Journal of Applied Phycology

# Biomass soaking treatments to reduce potentially undesirable compounds in the edible seaweeds sugar kelp (Saccharina latissima) and winged kelp (Alaria esculenta) and health risk estimation for human consumption

--Manuscript Draft--

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| **Manuscript Number:** | |
| **Full Title:** | Biomass soaking treatments to reduce potentially undesirable compounds in the edible seaweeds sugar kelp (Saccharina latissima) and winged kelp (Alaria esculenta) and health risk estimation for human consumption |
| **Article Type:** | Original Research |
| **Keywords:** | bioactive compounds; cadmium; edible seaweeds; iodine; inorganic arsenic; processing |
| **Corresponding Author:** | Pierrick Stévant, MSc<br>Møreforsking Ålesund<br>Ålesund, NORWAY |
| **Corresponding Author Secondary Information:** | |
| **Corresponding Author's Institution:** | Møreforsking Ålesund |
| **Corresponding Author's Secondary Institution:** | |
| **First Author:** | Pierrick Stévant, MSc |
| **First Author Secondary Information:** | |
| **Order of Authors:** | Pierrick Stévant, MSc |
| | Hélène Marfaing |
| | Arne Duinker |
| | Joël Fleurence |
| | Turid Rustad |
| | Ingrid Sandbakken |
| | Annelise Chapman |
| **Order of Authors Secondary Information:** | |
| **Funding Information:** | Norges Forskningsråd (244244) — Not applicable<br>Sparebanken Møre — Mr. Pierrick Stévant |
| **Abstract:** | Samples of cultivated edible kelps Alaria esculenta and Saccharina latissima were analysed for their cadmium, iodine and inorganic arsenic contents. The inorganic arsenic levels were low in both species but samples of A. esculenta had relatively high cadmium contents (up to 2.01 mg kg-1 DW), and iodine levels were high in S. latissima samples (up to 6568 mg kg-1 DW), exceeding the limits established by the French food safety authority for both elements. Simple soaking treatments in warm fresh water (32˚C) reduced the iodine in S. latissima and treatment of A. esculenta in hypersaline solution (2.0 M NaCl) reduced the relative cadmium content. However, both treatments affected the nutritional value of the biomass, illustrated by considerable variations in dry weight and losses of bioactive compounds (e.g. minerals, polyphenols, fucoxanthin). Health risks associated with the consumption of these seaweed species were estimated using risk factors based on established tolerable intake levels. The contribution of A. esculenta to dietary cadmium intake does not appear to pose a threat to the consumer while the daily consumption of S. latissima leads to excessive iodine intakes. The moderate consumption of these kelps will, on the other hand, improve the iodine status in iodine deficient populations. |

# Biomass soaking treatments to reduce potentially undesirable compounds in the edible seaweeds sugar kelp (*Saccharina latissima*) and winged kelp (*Alaria esculenta*) and health risk estimation for human consumption

Pierrick Stévant [a,b,1], Hélène Marfaing [c], Arne Duinker [d], Joël Fleurence [e], Turid Rustad [b], Ingrid Sandbakken [f], and Annelise Chapman [a]

[a] Møreforsking Ålesund AS, PO Box 5075, N-6021 Ålesund, Norway

[b] Norwegian University of Science and Technology NTNU, N-7491 Trondheim, Norway

[c] CEVA (Centre d'Etude et de Valorisation des Algues), B.P. 3, F-22610 Pleubian, France

[d] National Institute of Nutrition and Seafood Research (NIFES), PO Box 2029 Nordnes, 5817 Bergen, Norway

[e] Mer Molécule Santé, EA2160, Université de Nantes, BP 92208, F-44322 Nantes, France

[f] SINTEF Materials and Chemistry, N-7465 Trondheim, Norway

## Abstract

Samples of cultivated edible kelps *Alaria esculenta* and *Saccharina latissima* were analysed for their cadmium, iodine and inorganic arsenic contents. The inorganic arsenic levels were low in both species but samples of *A. esculenta* had relatively high cadmium contents (up to 2.01 mg $kg^{-1}$ DW), and iodine levels were high in *S. latissima* samples (up to 6568 mg $kg^{-1}$ DW), exceeding the limits established by the French food safety authority for both elements. Simple soaking treatments in warm fresh water (32˚C) reduced the iodine in *S. latissima* and treatment of *A. esculenta* in hypersaline solution (2.0 M NaCl) reduced the relative cadmium content. However, both treatments affected the nutritional value of the biomass, illustrated by considerable variations in dry weight and losses of bioactive compounds (e.g. minerals, polyphenols, fucoxanthin). Health risks associated with the consumption of these seaweed species were estimated using risk factors based on established tolerable intake levels. The contribution of *A. esculenta* to dietary cadmium intake does not appear to pose a threat to the consumer while the daily consumption of *S. latissima* leads to excessive iodine intakes. The moderate consumption of these kelps will, on the other hand, improve the iodine status in iodine deficient populations.

**Key words: bioactive compounds; cadmium; edible seaweeds; iodine; inorganic arsenic; processing**

[1] Corresponding author: email: pierrick.stevant@moreforsk.no, tel: +47 70 11 16 13

## 1. Introduction

Seaweeds are a major element of the human diet in Asian countries where a variety of species have long been recognized for their nutritional value as well as for their rich and unique flavours (Nisizawa et al. 1987; Mouritsen 2013). Although seaweeds have no tradition of being a significant food resource in Western societies, their use as a food item and as a functional ingredient has gained increasing interest over the past decades. This recent trend is supported by the nutritional and health benefits of including seaweeds in the diet, especially regarding the high content of dietary fibres (Dawczynski et al. 2007a; Rupérez and Saura-Calixto 2001), minerals, vitamins and trace elements in most relevant species (Rupérez 2002; MacArtain et al. 2007; Holdt and Kraan 2011; Cabrita et al. 2016). The protein quality (Fleurence 2004; Mæhre et al. 2014) and lipid profiles of the most commonly used species (Sánchez-Machado et al. 2004) are highly relevant for human food and animal feed applications. In addition, brown seaweeds, can be a rich source of polyphenols, a class of secondary metabolites with documented antioxidant activity (Wang et al. 2010), also described in the literature for their anti-allergenic properties (Fleurence and Ar Gall 2016). Likewise, fucoxanthin, a xanthophyll pigment abundant in kelp species, is a potent antioxidant (Fung et al. 2013) with anti-obesity and anti-diabetic effects (Maeda et al. 2005; Maeda et al. 2008). Thus, the potential for using seaweeds in pharmaceutical, nutraceutical and cosmetic applications.

Seaweed biomass can be cultivated at sea on a large scale and is considered an alternative food and feed source with great potential. In Europe, current efforts for the cultivation of macroalgae largely focus on kelp species, particularly sugar kelp (*Saccharina latissima*) and winged kelp (*Alaria esculenta*) because of potentially high biomass yields, valuable nutritional content (Kraan et al. 2000; Handå et al. 2013) as well as their culinary appeal (Chapman et al. 2015).

On the other hand, seaweeds, including edible kelp species, can accumulate toxic elements with potentially negative effects on human health. Here, both non-essential heavy metals (Caliceti et al. 2002), as well as essential elements, especially iodine (I), in excessive amounts (Ar Gall et al. 2004), may be problematic. Previous studies have reported high levels of arsenic (As) in its toxic inorganic form (Rose et al. 2007; Almela et al. 2006; Besada et al. 2009), cadmium (Cd) (Almela et al. 2006; Besada et al. 2009) and iodine (Dawczynski et al. 2007b; Desideri et al. 2016) in seaweed food products commercialized in Europe. Direct evidence for seaweed consumption being associated with clinical pathology is scarce (Cheney 2016). Exposure to toxic elements such as Cd can have negative health effects, including renal dysfunction and bone disease, even at low intake levels if consumed over a long period of time (Järup 2002) although such effects have never been associated to seaweed consumption. As in its inorganic form (iAs) is a known human carcinogen associated with liver, bladder, lung and skin cancers

(Hughes 2002). Excessive I intakes are known to affect the thyroid function, particularly in susceptible individuals, potentially resulting in hypo- or hyperthyroidism (Leung and Braverman 2014). At present, the EU has not established specific regulation addressing toxic elements in edible seaweeds. France is the only European country with defined limits of potentially toxic compounds in seaweeds to be used for human consumption (Mabeau and Fleurence 1993) although these limits are recommendations from the food safety authority and are not legally binding.

Previous studies reported simple soaking and washing processes to effectively reduce the levels of toxic elements in brown seaweeds such as As in hijiki (*Hizikia fusiformis*) using fresh water either at ambient or high temperatures (Hanaoka et al. 2001; Katayama et al. 2015). Likewise, immersing kelps in boiling water can considerably reduce their I levels (Lüning and Mortensen 2015; Zava and Zava 2011). However, nutritional compounds may be considerably reduced during these processes, e.g. minerals (Sugawa-Katayama and Katayama 2007), polyphenols (Cox et al. 2011) and vitamins (Amorim-Carrilho et al. 2014), although the extent of losses varies greatly among seaweed species, treatment temperature and duration. Water salinity is also an important factor influencing the uptake of toxic elements by marine biomass from their environment. Previous investigations have shown that Cd accumulation is inversely related to chloride concentration (i.e. water salinity) (Engel and Fowler 1979). Furthermore, chloride salt (NaCl and $CaCl_2$) solutions at 1.0 M were effective in desorbing Cd from the brown alga *Ecklonia maxima* (Stirk and van Staden 2002) thus, could be applied as a post-harvest treatment to selectively reduce high Cd levels in edible seaweeds.

The aim of the present study is to report on the content of potentially toxic compounds (i.e. Cd, iAs and I) in cultivated *A. esculenta* and *S. latissima*, and examine the effects of fresh water soaking treatments, as well as treatment temperature in *S. latissima*, on the samples' levels of these elements. The samples' content of nutritional compounds, including mineral fraction, carbohydrates, proteins, polyphenols and fucoxanthin, was also analysed prior to and after treatment. The biomass' surface colour was measured throughout treatments in order to monitor changes in the products' general appearance. The effects of soaking treatments at high salinities on the Cd level of *A. esculenta* is also investigated as a potential process to selectively reduce the Cd content in edible seaweeds. Finally, the levels of essential and non-essential toxic elements in both edible kelps are compared to established tolerable intake levels to provide information regarding potential health risks associated with their consumption.

