of 150  $\mu$ g day<sup>-1</sup> provided by EFSA [32] for adequate iodine intake (AI) for adults was used to calculate the amount of kelp (i. e. portion) that can be included in a food matrix to reach this limit. Likewise, 600  $\mu$ g day<sup>-1</sup> for adults (or 8.5  $\mu$ g (kg body weight (bw))<sup>-1</sup> day<sup>-1</sup>) was used to calculate the portion to reach the tolerable upper intake level (UL) for adults [33]. The nutrient contribution of W-SW-exposed kelp at 45 °C for 1 min and 4 min was estimated by comparing the amount of individual nutrients from portions of kelp ingredient to dietary reference intakes (DRI) established by the European parliament and the council of the European Union [34]. The toxicological risk was estimated by comparing dietary exposure levels of Cd and Hg from kelp samples (calculated from measured concentrations) to toxicological guideline values established by EFSA for each element [35,36]. No toxicological guideline has been established for Pb and calculation of the margin of exposure (MoE) based on benchmark doses (lower confidence limit) (BMDLs) for different endpoints is necessary for assessing the risks from dietary exposure [37]. The MoE is calculated following eq. 3 where

EED is the estimated exposure dose from consuming seaweeds. Both BMDL and EED are expressed in  $\mu\text{g} (\text{kg bw})^{-1} \text{day}^{-1}$ .

$$MoE = \frac{BMDL}{EED} \quad (3)$$

### 2.6. Data analysis

The statistical analysis of the results was performed using R (version 4.2.2, [38]). The results from the analysis of replicate samples were described as mean  $\pm$  standard deviation. For each kelp species and treatment (i.e. temperature), The main effect of W-SW treatment i.e. up to 8 min on the content of individual nutrients and FAAs were analysed by repeated measures analysis of variance (RM ANOVA, R function lmer, [39]), considering samples from replicate treatments as random factor. The fitted linear mixed effect model is summarized by the following formula:

$$\text{Variable} \sim \text{Treatment time} + (1|\text{repl.}) \quad (4)$$

The assumptions of homogeneity of variances and normal distribution were verified visually by inspecting residual and Q-Q plots respectively. The obtained *p*-values were adjusted using the Benjamini-Hochberg procedure to control for the false discovery under multiple testing. Post-hoc pairwise comparisons of Least Square Means (R function lsmeans, [40]) were performed following significant ANOVA results.

## 3. Results

### 3.1. Iodine content following warm seawater (W-SW) treatment

The iodine content of solid kelp samples, expressed as part of the freeze-dried dry weight (DW) of the samples, as well as water samples from W-SW treatment of *S. latissima* were analysed. The initial iodine content was higher in samples of cultivated *S. latissima* ( $5700 \pm 660 \text{ mg kg}^{-1} \text{ DW}$ ) compared to the samples of wild *A. esculenta* ( $510 \pm 45 \text{ mg kg}^{-1} \text{ DW}$ ) (Fig. 2). Considering W-SW treatments at  $45^\circ\text{C}$ , a significantly lower iodine content was achieved in *S. latissima* after 10 s compared to initial levels (LSmeans,  $p = 0.005$ ) while the earliest significantly lower value was measured at 1 min in *A. esculenta* (LSmeans,  $p = 0.003$ ). In *S. latissima*, the iodine concentration was  $1200 \pm 420$  and  $690 \pm 150 \text{ mg kg}^{-1} \text{ DW}$  after 1- and 2-min treatment respectively (i.e. 78 % and 88 % decrease from the initial level) and did not further decrease significantly following prolonged treatment (LSmeans,  $p > 0.960$ ). In *A. esculenta*, the decrease in iodine concentration was 51 % of initial levels after 2 min, and 88 % decrease was achieved after 8 min. W-SW treatment at  $35^\circ\text{C}$  did not significantly affect the iodine concentration of *S. latissima* (RM ANOVA  $F_{5, 12} = 0.85$ ,  $p = 0.660$ ). The background concentration of iodine in the seawater used for W-SW treatment of *S. latissima* was  $0.13 \pm 0.058 \text{ mg L}^{-1}$  (suppl. material S1). The concentrations measured in water samples from these treatments mirror the results described above i.e. low iodine concentrations were found in water samples from  $35^\circ\text{C}$ -treatments (max  $1.1 \pm 0.52 \text{ mg L}^{-1}$ ) compared to those from  $45^\circ\text{C}$ -treatments (max.  $20 \pm 2.0 \text{ mg L}^{-1}$ ) (suppl. material S1).

### 3.2. Nutrient and PTE concentrations and retention

The nutrient profile of *A. esculenta* and *S. latissima* including minerals, WSCs, and vitamins (B1, B9 and C) was analysed before and during W-SW treatment up to 8 min. Table 2 provides an overview of initial levels of individual nutrients in both kelps prior to W-SW exposure. The dry matter content of *A. esculenta* was almost twice that of *S. latissima*. Other notable differences between the two kelps include a higher K content in *S. latissima* as well as a two-fold higher WSC level compared to *A. esculenta*. Considering PTEs, comparable levels of As

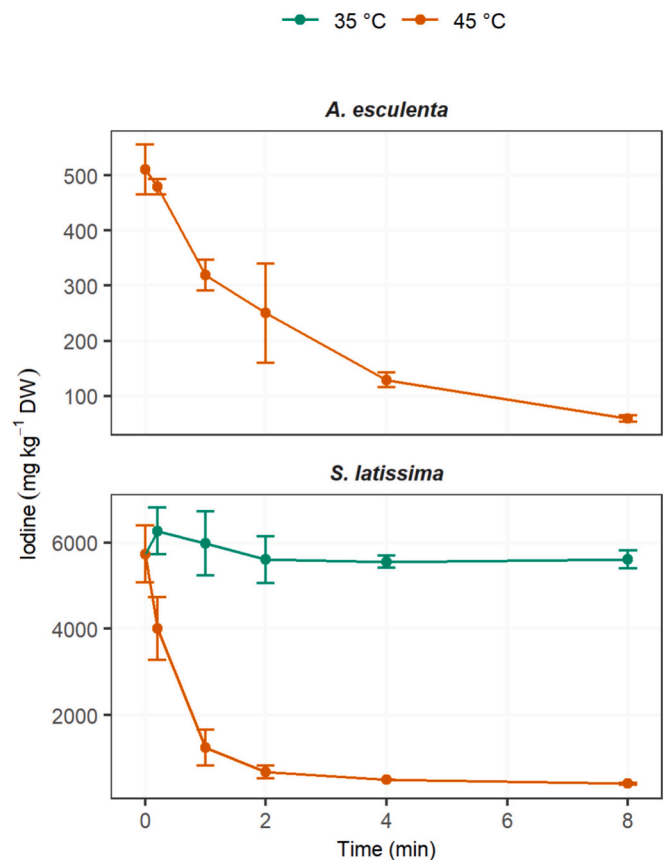


Fig. 2. Iodine concentration of *Alaria esculenta* and *Saccharina latissima* during warm seawater (W-SW) treatments. Values are given as mean  $\pm$  st. dev ( $n = 3$ ).

Table 2

Nutrient concentration of *Alaria esculenta* and *Saccharina latissima* prior to warm seawater (W-SW) treatment. Values are given as mean  $\pm$  st. dev ( $n = 3$ ).

