REPORT 2115 | Pierrick Stévant, Arne Duinker, Wenche Emblem Larssen, Johanna Liberg Krook, Ingri Mjelde Birkeland, Linn Anne Brunborg Bjelland, Annelise Chapman, Solbjørg Hogstad, Rita Nilsen McStay, Harald Sveier

METHODS FOR IODINE-REDUCTION OF SUGAR KELP TO PRODUCE SAFE, FLAVOURFUL AND NUTRITIOUS INGREDIENT TO THE FOOD INDUSTRY

Final report from the SensAlgae project

PROJECT TITLE	Methods for iodine-reduction of sugar kelp to produce safe, flavourful and nutritious ingredient to the food industry
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FOREWORD

This work was coordinated by Møreforsking and conducted between May 2020 and October 2021 in collaboration with the Institute of Marine Research, Orkla Foods Norge AS, Ocean Forest AS, Tango Seaweed AS and Mattilsynet (the Norwegian Food Safety Authority) as part of the SensAlgae Project.

The project was financed by the Regional Research Fund for the Møre & Romsdal region.

SUMMARY

The Norwegian seaweed sector based on cultivated kelp biomass (mostly the sugar kelp *S. latissima*) is growing rapidly. The high iodine content of *S. latissima* is a major challenge hampering the commercial development of food products containing kelp ingredients. During this project, the production and quality of iodine-reduced *S. latissima* and its potential as a food ingredient for the Nordic food industry was investigated. The objectives of the project were to i) identify processing methods to provide safe, nutritious and tasty ingredients from sugar kelp and ii) test one or more iodine-reduced ingredients from *S. latissima* in a commercial product from the food industry.

Both established (blanching with freshwater) and new methods (e.g. steam treatment, use of rotavapor, seawater blanching) were tested on either fresh, fermented or frozen/thawed biomass. Due to large variations in the iodine content of the start material of the different experiments, it has been difficult to achieve predictably low iodine content below 2000 mg kg^{-1} (dry weight, DW). Significant iodine reduction was only achieved by the addition and subsequent removal of liquid during the process. In contrast, no iodine reduction was achieved in processes where the liquid (i.e. added water and/or drip loss) was retained. This indicates that there is no evaporation of iodine (mainly present in the form of water-soluble iodide) under the tested conditions. Sufficient iodine reduction below 2000 mg kg^{-1} DW was achieved by the combination of seawater blanching and fermentation (850 mg kg^{-1} DW) suggesting that the iodine content reduction is mainly due to the iodide in the kelp leaching in the surrounding liquid. The analysis of additional seawater-blanched samples under commercial production settings involving a larger volume of seawater revealed an even lower iodine content (190 mg kg^{-1} DW). These results indicate that blanching using salt water has the potential to reduce the iodine content in *S. latissima* to safe levels for food applications. The kelp-to-water ratio (either fresh- or seawater) may be an important factor determining the extent of iodine-reduction during the process. Seawater-blanching both with and without subsequent fermentation limited dry matter (i.e. nutrient) losses compared to blanching in freshwater even though loss of minerals (especially potassium) was observed. This processing method will be investigated further in a second-stage research project.

The sensory properties (flavour/aroma and texture) of a selection of *S. latissima* ingredients were evaluated using a trained panel. Significant differences were measured among steam-treated, freshwater and seawater-blanched samples, as well as seawater-blanched then fermented, and *A. esculenta* samples (used as reference). All samples, except freshwater-blanched, had a fairly distinct umami flavour. This indicates that water-soluble flavour components (e.g. monosodium glutamate) are better retained in seawater-blanched samples compared to freshwater-blanched material. A product prototyping experiment demonstrated that the use of kelp ingredients even at low inclusion level (0.5 % of a portion) makes a significant contribution to the flavour of a typical commercial food product (dehydrated spinach soup). Specifically, the ingredients (fermented and seawater-blanched) contributed with saltiness highlighting their potential as salt replacement in the food industry.

CONTENTS

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 such as carbon emissions, freshwater use and decreased biodiversity. In this context, the aquaculture of macroalgae is considered being a part of the solution for the sustainable production of food and animal feed (Rotter et al. 2020). Macroalgal biomass can be cultivated on a large scale in coastal areas without need for chemicals and fertilizers, and without competing for freshwater or soil resources. If properly regulated, it will also provide ecosystem services, such as supporting biodiversity and mitigating CO2 emissions and eutrophication (Hasselström et al. 2018). The aquaculture of macroalgae, particularly the sugar kelp (*Saccharina latissima*) is in rapid development in Europe and specially in Norway (Stévant et al. 2017c).

Macroalgae are a rich source of nutrients, e.g. minerals, fibres, vitamins, trace elements and other health promoting compounds, providing benefits beyond basic nutrition (Holdt and Kraan 2011; Wells et al. 2017). The exploitation of wild macroalgal resources in Western societies has mainly focused on the industrial extraction of polysaccharides as gelling agents. The use of macroalgae for food items and as health promoting ingredients in the food industry has gained increasing interest over the past decades. Macroalgae are recognized as a source of bioactive compounds with applications in human nutrition such as functional ingredients (e.g. for salt replacement) and dietary supplements. A wide range of edible species are also prized for their unique flavours and are extensively used in everyday applications, particularly in Asia. Despite this, the Nordic and European food industry has not yet incorporated this resource in everyday products. This is partly due to knowledge gaps concerning efficient post-harvest methods suitable to provide safe, nutritious and flavourful ingredients from macroalgae in sufficient volumes to the food industry.

Macroalgae may accumulate potentially toxic elements (PTEs) such as non-essential metals (e.g. cadmium and inorganic arsenic) with negative effects on human health. The essential element iodine is occurring in high amounts in kelps especially *S. latissima* which may pose a health risk to the consumer when using the biomass in food applications (Stévant et al. 2017a; Roleda et al. 2018). Excessive iodine consumption may lead to thyroid dysfunction disorders (Miyai et al. 2008). At the same time, optimal iodine intake is important for foetal development and development of motoric skills in young children. Iodine deficiency is recognised in European population (Andersson et al. 2007), and macroalgae, especially kelps, could represent a dietary source of iodine which is relevant in a public health perspective.

Food processing techniques have the potential to minimize food safety risks. The iodine content of kelps may be reduced drastically (over 90 %) by heat treatment with tap water. However, this process is also associated with major losses of nutrients (e.g. minerals, vitamin C, phenolic compounds, free amino acids) (Stévant et al. 2017a; Nielsen et al. 2020). In food production, hurdle technology may be used to minimize food safety risks from the raw

material while retaining specific attributes (nutrients, flavour). Sustainable, as well as energy- and cost-efficient processing strategies must therefore be established to supply safe, nutritious, and flavourful ingredients from cultivated kelps to be attractive for the food industry.

The current trends on local, sustainable, healthy and vegetarian/vegan foods are in favour of macroalgae, but flavour needs to meet the consumers' demands. The cultivated kelp species *S. latissima* and *Alaria esculenta* have flavour and physicochemical characteristics that can be used to enhance the palatability of the food to which they are added (Mouritsen et al. 2012; Chapman et al. 2015). However, neophobia (Birch et al. 2019) and consumers' negative associations with macroalgae have been identified as major obstacles to a broader use in the Western diet (Mouritsen 2017). Flavour is a major factor determining the consumer acceptance of foods. The sensory characteristics of macroalgae are connected to their levels of flavour-active compounds (e.g. free amino acids, volatile compounds, minerals) which are affected during the processing and storage of the raw material (Bruhn et al. 2019; Stévant et al. 2020). At present, there are no established industrial standards connected to the sensory quality of macroalgae. It is therefore of prime importance to further the knowledge on how their sensory characteristics are impacted by relevant processes in commercial production.

The main objective of this study was to i) test and identify efficient methods to reduce the iodine content of cultivated *S. latissima*), ii) document changes in the sensory profile of the raw material following different processing methods and iii) to test the use of an iodine-reduced raw material as ingredient into a commercial product for the food industry.

2. MATERIAL & METHODS

2.1. TESTING OF DIFFERENT IODINE-REDUCING TREATMENTS ON SUGAR KELP

2.1.1. BLANCHING AND STEAMING TREATMENTS (EXPERIMENT 1)

Biomass of *S. latissima* was cultivated at Tango Seaweed AS (Herøy, Møre & Romsdal), harvested on June 4th, 2020, and transported to the laboratory (Møreforsking) within a few hours for further processing.

The water blanching treatment of 1 kg fresh samples was conducted in a 5L-water bath at 45 °C for 120 sec as reported suitable to effectively reduce the iodine content of *S. latissima* (Nielsen et al. 2020). Steam treatments were conducted by placing blades of sugar kelp in a compartment of a benchtop food steamer (Phillips HD 9140). Samples were taken after 5, 10 and 20 minutes of treatment. All treatments were performed in 3 replicates. Unprocessed fresh samples were taken as control. All samples were vacuum-packed and stored frozen until freeze-dying prior to iodine analysis. The moisture content (MC) of the samples was determined gravimetrically by drying ca. 5 g of sample at 105 °C until constant weight.

2.1.2. TESTING POTENTIAL IODINE-REDUCING TREATMENTS ON FERMENTED BIOMASS (EXPERIMENT 2)

Cultivated *S. latissima* at Ocean Forest AS (Austevoll, Vestlandet) was harvested in May 2020 and immediately conserved by lactic acid fermentation. A commercial mixture of lyophilized lactic acid bacteria (*Lactobacillus plantarum, Pediococcus acidilactici, Pediococcus pentosaceus*) was added to freshly harvested and shredded biomass maintained in anaerobic conditions resulting in a pH drop, below 4.0 within 24 h. The stabilized fermented *S. latissima* was transported and stored cold at Orkla's product development facility (Orkla Food Norge AS, Arna, Norway). A pH of 3.5 was measured from the fermented raw material.

Several food-processing methods were screened at the facility on September 22nd, 2020 to assess their potential as iodine-reducing treatments. Each process was tested on representative subsamples of fermented material consisting of solid pieces of macroalgal fronds and drip water (**fig. 1**).

- **Rotary evaporator (rotavapor)**: 200 mL of fermented macroalgae was placed in a rotavapor (fig. 1B). The effect of treatment temperature (45 and 80 °C), duration (within 60 min) and vacuum vs. no vacuum on the iodine content of the samples was tested.
- **Aeration while heating**: 200 mL of fermented material was transferred in a beaker placed in a water bath at 45 °C for 60 min. Aeration was provided by an aquarium pump connected to an air-stone placed in the beaker (fig. 1C).

- **Cooking**: 100 mL of fermented material was placed in a pot and cooked (ca. 100 °C) for 60 min (fig. 1D). Tap water was added at regular intervals during the process (ca. 130 mL in total) to compensate for evaporation and avoid burning of the samples.
- **Freeze-drying**: 150 mL of fermented material was freeze-dried.