## 2. Materials and methods

### *2.1. Fresh water soaking of A. esculenta and S. latissima*

Samples of *A. esculenta* and *S. latissima* were harvested from CEVA's cultivation site (latitude: 48.836362 N, longitude: -3.044157 W) at Pleubian, off the Northern coast of Brittany in France on May 27$^{th}$ and 28$^{th}$, and June 4$^{th}$ 2015. If epiphytic brown seaweeds (Ectocarpaceae) were observed on the distal part of some blades, these sections were cut off and discarded. The biomass was stored in mesh bags during boat transport to the laboratory. All biomass samples were received and treated within 2 h post-harvest. Batches of 5 kg of harvested seaweeds were transferred to tanks supplied with air bubbling and filled with 100 L of either fresh tap water at ambient temperature (FW, 16°C) or warm fresh water (WW, 32°C). Simultaneously, equivalent treatments with ambient temperature seawater (SW, 18°C) were conducted as controls. Samples of 500 g of seaweed biomass from the various treatments were analyzed for their chemical content (see below) prior to and after 1 h, 2 h, 6 h and 22 h soaking treatments.

### *2.2. Hypersaline treatments of A. esculenta*

Biomass of *A. esculenta* was harvested on May 16$^{th}$, 2016 from the same location and handled in the same way. In order to minimize the need for seaweed biomass and salt quantity, these treatments were conducted in a smaller scale than the fresh water soaking experiment. Batches of 1 kg of biomass were transferred to containers filled with 20 L of sodium salt (NaCl) solutions of different concentrations: 1.0 M, 2.0 M, and 0.5 M NaCl, the latter reflecting a saline marine environment (35‰ salinity) and used as a control. Samples of approximately 250 g of seaweed biomass were analyzed for their Cd content (see below) prior to and after 30 min, 1 h and 2 h soaking treatments. All treatments were performed in triplicate. Sampled blades were gently blotted to remove excess water, vacuum-packed and frozen until freeze-drying (C38L Cryorivoire), then ground to 250 µm (using a knife-mill) prior to chemical analyses. The dry weight (DW) was determined gravimetrically as the residue remaining after freeze-drying.

### *2.3. Analysis of potentially toxic elements*

***Cadmium (Cd)*** content was analyzed following a standard reference method (AOAC, 2000) samples were combusted overnight in a muffle furnace. The ashes were dissolved in nitric acid ($HNO_3$, 65%) under high heat and pressure using a laboratory microwave oven. The dissolved Cd was quantified by inductively coupled plasma atomic emission spectroscopy (ICP-OES, Perkin Elmer Optima 7300DV).

***Iodine (I)*** content in samples was analyzed following a standard method for the determination of I compounds in foodstuffs (BS EN 15111:2007). Samples were digested in a laboratory microwave with tetramethylammonium hydroxide (TMAH). After removing un-dissolved compounds, the nebulized sample was atomized and ionized in

an inductively coupled argon plasma. The ions were extracted from the plasma by a system of sampler and skimmer cones, separated in a mass spectrometer on the basis of their mass/charge ratio and determined using a pulse counting detector system.

***Inorganic arsenic (iAs)*** As compounds were extracted from the samples using diluted hydrochloric acid (HCl) following a standard procedure (BS EN 15517:2008). In acidic media, inorganic compounds of As form a volatile hydride with sodium borohydride whereas stable organic As compounds e.g. arseno-sugars, do not react. The iAs was determined by hydride generation atomic absorption spectrometry (HGAAS) at 193.7 nm (As line).

### *2.4. Health risk estimation*

The health risk related to the consumption of *A. esculenta* and *S. latissima* with their respective levels of potentially harmful elements was estimated following the approach described by Phaneuf et al. (1999). These authors considered the intake levels of contaminants for (i) an average daily consumption of 3.3 g seaweed (DW) and (ii) a maximum consumption in a single serving corresponding to 12.5 g seaweed (DW). The results obtained were compared to health-based guidance values for each element, established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) or the European Food Safety Authority (EFSA).

### *2.5. Nutritional value analysis*

***Ash content*** The samples' ash content was determined using a standard procedure (AFNOR, 1977) in which samples were combusted at 550°C for 12h in a laboratory muffle furnace. Ashes were quantified gravimetrically after combustion.

***Sodium (Na), Potassium (K) analysis*** Na and K contents were analyzed following an official reference method (AOAC, 2000) in which samples were combusted overnight in a muffle furnace. The ashes were solubilized in nitric acid ($HNO_3$, 65%) under high heat and pressure using a laboratory microwave oven. The Na and K of the solutions were quantified by inductively coupled plasma atomic emission spectroscopy (ICP-OES, Perkin Elmer Optima 7300DV).

***Carbohydrate analysis*** Neutral sugars (D-glucose, D-galactose, D-mannose, D-xylose, L-fucose, L-rhamnose), D-mannitol and uronic acid (D-glucuronique, D-mannuronic, poly-D-guluronic and poly-D-mannuronic) composition were determined by high-performance liquid chromatography (HPLC) analysis after depolymerization under methanol- acid hydrolysis reaction (methanolysis) as described by Quemener et al. (2000). Ground freeze-dried seaweed samples of 15 mg were transferred into 2mL MeOH–HCl solution, prepared by adding acetyl chloride in methanol (17/3 v/v, from pure solutions). Methanolysis was conducted at 100°C for

4 h, after which neutralization was achieved by adding silver carbonate (successively 100 mg then 50 mg) until pH reached 4-5. The solutions were evaporated at 47°C for 16 h, then dissolved in distilled water and filtered prior to HPLC analysis (Grace smart RP18, 5 µm, 4.6×250 mm). Chromatographic peaks were identified by comparison with high purity reference sugars purchased from Sigma-Aldrich (Steinheim, Germany) except for the poly-D-guluronic and poly-D-mannuronic standards prepared at CEVA's laboratory.

***Protein content*** Total nitrogen (N) was determined in ground freeze-dried samples using a CHNS-O elemental combustion system (Costech Instruments ECS 4010) at a temperature of approximately 1000°C, where the samples' N is converted to N gas/oxides. Results were expressed in gram N per 100 g of dried sample. A N-to-protein conversion factor of 5, recommended as suitable to predict the protein content of brown seaweeds (Angell et al. 2016), was used. Analyses were performed in triplicate.

***Polyphenolic content*** The polyphenolic content of algal extracts was determined colorimetrically using the Folin–Ciocalteu reagent according to the method of Ragan and Glombitza (1986). The extraction was performed using 250 mg of ground freeze-dried seaweed samples in 10 mL solvent (acetone/water, 80/20 v/v). The mixture was incubated for 1 h in the dark at room temperature. After decantation, the supernatant was recovered and re-extracted under the same conditions. Both supernatants were pooled prior to filtration (0.45 µm). The filtrate represented the seaweed sample extract. Then, 200 µL of seaweed extract was mixed with 1300 µL distilled water and 100 µL Folin-Ciocalteu reagent followed by the addition of $Na_2CO_3$ (29% w/w). After incubation at 45°C for 30 min in the dark, the absorbance was recorded at 760 nm using a UVIKON-XL spectrophotometer (Bio-Tek Instruments, USA), with phloroglucinol used as the standard reference (Sigma-Aldrich, Steinheim, Germany). A standard curve with serial phloroglucinol solutions (ranging from 0 to 100 µg $ml^{-1}$) was used for calibration. The polyphenol contents were expressed as gram phloroglucinol equivalent per 100 g of dried sample. Analyses were performed in duplicate with 10% relative uncertainty of measure.

***Fucoxanthin content*** The extraction of fucoxanthin from seaweed samples was carried out in ethanol/water solvent 60/40 for 2 h in ice bath protected from light (1% seaweed powder in solvent). After decantation, the seaweed sample residue was subjected to a second extraction following the same conditions. The supernatants were pooled prior to analysis. The fucoxanthin content in the extracts was determined by reversed phase HPLC in a YMC Carotenoid column (250 x 4.6 mm i.d. 5.5 µm particle size, INTERCHIM, France) with UV detection at 448 nm. Acetonitrile, methanol and water was used as mobile phase. A commercial fucoxanthin standard (C5753, Caroténature,) was used for quantification.

### *2.6. Surface color analysis*

The surface color of seaweed samples was analyzed by a computerized image technique known as computer vision system (CVS) as described by Girolami et al. (2013), using a digital camera (Canon EOS 60D) and a 35 mm lens (Canon EF 35 mm f/2) mounted in a black box supplied with standard illumination (6500 K) positioned at an angle of 45° from the sample to obtain uniform lighting. The color was analyzed quantitatively using Photoshop (Photoshop CC 2015, Adobe Systems Inc.) and expressed in CIE $L^*$ (whiteness or brightness), $a^*$ (redness/greenness) and $b^*$ (yellowness/blueness) coordinates, as described by Yam and Papadakis (2004). A minimum of three blades from each sample were photographed and the results averaged prior to calculating the total color difference ($\Delta E$) using Eq. (1), where $L^*_0$, $a^*_0$ and $b^*_0$ are color coordinates of the samples before treatment.

$$\Delta E = \sqrt{(L^* - L^*_0)^2 + (a^* - a^*_0)^2 + (b^* - b^*_0)^2} \quad (1)$$

### *2.7. Statistical analysis*

Raw data were pre-processed for descriptive statistics and results expressed as mean ± standard error (n = 3). All data sets from chemical and color analysis were tested for homogeneity of variances using the Levene's test. A repeated measures analysis of variance (RM ANOVA) at $p < 0.05$ was used to detect significant differences among storage duration treatments on parameters analyzed at all sampling times. The paired sample *t*-test was used to detect significant differences on parameters analyzed only at $t_0$ and at the end of soaking treatments. All statistical analyses were performed on R (R Development Core Team 2008).

## 3. Results

### *3.1. Potentially toxic elements in A. esculenta and S. latissima*

The harvested biomass was analysed for its initial content of potentially harmful compounds, i.e. I, Cd and iAs. The results are shown in table 1. Although both kelp species were grown at the same cultivation site, their respective content of both Cd and I was very distinct. High levels of Cd were found in *A. esculenta*, with almost 10-fold the levels found in *S. latissima.* In stark contrast, I levels in *S. latissima* were over 30 times higher than those found in *A. esculenta*. A difference in Cd content of *A. esculenta* was observed between the samples harvested in 2015 and 2016. Likewise, a temporal variation in I content was observed between samples of *S. latissima* harvested with one-week interval in 2015 (end of May, beginning of June). *A. esculenta* and *S. latissima* exceeded the recommended French limits for Cd and I content approximately by factor 4 and 3, respectively. The measured levels of iAs were relatively low in both species and below the threshold value of 3 mg $kg^{-1}$ DW.

**Table 1:** Initial content of the potentially harmful compounds Cd, I and iAs, expressed in mg $kg^{-1}$ DW of *A. esculenta* and *S. latissima* prior to soaking treatments. Values are given as mean ± standard error (n = 3).

| | *A. esculenta* (May 2015) | *A. esculenta* (May 2016) | *S. latissima* (May 2015) | *S. latissima* (June 2015) | Limit values (French recommendation) [a] |
|---|---|---|---|---|---|
| Cd | 2.01 ± 0.09 | 1.55 ± 0.20 | 0.22 ± 0.03 | 0.27 ± 0,01 | 0.5 |
| I | 213 ± 12 | - | 4898 ± 166 | 6568 ± 398 | 2000 |
| iAs | 0.22 ± 0.04 | - | 0.16 ± 0.02 | 0.23 ± 0.01 | 3 |

[a] Source: CSHPF (1999) and AFSSA (2009). Values expressed in mg $kg^{-1}$ DW.