Variable	Unit	<i>A. esculenta</i>	<i>S. latissima</i>
DW	% WW	16 $\pm$ 0.7	9 $\pm$ 1.0
<b>Minerals</b>			
I	mg kg <sup>-1</sup> DW	510 $\pm$ 45	5700 $\pm$ 660
Na	mg g <sup>-1</sup> DW	49 $\pm$ 1.5	50 $\pm$ 4.2
K	mg g <sup>-1</sup> DW	51 $\pm$ 6.3	100 $\pm$ 10
Mg	mg g <sup>-1</sup> DW	9.4 $\pm$ 0.15	7.4 $\pm$ 0.083
Ca	mg g <sup>-1</sup> DW	17 $\pm$ 0.78	7.4 $\pm$ 0.22
P	mg g <sup>-1</sup> DW	4.2 $\pm$ 0.18	0.98 $\pm$ 0.057
As	mg kg <sup>-1</sup> DW	45 $\pm$ 1.4	52 $\pm$ 4.6
Cu	mg kg <sup>-1</sup> DW	1.3 $\pm$ 0.34	4.1 $\pm$ 2.7
Fe	mg kg <sup>-1</sup> DW	50 $\pm$ 7.4	33 $\pm$ 4.8
Mn	mg kg <sup>-1</sup> DW	6.2 $\pm$ 0.17	3.7 $\pm$ 0.55
Zn	mg kg <sup>-1</sup> DW	57 $\pm$ 7.2	< LOQ
Ag	mg kg <sup>-1</sup> DW	< LOQ	0.053 $\pm$ 0.025
Cd	mg kg <sup>-1</sup> DW	1.5 $\pm$ 0.23	0.26 $\pm$ 0.025
Cr	mg kg <sup>-1</sup> DW	0.59 $\pm$ 0.15	0.49 $\pm$ 0.035
Hg	mg kg <sup>-1</sup> DW	< LOQ	0.010 $\pm$ 0.000
Ni	mg kg <sup>-1</sup> DW	< LOQ	0.37 $\pm$ 0.062
Pb	mg kg <sup>-1</sup> DW	0.34 $\pm$ 0.072	0.27 $\pm$ 0.17
Se	mg kg <sup>-1</sup> DW	0.083 $\pm$ 0.040	< LOQ
V	mg kg <sup>-1</sup> DW	0.29 $\pm$ 0.040	< LOQ
<b>WSC</b>			
Fucose	g (100 g) <sup>-1</sup> DW	0.66 $\pm$ 0.049	1.2 $\pm$ 0.031
Mannitol	g (100 g) <sup>-1</sup> DW	8.9 $\pm$ 1.1	18.5 $\pm$ 1.1
Glucose	g (100 g) <sup>-1</sup> DW	3.4 $\pm$ 0.13	6.1 $\pm$ 0.21
<b>Vitamins</b>			
Vit. B1 (Thiamine)	mg kg <sup>-1</sup> DW	4.0 $\pm$ 0.18	2.6 $\pm$ 0.29
Vit. B9 (Folic acid)	mg kg <sup>-1</sup> DW	9.4 $\pm$ 0.55	2.4 $\pm$ 0.039
Vit. C (Ascorbic acid)	mg kg <sup>-1</sup> DW	110 $\pm$ 10	92 $\pm$ 20

Abbreviations: dry weight (DW); wet weight (WW); water-soluble carbohydrates (WSC); Limit of quantification (LOQ).

were measured in both species while higher Cd levels were found in *A. esculenta*. Low Pb levels were detected in both species and traces of Hg were measured in *S. latissima* only. Both thiamine (vitamin B1) and folic acid (vitamin B9) levels of *A. esculenta* were superior to that of *S. latissima*.

Kelp may undergo changes in composition during W-SW treatment which can be the results of i) biomass (i.e. DW) loss, ii) uptake or release of water and iii) uptake of elements present in the seawater. The TR and RFs of individual nutritional compounds were calculated to account for the variations in both WW and DW of the samples during processing. The results are presented in Fig. 3 and Fig. 4 respectively. The summary of the main effect of W-SW treatments on the level of individual nutrients by RM ANOVA is available as suppl. material (S2) as well as the DW (S3) and content of the nutrients expressed as part of the samples' DW (S4–6). A higher TR (i.e. retention of DW) was observed in *A. esculenta* compared to *S. latissima* following exposure to W-SW at 45 °C (Fig. 3). After 1 min exposure at this temperature, the TR was  $0.84 \pm 0.021$  and  $0.57 \pm 0.15$  in *A. esculenta* and *S. latissima* respectively, and  $0.73 \pm 0.030$  and  $0.42 \pm 0.085$  respectively after 8 min. The TR tended to stabilize after 2 min W-SW treatments in both species. The DW of *A. esculenta* samples did not vary significantly during treatment (RM ANOVA,  $F_{5, 12} = 2.2, p = 0.165$ ) as opposed to the DW of *S. latissima* treated at 45 °C (RM ANOVA,  $F_{5, 12} = 4.1, p = 0.033$ ) (suppl. material S2–3). The TR of *S. latissima* exposed to W-SW at 35 °C remained stable and approx.1.0 throughout the treatment (Fig. 3).

Among the analysed macronutrients of the kelps, K and mannitol were the compounds associated with the lowest retention (Fig. 4). The RF of K was as low as 0.1 in *S. latissima* after 4 min W-SW treatment at 45 °C corresponding to  $18 \pm 1.1 \text{ mg g}^{-1}$  DW i.e. approximately 18 % of the initial concentration. The lowest K retention in *A. esculenta* was

observed after 8 min 45 °C-W-SW exposure (RF = 0.4), corresponding to  $32 \pm 2.9 \text{ mg g}^{-1}$  DW i.e. 62 % of the initial concentration. Comparable mannitol concentrations were measured in both kelps after 4 min treatment at 45 °C (suppl. material S7) despite a higher initial concentration in *S. latissima* compared to *A. esculenta* reflecting the low retention for this compound in the former species. Other macro-elements including Na, Mg, Ca as well as the carbohydrates fucose and glucose were well retained or increased, likely due to uptake from seawater (in the case of Na and Mg). Analysis of the seawater prior to treatment of *S. latissima* revealed a higher concentration of Na and Mg in seawater compared to the kelp (by a factor of 2.4 and 1.7 respectively) whereas K, Ca and P were more concentrated in the kelp (by a factor of 26, 1.9 and 24 respectively, suppl. material S8). The Na/K ratio increases more rapidly in *S. latissima* compared to *A. esculenta* (suppl. material S9) following greater Na uptake and K loss in the former kelp species.

Among micronutrients and trace elements, iodine was associated with the lowest retention in both species. Initial Cu concentrations were comparable in the two studied kelps but increased in *S. latissima* while it remained stable in *A. esculenta* (suppl. material S5). This is reflected by RFs for this element comprised between 0.6 and 0.7 in *A. esculenta* and consistently above 1.0 in *S. latissima* upon W-SW exposure at both 35 and 45 °C. The retention of Mn was low compared to other microelements in *A. esculenta*, in which initial concentrations were reduced by half after 4 min treatment. The retention of Cr in *A. esculenta* and Ag in *S. latissima* (both exposed to 35 and 45 °C W-SW) was low ( $\leq 0.4$ ) throughout treatments. Considering PTEs, the As content of both species and associated RFs decreased linearly during treatment at 45 °C (Fig. 4 and suppl. material S5), whereas the Cd and Pb levels did not vary significantly (suppl. material S2). After 1 min W-SW exposure at 45 °C the retention of thiamine (vitamin B1) was high (0.8) in both species while the retention of folic acid (vitamin B9) was higher in *A. esculenta* than in *S. latissima*. After 4 min at this temperature, the retention of vitamin B1 and B9 was quite similar in both kelps with a maximum of 50 % of initial levels measured in the samples (suppl. material S10). The levels of ascorbic acid (vitamin C) measured in *A. esculenta*, were approximately half of the initial levels after 4 min treatment (i.e.  $57 \text{ mg kg}^{-1}$  vs.  $110 \text{ mg kg}^{-1}$  initially) whereas concentrations in *S. latissima* were below limit of quantification. W-SW treatments for 1 min led to a generally high retention of minerals and vitamins, higher in *A. esculenta* compared to *S. latissima*.