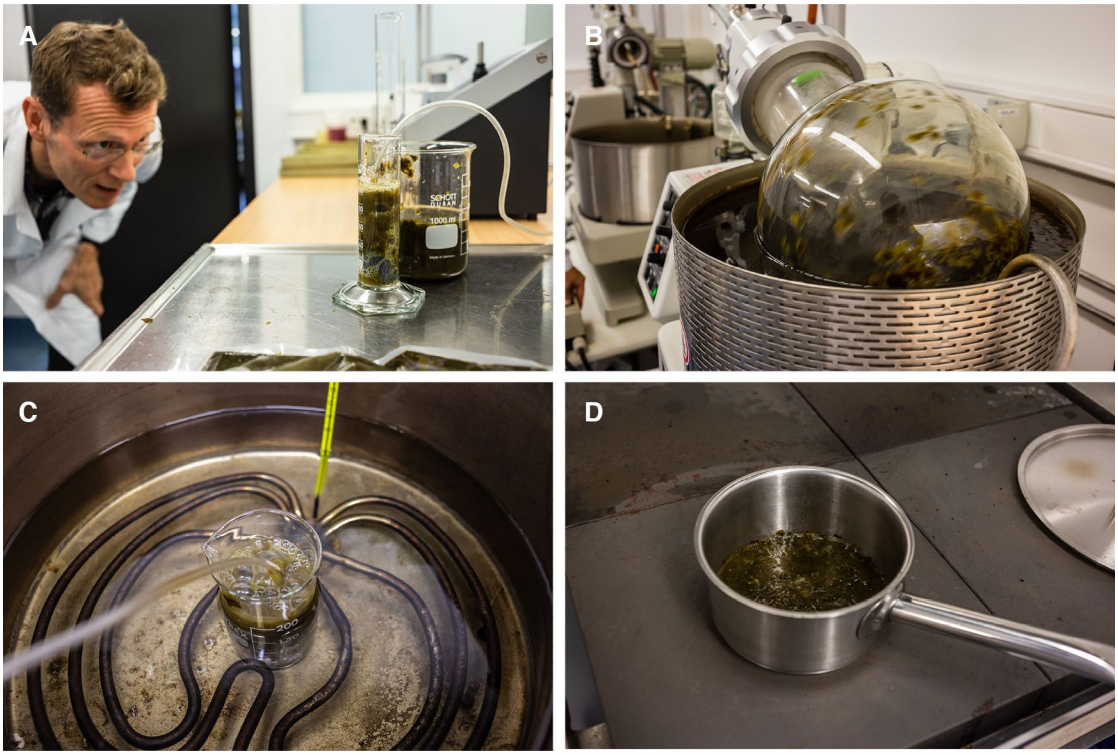

Fig. 1: Food processing methods screened at Orkla's product development facility (Torofabrikken, Arna, Norway): (A) fermented *S. latissima* was used as start material in (B) rotavapor treatment, (C) aeration treatment under heating and (D) cooking.

Samples of the original batch of fermented *S. latissima* were taken at the beginning and at the end of the experiment to detect any variation in the iodine content of the raw material once exposed to air. All samples produced were stored in a freezer and cryo-grinded prior to iodine analysis.

2.1.3. TESTING POTENTIAL IODINE-REDUCING TREATMENTS ON FROZEN AND THAWED BIOMASS AND MEASUREMENT OF PARTITIONING OF IODINE IN FROZEN/THAWED AND FERMENTED BIOMASS (EXPERIMENT 3)

Cultivated *S. latissima* at Seaweed Solutions AS (Frøya, Trøndelag) was harvested in May 2020, then vacuum-packed and frozen within few hours after harvest. The samples were transported to Orkla's product development facility than thawed prior to a new session of testing conducted on October 22nd, 2020. During this session, the same processes as described in the

previous section (experiment 2) were tested. In addition, the iodine content of frozen and thawed biomass from Seaweed Solutions AS and fermented *S. latissima* samples from Ocean Forest AS including both solid pieces of kelp fronds and drip water was measured.

2.1.4. COOKING OF FERMENTED BIOMASS (EXPERIMENT 4)

Fermented biomass of *S. latissima* from Ocean Forest AS including drip water was used in this experiment to study the fate of iodine during the cooking process. Tap water (200 mL) was added to approximately 50 g of fermented kelp and boiled for 15 min. The solid kelp material was then removed, and the broth was boiled for another 45 min while more water was added to prevent total evaporation. The total iodine in the initial raw material, in the boiled solid kelp and in the cooking broth were measured. The treatments were performed in 3 replicates.

An iodine speciation analysis of the fermented start material was performed at the Danish Technical University (DTU).

2.1.5. SEAWATER BLANCHING AND FERMENTATION OF WILD HARVESTED BIOMASS (EXPERIMENT 5)

Biomass of fresh *S. latissima* was harvested by Arctic Seaweed AS from wild beds near Misje (Western Norway) on February 2nd, 2021, then stored for one day in a mesh bag submerged in free-flowing seawater. On the following day, several batches of kelp biomass (5 kg) were blanched in seawater (20 L) at 45 °C for 120 sec. The same blanching water was used repeatedly. A blanching treatment following the same protocol, followed by fermentation using *L. plantarum* was conducted on another batch of wild *S. latissima* harvested few weeks later at the same location.

2.2. CHEMICAL ANALYSES

2.2.1. IODINE

Samples were added 1 mL tetrametylammonium hydroxyide (TMAH) and 5 mL deionized water before extraction at 90 °C ± 3 °C for 3 h. The samples were then diluted and centrifuged. Prior to quantification, the samples were filtered through a 0.45 µm single use syringe and disposal filter. Tellurium which was used as an internal standard to correct for instrument drift. Iodine concentration in the samples was determined by inductively coupled plasma-mass spectrometry (ICP-MS).

Iodine species were extracted by enzymatic extraction and subsequently determined by using ion chromatography coupled to inductively coupled plasma mass spectrometry (IC-ICPMS).

2.2.2. NUTRIENT CONTENT

A selection of samples was analysed by an accredited laboratory (ALS Laboratory Group Norway AS) for their basic nutrient profile. The variables analysed and corresponding methods are listed in **table 1**.

Table 1: Variables entering the nutritional profile analysed on a selection of *S. latissima* samples.

Variable	Analytical method
Moisture content (MC)	Drying of the samples at 105 °C until constant weight. Gravimetric determination of the samples' MC.
Ashes	Combustion of the dried samples in a muffle furnace at 590 °C for 12 h. Quantification of the ashes gravimetrically as the residue from combustion.
Fatty acids (FA) composition	Conversion of FAs into free FAs by saponification then into their methyl esters by treatment with methanolic acetyl chloride before extraction with heptane. Identification and quantification of saturated FAs (SFA), monounsaturated FAs (MUFA) and polyunsaturated FAs (PUFA) is achieved by gas chromatography using flame ionization detection (FID).
Proteins	Nitrogen is determined by complete combustion of the sample in the presence of oxygen using the Leco TruMac N analyser or the Elementar Rapid Max N Exceed analyser. The resulting gases pass through various filters to remove interfering gases/particles and nitrous oxide gases are reduced to nitrogen by means of a heated catalyst. The filtered gases are analysed using a thermal conductivity cell, with helium being used as the reference gas and the carrier gas. The output voltage that results is processed by the internal computer and converted to give the nitrogen content of the sample which is then converted into a protein value using a factor of 5 as previously reported suitable to predict the protein content of macroalgae (Angell et al. 2016).
Carbohydrates	The carbohydrate content (including fibres) was estimated by difference: Carb = 100 – (moisture + ash + FA + protein)

2.2.3. DETERMINATION OF METALS (INCLUDING CD, HG, PB, FE, ZN AND SE)

The metal content of the samples was determined following an internal standard method developed at the Institute of Marine Research (IMR method 197). Two parallels were weighed from each sample. The metals were determined by ICP-MS after decomposing in microwave oven as described by Julshamn et al. (2007). The method is accredited for cadmium (Cd), mercury (Hg), lead (Pb), zinc (Zn) and selenium (Se).

2.2.4. DETERMINATION OF MACRO MINERALS (NA, MG, K, CA AND P)

The macro-mineral content of the samples was determined following the IMR method 382. Two parallels were weighed from each sample. The concentrations of the macro minerals (Na, Mg, K, Ca and P) were determined by ICP-MS, after acid wet digestion in a microwave oven. The concentrations were determined using an external calibration (standard curve) and the method is accredited according to ISO 17025.

2.3. SENSORY ANALYSIS

A generic descriptive analysis (GDA) was used to characterize and compare the sensory profile of four samples obtained from *S. latissima* and a reference sample consisting of dried *A. esculenta* cultivated in Norway. The sensory panel consisted of 9 judges selected and trained according to the guidelines in ISO:8586 (2012). The samples were evaluated with regards to 10 sensory attributes (**table 2**) used in previous sensory evaluations of *S. latissima* (Stévant et al. 2018; Stévant 2019), describing flavour and odour in the one hand and texture on the other hand. The panel members were trained to the evaluation of two characteristic samples using a scale ranging from 0 to 9 (lowest to highest intensity). All samples were coded with three-digit numbers and evaluated in two duplicate sessions. Due to the restrictions for physically gathering a sensory panel from the COVID-19 pandemic, 3 out of 9 panelists participated to the evaluation online after receiving the samples. The sensory evaluation program RedJade (Tragon Corporation, Palo Alto, CA, USA) was used to collect sensory data. The sensory data was processed according to the General Data Protection Regulation (GDPR).

Table 2: Sensory attributes, and their definitions, associated to the samples assessed

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

2.4. FOOD PROTOTYPING

Selected kelp ingredients i.e. fermented *S. latissima* (from Ocean Forest AS, see section 2.1.2) and seawater-blanched (from Arctic Seaweed AS, section 2.1.5) were added to a commercial product, namely a dehydrated spinach soup (Toro spinatsuppe), in order to evaluate the flavour contribution of such ingredients into a commercial food formulation.

Each portion consisted in 19.75 g of dried soup base mixed with 1.9 dL water and 0.6 dL milk. The final weight of a portion of soup was approximately 270 g. In test products, kelp ingredients were added to the soup at 0.5 and 1 % inclusion levels from adding 0.13 and 0.26 g of dried kelp, equivalent to 1.3 and 2.6 g of rehydrated ingredient. These inclusion levels were determined based on the calculated dietary iodine contribution from these ingredients. The food prototypes were evaluated by 16 panellists in a tetrad test to detect perceptible differences between kelp-containing products and standard products without kelp ingredient. Assessors were given four samples i.e. two from one group (with kelp) and two from another group (control without kelp) and were instructed to sort the samples based on their similarity. The assessors were also asked to describe the samples during each repetition.

2.5. DATA ANALYSIS

The results from the analyses of replicate samples were expressed as mean ± standard deviation and analysed using R (version 4.1.0, R Development Core Team (2021)).

A one-way analysis of variance (ANOVA, R function aov) was used to detect significant differences ($p < 0.05$) among treatments *S. latissima* samples from experiment 1 (section 2.1.1) regarding the iodine and moisture content of the samples.

The sensory results were analysed using a mixed model ANOVA (R function lmer (Bates et al. 2015)). In this model, individual panellists were treated as random factors to detect significant differences in sensory profile (scores for each attribute) among samples. The Benjamini-Hochberg procedure was applied to control the false discovery rate under multiple testing. Tukey's honest significant differences (HSD) were computed for the pairwise comparison of samples. A principal component analysis (PCA, R function prcomp) based on covariance matrix (no scaling) was applied to visualize sensory profiles among samples.

The results from the evaluation of food prototypes using the tetrad test were analysed by binomial test (R function binom.test). The critical number of correct answers to conclude that samples were perceptibly different was fixed at 10 ($\alpha = 0.05$).

3. RESULTS

3.1. BLANCHING AND STEAMING TREATMENTS (EXPERIMENT 1)

The untreated control samples of *S. latissima* used in this experiment were characterized by a relatively high iodine content i.e. nearly 8000 mg kg^{-1} (**fig. 2**), compared to published data (Stévant et al. 2017a; Nielsen et al. 2020; Duinker et al. 2020). The blanching treatment (45 °C for 120 sec) significantly reduced the iodine content of the samples but not below the threshold value of 2000 mg kg^{-1} established by the French Food Safety Authority. The iodine levels of steam-treated samples, regardless of treatment time was not significantly different from those measured in untreated samples. Slightly lower average iodine levels were measured after longer treatments (10 and 20 min) compared to 5-min treatments. The MC content of *S. latissima* samples after stream treatment remained relatively stable compared to blanching which resulted in significantly higher MC than the control samples.

