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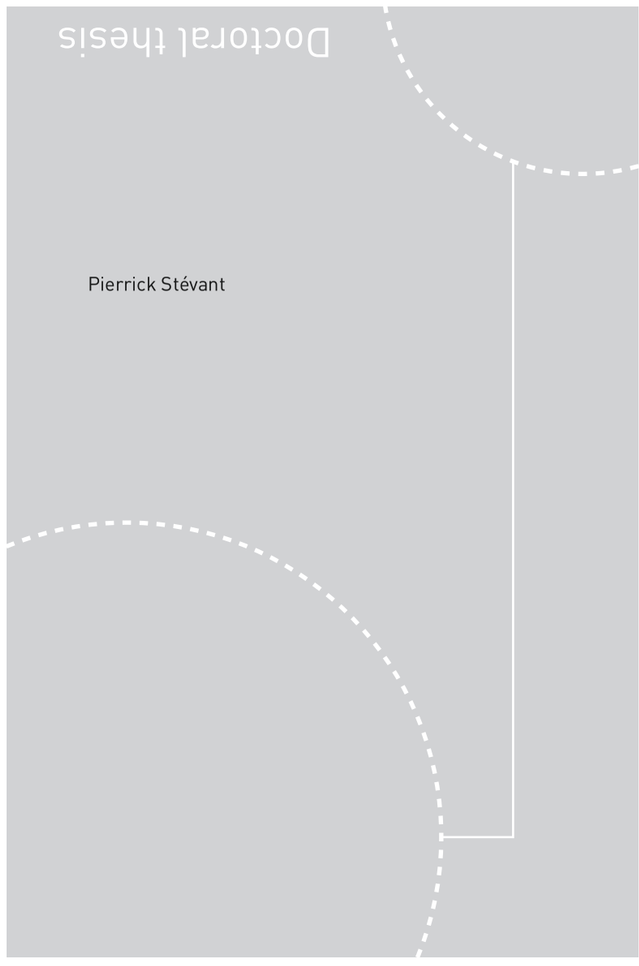
Pierrick Stévant

Seaweeds in food applications: Effects of processing on product quality

Doctoral thesis



Pierrick Stévant

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“Er mor ez eus danvez”

“The sea is a source of goodness” (Breton proverb)

Summary

Unlike in Asian countries, seaweeds have been mostly disregarded as food in Western societies in the past. They are now receiving increasing attention from Western consumers and the food industry following a growing demand for healthy and natural foodstuffs that can be produced in a sustainable way. Industrial seaweed cultivation is emerging in Europe and is expected to become a significant industry in some countries such as Norway, with multiple food and non-food applications for the produced biomass. However, efficient processing strategies providing optimal quality for the targeted products are yet to be established, currently limiting industrial developments. In food applications, the nutrient content, physico-chemical and sensory properties as well as product safety are of prime importance. This doctoral work aimed to evaluate the effects of relevant processing and storage methods on the food product quality from three major seaweed species of commercial interest in Europe: *Saccharina latissima*, *Alaria esculenta* and *Palmaria palmata*.

The results showed that the biomass from these species can be stored in seawater tanks after harvest without significant loss of nutrients. The temperature employed will determine the shelf-life of the biomass. While the nutrient content of seaweed samples air-dried in the range 25 to 70 °C was similar to that of freeze-dried samples, their physico-chemical properties were reduced due to shrinkage especially at high drying temperature. Freezing and subsequent thawing of *S. latissima* resulted in a considerable drip loss (over 40 % of the fresh biomass). Low pH (close to 4.0) was achieved during ensiling of this kelp even without the addition of lactic acid bacteria, but neither chopping the fresh biomass nor the initial addition of alginate lyase did affect the process of ensiling. It was shown that the sensory properties of edible seaweeds are affected by their storage conditions and that controlling these conditions i.e. increasing the moisture content to a certain level, may be used to develop rich and complex flavors compared to the characteristic marine flavors of dried samples. The particularly high iodine content of *S. latissima* was identified as the main food safety issue from the health risk estimation

of the three species. Simple processing methods (exposure to warm water or steam) effectively reduced the iodine content. This thesis also provides insights into the energy requirements related to the different methods studied as well as suggestions for future optimization of seaweed processing.

Sammendrag (summary in Norwegian)

I motsetning til store deler av Asia, har tang og tare ikke vært en viktig del av kosthold i vestlige samfunn. Denne ressursen får nå økende oppmerksomhet fra både vestlige forbrukere og næringsmiddelindustrien på grunn av en økende etterspørsel etter sunne og naturlige matvarer som kan produseres på en bærekraftig måte. Industriell taredyrking er en voksende næring i Europa. I Norge spåes dette å bli en betydelig industri i fremtiden med mange bruksområder (som f. eks. mat) for den produserte biomassen. Effektive prosesseringsmetoder som gir optimal kvalitet for de ulike produktene er ennå ikke etablert, noe som begrenser dagens utvikling av denne næringen. I mat er næringsinnholdet, fysikalsk-kjemiske samt sensorisk egenskaper og at produktene er trygge å spise, de viktigste faktorene. Målet med dette doktorgradsarbeidet var å studere effekten av relevante prosesserings- og lagringsmetoder på produktkvalitet som mat fra tre arter av kommersiell interesse i Europa: *Saccharina latissima*, *Alaria esculenta* og *Palmaria palmata*.

Resultatene viste at biomassen kan lagres i sjøvannstanker etter høsting uten betydelige tap av næringsstoffer. Lagringstemperatur er avgjørende for holdbarheten. Mens lufttørking i temperatur området 25 til 70 °C ikke påvirket prøvenes næringsinnhold i stor grad sammenlignet mot frysetørking, ble fysikalsk-kjemiske egenskapene reduserte på grunn av krymping under prosessen. Frysing og påfølgende opptining av *S. latissima* resulterte i betydelig drypptap (over 40% av fersk biomasse). Lav pH (ca. 4.0) ble oppnådd under ensilering av denne arten selv uten tilsetning av melkesyrebakterier, men hverken kutting av den ferske biomassen eller tilsetning av alginat lyase påvirket ensileringsprosessen. Det ble vist at de sensoriske egenskapene til tang og tare påvirkes av lagringsforhold og at kontroll av disse forholdene, dvs. å øke fuktighetsinnholdet til et visst nivå, kan brukes til å utvikle en mer kompleks sensorisk profil sammenlignet med de karakteristiske marine smaker av tørket tang og tare. En risikovurdering av bruk av de tre arter som mat, viste at det spesielt høye jodinnholdet i *S. latissima* kan utgjør en risiko for konsumenten. Enkle prosesseringsmetoder (eksponering for varmt vann

eller damp) reduserte jodinnholdet effektivt. Dette arbeidet også bidratt med ny kunnskap med hensyn til energikravene knyttet til de ulike metodene som studeres, samt forslag til optimalisering av makroalgeprosessering.

Acknowledgements

This work has been conducted at Møreforsking Ålesund AS and the Norwegian University of Science and Technology (NTNU), Department of Biotechnology and Food Science in the period September 2015 to February 2019.

This thesis was supervised by Prof. Turid Rustad (NTNU), Prof. Joël Fleurence (Univ. de Nantes) and Dr. Céline Rebours (Møreforsking Ålesund) whom I sincerely thank for their excellent guidance, invaluable inputs from their respective fields of expertise and constructive critiques throughout this work. Special thanks go to my initial co-supervisor Dr. Annelise Chapman before she went on the adventure of becoming a seaweed farmer, who is at the origin of this PhD project and has been a true source of inspiration during these past years.

I also thank my colleagues at Møreforsking Ålesund for creating a friendly working environment, for interesting discussions and for their inputs. Siri Stavrum and Thi Cam Huong Nguyen at the Department of Biotechnology and Food Science of NTNU and Emilie Ragueneau, Justine Dumay at the MMS laboratory of the University in Nantes and Turid Fylling Standal at Møreforsking Ålesund are deeply thanked for their technical help to analyse some of the samples. A part of the practical work was conducted at the CEVA in Brittany (France) where I felt like at home, thanks to Hélène Marfaing and the technical staff, *trugarez bras*! I wish to thank both Hélène and Dr. Arne Duinker (Havforskningsinstituttet, Bergen) for sharing their enthusiasm (and some tips and recipes) related to using seaweeds in everyday cooking.

Warm thanks also go to family and friends in France, Norway and elsewhere. To my beloved Ingrid for her unconditional support and our two children Miriam and August who came up to this world during this PhD period and taught me more important things than anything I could learn throughout this project.

Preface

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PROMAC is a multidisciplinary project coordinated by Møreforsking Ålesund AS which draws on key expertise from both Norwegian and international (France, Iceland, Sweden) research communities to create new knowledge for energy efficient and sustainable processing of macroalgae as human food and animal feed. The work presented in this thesis was conducted as part of the project's work package 2, focusing on primary processing methods of seaweed and direct food applications.

List of appendix papers

I. Stévant P, Marfaing H, Rustad T, Sandbakken I, Fleurence J, Chapman A (2017). Nutritional value of the kelps *Alaria esculenta* and *Saccharina latissima* and effects of short-term storage on biomass quality. *Journal of Applied Phycology* 29(5): 2417–2426.

II. Stévant P, Indergård E, Ólafsdóttir A, Marfaing H, Emblem Larssen W, Fleurence J, Roleda MY, Rustad T, Slizyte R, Nordtvedt TS (2018). Effects of drying on the nutrient content, physico-chemical and sensory characteristics of the edible kelp *Saccharina latissima*. *Journal of Applied Phycology* 30(4): 2587-2599.

III. Stévant P, Ólafsdóttir A, Dumay J, Fleurence J, Ingadóttir I, Jonsdóttir R, Ragueneau E, Rebours C, Rustad T (2019). Ageing gracefully? Semi-dry storage as a maturation process to develop the sensory characteristics of the red seaweed dusle (*Palmaria palmata*). Manuscript in preparation.

IV. Stévant P, Marfaing H, Duinker A, Fleurence J, Rustad T, Sandbakken I Chapman A (2017). 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. *Journal of Applied Phycology* 30(3): 2047-2060.

Author's contributions

All experiments described in the papers and included as non-published results (NPR) in this thesis, were planned by P. Stévant, with inputs from H. Marfaing and A. Chapman (**Paper I and IV, NPR 1 and 5**), E. Indergård and T.S. Nordtvedt (**Paper II, NPR 2**), A. Ólafsdóttir, C. Rebours, J. Fleurence and T. Rustad (**Paper III**), C. Rebours and T.S. Nordtvedt (**NPR 4 and 6**), and A. Ólafsdóttir and A. Chapman (**NPR 3**). The experiments, except the drying experiments described in **Paper III and NPR 2** were conducted by P. Stévant, with the assistance of H. Marfaing (**Paper I and IV, NPR 5**) and T.S. Nordtvedt (**NPR4**).

In all experiments, P. Stévant contributed to the analysis of the samples and was responsible for processing all the data and for the statistical analysis of the results. **Paper I – IV** were initiated and edited by P. Stévant following contributions from all co-authors.

Additional scientific contributions (not included in this thesis)

Peer-reviewed articles

Chapman AS, **Stévant P** & Emblem Larssen W (2015) Food or fad? Challenges and opportunities for including seaweeds in a Nordic diet. *Botanica Marina*, 58(6), 423-433.

Stévant P, Rebours C & Chapman A (2017) Seaweed aquaculture in Norway: recent industrial developments and future perspectives. *Aquaculture International*, 25(4), 1373–1390.

Scientific reports

Stévant P, Indergård E, Nordtvedt TS, Barnung T, Fylling Standal T, Rebours C (2018). Optimizing shelf-life of *S. latissima* using refrigeration and freezing storage. Møreforsking – Report MA 18-08.

Stévant P, Nordtvedt TS, Indergård E, Halfdanarson J, Baarset H, Rebours C (2019). Preliminary study on processing sugar kelp (*Saccharina latissima*) using superheated steam drying and steam pre-treatment. Møreforsking – Report MA 19-03.

A selection of oral presentations and posters in international and national conferences

Oral presentations

Stévant P, Marfaing H, Sandbakken I & Chapman A (2016). Effects of short-term storage and washing treatments on the nutritional value of edible seaweeds. 22nd International Seaweed Symposium (ISS), 19-24 June 2016, Copenhagen, Denmark.

Stévant P (2017). Energy-efficient processing of seaweeds in blue-green value chains and effects of primary processing on product quality. Sats Marint conference, 15-16 March 2017, Bergen, Norway.

Stévant P, Rebours C & Chapman A (2017) Seaweed aquaculture in Norway: recent industrial developments and future perspectives. 6th Congress of the

International Society for Applied Phycology (ISAP), 18-23 June 2017, Nantes, France.

Stévant P (2017). Potentially toxic compounds in seaweeds for human food. SIG Seaweed workshop, April 4th 2017, Trondheim, Norway.

Stévant P, Ólafsdóttir A (2018). Developing the sensory profile of seaweeds by maturation process. PROMAC final conference, 08-09 November 2018, Ålesund, Norway.

Posters

Marfaing M, **Stévant P** & Pierre R (2017). Applying ensilage process to brown seaweeds *Saccharina latissima* and *Laminaria digitata*; characterization and consequences for their applications. 6th Congress of the International Society for Applied Phycology (ISAP), 18-23 June 2017, Nantes, France.

Stévant P, Indergård E, Ólafsdóttir A, Marfaing H, Emblem Larssen W, Fleurence J, Roleda MY; Rustad T, Slizyte R, Nordtvedt TS (2018). Effekt av tørking på kvalitet av sukkertare (*Saccharina latissima*) til mat. Havbruk konferansen, 18-20 April 2018, Oslo, Norway.

Stévant P, Marfaing H, Duinker A, Fleurence J, Rustad T, Sandbakken I, Chapman A (2018). Tungmetaller og jod i makroalger: utgjør de en risiko for konsumenten? Havbruk konferansen, 18-20 April 2018, Oslo, Norway.

Abbreviations

AD	Air-drying
AL	Alginate lyase
ANOVA	Analysis of variance
a_w	Water activity
BC	Buffering capacity
CEVA	Centre d'Études et de Valorisation des Algues
DW	Dry weight
EAA	Essential amino acid
EU	European Union
€	Euro
EFSA	European Food Safety Authority
FAA	Free amino acid
FAO	Food and Agriculture Organization of the United Nations
FD	Freeze-drying
GC-MS	Gas chromatography – mass spectrometry
HPD	Heat pump drying
HPLC	High performance liquid chromatography
JECFA	Joint FAO/WHO expert committee on food additives
LAB	Lactic acid bacteria
Lp	*Lactobacillus plantarum*
MSG	Monosodium glutamate
NOK	Norwegian krone
NPR	Non-published result
OBC	Oil binding capacity
PCA	Principal component analysis
RM ANOVA	Repeated measures analysis of variance
R-PE	R-phycoerythrin
RSW	Refrigerated seawater
SES	Seaweed Energy Solutions
SC	Swelling capacity
SSD	Superheated steam drying
TDI	Tolerable daily intake
T_g	Glass transition temperature
TVC	Total viable counts
WBC	Water binding capacity
WHO	World Health Organization

Table of contents

1. Introduction

As the world population is expected to increase to over 9 billion people within the next 40 years, there is a growing pressure to produce more food and feed in a sustainable way. Increasing agricultural production is often associated with ecosystem deterioration such as deforestation, reduced biodiversity, and eutrophication of aquatic systems (FAO 2011). Strategies to meet this demand points towards value-chains linked to renewable resources, reduced environmental impacts including carbon emissions, to support global actions against climate change, and the complete utilization of the biomass produced. In this context, seaweeds (also referred to as "macroalgae") are considered a promising resource for the sustainable production of food and animal feed.

Seaweeds are a rich source of nutrients e.g. minerals, fibers, vitamins, trace elements, and other health promoting compounds providing benefits beyond basic nutrition (Holdt and Kraan 2011). A wide range of edible seaweeds are also prized for their unique flavors and widely used in everyday culinary applications, particularly in Asia. Although the exploitation of macroalgal resources in Western societies has mainly focused on the industrial extraction of polysaccharides as gelling agents (e.g. alginates), the use of seaweeds as a food item (e.g. sea vegetable, condiment) and health promoting ingredients in the food industry has gained increasing interest over the past decades. Seaweeds are recognized as a source of bioactive compounds with applications in human nutrition such as functional[1] ingredients and dietary supplements (nutraceuticals). The chemical content of seaweeds and associated health benefits from individual bioactive substances are well reviewed in the scientific literature (Holdt and Kraan 2011; Pereira 2011; MacArtain et al. 2007; Fleurence and Levine 2016; Wells et al. 2017).

[1] The term "functional" used in this thesis is associated with foods and food ingredients that provide health benefits (based on scientific evidence) beyond the provision of essential nutrients.

1. Introduction

Seaweed biomass can be cultivated on a large scale in coastal areas without competing for fresh water or soil resources and the production rates of some macroalgae species like kelps exceeds those of agricultural plant production (Bruhn et al. 2016). Norway has an extensive coastline as well as existing know-how on processing of marine raw materials and related infrastructures. Recent efforts from research, industry and public authorities have been devoted to developing a bio-economy based on the utilization of seaweed biomass. Future perspectives for industrial developments are positive (Stévant et al. 2017), in line with the European strategy driving "Blue growth" initiatives. Progress on cultivation technology includes the year-round production of spores and seedlings of sugar kelp (*Saccharina latissima*) (Forbord et al. 2012), and high production yields (150 - 200 ton wet weight ha^{-1} $year^{-1}$)[2] are expected (Broch et al. 2013; Handå et al. 2013; Wang et al. 2013; Broch et al. 2019).

Besides nutritional and functional benefits, seaweeds also have properties that may enhance the physico-chemical characteristics of foods e.g. water- and oil-binding capacities (WBC and OBC), swelling capacity (SC), color, textural properties as well as product shelf-life (Rioux et al. 2017; Roohinejad et al. 2017). In food applications, these parameters are important factors which can improve food formulations and the consumer acceptance of a product.

Macroalgae have also attracted considerable attention as a potential feedstock for multiple applications, including bio-fuels, -plastics and -chemicals, fertilizers, cosmetic and pharmaceutical products. Biorefinery concepts for cultivated seaweeds aiming at the complete utilization of the raw material and the valorization of both high-value products and waste streams are currently under development (Baghel et al. 2016).

However, the high water content of macroalgae (70 to 90%, Jensen 1993) and rapid decay once harvested (Enríquez et al. 1993) represents a challenge for conserving and transporting the biomass from harvesting to processing sites. In the case of using seaweeds in food applications, maintaining the nutrient content and enhancing

[2] The term "ton" used here and further in the text refers to metric ton

organoleptic properties (flavor, color, texture) as well as minimizing potential food safety issues of the product are of high priority. Optimized processing strategies to supply high quality products at acceptable costs are still missing. This has been identified by stakeholders as a major bottleneck for industrial developments (Skjermo et al. 2014; Stévant et al. 2017). There is therefore a strong need for detailed investigations into how the quality of seaweeds to be used in food applications is affected by processing methods and storage conditions suited for commercial production. Understanding the behavior of the seaweed biomaterial is a key to develop processing strategies that will maximize product quality and contribute to the development of value-chains based on macroalgal resource. Current needs in research and development from seaweed producers are directed towards energy efficient processing standards that will maintain or increase product quality, ensure consumer safety and maximize biomass utilization.

The following thesis answers the pressing demand from an emerging industry producing and using seaweed raw material in Norway and Europe, to study the effects of primary processes, i.e. post-harvest handling including short- and long-term preservation methods and storage conditions, on the quality of the biomass to be used in food applications. The content of macro- and micronutrients, physico-chemical properties and sensory characteristics along with levels of potentially toxic elements are in focus in the present work, as key factors governing the quality of seaweeds as food. Technical aspects and energy requirements related to the studied processes are also discussed. Although the scope of this study is limited to food applications, the generated knowledge on the process and products will also be relevant to other applications relying on this renewable bioresource.

This doctoral work consists of individual experiments investigating the effects of specific primary processes and storage methods on the overall quality of seaweeds to be used as food. The main results and outcomes of each study, including in-depth discussions are reported in **Paper I - IV**. Additional non-published results (**NPR 1 - 5**) obtained during the project are also included in this thesis to support the data from published papers and provide a comprehensive study of the topic.

2. Overall aim and specific research objectives

The main objective of this work has been to **study the effects of various primary processes relevant to the seaweed industry on the quality of three edible seaweeds species which are currently cultivated and/or harvested in Europe**, i.e. sugar kelp (*Saccharina latissima*), winged kelp (*Alaria esculenta*) and dulse (*Palmaria palmata*) (**fig. 1**). The term "primary processing" used in this study refers to post-harvest handling of the biomass including pre-treatments (e.g. soaking, heat treatment) as well as short- and long-term preservation methods (e.g. drying, freezing) and excludes fractionation processes of the biomass for further extraction of specific compounds. The quality is defined as the nutrient content and levels of bioactive substances, physico-chemical and organoleptic properties of the seaweed raw material measured by quantitative (chemical analyses) and qualitative methods (color and texture analyses, sensory evaluations).

Specific scientific objectives were formulated as follows:

- Can the post-harvest shelf-life of fresh seaweed biomass be prolonged by storage in seawater tanks without significant loss of nutritional compounds? (**Paper I, NPR 1**)
- Is the quality of edible seaweeds affected by the drying method and/or temperature levels commonly employed during processing? (**Paper II, NPR 2**)
- How stable is the quality of dried seaweeds during storage? Can the sensory characteristics of seaweed products be influenced and controlled by controlling the storage conditions? (**Paper III, NPR 3**)
- Can freezing and subsequent frozen storage extend the shelf-life of *S. latissima* without significantly affecting the quality of the biomass after thawing? (**NPR 4**)
- Does the initial addition of ferment and/or enzymes as well as a biomass chopping pre-treatment step improve the stability and quality of silages from *S. latissima*? (**NPR 5**)

- What are the health risks for the consumer associated with the presence of potentially toxic elements in edible seaweeds? Can food safety issues related to potentially undesirable compounds in edible seaweeds be remediated by simple processing steps? (**Paper IV, NPR 1, NPR 6)**

A multidisciplinary approach, combining methods from biochemistry, sensory science and process engineering, was used to answer these questions.

The following chapter provides background information relevant to this work, on the production and uses of macroalgae in food applications, with emphasis on the European and Norwegian contexts. The results obtained during this project are integrated into a general review presenting the status of knowledge on the nutrient content, physico-chemical and sensory properties of edible seaweeds as well as potential food safety issues related to their consumption (chapter 5), and the effects of primary processing and storage methods on product quality (chapters 6).

Fig. 1: Three seaweed species studied in this thesis. From left to right: *S. latissima*, *A. esculenta* and *P. palmata*.

3. Background: seaweed production and uses in food applications

3.1. Seaweed biology

Seaweeds belong to a diverse group of multicellular marine photosynthetic organisms. Seaweeds are classified into 3 broad groups (phyla) based on their pigmentation, namely brown, red and green, referred to by phycologists as Phaeophyceae, Rhodophyceae and Chlorophyceae respectively. Species differ considerably in their life cycle as well as their morphology and biochemical features both within and across phyla. Seaweeds can be found in all coastal areas from the uppermost level of the medio-littoral zone down to the lower limits of the euphotic zone where the available light limits their growth. As photosynthetic organisms, seaweed efficiently take up carbon dioxide (CO_2), bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}) from the ocean, along with utilizing light as the energy source to produce sugars and oxygen (O_2). They also take up organic and inorganic substances, e.g. nitrogen and phosphorus, to sustain their growth and biological functions. Macroalgae and especially kelp forests support complex food webs and provide ecosystem services such as habitat, food and shelter to a variety of organisms from all trophic levels, as well as nutrient cycling (Smale et al. 2013).

There are about 12 000 species of marine macroalgae described to date (www.algaebase.org). Of the three species of interest in this thesis, two are brown macroalgae, namely the kelps *A. esculenta* (“winged kelp”) and *S. latissima* (“sugar kelp”). Both are perennial organisms naturally growing in the subtidal zone, the former typically reaching 1 to 2 m length and the latter up to 4 m. *P. palmata* (“dulse”) is a red macroalgae characteristic of the intertidal and subtidal zones of exposed sites. The species is found attached on rocks or is epiphyte of large kelps. The fronds grow up to 50 cm. All three species are commonly found across the North Atlantic ocean (Cabioc'h et al. 2006).