### 3.3. Free amino acid content and retention

The FAA concentration of fresh kelp samples prior to and during W-SW treatment was analysed. The original FAA profile of each kelp prior to treatment is presented in Fig. 5. The RFs of individual and total FAAs were computed and are summarized in Fig. 6. The results for the main effect of W-SW treatments on FAAs from RM ANOVA is given as suppl. material (S11) along with the concentrations of total FAAs and selected most dominant FAAs for each species during treatment (S12–13).

The total FAA content was  $4.7 \pm 0.10 \text{ mg g}^{-1}$  in *A. esculenta* and  $10 \pm 1.5 \text{ mg g}^{-1}$  in *S. latissima*. The FAA profile of *A. esculenta* was dominated by alanine (55 % of the total). Glutamate and aspartate represented 8 and 6 % of the total (Fig. 5). In contrast, the FAA profile of *S. latissima* was more evenly distributed across several FAAs, tryptophan accounting for 16 % of the total, followed by phenylalanine (14 %) and alanine (14 %). Aspartate and glutamate accounted for 8 and 6 % of the total FAA respectively.

Considering W-SW exposure at 45 °C, the retention of FAAs was generally higher in *A. esculenta* compared to *S. latissima* (Fig. 6). After 1 min treatment, the total FAA content was comparable in both kelps i.e.  $4.1 \pm 0.31$  and  $3.8 \pm 0.88 \text{ mg g}^{-1}$  DW in *A. esculenta* and *S. latissima* respectively (suppl. material S12–S13). Glutamate was associated with high RFs in both species compared to other abundant FAAs (e.g. alanine). Some minor FAAs, e.g. glycine and isoleucine, were associated with relatively high RFs compared to others. The W-SW treatment of

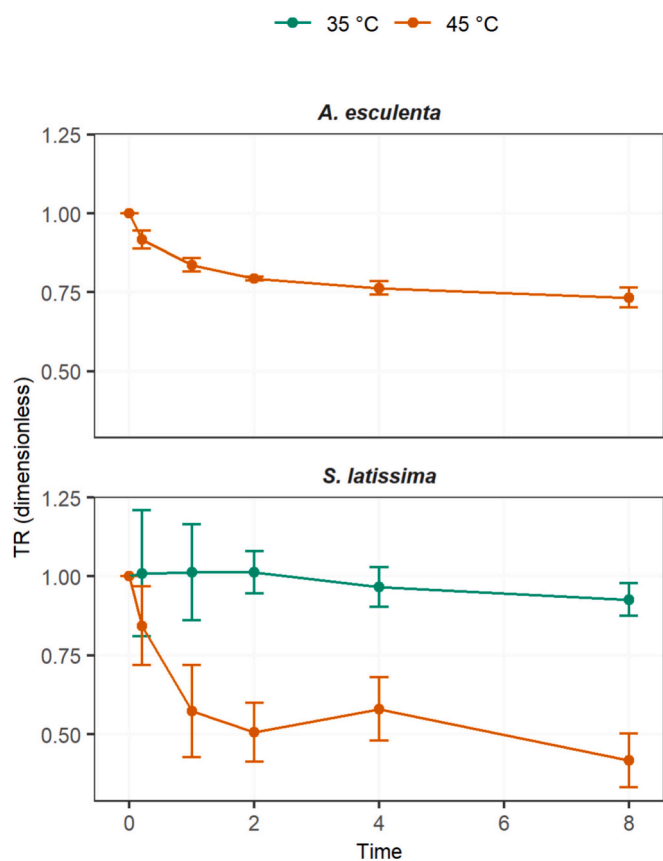


Fig. 3. Total retention (TR) of dry weight in *Alaria esculenta* and *Saccharina latissima* during warm seawater (W-SW) treatment. Values are given as mean  $\pm$  st. dev ( $n = 3$ ).

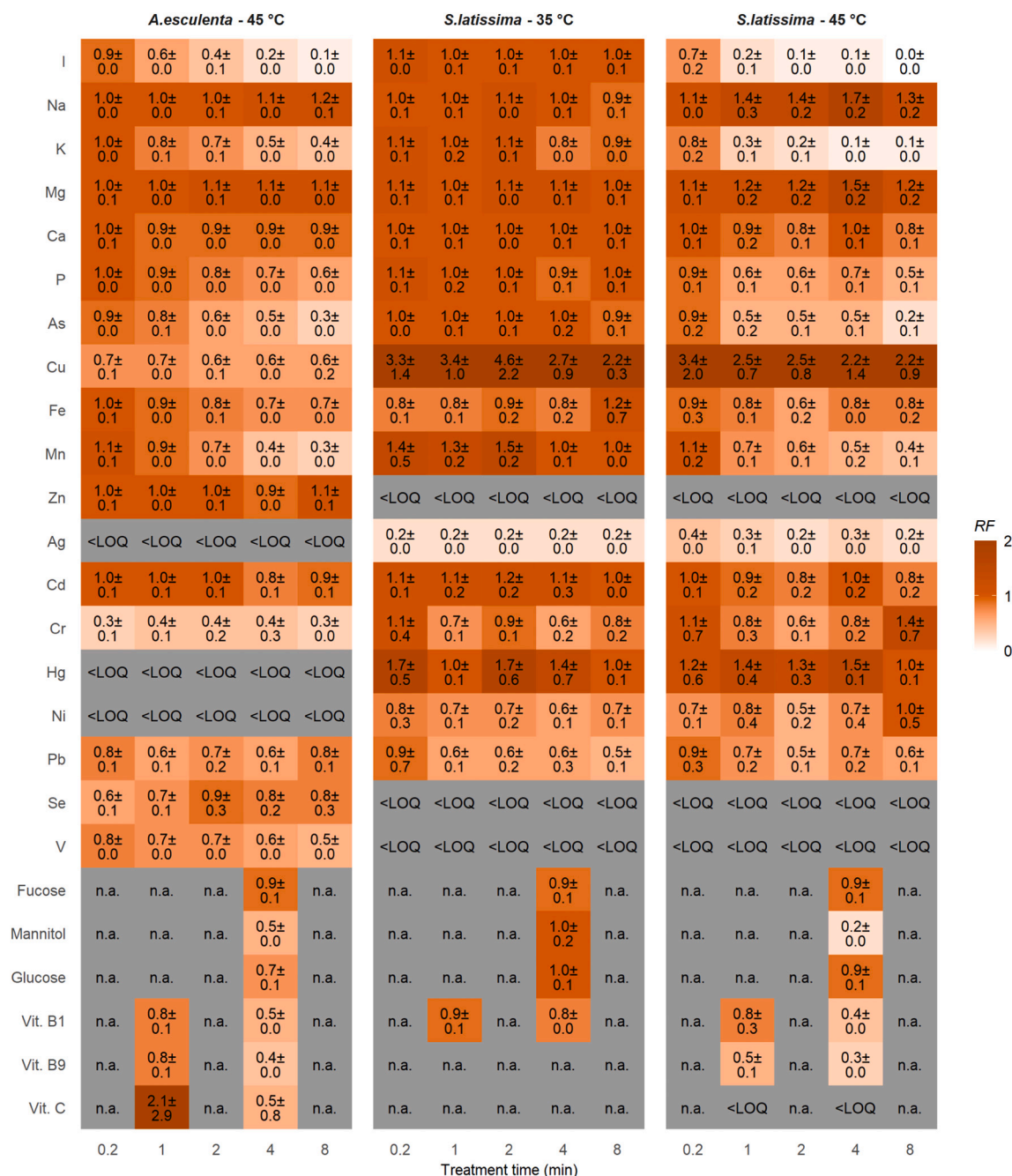


Fig. 4. Retention factors (RFs) of nutritional compounds of *Alaria esculenta* and *Saccharina latissima* during warm seawater (W-SW) treatment at 35 and 45 °C. Values are given as mean ± st. dev (n = 3). Abbreviations: limit of quantification (LOQ); not analysed (n.a.).