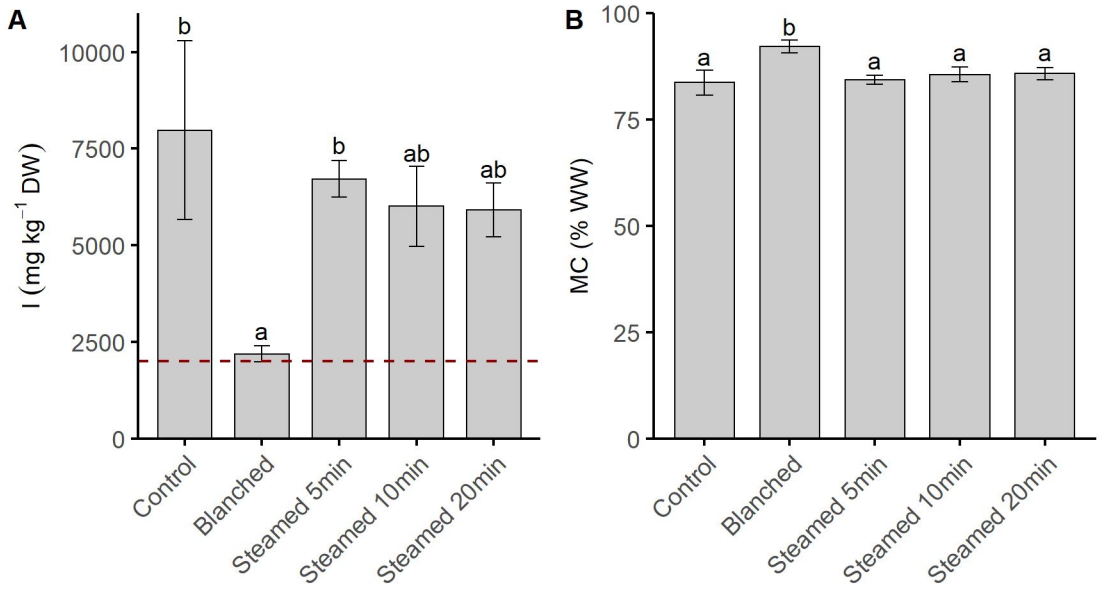

Fig. 2: Iodine (A) and moisture content (B) of unprocessed as well as blanched and steam-treated samples of *S. latissima*. The results are expressed relatively to the samples' dry weight (DW) and wet weight (WW) in plot A and B respectively. Values are given as mean ± standard deviation (n = 3 except for control samples, n = 7). Different subscript letters in the same row indicate significant differences among samples (Tukey HSD, $p < 0.05$). The red dashed line represents the threshold value of 2000 mg kg^{-1} iodine in macroalgae established by the French Food Safety Authority.

3.2. TESTING POTENTIAL IODINE-REDUCING TREATMENTS ON FERMENTED BIOMASS (EXPERIMENT 2)

The control samples of this experiment consisted of fermented biomass of *S. latissima* including both the solid (i.e. the kelp fronds) and the liquid fraction (i.e. the drip water). None of the heating treatments tested in this experiment (**fig. 1**) including the use of rotavapor (at 45 and 80 °C) with and without vacuum, a 60 °C water bath with aeration and a 60-min cooking treatment successfully reduced the iodine content of *S. latissima* (**fig. 3**). No variation was detected in the iodine content of the untreated fermented raw material at the beginning and at the end of the experiment indicating that no iodine evaporation occurred in the original batch once exposed to air.

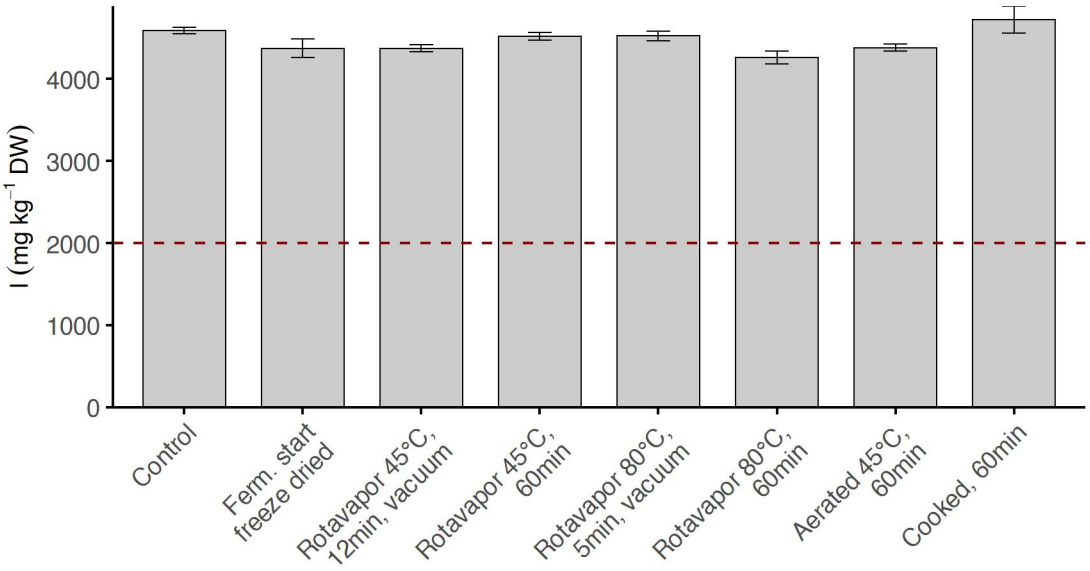

Fig. 3: Iodine content of fermented *S. latissima* samples following different treatments. Values are given as mean ± standard deviation (n = 3). The red dashed line represents the threshold value of 2000 mg kg^{-1} iodine in macroalgae established by the French Food Safety Authority.

3.3. TESTING POTENTIAL IODINE-REDUCING TREATMENTS ON FROZEN AND THAWED BIOMASS AND MEASUREMENT OF PARTITIONING OF IODINE IN FROZEN/THAWED AND FERMENTED BIOMASS (EXPERIMENT 3)

In this experiment, the same treatments as those tested in experiment 2 (see previous section) were applied to frozen and thawed samples of *S. latissima*. The control untreated samples were freeze-dried from frozen state i.e. without thawing. As observed in the previous experiment, no iodine reduction was achieved from these treatments on frozen and thawed samples (**fig. 4**).

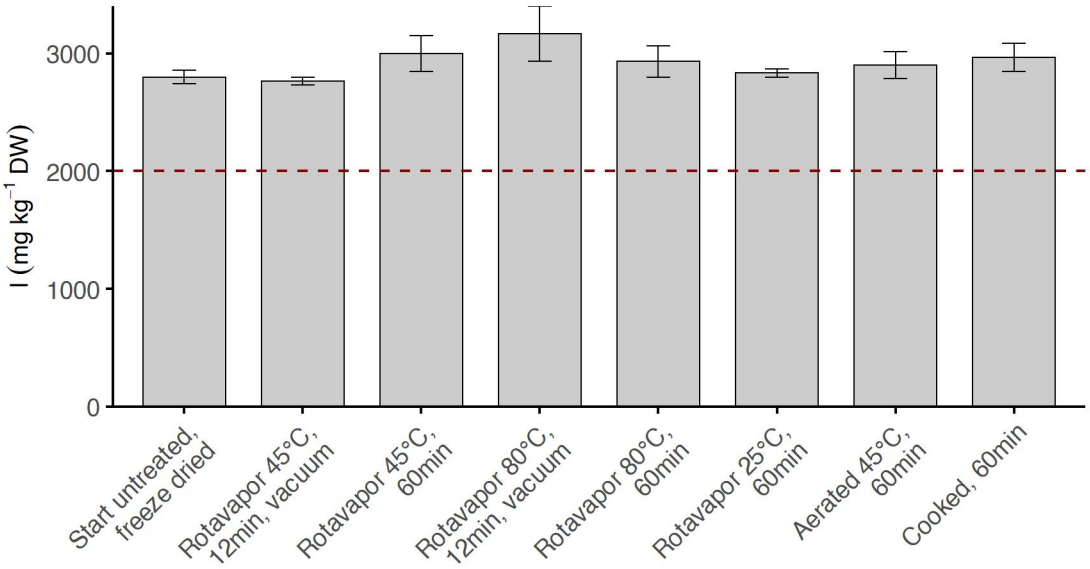

Fig. 4: Iodine content of frozen and thawed samples of *S. latissima* following different treatments. Values are given as mean ± standard deviation (*n* = 3). The red dashed line represents the threshold value of 2000 mg kg^{-1} iodine in macroalgae established by the French Food Safety Authority.

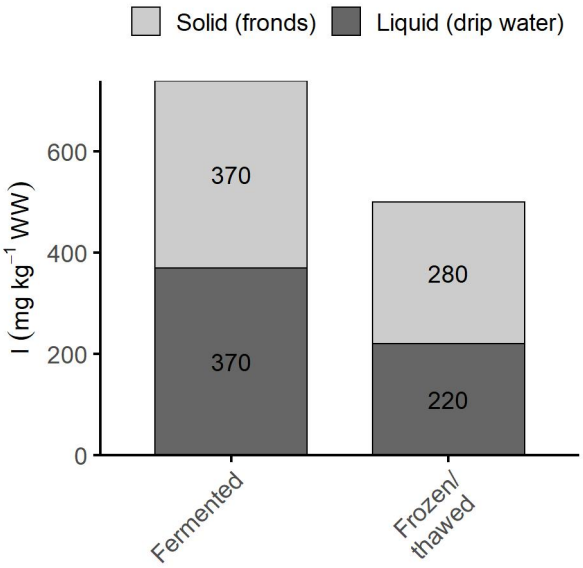

Fig. 5: Iodine content measured in both the solid and liquid fractions of fermented and frozen/thawed samples of *S. latissima*. The results are from single measurements and expressed relatively to the WW of the samples.

The results from the preliminary study of the partitioning of the iodine between the solid and liquid fractions of fermented *S. latissima* in the one hand and frozen and thawed biomass on the other hand, is presented in **fig. 5**. In both samples, the iodine was found to be evenly distributed across the solid fronds and the liquid fraction.

3.4. COOKING OF FERMENTED BIOMASS (EXPERIMENT 4)

A third cooking experiment was performed with focus on dissolved iodide in the broth after boiling. Only 14 % of the initial amount of iodine contained in the fermented start material was left in the solid kelp after boiling for 15 min (**fig. 6**). It should be noted that the sum of the iodine measured in the solid kelp and in the broth after boiling for 15 min accounts for 90 % of the total initial iodine content. This may be due to part of the iodine (i.e. 10 %) being lost by evaporation. No substantial iodine reduction was achieved from boiling the broth for another 45 minutes compared to the levels measured after 15 min boiling. The concentration of iodine in the kelp is the same as in the broth (i.e. 117 and 124 µg g^{-1} WW respectively). Hence, it can be expected that addition of larger volume of tap water would result in lower concentrations in the broth and solid kelp.

The iodine content of the solid kelp following 15-min boiling was 2 025 ± 110 mg kg^{-1} DW. An iodine speciation analysis of the initial fermented *S. latissima* revealed that 93 % of the iodine is present in the form of iodide, both in fermented and fresh frozen kelp. Other species of iodine were below the limit of quantification.

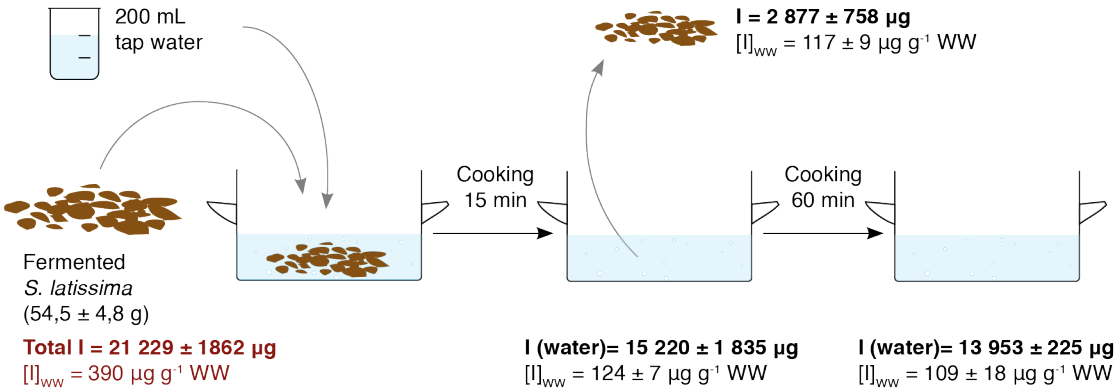

Fig. 6: Schematic summary of a cooking experiment of fermented *S. latissima*. The fate of iodine during the process was monitored by measuring the total iodine (in bold) and iodine concentration ($[I]_{ww}$) in the initial raw material as well as in the cooked solid kelp fronds and in the broth. Water was added during the process to prevent total evaporation. Values are given as the mean of replicate samples (n = 3 ± standard deviation).