3.2. Global seaweed production

Global figures show that most of the macroalgal biomass harvested is produced through aquaculture (FAO 2018a). The latest released FAO aquaculture statistics (2018a) for the year 2016 reports a global aquaculture production of 110.2 million ton (wet weight) of aquatic biomass, of which 30.1 million ton were aquatic plants, mostly consisting (over 95 %) of marine macroalgae, with a commercial value of 10.2 billion €[3]. The global seaweed aquaculture production expanded in average at 8 % per year over the past decade, mostly in Asian countries (> 99 %), i.e. China and Indonesia (accounting for over 86 % of the global production), The Philippines, South and North Korea, and Japan (FAO 2018a). As autotrophic organisms, macroalgae utilize dissolved organic and inorganic substances in combination with light for growth and do not require feed nor fertilizer. They can be cultivated on large scales in coastal areas with little or no demand on fresh water resources in their production cycles, offering an alternative to the production of terrestrial crops.

As part of the culinary culture in Japan, Korea and China, seaweeds have been grown and harvested for centuries (Mumford and Miura 1988). Modern cultivation techniques were developed since the early 1950s and have improved dramatically, benefiting from advances in the fields of marine ecology, taxonomy, physiology and genetics (Kim et al. 2017). There is a large variety of cultivation techniques employed depending on the biology and life cycle of the species, ranging from ropes or nets seeded with spores in open-water systems, to onshore cultivation ponds or tanks. Seaweed aquaculture production is dominated by relatively few species of brown (*Saccharina japonica* and *Undaria pinnatifida*), and red seaweeds (*Eucheuma* spp, *Kappaphycus alvarezii*, *Gracilaria* spp, and *Pyropia* species (formerly *Porphyra*) (FAO, 2018b; **fig. 2**).

Wild seaweed biomass is also harvested for industrial purposes although it only represents 3.5 % by volume of the total 31.2 million ton of wild-collected and cultivated aquatic plants combined (FAO 2018a). In Europe, the kelp species *Laminaria*

[3] The original value is given in US$. The exchange rate of 1 US$ = 0.8818 € was used

hyperborea and *Laminaria digitata* are harvested mechanically mainly in Norway and France (ca. 120 000 ton and 49 000 ton for both species respectively for the year 2016; FAO, 2018b), to supply the alginate industry. This activity is somewhat controversial since the removal of and interference with natural habitats (i.e. kelp forests) has the potential to affect local biodiversity and ecosystem integrity (Lorentsen et al. 2010).

The vast majority (ca. 83 %) of both the cultivated and wild harvested seaweeds are consumed by humans, either as a direct food source (sea vegetables) or as a food additive, i.e. phycocolloids (agar, carrageenan, alginates) while the remainder is used as fertilizers, animal feed additives, and in medical and biotechnological applications (McHugh 2003; Craigie 2011; Buschmann et al. 2017).

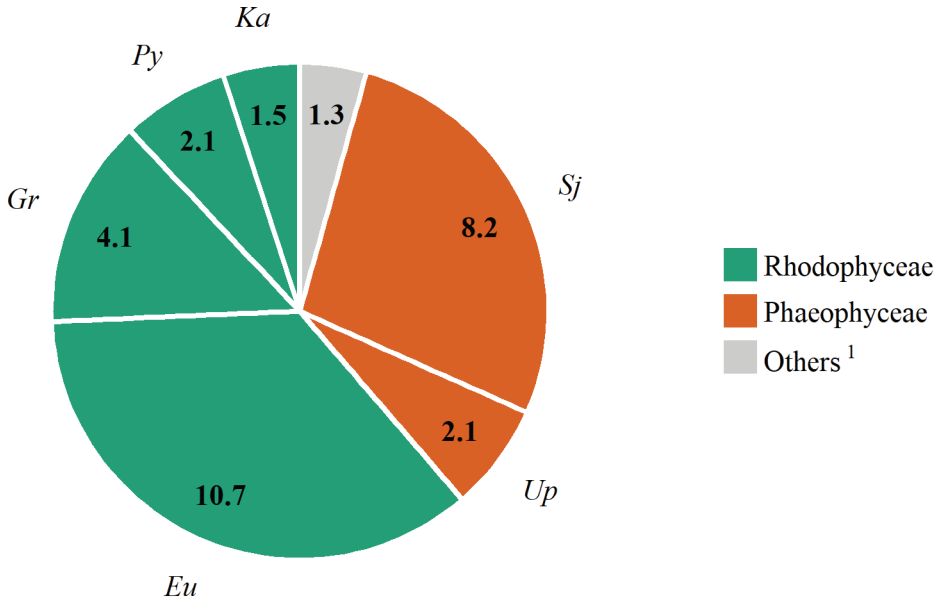


Fig. 2: Production of the main seaweed species by aquaculture for the year 2016 (in million-ton wet weight). *Sj*: *Saccharina japonica* (*konbu*), *Up*: *Undaria pinnatifida* (*wakame*), *Eu*: *Eucheuma* spp. (Eucheuma seaweeds), *Gr*: *Gracilaria* spp. (*Ogonori*), *Py*: *Pyropia* spp. (*nori*), *Ka*: *Kappaphycus alvarezii* (Elkhorn sea moss). Data from FAO (2018b). [1] Includes Chlorophyceae and unspecified seaweeds.

3.3. Seaweed cultivation in Europe and Norway

Aquaculture of macroalgae is rare outside Asia. According to FAO (2018b), less than 1 500 tons of seaweeds were cultivated in Europe in 2016, representing only 0.005 % of the total cultivated volume globally, with a value of 2.4 million €. Although seaweed aquaculture is a relatively new industry in Europe and North America, the Western demand for seaweeds is increasing following recent trends promoting sustainable sources of proteins, functional and textural ingredients in the food industry as well as healthy food supplements to consumers. The potential for using seaweeds in various industrial applications, i.e. food (Wells et al. 2017; Rioux et al. 2017) and non-food (Hellio et al. 2001; Bruhn et al. 2011), has stimulated the interest to cultivate biomass during the last two decades. In addition, seaweed cultivated in proximity to fish farms in so-called Integrated Multitrophic Aquaculture systems (IMTA) represent a practical solution for mitigating the negative effects of fish farming wastes by utilizing dissolved excess nutrients as a valuable resource for macroalgal production (Chopin et al. 2001; Troell et al. 2009; Chopin et al. 2012). Along with recent focus on aquaculture developments and sustainability, the increased yield of biomass produced on a single site in IMTA systems is also a driver for upscaling seaweed cultivation in Europe (Sanderson et al. 2012; Wang et al. 2012b; Handå et al. 2013; Fossberg et al. 2018).

Currently, pilot-scale and pre-commercial seaweed farming projects largely focuses on kelp species, primarily *S. latissima* and *A. esculenta*, due to their phytochemical content and ability to achieve high biomass yields in short time (Peteiro et al. 2016; Broch et al. 2019). *P. palmata* is valued for its favorable nutrient profile and gastronomic potential (Mouritsen et al. 2013). Cultivation protocols for the commercial production of this species are under development. Joint efforts of both research and private sectors have successfully developed the cultivation technology for kelp species based on their life-cycle (Forbord et al. 2012). Efforts are now being directed at developing efficient farming strategies and marine technology to mechanize seedling deployment and biomass harvest and reduce the need for maintenance. However, biofouling by epiphytes is a major constraint for the year-round cultivation of kelps. Encrusting fouling by bryozoans leads to extensive losses of biomass and considerable quality deterioration,

forcing producers to harvest in May-June, before the onset of fouling (Forbord et al. 2012; Førde et al. 2016).

Norway has a long and complex coastline characterized by fjords and skerries, which is highly suited for aquaculture. The country is leading the global production of salmon and trout (1.32 million tons were farmed in 2016; Statistisk Sentralbyrå, 2017) and has competence and infrastructures related to the production and processing of marine raw material. In recent years, seaweed cultivation has been an active area of research in Norway, as part of the national strategy to develop a bio-economy based on renewable biomass production (Skjermo et al. 2014). Since the first commercial permit for macroalgal cultivation at sea in 2014, the total area allocated to seaweed farming has increased rapidly, following the interest of private stakeholders to develop an industry based on cultivated biomass (Stévant et al. 2017) (**table 1**). Although this potential by far exceeds the real national production output, given that most companies involved in this activity are at an experimental start-up phase, this rapid evolution reflects the commitment of both public authorities and private actors to develop seaweed aquaculture.

Products with a relatively high market value such as food and food ingredients are predicted to play an important role in creating value from Norwegian cultivated seaweeds (Skjermo et al. 2014; Stévant et al. 2017). New food products containing seaweeds have been released regularly in Europe since 2011, with a 147 % increase between 2011 and 2015 (Mintel 2016) reflecting the consumer's interest for this resource. A seaweed industry relying on the use of cultivated biomass is emerging and expected to grow in Europe and Norway based on the demand for sustainable nutrient-rich foods in developing as well as in developed countries. Establishing efficient post-harvest logistics and biomass processing steps have been identified as prerequisites to fulfill this potential (Skjermo et al. 2014).

Table 1: Statistics from seaweed cultivation in Norway between 2014 and 2018 (as per 13.02.2019). Table updated from Stévant et al. 2017. Source: Directorate of Fisheries (2018).

	2014	2015	2016	2017	2018
Number of seaweed cultivation sites at sea	10	28	41	55	71
Total area allocated to seaweed cultivation at sea (ha)	96	214	296	475	738
Seaweed production output (ton wet weight)	na	51	60	145	na
Commercial value (1000 €) [a]	na	18	94	72	na
Number of companies involved	6	15	21	31	38

[a] Values initially expressed in Norwegian krone (NOK). The exchange rate of 1 NOK = 0.1026 € was used.

3.4. Current trends in using seaweeds in food

Historical uses of seaweeds for food by coastal populations can be traced several millennia back in time (Dillehay et al. 2008; Mouritsen 2013). According to Zemke-White and Ohno (1999) there are at least 145 species of brown, red and green seaweeds with records of use as human food worldwide. Nevertheless, the use of macroalgae as staple food remains limited to Eastern and South-Eastern Asia, where a variety of edible species enters the composition of the daily diet. In these countries, seaweeds are prized for their nutritional properties as well as rich and unique flavors. The most commonly consumed species include *S. japonica* (*konbu*), *Pyropia* spp. (*nori*), *U. pinnatifida* (*wakame*), *S. fusiforme* (*hijiki*) and *Monostroma* spp. (*aonori*). They are consumed either fresh, salted or dried, often in soups, salads, sushi or snacks (Fleurence 2016). Due to the lack of reliable data, estimating the seaweed consumption per country is a difficult task. The average daily consumption of seaweeds per capita in Japan has been estimated

and reported to be between 3.3 to 5.3 g (DW) per day (Darcy-Vrillon 1993; Matsumura 2001).

Seaweeds occur with high abundance and diversity along the coasts of Europe and have not been a significant food source in Western societies throughout the past centuries. Traditions for eating seaweeds have been limited to coastal Atlantic communities in Brittany, Ireland, Iceland, Wales and Norway (Kristjánsson 1980; Rhatigan 2009; Mouritsen 2013). However, seaweeds have enjoyed an increasing interest from Western consumers during the past decades, because of the popularity of Asian dishes as well as the renewal of ancient traditions in coastal European countries. Seaweeds are now sold to restaurants or directly to the consumer as sea vegetables and condiments or included in a large variety of food products e.g. canned products, butter, soups, alcoholic drinks (Le Bras et al. 2015; Fleurence 2016). In France, following the increasing popularity of seaweeds as food during the 1980s, the national food safety authority established a list of macroalgae species authorized as sea vegetables and condiments on the market (Mabeau and Fleurence 1993; CEVA 2014) (**table 2**). As part of a specific regulation, these products must meet certain criteria regarding their levels of potentially toxic elements (see section 5.4).

In Scandinavia, the renewal of the Nordic Cuisine fronted by avant-garde restaurants and based on locally available natural ingredients, stimulated the interest for including seaweeds in cooking. Recent studies conducted in Norway and Denmark highlighted the potential of locally available seaweed species to be included in traditional recipes and everyday culinary applications as means to enrich the overall flavor and to provide texture to food (Mouritsen et al. 2012; Chapman et al. 2015) (**fig. 3**). While the consumption of seaweeds in Asia is based on culinary traditions and largely depends on the organoleptic properties and the price of the product, the renewed interest for this resource from Western markets bears the fact that seaweeds are regarded as healthy food ingredients with a strong consumer preference towards organic, sustainable and fair-trade products (Buschmann et al. 2017; Birch et al. 2018b).

Table 2: Macroalgae species authorized in France for human consumption (Mabeau and Fleurence 1993; CEVA 2014).

Scientific name	Common name
Brown seaweeds	
Alaria esculenta	Winged kelp
Ascophyllum nodosum	Egg wrack
Fucus vesiculosus	Bladder wrack
Fucus serratus	Toothed wrack
Himanthalia elongata	Sea spaghetti
Laminaria digitata	Oarweed
Saccharina latissima	Sugar kelp
Saccharina japonica	*Konbu*
Undaria pinnatifida	*Wakame*
Red seaweeds	
Chondrus crispus	Irish moss
Gracilaria verrucosa	*Ogonori*
Lithothamnion calcareum	Maërl
Palmaria palmata	Dulse
Pyropia / *Porphyra* spp [a]	Laver, *nori*
Green seaweeds	
Ulva spp [b]	Sea lettuce

[a] Includes the species *P. umbilicalis*, *P. tenera*, *P. yezoensis*, *P. dioica*, *P. pupurea*, *P. laciniata* and *P. leucostica*. [b] Includes species of the former *Enteromorpha* genus.

Seaweeds can be included in a wide range of food preparations as versatile and delicious whole foods (e.g. fresh sea vegetables, dried flakes) or in the form of extracts where

single or multiple bioactive compounds recovered from the biomass can be used for specific purposes in the food industry. In spite of the increasing awareness of this potential from the consumer (Marfaing et al. 2009), eating seaweed is still regarded as an exotic practice. Food neophobia has been identified as a major obstacle for consuming seaweeds (Birch et al. 2018a). Generally, the quality requirements for the commercial success of natural food products include flavor and nutritional value, along with color, mouthfeel, freshness, cleanliness as well as traceability and ethical requirements related to the values that condition consumer behavior (Peri 2006).

Fig. 3: Examples of food dishes containing seaweeds prepared during a cooking workshop in Norway described in Chapman et al. (2015): A) cod with leeks and sugar kelp (*S. latissima*), and a white butter sauce with dulse. B) marinated halibut with oarweed (*L. digitata*). C) dulse (*P. palmata*) tagliatelle and chicken breast with sugar kelp crust. D) clip fish brandade with dulse.

There is therefore a need for developing a range of tasty, nutritious, safe and convenient seaweed food products, attractive to Western markets, to support the emerging industry based on macroalgae in Europe. This goal can be achieved by optimizing processing methods to provide high quality ingredients to be used in food applications and develop adapted market strategies. In addition, the legislation for this type of products is underdeveloped in Europe and needs to be properly established, based on scientific data related to food safety issues and levels of potentially undesirable compounds in seaweeds.

4. Methodology

A detailed description of the methodology employed in each individual study is given in the Material and Methods section of the respective papers. The following sections describe the methodology used to collect the data related to unpublished research conducted throughout the project.

4.1. Study of the chemical composition of *P. palmata* and effects of seawater storage (NPR 1)

Biomass collection – Wild biomass of *P. palmata*, free of epiphytes, was harvested in May 2016 at Pleubian in Britany and received the same day at the Centre d'Étude et de Valorisation des Algues (CEVA)'s laboratory. The seaweeds were rinsed with seawater (filtered at 10 µm) to remove associated organisms.

Seawater storage – The same experimental conditions (stocking density, water mixing) as described in **Paper I** to investigate seawater storage of *A. esculenta* and *S. latissima*, were applied to the *P. palmata* biomass. However, the temperature of the filtered seawater (10 µm) supplied to the tanks was lower than the temperature measured during the experiment on kelps (13.5 ± 0.0 °C vs 18.0 ± 1.0 °C reported in **Paper I**). Samples of 500 g of seaweed biomass were analyzed for their chemical content both prior to (t_0) and after 22-h storage. The initial sampling at t_0 corresponds to biomass arrival at the laboratory. Samples were taken after 1, 2 and 6-h storage for chemical analyses. The storage treatment was performed in triplicate and the samples were freeze-dried and analyzed for their dry weight (DW), ash, sodium (Na), potassium (K) and protein contents as well as carbohydrate profile using the methods described in **Paper I**. The content in R-phycoerythrin (R-PE) pigment was analyzed as described in **Paper III**. The samples were also analyzed for their levels of potentially undesirable compounds, i.e. iodine (I), cadmium (Cd) and inorganic arsenic (iAs) as described in **Paper IV**. Repeated

measures analysis of variance (RM ANOVA) was used to detect differences in quality parameters over time using the same method as in **Paper I**.

4.2. Effects of drying on the chemical composition and physico-chemical characteristics of *P. palmata* (NPR 2)

Biomass collection – Wild biomass of *P. palmata* was harvested on May 8, 2017 in Ålesund, Norway, and immediately transported to the laboratory where epiphytes were discarded. The seaweeds were rinsed with seawater (20 µm, protein skimmer- and UV-treated).

Drying treatments – Air-drying (AD) treatment at 40 °C in a shelf dryer and freeze-drying (FD) were performed as described in the corresponding study performed on *S. latissima* (**Paper II**). AD at 70 °C was performed in a drying cabinet. All treatments were performed in three replicates using approximately 1 kg of seaweed biomass per replicate. Dried samples were vacuum-packed and dispatched for further analyses. DW and ash content of the samples as well as their physico-chemical and color properties were analyzed using the methods described in **Paper II**. The water-soluble fraction of the carbohydrates and proteins, including the R-PE pigment, as well as the lipid content of the samples were determined using the methods described in **Paper III**. ANOVAs were used to detect differences in individual quality parameters and a principal component analysis (PCA) based on covariance matrix was used to visualize differences in color profiles among sample groups as described in **Paper III**.

4.3. Preliminary study on seaweed maturation: effect of moisture content during storage on the sensory characteristics and free amino acid content of *S. latissima* (NPR 3)

Sample handling and sensory analysis – Samples of *S. latissima* air-dried at 25 and 70 °C from the drying experiment reported in **Paper II** were used in this study. The moisture content of the dried samples was determined gravimetrically and the biomass from each drying temperature group was divided into 2 groups: a dried control (labelled 25-D and 70-D) and a test group (labelled 25-M and 70-M). At the start of the experiment, the test samples were partially rehydrated by spraying the required amount of water (unfiltered tap water) to achieve 20 % moisture content in the samples, as preliminary tests of storage at this moisture level did not reveal any signs of spoilage. All samples (i.e. from both test and control groups) were sealed in polyethylene bags and stored in the dark at a constant temperature of 12 °C for 97 days until their sensory evaluation. The DW of the samples was measured again prior to the evaluation. The experimental protocol employed during this study is illustrated in **fig. 4**.

The 4 sample groups (25-D, 70-D, 25-M and 70-M) were characterized by a descriptive test (ISO:13299 2003) using a trained panel consisting of eight judges as described in **Paper II**. The results from the sensory evaluation of the dried samples (25-D and 70-D) are presented in **Paper II**. This additional set of data reports the results obtained from the matured groups (25-M and 70-M) and provides a comparison with those of the control dried groups. The same set of 13 sensory attributes as presented in **Paper II** were used here. These attributes are listed and described in **table 3**. The statistical analysis of the sensory results were performed on R software (R Development Core Team 2018) using a mixed model ANOVA (sensmixed function) from the SensMixed package (Kuznetsova et al. 2018) with individual panelists treated as random factor. Drying temperature and moisture levels during storage were treated as fixed factors. A PCA (R function prcomp) based on covariance matrix was applied to visualize differences in sensory profiles among sample groups.

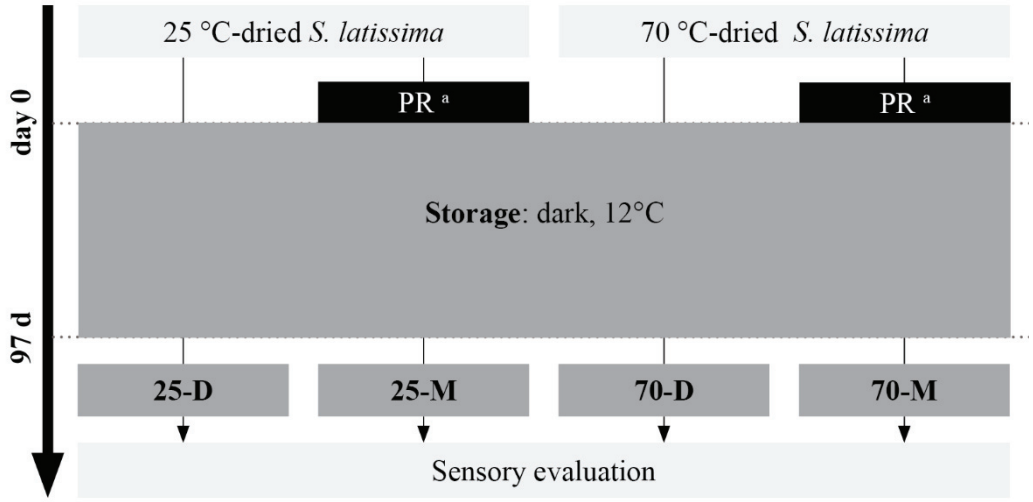


Fig. 4: Experimental design to study the effects of drying temperature and storage conditions i.e. low (D) and moderate (M) moisture content on the sensory properties of *S. latissima*. [a] PR: partial rehydration of the samples to 20 % moisture content.

Sample handling and free amino acid (FAA) analysis – Samples of *S. latissima* air-dried at 40 °C (**Paper II**) were used in this experiment. These samples were divided into a control group and a matured group. The samples from the matured group were split into 3 sub-groups which were partially rehydrated to approximately 20 % moisture and stored in a semi-dry state, under the conditions described above, for different periods (**fig. 5**). All treatments were performed in triplicates.

The FAA content and profile of the samples was determined using the method described in **Paper II** and **III**. A one-way ANOVA was performed on R software (ANOVA, R function aov) to detect differences among sample from different treatments regarding their total FAA levels, after testing for homogeneity of variances (Levene's test). A Tukey's honest significant difference (HSD) test was used for post-hoc comparisons of significant ANOVA results. A PCA (R function prcomp) based on covariance matrix was used to differentiate the samples based on their FAA profile.

Table 3: Sensory attributes, and their definitions, associated to the *S. latissima* samples.

Sensory attribute	Label	Scale anchors	Definition
Aroma			
Fresh sea	A – fresh sea	none || much	Fresh sea odor
Fermented	A – fermented	none || much	Fermented odor, pungent, marmite, matured cheese, cured
Hay	A – hay	none || much	Dry hay, green tea
Flavor			
Salty	F – salty	none || much	Salty taste
Fresh sea	F – fresh sea	none || much	Sea flavor
Fermented	F – fermented	none || much	Fermented flavor, matured cheese, marmite, cured
Hay	F – hay	none || much	Fresh hay, green tea
Umami	F – umami	none || much	Umami, meat stock, brown crab meat
Bitter	F – bitter	none || much	Bitter taste
Texture			
Crispy	T – crispy	cohesive || crispy	During first bites, how crispy is the sample
Chewy	T – chewy	tender || chewy	While chewing, chewy: difficult to disintegrate
Viscous	T – viscous	thin || viscous	Viscous, slimy, porridge like
Dissolves	T – dissolves	None || much	Dissolves or melts easily in mouth while chewing

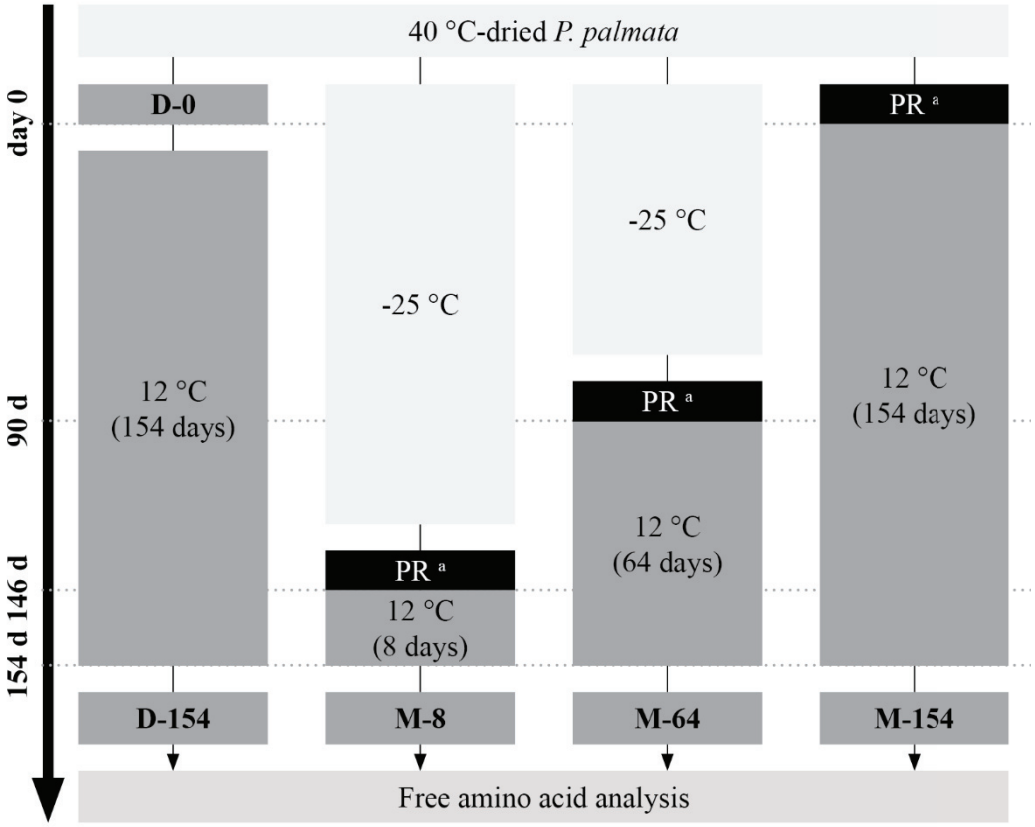


Fig. 5: Experimental design to study the effects of storage conditions i.e. low (D) and moderate (M) moisture content on the FAA content of *S. latissima*. [a] PR: partial rehydration of the samples to 20 % moisture content.