### *3.2. Fresh water soaking treatments of A. esculenta and S. latissima*

The effects of fresh water soaking treatments on the chemical composition of both kelp species were assessed, with emphasis on the Cd content of *A. esculenta* and the I content of *S. latissima*. The results following fresh water treatments (FW and WW) are compared to those obtained from seawater (SW) soaking treatments conducted under similar conditions.

The initial DW was relatively similar in both species as well as DW losses during SW treatment (fig. 1). Higher losses were observed in *S. latissima* than in *A. esculenta* following FW soaking, mainly during the first 2 h of treatment (67% and 49% DW losses in both species respectively after 2 h). The WW treatment did not lead to lower DW in *S. latissima* samples compared to the biomass treated in FW (5.2 and 5.3% DW in the samples after 2 h WW and FW treatments respectively). However, an increase in DW was observed in FW-treated samples at the end of the treatment and not in samples treated in WW. Expressing the results from chemical analyses as part of the DW of the biomass reflects on the relative proportions of each compound analyzed and does not highlight their absolute variation throughout the treatment period when significant losses of dry matter occur. The DW in both species was significantly reduced by all treatments (RM ANOVA, $p < 0.05$) except SW soaking of *S. latissima*, probably due to the relatively high variation in the results of DW analysis at $t_0$ among replicates. A decrease in DW can be the result of a release of compounds and/or water uptake from the biomass during the process. In the case of fresh water soaking treatments, water uptake is expected to contribute to the DW reduction, due to the osmotic potential between the blades and the soaking water. Hence, the variations in the relative content of potentially harmful elements as well as bioactive compounds, expressed as part of the DW of the biomass, will be further discussed along with the variations in DW throughout treatments.

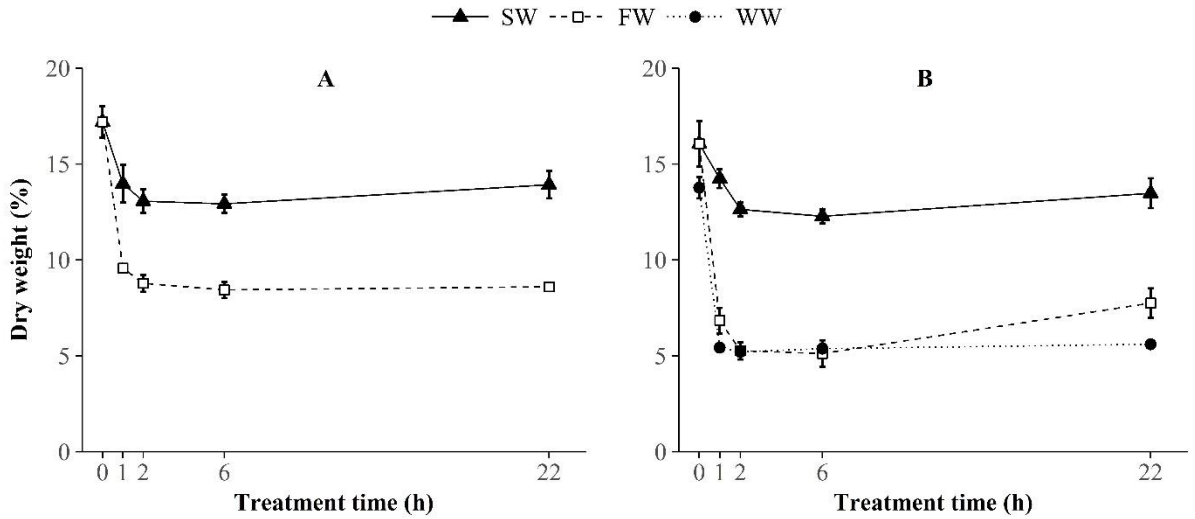


**Fig. 1:** Variations in dry weight of *A. esculenta* (A) and *S. latissima* (B) during soaking treatments in seawater (SW), fresh water (FW) and warm fresh water (WW). Values are given as mean ± standard error (n = 3).

A significant increase in the relative Cd content of *A. esculenta* samples was observed during FW soaking treatment (RM ANOVA, $p < 0.05$, fig. 2) while levels remained stable in SW. The Cd content was increased to 2.9 ± 0.2 mg $kg^{-1}$ DW after 22 h soaking in FW. The relative I content of *S. latissima* remained stable throughout both FW and SW treatments whereas soaking in WW rapidly reduced the I in the samples to below the threshold value of 2000 mg $kg^{-1}$ DW (fig. 3).

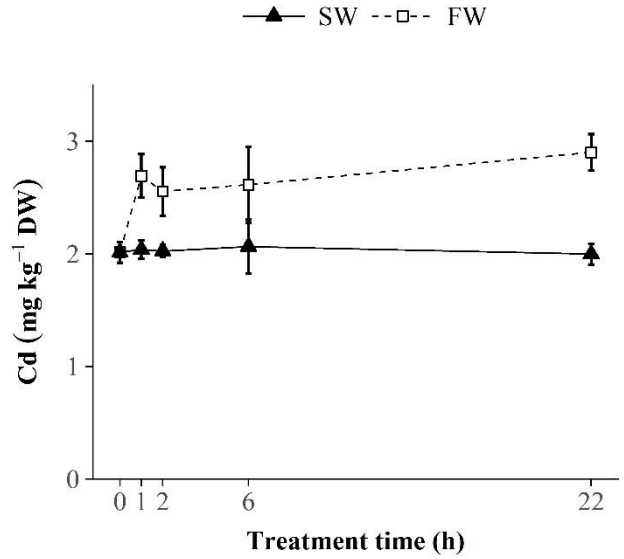


**Fig. 2:** Variations in Cd content in *A. esculenta* during soaking treatments in seawater (SW) and fresh water (FrW). Values are given as mean ± standard error (n = 3).

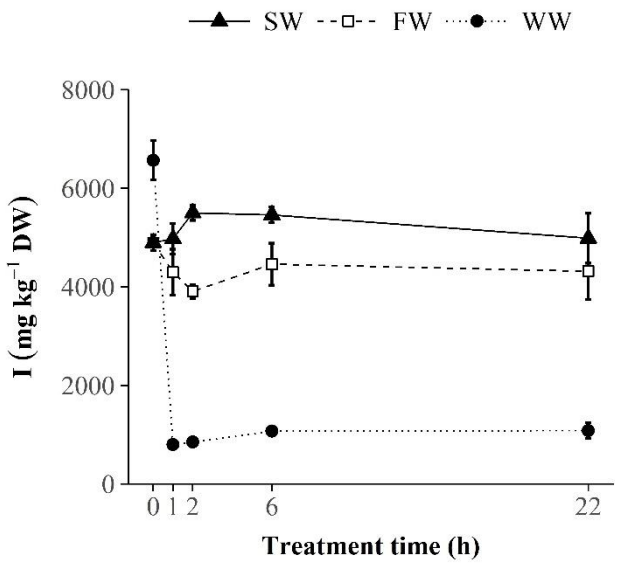


**Fig 3:** Variations in I content of *S. latissima* during soaking treatments in seawater (SW), fresh water (FW) and hot fresh water (WW). Values are given as mean ± standard error (n = 3).

Table 2 summarizes variations in the relative composition in bioactive compounds in both kelps, after 22 h soaking treatments, as well as changes in surface color ($\Delta E$). Both fresh water soaking treatments (FW and WW) resulted in lower mineral and higher carbohydrate contents as compared to initial values measured in the *A. esculenta* and *S. latissima* samples. Among carbohydrates, the relative alginate content increased, while the mannitol levels were reduced following FW soaking, particularly in *S. latissima*. Mannitol was not detected in *S. latissima* samples treated in WW. The relative protein content slightly increased in both kelps following fresh water treatments (FW and WW). A higher relative fucoxanthin content was observed in *A. esculenta* following 22 h soaking in FW while the levels of this pigment decreased in *S. latissima* following FW and WW treatments.

The total colour variation ($\Delta E$) of the samples' surface was recorded in order to monitor changes in the seaweed's appearance throughout treatments. The $\Delta E$ parameter reflects the variation in each of the three chromatic coordinates ($L^*$, $a^*$ and $b^*$) during treatment as compared to initial values measured at $t_0$. In both *A. esculenta* and *S. latissima*, relatively similar and moderate color variations were observed between SW- and FW-treated samples after 22 h soaking. Large variations in surface color were observed in WW-treated samples of *S. latissima*. Large increases in $L^*$ and $b^*$ (i.e. increased lightness and yellowness) and decrease in $a^*$ (increased greenness) were responsible for this severe alteration in product appearance (data not presented).

**Table 2:** Chemical composition of the seaweed biomass prior to ($t_0$), and after 22 h soaking treatments as well as variation in the surface color of the samples ($\Delta E$). Concentrations are expressed in g $(100\ g)^{-1}$ DW, except for the fucoxanthin content expressed in mg $kg^{-1}$ DW and the dimensionless $\Delta E$. Values are given as mean ± standard error (n = 3).