3.5. SEAWATER BLANCHING AND FERMENTATION OF WILD HARVESTED BIOMASS (EXPERIMENT 5)

Seawater blanching was tested on wild harvested biomass of *S. latissima* using the same protocol as the one used in the experiment 1, i.e. 45 °C during 120 min. Another batch of *S. latissima* was also harvested to test the combination of seawater blanching followed by a fermentation process using *L. plantarum*. Lower iodine content was achieved by seawater blanching compared to the control sample although not below 2000 mg kg^{-1} DW (**Table *3***). Seawater blanching followed by fermentation resulted in an iodine content below this threshold. The MC of both samples remained relatively stable compared to the untreated control. It should be noted that these fermented samples underwent a different commercial fermentation process than the samples used in experiment 2-4. The process in experiment 5 involved the use of freshwater containing lactic acid bacteria and fermentable sugars used as inoculum. However, information regarding the amount of kelp and freshwater was not available from the producer of these samples. The fluid is discarded at the end of the process which may be an important factor to explain the large difference in iodine content with the seawater-blanched samples. This is opposed to the other commercial fermentation process in this study (experiment 2-4) in which the effluent produced during the process is kept, and no additional water is added to inoculate lactic acid bacteria.

Table 3: Iodine (I, expressed in mg kg^{-1} DW) and moisture content (MC, expressed in % WW) of unprocessed (control) and processed *S. latissima* samples. Values are given as mean of duplicate measurements or as single measurement.

	Fresh (*n* = 1)	**Seawater-blanched (*n* = 2)**	**Seawater-blanched, fermented [1] (*n* = 1)**
I	5580	2310	850
MC	91.04	91.34	92.47

[1] These samples were produced from a different batch harvested a few weeks later than the one used for the control and seawater blanching, which may introduce a bias in comparing these samples with the two other groups.

Additional samples obtained from the same commercial kelp producer including seawater-blanched *S. latissima* and *A. esculenta* during 4-6 min at 45 °C at a pre-commercial scale were also analysed. This treatment involved a considerably larger volume of water compared to the seawater blanching procedure described above. The iodine content of these samples i.e. 190 and 479 mg kg^{-1} DW respectively in *S. latissima* and *A. esculenta* indicates that significant iodine reduction to below 2000 mg kg^{-1} DW can be achieved from seawater blanching and that the extent of the reduction may depend on the water volume.

3.6. CHEMICAL COMPOSITION OF SELECTED PROCESSED SAMPLES OF *S. LATISSIMA* AND DRIED *A. ESCULENTA*

Selected samples from experiments described above were analysed for their nutritional content and levels of PTEs. The selection includes:

- Untreated samples (hereafter referred to as "control 1"), blanched and steamed (20 min) samples of *S. latissima* from experiment 1,
- Untreated samples (hereafter referred to as "control 2"), seawater-blanched and seawater-blanched then fermented samples of *S. latissima* from experiment 5,
- Dried samples of cultivated *A. esculenta* which were used as a reference.

The moisture content of fresh samples of cultivated *S. latissima* from the experiment 1 (i.e. control 1 samples, harvested in June 2020) was considerably lower than the levels measured in wild harvested biomass (control 2 samples, harvested in February 2021) (**table 4**). Hence, the total dry matter content, reflecting the proximate composition excluding moisture i.e. the total nutrient content of the samples, was approximately twice as high in the control 1 compared to the control 2 (16.29 and 8.96 g 100 g^{-1} DW respectively). This trend as well as the differences in ash, protein, FA and carbohydrate content between the control sample reflects the seasonal variations in the chemical composition of *S. latissima* as previously reported in the literature (Schiener et al. 2015). Generally, the chemical composition of the initial samples reported in **table 4** is in the range of the values reported for this species (Schiener et al. 2015; Stévant et al. 2017b). The basic nutrient profile of the dried *A. esculenta* sample, used as reference in this study was relatively similar to those of *S. latissima* samples.

The moisture content of freshwater-blanched samples increased compared to the control indicating the release of soluble compounds during the process combined with water uptake due to the osmotic potential between the blades and the soaking water (Stévant et al. 2017a) (**table 4**). Since the nutrient composition expressed relatively to the DW of the samples does not account for the loss of biomass which may occur during the treatments, the overall losses of nutrients were estimated relatively to the WW of the samples, based on the model of Nielsen et al. (2020). In the study of Nielsen et al. (2020), true retention factors for each individual compound analysed were calculated. This factor accounts for the variations in total fresh weight of the samples during processing treatments, hence reflects the absolute loss of nutrients. Although true retention factors were not calculated in this study, the mass balance for the main nutrients were estimated based on the WW of the samples, accounting for the variations in moisture content following treatments. The blanching and steam treatments led to 52 % and 13 % nutrient losses respectively (**fig. 7**). Mainly ashes and carbohydrates were lost during blanching while the protein and FA remained stable. Only minor nutrient losses (3 %) were observed from blanching in seawater and the subsequent fermentation following seawater blanching resulted in 16 % loss of nutrients. A reduction in ash content accounted for most of the losses from the latter treatment.

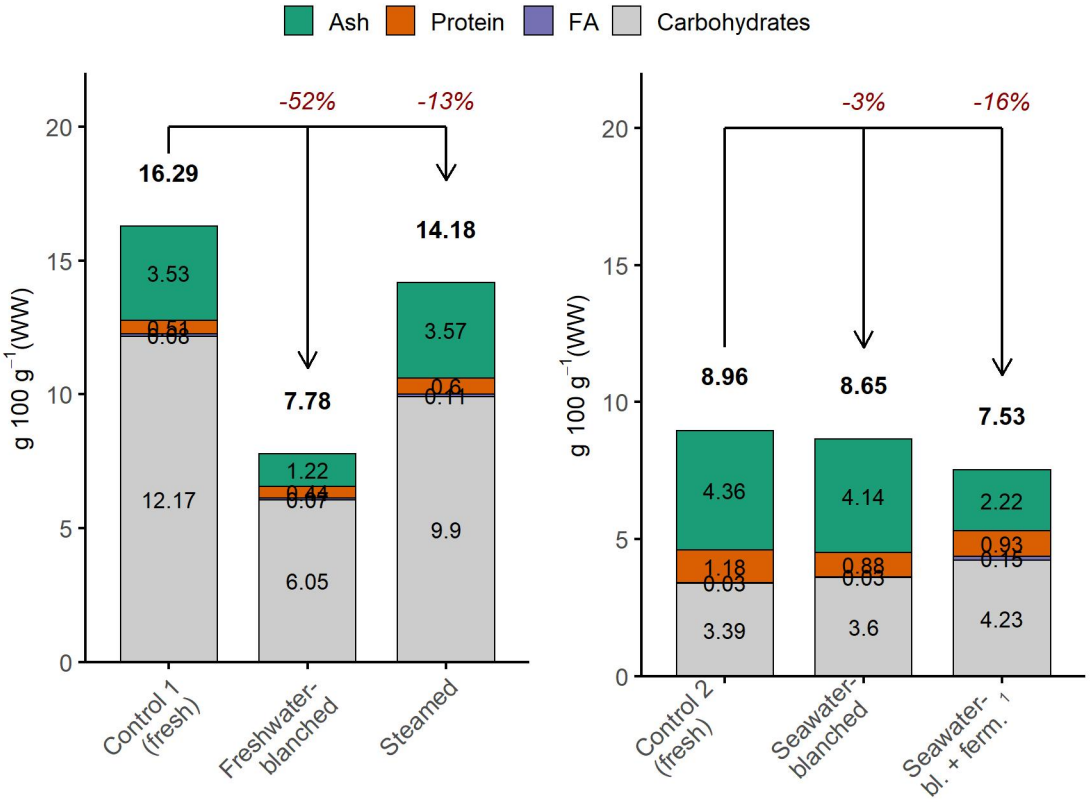

Fig. 7: Mass balances for the proximate composition relative to the WW of *S. latissima* for each treatment compared to the control samples. The values in bold above the bars indicate the total dry matter content i.e. the sum of ash, protein, FA and carbohydrates and the percentages in red indicate the total loss of these nutrient. [1] These samples were produced from a different batch harvested a few weeks later that the one used for the control and seawater blanching, which may introduce a bias in comparing these samples with the two other groups.

The FA content of the samples was low (**table 4**) as generally reported in macroalgae including kelp species. However, the proportion of unsaturated FA (MUFA + PUFA) was relatively high i.e. ranging from 57 to 66 % in control samples (in control 2 and 1 respectively) and increasing to about 75 % in steamed and freshwater-blanched samples (expressed relatively top the DW of the samples), and to about 80 % in seawater-blanched samples, both with and without subsequent fermentation.

The mineral and metal profile of the samples was analysed. The results, expressed relatively to the DW of the samples, are presented in **table 5**. The macrominerals analysed (i.e. Ca, K, Na, Mg, P) reacted differently to the treatments tested. From the experiment 1, the content of individual macromineral elements of steamed samples was relatively similar to the levels measured in the control samples. The Ca content of freshwater-blanched samples increased by 80 % compared to the levels measured in the fresh control samples, whereas the Na and K levels decreased by 50 % and 40 % respectively. These observations reflect the mass balance

(relatively to the WW) of the macromineral content of these samples showing major losses of Na and K from freshwater blanching and only a minor reduction in Ca (**fig. 8**).

The K content also decreased following seawater blanching (experiment 5) but in lower proportions compared to freshwater blanching. However, the subsequent fermentation to seawater blanching drastically reduced the K content compared to the initial levels (**table 5**, **fig. 8**). The Na content increased relatively following seawater blanching then decreased again after fermentation to a level similar to that of fresh samples. A similar pattern was observed for the Mg levels of samples from the experiment 5. The P levels decreased in both treatments tested in experiment 5 whereas they remained stable in blanched and steamed samples compared to the control in experiment 1.

Overall, the treatments tested in the experiment 1 did not affect the Na/K ratio of *S. latissima* to a great extent i.e. the Na/K ratio of both blanched and steamed samples was 0.5 and 0.6 in the control. On the other hand, seawater blanching (Na/K = 0.7) and subsequent lactic acid fermentation (Na/K = 1.9) increased this ratio compared to the control (Na/K = 0.4).

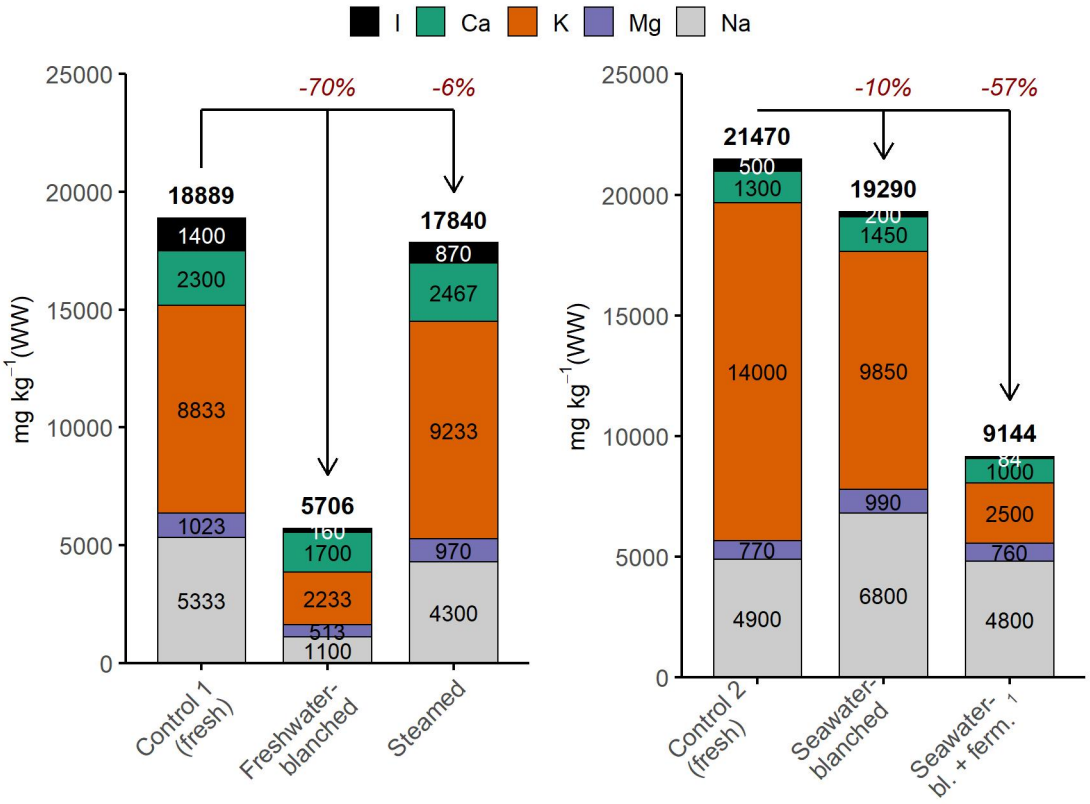

Fig. 8: Mass balances for the macrominerals (Ca, K, Mg and Na) and iodine relative to the WW of *S. latissima* for each treatment compared to the control samples. The values in bold above the bars indicate the sum of the five elements and the percentages in red indicate the total loss of macrominerals. [1] These samples were produced from a different batch harvested a few

weeks later that the one used for the control and seawater blanching, which may introduce a bias in comparing these samples with the two other groups.