*S. latissima* at 35 °C did not result in considerable losses of FAAs reflected by high RFs across the main FAAs (Fig. 6). It should be noted that a two-fold increase in glutamate levels was measured in *S. latissima* samples exposed to W-SW at 35 °C during 1 and 4 min (suppl. material S13).

### 3.4. Food safety and nutritional contribution

W-SW treatment reduced the iodine concentration (690 mg kg<sup>-1</sup> DW) of *S. latissima* below the recommended French guideline for food (2000 mg kg<sup>-1</sup> DW) within 1 min at 45 °C, but not at 35 °C. *Alaria esculenta* was initially (before treatment) below that recommendation. Based on the measured iodine concentrations of *A. esculenta* and *S. latissima* samples in this study, the amounts of the corresponding kelp ingredient to cover the AI and UL values for iodine exposure were

calculated. Table 3 shows that very limited amounts of *S. latissima*, both untreated and W-SW-treated (at 45 °C) may be consumed to cover the AI and UL for iodine, although treatment for 1 min allows using 5 times more material to cover these values compared to untreated samples. Using *A. esculenta* from a similar treatment allows more kelp to be included in a food matrix to cover the AI and UL due to lower iodine concentration. Following 1 min W-SW treatment at 45 °C, 1.9 g and 0.50 g of *A. esculenta* and *S. latissima* respectively covers the UL for daily iodine intake while 4 min treatment allows intake of 4.6 and 1.2 g of each species respectively.

The mineral and vitamin contribution of W-SW-treated *A. esculenta* (ingredients A and B) and *S. latissima* (ingredients C and D) was calculated and compared to DRIs for each individual nutrient of interest (Table 4). The contributions were calculated in a scenario where iodine

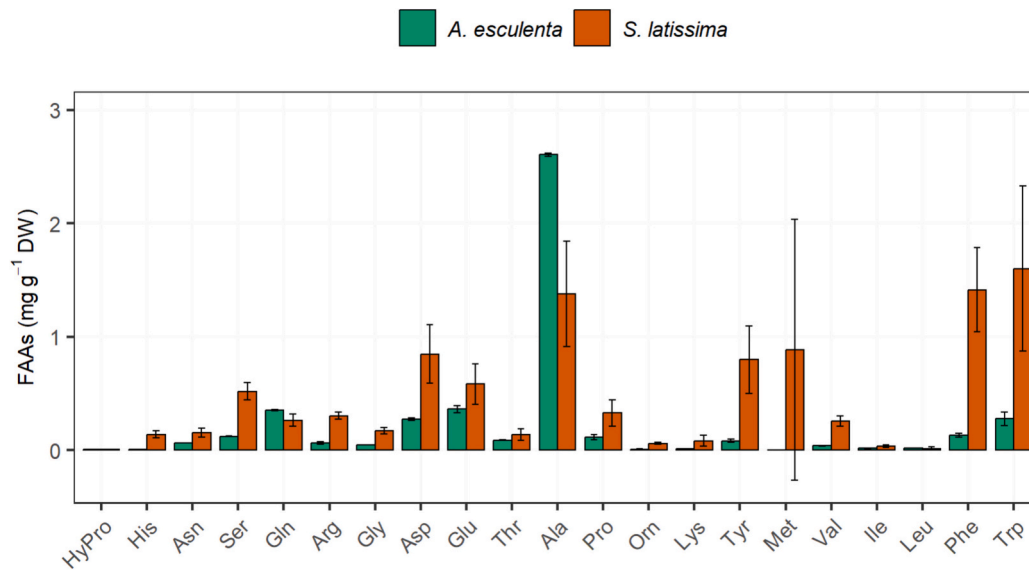


Fig. 5. Free amino acid (FAA) profile of *Alaria esculenta* and *Saccharina latissima* prior to warm seawater (W-SW) treatment. Values are given as mean  $\pm$  st. dev ( $n = 3$ ).

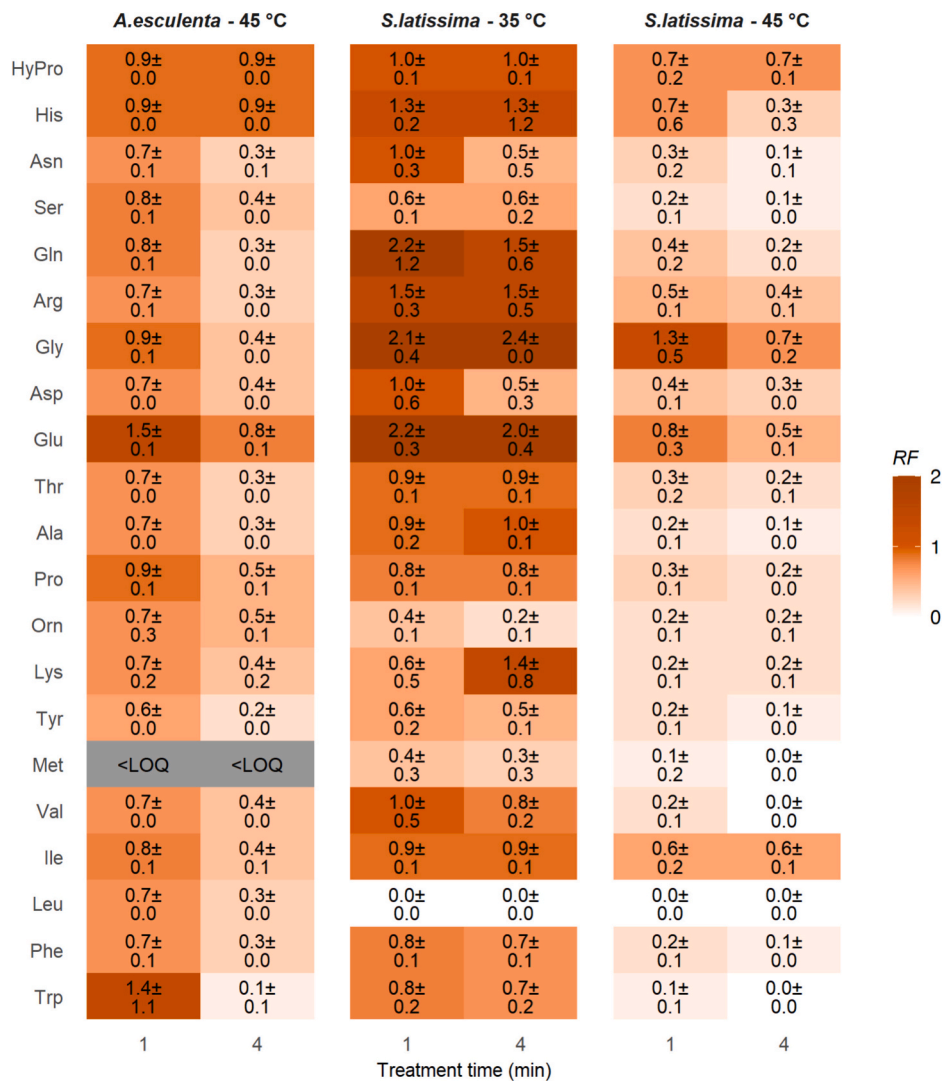


Fig. 6. Retention factors (RFs) of free amino acids of *Alaria esculenta* and *Saccharina latissima* during warm seawater (W-SW) treatment at 35 and 45 °C. Values are given as mean  $\pm$  st. dev ( $n = 3$ ). Abbreviations: limit of quantification (LOQ).