| | *A. esculenta* | | |
|---|---|---|---|
| | **$t_0$ [a]** | **SW [a] t = 22 h** | **FW t = 22 h** |
| Dry weight (%) | 17.2 ± 0.8 | 13.9 ± 0.7 * | 8.6 ± 0.1 * |
| *Minerals* | | | |
| Ash | 24.2 ± 1.4 | 27.0 ± 1.6 | 13.4 ± 0.8 * |
| Na | 3.92 ± 0.23 | 5.21 ± 0.20 * | 0.93 ± 0.03 * |
| K | 4.2 ± 0.3 | 4.4 ± 0.5 | 2.1 ± 0.3 * |
| *Carbohydrates* | | | |
| Total carbohydrates | 40.7 ± 1.5 | 37.7 ± 1.5 | 45.5 ± 1.5 |
| Alginate | 19.9 ± 0.5 | 18.6 ± 0.4 | 26.7 ± 0.9 * |
| Mannitol | 10.5 ± 0.4 | 10.4 ± 0.3 | 6.6 ± 0.7 |
| Glucose | 8.5 ± 1.9 | 7.5 ± 1.4 | 10.1 ± 1.8 |
| Fucose | 1.25 ± 0.03 | 0.98 ± 0.04 * | 1.76 ± 0.14 |
| Proteins | 10.5 ± 0.2 | 9.9 ± 0.1 | 12.7 ± 0.2 * |
| Polyphenols | 3.43 ± 0.08 | 2.55 ± 0.09 * | 2.93 ± 0.29 |
| Fucoxanthin | 871 ± 53 | 829 ± 45 | 1052 ± 114 |
| $\Delta E$ | - | 11 ± 3 | 8 ± 2 |

| | *S. latissima* | | | *S. latissima* | |
|---|---|---|---|---|---|
| | **$t_0$ [a]** | **SW [a] t = 22 h** | **FW t = 22 h** | **$t_0$** | **WW t = 22 h** |
| Dry weight (%) | 16.1 ± 1.2 | 13.5 ± 0.8 | 7.7 ± 0.8 * | 13.8 ± 0.6 | 5.6 ± 0.2 * |
| *Minerals* | | | | | |
| Ash | 26.2 ± 2.6 | 30.0 ± 2.1 | 16.7 ± 0.2 | 30.5 ± 1.1 | 15.5 ± 0.2 * |
| Na | 3.6 ± 0.2 | 4.3 ± 0.2 | 1.2 ± 0.1 * | 3.85 ± 0.04 | 0.97 ± 0.02 * |
| K | 6.5 ± 1.1 | 7.2 ± 0.8 | 2.7 ± 0.1 | 8.17 ± 0.42 | 1.86 ± 0.03 * |
| *Carbohydrates* | | | | | |
| Total carbohydrates | 46.1 ± 2.63 | 40.0 ± 1.0 | 49.6 ± 2.1 | 39.6 ± 1.0 | 47.1 ± 1.8 |
| Alginate | 21.5 ± 0.5 | 23.1 ± 1.5 | 38.2 ± 0.8 * | 21.0 ± 0.5 | 41.1 ± 0.9 * |
| Mannitol | 17.6 ± 1.2 | 12.3 ± 2.2 | 3.1 ± 0.8 * | 14.8 ± 1.1 | n.d. |
| Glucose | 5.0 ± 2.0 | 2.8 ± 0.6 | 4.3 ± 0.4 | 1.9 ± 0.4 | 1.3 ± 0.1 |
| Fucose | 0.76 ± 0.03 | 0.89 ± 0.07 | 1.50 ± 0.00 * | 0.89 ± 0.07 | 1.75 ± 0.03 * |
| Proteins | 10.6 ± 0.3 | 11.6 ± 0.2 | 12.7 ± 0.7 * | 11.3 ± 0.3 | 12.6 ± 0.2 * |

| | | | | | |
|---|---|---|---|---|---|
| Polyphenols | 0.69 ± 0.04 | 0.49 ± 0.04 * | 0.22 ± 0.01 * | 0.44 ± 0.02 | 0.26 ± 0.01 * |
| Fucoxanthin | 431 ± 19 | 360 ± 27 | 343 ± 24 * | 526 ± 27 | 201 ± 34 * |
| $\Delta E$ | - | 6 ± 2 | 7 ± 1 | - | 27 ± 1 |

The symbol * indicates a significant different level of a compound measured after 22 h storage as compared to the initial value measured at $t_0$ (paired sample *t*-test, $p < 0.05$).

[a] data published in Stévant et al. (2017)

### *3.3. Hypersaline bath treatments of A. esculenta*

The effects of hypersaline soaking treatments of different NaCl concentrations i.e. 1.0 and 2.0 M and a control reflecting the NaCl concentration in fully saline marine environment (0.5 M), on the Cd content of *A. esculenta* were investigated. Both hypersaline treatments (1.0 and 2.0 M) resulted in slightly increased DW in the samples along with significant increase in minerals (Na and ash, table 3). A steady reduction of Cd was observed during soaking in 2.0 M NaCl (fig 4) which was significant (RM ANOVA, $p < 0.05$), although the Cd content of *A. esculenta* was not reduced to below the threshold value of 0.5 mg $kg^{-1}$ DW. Conversely, the relative Na content following soaking treatments at this concentration reached almost 3 times the initial level measured in the samples. Large variations in surface color were observed from samples soaked in hypersaline solutions as compared to the control treatment at 0.5 M NaCl.

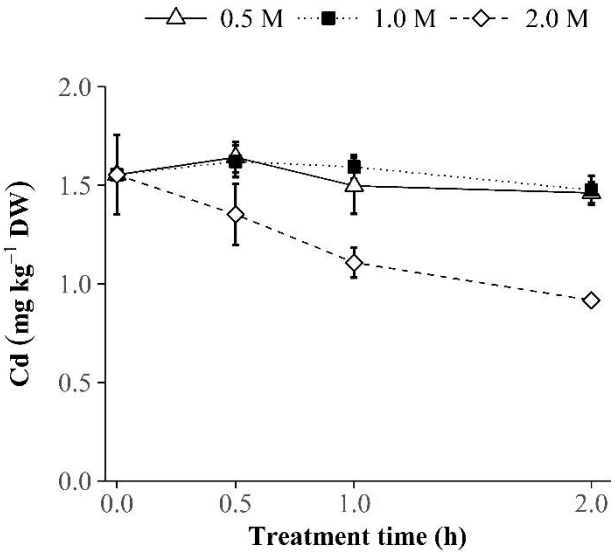


**Fig. 4:** Variations in Cd content in *A. esculenta* during soaking treatments in 0.5 M, 1.0 M and 2.0 M NaCl solutions. Values are given as mean ± standard error (n = 3).

**Table 3:** Total variation in dry weight, Cd, ash, Na and surface color ($\Delta E$) analyzed after 2 h soaking treatments in 0.5 M, 1.0 M and 2.0 M NaCl. Values are given as mean ± standard error (n = 3).

| | *A. esculenta* | | | |
|---|---|---|---|---|
| | **$t_0$** | **0.5 M t = 2 h** | **1.0 M t = 2 h** | **2.0 M t = 2 h** |
| Dry weight (%) | 13.4 ± 1.3 | 10.9 ± 0.1 | 14.4 ± 0.2 | 15.4 ± 0.2 |
| Cd (mg $kg^{-1}$ DW) | 1.55 ± 0.20 | 1.46 ± 0.05 | 1.48 ± 0.07 | 0.92 ± 0.02 * |
| Ash (g (100 g)$^{-1}$ DW) | 29.3 ± 1.7 | 35.0 ± 1.0 * | 39.9 ± 0.5 * | 45.1 ± 0.7 * |
| Na (g (100 g)$^{-1}$ DW) | 4.69 ± 0.18 | 6.36 ± 0.01 | 9.44 ± 0.05 * | 12.72 ± 0.13 * |
| $\Delta E$ | - | 9 ± 3 | 18 ± 2 | 19 ± 9 |

The symbol * indicates significant variations of a parameter during treatment (RM ANOVA, $p < 0.05$).

### *3.4. Health risk estimation*

The risks for human health from the consumption of *A. esculenta* and *S. latissima* was estimated based on the maximum levels of Cd, I and iAs found in each species. Daily intakes of potentially harmful elements were determined based on a daily consumption range of either 3.3 g dry seaweed at the moderate end and a large serving of 12.5 g representing a large intake. This approach was suggested by Phaneuf et al. (1999) and further developed by Desideri et al. (2016) in a similar study. The results are listed in table 4 along with risk estimators for each element established by international authorities. A tolerable weekly intake (TWI) of 2.5 µg Cd per kg body weight (bw) was established by the EFSA (2012), corresponding to a maximum daily dose of 0.025 mg $day^{-1}$ for a 70 kg adult. The maximum daily dose of 1.19 mg $day^{-1}$ for I (for a 70-kg person) was determined from the provisional maximum tolerable daily intake (PMTDI) of 0.017 mg I (kg bw)$^{-1}$ $day^{-1}$ indicated by the JECFA (WHO 1989). In the case of iAs, the EFSA panel on contaminants in the food-chain (CONTAM panel) identified a benchmark dose (95% lower confidence limit) corresponding to 1% increased risk of cancer ($BMDL_{01}$), between 0.3 and 8 µg iAs (kg bw)$^{-1}$ $day^{-1}$ (EFSA CONTAM panel 2009). The lower limit of this range was used as a reference, corresponding to a maximum daily dose of 0.021 mg iAs $day^{-1}$ for a 70-kg adult.

The daily consumption of 3.3 or 12.5 g of dried *A. esculenta*, in which high levels of Cd were registered, corresponds to daily intakes of 0.007 mg and 0.025 mg of this toxic metal, contributing to 27 and 101% of the tolerable daily dose respectively. Similarly, the I intake from this kelp contributes to 59 and 224% of the tolerable daily dose, whereas the iAs intake represented 3 and 13% of the established limit. Following the consumption of *S. latissima*, average and large daily servings correspond to 21.7 mg and 82.1 mg I respectively, exceeding the

tolerable daily dose by 18 and 69 times. The contribution to the Cd intake from *S. latissima* was 4 and 14% (from 3.3 and 12.5 g dried seaweed respectively) of the tolerable daily doses. Similarly, the contribution to iAs intake from this species was 4 and 14%, respectively, of the indicated maximum daily dose.

**Table 4:** Daily dose of potentially harmful compounds from the consumption of *A. esculenta* and *S. latissima* following their maximum concentrations of Cd, I, and iAs. Daily doses from risk estimators are based on TWI, PMTDI and for a 70 kg adult.

| Element | Species | Maximum concentration (mg $kg^{-1}$ DW) | Daily dose for 3,3 g consumption (mg $day^{-1}$) | Daily dose for 12,5 g consumption (mg $day^{-1}$) | Daily dose from risk estimators (mg $day^{-1}$) |
|---|---|---|---|---|---|
| Cd | *S. latissima* | 0.27 | 0.0009 | 0.0034 | 0.025 [a] |
| | *A. esculenta* | 2.01 | 0.007 | 0.025 | |
| I | *S. latissima* | 6568 | 21.7 | 82.1 | 1.19 [b] |
| | *A. esculenta* | 213 | 0.7 | 12.7 | |
| iAs | *S. latissima* | 0.23 | 0.0008 | 0.0029 | 0.021 [c] |
| | *A. esculenta* | 0.22 | 0.0007 | 0.0027 | |

[a] from TWI (EFSA 2012), [b] from PMTDI (WHO 1989) and [c] from $BMDL_{01}$ (lower bound) (EFSA CONTAM panel 2009)

## 4. Discussion

### *4.1. Potentially toxic elements in A. esculenta and S. latissima*

Despite a number of studies reporting on seaweeds' bioactive compounds and their associated nutritional benefits (Déléris et al. 2016; Mabeau and Fleurence 1993; MacArtain et al. 2007; Holdt and Kraan 2011), relatively high levels of potentially undesirable compounds, namely Cd and I, were measured in *A. esculenta* and *S. latissima* respectively. At present, specific regulations for the levels of toxic elements in edible seaweeds do not exist in Europe. However, French authorities have established recommendations for the levels of potentially toxic compounds in seaweed food products (Mabeau and Fleurence 1993). *A. esculenta* and *S. latissima* would fail to comply with the recommended maximum levels of Cd (0.5 mg $kg^{-1}$ DW) and I (2000 mg $kg^{-1}$ DW) respectively. On the other hand, both species appear to be a good source of nutritional compounds e.g. alginates (regarded as dietary fibres), proteins, minerals (particularly low Na/K ratios) and fucoxanthin pigment in the case of *A. esculenta* as previously described by Stévant et al. (2017). These results highlight the potential of both kelps to be used as a functional food ingredient.