4.4. Preliminary study on the frozen storage and thawing of *S. latissima* (NPR 4)

Biomass collection – Cultivated biomass of *S. latissima* was harvested at Seaweed Energy Solution (SES) at Frøya on June 7, 2017, then transported to SINTEF's laboratory facilities, where the seaweeds were stored overnight in a tank (2 m^3) provided with a seawater (9 °C) flow-through system.

Freezing and thawing – Individual bags containing at least 3 kg of *S. latissima* blades were prepared and weighed. The samples were frozen at -25 °C using impingement freezing technology (Frigoscandia, ADVANTEC) then stored in a freezer warehouse at -25 °C. Sample bags were taken after 1, 3, 6 and 11-months storage, then either thawed in a laboratory incubator maintained at 20 °C during 24 h or in running tap water at 13 °C and 1 L min^{-1} during 10 h. Both thawing methods at each sampling time were tested in triplicates. The samples were analyzed for their color, texture, drip loss, dry matter content and microbial load.

Quality parameters – The DW of the solid fraction of the samples was determined gravimetrically as the residue remaining after drying in a laboratory oven at 105 °C for 24 h. Each measurement was performed in triplicate. The surface color of the samples was analyzed using the method described in **Paper I - IV**.

The texture of thawed *S. latissima* biomass was analyzed by measuring the tensile strength of the blades using a TA.XT plus texture analyzer (Stable Micro Systems Ltd, Godalming, UK) following the method adapted from Choi et al (2012a). 10 cm-long sample blades of *S. latissima* were cut ca. 3 cm over the meristematic zone (basal part over the blade stipe) and fastened on a perforated plate mounted on the texture analyzer. The peak load (maximum resistance) during perforation of individual blades was measured in tension mode (5.0 g trigger load, 1 mm s^{-1} crosshead speed) using a P5/S spherical probe and expressed in gram. A minimum of 5 blades from each sample were analyzed.

The drip loss following freezing and thawing reflects the amount of liquid lost from the seaweed blades after thawing and was calculated as following:

$$\frac{W_L}{W_i} \times 100$$

Where W_L is the weight of the liquid fraction from the thawed samples and W_i is the initial weight of the samples.

The microbial load (aerobic bacteria, coliforms, yeasts and molds) of thawed *S. latissima* samples was analyzed using the total viable count (TVC) method described in **Paper III**.

Statistical analysis – Statistical analyses were performed on R software including functions from the nlme package (Pinheiro et al. 2017). Raw data were pre-processed for descriptive statistics and the results expressed as mean ± standard error ($n = 3$). The difference among thawing treatments (between-subject variable) over time (within-subject variable) in quality parameters were analyzed by repeated measures analysis of variance (RM ANOVA, R function lme) at $p < 0.05$.

4.5. Preliminary study on ensiling *S. latissima*: effects of biomass pre-treatment and inoculation on the ensiling process (NPR 5)

Biomass collection and inoculant preparation – Fresh biomass of *S. latissima* was harvested in May 2016 from a commercial cultivation site (Algolesko, Brittany, France). No visible epiphytes were present on the surface of the blades. Freeze-dried *Lactobacillus plantarum* (PAL LP 3233, Standa, France) was rehydrated in a normal saline solution (NaCl 0.90 %) at ambient temperature during 30 min. The enzyme used as inoculum was alginate lyase from *Pseudomonas alginovora* purified at CEVA's laboratory facilities.

Ensiling – The fresh biomass of *S. latissima* was split into 4 batches corresponding to different treatment groups. The effect of (i) biomass pre-treatment i.e. whole vs chopped and (ii) silage inoculant i.e. no inoculum (control) vs. *L. plantarum* (*Lp*) vs. *Lp* + alginate lyase (AL), on the preservation efficiency of *S. latissima* was investigated. The biomass from the pre-treated group was chopped into 1 cm pieces in a knives-mill (Urschel, Comitrol Processor Model 3600). 3 of the 4 groups were added a solution containing *L. plantarum* at a ratio of 10^6 CFU g^{-1} fresh seaweed as suggested to be optimal by the ferment manufacturer. In addition, one group was added a solution containing AL

(diluted in seawater, pH optimum: 7.6) at a ratio of 0.16 g enzyme (850 enzyme unit (U) per g) per 10 kg fresh seaweed as reported suitable for the hydrolysis of alginates from kelp raw material (H. Marfaing pers. comm.). After thorough mixing with their inoculum, the seaweeds were transferred into plastic bags and compressed manually to limit contact with air. The samples were then sealed and stored at ambient temperature (18.4 ± 0.1 °C). As many bags as sampling points were prepared to avoid disrupting the silage process from opening the bags during sampling. Samples from each group were taken after 5, 15, 48 and 103 days of storage for monitoring the silage quality including pH and effluent formation, determined as the ratio between the liquid fraction and the total sample weight (w/w). The samples were then vacuum-packed and frozen until FD (C38L Cryorivoire) then ground to 250 µm (using a laboratory-mill) prior to chemical analyses. The DW was determined gravimetrically as the residue remaining after FD. All treatments were performed in triplicates.

Total carbohydrates – Neutral sugars (D-glucose, D-galactose, D-mannose, D-xylose, L-fucose, L-rhamnose), D-mannitol and uronic acids (D-glucuronic, D-mannuronic, poly-D-guluronic and ploy-D-mannuronic) composition were determined by high-performance liquid chromatography (HPLC) analysis after depolymerization under methanol- acid hydrolysis reaction (methanolysis; Quemener et al. 2000) as described in **Paper I, II and IV**. Due to interfering peaks of glucose and mannitol in these samples from the HPLC, both compounds, as well as organic acids, were quantified using another method in which 100 mg of ground freeze-dried samples were extracted in 10 mL H_2SO_4 solution (pH 1.5), prepared by adding 1 mL of a concentrated H_2SO_4 solution (> 95%) in 1 L ultrapure water, during 30 min at ambient temperature. The extracts were filtered (filter paper MN 616) prior to HPLC analysis on a Rezex ROA-H^+ column (column temperature: 60 °C) at 0.6 mL min^{-1}, with UV detection at 210 nm. Chromatographic peaks obtained from this method and the HPLC following methanolysis were identified by comparison with high purity reference standards for organic acids, sugars and uronic acids purchased from Sigma-Aldrich (Steinheim, Germany) except for poly-D-guluronic and poly-D-mannuronic prepared at CEVA's laboratory. The sum of guluronic and mannuronic acids (known as G- and M-units) measured in the samples, which are the

monomeric units composing alginate, was used to quantify the alginate content. The laminaran content of the samples was quantified by the glucose levels measured in the extracts. The results were expressed as % of the DW.

Statistical analysis – Statistical analyses were performed on R software including functions from the nlme package (Pinheiro et al. 2017). Raw data were pre-processed for descriptive statistics and the results expressed as mean ± standard error (n = 3). A RM ANOVA (R function lme) with one within-subjects factor (time), and two between-subjects factors (pre-treatment, inoculant), was used to test differences in silage quality during the experiment and between treatments.

4.6. Effects of steam blanching on the iodine content of *S. latissima* (NPR 6)

Biomass collection and steam blanching treatment – Biomass of *S. latissima* was harvested at Seaweed Energy Solutions (SES) at Frøya, Norway, in May 2018, then transported within a few hours to the processing plant (HitraMat). Blades of *S. latissima* were placed in a compartment of a benchtop food steamer (Phillips HD 9140). Samples were taken prior to and after 1-, 3- and 10-min treatments, vacuum-packed and frozen until FD, for further analysis of the I content. The temperature measured in the compartment was 95 °C. The treatment was performed in 3 replicates. The DW of the fresh samples was determined gravimetrically as the residue remaining after FD.

Iodine content – The determination of I in the samples was based on the colorimetric Sandell-Kolthoff-reaction depending on the reduction of cerium (IV) sulfate by arsenite in the presence of iodide (I^-) (Yaping et al. 1996). Dried seaweed samples were burned at 1000 °C, to convert all inorganic and organic I species to I^-. The residues were solubilized in deionized water and sodium arsenite ($NaAsO_2$) was added to the solutions in microplates. After the addition of cerium (IV) sulfate $Ce(SO_4)_2$ and shaking, the microplates were allowed to stand for 20 min away from ambient light. The absorbance

of the remaining Ce (IV), representing the amount of I in the samples, was measured in a spectrophotometer at 436 nm and compared to a standard curve.

Statistical analysis – Repeated measures analysis of variance (RM ANOVA) was used to detect differences in I in the samples over time as described in **Paper I**.

5. Seaweeds as a source of nutrients, flavors and texture

5.1. Chemical composition and nutritional benefits of seaweeds

This section provides an overview of the nutritional features and associated health benefits of seaweeds, relevant in the context of this thesis. More detailed information can be found in the cited literature. **Table 4** summarizes data collected during this project (**Paper I**, **NPR 1**) and reported in the literature for the chemical composition of *S. latissima*, *A. esculenta* and *P. palmata* sampled along the coast of Europe. Since critical factors influencing these results, such as seasonality and analytical methods employed, were not considered, the data presented in **table 4** may only provide nutrient levels as a snapshot.

Although seaweeds belong to a diverse group of photosynthetic marine organisms, with a variable chemical composition depending on species, season and habitat, most species are characterized by high levels of carbohydrates (notably cell-wall structural and storage polysaccharides), typically between 40 and 60 % DW, and low lipid levels, generally in the range 1 to 4.5 % DW (Holdt and Kraan 2011; Pereira 2011). A large part of the macroalgal polysaccharides are not digested by humans and are therefore regarded as dietary fibers. Hence, seaweeds contain few calories. The polysaccharides differ among classes of macroalgae. Alginates, fucoidans and cellulose constitute the cell-wall of brown species, while sulfated galactans (agar and carrageenans), xylans and mannans dominate in red seaweeds. Cellulose, xylans and mannans are characteristic of the cell walls of green seaweeds (Lahaye and Kaeffer 1997; Murata and Nakazoe 2001). Alginates extracted from brown macroalgae, as well as agar and carrageenans from red species are used extensively as gel-forming additives in the food, pharmaceutical and biotechnological industries worldwide (Bixler and Porse 2010). From a nutritional viewpoint, dietary fibers provide bulk to feces, holds water, reduce transit time and modulate the gastrointestinal microbiota (Thebaudin et al. 1997). Scientific evidence

suggests that soluble dietary fibers such as alginates has beneficial physiological effects on both colonic and cardiovascular health as well as reduced intestinal absorption of metabolizable nutrients and increased satiety (Brownlee et al. 2005). Fucoidans, a group of sulfated fucose-rich polysaccharides present in the cell wall of brown seaweeds, are also considered as soluble dietary fibers. Recent findings suggesting the potential therapeutic properties of fucoidans, including anti-inflammatory, anti-coagulant activities and anti-tumoral activities (Ale and Meyer 2013) attracted research teams and private stakeholders to undertake comprehensive investigations for potential applications of the molecule in the pharmaceutical industry. Fucoidan is already commercially available as dietary supplement. Relatively low fucoidan contents are reported in *A. esculenta* and *S. latissima* (**table 4**) compared to other brown seaweeds, e.g. 13 g $(100\ g)^{-1}$ DW in *F. vesiculosus* (Ale et al. 2011) suggesting that these two kelps may not be a major source of fucoidans.

The main storage carbohydrates in kelps species consists of laminaran and mannitol, while floridoside is the main reserve product in red macroalgae. Although these metabolites only have few potential food applications reported, they may contribute significantly to the amount of dietary fibers found in edible seaweeds. Specific methods for the quantification of dietary fibers in seaweeds are established (Lahaye 1991) but were not employed during this project. However, high levels of alginates and, to a lesser extent, laminaran in *A. esculenta* and *S. latissima*, and xylans in *P. palmata* support earlier studies highlighting these species (and seaweeds in general) as a rich source of dietary fibers (Lahaye and Kaeffer 1997).

Table 4: Chemical composition of *S. latissima*, *A. esculenta* and *P. palmata* sampled along the coast of Europe (from Brittany to Northern Norway). Values in bold were obtained during this study.

	S. latissima	***A. esculenta***	***P. palmata***
Moisture content (% wet weight)	**84** [a] 81 – 91 [c] 83 – 90 [d] 81 [e]	**83** [a] 76 – 88 [d]	**88** [b] 84 [e] 82 [f] 80 – 88 [g]
Carbohydrates			
Total (% DW)	**46** [a] 10 – 61 [c] 42 – 77 [d] 27 [e]	**41** [a] 57 – 72 [d]	**25** [b] 38 [e] 42 – 64 [g]
Alginate / alginic acid (polymer of D-mannuronic and L-guluronic acid) (% DW)	**21** [a] 8 – 30 [c] 16 – 30 [d] 24 [e]	**20** [a] 25 – 33 [d]	
Fucoidan (sulphated fucose-rich polysacch.) (% DW)	**1** [a] 1 [e] 2 – 6 [h]	**1** [a]	
Cellulose (% DW)	10 – 14 [d]	12 – 13 [d]	
Laminaran (β-(1→3)-D-glucan with some β-(1→6)-linked branches) (% DW)	**5** [a] 3 – 23 [c] 1 – 14 [d]	**9** [a] 2 – 17 [d]	
Mannitol (% DW)	**18** [a] 1 – 19 [c] 10 – 25 [d] 1 [e] 5 – 10 [i]	**10** [a] 10 – 17 [d]	0.9 [e]
Xylans (% DW)			**19** [b] 23 [e] 24 – 35 [g]
Floridoside (α-D-galactopyranosyl-(1-2)-glycerol) (% DW)			3 – 25 [g]

Protein (% DW)	**11** [a] 5 – 10 [d] 11 [e] 6 – 11 [i]	**10** [a] 9 – 12 [d] 9 [f]	**18** [b] 16 [e] 12 [f] 14– 24 [g]
Lipids (mg g^{-1} DW)	1.7 – 3.9 [i]	13 [f]	12 [e] 13 [f]
Minerals			
Ash	**26** [a] 22 – 40 [d]	**24** [a] 21 – 33 [d] 25 [f]	**35** [b] 42 [f] 15 – 27 [g]
Na (mg g^{-1} DW)	**36** [a] 20 – 39 [d] 24 [j]	**39** [a] 27 – 40 [d]	**26** [b] 11.2 – 22.5 [g] 3.2 [j]
K (mg g^{-1} DW)	**65** [a] 100 [j] 17 – 65 [d]	**42** [a] 7 – 30 [d]	**106** [b] 47.7 – 86.6 [g] 28 [j]
Ca (mg g^{-1} DW)	4 – 23 [d] 17 [j]	9 – 15 [d] 8 [f]	3.6 [f] 2.4 – 7.9 [g] 2.5 [j]
Mg (mg g^{-1} DW)	4 – 5 [d] 7.7 [j]	7 – 8 [d] 8.7 [f]	5.3 [f] 2.3 – 3.2 [g] 1.2 [j]
Polyphenols (mg g^{-1} DW)	**7** [a] 2 – 7 [d] 4 – 12 [i] 5 – 15 [k]	**34** [a] 3 – 15 [d] 14 – 61 [k]	2 – 6 [k]
Pigments			
Fucoxanthin (mg g^{-1} DW)	**0.4** [a] 0.6 – 0.8 [l] 0.5 [m]	**0.9** [a] 0.9 [m]	
R-Phycoerythrin (mg g^{-1} DW)			**2** [b] 5 – 18 [n]

[a] **Paper I**; [b] **NPR 1**; [c] Manns et al. (2017); [d] Schiener et al. (2015); [e] Jard et al. (2013); [f] Mæhre et al. (2014); [g] Rødde et al. (2004); [h] Bruhn et al. (2017); [i] Veide Vilg et al. (2015); [j] Biancarosa et al. (2018); [k] Roleda et al. (2019); [l] Boderskov et al. (2016); [m] Shannon and Abu-Ghannam (2017); [n] Guihéneuf et al. (2018)

The protein content is generally low in brown macroalgae (3 – 15 % DW), moderate in green (9 – 26 % DW), and high in red seaweeds (up to 47 % DW) (Fleurence 1999). While seaweeds are also regarded as a potential alternative protein source in human and animal nutrition, only few species reach similar protein levels than those of typical protein-rich foods, e.g. pulses, beans and soy (ca. 20 - 35 g $(100g)^{-1}$) (Fleurence 1999). Despite lower protein contents than those of *P. palmata* (**Paper I – II, NPR 1)** (**table 4**), the kelps *S. latissima* and *A. esculenta* are associated with higher biomass yields and can be cultivated at sea on a large scale. These species may therefore represent a larger potential for the provision of proteins in the future. Macroalgal proteins are usually of high quality, i.e. they contain all essential amino-acids (EAA) required in human nutrition (Fleurence 2004; Dawczynski et al. 2007a; Mæhre et al. 2014). However, the amino-acid profile of a given species may vary considerably depending on the geographical location and season of harvest. As reported in **Paper II**, chemical scores of 100 % are associated with dried samples of *S. latissima* cultivated in Norway and harvested in May (i.e. all EAA are present in sufficient amounts with regard to human nutritional requirements) while scores ranging from 39 to 52 % are reported in the literature from samples of the same species cultivated in Denmark and harvested in the same period (i.e. one or several EAA are limiting) (Marinho et al. 2015). Despite remarkable amino-acid profiles, the protein digestibility of seaweeds is generally limited by structural polysaccharides (alginates, agar, carrageenan, xylans) (Fleurence et al. 2012) as well as phenolic compounds in the case of brown species (Wong and Cheung 2001b). Processing steps such as boiling (Mæhre et al. 2015), washing and fermentation using a *Trichoderma* strain (Marrion et al. 2003) has been shown to improve the digestibility of proteins from *P. palmata*, as a results of the partial removal or degradation of xylans. Extraction yields of proteins from *P. palmata* can also be improved by an enzymatic pre-treatment of the biomass using xylanase (Joubert and Fleurence 2008; Bjarnadóttir et al. 2018).

Seaweeds are known to be a rich source of both macro-minerals (Na, K, Ca and Mg) and trace elements (e.g. I, Fe, Mn, Zn) (Dawczynski et al. 2007b). While the mineral content of land vegetables does not exceed 20 % DW, the ash, and thereby, the mineral content

of seaweeds largely exceeds those levels (Rupérez 2002; MacArtain et al. 2007) and can reach 40 % DW in the three studied species (**table 4**). Moreover, macroalgae often contain equal or higher levels of K compared to Na, which is interesting in a nutritional perspective given that diets rich in Na (i.e. with a high Na/K ratio) are associated with health risks, such as high blood pressure and cardiovascular diseases (Perez and Chang 2014). 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). In this context, edible seaweeds are regarded as potential functional ingredients for salt replacement. The inclusion of seaweeds in food products even at low levels (5 % and below) results in healthier mineral profiles (i.e. lower Na/K ratios) (López-López et al. 2009a; López-López et al. 2009c; Hotchkiss 2009; Circuncisão et al. 2018). Processed foods like meat, bread, sauces and condiments, are often characterized by high Na/K ratios (over 5.0) (O'Halloran et al. 2016; Circuncisão et al. 2018) while those from seaweeds typically lies below 1.5 (with some exceptions) (Dawczynski et al. 2007b; Jard et al. 2013). The Na/K ratio recommended by the World Health Organization (WHO) is close to 1.0, so the consumption of food products with this proportion or below should be considered for healthy cardiovascular purposes. The Na/K ratios of *P. palmata* and *S. latissima* measured during this project were particularly low, i.e. 0.25 (**NPR 1**) and 0.56 (**Paper I**) respectively, highlighting the potential of these two species as salt replacing ingredients. While most minerals are essential in human nutrition, some are toxic in varying degrees. Seaweeds are an excellent source of iodine (I) and has been used in Asia as dietary supplement to prevent goiter (Wells et al. 2017). However, excessive I intakes can have negative consequences on human health. This topic is further discussed in section 5.4.

It is well accepted that cellular oxidative stress due to the excessive production of reactive oxygen species (and related to poor dietary habits), leads to a wide range of degenerative diseases such as cardiovascular diseases and cancers. There are numerous scientific reports showing that macroalgae are a good source of a variety of antioxidant compounds. These compounds include polyphenols (Wang et al. 2012a), particularly abundant in brown macroalgae, sulfated polysaccharides (e.g. fucoidans) (Cornish and

Garbary 2010), vitamins (B_{12}, C, E, D, provitamin A) (Mabeau and Fleurence 1993), carotenoid pigments in brown seaweeds, e.g. fucoxanthin (Fung et al. 2013) and phycobilin pigments in red species (Sekar and Chandramohan 2008).

Owing to its unique structure containing an unusual allenic bond combined to an epoxide and hydroxyl groups, fucoxanthin has received significant attention for its remarkable bioactivities including anti-obesity and inhibitory effects on the growth of cancerous cells (Maeda et al. 2008; Nakazawa et al. 2009). Fucoxanthin is currently extracted and purified from *S. japonica* and commercialized as functional ingredient for the food and cosmetic industries (Oryza 2015). Fucoxanthin supplements are recognized safe by the European Food Safety Authority (EFSA) and the United State Food and Drug Administration (FDA). The fucoxanthin content of *A. esculenta* measured in this study was higher than that of *S. latissima* (**Paper I – II**) although the levels measured in both species were in the range of those found in *S. japonica* and *U. pinnatifida*, also suggested as raw material for the commercial extraction of fucoxanthin (Kanda et al. 2014; Sivagnanam et al. 2015).

In addition to the health benefits associated to the antioxidant substances from macroalgae, brown seaweed extracts rich in fucoxanthin and polyphenols were found to be potent ingredients to inhibit lipid peroxidation in food systems thus, extend product shelf-life (Sasaki et al. 2008; Wang et al. 2010).

The water-soluble chromoprotein R-PE is a major photosynthetic pigment in red macroalgae which is used commercially as a natural protein dye in the food and cosmetic industries (Dumay et al. 2014). *P. palmata* has been identified as a good source of R-PE (Galland-Irmouli et al. 2000; Guihéneuf et al. 2018) although the levels measured in this work, i.e. 2.2 and 16.9 mg g^{-1} DW in **NPR 1** and **Paper III** (measured in initial dried samples), revealed important variations across harvesting season (samples from **NPR 1** and **Paper III** were harvested in May and November respectively, in North Brittany, France). Generally, light and nutrient availability during growth affect the levels of macroalgal pigments, including fucoxanthin (Boderskov et al. 2016) and R-PE (Galland-Irmouli et al. 2000; Guihéneuf et al. 2018). Hence, the pigment production from

seaweeds may be optimized by controlling the growth conditions during biomass cultivation.