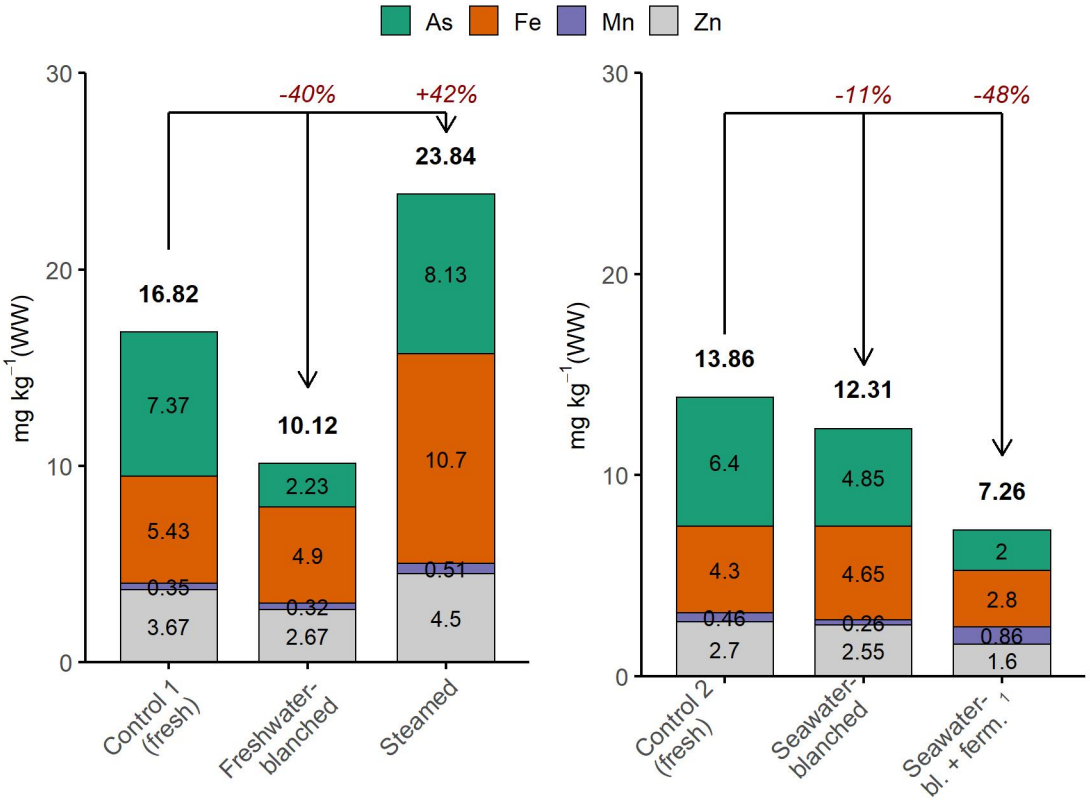

Fig. 9*:* Mass balances for the selected microminerals (As, Fe, Mn and Zn) relative to the WW of *S. latissima* for each treatment compared to the control samples. The values in bold above the bars indicate the sum of the four elements and the percentages in red indicate the total loss of these microminerals. [1] These samples were produced from a different batch harvested a few weeks later that the one used for the control and seawater blanching, which may introduce a bias in comparing these samples with the two other groups.

Overall, the seawater blanching did not appear to affect microminerals and trace elements significantly as their relative proportions remained quite stable compared to the control (**fig. 9**, **fig. 10**). Subsequent fermentation as well as freshwater blanching and steaming treatments affected the levels of microminerals and trace elements relatively to their respective control. In freshwater-blanched samples, the decrease in micromineral generally reflects the ash content reduction compared to fresh control samples (**fig. 7**). However, the arsenic (As) was reduced to a greater extent compared to other elements (i.e. Fe, Mn and Zn). The relative proportion of trace elements was greater in blanched samples compared to the control (**fig. 10**) indicating a higher retention of these elements, and particularly copper (Cu), compared to

other nutrients following this treatment. Likewise, Cu and iron (Fe) (**fig. 9**) appear to be well retained following steam treatments.

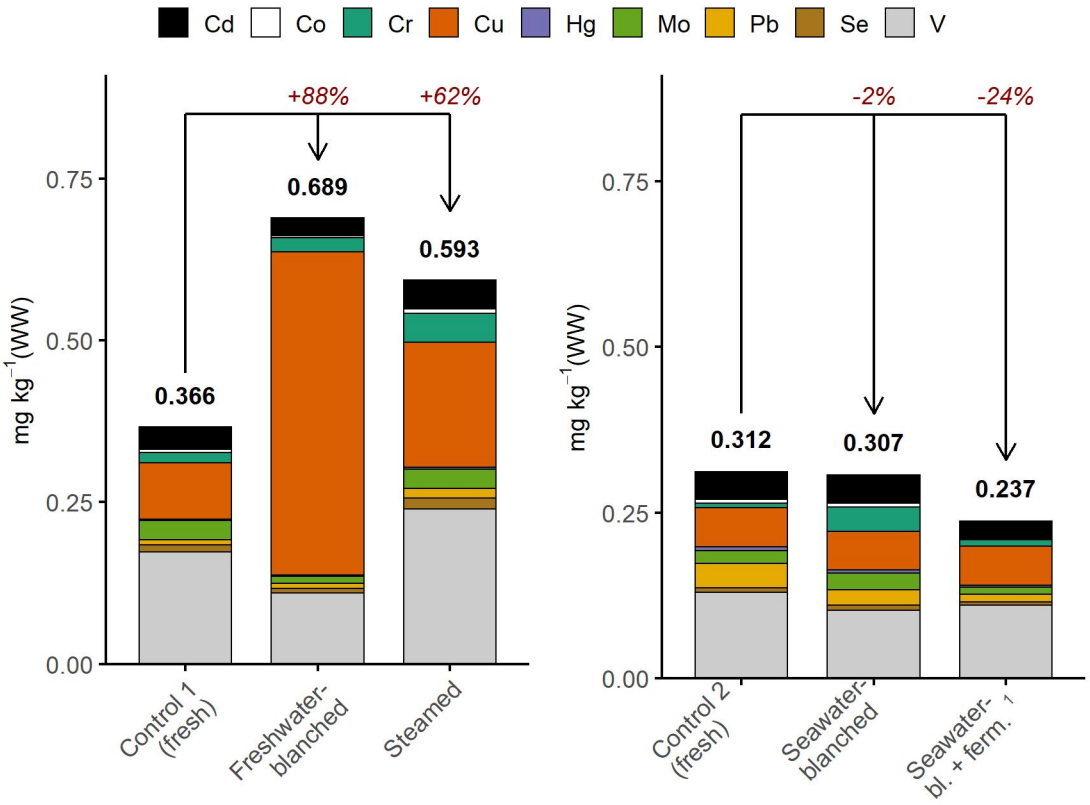

Fig. 10: Mass balances for selected trace elements relative to the WW of *S. latissima* for each treatment compared to the control samples. The values in bold above the bars indicate the sum of the trace elements and the percentages in red indicate the total loss of these microminerals. [1] These samples were produced from a different batch harvested a few weeks later that the one used for the control and seawater blanching, which may introduce a bias in comparing these samples with the two other groups.

Table 4: Basic nutrient content (expressed in g 100 g^{-1} DW) of selected samples of *S. latissima* (*SL*) and *A. esculenta* (*AE*). Values are given as mean of replicate measurements (± standard deviation) or as single measurement.

	Samples	Moisture [1]	Ash	Protein	Total FA	SFA	MUFA	PUFA	Carbohydrates
Exp. 1	*SL*-Control 1 (*n*=1)	83.71 ± 2.93	21.7	3.15	0.47	0.16	0.08	0.23	74.7
	SL-Freshwater-blanched (*n*=3)	92.23 ± 1.48	15.67 ± 1.03	5.62 ± 0.22	0.84 ± 0.05	0.23 ± 0.01	0.12 ± 0.01	0.49 ± 0.03	76.4 ± 1.2
	SL-Steamed (*n*=3)	85.81 ± 1.41	25.19 ± 1.49	4.23 ± 0.13	0.81 ± 0.09	0.20 ± 0.02	0.11 ± 0.01	0.50 ± 0.05	68.7 ± 1.4
Exp. 5	*SL*-Control 2 (*n*=1)	91.04	48.69	13.15	0.30	0.13	0.08	0.09	37.9
	SL-Seawater blanched (*n*=1)	91.35	47.87	10.13	0.40	0.09	0.06	0.26	41.6
	SL-Seawater blanched + ferm. (*n*=1) [2]	92.47	29.49	12.36	1.98	0.41	0.28	1.28	56.2
	AE-Dried (*n*=1)	5.43	35.50	10.41	0.42	0.13	0.09	0.20	51.05

[1] expressed in % WW; [2] These samples were produced from a different batch harvested a few weeks later that the one used for the control and seawater blanching, which may introduce a bias in comparing these samples with the two other groups.

Table 5: Mineral and metal profile (in mg kg^{-1} DW) of selected samples of *S. latissima* (*SL*) and *A. esculenta* (*AE*). Values are given as mean of replicate measurements (± standard deviation) or as single measurement.

Samples	I	Ca	K	Mg	Na	P	Ag	As	iAs	Cd
SL-Control 1 (*n*=3)	**7977 ± 2317**	14 421 ± 935	55 348 ± 2 142	6 421 ± 557	33 488 ± 3 696	1 232 ± 86	b.d.l.	46 ± 2	0.067 ± 0.00	0.21 ± 0.02
SL-Freshwater-blanched (*n*=3)	**2189 ± 209**	26 007 ± 1 963	34 146 ± 1 809	7 838 ± 272	16 830 ± 1 042	1 280 ± 79	0.06 ± 0.01	34 ± 2	0.063 ± 0.00	0.42 ± 0.06
SL-Steamed (*n*=3)	**5916 ± 699**	15 764 ± 1 133	59 018 ± 5 502	6 198 ± 429	27 513 ± 2 570	1 298 ± 76	b.d.l.	52 ± 1		0.28 ± 0.01
SL-Control 2 (*n*=1)	**5580**	14 509	156 250	8594	54 688	3 795	b.d.l.	71		0.47
SL-Seawater blanched (*n*=2)	**2310 ± 227**	16 752 ± 549	113 804 ± 1 536	11 438 ± 96	78 566 ± 1 019	2 773 ± 11	b.d.l.	56 ± 2		0.49 ± 0.00
SL-Seawater blanched + ferm. (*n*=1) [1]	**850**	13 280	33 201	10 093	63 745	1 859	b.d.l.	27		0.35
AE-Dried (*n*=1)	**1163**	20 091	95 165	8 988	44 412	3 384	b.d.l.	62		1.05

b.d.l.: below detection limit; [1] These samples were produced from a different batch harvested a few weeks later that the one used for the control and seawater blanching, which may introduce a bias in comparing these samples with the two other groups.