is a limiting factor for the inclusion of kelp in food i.e. the amount of kelp ingredient (A – D) providing a maximum intake of 600 µg iodine per day. Despite relatively similar mineral profiles of W-SW-exposed kelp, higher allowed portions of *A. esculenta* (due to lower iodine levels) makes higher mineral contributions than comparable ingredients from *S. latissima* (i.e. A vs. C and B vs. D). However, the highest portion of W-SW-treated *A. esculenta* (4.6 g of B) only makes a nutritionally relevant dietary contribution (> 15 % of DRI) of Na, which is not lacking in the diet for most parts of the world. Other notable contributions (≥10 % of DRI) include Ca (10 %), Mg (15 %) and vitamin B9 (10 %). A portion of W-SW (45 °C)-treated *S. latissima* for 4 min (1.2 g of D) only makes a significant contribution of Na (23 % of DRI). The contribution of other minerals and vitamins from portions of *S. latissima* were below 5 % of DRI. The levels of minerals and vitamins expressed per 100 µg iodine reveals that *A. esculenta* is more nutritious than *S. latissima* in a scenario of limiting iodine and that W-SW increases mineral and vitamin levels (per unit iodine) in these kelps (Fig. 7).

The results from the chemical risk assessment of the kelp ingredients regarding exposure to Cd and Hg are presented in Table 5. The Cd exposure of portions of W-SW-treated *A. esculenta* ranged from 12 to 25 % of TDI. Cd exposure from *S. latissima* was considerably lower due to smaller portions and lower Cd concentrations compared to *A. esculenta* samples. The exposure to Hg from portions of all four ingredients were below 5 % of established thresholds by EFSA.

No TDI has been established for Pb and calculation of MoEs based on BMDLs is necessary for a risk assessment. According to the EFSA CONTAM Panel, an MoE of 10 or greater would be sufficient to ensure that there was no appreciable risk regarding the different endpoints, and “even at MoEs greater than 1 the risk would be very low” [37]. Based on the established BMDL<sub>10</sub> intake value of 0.63 µg (kg bw)<sup>-1</sup> day<sup>-1</sup> for nephrotoxicity [37] and a maximum daily exposure dose from seaweeds of 0.014 µg (kg bw)<sup>-1</sup> day<sup>-1</sup> for a 70-kg adult (derived from consuming 4.6 g of ingredient B contributing 1.0 µg Pb), the MoE is 35. For 4 to 6 year-old children, a lower UL of 250 µg of iodine per day is advised [19],

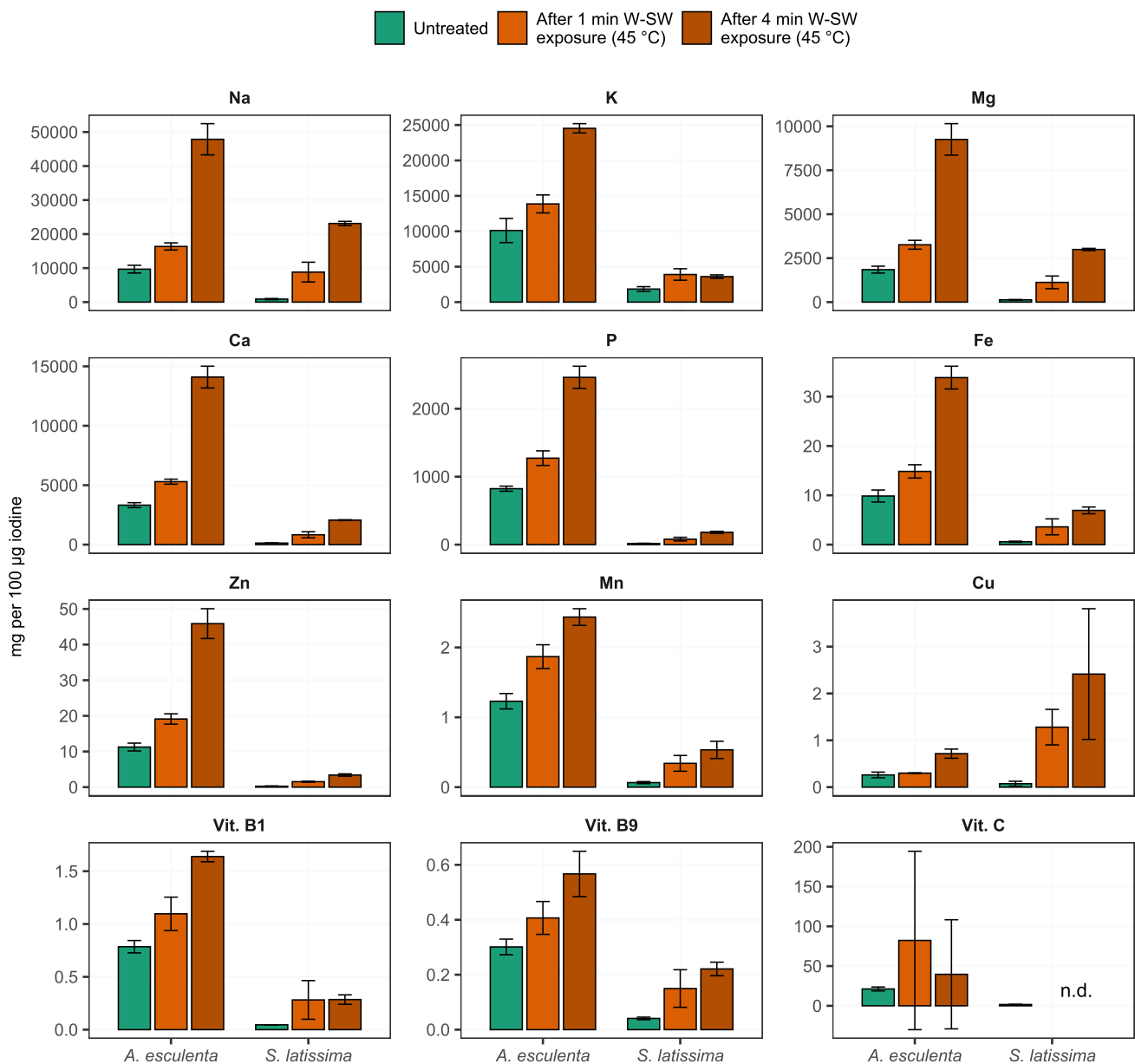


Fig. 7. Mineral and vitamin contribution of untreated and warm seawater (W-SW)-treated *Alaria esculenta* and *Saccharina latissima* expressed per 100 µg of iodine. Values are given as mean ± st. dev (n = 3). Abbreviation: n.d. (no data).



hence a lower amount of kelp ingredient should be considered in the risk assessment. This level of iodine is covered by consuming 1.9 g W-SW-treated *A. esculenta* (ingredient B), which will contribute with 0.42 µg Pb. Given the BMDL<sub>01</sub> of 0.5 µg (kg bw)<sup>-1</sup> day<sup>-1</sup> for developmental neurotoxicity in children [37] and a body weight of 20 kg, the MoE is 24. These exposure levels can be regarded as low and of no appreciable risk.