The bioactivities from macroalgal metabolites have been tested mainly within *in vitro* or animal models and limited clinical data currently exists to substantiate the positive claims related to individual compounds on human health. However, there are increasing evidences that the consumption of seaweeds is associated with health and nutritional benefits. Studies conducted in Japan positively correlated the typical Japanese dietary pattern which includes the daily consumption of seaweeds (3.3 to 5.3 g day^{-1}, see section 3.4) with decreased cardiovascular diseases (Iso and Kubota 2007; Shimazu et al. 2007). In another study based on a clinical trial, the daily consumption of seaweeds was proposed as a factor explaining the lower postmenopausal breast cancer incidence and mortality rates in Japan (Teas et al. 2013). Evidence from the Nutrition and Health Surveys in Taiwan associated several foods, including seaweed, with limiting the increase in metabolic syndrome prevalence among women (Yeh et al. 2011).

Although macroalgae are a rich source of essential nutrients, they only contain few calories and thus, can only constitute a small part of the diet. Consuming small amounts of a variety of edible species regularly is suggested as an optimal approach to introduce edible seaweeds to Western diets (Cornish et al. 2015). Moreover, seaweeds can be used in the food industry either as a whole or in the form of extract to improve the nutritional and functional properties of food products.

5.2. Physico-chemical, textural and color properties

Primarily, purified macroalgal polysaccharides i.e. alginates extracted from brown macroalgae and agar and carrageenans from red seaweed, are hydrocolloids that are used extensively as gel-forming additives, clarifying, emulsifying and stabilizing agents in the food and pharmaceutical industries (Bixler and Porse 2010). Their behavior in a food matrix is governed by their structural characteristics and the conditions in which they are used (e.g. pH, temperature) (Helgerud et al. 2010). In recent years, several studies

investigated the inclusion of whole seaweeds (powdered or flaked) or seaweed extracts in various food matrices and reported the effects on the physico-chemical and textural properties of the resulting products (see Roohinejad et al. 2017 and references therein). The results from different studies concluded that the addition of seaweed powder from the brown macroalgae *Himanthalia elongata*, *U. pinnatifida* or *S. japonica* at levels typically ranging from 1 to 10 % generally improved the WBC and OBC of meat products (pork and beef patties, frankfurters) and their texture (Cofrades et al. 2008; López-López et al. 2009b; Choi et al. 2012b; Cox and Abu-Ghannam 2013).

Table 5: WBC, OBC and SC of freeze-dried *S. latissima* and *P. palmata* (data from **Paper II** and **NPR 2**). WBC and OBC are expressed in gram water and gram oil per gram dried sample respectively, and SC is expressed in milliliter per gram dried sample.

	S. latissima	***P. palmata***
WBC	7.2 ± 0.2	7.1 ± 0.3
OBC	6.1 ± 0.1	5.0 ± 0.5
SC	10.2 ± 0.4	6.7 ± 0.3

In the present work, the WBC, OBC and SC of *S. latissima* and *P. palmata* exposed to different drying treatments (**Paper II**, **NPR 2**) and storage conditions (**Paper III**) were studied. The results are reported in the next chapter. The values obtained in **Paper II** and **NPR 2** for freeze-dried samples are summarized in **table 5** for comparison purposes. Both WBC and OBC were similar in the two species. WBC depends largely on the content and structure of insoluble fibers present in the raw material (Lahaye and Kaeffer 1997) while OBC may be related to the levels of non-polar residues in the protein fraction as well as the hydrophilic nature of charged polysaccharides such as alginates and xylans (Fleury and Lahaye 1991; Rupérez and Saura-Calixto 2001). The measured

SC was higher in *S. latissima* than in *P. palmata* samples. Similar observations were made by Rupérez and Saura-Calixto (2001) who measured higher SC in kelp species (*Laminaria* sp., *U. pinnatifida*) compared to red seaweeds (*Pyropia* sp., *C. crispus*), which may be related to the presence of alginates in the former group and their ability to increase volume when exposed to an appropriate solvent.

From a technological viewpoint, these properties are interesting since they reflect the ability of an ingredient to prevent water losses, improve adhesion, stabilize emulsions and provide body to foods. As observed by Chapman et al. (2015), the inclusion of *S. latissima* flakes altered the viscosity of desserts (pancake dough and chocolate ice-cream) and resulted in increased body of the final dishes. These findings confirm the potential of edible seaweeds, and especially kelps, to improve the texture of foods.

Color is a critical quality attribute for the acceptability and palatability of foods. The characteristic color of kelps is due to the abundance of carotenoid pigments, mainly fucoxanthin, but also β-carotene and violaxanthin, chlorophyll pigments (Chl *a*, Chl *c*) and secondary metabolites (e.g. polyphenols). Several studies previously reported drastic color changes of brown edible seaweeds from olive-brown to green upon heating (Cox et al. 2011; Blikra et al. 2018) giving the material the aspect of green vegetables. This color was considered more attractive to the Western consumer, as a bright, vegetable-associated color may lower the threshold for including seaweeds in the diet. Color change caused by thermal processes are often the result of distinct enzymatic and non-enzymatic reactions (Bonazzi and Dumoulin 2011). Changes in color and physico-chemical properties of seaweeds following different processes were monitored during this study. The results are discussed in chapter 6.

5.3. Sensory properties

The use of seaweeds as food ingredients is of indubitable interest from the standpoints of both nutrition and food technology. However, seaweeds also have a large and unexploited potential with applications in the field of gastronomy due to their

organoleptic properties. In Asia, the variety of edible species consumed are recognized for the particular flavors and textures they bring to food dishes (Mouritsen 2013). The most characteristic flavor from seaweeds reported in the literature is umami.

Umami is the fifth basic taste (along with sweet, salty, sour and bitter) which was first described by Kikunae Ikeda in 1909 as brothy, meaty and savory (*umai*) (Ikeda 2002). Ikeda associated this taste to the traditional broth (*dashi*) used as soup base and prepared from the Japanese kelp *konbu* (*S. japonica*), and to the notably high amount of monosodium glutamate (MSG) present in free chemical form in the broth. MSG produced from fermentation processes of terrestrial plant material (e.g. sugar cane) is nowadays widely used in the food industry as flavoring additive. In the Japanese culinary tradition, the umami from *konbu* and *dashi* is used to flavor soups and other dishes (e.g. vegetables) resulting in meals with increased palatability and relatively low sugar, salt and fat (Mouritsen and Styrbæk 2014).

Due to high free glutamate contents (Ninomiya 1998), seaweeds are suggested as a potential source of umami, to be used in everyday culinary applications and in the food industry. However, large variations in free glutamate are observed among species (Mouritsen et al. 2012; Mouritsen et al. 2018) and the perception of umami not only depends on the glutamate content but also on other molecules, e.g. guanylate and inosinate (Ninomiya 1998), pyroglutamate peptides, succinyl amino-acids (Zhao et al. 2016), as well as other flavors influencing the overall taste experience (Mouritsen et al. 2018). Recently, enzymatic fermentation processes using seaweed raw material as substrate were successfully tested and proposed to produce flavor-rich extracts (Laohakunjit et al. 2014; Uchida et al. 2017).

Although the sensory science related to macroalgae in Western cultures is still in its infancy, recent studies attempted to characterize the sensory profiles of naturally available seaweeds species (Peinado et al. 2014; Chapman et al. 2015; López-Pérez et al. 2017; Mouritsen et al. 2018). In comparative studies where several macroalgae species common to the coast of Scandinavia were screened for their potential in culinary applications, *P. palmata* was distinguished and considered a promising ingredient

providing umami flavor in a wide range of dishes such as ice cream, bread and pasta (Mouritsen et al. 2012; Chapman et al. 2015). The same species was described by fresh marine aromas when evaluated in its fresh form (Le Pape et al. 2002) while it is associated with fish and fish meal notes in its dry form (Michel et al. 1997; López-Pérez et al. 2017). Similar associations (i.e. dried fish flavor and fish skin aroma) were found from the sensory evaluation of dried *P. palmata* samples during this project (**Paper III**). Saltiness along with sour, bitter and green notes are characteristic of dried *S. latissima* (Chapman et al. 2015; López-Pérez et al. 2017). In contrast, dried samples of the same species evaluated by a sensory panel in **Paper II** were associated with intense saltiness along with fresh marine aromas and flavors, highlighting the variations in organoleptic profiles of a given species. This may be due to e.g. intra-specific variations in chemical composition across geographical locations and the physiological state of the plant, or to the differences in sample handling and preparation across studies. Other kelp species such as *A. esculenta* and *L. digitata* are characterized as relatively less salty, sweeter and are associated with milder tastes (Chapman et al. 2015).

The sensory characteristics of seaweeds are attributed to their levels of flavor-active compounds including FAA, volatile compounds and minerals (Michel et al. 1997; Mouritsen et al. 2012; Peinado et al. 2014; López-Pérez et al. 2017; Mouritsen et al. 2018), which are likely to be affected by processing steps of the raw material. There are few studies reporting on the effects of biomass post-harvest treatments such as drying (Michel et al. 1997), freezing (Le Pape et al. 2002), washing and cold storage (Liot et al. 1993) on the seaweed flavors and aromas, but in general, the effects of primary processing treatments and storage conditions on the organoleptic quality of edible seaweeds remain poorly understood. The effect of drying within a range of temperatures commonly used in seaweed processing (25 to 70 °C), on the sensory characteristics of *S. latissima* was investigated during this project. The results are presented and discussed in **Paper II** and sections 6.2 and 6.3 of this thesis.

After harvest, *konbu* is sun-dried to prevent rapid spoilage by microorganisms and aged in cellars, usually two years and up to ten years, to fade the strong marine taste in favor

of mild, rich and savory flavors (Mouritsen and Styrbæk 2014). A white precipitate consisting of salts, mannitol and free glutamate is observed on the surface of the dried and aged *konbu* blades, providing a combination of salty, sweet and umami flavors (Mouritsen et al. 2012). A similar precipitate was produced during drying of bull kelp (*Nereocystis leutkeana*) blades (Mouritsen et al. 2018). The precipitate contained high amounts of minerals (particularly K) whereas its FAA content was lower than in the kelp blades. Scientific data to describe the changes in chemical composition during the maturation process of *konbu* is lacking.

Historical records from Iceland depict the collection of dulse from the shore by gatherers since the 700s. The seaweed was handpicked during the summer, rinsed and spread over the fields for sun-drying. A white precipitate (*hneita*) tasting both salty and sweet, forming on the surface of the fronds during drying, was indicative of the quality of the seaweed. Storing the material in closed barrels for several months increased the precipitate formation and the value of the seaweed as food which was also used as a trading commodity (Kristjánsson 1980).

While there are a large number of studies investigating processes to optimize the extraction of a single or multiple high-value compounds from macroalgal biomass, the processes of enhancing the sensory characteristics of seaweeds has not been addressed scientifically. As pointed out by Mouritsen (2017; 2018) product flavor is the main factor governing consumer acceptance and a key issue to sustain the health food movement based on edible seaweeds currently ongoing in Europe. Moreover, seaweeds are unfamiliar food items (Birch et al. 2018a) and also suffer the burden of being associated with decomposing beach-cast biomass, limiting their attractivity to a significant part of Western consumers (Le Bras et al. 2014; Mouritsen 2017). There is therefore a strong need to identify processes to produce flavor-rich foods and ingredients from macroalgae attractive to both Western consumers and the food industry.

Based on the observations described above, an original methodology was designed to test the hypothesis that the moisture level during dry storage affects the organoleptic properties of the raw material and that controlling this parameter may be employed to

develop a variety of sensory profiles from edible seaweeds. Changes in the sensory properties of *S. latissima* (**NPR 3**) and *P. palmata* (**Paper III**) stored in a dried and so-called semi-dried state (also referred to as "matured") at different durations, were monitored. Maturing the samples, i.e. increasing their moisture content to ca. 20 % and storing them at 12 °C in the dark, produced distinct flavors compared to dry samples (containing about 5 % moisture) stored under the same conditions. The results are reported and discussed in **Paper III** and the section 6.4 of this thesis.

5.4. Potential food safety issues

Besides being a rich source of bioactive substances, seaweeds may also accumulate toxic elements with potentially negative effects on human health. Here, both non-essential metals (Almela et al. 2006; Rose et al. 2007; Besada et al. 2009; Desideri et al. 2016) as well as essential elements, especially I, in excessive amounts (Bouga and Combet 2015; Lüning and Mortensen 2015; Desideri et al. 2016) may be a problem in the context of using seaweeds in human nutrition. The concentration of potentially toxic elements depends mainly on the bioavailability of the element in the surrounding water and the uptake capacity of the species (Besada et al. 2009). However, intra-specific variations in metal and I content are reported and associated to differences among harvesting sites and across seasons (Ar Gall et al. 2004; Morrison et al. 2008; Duinker 2014).

Previous studies have reported high levels of arsenic (As) in its inorganic form (iAs) (Almela et al. 2006; Rose et al. 2007; Besada et al. 2009), cadmium (Cd) (Almela et al. 2006; Besada et al. 2009) and I (Dawczynski et al. 2007b; Desideri et al. 2016) in seaweed food products commercialized in Europe. However, direct evidence for seaweed consumption being associated with clinical pathology worldwide is scarce (Cheney 2016). The health risks associated with eating seaweeds depends on the levels of toxic elements in the product, the quantity ingested over time and the compounds' bioavailability in the human body. The levels of iAs, Cd and I in *S. latissima* and *A. esculenta* were measured and discussed in the perspective of including these species

in Western diets (**Paper IV**). The levels of these three elements were also measured in *P. palmata* (**NPR 1**).

At present, 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; CEVA 2014) (**table 6**). The European Union (EU) is in the process of establishing a specific regulation in this regard but currently has only defined limits for selected heavy metals in dietary supplements consisting exclusively or mainly of dried seaweeds (EU No 629/2008 2008). Levels of potentially undesirable compounds typically found in seaweeds, measured in *S. latissima*, *A. esculenta* and *P. palmata* during this project (**Paper IV**, **NPR 1**) and reported in the literature is summarized in **table 6**. Scrutiny of the table shows that in the case of these three species, mainly the high I content of *S. latissima* and Cd content of *A. esculenta* exceed the limits established by the French food safety authority and may pose a problem in the context of using these species in food applications. The Cd content of *S. latissima* and *P. palmata* may slightly exceed the maximum value allowed by the French regulation although the levels measured in the present study were below the recommended French limit.

Simple treatments to reduce the levels of I and Cd in *S. latissima* and *A. esculenta* respectively were proposed and their effects on the quality of the raw material to be used in food applications is reported in **Paper IV**. Soaking treatments in warm (32 °C) fresh water reduced the I content of *S. latissima* (see section 6.6) while hypersaline treatments at 2.0 M NaCl (like a brining treatment) reduced the Cd content of *A. esculenta*. The levels of other heavy metals which may accumulate in the marine food chain such as lead (Pb) and mercury (Hg) are reported low in seaweeds (**table 6**) hence, were not analyzed during this study.

Table 6: Levels of selected potentially toxic compounds in *S. latissima*, *A. esculenta* and *P. palmata* sampled along the coast of Europe (from Brittany to Northern Norway) and their maximum authorized levels in France and the EU. Values are expressed in (in mg kg^{-1} DW). Values in bold were obtained during this study.

	Species			**Limit values**	
	S. latissima	***A. esculenta***	***P. palmata***	**France [a, b]**	**EU [c]**
iAS	**0.16 – 0.23 [d]** 0.25 [f] 0.03 – 0.07 [g] 0.39 [h]	**0.22 [d]** 0.05 [f]	**0.18 [e]** 0.02 [f] 0.05 [g]	3	
Cd	**0.22 – 0.27 [d]** 0.59 [f] 0.28 – 0.46 [g] 0.60 [i] 0.13 [h]	**1.55 – 2.01 [d]** 2.5 [f] 1.58 [i] 3.4 [j]	**0.06 [e]** 0.37 [f] 0.12 – 0.26 [g] 0.83 [i] 0.48 [j]	0.5	3.0
Hg	0.01 [f] 0.01 – 0.02 [g] 0.033 [i] 0.03 [h]	0.005 [f] 0.058 [i] < 0.005 [j]	0.003 [f] ≤ 0.01 [g] 0.063 [i]	0.1	0.1
Pb	0.21 [f] 0.19 – 0.72 [g] 0.18 [i, h]	0.14 [f] 0.25 [i]	0.14 [f] 0.09 – 1.12 [g] 0.16 [i]	5	3.0
Sn	na	na	na	5	
I	**4898 – 6568 [d]** 4600 [f] 2103 – 3378 [g] 420 – 3965 [k] 1556 – 7208 [l]	**213 [d]** 380 [f] 220 [j] 180 – 1070 [l]	**74 [e]** 220 [f] 54 – 414 [g] 260 [j] 72 – 293 [l]	2000	

na: no data available

[a] Mabeau and Fleurence (1993); [b] CEVA (2014); [c] EU No 629/2008 (2008); [d] **Paper IV**; [e] **NPR 1;** [f] Biancarosa et al. (2018); [g] Duinker (2014); [h] Maulvault et al. (2015); [i] Roleda et al. (2019); [j] Mæhre et al. (2014); [k] Lüning and Mortensen (2015); [l] Roleda et al. (2018)

Cd naturally occurs in soil, water and sediments but is found to accumulate in land plants and marine environments due to anthropogenic activities. 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). Such effects have not been associated with seaweed consumption so far. A relatively high Cd content in *A. esculenta*, exceeding the French limit, is reported in **Paper IV**. This observation supports comparable values reported in the literature (**table 6**), reflecting the affinity of this species to Cd. The role of cell-wall polysaccharides such as alginates in the sequestration of heavy metals, have been demonstrated (Davis et al. 2003). However, the affinity for divalent cations (Cd^{2+}) also depends on the alginate structure, i.e. the ratio of its constitutive monomers β-D-mannuronic acid (M-block) and α-L-guluronic acid (G-block). Early studies showed that this affinity increased with the proportion of guluronic acid (Haug 1961) which may explain the differences in Cd accumulation between *A. esculenta* and other brown macroalgae. After ingestion, the food undergoes a series of chemical and physical reactions that can modify the amount of a compound that reaches the systemic circulation (bioavailability). Although the chelation of Cd by seaweed dietary fibers suggests a low bioavailability of the metal in the human body, pH influences the mechanisms of proton exchange and Cd may be released from alginates at low pH (Stirk and van Staden 2002) as in contact with gastric fluids. Based on the Cd levels measured in *A. esculenta* during this project as well as established tolerable intake levels for this element and background exposure levels from the European population, the health risk associated with a moderate consumption of this kelp (3.3 g DW day^{-1}) was considered low (**Paper IV**). However, the behavior of macroalgal Cd in the human body must be studied to confirm this result.

In general, the total amount of As ingested by humans depends greatly on the proportion of seafood in the diet since As concentration is higher in marine compared to terrestrial biomass (Phillips 1990). In macroalgae, As is present mainly in organic forms (arsenosugars and methyl derivates) while inorganic forms (e.g. arsenate and arsenite) occur at lower levels (Almela et al. 2006). In general, organic forms of As exert low or no toxicity, while inorganic forms are undoubtedly the most hazardous, being associated

with liver, bladder, lung and skin cancers (Hughes 2002). Generally, brown seaweeds display higher levels of As and iAs compared to species of the two other groups (Almela et al. 2006) although none of the species analyzed in this study exceeded the limit value for iAs (**table 6**). The elevated levels of iAs in some macroalgae, especially in *S. fusiforme* (*Hijiki*, 42 – 117 mg kg^{-1} DW) (Almela et al. 2006) has raised concern among national and international authorities since even the consumption of small amounts of this seaweed may considerably exceed the tolerable daily intake (TDI) established for this element by the WHO (150 µg iAs day^{-1} for an adult weighing 70 kg, WHO 1989). Recently, the value of 20 mg iAs kg^{-1} DW was reported from *L. digitata*, an edible species, harvested in Norway (Maulvault et al. 2015) raising concern for consumer safety. Using an *in vitro* gastrointestinal digestion procedure, Garcia-Sartal et al. (2012) concluded that only a limited fraction (12 – 16 %) of the total As in dried seaweed samples (*U. pinnatifida*, *Laminaria ochroleuca*, *P. umbilicalis*, *U. rigida*) was bioavailable. Although a cooking process efficiently reduces the total As and iAs levels, cooking also increases the bioaccessibility (i.e. the quantity released in the gastrointestinal tract available for absorption) of the remaining fraction (Laparra et al. 2003). As explained in the discussion section of **Paper IV**, the current dietary exposure to iAs in the European population is quite high and overlaps the critical range of exposure for a 0.5 % increased incidence of lung cancer. Therefore, the inclusion of foods with high levels of iAs in the Western diet should be avoided.

Iodine (I) is an essential micromineral involved in the synthesis of thyroid hormones which play a key role in fetus growth, brain development of children and regulates metabolic functions. On the other hand, excessive I intakes are known to affect the thyroid function, particularly in susceptible individuals (elderly, fetuses and neonates), potentially resulting in hypo- or hyperthyroidism (Leung and Braverman 2014). Brown macroalgae generally contain high levels of I, with kelp species being the strongest I accumulators among all living systems (Ar Gall et al. 2004). The I level measured in *S. latissima* in this study largely exceeds the threshold value of 2000 mg kg^{-1} DW allowed in dried seaweed products (**table 6**). The I bioavailability from another kelp species (*L. hyperborea*) in the human body was reported to be between 62 and 90 %

depending on the I status of the individual (Aquaron et al. 2002) and lower than the bioavailability of pure mineral I (96% from KI). A recent study using different *in vitro* approaches indicates that up to 82 % of the I from cooked *konbu* is available for absorption (Domínguez-González et al. 2017). The results from another *in vitro* assay suggested the role of the seaweed cell-wall polysaccharides in delaying the I absorption, resulting in a slower I release from seaweed ingredients compared to foods enriched with KI (Combet et al. 2014). Based on the I level measured in this study an average daily consumption of 3.3 g DW of *S. latissima* will contribute to an oversupply of dietary I, (1800 % of the TDI) whereas the same amount of *A. esculenta* will provide this element within the recommended limit (59 % of the TDI) (**Paper IV**). Based on the I content of *P. palmata* (74 mg kg^{-1} DW, **NPR 1**) and the same consumption pattern, this species will contribute to 21 % of the TDI, hence represents a safe source of I. As reported in the present work (**Paper IV**) and previous studies, the I content of edible seaweeds is influenced by processing and cooking of the raw material (Lüning and Mortensen 2015; Nitschke and Stengel 2016) making the assessment of seaweed-I dietary exposure difficult. There is therefore a need to gain knowledge and differentiate between the total amount of I in seaweeds and the amount ingested after processing and cooking of the raw material.

By combining information from dietary surveys and I analysis in urine samples, Zava and Zava (2011) estimated the Japanese I intake between 1 and 3 mg day^{-1}, far above the recommended daily intake of 150 μg day^{-1} for adults (WHO 1989). The joint FAO/WHO expert committee on food additives (JECFA) established a maximal tolerable intake of 1 mg I day^{-1} (WHO 1989) while the value of 0.6 mg day^{-1} is defined by the European Food Safety Authority (EFSA) as the limit (EFSA 2006). This reflects the lack of consensus among expert committees regarding the toxicity of this element. Several cases of thyrotoxicosis (an excess of thyroid hormones in the body) have been reported associated with the daily consumption of kelp-containing dietary supplements (Leung and Braverman 2014). However, a large proportion of Eastern Asians ingest large amounts of I (Zava and Zava 2011), without any reported adverse effects. Nevertheless, the physiological response to I excess vary among individuals and largely depends on

previous intakes (Aquaron et al. 2002). On the other hand, I deficiency disorders affects 30 % of the world population and is the main source of preventable mental retardation. Europe is the region with the most occurrences of I deficiency and statistics indicate that 52 % of Europeans have insufficient intakes of I (de Benoist et al. 2008).

The risks associated with eating seaweeds must be evaluated with respect to realistic consumption patterns. Despite the increasing interest for sea vegetables, seaweeds remain an unusual ingredient in Western diets (Le Bras et al. 2014; Birch et al. 2018a). The conclusion from the health risk estimation presented in **Paper IV**, is that the consumption of one to two meals containing seaweeds per week (corresponding to 1 g DW day^{-1}), and not exclusively kelp species, appears a more plausible scenario to estimate the dietary exposure of the European population to potentially toxic compounds from seaweeds. To this extent, the inclusion of seaweeds in the diet could support current efforts to improve the I status among European populations. However, some groups such as vegetarians and vegans, may eat seaweeds more frequently and should be informed of the potential risks. Similarly, including seaweeds containing high amounts of potentially toxic compounds in staple foods that are likely to be consumed every day, such as bread, may cause a health risk for regular consumers of such products. Gaining knowledge on the behavior of these compounds, especially I, in food matrices upon cooking, is necessary to properly assess the risks. The surveillance of potentially toxic elements in edible and commercialized seaweeds and adequate product labelling (Bouga and Combet 2015) is essential to ensure consumer safety and sustain the credibility of seaweeds as healthy ingredient in human nutrition.