*A. esculenta* accumulated approximately ten times more Cd than *S. latissima* cultivated at the same location indicating a high affinity for this element in this species. High concentrations of Cd have been reported previously for the same species (Mæhre et al. 2014) as well as in other edible seaweeds such as *Laminaria digitata*, *Porphyra umbilicalis* (Desideri et al. 2016) and *Undaria pinnatifida* (Almela et al. 2006; Besada et al. 2009). Cd naturally occurs in soil, water and sediments but is found to accumulate in land plants and marine environments due to anthropogenic activities. It should be noted that the area of the cultivation site is considered in good environmental condition regarding the presence of heavy metals. There are several reports highlighting the high heavy metal (including Cd) adsorption potential of brown seaweed species and extracts (Stirk and van Staden 2000; Davis et al. 2003). A review from Davis et al. (2003) emphasizes the role of the carboxyl groups of cell wall polysaccharides such as alginate and fucoidan in the biosorption of heavy metals. However, the alginate and fucose (main fucoidan monomer) contents of both species were relatively similar (19.9, 21.5 g $(100g)^{-1}$ DW alginate and 1.25, 0.76 g $(100g)^{-1}$ DW fucose in *A. esculenta* and *S. latissima* respectively). This suggests that other factors such as differences in alginate structure, may explain the contrast in Cd levels between these kelp species. Chronic toxicity from Cd intake is associated with kidney dysfunction, bone diseases and some form of cancer (Järup 2002), although none of these has been reported related to seaweed consumption.

High concentrations of I were found in *S. latissima*, more than thirty times higher than in *A. esculenta*. These levels reflect results reported in previous studies showing kelp species, including *S. latissima* to be the strongest I accumulators among all living systems (Küpper et al. 1998; Ar Gall et al. 2004). Similarly high contents were reported from samples of the same species harvested in Brittany (Lüning and Mortensen 2015) although large intra-specific variations are reported in the literature depending on the origin of the biomass and growth conditions (Lüning and Mortensen 2015; Yeh et al. 2014; Teas et al. 2004; Dawczynski et al. 2007b). A study of Ar Gall et al. (2004) monitored the variability in I contents of different populations of *L. digitata* across Europe, following different size class, throughout a seasonal cycle. The authors found higher contents in young blades (up to 4.5% DW), and higher in autumn and winter as compared to levels measured during spring and summer. In *Laminaria* species, I is mainly water-soluble and found as iodide ($I^-$) (Hou et al. 1997). I is an essential micromineral involved in the synthesis of the thyroid hormones. However, exposure to high levels can cause thyroid dysfunctions with symptoms similar to those associated with I deficiency (Crawford et al. 2010; Leung and Braverman 2014), as well as increased cancer risks in postmenopausal women (Michikawa et al. 2012). The I level of *A. esculenta* was moderate and below the maximum level of 2000 mg $kg^{-1}$ DW as reported in the literature (Mæhre et al. 2014; Teas et al. 2004).

### *4.2. Biomass soaking treatments to remove potentially toxic elements*

In this study, the chemical composition of *A. esculenta* and *S. latissima* was clearly altered as a result of soaking treatments illustrated primarily by substantial DW reductions in both species. However, the results from this study could not confirm whether this reduction was caused by (i) the release of nutritional compounds from seaweed biomass or (ii) water uptake during treatments as a consequence of osmosis. Stévant et al. (2017) suggested the action of both water uptake and the stress-induced exudation of bioactive compounds e.g. mannitol, laminaran, fucoidan and polyphenols following harvesting and SW storage of the same biomass. A methodology including the analysis of the soaking water which should contain the leaked compounds along with fresh weight measurements of individual blades throughout treatment, will allow a more precise estimation of the mass balance between the seaweed biomass and the soaking water during the process. In the case of fresh water soaking treatments (FW and WW), water uptake is likely playing an important role in the DW reduction, due to the high osmotic potential between the blades and the soaking water, as compared to SW treatments. Both FW and WW treatments clearly affected the integrity of *S. latissima* blades, on which blisters were observed. Although the tensile strength of the biomass was not measured in this study, the texture of these samples clearly differed from those treated in SW for the same species indicating major structural degradations of the biomass, which were not observed in *A. esculenta*. These observations may explain the higher DW losses measured in *S. latissima* than in *A. esculenta.* An important part of the reduction in DW following fresh water treatment of both species was also likely due to the diffusion of minerals out of the biomass. Conversely, the combined effect of water losses and Na uptake is likely responsible for the DW increase resulting from the treatment of *A. esculenta* in hypersaline solutions.

Soaking *A. esculenta* in FW resulted in 49% loss of DW along with the relative increase in the levels of Cd and carbohydrates constituting the intercellular matrix, i.e. alginate as well as glucose and fucose (reflecting the laminaran and fucoidan levels respectively). These results support earlier observations indicating that Cd in brown seaweeds is mainly bound to alginates and fucoidan (Davis et al. 2003). Hypersaline soaking treatments in 2.0 M NaCl significantly reduced the Cd levels in *A. esculenta*, which is comparable to the results obtained by Stirk and van Staden (2002) who recovered 80% of the Cd from contaminated powder of *Ecklonia maxima* after 2h bath treatment in 1.0 M NaCl. Although no effect on the Cd content after treatment at this concentration was achieved in this study, the metal ions from powdered biomass is likely more readily available for ion-exchange than from whole blades. These findings are supported by environmental observations along with experimental data showing

that Cd accumulation in marine organisms is inversely correlated to seawater salinity due to the complexation of free Cd ions with chloride ions (Engel and Fowler 1979). Hypersaline treatments can potentially reduce the Cd content in *A. esculenta* to levels below the threshold value established for dried seaweed products. However, further soaking or rinsing treatments may be necessary to reduce the Na levels achieved (12.7 g $(100g)^{-1}$ DW). In addition, large variations in the surface colour of samples soaked in hypersaline solutions suggest a strong impact of these treatments on the product's pigment content. Alternatively, longer soaking treatments at lower NaCl concentrations (slightly above 0.5 M) may reduce the biomass' Cd content while limiting the Na intake.

Soaking treatment of *S. latissima* in FW at 16˚C resulted in substantial losses of minerals (ashes, Na, K) but did not affect the relative I content of the samples which remained over the threshold value established by the French food authority. A similar treatment at 32˚C (WW) reduced the relative I content after 1 h to acceptable levels (800 mg $kg^{-1}$ DW). This result is in agreement with those obtained from *Laminaria digitata* for which canning (hot water treatment at 120°C) strongly decreased the I level of the seaweed to values below 500 mg $kg^{-1}$ DW (Fleurence, unpublished data). Likewise, boiling treatment in tap water was reported to reduce the I level of *S. latissima* by 70% (Lüning and Mortensen 2015). A review from Zava and Zava (2011) also reports on cooking processes to remove I from kelps. However, kelps are often used to flavour soup stocks from which the seaweed is removed after boiling, resulting in an I-rich broth. Along with reducing I, WW treatment also strongly affected the levels of bioactive compounds measured in this study i.e. minerals, mannitol and polyphenols, and hence severely compromised the biomass' nutritional value. Similarly, blanching treatments i.e. immersing fresh seaweed in boiling water for a short time, are commonly applied to some fresh brown seaweeds to be used as food, e.g. wakame (*Undaria pinnatifida*) and thongweed (*Himanthalia elongata*), as a mean to improve product palatability, including colour and texture (Cox et al. 2011). However, blanching also reduces the seaweed polyphenol content and radical scavenging activity (Cox et al. 2011) as well as vitamin levels (Amorim-Carrilho et al. 2014). Despite substantial DW losses, FW treatments of *S. latissima* only affected the samples' surface colour moderately. In contrast, large variations in colour were observed in WW-treated samples reflecting the impact of temperature on the pigment structure and content of the biomass. Similar colour changes (i.e. lighter and greener) following blanching of *H. elongata* were observed by Cox et al. (2011) who considered the final product to be visually more attractive.

### *4.3. Health risk estimation*

Although the levels of Cd found in *A. esculenta* exceed the upper limits specified by the French food safety authority (0.5 mg $kg^{-1}$ DW), an average consumption (3.3 g) will contribute to 27% of the tolerable daily intake,

derived from the tolerable weekly intake (TWI) of 2.5 µg (kg bw)$^{-1}$ week$^{-1}$ (EFSA 2012) giving 25 µg per day for a 70-kg person (corresponding to 175 µg week$^{-1}$). Large daily servings (12.5 g) will reach the limit for this element established by the EFSA. The dietary exposure of the European adult population has been estimated to 1.7 µg (kg bw)$^{-1}$ per week (EFSA 2012), corresponding to 119 µg per week for a 70-kg person, leaving a margin of 56 µg per week to the TWI for this person. With a daily dose of 3.3 g dry seaweed contributing with 49 and 6.3 µg Cd per week for *A. esculenta* and *S. latissima*, respectively, this is within the margin between the estimated exposure and the TWI. It should be noted that this limit is 2.3 times lower than the maximum tolerable daily intake of Cd derived from the provisional tolerable monthly intake (PTMI) of 25 µg Cd (kg bw)$^{-1}$ established by the JEFCA (WHO 2013) i.e. 58 µg day$^{-1}$ for a 70-kgadult. Moreover, the maximum value established in France for Cd in seaweed is considerably lower than the maximum values allowed in seafood (0.5 mg kg$^{-1}$ wet weight for crustaceans and 1 mg kg$^{-1}$ wet weight for bivalve molluscs and cephalopods) as well as food supplements consisting of dried seaweeds (3 mg kg$^{-1}$ wet weight) by the European Union (EU No 488/2014). As seaweeds are not traditionally consumed in Europe, other types of seafood would contribute to a larger extent to dietary Cd exposure. The results from a national study of the consumption of edible seaweeds in France revealed that few seaweed consumers (i.e. individuals who eat food products explicitly containing seaweed at least once a year) eat seaweeds more than once a week (Le Bras et al. 2014). Hence, the health risk estimation related to the dietary exposure to potentially toxic elements from seaweeds based on the daily consumption of 3.3 g (DW) is likely conservative regarding actual consumption practices in Europe. The perspective of a consumption based on 1 to 2 meals weekly (corresponding to 1 g DW day$^{-1}$) appears a more realistic scenario.