## 4. Discussion

### 4.1. Iodine reduction

The high iodine concentration of kelp species is considered a limit to their inclusion in every-day food products. Recent reports have raised concern about the frequent consumption of kelp-containing food products based on estimated iodine exposures per servings and potentially negative health effects associated with excessive iodine intakes [11,12,17,41,42]. In the present study, the iodine content of cultivated *S. latissima* was approximately 10 times higher compared to wild-harvested *A. esculenta*. The measured iodine levels in both kelp species were consistent with the range of values reported in the literature [12,24,43,44]. W-SW exposure at 45 °C significantly decreased the iodine content of *A. esculenta* after 1 min, and *S. latissima* after 10 s. Under these conditions, the loss of iodine was generally higher than losses of other nutrients, reflected by lower RFs for iodine compared to other nutrients analysed. Following 1 min exposure to W-SW at 45 °C, the iodine content of *A. esculenta* and *S. latissima* was respectively 38 % and 78 % of their initial levels. A minimum of 1 min treatment at 45 °C was necessary to decrease the concentration of iodine in *S. latissima* below the French recommendation for food, whereas 35 °C was not enough at any duration. For *A. esculenta*, W-SW treatment was not necessary safety wise, since the initial concentration was already below this threshold. After 2 min treatment, the achieved reduction was 51 % and 88 % in the two kelp species respectively. Similar reductions in *S. latissima* were achieved by Nielsen et al. [23] using freshwater at 45 °C for 2 min and by Wirenfeldt et al. [45] using seawater at 80 °C for 2 min. Kelp-to-water ratios in these studies are comparable to the ratio of the present study (2.5 kg in 40 L). A lower iodine content reduction (58 % from seawater exposure for 2 min at 45 °C was reported by Krook et al. [24] using 5 kg *S. latissima* in 20 L. This suggests that the kelp-to-water ratio is a critical factor affecting the iodine content reduction during processing of kelps.

Iodine is an inorganic antioxidant in kelps which is taken up from the surrounding seawater and accumulated mainly in the form of water-soluble iodide (I<sup>-</sup>) [46]. The efflux of iodine into the surrounding water or air is a natural response mechanism to oxidative stress in kelps. The localization of iodine in apoplasmic spaces of peripheral tissues [47] can explain its rapid release upon exposure to heat stress during treatment, whereas other phytochemical compounds (e.g. minerals and vitamins) which are mainly found in the cytoplasm or bound to cell-wall compounds, may not be as readily released. Stress response in kelps resulting in efflux of iodine involves vanadium-dependent haloperoxidases [48]. These enzymes purified from *S. latissima* are reported stable between 25 and 50 °C with maximum iodoperoxidase activity at 40 °C [49]. This may partly explain the contrast between the rapid iodine efflux observed in *S. latissima* exposed to W-SW at 45 °C and stable iodine levels observed at 35 °C. Considerable iodine content reduction in *S. latissima* exposed to freshwater at moderate temperature (33 °C) has been reported although it requires longer exposure (22h) [22]. A similar effect of W-SW at 35 °C used in this present experiment is expected at treatment time longer than 8 min.

### 4.2. Nutrient retention

The initial DW of wild-harvested *A. esculenta* in April was approximately twice as high as that of cultivated *S. latissima* harvested in May. This likely reflects interspecific and seasonal variation in moisture

content and chemical composition of kelps [50] as well as differences between wild-harvested and cultivated biomass [51]. Since the data in this study are mainly presented as part of the samples' DW, it is worth noticing that initial concentrations that are twice as high in *S. latissima* compared to *A. esculenta* will be the same on a WW basis, as for the monosaccharides analysed (Table 2). The total retention of DW upon W-SW treatment at 45 °C was higher in *A. esculenta* compared to *S. latissima*. W-SW treatment at 35 °C for up to 8 min did not affect the solid content of *S. latissima* as reflected by TRs and RFs of individual nutrients analysed close to 1.0 (Fig. 3, Fig. 4). In both kelps, nutrient losses upon W-SW exposure at 45 °C mainly concerned minerals (predominantly K) and carbohydrates (specially mannitol) confirming recent results from similar treatments of *S. latissima* using fresh water (2 min at 60 °C) [52]. Losses of K paired with high retention of other macrominerals (i.e. Ca and Mg) and uptake of Na upon W-SW exposure of *S. latissima* at 45 °C was also reported by Krook et al. [24]. Both Na and K as well as the storage carbohydrate mannitol are involved in osmotic adjustments in algal cells in response to salinity changes. These small compounds are therefore rapidly released from kelps upon stress. Comparable losses of K and mannitol were measured in *S. latissima* upon short exposure to W-SW (this study) and fresh water [52]. Therefore, heat stress (> 35 °C) appears to play an important role in the release of these osmolytes and may involve active mechanisms (e.g. release of K through ion channels, active release and/or catalysation of mannitol). Significant reduction of Na, K and mannitol levels in *S. latissima* and *A. esculenta* following exposure to fresh water have also been reported from longer treatments (up to 22 h) [22], indicating that compound release from osmotic stress is a longer process. Losses of K and uptake of Na during W-SW treatment were higher in *S. latissima* compared to *A. esculenta* suggesting a greater permeability of the former species.

The concentrations of fucose measured in the kelps reflect their content in fucoidan, a diverse group of sulphated polysaccharides [53]. Glucose in kelps is mainly found in the form of cellulose (insoluble form) or as the main constituent of laminarin, a soluble storage glucan found to accumulate in kelps during summer and autumn [50]. The glucose levels measured in this study represent the laminarin content of the samples. Both fucoidan and laminarin, along with alginates (not analysed in the present study), constitutes the soluble fraction of dietary fibres in kelps [54]. Recent reports suggest the prebiotic effect of these kelp polysaccharides modulating gut microbiota with positive impacts on the host's health condition [55,56]. Both fucoidan and laminarin were well retained upon W-SW treatments as observed following freshwater exposure [22,52]. The protein content was not analysed in the present study but is relatively low in *A. esculenta* and *S. latissima* (7 to 10 % DW) [50,57]. Krook et al. [24] reported a relatively high protein retention

**Table 3**

Quantity of kelp ingredient to cover iodine exposure thresholds established by the European Food and Safety Authority (EFSA) [19], based on measured iodine concentrations in original (untreated) kelp samples and warm seawater- (W-SW)-treated samples.

Sample	Iodine conc. (mg kg <sup>-1</sup> DW)	g to cover the AI (150 µg day <sup>-1</sup> )	g to reach the UL (600 µg day <sup>-1</sup> )
<i>Alaria esculenta</i>			
Untreated	510	0.29	1.2
W-SW-treated (45 °C), 1 min	320	0.47	1.9
W-SW-treated (45 °C), 4 min	130	1.2	4.6
<i>Saccharina latissima</i>			
Untreated	5700	0.03	0.10
W-SW-treated (45 °C), 1 min	1200	0.12	0.50
W-SW-treated (45 °C), 4 min	510	0.29	1.2

Abbreviations: concentration (conc.); adequate intake level (AI); upper intake level (UL); dry weight (DW).

**Table 4**

Mineral and vitamin (B1, B9 and C) contributions of warm seawater- (W-SW)-treated *Alaria esculenta* (ingredients A and B) and *Saccharina latissima* (ingredients C and D) compared to daily reference intakes (DRI). Ingredients A and B corresponds to W-SW-exposed *A. esculenta* for 1 and 4 min respectively while C and D are *S. latissima* exposed to W-SW for 1 and 4 min.