The available data in the scientific literature related to seaweed as a potential source of microbial contamination is limited. However, few reports have reviewed the knowledge on microbiology and safety regarding seaweed consumption, and concluded that although very little was studied, there was no reason of concern (Hendriksen and Lundsteen 2014; Duinker et al. 2016). Generally, the indigenous microbiota of marine and brackish environments is not considered to have a direct pathogenic potential in humans (except for some bacteria from the *Vibrio* genus). Potential risk could arise from

the occurrence of intestinal pathogens (*Escherichia coli*) and other environmental bacteria such as those from the genus *Bacillus*, *Clostridium*, *Salmonella*, *Listeria* and *Staphylococcus*, originating from waste water outlets, land run-offs or contamination during harvest and processing (Duinker et al. 2016). Bacterial counts conducted on raw and cooked *S. latissima* and *A. esculenta* did not detect coliforms, pathogenic *Vibrio* spp. or *Listeria monocytogenes* (Blikra et al. 2018). However, the isolation of potentially toxin-producing spore-forming bacteria (*Bacillus pumilus* and *Bacillus lichenformis*) in both raw and heat-treated seaweeds suggested that the growth of these microorganisms need to be controlled during handling and storage of the seaweeds. The authors suggested a heat treatment of 15 min at 95 °C for a 3-log reduction of these bacteria (Blikra et al. 2018). Maximum allowed bacterial levels have also been defined by the French food safety authority (Mabeau and Fleurence 1993; CEVA 2014) although these only apply to dried seaweeds (**table 7**).

Table 7: Microbiological criteria for dried seaweed products established by the French food safety authority (CEVA 2014).

Bacteria	**Limit (CFU g^{-1})**
Mesophilic aerobic bacteria	$\leq 10^5$
Fecal coliforms	$\leq 10^1$
Sulphite-reducing anaerobic bacteria	$\leq 10^2$
Staphylococcus aureus	$\leq 10^2$
Clostridium perfringens	≤ 1
Salmonella sp.	None in 25 g

The loads of aerobic bacteria, coliforms, yeasts and molds of fresh and thawed *S. latissima* after freezing (**NPR 4**), as well as dried and matured *P. palmata* samples (**Paper III**) were analyzed in this study. The levels of aerobes, yeasts and coliforms were below the established limits in all samples. Some molds were detected in dried samples of *P. palmata* and not in semi-dried, matured samples of the same batch (**Paper III**). Some fungal species (mainly of the genera *Aspergillus*, *Cladosporium* and *Penicillium*) originating from marine environments have been isolated from salted food and may persist even when the water activity (a_w) is low and prohibitive to the growth of most microorganisms (Biango-Daniels and Hodge 2018). Since some of these strains have been involved in food spoilage, their presence on edible seaweeds must be avoided. A higher a_w in the matured samples of *P. palmata* allows the growth of a larger diversity of microorganisms which can outcompete the fungal strains present on the dried samples. This can explain the absence of molds in the matured samples of *P. palmata* (**Paper III**).

6. Primary processing and storage of seaweeds

The interest in commercial cultivation of macroalgae to be used in food applications is growing rapidly in Europe. However, the high-water content of the biomass (**table 4**) represents a challenge for conserving and transporting large volumes for industrial scale production. Macroalgae are characterized by a rapid decay once harvested (Enríquez et al. 1993) and thus, requires appropriate preservation methods to maintain biomass quality and ensure product safety. The conditions of cultivation at sea in coastal areas, namely the onset of biofouling, is another difficulty forcing the producers to harvest the biomass during spring and early summer. Nevertheless, a year-round supply of biomass is necessary to meet an increasing consumer demand and to sustain the emerging industry relying on cultivated seaweeds. Hence, efficient processing and storage strategies need to establish to meet this growing demand, avoid spoilage of the biomass and achieve optimal product quality to food applications.

As reviewed in the previous chapter, maintaining the nutrient content and enhancing organoleptic properties (flavor, color, and texture) as well as minimizing potential food safety issues are of high priority. Food preservation techniques such as drying and freezing are commonly used to stabilize seaweed biomass but may also affect the characteristics of the raw material and its content of nutritional compounds (Wong and Cheung 2001a; Gupta et al. 2011; Sappati et al. 2017). Alternative post-harvest treatments to increase shelf-life of fresh seaweeds include short-term storage in seawater and cold storage (Liot et al. 1993). However, the preservation of aquatic raw material such as macroalgae, is energy demanding (van Oirschot et al. 2017). Naturally available energy sources, e.g. solar energy such as in tropical areas (Chan et al. 1997) or geothermal energy in Iceland (Hallsson 1992) can be used for commercial primary processing of seaweeds. Sun-drying does not require fossil energy but is weather dependent and requires large areas to process significant volumes (Milledge and Harvey 2016b). In Europe, large amounts of surplus heat are available from various industrial processes and integrated models using this secondary energy source are suggested for

seaweed processing (Philis et al. 2018). Ensiling is also regarded as a promising alternative method with a low energy input (Milledge and Harvey 2016a; Cabrita et al. 2017).

In contrast to the rapid development of seaweed cultivation technology, knowledge remains limited regarding the effects of different preservation techniques on the biomass quality of relevant species in Europe, for direct use in food applications or in biorefinery processes for the valorization of multiple products. Best storage procedures must be determined for each species and product along with establishing adapted methods to evaluate product shelf-life.

6.1. Seawater storage

Primary processes of harvested seaweeds using convective air-drying (AD) can effectively stabilize the biomass but usually has a limited capacity to process large volumes and may be difficult to implement close to harvesting sites. Short-term storage in seawater is a standard practice to extend the post-harvest shelf-life of the seaweeds and for the supply of fresh biomass, or prior to further processing. Paull and Chen (2008) reported that the shelf-life of fresh *Gracilaria* spp. from Hawaii can be extended from 4 up to nearly 30 days by submerging the biomass in seawater at 15 °C in the dark. Similarly, the storage of *P. palmata* in artificial seawater at 4 °C can be used to preserve the characteristic aromas of freshly harvested fronds up to 15 days (Le Pape et al. 2002). The seawater storage of newly harvested kelp is currently regarded as a solution to handle large biomass volumes in commercial cultivation operations. However, knowledge remains limited regarding the effects of this technique on the raw material and their consequences on further processing steps and the quality of the final product.

During the industrial handling of seaweed biomass to be used in food applications, losses of bioactive and nutritious compounds must be minimized throughout the processing chain. The study reported in **Paper I** was designed in the context of an increasing interest for large-scale cultivation of kelps in Europe and Norway, and the subsequent need for

efficient methods to maintain the quality and increase the shelf-life of the raw material immediately after harvest. It is the first attempt to characterize the changes occurring in the biomass of cultivated kelps (*A. esculenta* and *S. latissima*) after harvest during storage in seawater tanks.

Only moderate changes in the chemical composition of both species were observed in this study, namely a slight increase in minerals and decrease in carbohydrates, more pronounced in *S. latissima*. Since these changes could not alone explain the decrease in DW observed (**fig. 6**), it was concluded that water uptake likely occurred during the first 2-h of storage. Seawater storage appears to be an acceptable method to extend the shelf-life of fresh kelps although a fraction of the low molecular-weight soluble carbohydrates (mannitol, laminaran) and polyphenols may be lost during the process. A reduction in the content of fermentable sugars would negatively affect a subsequent ensiling process (see section 6.5).

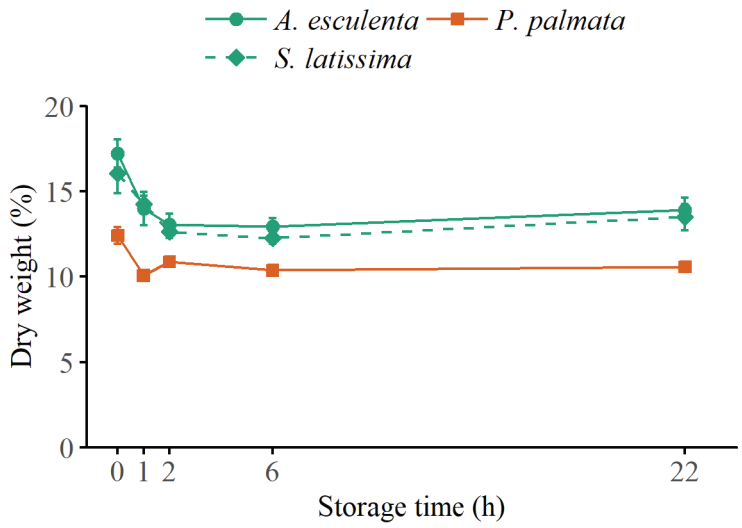


Fig. 6: Changes in dry matter content of *A. esculenta*, *S. latissima* (**Paper I**) and *P. palmata* (**NPR 1**) stored in seawater tanks. Values are given as mean ± standard error ($n = 3$).

The same experiment was conducted on *P. palmata* (**NPR 1**). The table in **suppl. material 1** show stable levels of the macronutrients analyzed in the samples after 22 h of storage in seawater compared to initial levels. Only the photosynthetic pigment R-PE could not be detected in the samples at the end of the experiment. These results suggest that the decrease in DW, occurring during the first hour of storage (**fig. 6**) is mainly due to water uptake during storage and that *P. palmata* can be stored in seawater tanks at this temperature (13.5 °C) for a 22-h period without significant changes in nutrient content. This species is growing in the intertidal zone and the samples were hand-picked at low tide. Thus, it is likely to be less sensitive to stress induced by air exposure between harvest and seawater storage, compared to the two studied kelps species. Water uptake during storage will however entail longer processing times if drying is the method chosen to obtain a stable product.

The relatively short storage duration (22 h) and the unique temperature level tested (i.e. ambient seawater temperature outside the CEVA's facility, which was higher than usual when conducting tests on the kelp species) are the main limitations of this study. Chilling storage using refrigerated seawater (RSW) is a widely used technique to extend the shelf-life of fish and other seafood products. The technique consists in using temperature near or marginally below 0 °C. Under these conditions the temperature in the product remains low and stable, maintaining freshness and suppressing the growth of spoilage microorganisms, without the formation of ice crystals and tissue disruption (Piñeiro et al. 2004). No report is currently available in the literature on the use of this technique for seaweed preservation. In a preliminary experiment conducted at Seaweed Energy Solutions (SES) using RSW at -0.5 °C instead of 7 °C (usual seawater temperature in May at SES), the shelf-life of *S. latissima* was prolonged from 7 to 14 days (J. Funderud, pers. comm.). However, sufficient water circulation is critical to maintain a low and homogenous temperature in the tank and avoid spontaneous fermentation processes of the kelp biomass (Stévant et al. 2018).

In another experiment, the total energy demand of RSW storage of *S. latissima* at 2 °C for 14 days with a water flow of 24 L min^{-1} was estimated at 24.7 kWh ton^{-1} fresh

seaweeds (Stévant et al. 2018). Based on the estimate of 0.4 NOK kWh^{-1} as the average electricity price for the industry in Norway (Statistisk Sentralbyrå 2018b) the cost of this operation is 9.9 NOK ton^{-1} (1.0 € ton^{-1}), making this technology attractive to extend the shelf-life of seaweeds at a relatively low cost. However, the investment costs associated with RSW unit is relatively high (T. Nordtvedt, pers. comm.). Further investigation of the biomass quality, including the microbial load, during storage of kelps using RSW systems is under current investigation.

6.2. Drying

Drying is a common method for the stabilization of wet biomass including macroalgae. Drying is a mass transfer process that removes moisture from the product and reduces the water activity (a_w), thus preserving the product by avoiding microbial growth and limiting the rate of chemical reactions. In addition, the weight and volume of the material are substantially reduced, minimizing the packaging, storage and transportation costs. A large part of seaweed food products in Europe are sold in dry form (Le Bras et al. 2014) because they are versatile and convenient for the consumer. Dried seaweeds can also be used in further industrial processes.

Seaweeds are typically preserved using sun-drying or hot-air convection drying (AD) methods. The phytochemical content (Chan et al. 1997; Gupta et al. 2011; Ling et al. 2015) and physico-chemical properties (Tello-Ireland et al. 2011) of the material may be affected by the method employed with consequences on the product's overall quality as food and on extraction yields of valuable compounds. However, the reported effects of different drying methods and temperatures on the product quality vary depending on seaweed species. The consequences of drying on product quality from the three species of interest in this study has not been thoroughly investigated and existing knowledge on drying of seaweeds may not be applicable to these species. Furthermore, the sensory properties of the products have usually not been included in previous studies evaluating the effects of drying treatments on seaweeds. Only Michel et al. (1997) reports the changes in organoleptic profiles between fresh and dried *P. palmata* and *Ulva* spp. at

60 °C. The thorough investigation of drying treatments of the main commercial seaweed species in Europe and their impacts on food product was undertaken.

Sun-drying is the preferred method for preserving seaweed biomass in tropical and sub-tropical environments since it does not require additional electrical power. However, it also implies long drying times (several days) and leaching of nutrients and oxidative degradations following long exposure to air (Chan et al. 1997; Ling et al. 2015). Generally, due to the absence of liquid water and the low temperatures during the process of FD biomaterials, the rate of most reactions responsible for product deterioration are very low, resulting in high quality food products (Bonazzi and Dumoulin 2011). Nevertheless, because of the high equipment and operating costs of FD, convective AD using ovens is often preferred and regarded as a viable method to process significant amounts of biomass in a shorter time.

As part of this doctoral project, a drying experiment was conducted to study the effects of AD treatments in the range of temperatures commonly used to process seaweeds (25 to 70 °C) and compared to FD, on the nutrient content, physico-chemical and sensory properties of the main commercial seaweed species in Europe, i.e. *S. latissima* (**Paper II**). Samples of all AD treatments (25, 40 and 70 °C) exhibited similar levels of macronutrients compared to those of freeze-dried samples. No clear trend in the fucoxanthin content of *S. latissima* among sample groups could be observed, due to the variations observed within groups, although higher levels were measured in samples air-dried at 70 °C. In general, carotenoids, including fucoxanthin are considered to be sensitive to heat, oxygen, light and low pH (Ho et al. 2008; Mise et al. 2011). However, higher fucoxanthin recovery yields are reported from *F. vesiculosus* samples air-dried at 40 °C compared to samples processed at lower temperatures (25 °C and freeze-dried) (Silva et al. 2019). Color differences observed in the present study i.e. greener samples air-dried at 40 and 70 °C compared to samples treated at 25 °C and freeze-dried, can be explained by the dissociation of the fucoxanthin pigment from light-harvesting complexes of chlorophylls, more pronounced at temperatures above 25 °C.

No differences in polyphenols among treatments were measured. Gupta et al. (2011) reported an overall reduction in the total phenolic content of *H. elongata* after drying, which was more pronounced at lower temperatures (in a range 25 to 40 °C), probably due to longer drying times (i.e. a prolonged oxidative stress) compared to high temperatures. In contrast, increasing temperatures in the range 35 to 70 °C results in a decrease in total phenolic content and antioxidant activity of *F. vesiculosus* (Moreira et al. 2016), highlighting the complexity of the response to high temperature. This response may involve the release of phenolic substances from cell-wall polysaccharides upon drying, binding to other substances, such as proteins, and thermal degradation by oxidative enzymes. A similar reduction in antioxidant activity of the red alga *Gracilaria chilensis* at high drying temperatures was observed by Tello-Ireland et al. (2011), along with the thermal denaturation of photosynthetic pigments (e.g. R-PE).

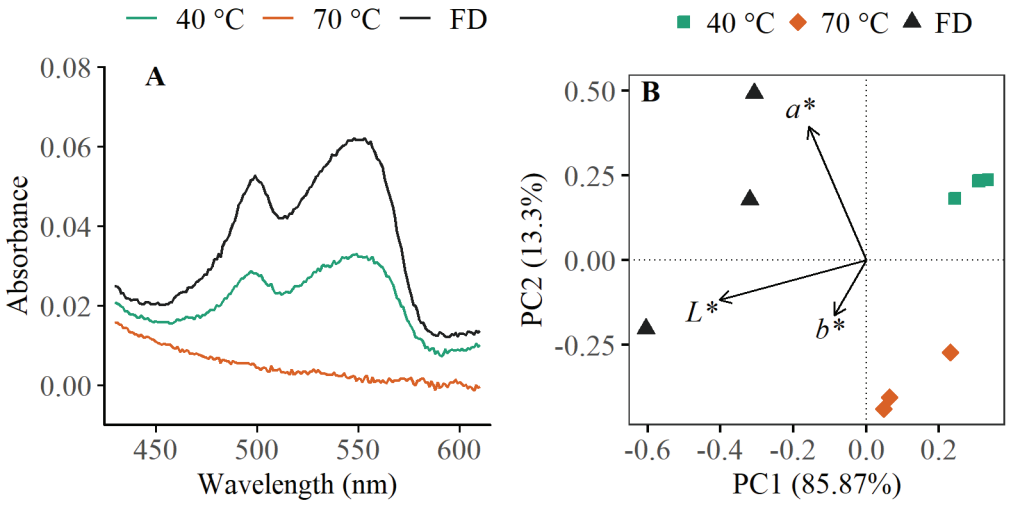


Fig. 7: A) Absorption spectra of R-phycoerythrin (R-PE) from *P. palmata* extracts from air-dried samples at 40 and 70 °C and freeze-dried (FD). B) PCA biplot (1st and 2nd principal component axis) obtained from the color analysis of the dried *P. palmata* samples (**NPR 2**). Vectors indicate loadings representing the variation in individual color coordinate (*L**, *a** and *b**) among all samples.

The results from the drying experiment of *P. palmata* conducted in this study (**NPR 2**) clearly showed the denaturation of R-PE from AD at 40 and 70 °C compared to FD, as illustrated by the reduced absorption spectra of extracts from air-dried samples, especially at high temperature (**fig. 7.A**). Differences in the color of the samples, i.e. darker and decreased redness (decreased *L** and *a**, **fig. 7.B**) of air-dried samples at increasing temperatures compared to freeze-dried samples, supported the results from pigment analysis. This observation is opposed to the recent results of Silva et al. (2019) who found that mild thermal drying of *Gracilaria* sp. (up to 40 °C) do not impair the recovery of R-PE, compared to freeze-dried or fresh samples. AD treatments also affected the content of soluble proteins from aqueous extracts of *P. palmata* compared to freeze-dried samples (**suppl. material 2**). This result may be due to the thermal denaturation and/or aggregation of proteins upon AD particularly at high temperatures, affecting their solubility.

From the drying experiment of *S. latissima* (**Paper II**), mainly the physico-chemical properties (OBC, SC) of air-dried samples were altered, especially at high drying temperatures. This result was explained by changes of the microstructure, due to product shrinkage and reduced porosity. Similar alterations of *S. latissima* were also observed in a recent study by Sappati et al. (2017) who reported higher rates of shrinkage at increasing drying temperatures (40 and 70 °C), explained by higher temperature difference above the glass transition temperature (T_g) during the drying process, maintaining the product in a rubbery state and increasing its viscoelastic behavior. Reduced hydration properties due to product shrinkage following air-drying were also observed on *P. palmata* (**NPR 2**, **suppl. material 3**) and other seaweed species (Tello-Ireland et al. 2011; Chenlo et al. 2017). Heterogeneity of the granulometry after pulverization and prior to extraction of *P. palmata* samples from different drying treatments was also noticed. A finer powder was obtained from freeze-dried samples compared to air-dried (**NPR 2**), which can be the direct consequence of a reduced porosity. These alterations affect the quality of the product as food, and may also reduce solvent penetration, potentially leading to lower extraction yields of valuable compounds from seaweed biomass. It should be noted that AD at high rates (at high temperatures

and/or high air velocity) may lead to the formation of a hard layer at the surface of seaweeds (case-hardening), especially on kelp blades, limiting the moisture diffusion from the core to the outside of the product.

Drying clearly affects the sensory profile of seaweeds when compared to fresh material (Michel et al. 1997). Michel et al (1997) reported a decrease in aromatic compounds analyzed by gas chromatography and mass spectrometry (GC-MS) from air-dried samples of *P. palmata* and *Ulva* sp. at 60 °C compared to fresh samples while the relative proportions between these compounds remained relatively unchanged. In the same study, drying at 150 °C induced drastic changes including the alteration of the color and aromatic perception of the products, and the formation of low-molecular-weight volatile compounds at the expense of long-chain fatty acids and aldehydes (Michel et al. 1997). These effects were explained by the combination of Maillard and oxidative reactions occurring at high temperature. On the contrary, the sensory analysis of *S. latissima* reported in **Paper II** did not detect significant differences between samples dried at high (70 °C) and low temperatures (25 °C) suggesting that no drastic changes in the composition of flavor-active compounds, from thermally-induced reactions, occur in this temperature range in *S. latissima*. The characteristic flavor of many processed foods is often the result of such reactions (e.g. Maillard reactions and Strecker degradations) potentially leading to desired flavor profiles (Lindsay 2008). For instance, AD of black tea leaves at 85 °C causes an overall loss of aromatic constituents giving the product its typical aromas (Sanderson and Graham 1973). Some desirable flavors may also be obtained from edible seaweeds processed at higher temperature levels than tested in **Paper II**.

While AD at low temperatures resulted in a somewhat better product quality (i.e. preservation of heat sensitive bioactive substances, less shrinkage) from *S. latissima* and *P. palmata* compared to high temperatures, it is also associated with slower drying rates, which can limit the possibility for processing large biomass quantities. Large-scale drying of seaweed biomass at low temperature may require technical adaptations of the drying systems to optimize the process e.g. increased air velocity, dehumidification of

inlet air, fluidization of the seaweed material to increase surface contact with the drying medium. However, dewatering of kelp biomass using convective AD methods is an energy-intensive process, thus reducing the environmental sustainability and cost-effectiveness of the value-chain (van Oirschot et al. 2017).

The costs of drying seaweeds using conventional open systems at 70 °C such as the shelf dryer used in **Paper II** were calculated and estimated to 7200 kWh ton^{-1} dry product (containing 10 % moisture) (Nordtvedt et al. manuscript in prep.), representing a cost of 2908 NOK ton^{-1} dry seaweeds (298 € ton^{-1}). This figure is quite high, limiting the commercial outputs from this process to high-value products. For a direct comparison with other preservation methods investigated in the present work such as freezing (section 6.4), the energy requirements of drying can be estimated per ton wet biomass based on these figures. Given an initial biomass containing 90 % moisture, drying 1000 kg of seaweeds to 10 % moisture requires the removal of 890 kg water, resulting in 110 kg dried product. The energy requirement is then estimated to 7200 x 0.11 = 792 kWh ton^{-1} wet seaweed biomass and the associated costs 317 NOK ton^{-1} (33 € ton^{-1}), highlighting the need to reduce the energy costs of seaweed processing.

Alternative technologies such as heat pumps and superheated steam drying (SSD) are gaining priority in the food processing industry as potential replacement technologies to increase the energy efficiency of drying processes. A heat pump transfers thermal energy from a heat source at low temperature (e.g. 0 to 40 °C, at the evaporator) to heat sinks at higher temperature (condenser) using a refrigerant. During AD using a heat pump, the latent energy (from the evaporation of water from the product) is recovered (via the evaporation and recompression of the refrigerant) and transferred back to the recirculated drying air which is re-heated at the desired drying temperature. Strømmen et al. (2002) estimated the energy consumption of heat pump drying (HPD) systems to be reduced by 60 to 80 % compared to conventional dryers operating at the same temperature.

SSD is also associated with increased efficiency compared to convective AD methods. This technology involves the use of recirculated superheated steam instead of hot air, as drying medium to supply heat to the product and remove the evaporated water. The

higher capacity and thermal conductivity of superheated steam leads to higher drying rates (Mujumdar 2014). The exhaust steam can be used as a heat source to improve the energy efficiency of the system. In addition, nutrient losses due to oxidation are avoided and mechanical stress (shrinkage) of the product is limited (Sehrawat et al. 2016). This technology applied to drying kelps was tested by Jia et al. (2018), who reported a reduction of the energy input by nearly 50 % from a SSD system bearing exhaust heat recovery unit, compared to an air-dryer. The possibilities for using SSD technology for processing cultivated kelps in Norway and effects on product quality are currently under investigation.

Industrial excess heat (e.g. from a waste incineration plant) could also be used as an alternative to conventional energy sources and contribute to increase sustainability in the seaweed processing chain (Philis et al. 2018).