Table 5 (continued): Mineral and metal profile (in mg kg^{-1} DW) of selected samples of *S. latissima* (*SL*) and *A. esculenta* (*AE*). Values are given as mean of replicate measurements (± standard deviation) or as single measurement.

Samples	Co	Cr	Cu	Fe	Hg	Mn	Mo	Ni	Pb	Se
SL-Control 1 (*n*=3)	0.03 ± 0.00	0.10 ± 0.01	0.55 ± 0.04	34 ± 2	0.01 ± 0.00	2.17 ± 0.06	0.19 ± 0.01	b.d.l.	0.05 ± 0.00	0.07 ± 0.01
SL-Freshwater-*b*lanched (*n*=3)	0.05 ± 0.00	0.34 ± 0.02	7.67 ± 0.81	75 ± 9	0.03 ± 0.00	4.94 ± 0.32	0.15 ± 0.01	0.11 ± 0.16	0.12 ± 0.01	0.11 ± 0.02
SL-Steamed (*n*=3)	0.04 ± 0.01	0.29 ± 0.09	1.24 ± 0.17	69 ± 11	0.02 ± 0.00	3.26 ± 0.24	0.19 ± 0.01	0.09 ± 0.13	0.10 ± 0.02	0.10 ± 0.00
SL-Control 2 (*n*=1)	0.07	0.08	0.66	48	0.06	5,13	0.22	b.d.l.	0.41	0.07
SL-Seawater blanched (*n*=2)	0.06 ± 0.01	0.41 ± 0.13	0.67 ± 0.01	54 ± 4	0.05 ± 0.01	2.95 ± 0.17	0.29 ± 0.06	0.29 ± 0.06	0.28 ± 0.08	0.08 ± 0.00
SL-Seawater blanched + ferm. (*n*=1) [1]	0.03	0.13	0.78	37	0.04	11.42	0.13	b.d.l.	0.16	0.07
AE-Dried (*n*=1)	b.d.l.	b.d.l.	9.31	116	b.d.l.	7.82	b.d.l.	b.d.l.	0.21	0.14

b.d.l.: below detection limit; [1] These samples were produced from a different batch harvested a few weeks later that the one used for the control and seawater blanching, which may introduce a bias in comparing these samples with the two other groups.

Table 5 (continued): Mineral and metal profile (in mg kg^{-1} DW) of selected samples of *S. latissima* (*SL*) and *A. esculenta* (*AE*). Values are given as mean of replicate measurements (± standard deviation) or as single measurement.

Samples	V	Zn
SL-Control 1 (*n*=3)	1.09 ± 0.07	23 ± 1
SL-Freshwater-blanched (*n*=3)	1.68 ± 0.06	41 ± 1
SL-Steamed (*n*=3)	1.53 ± 0.12	29 ± 2
SL-Control 2 (*n*=1)	1.45	30
SL-Seawater-blanched (*n*=2)	1.18 ± 0.08	29 ± 1
SL-Seawater-blanched + ferm. (*n*=1) [1]	1.46	21
AE-Dried (*n*=1)	0.67	51

[1] These samples were produced from a different batch harvested a few weeks later that the one used for the control and seawater blanching, which may introduce a bias in comparing these samples with the two other groups.

3.7. SENSORY PROFILING OF SELECTED PROCESSED SAMPLES OF *S. LATISSIMA* AND DRIED *A. ESCULENTA*

The sensory characteristics including aroma, flavour and texture qualities of selected *S. latissima* samples obtained from different processes were evaluated by nine trained panel members. The evaluation included freshwater-blanched and steamed samples (experiment 1) and seawater-blanched samples both with and without subsequent fermentation. Dried commercial samples of *A. esculenta* cultivated in Norway were used as a reference. All samples were assessed in dried powder form. The sensory profile of the samples is illustrated by the plot in **fig. 11**. Differences in sensory characteristics obtained from different processes and between sample replicates are illustrated by the PCA biplot in **fig. 12**.

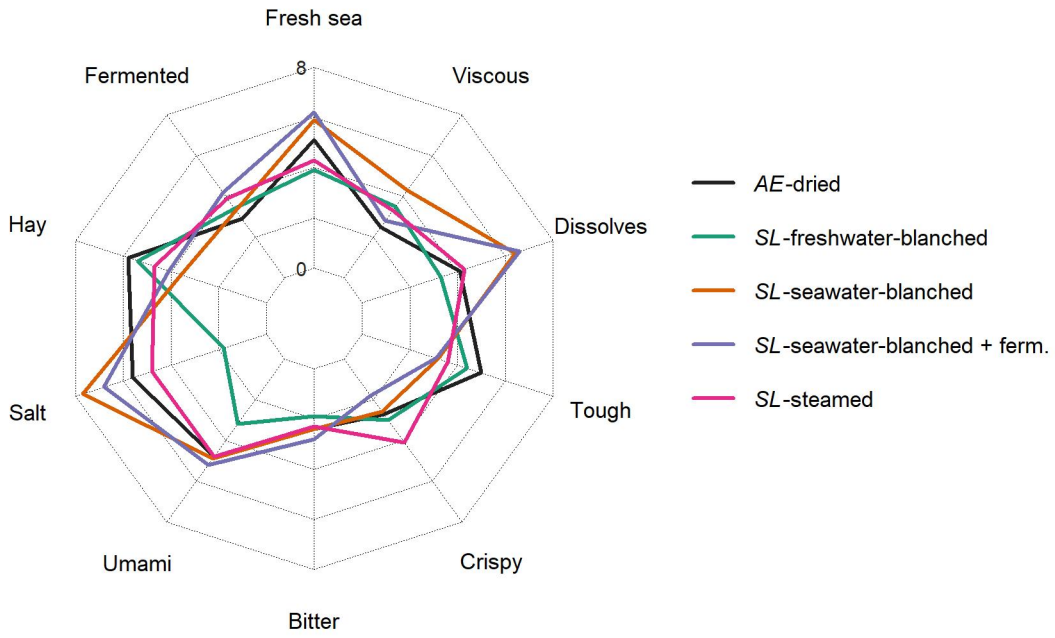

Fig. 11: Mean sensory scores (on a scale from 0 to 9) from the descriptive analysis of four samples from *S. latissima* (*SL*) and one from *A. esculenta* (*AE*) by panellist (n = 9) during repeated sensory sessions (n = 2) and based on 10 sensory attributes.

Significant differences were detected across samples regarding most of the sensory attributes except for bitterness, associated with low scores, fermented flavour and aroma, as well as viscous texture (**table 6**). Both seawater-blanched samples of *S. latissima* (with and without subsequent fermentation) were characterized by a relatively intense salty taste as well as fresh sea aroma and flavour compared to the other samples. They also had a characteristic dissolving mouthfeel when chewing. Dried *A. esculenta* and freshwater-blanched *S. latissima* are associated with hay flavour and aroma as well as a tough texture. The latter sample had the lowest scores associated with saltiness and umami flavour. The steamed samples of *S. latissima* were regarded as neutral compared to other samples. Yet, the intensity of umami flavour of these samples was comparable to those of seawater-blanched *S. latissima* and dried *A. esculenta*.

Table 6: Mean sensory scores on a scale from 1 to 9 (± standard deviation) from the descriptive analysis of four ingredients from cultivated *S. latissima* and one from cultivated *A. esculenta* by panellist (n = 9) during repeated sensory sessions (n = 2) and based on 10 sensory attributes.

Sensory attribute	***AE*-Dried**	***SL*-FW-blanched**	***SL*-SW-blanched**	***SL*-SW-blanched + ferm.**	***SL*-Steamed**	***p*-value**
Fresh sea	5.1 ± 1.8 ^{ac}	3.9 ± 1.7 ^{a}	5.9 ± 2.1 ^{bc}	6.2 ± 1.8 ^{c}	4.3 ± 1.9 ^{ab}	**0.002**
Fermented	2.9 ± 1.5 ^{a}	3.4 ± 1.7 ^{a}	3.3 ± 1.5 ^{a}	4.2 ± 1.6 ^{a}	3.9 ± 1.9 ^{a}	0.146
Hay	5.8 ± 2.1 ^{c}	5.4 ± 1.9 ^{bc}	3.6 ± 2.0 ^{a}	4.1 ± 1.6 ^{ab}	4.7 ± 2.1 ^{ac}	**0.002**
Salt	5.6 ± 1.3 ^{b}	1.8 ± 0.8 ^{a}	7.7 ± 1.1 ^{c}	6.8 ± 1.3 ^{c}	4.8 ± 1.5 ^{b}	**0.000**
Umami	4.8 ± 1.5 ^{b}	3.2 ± 2.1 ^{a}	4.9 ± 1.7 ^{b}	5.2 ± 1.8 ^{b}	4.8 ± 1.9 ^{b}	**0.002**
Bitter	2.4 ± 1.7 ^{a}	1.9 ± 1.4 ^{a}	2.4 ± 1.5 ^{a}	2.8 ± 1.6 ^{a}	2.3 ± 1.2 ^{a}	0.412
Crispy	2.7 ± 1.5 ^{ab}	3.0 ± 1.7 ^{bc}	2.6 ± 1.4 ^{ab}	1.8 ± 0.7 ^{a}	4.1 ± 2.1 ^{c}	**0.000**
Tough	5.0 ± 2.4 ^{b}	4.4 ± 2.1 ^{ab}	3.2 ± 1.8 ^{a}	3.1 ± 1.7 ^{a}	3.6 ± 1.9 ^{ab}	**0.003**
Dissolves	4.1 ± 2.0 ^{a}	3.3 ± 1.7 ^{a}	6.4 ± 1.1 ^{b}	6.6 ± 1.5 ^{b}	4.3 ± 2.1 ^{a}	**0.000**
Viscous	2.5 ± 1.5 ^{a}	3.5 ± 2.0 ^{a}	4.3 ± 2.4 ^{a}	2.8 ± 1.2 ^{a}	3.3 ± 2.1 ^{a}	0.050

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

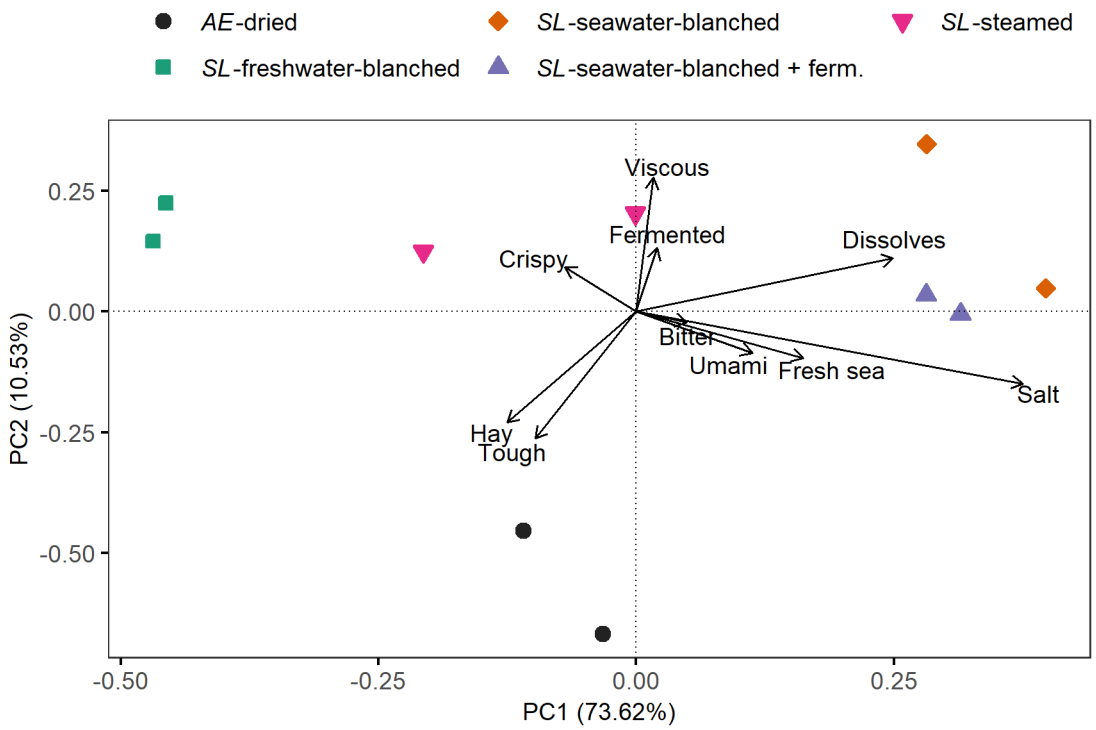

Fig. 12: Biplot (1st and 2nd principal component axes) obtained from the principal component analysis of sensory scores (mean scores over 9 panellists for each repeated sensory session, no scaling applied) from the descriptive analysis of four ingredients from cultivated *S. latissima* (*SL*) and one from cultivated *A. esculenta* (*AE*).