	DRI	<i>Alaria esculenta</i>			<i>Saccharina latissima</i>		
		Treatment time 1 // 4 min					
		Conc. in samples A // B (mg kg <sup>-1</sup> DW)	Amount <sup>a</sup> in 1.9 g A // 4.6 g B	% of DRI (from A // B)	Conc. in samples C // D (mg kg <sup>-1</sup> DW)	Amount <sup>a</sup> in 0.5 g C // 1.2 g D	% of DRI (from C // D)
I	150 µg	320 // 130	608 // 593 µg	405 // 396	1200–510	600 // 612 µg	400 // 408
Na	600 mg	52,000 // 61,000	99 // 281 mg	16 // 47	101,000 // 117,000	51 // 140 mg	8.4 // 23
K	2000 mg	44,000 // 32,000	84 // 147 mg	4.2 // 7.4	46,000 // 18,000	23 // 22 mg	1.2 // 1.1
Mg	375 mg	10,000 // 12,000	19 // 55 mg	5.1 // 15	13,000 // 15,000	6.5 // 18 mg	1.7 // 4.8
Ca	800 mg	17,000 // 18,000	32 // 83 mg	4.0 // 10	10,000 // 10,000	5.0 // 12 mg	0.6 // 1.5
P	700 mg	4000 // 3000	7.6 // 14 mg	1.1 // 2.0	930 // 920	0.47 // 1.1 mg	0.07 // 0.16
Cu	1000 µg	0.95 // 0.92	1.8 // 4.2 µg	0.18 // 0.42	15 // 12	7.5 // 15 µg	0.75 // 1.5
Fe	14 mg	47 // 43	0.09 // 0.20 mg	0.64 // 1.4	41 // 35	0.02 // 0.04 mg	0.15 // 0.30
Mn	2 mg	5.9 // 3.1	0.01 // 0.01 mg	0.56 // 0.72	3.9 // 2.72	0.002 // 0.003 mg	0.10 // 0.16
Zn	10 mg	61 // 59	0.12 // 0.27 mg	1.2 // 2.7	< LOQ	–	–
Cr	40 µg	0.27 // 0.29	0.51 // 1.3 µg	1.3 // 3.3	0.64 // 0.15	0.32 // 0.18 µg	0.80 // 0.45
Se	55 µg	0.06 // 0.08	0.11 // 0.37 µg	0.21 // 0.67	< LOQ	–	–
Vit. B1	1.1 mg	3.5 // 2.1	0.01 // 0.01 mg	0.60 // 0.88	3.1 // 1.5	0.002 // 0.002	0.14 // 0.16
Vit. B9	200 µg	7.8 // 4.5	15 // 21 µg	7.4 // 10	1.7 // 1.1	0.86 // 1.4	0.43 // 0.68
Vit. C	80 mg	250 // 57	0.48 // 0.26	0.59 // 0.33	< LOQ	–	–

<sup>a</sup> The quantities of kelp ingredient (dried) in each portion corresponds to the amounts of *A. esculenta* and *S. latissima* covering the upper intake level (UL) for iodine (see Table 3). Abbreviations: concentration (conc.); limit of quantification (LOQ); dry weight (DW).

**Table 5**

Cadmium (Cd) and mercury (Hg) contribution of warm seawater (W-SW)-treated *Alaria esculenta* (ingredients A and B) and *Saccharina latissima* (ingredients C and D) compared to tolerable daily intakes (TDI) for a 70-kg adult. Ingredients A and B corresponds to W-SW-exposed *A. esculenta* for 1 and 4 min respectively while C and D are *S. latissima* exposed to W-SW for 1 and 4 min.

	TDI	<i>Alaria esculenta</i>			<i>Saccharina latissima</i>		
		Treatment time 1 // 4 min					
		Conc. in samples A // B (mg kg <sup>-1</sup> DW)	Amount <sup>a</sup> in 1.9 g A // 4.6 g B	% of TDI (from A // B)	Conc. in samples C // D (mg kg <sup>-1</sup> DW)	Amount <sup>a</sup> in 0.5 g C // 1.2 g D	% of TDI (from C // D)
Cd	0.025 mg <sub>b</sub>	1.6 // 1.4	3.1 // 6.2 µg	12 // 25	0.35 // 0.35	0.18 // 0.42 µg	0.69 // 1.7
Hg	0.013 mg <sub>c</sub>	< LOQ	–	–	0.02 // 0.02	0.01 // 0.02 µg	0.08 // 0.18

Abbreviations: concentration (conc.); limit of quantification (LOQ); dry weight (DW).

<sup>a</sup> The quantities of kelp ingredient (in dried form) in each portion corresponds to the amounts of *A. esculenta* and *S. latissima* covering the upper intake level (UL) for iodine (see Table 3).

<sup>b</sup> From tolerable weekly intake (TWI) [35]

<sup>c</sup> From TWI [36]

from a commercial W-SW treatment (45 °C for 2 min) of *S. latissima* comparable to the results of Nielsen et al. [23] from an experimental study using freshwater under the same conditions (RF = 0.63). Losses of proteins in the present study are therefore assumed to be comparable to those found in the cited literature.

The retention of selected water-soluble vitamins following W-SW treatments was investigated. The level of ascorbic acid (vitamin C) measured in *A. esculenta* before treatment was comparable to the values reported in the literature for other kelp species including *S. latissima* [45,58,59]. The retention of vitamin C in *A. esculenta* exposed to W-SW at 45 °C for 4 min was ca. 50 % compared to initial levels. This is higher than the results reported by Wirenfeltd et al. [45] following blanching of *S. latissima* (both in freshwater and seawater, at ca. 80 °C for 2 min) which resulted in the complete removal of vitamin C. The same authors also reported a considerable decrease in vitamin C levels during the refrigerated storage (at 2.8 °C) of *S. latissima* for 13 days, highlighting the sensitivity of this compound to heat, light and oxygen during processing. To the authors' knowledge, there are no other studies analysing the fate of vitamins B (B1 and B9) in seaweeds upon processing. The

retention of thiamine (vitamin B1) and folic acid (vitamin B9) upon W-SW exposure of *A. esculenta* and *S. latissima* at 45 °C was similar to the retention of vitamin C in *A. esculenta* i.e. their content was reduced by half or more after 4 min of exposure. The sensitivity of vitamins B1 and B9 to heat is well established and characterized by increasing rates of degradation at increasing temperatures [60,61]. This is supported by a lower RF for vitamin B1 in *S. latissima* exposed to W-SW at 45 °C compared to 35 °C. It should be noted that the RFs for the vitamins analysed was high in kelp samples treated in W-SW for 1 min (i.e. > 0.8, except 0.6 for vitamin B9 in *S. latissima* at 45 °C).

#### 4.3. FAA retention

Seaweeds have a large and unexploited potential in gastronomy and food applications due to their organoleptic properties [8,9]. FAAs are major flavour-active compounds in foods [62]. Due to their relatively high content in free glutamate, seaweeds including kelps, are suggested as a source of umami flavour with potential in the food industry and every-day culinary applications. The initial FAA profile of *A. esculenta*,

dominated by alanine, was similar to reported profiles for this kelp species [63] whereas the levels of specific FAAs i.e. tryptophan, phenylalanine, methionine, tyrosine and serine were particularly high in *S. latissima* compared to published results [63,64]. Levels of free glutamate and aspartate (also contributing to umami flavour) were lower than those reported in the literature cited above.

W-SW exposure at 45 °C led to significant losses of FAAs in both kelps although to a higher extent in *S. latissima* compared to *A. esculenta*. High variations were observed in the retention of individual FAAs. Glutamate was highly retained in both species. Free glutamate (i.e. the anionic form of glutamic acid) naturally combines with sodium cations to form a salt, monosodium glutamate. Therefore, the conditions upon W-SW exposure resulting in sodium uptake appears favourable to the retention of glutamate, hence umami flavour. This is supported by significantly higher umami scores from seawater-treated *S. latissima* compared to freshwater-treated material under similar conditions, in a recent sensory analysis [24]. However, Wirenfeldt et al. [45] reported the complete removal of glutamate and aspartate both in seawater- and freshwater-blached samples of *S. latissima*. Higher temperatures (80 °C) compared to those tested in the present work may explain differences in glutamate retention across the two studies. The perception of umami in kelp does not only depend on glutamate and aspartate levels but is also affected by other flavour compounds (e.g. volatile compounds and other FAAs) [65] together with other flavours influencing the overall taste experience. The results from the recent sensory evaluation of *A. esculenta* and *S. latissima* following different processing treatments revealed similar sensory profiles (including perceived umami intensity) of W-SW treated kelps (at 45 °C for 2 min) compared to their untreated controls (Stévant et al. in prep). These results indicate that iodine-reduced kelps from W-SW treatment can contribute with flavour in food formulations, supporting the results of Krook et al. [24] who reported a perceptible flavour contribution of W-SW treated *S. latissima* in a commercial dehydrated spinach soup even at low inclusion level (0.5 %) compared to the same soup without kelp ingredient.