6.3. Dry and semi-dry storage

It has long been recognized that the shelf-life of a food product can be predicted by the amount of unbound water (i.e. a_w) available for microbial growth and many other degradative reactions. The stability of a stored biomaterial is also affected by its diffusional properties, directly related to its T_g and water content (Reid and Fennema 2008). During processing such as drying, as the water content and temperature change, the food undergoes complex phase and state changes influencing the molecular mobility and thus the rate of most chemical and physical processes occurring in the product. These changes are illustrated by a simplified temperature-composition state diagram for a binary system (water and the dominant solute of the system, **fig. 8**) (Rahman 2006; Reid and Fennema 2008).

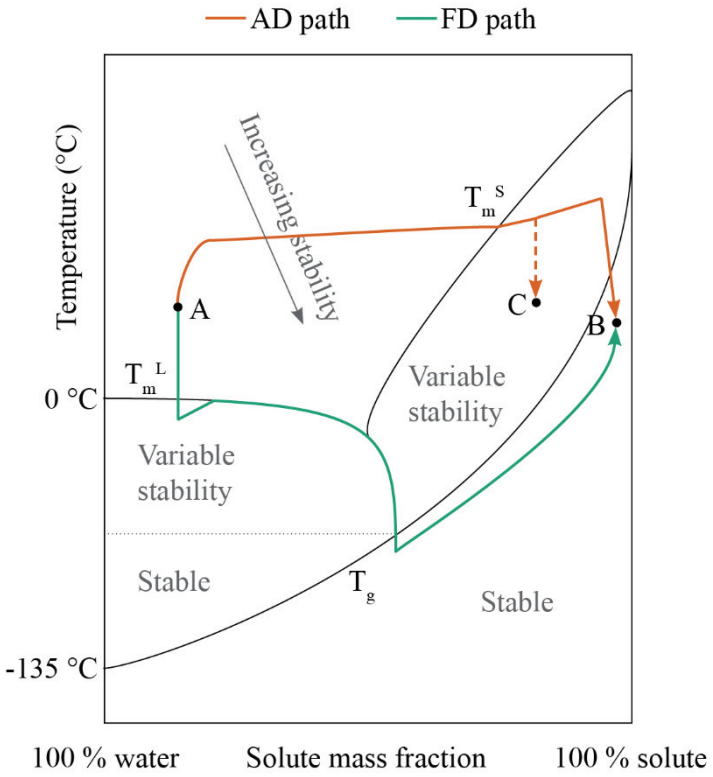


Fig. 8: State diagram of a binary system showing the potential product stabilities in different zones and paths for air-drying (AD) and freeze-drying (FD). T_m^S is the solubility curve, T_m^L is the melting point curve and T_g, the glass transition curve. (Adapted from Reid and Fennema 2008).

During convective AD, the temperature of the product (A) increases and moisture is removed until the system passes the solubility curve (T_m^S) and becomes supersaturated, also described as a rubbery state. Drying beyond the dry bulb air temperature results in the product falling under the T_g curve after cooling (B) and turning into a metastable, amorphous glassy state. Limited drying followed by cooling leads to product C above T_g which is characterized by a higher molecular mobility (reduced stability) compared to B. During FD, the raw material A is cooled down leading to the formation of ice and the supersaturation of the solutes in the unfrozen fraction of the product. As the

temperature is lowered down to below T_g the supersaturated unfrozen phase turns into a glassy state, and ice crystals are removed by sublimation. State diagrams can be used to predict the stability of foods during storage. Generally, food products are more stable at and below T_g and the higher the temperature above T_g during processing and storage, the higher the deterioration or reaction rates (Rahman 2006).

Obtaining a stable dry product is critical in applications where seaweeds are used as a source of bioactive substances that are sensitive to degradations over time (e.g. vitamins, polyphenols, pigments) (Gupta et al. 2011; Munier et al. 2013; Lage-Yusty et al. 2014). As illustrated by the **fig. 8**, a low moisture content and low storage temperatures, below T_g, will provide optimal stability. In other cases, storage conditions that allow endogenous reactions within the product can contribute to develop favorable sensory characteristics. Such cases were exemplified earlier (section 5.3) with the maturation of *konbu*, and presumably a similar process occurring upon storage of *P. palmata* in ancient times in Iceland. In both cases, the seaweeds were sun-dried, typically resulting in products with higher levels of moisture than when using air ovens (Chan et al. 1997). While the growth of spoilage microorganisms remains limited, a higher moisture content may promote the activity of endogenous enzymes and other reactions (hydrolysis of proteins, carbohydrates, oxidation of lipids) leading to the formation of flavor compounds such as FAA, mono- and oligosaccharides and the formation of volatile compounds. Changes of this kind are typically observed during the maturation (also referred to as “curing” or “ripening”) of other food products such as ham, cheese and tomatoes (Ninomiya 1998; Petrova et al. 2015). However, the present knowledge on the factors controlling the sensory characteristics of edible seaweeds is anecdotal and mostly experience-based.

Based on the observations described above, an original methodology was designed to test the hypothesis that the moisture level during dry storage affects the organoleptic properties of the raw material and that controlling this parameter may be employed to develop a variety of sensory profiles from edible seaweeds. Changes in the sensory properties of *S. latissima* and *P. palmata* stored in a dried and so-called semi-dried state

(i.e. "matured") under controlled conditions (12 °C in darkness) were monitored over time, along with other alterations of their chemical content (**NPR 3** and **Paper III**).

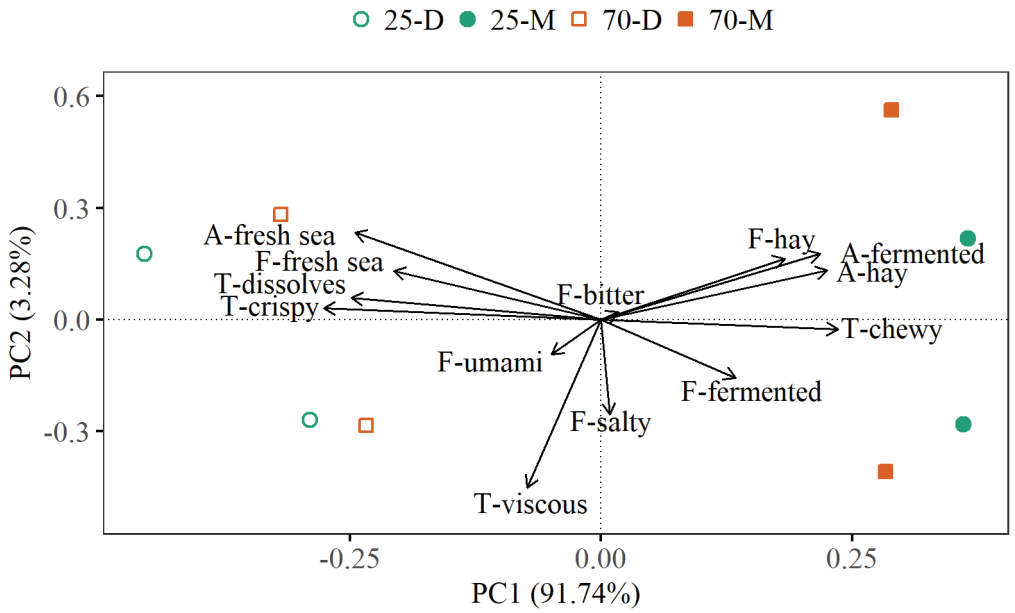


Fig. 9: PCA biplot (1st and 2nd principal component axes) obtained from the descriptive sensory analysis of *S. latissima* samples after drying at 25 and 70 °C and storage in dry (D) and semi-dry state (matured, M) over a period of 97 days (**NPR 3**). Average scores over panelists (n = 8) were used for the PCA. Vectors indicate loadings representing the variation in intensity for individual sensory attributes including aroma (A), flavor (F) and texture (T) characteristics, among all samples.

The sensory characteristics of dried *S. latissima* samples at 25 and 70 °C (25-D and 70-D) and matured samples (25-M and 70-M) stored for 97 days, were evaluated in their original form (i.e. dried and semi-dried) by eight trained panel members, based on 13 selected attributes listed in **table 3** (**NPR 3**). The DW of the dried samples was 5.9 ± 0.2 % and 4.0 ± 0.5 % (for 25-D and 70-D respectively), and 17.6 ± 0.1 % and 15.3 ± 0.2 % for the matured samples (25-M and 70-M respectively). The statistical analysis of

the results from the sensory evaluation revealed no significant effect of the drying temperature on the sensory attributes across sample groups (25 and 70 °C, **suppl. material 4**). In stark contrast, highly significant differences in aroma, flavor and texture properties were detected between samples of different moisture levels during storage (D and M samples, ANOVA: $p < 0.001$) except for the flavor attributes "salty", "bitter" and "umami", as well as the texture attribute "viscous" (**suppl. material 4**). These results are confirmed by the multivariate analysis of the scores by PCA, illustrated in **fig. 9**. The first axis of the PCA biplot (PC1) explains almost 92 % of the variance observed in the dataset. PC1 discriminates the dried samples from the matured ones, the first group being associated with fresh marine aromas and flavors while these characteristics faded in the second group in favor of fermented and green notes, related to "hay" or "green tea" flavors and aroma. All samples were characterized by intense saltiness, low bitterness and a moderate umami flavor.

The differences in texture observed in this preliminary study (regarding the attributes "crispy", "chewy" and "dissolves", **fig. 9**) were associated with the heterogeneity of the sample groups during the sensory evaluation, i.e. D-samples were evaluated in their dry form and M-samples in their semi-dry form, limiting their comparison with respect to texture attributes. To study the effects of the moisture content during storage on the product texture, both D- and M-samples of *P. palmata* were evaluated in semi-dry form in **Paper III**.

Using another set of dried *S. latissima* samples (dried at 40 °C), the FAA content of dried (D) and matured (M) samples during a 154-days storage period was analyzed. The total FAA content increased in all samples during storage (**fig. 10**.**A**). Similar levels of FAA were measured in both D- and M-samples after 154 days of storage. After partial rehydration to 20 % moisture, the total FAA content of M-samples increased rapidly after 8 days compared to initial levels (D-0), reached a maximum at 64 days and decreased to comparable levels measured after 8 days. The accumulation of FAA and small peptides is typically observed during the ripening process of fruits, ageing of cheese and curing of meat as a result of the proteolysis by endogenous enzymes and/or

microorganisms (Cordoba et al. 1994; Ninomiya 1998; Zhao et al. 2016). Secondary reactions usually involve the conversion of FAA into flavor-active amino acid derivatives from different enzymatic and non-enzymatic reactions such as Strecker and Maillard reactions (Zhao et al. 2016). This can explain the decrease in FAA in M-samples measured between 64 and 154 days of storage.

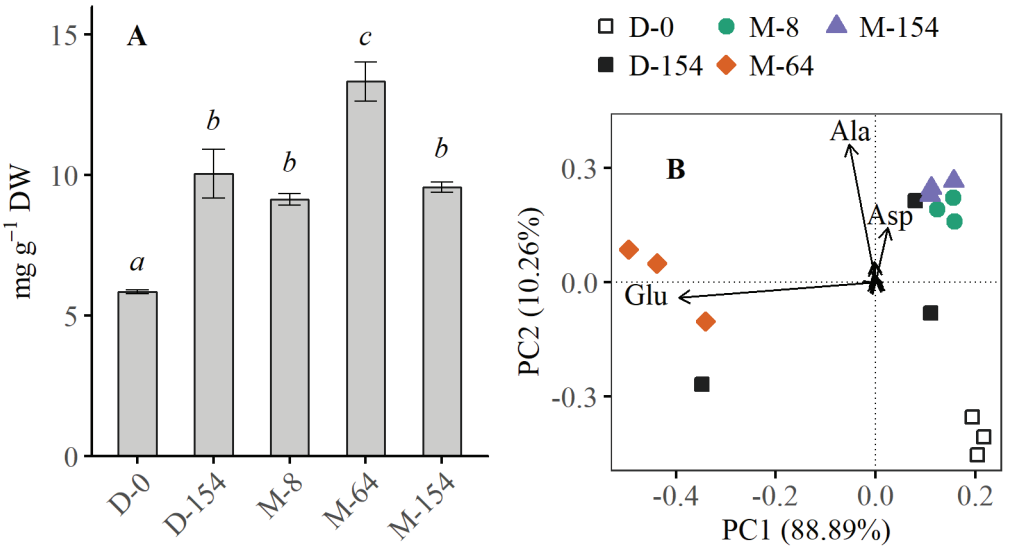


Fig. 10: A) Total free amino acid (FAA) content of *S. latissima* samples after dry (D) and semi-dry, matured (M) storage over a period of 154 days (**NPR 3**). Different letters indicate significant differences among sample groups ($p < 0.05$). B) PCA biplot (1st and 2nd principal component axes) obtained from the analysis of the FAA profile of the samples. Vectors indicate loadings representing the variation in the content of individual FAA among the samples.

Alanine, glutamate and aspartate were the main FAA present in the initial dried *S. latissima* samples (D-0) representing over 70 % of the total FAA content. The analysis of the FAA profiles by PCA reveals clear differences between the initial D-0 and stored samples (D-154, M-8, M-64 and M-154, **fig. 10.B**). Higher levels of free glutamate were

measured in stored samples (both dried and semi-dried) compared to D-0, with a maximum detected in matured samples at 64 days of storage, suggesting this compound to be involved in secondary reactions (after 64 days) as mentioned previously. The metabolism of glutamate during the fermentation of foods is well studied and may lead to the formation of flavor-active compounds (e.g. pyroglutamate peptides, succinyl glutamate) also involved in the perception of umami (Zhao et al. 2016). However, umami was not identified as a characteristic flavor in any of the *S. latissima* samples from the sensory evaluation described previously (**fig. 9**). This can be linked to the relatively low levels of free glutamate measured in the present samples (reaching a maximum of 6.4 ± 0.3 mg g^{-1} DW in M-64 samples) compared to the levels reported in *konbu* (ca. 15 mg g^{-1} DW) (Ninomiya 1998; Mouritsen et al. 2012) as well as the dominance other flavors such as an intense saltiness. Free alanine is also a known flavor-active substance in different types of seafood including seaweeds (e.g. *wakame* and *nori*) (Ninomiya 1998). In the present study, this compound was found more abundant in all stored samples of *S. latissima* regardless of their moisture content, compared to the initial samples prior to storage (**fig. 10B**).

The results from this experiment revealed changes in the FAA content and composition of *S. latissima* during storage, both in dried and semi-dried form. A difference in the moisture content of the control dried samples at the beginning and the end of the experiment (D-0 and D-154) was measured (i.e. 4.4 ± 0.2 % and 8.7 ± 0.1 % moisture respectively), which may have affected the stability of these samples. Therefore, the design of the following experiment conducted on *P. palmata* was modified to provide a better control over the stability of the dried (control) samples and after the process of maturation before chemical and sensory analyses, i.e. by FD and storage at -80 °C (see fig.1 of **Paper III**).

The sensory characteristics, chemical composition, physico-chemical and color parameters, and microbial status of dry (D) and semi-dried, matured (M) samples of *P. palmata* during a 126-days storage period at 12 °C were analyzed. The results are reported in **Paper III**. Strong marine flavors and aromas (“seaweed” and “fishy”), were

identified from the sensory evaluation of the D- and shortly matured samples (M-12) as well as a tough and crunchy texture. On the other hand, the samples undergoing a longer maturation (M-61, M-126) were characterized by "hay" and sweet aromas, complex flavors ("processing" flavor attribute) and a tender texture (**fig. 11**). The attribute "flavor richness" which includes the perception of umami (see table 1 from **Paper III**), was also more pronounced in the matured samples although this trend was not significant.

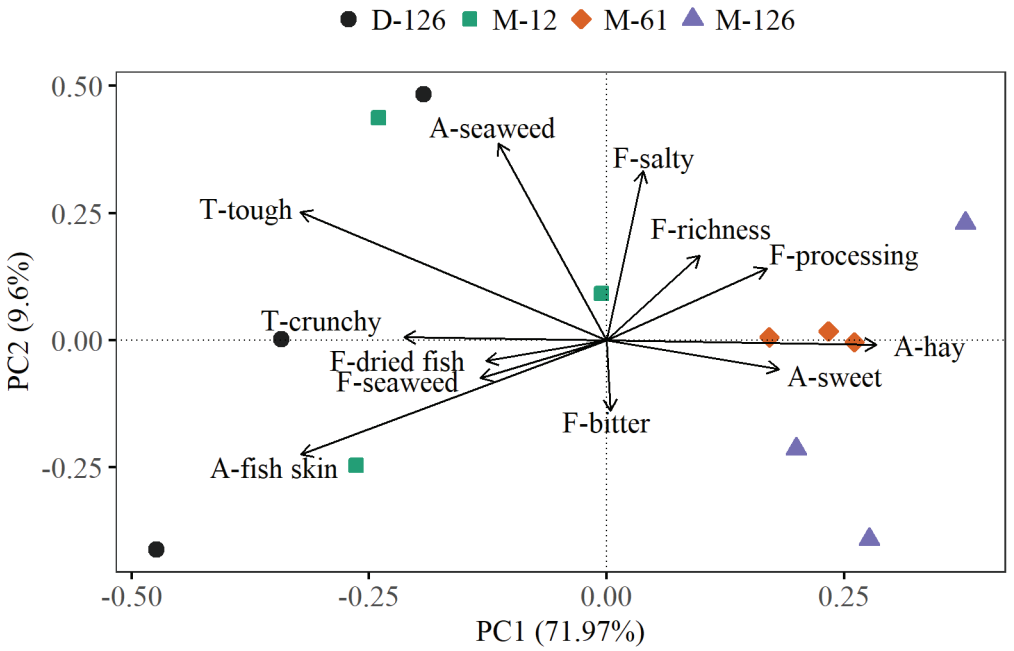


Fig. 11: PCA biplot (1st and 2nd principal component axes) obtained from the descriptive sensory analysis of *P. palmata* samples after dry (D) and semi-dry, matured (M) storage over a period of 126 days (reproduced from **Paper III**). Average scores over panelists (n = 9) were used for the PCA. Vectors indicate loadings representing the variation in intensity for individual sensory attributes including aroma (A), flavor (F) and texture (T) characteristics, among all samples.

M-61 was the group with the highest amount of soluble sugars although no significant differences distinguishing M- from D-samples could be identified from these results. Increasing amounts of soluble sugars can result from the hydrolysis of cell-wall constituents, mainly xylans in *P. palmata*, during the maturation process. This would correlate with increased sweet aromas and a more tender texture of the M- compared to the D-samples. The degradation products of such reaction may be oligosaccharides which are not detected by the chosen analytical method. The decrease in WBC and OBC in all samples also suggests that structural polysaccharides are affected during the process of maturation and to a certain extent, during dry storage. The soluble fraction of the proteins, including the R-PE pigment, decreased during storage of the matured samples and not in the dried control samples. This indicates that higher moisture levels in *P. palmata* allows endogenous reactions involving proteins to occur at higher rates. The FAA and volatile compounds profile of the samples as well as their protein and carbohydrate molecular weight distribution are under current investigation. The results may give evidences of the endogenous chemical and enzymatic reactions occurring during the maturation process.

The maturation process in this study can be compared to ageing or ripening processes commonly employed in a wide range of foodstuffs. The food products obtained from these processes are highly valued by consumers worldwide for their rich and complex flavors. The changes occurring during the process are the results of spontaneous chemical reactions (e.g. hydrolysis, Maillard) or the metabolic activity of microorganisms or enzymes either added or initially present in the raw material (Zhao et al. 2016). Increasing the moisture content (and thus the a_w) of dried *S. latissima* and *P. palmata* samples to ca. 20 %, as a mean to promote endogenous reactions during storage under controlled conditions, affected significantly the sensory profile of the products compared to dried material (containing ca. 5 % moisture). The characteristic marine flavors and aromas of the dried seaweeds faded, as described from the maturation of *konbu* in Japan (Mouritsen and Styrbæk 2014), while a variety of other flavors arose during storage. However, no salt precipitate clearly formed on the surface of these samples as observed on stored *konbu* (Mouritsen and Styrbæk 2014) and *P. palmata* in

Iceland (Kristjánsson 1980). Further results from the chemical characterization of *P. palmata* samples will provide a basis to identify and describe the formation of flavor-active compounds from this species of commercial interest. Understanding the biochemical and chemical patterns involved in flavor development will enable the optimization of processing and storage conditions of edible seaweeds to achieve tastier food products attractive to Western consumers and sustain the current movement promoting seaweeds as natural and healthy food ingredients.

6.4. Freezing and frozen storage

Freezing is another common preservation method of food. By changing the physical state of the liquid water in food into ice below freezing temperatures, the growth of microorganisms is stopped and the rate of biochemical reactions governing food deterioration is limited. The temperature is further reduced to storage level, often below -18 °C. The loss of quality in frozen foods depends primarily on the composition of the food, storage temperature and duration, as well as thawing procedures (Rahman and Velez-Ruiz 2007).

Many studies have focused on improving the quality of frozen biomaterials (e.g. fruits, meat, fish) however, few studies have been focusing on optimizing the quality of seaweeds using this process. Choi and al. (2012a) suggested freezing at -30 °C in polyethylene bags containing 50 % seawater then thawing in running tap water for 6 h as a simple method for the long-term storage of freshly harvested *wakame* (*U. pinnatifida*), preventing significant losses of quality (texture, color, bacterial development) compared to the fresh material. However, using seawater as a freezing medium will considerably increase the energy requirements of the process along with the costs associated with the transport and storage of the frozen material in a commercial setting. Some effects of freezing on the quality of red seaweeds have been reported. Le Pape et al. (2002) concluded that the freezing process significantly affected the sensory characteristics of *P. palmata* which developed green aromas (hay, tea, cut grass), compared to samples kept in artificial seawater at 4 °C, which preserved original fresh

and marine aromas. There are currently no reports on the effects of freezing and thawing methods on the food quality of the kelp species cultivated in Europe.

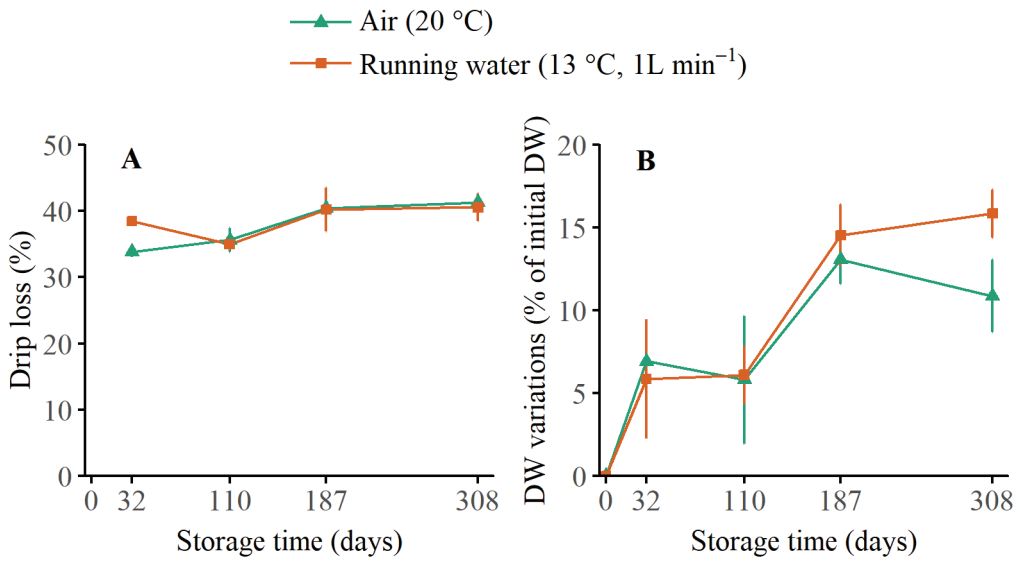


Fig. 12: Variations in A) DW and B) drip loss of *S. latissima* following freezing storage and two thawing methods (**NPR 4**). Values are given as mean ± standard error (n = 3).

The quality of frozen *S. latissima* cultivated in Norway, stored over a 10 months-period and thawed using two different methods was investigated (**NPR 4**). A considerable drip loss was observed in both groups at each sampling time, exceeding 40 % of the initial weight of the samples (**fig. 12.A**). The initial DW of the biomass was 9.29 % and increased to 10.2 ± 0.2 % and 10.8 ± 014 % (after thawing in air and running water respectively) in the remaining solid fraction of the samples after 10 months of storage (**fig. 12.B**). Neither the variations in DW nor the drip loss were significantly affected by the thawing method employed (**table 8**), i.e. between a rapid thawing in running water or a slow thawing in air. The extent of the losses and the aspect of the liquid fraction from thawed samples, i.e. a viscous and brown solution, suggest that the fluids lost during the process also contained phytochemical compounds. Further analyses are needed to identify the content of this liquid fraction.