Commercial samples of fermented *S. latissima* used in experiments 2-4 (i.e. without prior seawater-blanching step) were analysed in a separate sensory evaluation at a later stage. The samples were evaluated following the same methodology and attributes as above (results not shown). The sensory profile of these samples was relatively similar to those of seawater-blanched and fermented samples, although they were more bitter and had less hay flavour. The panellist also commented that the samples had a characteristic acidic taste, which was not included in the list of attributes.

3.8. FOOD PROTOTYPING & PRODUCT TESTING

Seawater-blanched as well as fermented samples were selected to be tested in a food prototyping experiment. The levels of inclusion i.e. 0.13 and 0.26 g of dried ingredient making up 0.5 and 1 % of the final soup portion) were determined based on the dietary iodine contribution of the seawater-blanched sample (containing 2310 mg iodine kg^{-1} DW). These levels cover respectively 50 and 100 % of the Upper Intake Level (UL) of 600 µg iodine day^{-1} for adults. It should be noted that the fermented kelp ingredient used in this experiment

(containing 4856 mg iodine kg^{-1} DW) at the same inclusion levels will cover approximately 100 and 200 % of the UL.

Overall, all spinach soups with added kelp ingredients were significantly discriminated by the panel from the control (standard spinach soup without kelp, **table 7**). The soup with fermented *S. latissima* at the lowest inclusion level was grouped correctly by the fewest assessors (10 of 16), which indicates that it was the most similar to the standard. The soup with seawater-blanched kelp at the highest inclusion level was grouped correctly by most panellists (13 of 16), suggesting that it differed most from the standard.

Table 7: Results from the assessment of food prototypes (commercial spinach soup with *S. latissima* ingredients) vs. a control (commercial spinach soup without kelp) in a tetrad test by 16 panellists.

Tested product	Inclusion level	Correct answers	*p*-value
Soup with seawater-blanched *SL*	0.5 %	11	**0.006**
Soup with seawater-blanched *SL*	1 %	13	**0.000**
Soup with fermented *SL*	0.5 %	10	**0.017**
Soup with fermented *SL*	1 %	11	**0.006**

Significant results from a binomial test ($p < 0.05$, in bold) indicate a significant perceptible difference between the prototype and the control.

Generally, the comments from panellists about the soups containing ingredients from *S. latissima* were not unambiguous as impressions of better/more flavour and less/off-flavour were equally mentioned. However, 3 of 16 and 5 of 16 assessors associated fermented and seawater-blanched *S. latissima* added at 1 % inclusion level with more intense saltiness compared to the control. The kelp-containing spinach soups were not compensated for salt compared to the control. According to the nutrient content given by the manufacturer, this commercial soup contains 1.7 g salt (NaCl) per portion.

4. DISCUSSION

The primary conclusions that can be derived from this study are the following:

- Blanching using either freshwater or seawater reduced the iodine content of *S. latissima* but not below the limit value of 2000 mg kg^{-1} DW under the conditions tested (1 kg in 5 L, 45°C for 120 min). Commercial samples of seawater-blanched *S. latissima* using a longer treatment time and a higher volume of seawater contained a considerably lower level of iodine (190 mg kg^{-1} DW).
- Seawater blanching followed by lactic acid fermentation also achieved a low level of iodine (850 mg kg^{-1} DW).
- Drip losses during the process of fermentation and freeze/thawing may contribute to iodine level reduction due to the iodide leaching into the liquid fraction. A higher volume of inoculated fluid during the fermentation may provide a greater iodine level reduction.
- No iodine level reduction was achieved from other treatments involving high temperatures and in which the liquid phase was retained. This means that the iodine, mainly present in the form of iodide (I^-) does not evaporate under these conditions.
- Blanching using seawater provides better nutrient retention (i.e. ashes, proteins, FA, carbohydrates) compared to similar treatments using freshwater leading to significant losses of carbohydrates and minerals.
- Fermentation after seawater blanching leads to some loss of nutrients, especially macro- and microminerals. The resulting Na/K ratio increased to 1.9 compared to 0.3 in the control fresh samples. However, the ratio of several minerals to iodine is favourable in seawater-blanched and fermented kelp, providing an interesting product for the food industry. This will be followed up in further studies involving the analysis of other nutrients.
- Processing methods for iodine reduction resulted in distinct sensory profiles including flavour and texture attributes.
- The inclusion of either fermented or seawater-blanched *S. latissima* to a commercial spinach soup, even at a relatively low inclusion level (0.5 % of the final portion) contributes to a perceptible difference in sensory properties from the standard soup. Saltiness appears to be one of the main flavour contributions to the product highlighting the potential of kelp ingredients to be used as salt replacement in the food industry.

4.1. IODINE IN KELPS

Brown algae of the Laminariales order (which includes *S. latissima*) are the strongest accumulators of iodine among living organisms. The iodine in kelps is mainly stored in the extracellular matrix of peripheral tissues (Verhaeghe et al. 2008) acting as an antioxidant (Küpper et al. 2008). The uptake is catalysed by extracellular enzymes (vanadium

haloperoxidases) (Leblanc et al. 2006). In adult sporophytes of *Laminaria digitata*, the meristematic tissue (basal part of the plant) tend to have a lower iodine content than the distal part of the frond consisting of older tissue (Küpper et al. 1998). Speciation studies demonstrated that iodine in kelps is mainly stored as an inorganic water-soluble form (up to 90 %), namely iodide (I^-) (Hou et al. 1997; Verhaeghe et al. 2008). Similarly, 93 % of the total iodine was present in the form of iodide in fresh and fermented *S. latissima* samples, in the present study. The remaining iodine forms (ca. 10 %) consists of iodate (IO_3^-) and organic iodine i.e. iodinated amino-acid residues (monoiodotyrosine and diiodotyrosine) (Hou et al. 1997), which were not detected in the present samples. Upon oxidative stress from gaseous oxidants (O_3) at low tide under natural conditions, part of the iodide is released into the coastal atmosphere in the form of molecular iodine (I_2) to form aerosol particles.

The iodine content of kelps is highly variable depending on the season, location and physiological state of the plant (Ar Gall et al. 2004; Lüning and Mortensen 2015). Iodine contents ranging from 670 to 10 000 mg kg^{-1} DW are reported from *S. latissima* (Duinker et al. 2020).

4.2. EFFECT OF PROCESSING METHODS ON THE IODINE CONTENT OF *S. LATISSIMA*

The first objective of this study was to test the potential of various processing methods for iodine level reduction in *S. latissima*. There are several reports of successful attempts of lowering the iodine content in this species following rehydration of dried material (Correia et al. 2021), boiling treatments (Lüning and Mortensen 2015; Correia et al. 2021; Jordbrekk Blikra et al. 2021b) and blanching procedures in freshwater involving temperatures in the range 30 to 80 °C (Stévant et al. 2017a; Nielsen et al. 2020). In the present study, the temperature (45 °C) and treatment time (120 sec) of blanching were selected based on the results of Nielsen et al. (2020) who measured an iodine level reduction of over 90 % of the initial iodine content (i.e. 4605 mg kg^{-1} DW) of fresh *S. latissima* under these conditions. However, the reduction achieved in the experiment 1 (i.e. 72.6 % of the initial iodine measured in control samples, **fig. 2**) failed to reach iodine levels in blanched samples to below 2000 mg kg^{-1} DW as recommended by the French food safety authority (Mabeau and Fleurence 1993; CEVA 2019). A much higher initial iodine content of *S. latissima* (7977 mg kg^{-1} DW) in this study compared to those measured by Nielsen et al. (2020) (also from cultivated biomass in Norway) and a higher kelp-to-water ratio i.e. 1 kg (WW) in 5 L vs 150 g in 5 L in Nielsen et al. (2020) may explain the limited effect of blanching in this particular experiment.

One of the sub-objectives of this study was to identify alternative methods to selectively reduce the iodine content of the material while limiting losses of soluble nutrients. Hence, treatment methods involving exposure to heat and limited amount of freshwater were tested. The steam treatment tested in the experiment 1 only had a limited effect on the iodine content of *S. latissima* (**fig. 2**) as observed in a previous experiment (Stévant 2019) even at longer treatment time (20 min) as tested in this study. Iodine evaporation may occur in the form of elemental iodine (I^2, yellow-red gas) or hydrogen iodide (HI, colourless gas) and could represent a way to selectively reduce the iodine content of kelps. Evaporation is seen during

acid degradation of samples prior to metal analysis, at very low pH. Iodine is hence analysed under alkaline conditions. Loss of nitrogen is also observed during the analysis of iodine using enzymes prior to alkaline analysis at the Institute of Marine Research. Evaporation of iodine seemed to occur during frying and cooking of kelp and kelp broth in previous studies (Duinker et al. 2020). However, no iodine content reduction was measured from the methods tested in experiment 2 and 3 including rotavapor and aeration treatments under heating and a cooking treatment in which small volumes of water were added to compensate for evaporation (**fig. 3**, **fig. 4**). This suggests that the iodide does not undergo chemical reactions resulting in the formation of gaseous iodine species under these conditions which would have led to a selective reduction of the iodine content in *S. latissima*. Rather, the results from the last cooking experiment of this study suggests that the iodine content reduction is mainly due to the water-soluble iodide leaching into the surrounding liquid (**fig. 6**). This hypothesis is supported by the preliminary study of the partitioning of the iodine among the solid (kelp fronds) and liquid fraction (drip water) from both fermentation and freeze/thawing processes, revealing the relatively equal distribution of the iodine across these fractions (**fig. 5**). Effluent formation has been documented previously from these processes and may represent up to 40 % of the wet weight of the initial biomass (Stévant 2019). Structural alterations of the kelp frond following fermentation as well as freeze/thawing may therefore contribute to the release of the iodine.

Küpper et al. (2008) demonstrated that iodide is released in the surrounding seawater upon oxidative stress caused by alginate-degrading bacteria in a biofilm. Alginate depolymerisation has been observed from the lactic acid fermentation of *Laminaria digitata* (H. Marfaing, unpublished results), which may facilitate the release of iodine during the process. However, the fermented commercial batch of *S. latissima* used as start material were high in iodine (i.e. 4375 mg kg^{-1} DW measured in the experiment 2, **fig. 3**). Data on the initial iodine content prior to fermentation is unfortunately not available for comparison. However, these results suggest that low pH (3.5 in these fermented samples) and oxidation from microbial activity does not significantly affect the iodine content of *S. latissima*. The fermentation process following seawater blanching (experiment 5) from another kelp producer uses a different method involving a higher volume of inoculum solution. This seems to be an important factor explaining the greater iodine content reduction achieved from this fermentation method. However, these results were mainly obtained from single measurements. Repeated measurements and technical replication may confirm this observation. Additionally, innovative technology such as ultrasound and pulse electric field, possibly combined with heating treatments may be used to reduce the iodine content of *S. latissima* (Noriega-Fernández et al. 2021; Jordbrekk Blikra et al. 2021a) and should be further investigated.