#### 4.4. Food safety

Seaweeds, including iodine-rich kelp species (*Laminaria japonica*) are widely consumed in Eastern Asia as part of culinary traditions, with reported average daily consumptions ranging from 4.0 to 10.4 g DW in China, the Republic of Korea and Japan respectively [66,67]. The consumption of seaweeds in Western countries is more recent and supported by an increasing consumer interest for Asian dishes as well as sustainable, healthy and vegetarian/vegan foods. However, the high content of iodine in kelp species may expose the consumer to excessive iodine intakes and is therefore considered a potential food safety hazard [10–12]. Optimal iodine intake is important for the synthesis of thyroid hormones which are essential for normal growth and development in young children as well as metabolic regulation. Considering the recommended iodine intake of 150 µg day<sup>-1</sup> for adults and the UL of 600 µg day<sup>-1</sup> (or 8.5 µg (kg bw)<sup>-1</sup> day<sup>-1</sup>), only small amounts of dried *A. esculenta* and *S. latissima* should be consumed to prevent from exceeding these limits as confirmed in this study. The present results show that applying short treatments with W-SW at 45 °C for 1 to 4 min significantly reduces the iodine content of kelps, allowing for a greater inclusion in foods. According to the analysis and chemical risk assessment of seaweeds from EFSA, the consumption of iodine-rich species such as *S. latissima* once a week (i.e. 5 g DW) would not pose a threat to the general healthy population but may increase the risk of negative health outcomes in susceptible individuals e.g. elderly, fetuses and neonates and individuals with pre-existing thyroid disorders [10]. The assessment warrants for a more detailed evaluation of the risks for these subgroups regarding the quantity and frequency of kelp consumption. Ficheux et al. [68] recently estimated the iodine exposure among the French population from consuming seaweed-containing food, based on a probabilistic model including data from a consumer survey (estimate of seaweed

consumption frequency, quantities consumed per food intake). Considering the high exposure scenario from this model (i.e. 95th percentile exposure), the iodine exposure from consuming seaweeds (including but not only kelp species) is 3.28 µg (kg bw)<sup>-1</sup> day<sup>-1</sup> making these food ingredients important contributors of dietary iodine. Given a background exposure of 2.1 µg (kg bw)<sup>-1</sup> day<sup>-1</sup> from consuming other food types, the total exposure (5.4 µg (kg bw)<sup>-1</sup> day<sup>-1</sup>) remains below the UL. The inclusion of kelp in manufactured food products with broad distribution may result in a substantial increase in iodine exposure. W-SW exposure appears therefore as an appropriate processing step limiting the risk of excessive iodine exposure from consuming kelp-containing food. On the other hand, iodine-reduced kelp ingredient may be a dietary source of iodine to population groups at risk of iodine deficiency such as vegetarians, vegans and pescatarians [69]. Safe consumption of kelp requires that iodine concentrations remain within tolerable amounts per serving and that information on the iodine content of food products is available to the consumer.

Other PTEs measured in the kelp samples in this study, including As, Cd, Hg and Pb were in the range of reported levels in the literature for *A. esculenta* and *S. latissima* [44,51,63]. The total As content greatly decreased in both kelps as a result of W-SW treatments. The fraction of inorganic As (i.e. the most toxic form of As) was not determined in the present study but has been reported to be below 1 % of total As in kelps [70]. Only marginal or no decrease in Cd, Hg and Pb were observed in both species upon processing in W-SW. According to the chemical risk assessment in this study, the exposure to Hg and Pb from iodine-reduced kelp is low and does not pose a risk to the consumer, supporting the conclusions from earlier assessments [10,68]. However, the Cd contribution of ingredients from *A. esculenta* ranged between 12 and 25 % of the TDI which can be considered of toxicological concern. The average dietary exposure of the European population to Cd ranges from 1.50 to 2.23 µg (kg bw)<sup>-1</sup> week<sup>-1</sup> (min. - max.) which is close to the TWI of 2.5 µg (kg bw)<sup>-1</sup> week<sup>-1</sup> [35] indicating that additional sources of dietary Cd exposure should be avoided. However, Cd in kelps is mainly bound to alginates (soluble fibres) suggesting a low bioavailability in the human body. This is supported by the results of Fjære et al. [71] who reported the excretion of 93 % of ingested Cd in rats fed with *S. latissima*.

#### 4.5. Nutritional considerations

Seaweeds including the two studied kelp species are commonly referred to as a source of vitamins and minerals [6,7], however not a rich source of vitamin C compared to vegetables, according to Nielsen et al. [59]. In the present study, the nutritional quality of *A. esculenta* and *S. latissima* exposed to W-SW at 45 °C was assessed based on their contribution in minerals and vitamins (B1, B9 and C), relatively to the DRIs for these nutritional compounds per portion. The portion size for each kelp ingredient was calculated so that the iodine per intake does not exceed 600 µg. Portion size ranged between 1.9 and 4.6 g of W-SW-rinsed *A. esculenta* (for 1 and 4 min respectively) and 0.5 and 1.2 g of *S. latissima* processed under the same conditions. Higher portions of *A. esculenta* (due to lower iodine levels) makes a higher nutritional contribution compared to *S. latissima*. However, the largest portion of processed *A. esculenta* (4.6 g) only contributes with meaningful amount (> 15 % of DRI) of Na. Other notable nutrient contributions (≥ 10 % of DRI) of W-SW-exposed *A. esculenta* includes Mg, Ca, K and vitamin B9. A recent assessment of the nutritional contribution of *S. latissima* was published based on available data for the content of macro- and micronutrients from the literature [72]. The authors concluded that a 5 g-portion of this species provides nutritionally relevant contributions of Fe, Se and Ca (along with iodine contribution of toxicological concern) but does not provide meaningful amounts of other nutrients including protein fat and fibres.

## 5. Conclusions

W-SW treatment is a simple step which may be implemented close to kelp harvesting sites to reduce the risk of excessive dietary iodine exposure from using kelp ingredients in large-scale food applications. According to the present results, the amount of kelp that should be eaten per day that does not exceed the UL for iodine ( $600 \mu\text{g day}^{-1}$ ) only makes limited contributions of other nutrients. Both species may represent a superior source of dietary iodine to maintain adequate iodine status of population groups at risk of deficiency (e.g. vegetarian and vegans). Increased knowledge regarding potentially negative health effects of an increased iodine exposure from kelp-based food as well as appropriate product labelling are warranted for sustainable industrial developments using kelp biomass in food applications.

## CRedit authorship contribution statement

**Pierrick Stévant:** Writing – review & editing, Writing – original draft, Visualization, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Cecilie Bay Wirefeldt:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Janne Stangeland:** Writing – review & editing, Investigation. **David Cohen:** Writing – review & editing, Methodology, Conceptualization. **Jens J. Sloth:** Writing – review & editing, Data curation, Conceptualization. **Susan Løvstad Holdt:** Writing – review & editing, Data curation, Conceptualization. **Arne Duinker:** Writing – review & editing, Resources, Methodology, Investigation, Formal analysis, Conceptualization.

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## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.algal.2025.103969>.

## Data availability

Data will be made available on request.

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