Table 8: Results (*p*-values only) from RM ANOVAs for individual quality parameters measured on *S. latissima* samples following freezing and two thawing methods (**NPR 4**). Significant results ($p < 0.05$) are indicated in bold.

Independent variable	Thawing	Time	Thawing * Time
Dependent variable			
DW	0.467	**< 0.001**	0.215
Drip loss	0.591	**0.002** [a]	0.316
Color (ΔE)	0.399	0.109 [a]	0.329
Texture (peak load)	0.163	0.692	**< 0.001**

Independent variables are (i) thawing (2 levels: air, running water), (ii) time (5 levels: 0, 32, 110, 187, 308 days, except for [a], 4 levels: 32, 110, 187 308). Within-group replication: $n = 3$. Significant results ($p < 0.05$) are indicated in bold.

Drip loss reflects structural damages within a biomaterial and is influenced by several factors intrinsic to the food product and the conditions of both the freezing and thawing processes. In plant tissue, ice generally forms in the extracellular matrix because cell walls constitute a physical barrier to crystal growth. This leads to an increase in solute concentration in the unfrozen portion of the matrix during the freezing process (Reid and Barett 2004; Rahman and Velez-Ruiz 2007) as illustrated by the first stage of the FD process presented in **fig. 8**. Due to the osmotic potential between the intra- and extracellular spaces, water migrates out of the cell contributing to the growth of extracellular ice, resulting in cell dehydration and shrinkage, and subsequent membrane damage. This water does not return to the cell upon thawing, resulting in drip loss. In general, slow freezing produces frozen food of lower quality due to the formation of large ice crystals causing greater mechanical damage to cell walls (Sun and Li 2003) and allowing extensive water movement due to osmotic pressure. Rapid freezing produces

smaller ice crystals and limits the osmotic transfer of water due to the intracellular formation of ice. The impingement freezing technology used in this study is associated with high freezing rates comparable to those provided by cryogenic equipment. This technology consists in directing high velocity cold air jets creating turbulence around the product surface hence, breaking its insulating boundary layer (Salvadori and Mascheroni 2002). However, the threshold for distinguishing fast from slow freezing is system dependent. Although the contribution of each mechanism, i.e. crystal growth and osmotic pressure, to explain the observed drip loss cannot be quantified from these results, the high mineral content of *S. latissima* (25 to 45 % DW, harvested in May-June) (**Paper I - II)**, suggests that osmotic damage plays a major role in the structural alteration of the biomass. In addition, freezing slows down, but does not stop the biochemical reactions within a biomaterial and both enzymatic and non-enzymatic changes occur but at slower rates. As a consequence of cell rupture, enzyme systems may be released (Rahman and Velez-Ruiz 2007) and affect the structure of the seaweed as well as its characteristics as a food product, e.g. vitamins and proteins degradation. Such reactions may account for the slight but significant increase in drip loss with time of frozen storage (**fig. 12**, **table 8**).

The variations in the surface color of the samples (defined by the color coordinates L^*, a^* and b^*) were recorded and the total color variation (ΔE) reflected variation in each of the three coordinates during storage as compared to the initial values measured on the samples prior to freezing. No significant differences in ΔE were detected neither across sampling times nor thawing methods (**table 8**).

Generally, the variation across the a^* (redness/greenness), and to a lesser extent the b^* (yellowness/blueness) color coordinate accounted for the largest part of the total color variation. The frozen and thawed samples were characterized by lower a^* values reflecting a greener hue compared to the initial fresh samples (**fig. 13.A**). Fucoxanthin is an important compound responsible for the coloration of brown macroalgae. The increased greenness of the frozen and thawed samples could be the result of the degradation and/or the dissociation of the fucoxanthin from the light-harvesting

complexes (as hypothesized previously from the air-drying experiment in section 6.2), the green color characteristic of the chlorophylls then becoming more visible.

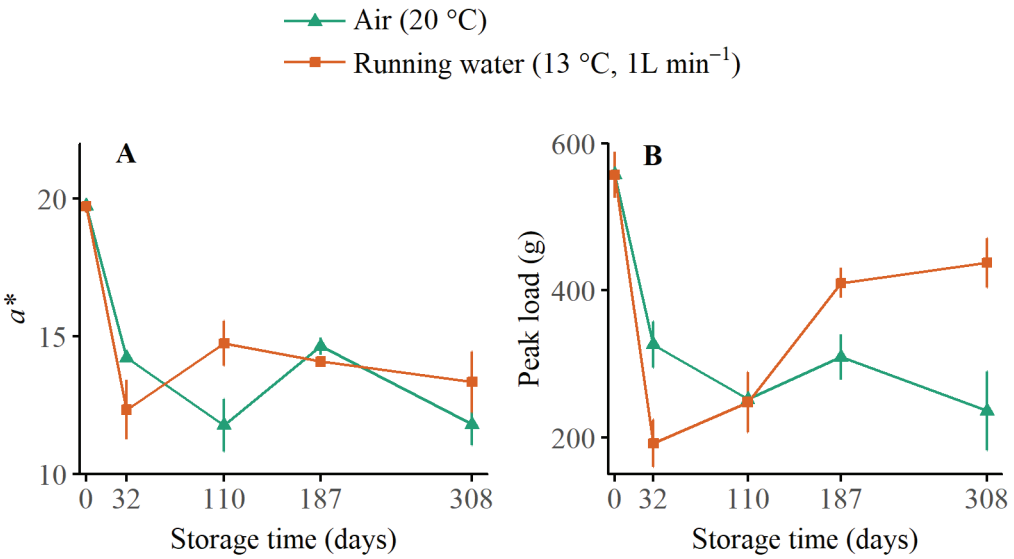


Fig. 13: Variations in A) *a** (redness/greenness) and B) tensile strength (peak load) of *S. latissima* following freezing storage and two thawing methods (**NPR 4**). Values are given as mean ± standard error (n = 3).

The tensile strength is a direct measure of the firmness of a product and has been used to monitor the quality of a wide range of biomaterials including seaweeds (Tello-Ireland et al. 2011; Choi et al. 2012a; Cox et al. 2012). In this study, the tensile strength of *S. latissima*, i.e. the force required to disrupt the blade, was significantly reduced by the freezing process, regardless of the thawing method employed (**fig. 13.B, table 8**). The decrease in tensile strength is most likely the result of cell damage and extensive structural alteration. In kelp species, cell walls are composed of alginates, cellulose, fucans and proteins. The alginate is the main skeletal compound of the intercellular matrix in brown algae, giving the plant both mechanical strength and flexibility. The loss of tensile strength in *S. latissima* observed in this study, may be the result of the

degradation of the alginate matrix. Furthermore, the viscosity of the liquid fraction indicates the release of large molecules, possibly alginate oligomers and fucoidans through drip losses.

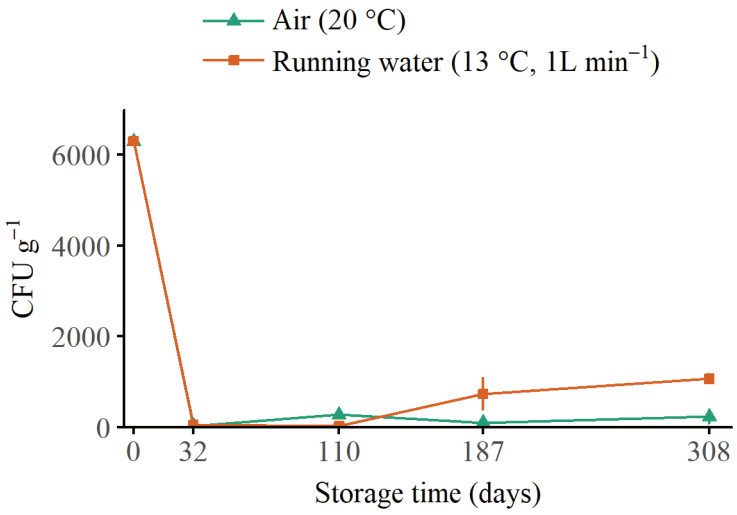


Fig. 14: Microbial load (total aerobes) of *S. latissima* following freezing storage and two thawing methods (**NPR 4**). Values are given as mean ± standard error (n = 3).

Frozen and thawed *S. latissima* samples were analyzed for their microbial load, including the total number of aerobes, yeasts and molds, and coliforms, using standard culture-based techniques in food control. The results were compared to those from fresh samples after harvest. Freezing greatly reduced the microbial load of *S. latissima* which remained stable at low levels during the entire storage period (**fig. 14**). Yeasts and molds were detected in low number in the initial biomass (< 100 CFU g^{-1}) but were not observed in the frozen and thawed samples (< 10 CFU g^{-1}). No coliforms (< 10 CFU g^{-1}) were detected in any of the analyzed samples. In vegetables, freezing causes the apparent death of 10 to 60 % of the viable microbiota, a percentage that gradually increases with time of frozen storage (Rahman and Velez-Ruiz 2007). There is considerable variation in the ability of bacteria to resist damage by freezing. Freeze injury of bacterial cells

involves damage to the membrane from crystallization, especially upon rapid cooling, and osmotic stress caused by extracellular ice (Archer et al. 1995). Osmotic stress probably played an important role in the large reduction of the bacterial load of *S. latissima* samples in this study, given the high mineral content reported in this kelp species (**Paper I - II**). The main concern regarding food safety, come from micro-organisms that are likely to survive the freezing treatment and to grow following thawing of the product, such as spores of the genus *Clostridium* and *Bacillus* (Duinker et al. 2016). *Bacillus* species were isolated by Blikra et al. (2018) from raw, frozen and heat-treated samples of *A. esculenta* and *S. latissima* most likely from a contamination during handling.

The energy requirements associated with freezing and storage of *S. latissima* in this experiment were calculated and reported in Stévant et al. (2018). The electrical demand associated with the freezing process was estimated at 38 kWh ton^{-1} (wet biomass) while the energy use for the frozen storage of the biomass at -25 °C over the entire period (308 days) was 123 kWh ton^{-1}. The sum of both the freezing and frozen storage represented a cost of 64.5 NOK ton^{-1} (derived from 0.4 NOK kWh^{-1}) and corresponding to 6.6 € ton^{-1}. This preservation method for seaweed biomass is therefore less energy-intensive and more cost-efficient than the conventional air-drying techniques described in section 6.2.

While optimal freezing protocols of foods aim at maintaining tissue integrity, textural and sensory attributes of the biomaterial, radical alterations such as extensive cell rupture, may also facilitate the extraction of intracellular compounds such as proteins and pigments, limiting the needs for enzymatic treatments to degrade cell-wall polysaccharides. The characterization of both the solid and liquid fractions from thawed *S. latissima* is scheduled and will give information on the possible advantage of implementing this process as a pre-treatment step for the recovery of multiple compounds on a commercial scale. Optimizing the conditions during the freezing processes of *S. latissima*, aiming to i) maintain the quality and integrity of the product as food and ii) facilitate the extraction of valuable phytochemical substances, will be determined in a future study.

6.5. Ensiling

Anaerobic fermentation techniques (referred to as ensiling) are preservation methods commonly applied to forage in agriculture. Ensiling of macroalgal biomass is currently an active area of research as this method offers low energy solutions to processing and storage of large biomass volumes (Herrmann et al. 2015). The aim of ensiling is to lower the pH of the seaweed biomass (typically below 4.0) to inhibit the growth of spoilage microorganisms (e.g. clostridia, yeasts, molds). Ensiling of plant material is typically achieved directly by the addition of an organic acid or indirectly by adding a population of lactic acid bacteria (LAB) to the biomass. The LAB will then convert available fermentable sugars to produce lactic acid. Ideally, the biomass to be ensiled should have a sufficient amount of water soluble carbohydrates (fermentable sugars), an adequate dry matter content (measured as the DW of the biomass), a low buffering capacity (BC), defined as the biomass resistance to change in pH, as well as LAB populations already present (Weißbach 1996).

The chemical composition of macroalgae i.e. low dry matter content, high BC related to high anion levels (e.g. chloride and sulfate) and variable contents in fermentable sugars, appears to be a challenge for proper ensiling, with variable results across species (Herrmann et al. 2015; Cabrita et al. 2017). High levels of polyphenols are also known to inhibit microbial activity and is an important factor restricting the anaerobic fermentation process in polyphenol-rich macroalgae species (Moen 1997). The kelp *S. latissima* showed superior fermentation potential in anaerobic conditions compared to other species, and produced lactic acid from native LAB populations (Herrmann et al. 2015). The addition of LAB as inoculant prior to ensiling only had minor effects on the fermentation process of this species (Cabrita et al. 2017). In another study, the addition of LAB such as *Lactobacillus plantarum* (*Lp*), and *L. brevis,* significantly delayed the growth of spoilage microorganisms in *U. pinnatifida* (Uchida et al. 2004).

However, due to limited control of the fermentation process and unpredictable quality of the produced silage, the implementation of this technique at a commercial scale to preserve seaweed biomass remains limited. This may be a consequence of the variability

in the content of water-soluble carbohydrates (laminaran and mannitol in brown seaweeds) as substrates for the lactic acid fermentation, low initial number of LAB and limited access to the substrate.

Table 9: Results (*p*-values only) from the RM ANOVAs of individual variables measured during the ensiling of *S. latissima* using different pre-treatments (PT) and inoculum (I) (**NPR 5**). Significant results ($p < 0.05$) are indicated in bold.

Independent variable	**PT**	**I**	**Time**	**PT * Time**	**I * Time**
Dependent variable					
pH	0.860	**0.042**	**< 0.001** [a]	0.626	0.925
Effluent formation	**0.016**	0.191	0.164 [a]	0.747	0.672
DW	0.496	0.588	0.232	0.866	0.662
Mannitol	0.176	0.584	**< 0.001**	0.755	0.853
Lactic acid	0.288	0.095	**< 0.001**	0.300	0.573
Fucose	0.095	0.829	0.172	**0.040**	0.681
Glucose	0.235	0.890	0.759	0.066	0.948
Alginate	0.167	0.541	0.689	0.487	**0.031**

Independent variables are (i) PT (2 levels: whole, chopped), (ii) I (3 levels: no inoculation, *Lp*, *Lp* + AL) and (iii) time (5 levels: 0, 5, 15, 48, 103 days, except for [a], 4 levels: 5, 15, 48, 103 days). Within-group replication: $n = 3$.

Chopping the raw material increases the surface area to volume ratio which may enhance the substrate availability to the fermenting microbiota. As whole kelp blades have higher chances of entrapping air and thus promote the growth of undesirable aerobic microorganisms, chopping the biomass into smaller pieces prior to ensiling may also

provide more stable anaerobic conditions during the process. The alginate matrix may limit the diffusion of mannitol and thus the substrate availability to the microbiota. Therefore, the partial degradation of alginates by alginate lyase (AL) may increase the rate of fermentation and promote a more rapid acidification of silages from *S. latissima*. Since the optimum pH of this enzyme is 7.6, the potential advantage of this inoculum would be limited to the initial phase of ensiling, the activity of AL being suppressed at pH 5 (Moen 1997), but may affect the subsequent stability of the silage. As part of this project, a preliminary study on ensiling *S. latissima* cultivated in Brittany, France, investigated the effects of biomass pre-treatment (i.e. chopping) and inoculum (i.e. no inoculum, *Lp*, *Lp* + AL) on the ensiling process (**NPR 5**). The quality of the produced silages was monitored over the course of 103 days. The results are presented in **table 9**.

The pH in the samples inoculated with *Lp* and *Lp* +AL decreased rapidly during the first 15 days of ensiling then stabilized at pH values between 4.0 and 4.2 during the rest of the experiment (**fig. 15.A**). In the untreated control samples, the acidification was delayed and at 48 days, the pH reached comparable values to those measured in the other groups (4.3 ± 0.1). The chopping pre-treatment did not affect silage acidification. These results present similar reductions in pH as observed during previous ensiling experiments of *S. latissima* (Herrmann et al. 2015; Cabrita et al. 2017; Sandbakken et al. 2018). The effluent production during the process was high in all samples, ranging from 17 to 28 % of the original ensiled biomass (**fig. 15.B**). Relatively lower amounts of effluents were measured from chopped samples at 15 and 48 days of storage but similar levels were observed in all sample groups at the end of the storage period. As reported by previous authors, a high effluent production leads to nutrient loss (minerals, water soluble carbohydrates) from the solid fraction of the silage and is typically observed when ensiling biomass with a low initial dry matter content (Herrmann et al. 2015). Pre-treatments such as wilting (Cabrita et al. 2017) and screw-pressing (Gallagher et al. 2017) can increase the DW of seaweeds and reduce the effluents upon ensiling.

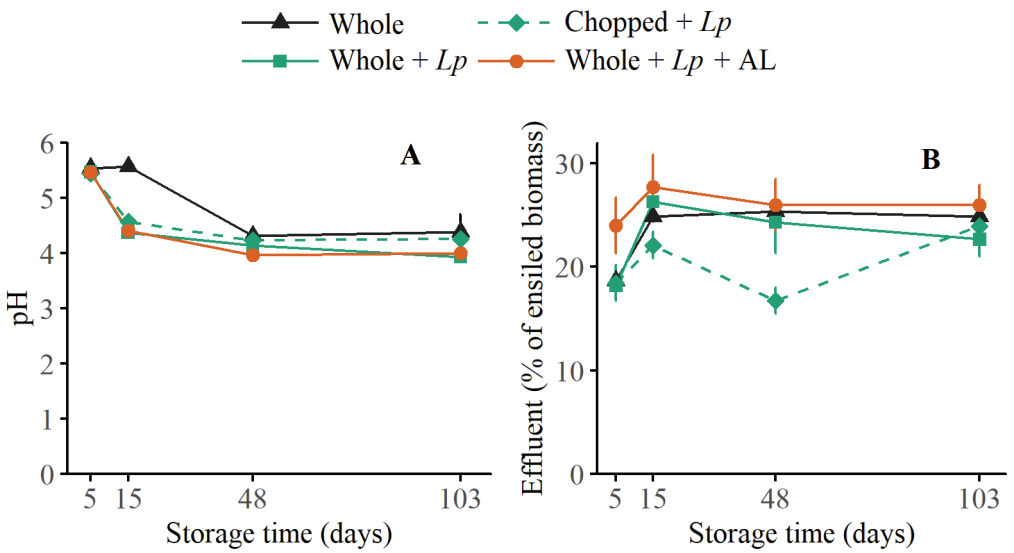


Fig. 15: Evolution of A) pH and B) effluent formation during ensiling of *S. latissima* under different conditions (**NPR 5**). Values are given as mean ± standard error (n = 3).

The evolution of the pH in all sample groups mirrored the increase in lactic acid (**fig. 16.A**). The initial glucose content was very low (< 1 % DW) and did not vary during the experiment suggesting the absence of laminaran in the samples. Instead, the sugar alcohol mannitol was the only carbon source for the fermentation and decreased during the experiment as lactic acid was produced (**fig. 16.B**). Butyric acid was not detected in any of the samples suggesting that clostridial growth and activity were inhibited during the process. The production of acetic acid was only detected in the untreated control samples (up to 1.4 % DW after 103 days). This observation may reflect the dominance of a population of heterofermentative bacteria during the ensiling process, initially present in the biomass, and leading to the production of both lactic and acetic acids, and possibly ethanol (not measured in these samples), as observed in earlier studies from the anaerobic fermentation of untreated *S. latissima* (Herrmann et al. 2015; Sandbakken et al. 2018). The sole production of lactic acid in other sample groups reflects the homofermentative activity of *Lp* added to the biomass. However, some molds were

observed at the surface of some sample replicates, independently of their group, which may be due to poor control over the anaerobic conditions.

The results from this preliminary study show that the chopping pre-treatment has no effect on the process of ensiling *S. latissima* (**table 9**). The inoculation of the biomass with *Lp* provides adequate acidification more rapidly than in untreated samples. However, the addition of AL did not result in an increased or more rapid acid production in the silage suggesting that the alginate matrix of *S. latissima* do not limit the substrate availability to the fermenting microbiota.

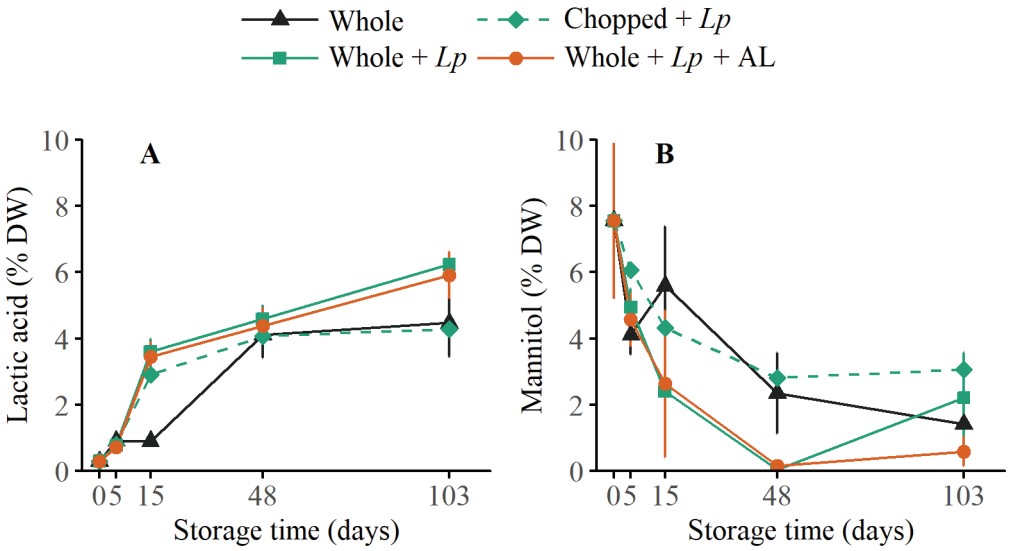


Fig. 16: Evolution of A) the lactic acid and B) mannitol contents during ensiling of *S. latissima* under different conditions (**NPR 5**). Values are given as mean ± standard error (n = 3).

The results from a simultaneous study conducted on *L. digitata* showed an increasing depolymerization of the alginates over time during ensiling without the initial addition of AL, and both with and without the addition of *Lp* (H. Marfaing, unpublished results), indicating the presence of inherent hydrolytic enzymes capable of degrading alginates to

oligomers. This supports the results from previous research activities in Norway reporting the severe depolymerization of alginates from the anaerobic fermentation of kelp species including *S. latissima* inoculated with a mixture of previously fermented seaweeds and cattle manure (Østgaard et al. 1993; Moen 1997). Oligoalginates are particularly interesting compounds in the context of human and animal nutrition, with reported prebiotic activities (O'Sullivan et al. 2010).

Ensiling is currently regarded as a promising low-cost alternative for the large-scale preservation of macroalgal biomass, although the process is yet to be optimized. LAB species of terrestrial origin such as *Lp* are, in some cases, efficient to produce marine silages (Uchida et al. 2004). Halotolerant strains of LAB and other microorganisms (e.g. yeasts) initially present on the biomass may be more adapted to the purpose of anaerobic fermentation of kelps. Future research focusing on the characterization and isolation of such microorganisms is envisaged. Moreover, technical adaptations of current ensiling systems are necessary to maintain anaerobic conditions and provide efficient solutions for the transport and storage of seaweed silage.

Fermentation may not be considered a relevant process in the context of using macroalgae in direct food applications due to the risk of contamination by spoilage microorganisms, but rather in industrial processes where bioactive ingredients are extracted and can be used as nutraceuticals. However, recent work from Uchida et al. (2014; 2017) opened the possibility for producing novel foods from the lactic fermentation of seaweeds. The benefits of fermented macroalgae in food applications (e.g. prebiotic) should be investigated in future studies.

6.6. Washing and blanching pre-treatments

Preservation processes of foods aim to retain or improve the inherent quality of the raw material. In some cases, physical and chemical pre-treatments are used before applying primary processing methods (e.g. drying, freezing, ensiling) as a mean to improve product quality and process efficiency.