Moreover, the binding of Cd from seaweeds to dietary fibres (alginate) suggests a rather low bioavailability in the human body. However, Stirk and van Staden (2002) found that Cd desorption from algal biosorbant was effective at pH below 2.1, meaning that the Cd could be released in contact with gastric fluids. The desorption of Cd by protons is a reversible exchange, and knowledge is missing regarding the behaviour of algal Cd in the human intestine. The bioavailability of Cd from seaweeds and subsequent accumulation in the human body must be closely examined since toxicity at relatively low exposure levels have also been reported (Järup 2002).

Regarding the health risk estimation, an average daily consumption of 3.3 g *S. latissima* exceeds the nutritional recommendations for I intake by far. It should be noted that a large serving (12.5 g) of dried *A. esculenta* also contributes to an excessive intake of I (2.2 times). The physiological response to an oversupply of I differs individually and depends on previous and current intakes (Dawczynski et al. 2007b). A study from Aquaron et al.

(2002) reported the I bioavailability to vary among seaweed species and among groups of individuals with different I status. The observed I bioavailability from *Laminaria hyperborea* for I-sufficient women (90%) was significantly higher than for I-insufficient women (62%). The results from *in vitro* bioavailability assays suggested the role of the seaweed polysaccharide matrix in delaying the I absorption, hence a slower I release from seaweed ingredients compared to foods enriched with KI (Combet et al. 2014).

Excess I exposure generally does not result in clinical symptoms since it is generally excreted when storage is repleted, and cases of acute I poisoning are rare. However, sensitive groups (e.g. I-deficient people, individuals with pre-existing thyroid disorders, elderly people, foetuses and neonates) may develop thyroid complications including hypo- and hyperthyroidism (Dawczynski et al. 2007b; Zava and Zava 2011; Leung and Braverman 2014; Crawford et al. 2010). Several studies reported increase of serum levels of thyroid-stimulating hormone (TSH) following a long-term daily ingestion of kombu (*Saccharina japonica*) or kombu supplement, which resulted in the suppression of the thyroid function (Miyai et al. 2008; Inui et al. 2010). However, the TSH levels returned to normal and the thyroid function was recovered shortly after discontinuing the kombu ingestion. Most people are unaffected by excess intakes of I but for those who are affected, the amount of I required to cause adverse effects is highly individual (Pennington 1990). Hence, the lack of consensus among expert committees i.e. the JECFA established a maximal tolerable intake of 1 mg I $day^{-1}$ for adults (WHO 1989) while the value of 0.6 mg $day^{-1}$ is set by the European Food Safety Authority and derived from clinical studies showing no adverse effects on human exposed to 1.8 mg I $day^{-1}$ (EFSA 2006).

Worldwide, I deficiency is a major threat and approximately 2 billion people are estimated to have inadequate I intakes even though salt iodization programs have had a large impact on global I nutrition. Despite national and international efforts to increase the dietary I intake, Europe is still the continent with highest prevalence of I deficiency, which is regarded as a major cause of preventable brain damage (Andersson et al. 2007). The moderate consumption of edible kelps has the potential to improve the I status of the European population.

In this study, only the inorganic forms of As were analysed, as they are known to be more toxic than organic forms (Hughes 2002) and have been identified as a potential issue in some seaweed species (Almela et al. 2006; Besada et al. 2009; Rose et al. 2007). However, the values found in these two edible kelps were low and either comparable or lower than other published results for Laminariales species (Almela et al. 2006; Diaz et al. 2012) and did not contribute to elevated dietary exposures to iAs based health risk estimators. iAs can accumulate in marine environments because of anthropogenic activities as well as from natural sources e.g. the erosion of arsenic-

bearing rocks and sediments. Thus, variability in the total and iAs contents can be expected within species among harvesting locations.

The exposure to iAs in the European population is already quite high, with contributions from grain based processed products, rice and milk (EFSA 2014). The EFSA found that the dietary exposure to iAs among all surveys in the adult population (including adults, elderly and very elderly) ranged from 0.09 to 0.38 µg (kg bw)$^{-1}$ day$^{-1}$ (min lower bound - max upper bound) for the mean dietary exposure, and from 0.14 to 0.64 µg (kg bw)$^{-1}$ day$^{-1}$ (min lower bound - max upper bound) for the 95$^{th}$ percentile dietary exposure (EFSA 2014). Based on epidemiological studies, the JECFA identified a range of exposure values for the 95% lower confidence limit of the benchmark dose for 0.5% increased incidence of lung cancer ($BMDL_{0.5}$) of 3.0 µg (kg bw)$^{-1}$ day$^{-1}$ (2-7 µg (kg bw)$^{-1}$ day$^{-1}$ based on the range of estimated total dietary exposure) (WHO 2010). Prior to that, the EFSA CONTAM Panel established a range of $BMDL_{01}$ between 0.3 and 8 µg (kg bw)$^{-1}$ day$^{-1}$ for a 1% increased risk of lung, skin and bladder cancers, as well as skin lesions (EFSA CONTAM panel 2009). The lower limit of this range was used as a reference to establish a maximum tolerable daily intake in the risk estimation of the present study. Since the range of exposure overlaps with the range of BMDLs, care should be taken regarding the consumption of foods with a high level of iAs. Given a relatively high daily dose of 3.3 g of dry seaweed as for the Japanese population, contributing with 0.8-0.7 µg of iAs per day, corresponding to 0.01 µg per kg body weight per day for a 70-kg person, from both kelp species, this would correspond to a 7% increased exposure relative to the lower limit 95th percentile dietary exposure 0.14 µg day$^{-1}$. The intake from kelp would hence add to the general dietary intake, which already poses health risk, and 7% may be regarded as a significant increase. However, as the European intake would realistically be significantly lower than in Japan, these kelps species should not be considered as a potential source of increased exposure to iAs.

## 5. Conclusion

The results from this study show that both *A. esculenta* and *S. latissima* harvested in France in late spring are a rich source of nutritional compounds, particularly dietary fibres, minerals and proteins, which is in accordance with earlier reports in the literature for the same species harvested at different seasons and locations (see references in Holdt and Kraan, 2011). However, these edible kelps also contained potentially undesirable compounds, namely Cd and I, at levels exceeding the recommended limit values established by the French food safety authority. Health risks associated with eating seaweeds depends on the products' content of toxic elements, the quantity ingested over time and the compounds' bioavailability in the human body. The particularly high I content of *S. latissima*,

could have negative consequences on human health, especially in sensitive individuals, if this seaweed is ingested regularly over an extended period. However, the health risk estimation based on the average daily ingestion of 3.3 g and large serving of 12.5 g dried kelp, as used in previous studies (Desideri et al. 2016; Phaneuf et al. 1999) appears rather unrealistic in the perspective of a broader consumption in Europe. Although seaweeds receive increasing consumer acceptance, it is still regarded as an exotic food item. The consumption of one to two meals containing seaweeds – and not exclusively kelp species – appears more plausible to estimate dietary exposure to potentially toxic compounds. To this extent, the inclusion of seaweeds, and particularly *A. esculenta* and *S. latissima*, in the diet could support current efforts to improve I status among European populations, while the measured values for Cd and iAs in these species would not pose a threat to the consumer. On the other hand, the consumption of health supplements from kelps on a daily basis may give high intake of I (Inui et al. 2010), and possibly other toxic elements if doses are high.

Alternatively, simple processing methods can effectively reduce the I content in edible kelp. Soaking treatments in warm water can be applied immediately after harvest of the biomass or prior to consuming fresh or dried products. The results of this study also highlight the difficulties of selectively reducing the levels of toxic compounds from seaweed biomass, without simultaneous significant alterations of the products' nutritional value. Although a reduced nutritional value might not be a problem in some products, it is not acceptable in most food and feed applications where seaweed raw material is used for its content in bioactive compounds (e.g. mineral profile, antioxidants). The optimal conditions for soaking treatments (i.e. time, water temperature, salinity) should be further investigated, as well as alternative processes which can reduce the levels of toxic compounds while maintaining the seaweeds' nutritional value.

At present, only a few countries have established specific regulations for the use of seaweeds in human food including limit values for relevant toxic elements. The surveillance of potentially undesirable compounds in edible and commercialized seaweeds along with further investigation of the potentially negative effects related to their consumption is essential. The development of adapted regulations regarding edible seaweeds as well as appropriate product labelling will ensure consumer safety and the sustainable development of a growing seaweed industry.

**Acknowledgements**

This work was conducted as part of the PROMAC project (244244), funded by the Research Council of Norway, and part of the Sustainable Innovation in Food- and Bio-based Industries Programme. Pierrick Stévant was

supported by a doctoral fellowship from Sparebanken Møre. Thanks are due to the CEVA's laboratory and pilot facility staff for valuable technical assistance.

**Conflict of Interest**

The authors declare that they have no conflict of interest.

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Figure 1

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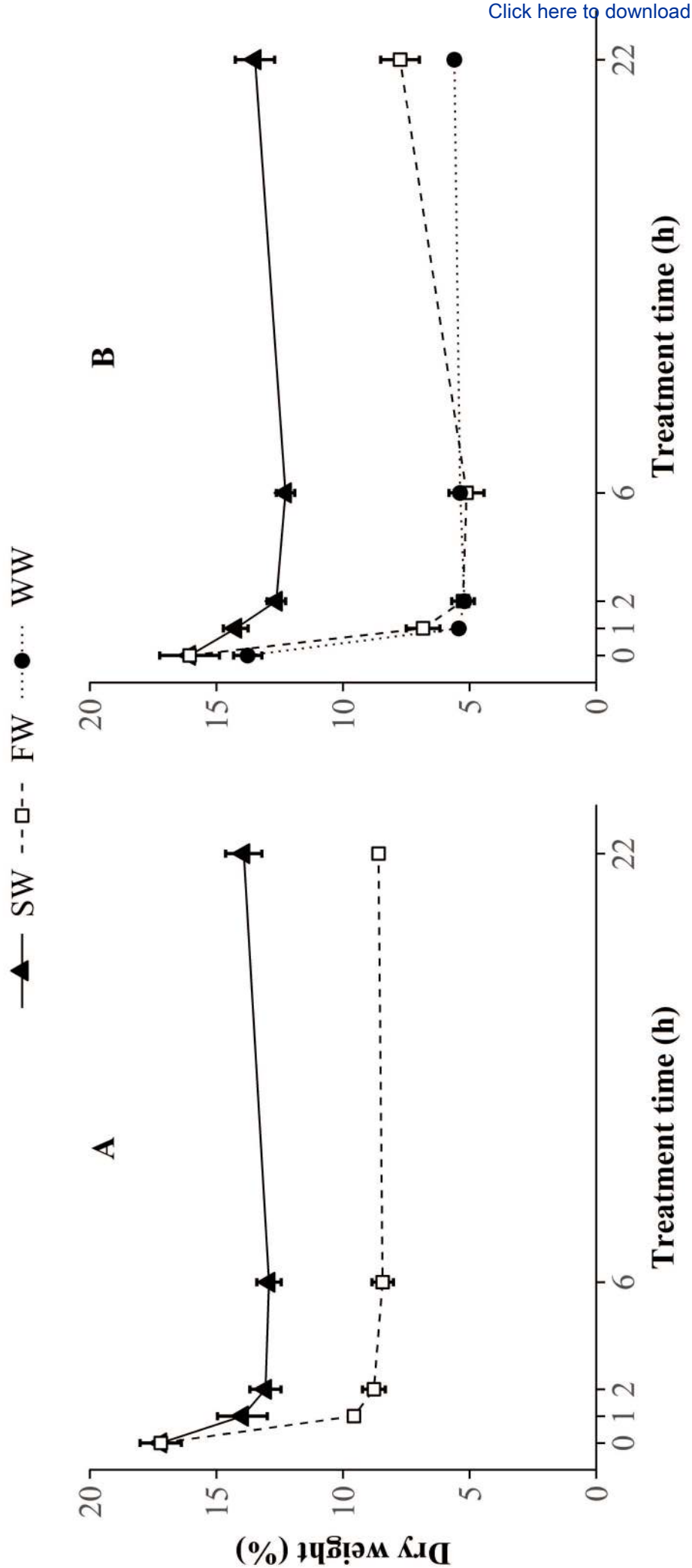