4.3. FOOD SAFETY ISSUES FROM INCLUDING KELPS IN COMMERCIAL PRODUCTS

In Western countries, macroalgae are increasingly recognized as a promising resource for the sustainable production of food. In Norway, the macroalgae production sector based on biomass cultivation of sugar kelp (*S. latissima*) and winged kelp (*A. esculenta*) is growing rapidly (Stévant et al. 2017c). The high iodine content of these kelp species and the uncertainty

regarding potential health risks are a major challenge for a broad use of kelps in food applications. Iodine is an essential element for the production of thyroid hormones i.e. triiodothyronine (T3) and thyroxine (T4) which are involved in cell metabolism, growth and reproduction, and in the development of the central nervous system (Andersson et al. 2007; de Benoist et al. 2008). Iodine deficiency is a global problem affecting one third of the global population, causing metabolic disorders and mental retardation. This problem is particularly widespread in Europe including Norway (Andersson et al. 2007; Lazarus 2014; Brantsæter et al. 2018). On the other hand, excessive iodine intakes can affect the thyroid function with potentially negative health effects primarily in susceptible individuals e.g. those with pre-existing thyroid disease, the elderly, foetuses and neonates (Leung and Braverman 2014). Toxicity reports of excessive iodine intake from kelp consumption describe the suppression of the thyroid function as a result of elevated serum levels of thyroid-stimulating hormone (TSH) leading to clinical symptoms similar to those caused by iodine deficiency (Miyai et al. 2008; Inui et al. 2010). The thyroid function returned to normal after discontinuing the consumption of kelp.

Table 8: Iodine intake in % of UL from the consumption of *S. latissima* and *A. esculenta* based on the iodine contents measured during this study.

	Sample	Iodine content (mg kg^{-1} DW)	% of UL (600 µg day^{-1}) from 1 g (DW) sample
Exp.1	*SL*-Control 1	7977	1330 %
	SL-Blanched	2189	365 %
	SL-Steamed	5916	986 %
	SL-Control 2	5582	930 %
Exp. 5	*SL*-Seawater-blanched	2310	385 %
	SL-Seawater-blanched + ferm.	850	142 %
	AE-Dried	1163	194 %

Based on the recommendations from the Scientific Committee on Food (SCF 2002), the European Food Safety Authority (EFSA) has provided dietary values for adequate iodine intake levels of 150 µg day^{-1} for adults, 70 µg day^{-1} for infants, 90 to 130 µg day^{-1} for children and 200 µg day^{-1} for pregnant and lactating women (EFSA 2014b). The UL of 600 µg iodine day^{-1} for adults including pregnant and lactating women was adopted based on dose-response studies conducted between 1978 and 1988 from intakes of up to 1800 µg day^{-1} showing no clinical adverse effects. An uncertainty factor of 3 was chosen to derive the UL (SCF 2002). The SCF also noted that an UL is not a threshold of toxicity and may be exceeded for short periods without an appreciable risk to the health of the individuals concerned. Nevertheless, including kelp ingredients in food products, even in relatively small proportions (1 g dry) would exceed the UL up to 13 times based on the results obtained in this study (**table 8**). These results support the conclusions of previous studies (Stévant et al. 2017a; Afonso et al. 2020; Aakre et

al. 2021). This is a major challenge in the perspective of a large distribution of kelp-containing food products. Some products that are likely to be eaten regularly (e.g. bread) may expose consumers to frequent excessive iodine intakes (Banach et al. 2020). This issue is reflected in a recent study involving human subjects revealing an excessive iodine status among macroalgae consumers following dietary intake (Aakre et al. 2020).

Processed kelp ingredients such as seawater-blanched then fermented will provide lower iodine intakes compared to untreated samples thus limiting the risks (**table 8**). Optimizing processing methods to selectively reduce the iodine content paired with appropriate product labelling will lower the risks associated with the consumption of kelp. Further studies are required to examine the thyroid function of frequent consumers of kelp-containing foods and the potentially adverse effects of long-term exposure to iodine from kelp.

Table 9: Potentially toxic element (in mg kg^{-1} DW) measured in *S. latissima* (*SL*) and *A. esculenta* (*AE*) samples in this study.

	Range of values found in this study (table 5)	**Limit values [1]**
iAs	0.063 - 0.067 (*SL*)	3
Cd	0.21 – 0.49 (*SL*) 1.05 (*AE*)	0.5
Hg	0.01 – 0.06 (*SL*) B.d.l. (*AE*)	0.1
Pb	0.05 – 0.41 (*SL*) 0.21 (*AE*)	5

B.d.l.: below detection limit; [1] French recommendation (CEVA 2019).

Macroalgae may accumulate other PTEs. Levels of Cd and inorganic As in several species have been identified as potential food safety issues (Duinker et al. 2020). The levels of PTEs from samples analysed in this study were below the limits recommended by the French Food safety Authority for seaweed products except for Cd levels in *A. esculenta* which are above the limit and close to the limit in *S. latissima* (**table 9**). 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 25 µg day^{-1} for a 70-kg adult (175 µg $week^{-1}$). The intake of 1 g dried *A. esculenta* (containing 1.05 µg g^{-1} DW) will contribute to 4 % of the maximum daily dose. Besides, the dietary background exposure of the European population from various food sources has been estimated to 1.7 µg per kg bw per week (EFSA 2012), corresponding to 119 µg per week for a 70-kg adult, leaving a safety margin of 56 µg per week to the TWI. Thus, the ingestion of *A. esculenta* based on realistic consumption scenario (one to two meals weekly corresponding to 1 g DW day^{-1}) does not pose a threat to the consumer considering Cd exposure, as previously concluded by Stévant et al. (2017a). The EFSA also reported a relatively high dietary exposure to iAs in the European population with contribution from grain-based products, rice and milk (EFSA 2014a). Therefore, introducing new sources of dietary exposure to iAs must be avoided.

However, the kelps *S. latissima* and *A. esculenta* were not considered as a potential source of exposure to iAs based on measured levels (higher than those measured in this study) and realistic consumption scenario (Stévant et al. 2017a). There are currently no regulatory limits for the levels of PTEs in edible macroalgae. The EFSA is now addressing levels of iodine and heavy metals in macroalgae to support the European Commission in establishing adapted regulations.

4.4. RETENTION OF NUTRIENTS AND IMPACTS ON FLAVOUR

Processing steps may affect the nutrient content of foods as a result of e.g. oxidative reactions and osmotic processes. Kelp species contain valuable nutrients and bioactive compounds (Holdt and Kraan 2011; Stévant et al. 2017b; Afonso et al. 2020) and can be used as ingredients to improve the nutrient profile of commercial food products. In addition, edible macroalgae including kelps are a source of unique flavours and textures that can be combined to a wide variety of food formulations and improve their overall palatability (Chapman et al. 2015; Figueroa et al. 2021). Umami is the most characteristic flavour from kelps and is attributed to the presence of non-volatile (primarily monosodium glutamate) and volatile compounds in the raw material (Figueroa et al. 2021; Milinovic et al. 2021). Therefore, the choice of processing route to produce food ingredients from kelps should aim at retaining the nutrients and flavour-active compounds present in the raw material.

The processing methods tested in this study affected the nutrient profile of the samples in different ways. Freshwater blanching resulted in a loss of soluble nutrients, primarily minerals and carbohydrates (**fig. 7**) while FAs and proteins are retained and up-concentrated in the biomass) as reported in previous studies (Stévant et al. 2017a; Nielsen et al. 2020). A significantly lower umami response from freshwater-blanched *S. latissima* was also observed from the sensory evaluation (**table 6**) suggesting that water-soluble compounds (e.g. monosodium glutamate and aspartate) are partially lost during the process as measured by Nielsen et al. (2020). The detailed analysis of the mineral profile of the samples reveals that macro-, micro and trace mineral elements are unevenly affected by this treatment. A large fraction of Na and K initially present in the samples are lost while the relative proportions of other elements such as Ca, Mg, Cd, Cu and Fe remain stable or increases (**table 5**, **fig. 8-10**). The metal chelation properties of cell-wall polysaccharides e.g. the affinity of alginate for divalent cations (Davis et al. 2003), is likely an major factor governing mineral retention during freshwater blanching.

A similar blanching procedure using seawater resulted in a higher nutrient retention compared to freshwater treatment (**fig. 7**), due to a lower osmotic potential between the kelp fronds and the blanching water. However, the relative proportion of macrominerals was altered during the process i.e. Na, Mg and Ca increased and K decreased (**fig. 8**). This is reflecting the intense saltiness of seawater-blanched samples compared to the other treatments (**Table 6**). Increasing the Na/K ratio would lower the quality of kelp to be used as salt substitute in food formulations. Further work on process optimization with particular emphasis on seawater volume, temperature and treatment time is required.

Kelp stabilization by fermentation implies a pH reduction typically to below 4.0 to inhibit the growth of spoilage microorganism (e.g. clostridia, yeasts, moulds). The pH generally affects the interaction between macromolecules and smaller compounds (e.g. metal ions, free amino acids, peptides). Future studies should focus on the effect of acidic environments during kelp processing on the retention of compounds of interest. The large volume of freshwater used for inoculating seawater-blanched *S. latissima* with lactic acid bacteria in experiment 5 is most likely a major factor contributing to the overall loss of minerals in these samples compared to the fresh control (**fig. 7-10**). Due to low requirements for energy and advanced equipment, lactic acid fermentation is becoming increasingly relevant among kelp producers to quickly stabilize large quantities of biomass (Stévant and Rebours 2021). Optimizing process conditions (e.g. bacterial strains to be used as inoculum, water volume) should be prioritized in later studies.

4.5. FOOD PRODUCT DEVELOPMENT WITH KELP INGREDIENTS

The food prototype experiment in this study aimed to estimate the flavour contribution of kelp ingredients to a typical commercial product. The results from the tetrad tests showed a significant effect of fermented as well as seawater-blanched *S. latissima* on the sensory characteristics of a commercial spinach soup (**table 7**). The hedonic effect of kelp ingredients in a variety of food products should be tested in future research.

The potential of macroalgae including kelps to improve the technological and sensory properties of commercial food products has been reported in numerous studies (Jiménez-Colmenero et al. 2010; Choi et al. 2012; Roohinejad et al. 2017; Akomea-Frempong et al. 2021). Despite multiple macroalgae-containing food products on the European market (Le Bras et al. 2015; Fleurence 2016), the distribution of these products remains limited. However, the growing interest from Western consumers for natural and sustainable food supports a broader use of ingredients from macroalgae in the food industry.

Hypertension is one of the most preventable cause of cardiovascular diseases and strokes and is associated with a high dietary Na/K (Perez and Chang 2014). Processed foods like meat, bread, sauces and condiments, are often characterized by high Na/K ratios (over 5.0) (O'Halloran et al. 2016) while the World Health Organisation (WHO) recommends a ratio close to 1.0 to maintain cardiovascular health. Reducing dietary Na-salt (NaCl) intakes is of high priority in Western societies and several national and European strategies have been implemented to reduce the use of NaCl in the food industry (EU 2009). Kelps such as *S. latissima* are characterized by a salty flavour (Chapman et al. 2015; Stévant et al. 2018) and a low Na/K ratio i.e. typically around 0.5 (Stévant et al. 2017b). Although the mineral profile may be affected during processing of the raw material, the Na/K ratio of seawater-blanched *S. latissima* remained low (0.7, **table 5**). Comments from panellists participating in the tetrad tests in the present study confirmed the salty taste contribution of the kelp ingredients to the tested food prototype (**Table 6**). These results highlight the potential of kelps to be used as functional ingredients for salt replacement in the food industry.

The recent interest for macroalgae as food in Western countries bears the fact that macroalgae are regarded as healthy food ingredients with a strong consumer preference towards organic and sustainable products (Birch et al. 2019; Moss and McSweeney 2021). However, current market barriers are related to food safety, the lack of quality standards and food neophobia (Birch et al. 2019; Jordbrekk Blikra et al. 2021a). The diversity of edible species and processing methods provide opportunities to include macroalgae in a wide range of commercial food products. Furter investigation of the attitudes and preferences from the consumer for seaweeds as food are necessary to design tailored processes and products. Sustainability throughout the entire value-chain together with high product quality and food safety will meet consumer requirements and increase the chances of product success. This is a key to realize the potential of macroalgae to contribute to increased sustainability and health-promoting compounds in the food sector.

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