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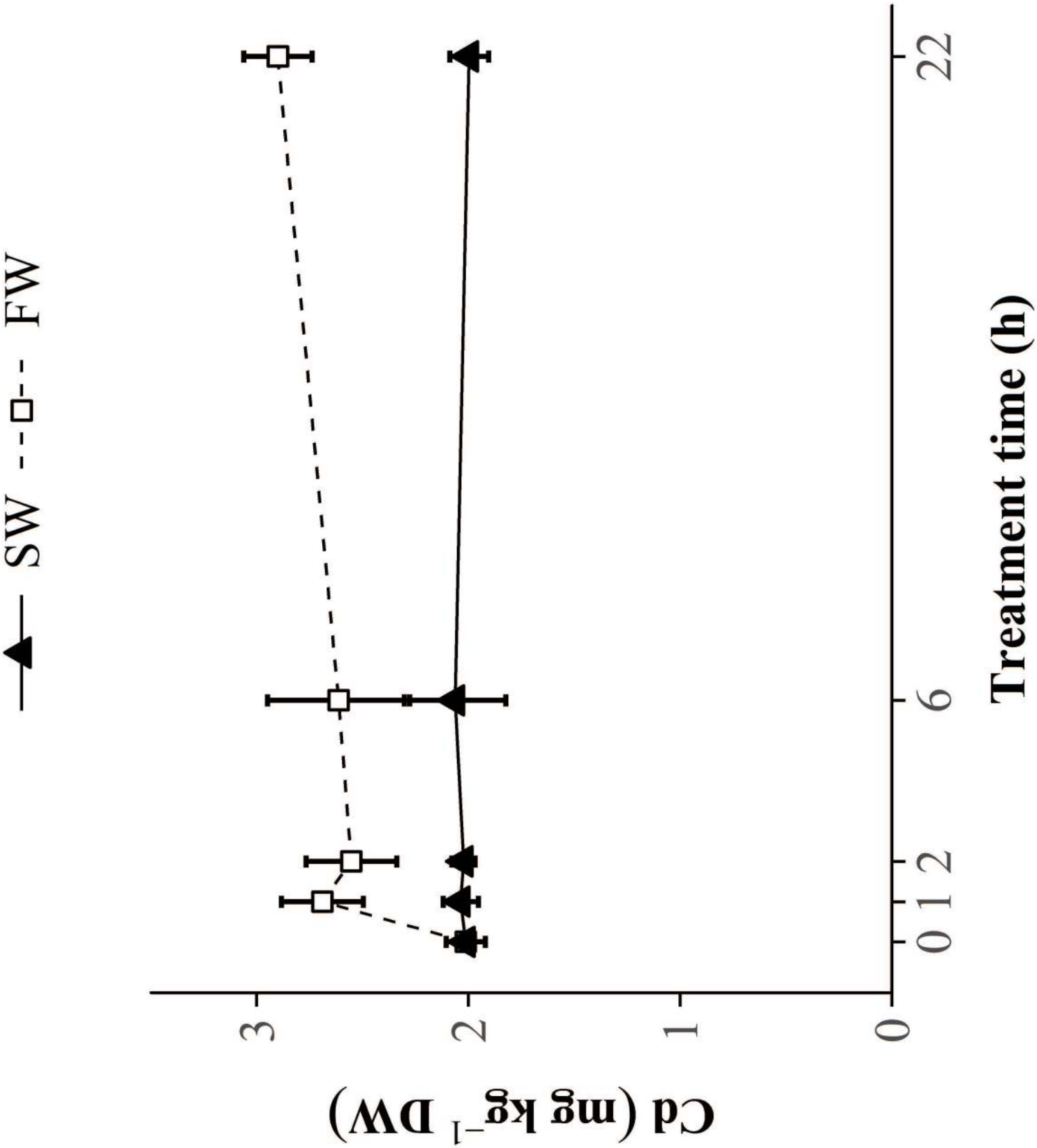

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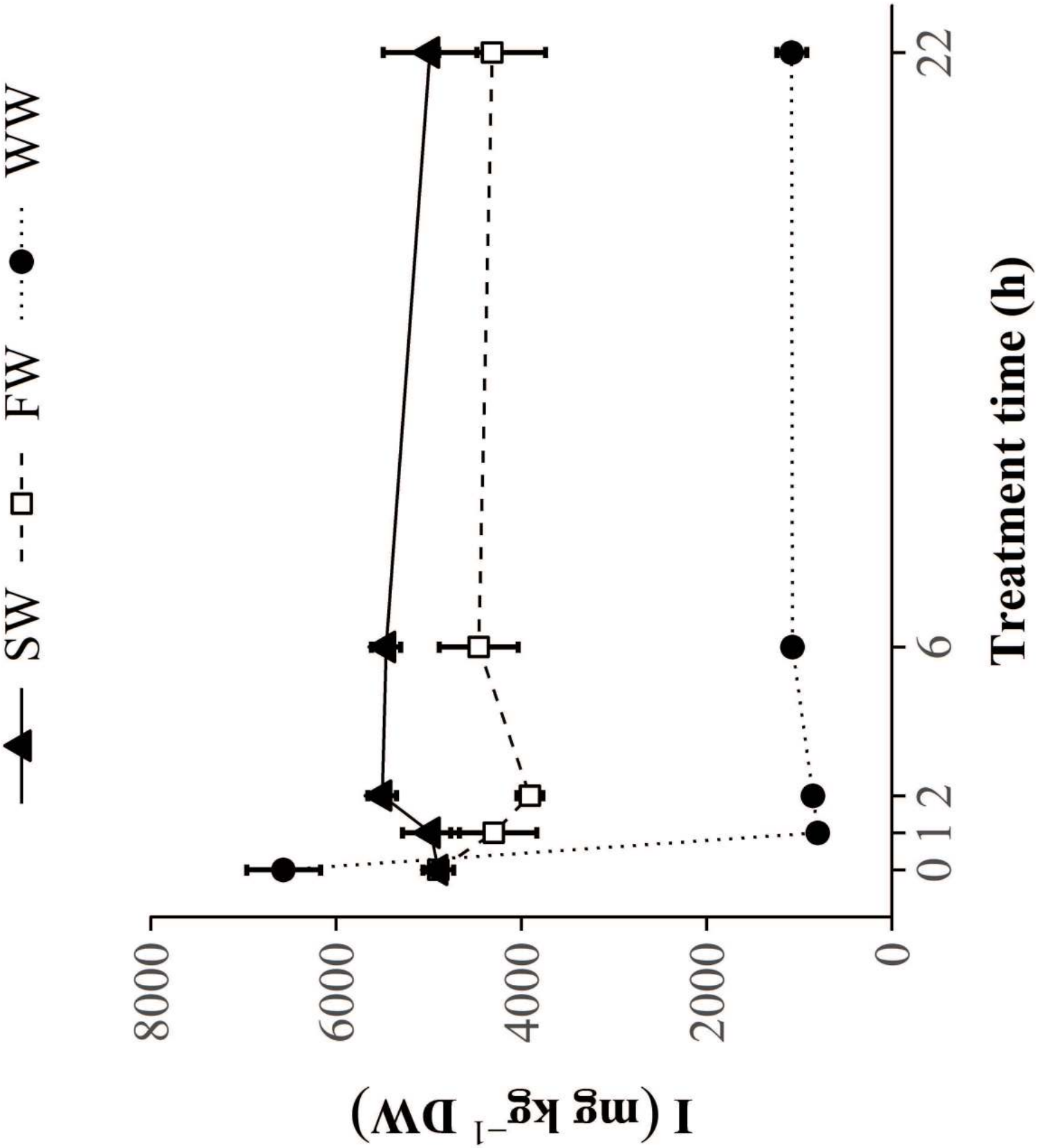

Figure 4

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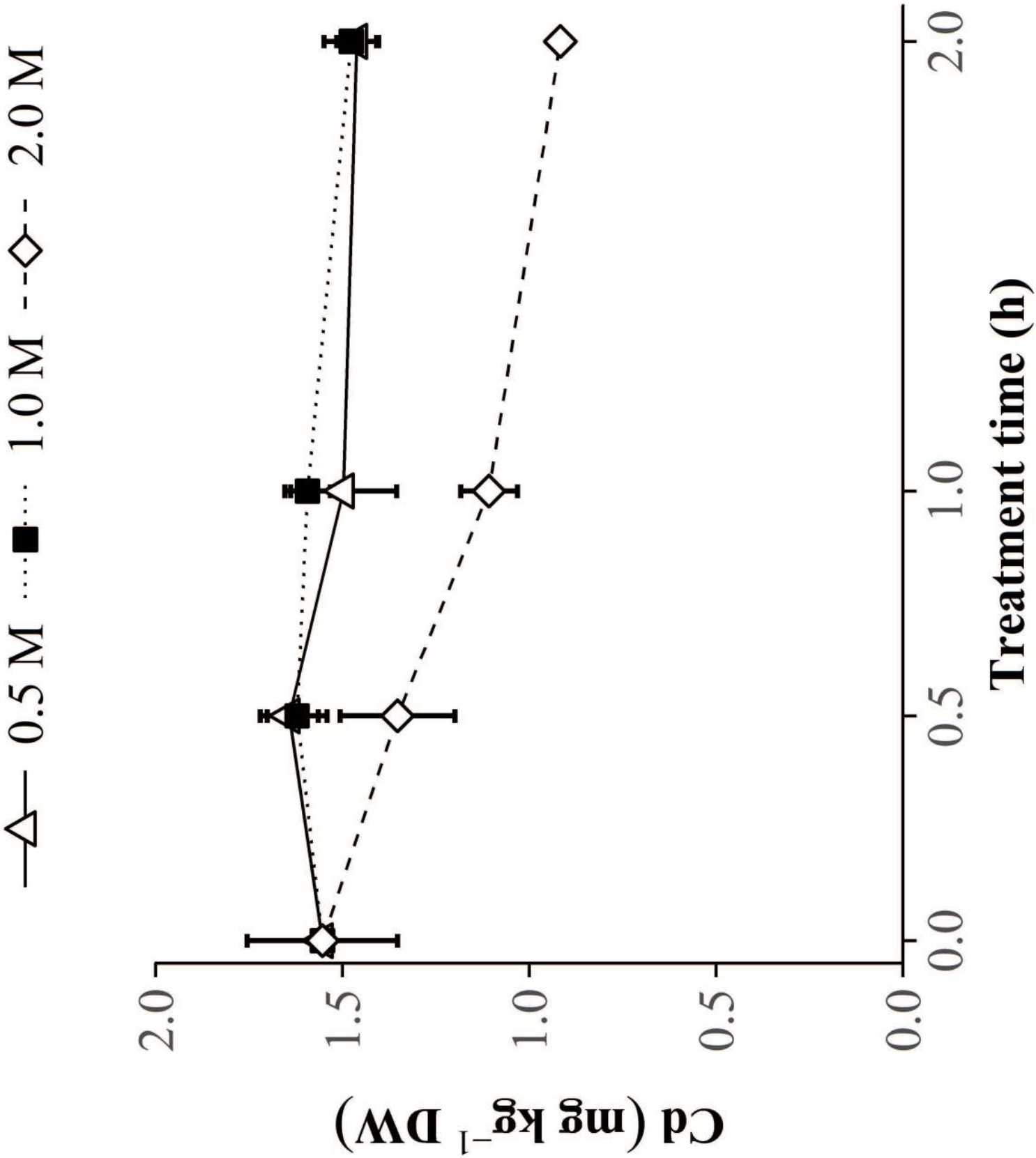

**Table 1:** Initial content of the potentially harmful compounds Cd, I and iAs, expressed in mg $kg^{-1}$ DW of *A. esculenta* and *S. latissima* prior to soaking treatments. Values are given as mean ± standard error (n = 3).

| | *A. esculenta* (May 2015) | *A. esculenta* (May 2016) | *S. latissima* (May 2015) | *S. latissima* (June 2015) | Limit values (French recommendation) [a] |
|---|---|---|---|---|---|
| Cd | 2.01 ± 0.09 | 1.55 ± 0.20 | 0.22 ± 0.03 | 0.27 ± 0,01 | 0.5 |
| I | 213 ± 12 | - | 4898 ± 166 | 6568 ± 398 | 2000 |
| iAs | 0.22 ± 0.04 | - | 0.16 ± 0.02 | 0.23 ± 0.01 | 3 |

[a] Source: CSHPF (1999) and AFSSA (2009). Values expressed in mg $kg^{-1}$ DW.

**Table 2:** Chemical composition of the seaweed biomass prior to ($t_0$), and after 22 h soaking treatments as well as variation in the surface color of the samples ($\Delta E$). Concentrations are expressed in g $(100\ g)^{-1}$ DW, except for the fucoxanthin content expressed in mg $kg^{-1}$ DW and the dimensionless $\Delta E$. Values are given as mean ± standard error (n = 3).

| | *A. esculenta* | | |
|---|---|---|---|
| | **$t_0$ [a]** | **SW [a] t = 22 h** | **FW t = 22 h** |
| Dry weight (%) | 17.2 ± 0.8 | 13.9 ± 0.7 * | 8.6 ± 0.1 * |
| *Minerals* | | | |
| Ash | 24.2 ± 1.4 | 27.0 ± 1.6 | 13.4 ± 0.8 * |
| Na | 3.92 ± 0.23 | 5.21 ± 0.20 * | 0.93 ± 0.03 * |
| K | 4.2 ± 0.3 | 4.4 ± 0.5 | 2.1 ± 0.3 * |
| *Carbohydrates* | | | |
| Total carbohydrates | 40.7 ± 1.5 | 37.7 ± 1.5 | 45.5 ± 1.5 |
| Alginate | 19.9 ± 0.5 | 18.6 ± 0.4 | 26.7 ± 0.9 * |
| Mannitol | 10.5 ± 0.4 | 10.4 ± 0.3 | 6.6 ± 0.7 |
| Glucose | 8.5 ± 1.9 | 7.5 ± 1.4 | 10.1 ± 1.8 |
| Fucose | 1.25 ± 0.03 | 0.98 ± 0.04 * | 1.76 ± 0.14 |
| Proteins | 10.5 ± 0.2 | 9.9 ± 0.1 | 12.7 ± 0.2 * |
| Polyphenols | 3.43 ± 0.08 | 2.55 ± 0.09 * | 2.93 ± 0.29 |
| Fucoxanthin | 871 ± 53 | 829 ± 45 | 1052 ± 114 |

| | | | |
|---|---|---|---|
| $\Delta E$ | - | 11 ± 3 | 8 ± 2 |

| | *S. latissima* | | | *S. latissima* | |
|---|---|---|---|---|---|
| | | **SW** [a] | **FW** | | **WW** |
| | **$t_0$** [a] | **t = 22 h** | **t = 22 h** | **$t_0$** | **t = 22 h** |
| Dry weight (%) | 16.1 ± 1.2 | 13.5 ± 0.8 | 7.7 ± 0.8 * | 13.8 ± 0.6 | 5.6 ± 0.2 * |
| *Minerals* | | | | | |
| Ash | 26.2 ± 2.6 | 30.0 ± 2.1 | 16.7 ± 0.2 | 30.5 ± 1.1 | 15.5 ± 0.2 * |
| Na | 3.6 ± 0.2 | 4.3 ± 0.2 | 1.2 ± 0.1 * | 3.85 ± 0.04 | 0.97 ± 0.02 * |
| K | 6.5 ± 1.1 | 7.2 ± 0.8 | 2.7 ± 0.1 | 8.17 ± 0.42 | 1.86 ± 0.03 * |
| *Carbohydrates* | | | | | |
| Total carbohydrates | 46.1 ± 2.63 | 40.0 ± 1.0 | 49.6 ± 2.1 | 39.6 ± 1.0 | 47.1 ± 1.8 |
| Alginate | 21.5 ± 0.5 | 23.1 ± 1.5 | 38.2 ± 0.8 * | 21.0 ± 0.5 | 41.1 ± 0.9 * |
| Mannitol | 17.6 ± 1.2 | 12.3 ± 2.2 | 3.1 ± 0.8 * | 14.8 ± 1.1 | n.d. |
| Glucose | 5.0 ± 2.0 | 2.8 ± 0.6 | 4.3 ± 0.4 | 1.9 ± 0.4 | 1.3 ± 0.1 |
| Fucose | 0.76 ± 0.03 | 0.89 ± 0.07 | 1.50 ± 0.00 * | 0.89 ± 0.07 | 1.75 ± 0.03 * |
| Proteins | 10.6 ± 0.3 | 11.6 ± 0.2 | 12.7 ± 0.7 * | 11.3 ± 0.3 | 12.6 ± 0.2 * |
| Polyphenols | 0.69 ± 0.04 | 0.49 ± 0.04 * | 0.22 ± 0.01 * | 0.44 ± 0.02 | 0.26 ± 0.01 * |
| Fucoxanthin | 431 ± 19 | 360 ± 27 | 343 ± 24 * | 526 ± 27 | 201 ± 34 * |

| | | | | | |
|---|---|---|---|---|---|
| $\Delta E$ | - | 6 ± 2 | 7 ± 1 | - | 27 ± 1 |

The symbol * indicates a significant different level of a compound measured after 22 h storage as compared to the initial value measured at $t_0$ (paired sample *t*-test, $p < 0.05$).

[a] *data published in Stévant et al. (2017)*

**Table 3:** Total variation in dry weight, Cd, ash, Na and surface color ($\Delta E$) analyzed after 2 h soaking treatments in 0.5 M, 1.0 M and 2.0 M NaCl. Values are given as mean ± standard error (n = 3).

| | *A. esculenta* | | | |
|---|---|---|---|---|
| | | **0.5 M** | **1.0 M** | **2.0 M** |
| | **$t_0$** | **t = 2 h** | **t = 2 h** | **t = 2 h** |
| Dry weight (%) | 13.4 ± 1.3 | 10.9 ± 0.1 | 14.4 ± 0.2 | 15.4 ± 0.2 |
| Cd (mg $kg^{-1}$ DW) | 1.55 ± 0.20 | 1.46 ± 0.05 | 1.48 ± 0.07 | 0.92 ± 0.02 * |
| Ash (g (100 g)$^{-1}$ DW) | 29.3 ± 1.7 | 35.0 ± 1.0 * | 39.9 ± 0.5 * | 45.1 ± 0.7 * |
| Na (g (100 g)$^{-1}$ DW) | 4.69 ± 0.18 | 6.36 ± 0.01 | 9.44 ± 0.05 * | 12.72 ± 0.13 * |
| $\Delta E$ | - | 9 ± 3 | 18 ± 2 | 19 ± 9 |

The symbol * indicates significant variations of a parameter during treatment (RM ANOVA, $p < 0.05$).

**Table 4:** Daily dose of potentially harmful compounds from the consumption of *A. esculenta* and *S. latissima* following their maximum concentrations of Cd, I, and iAs. Daily doses from risk estimators are based on TWI, PMTDI and for a 70 kg adult.

| **Element** | **Species** | **Maximum concentration (mg kg$^{-1}$ DW)** | **Daily dose for 3,3 g consumption (mg day$^{-1}$)** | **Daily dose for 12,5 g consumption (mg day$^{-1}$)** | **Daily dose from risk estimators (mg day$^{-1}$)** |
|---|---|---|---|---|---|
| Cd | *S. latissima* | 0.27 | 0.0009 | 0.0034 | 0.025 [a] |
| | *A. esculenta* | 2.01 | 0.007 | 0.025 | |
| I | *S. latissima* | 6568 | 21.7 | 82.1 | 1.19 [b] |
| | *A. esculenta* | 213 | 0.7 | 12.7 | |
| iAs | *S. latissima* | 0.23 | 0.0008 | 0.0029 | 0.021 [c] |
| | *A. esculenta* | 0.22 | 0.0007 | 0.0027 | |

[a] from TWI (EFSA, 2012), [b] from PMTDI (WHO, 1989) and [c] from $BMDL_{01}$ (lower bound) (EFSA CONTAM panel, 2009)

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