A washing step is often necessary after harvesting seaweeds to remove impurities (sand, organic matter) and associated organisms which may be present on the biomass. Either seawater or fresh (tap) water are used for this purpose. The results obtained during this study show little or no effects of seawater soaking treatments on the quality of the kelps *A. esculenta* and *S. latissima* (**Paper I**), and *P. palmata* (**NPR 1**). Liot et al. (1993) reported a faster degradation of *P. palmata* and *Ulva* sp. washed with fresh water compared to seawater, prior to storage at 4 °C. In this case, the osmotic stress from fresh water exposure resulted in the reduction of the native microbial flora, promoting the settlement and spreading of spoilage microorganisms. In the present study, soaking treatments in fresh water at ambient temperature were tested as a mean to reduce the levels of potentially undesirable compounds, namely Cd and I, in *A. esculenta* and *S. latissima* respectively (**Paper IV**). This treatment failed to reduce the Cd and I levels of the samples while soluble compounds (minerals and mannitol) decreased rapidly. Treating edible seaweeds with fresh water prior to freezing may reduce drip losses after thawing by reducing the osmotic potential between intra- and extracellular spaces and limiting the damage to cell membranes upon crystal formation and growth. On the other hand, such treatments will affect the amount of fermentable sugars e.g. mannitol, and the microbiota associated with the raw material. A washing pre-treatment step with fresh water should therefore be avoided prior to ensiling.

Blanching refers to the heat treatment of foods, usually in boiling water or steam, to inactivate endogenous enzymes responsible for quality alterations such as oxidation of nutrients, changes in color and texture, and development of off-flavors. Blanching pre-treatments are commonly employed in the vegetable industry to fix the color and prepare the products for freezing and canning. This process can be applied to brown seaweeds to improve product palatability, including color and texture (Cox et al. 2011; Blikra et al. 2018) but it also reduces the polyphenol content, antioxidant capacity (Cox et al. 2011) and vitamin levels (Amorim-Carrilho et al. 2014). Like fresh water soaking treatments, blanching reduces the total soluble solid content and may improve the quality of frozen seaweeds by limiting drip losses. Previous studies showed that the I content of *S. latissima* is rapidly reduced upon exposure to boiling water (Lüning and Mortensen

2015; Nitschke and Stengel 2016). A soaking experiment conducted during this project on the same species showed that most of the I (nearly 90 %) is lost after exposure to fresh water at 32 °C for 1 h (**Paper IV**). The application of such treatments at moderate temperatures compared to boiling treatments entails lower energy requirements and is therefore advantageous in commercial production settings. Shorter treatment time may also result in acceptable I levels and should be tested in future studies.

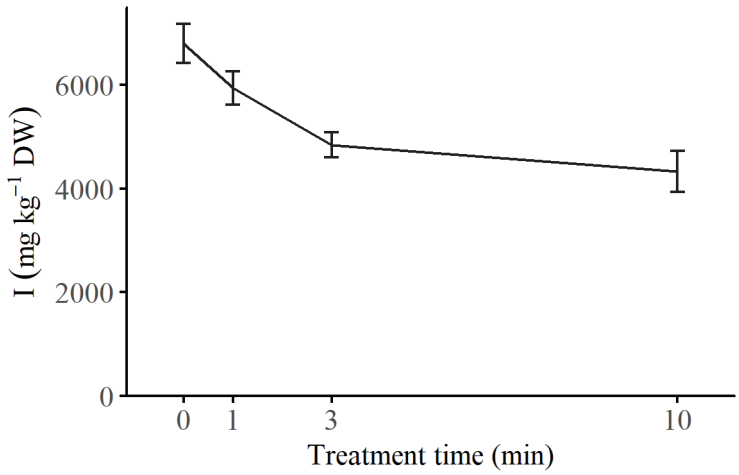


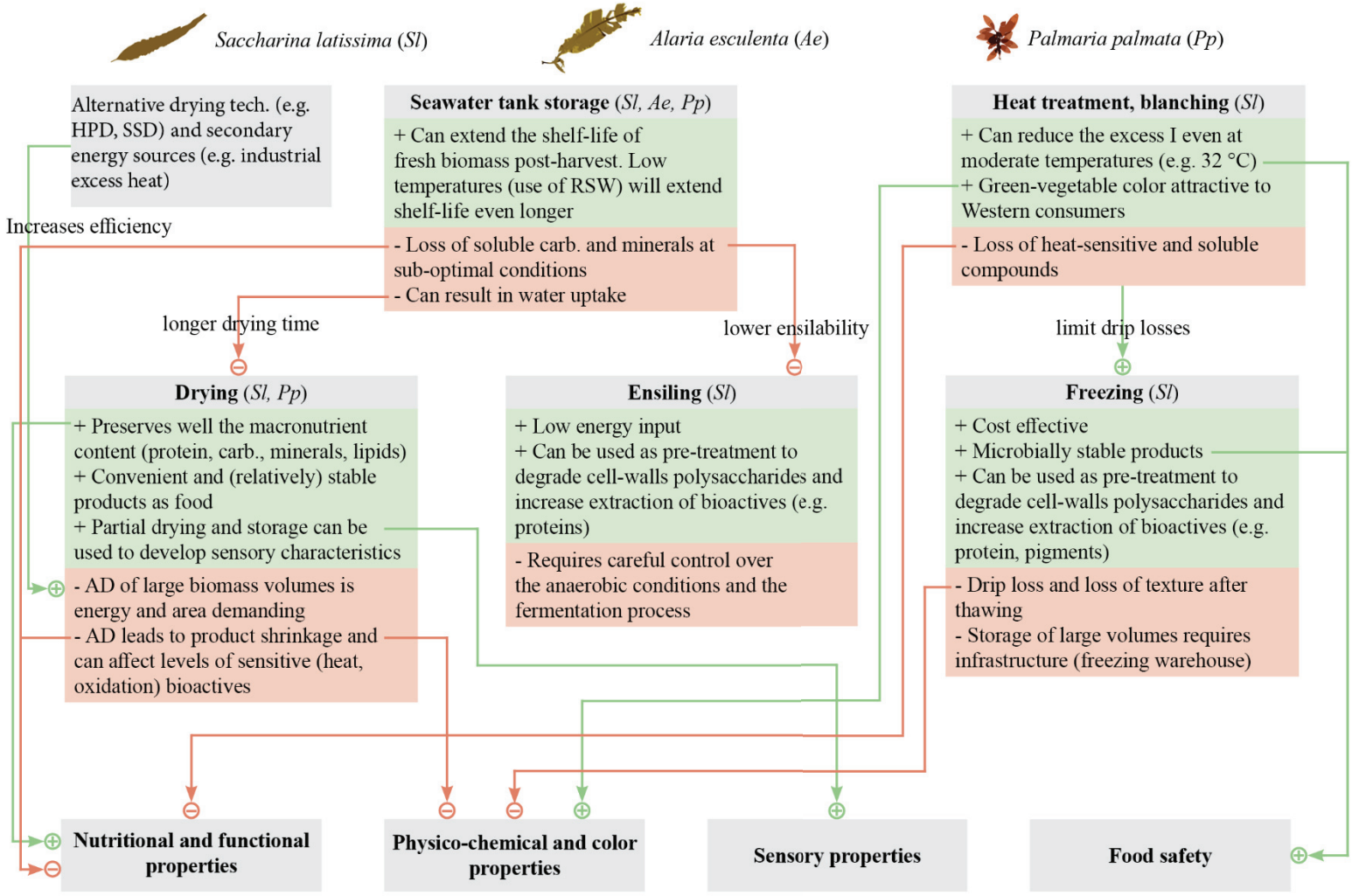
Fig. 17: Iodine content of *S. latissima* during steam blanching treatments (**NPR 6**)

Some of the detrimental effects of blanching seaweeds, i.e. loss of soluble compounds, may be avoided by using steam instead of hot fresh water. The effect of steam blanching on the I content of *S. latissima* was tested in this study (**NPR 6**). A significant reduction of the I was measured during the treatment (**fig. 17**, RM ANOVA, $p = 0.005$). However, the I level was not reduced to below the threshold value of 2000 mg kg^{-1} DW after 10 min as opposed to the soaking treatment in warm water tested in **Paper IV**. Longer steam exposure may reduce the I to below this limit. The chemical analysis of water-soluble compounds was not conducted in this preliminary experiment. However, the DW of the fresh steamed and control samples at t_0 was between 8.1 and 9.1 % and did not vary

significantly during the treatment (RM ANOVA, $p = 0.314$). This result suggests that the nutrient composition of these samples remained relatively stable, compared to the drastic reduction in DW and water-soluble substances measured in *S. latissima* samples following exposure to hot fresh water (**Paper IV**).

6.7. Summary of the results

The effects of different primary processing and storage methods on the food quality of *S. latissima*, *A. esculenta* and *P. palmata* have been investigated in this study. In the context of a commercial production, the combination of different treatments may be of advantage to optimize product quality or should be avoided in other cases. The suitability of a method not only depends on the quality of the final product but also on energy requirements and associated processing costs. These critical factors were therefore estimated. The results from this project are summarized by the schematic illustration presented on the next page. These findings will benefit the emerging seaweed industry in Norway and Europe and contribute to establishing efficient processing strategies to achieve optimal product quality from seaweeds to be used in food applications.


Saccharina latissima (Sl)
Alaria esculenta (Ae)
Palmaria palmata (Pp)
Alternative drying tech. (e.g. HPD, SSD) and secondary energy sources (e.g. industrial excess heat)
Increases efficiency
Seawater tank storage (Sl, Ae, Pp)
+ Can extend the shelf-life of fresh biomass post-harvest. Low temperatures (use of RSW) will extend shelf-life even longer
- Loss of soluble carb. and minerals at sub-optimal conditions
- Can result in water uptake
longer drying time
lower ensilability
Heat treatment, blanching (Sl)
+ Can reduce the excess I even at moderate temperatures (e.g. 32 °C)
+ Green-vegetable color attractive to Western consumers
- Loss of heat-sensitive and soluble compounds
limit drip losses
Drying (Sl, Pp)
+ Preserves well the macronutrient content (protein, carb., minerals, lipids)
+ Convenient and (relatively) stable products as food
+ Partial drying and storage can be used to develop sensory characteristics
- AD of large biomass volumes is energy and area demanding
- AD leads to product shrinkage and can affect levels of sensitive (heat, oxidation) bioactives
Ensiling (Sl)
+ Low energy input
+ Can be used as pre-treatment to degrade cell-walls polysaccharides and increase extraction of bioactives (e.g. proteins)
- Requires careful control over the anaerobic conditions and the fermentation process
Freezing (Sl)
+ Cost effective
+ Microbially stable products
+ Can be used as pre-treatment to degrade cell-walls polysaccharides and increase extraction of bioactives (e.g. protein, pigments)
- Drip loss and loss of texture after thawing
- Storage of large volumes requires infrastructure (freezing warehouse)
Nutritional and functional properties
Physico-chemical and color properties
Sensory properties
Food safety

7. Conclusions and future work

Macroalgae are a rich source of nutritional and bioactive compounds and have technological and sensory properties that can be used in the food industry and in culinary innovations. This doctoral work contributed to advancing knowledge related to primary processes and storage of three major edible seaweed species of commercial interest in Europe (*S. latissima*, *A. esculenta* and *P. palmata*) and their effects on product quality.

1. Seawater storage – The shelf-life of freshly harvested seaweeds can be prolonged by storage in seawater tanks with only moderate changes in the nutrient content of the biomass. The temperature employed during the process will determine the shelf-life of the biomass. Chilling storage using RSW technology operating at temperatures close to 0 °C could be applied to the commercial production of kelps at sea where large volumes of biomass are typically harvested in a short period, prior to further processing or to supply fresh raw material of high quality. The effect of RSW storage on the biomass quality and the practical advantages of using this method are in focus in a current project.

2. Drying methods – The quality of *S. latissima* and *P. palmata* air-dried at different temperatures (25, 40 and 70 °C) were compared to that of freeze-dried samples. While no differences were detected among samples regarding their levels of macronutrients (proteins, minerals, carbohydrates, lipids) and sensory characteristics (air-dried samples of *S. latissima* at 25 and 70 °C were compared), AD especially at high temperatures affected the physico-chemical properties of the material (WBC, OBC, SC) due to product shrinkage. The amount of soluble proteins extracted from these samples was also reduced compared to freeze-dried samples. Besides, conventional AD methods are energy-intensive, lowering the profitability and sustainability of the value-chain and limiting their use to process large amounts of biomass. Alternative drying technologies such as SSD and HPD have the potential to improve process efficiency and product quality and are under current investigation.

3. Freezing and thawing – Considerable drip loss (over 40 % of the fresh biomass) was measured in the samples following freezing using impingement technology, frozen storage and subsequent thawing. This severely compromised the quality of the product to be used in direct food applications (sea vegetables). No differences in product quality were detected between rapid and slow thawing. Pre-treatment such as blanching and steam exposure may remediate this problem. On the other hand, increasing drip loss by freezing may be used advantageously as a pre-treatment for further biomass fractionation and the subsequent recovery of nutritional and bioactive substances from seaweeds. Ongoing research activities from the author are investigating the optimization of both freezing and pre-treatment processes.

4. Ensiling – This method has the potential to stabilize high amounts of kelp biomass with a low energy input. However, the high-water content of the biomass and high BC represents a challenge to produce stable silage under anaerobic conditions. Neither a chopping pre-treatment of the fresh biomass nor the initial addition of alginate degrading enzyme (AL) affected the ensiling process of *S. latissima*. Adequate acidification was achieved both in samples inoculated with LAB and in control samples receiving no inoculum, although the decreased in pH was delayed in the control group. This highlights the ability of microorganisms naturally present on the biomass to convert fermentable sugars and provide stable conditions for the conservation of kelps. The characterization and isolation of these microorganisms as well as the practical implementation of ensiling kelp at a commercial scale should be investigated in future studies.

5. Sensory properties of seaweeds – The sensory quality is a major factor determining the consumer acceptance of a new product. The changes in sensory properties of *S. latissima* and *P. palmata* during storage under controlled conditions were described in this study. In both species, increasing the moisture content of the dried samples from approximately 5 to 20 %, faded the characteristic marine flavors and aromas and developed more complex flavors during storage. These results are comparable to the changes occurring during the maturation of *konbu* in Japan. The FAA analysis in *S. latissima* samples suggested that endogenous proteolysis, among other reactions, are

involved in the flavor development of the samples. The results from ongoing analyses of flavor-active compounds in *P. palmata* samples may give further evidences of the chemical and enzymatic reactions occurring during the process. This work provides a basis to further investigate the flavor development of edible seaweeds and to optimize biomass processing and storage methods. Producing flavor-rich and attractive ingredients from seaweeds will increase the consumer acceptance of these products.

6. Food safety – Potentially undesirable compounds, primarily I in excessive amount in *S. latissima*, were reported in this study. Health risks associated with the consumption of this species were estimated based on measured I levels and established tolerable intake levels. While the daily consumption of *S. latissima* leads to excessive I intakes and potentially negative health effects, a moderate consumption of seaweeds (not only high I-containing kelps) can improve the I status in generally I-deficient populations in Europe. Alternatively, simple processing methods based on heat treatments (soaking, steam exposure) can effectively reduce the I content of *S. latissima*. The monitoring of potentially undesirable compounds in edible and commercialized seaweeds along with further investigation of the potentially negative effects related to their consumption is essential to ensure consumer safety.

Conclusion and general views – Seaweeds represent highly nutritious seafood products with the potential for becoming more central elements in Western diets than currently realized. In the context of using seaweeds in commercial applications, the choice of a process will be driven by the nature of the final product (bulk, high value), the associated costs, the biomass quantity involved and logistic considerations. The use of seaweeds in food applications is expected to create value and support the development of a growing seaweed industry in Norway and Europe. The provision of affordable, high quality and safe products from edible seaweed species to the consumer and the food industry is a key to sustain this development. Nevertheless, expert knowledge regarding markets and prices for different seaweed products is required to establish market strategies. Successful product developments along with efficient logistic solutions throughout the entire value-chain will build competitiveness of the seaweed sector.

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9. Supplementary material

Suppl. material 1: Chemical composition of *P. palmata* samples (in g (100 g)$^{-1}$ DW unless stated otherwise) prior to (t0) and after 22-h storage in seawater tanks (**NPR 1**). Values are given as mean ± standard error (n = 3).

	t_0	**t = 22 h**
Dry weight (%)	12.4 ± 0.5	10.6 ± 0.5
Minerals		
Ash	35.0 ± 1.4	36.6 ± 1.2
Na	2.60 ± 0.35	3.48 ± 0.01
K	10.6 ± 0.1	12.0 ± 0.5
Na/K	0.25 ± 0.03	0.29 ± 0.01
Carbohydrates		
Total carbohydrates	24.6 ± 0.8	24.8 ± 0.3
Xylose	19.1 ± 0.9	19.6 ± 0.3
Galactose	5.0 ± 0.2	4.7 ± 0.1
Glucose	0.60 ± 0.05	0.54 ± 0.02
Proteins		
Total protein	17.9 ± 0.4	16.8 ± 0.5
R-Phycoerythrin (mg g^{-1} DW)	2.2 ± 0.4	b.d

b.d.: below detection.

Suppl. material 2: Chemical composition of *P. palmata* air-dried at 40 and 70 °C and freeze-dried (FD). Values are given as mean ± standard error (n = 3). Different superscript letters indicate significant differences among drying treatments ($p < 0.05$) (**NPR 2**).

	40 ˚C	**70 ˚C**	**FD**
Residual moisture (%)	4.9 ± 0.3 [b]	3.2 ± 0.3 [c]	7.0 ± 0.5 [a]
Ash [1]	50.6 ± 0.7 [a]	39.1 ± 3.5 [b]	45.1 ± 0.8 [ab]
Soluble carbohydrates [2]	23.3 ± 4.5 [a]	37.1 ± 2.4 [a]	23.0 ± 6.6 [a]
Lipids [1]	2.3 ± 0.1 [a]	2.9 ± 0.2 [a]	2.4 ± 0.1 [a]
Soluble proteins [2]	9.7 ± 0.6 [ab]	5.3 ± 0.2 [b]	15.8 ± 3.1 [a]
R-PE [2]	4.0 ± 0.2 [b]	0.2 ± 0.0 [c]	7.5 ± 1.2 [a]

[1] in g (100 g)$^{-1}$ DW

[2] in mg g^{-1} DW

Suppl. material 3: Physico-chemical properties of *P. palmata* samples following oven-drying at 40, 70˚C and freeze-drying (FD). WBC and OBC are expressed in g water and g oil per g dried sample respectively, and SC is expressed in ml per g dried sample. Values are given as mean ± standard error (n = 3). Different superscript letters indicate significant differences among drying treatments ($p < 0.05$) (**NPR 2**).

	40 ˚C	**70 ˚C**	**FD**
WBC	6.0 ± 0.1 ^{b}	4.6 ± 0.1 ^{c}	7.1 ± 0.3 ^{a}
OBC	3.6 ± 0.1 ^{b}	2.4 ± 0.2 ^{b}	5.0 ± 0.5 ^{a}
SC	5.8 ± 0.4 ^{a}	4.0 ± 0.2 ^{b}	6.7 ± 0.3 ^{a}

Suppl. material 4: Results (F-test from the fixed effects only) from a 2-way mixed model ANOVA for individual sensory attributes from the descriptive analysis of *S. latissima* samples dried at low (25 °C) and high (70 °C) temperatures and stored under different moisture contents (2 levels: dried, semi-dried) (**NPR 3**).

	Temperature	**Storage**	**Temp. : Storage**
Aroma			
Fresh sea	0.15	**52.21** ***	0.90
Fermented	0.86	**37.82** ***	**4.67** *
Hay	1.80	**70.79** ***	0.01
Flavors			
Salty	0.03	0.03	0.01
Fresh sea	1.10	**52.66** ***	1.39
Fermented	0.43	**18.01** ***	2.09
Hay	0.01	**45.52** ***	0.53
Umami	0.84	1.52	0.35
Bitter	0.17	0.25	3.09
Texture			
Crispy	0.24	**65.45** ***	0.09
Chewy	0.10	**36.89** ***	0.34
Viscous	0.00	1.44	0.60
Dissolves	1.81	**80.88** ***	**6.02** *

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Paper I

Paper II

Paper III

This paper is awaiting publication and is not included in NTNU Open

Paper IV

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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

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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} dry weight (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 nutrient content of the biomass, illustrated by considerable variations in DW and the content 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.

Keywords Bioactive compounds · Cadmium · Edible seaweeds · Iodine · Inorganic arsenic · Processing

Introduction

Seaweeds are a major element of the human diet in Asian countries where a variety of species have long been recognised for their nutritional value as well as for their rich and unique flavours (Nisizawa et al. 1987; Mouritsen 2013, 2017). 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 (Rupérez and Saura-Calixto 2001; Dawczynski et al. 2007a), minerals, vitamins and trace elements in most relevant species (Rupérez 2002; MacArtain et al. 2007; Holdt and Kraan 2011; Cabrita et al. 2016, Wells et al. 2017). 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 seaweed, 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.

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2005; Maeda et al. 2008). The abundance of bioactive substances in many species makes seaweeds an attractive raw material with multiple applications in pharmaceutical, food and cosmetic industries.

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 nutrient content (Kraan et al. 2000; Handå et al. 2013) and 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 (iAS, Almela et al. 2006; Rose et al. 2007; 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 commercialised in Europe. However, 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 with seaweed consumption. iAs is a known human carcinogen associated with liver, bladder, lung and skin cancers (Hughes 2002). Excessive I intakes are known to affect thyroid function, particularly in susceptible individuals, potentially resulting in hypo- or hyperthyroidism (Leung and Braverman 2014). At present, the EU has not established specific regulations 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 (Zava and Zava 2011; Lüning and Mortensen 2015; Nitschke and Stengel 2016). However, the nutrient content 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, it 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 elements (i.e. Cd, iAs and I) in cultivated *A. esculenta* and *S. latissima* and investigate soaking treatments as simple methods to reduce the levels of these elements in the biomass. Fresh water treatments at different temperatures were tested on *S. latissima*, in which high I contents are expected (Teas et al. 2004; Lüning and Mortensen 2015). Because relatively high Cd levels were reported in *A. esculenta* (Mæhre et al. 2014), the effect of soaking treatments at different salinities was investigated in this particular species, as a potential process to selectively reduce Cd levels in kelps. The content of nutritional substances in the samples, including mineral fraction, carbohydrates, proteins, polyphenols and fucoxanthin, was also analysed prior to and after treatments. The surface colour of algal thalli was measured throughout treatments in order to monitor changes in the products' general appearance. Finally, the levels of Cd, iAs and I in both edible kelps are compared to established tolerable intake levels to provide information regarding potential health risks associated with their consumption.

Materials and methods

Fresh water soaking of *Alaria esculenta* and *Saccharina latissima*

Mature adult thalli of *A. esculenta* and *S. latissima* were harvested from CEVA's cultivation site (lat, 48.836362 N, long, −3.044157 W) at Pleubian, off the northern coast of Brittany in France on May 27 and 28 and June 4, 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 tap water (WW, 32 °C). Simultaneously, equivalent treatments with seawater at ambient temperature (SW, 18 °C) filtered at 10 μm, 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, 2, 6 and 22 h soaking treatments.

Hypersaline treatments of *A. esculenta*

Biomass of *A. esculenta* was harvested on May 16, 2016 from the same location and handled the same way as described in the previous protocol. In order to minimise the need for seaweed biomass and salt quantity, these treatments were conducted on a smaller scale than the fresh water soaking experiment. Batches of 1 kg of biomass were transferred to containers filled with 20 L of NaCl solutions of different concentrations: 1.0, 2.0 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.

Analysis of potentially toxic elements

Cadmium (Cd) content was analyzed following a standard reference method (AOAC 2000) in which 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 was analyzed following a standard method for the determination of I compounds in foodstuffs (BS EN 15111:2007 2017). Samples were digested in a laboratory microwave with tetramethylammonium hydroxide (TMAH). After removing undissolved compounds, the nebulised sample was atomised and ionised 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) compounds were extracted from the samples using diluted hydrochloric acid (HCl) following a standard procedure (BS EN 15517:2008 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).

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 of seaweed (DW) and (ii) a maximum consumption in a single serving corresponding to 12.5 g of 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).

Analysis of the nutritional content

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

Sodium and potassium analysis Sodium (Na) and potassium (K) contents were analyzed following an official reference method (AOAC 2000) in which samples were combusted overnight in a muffle furnace. The ashes were solubilised 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-glucuronic, D-mannuronic, poly-D-guluronic and poly-D-mannuronic) composition were determined by high-performance liquid chromatography (HPLC) analysis after depolymerisation under methanol acid hydrolysis reaction (methanolysis) as described by Quemener et al. (2000). Ground freeze-dried seaweed samples of 15 mg were transferred into 2 mL 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 neutralisation was achieved by adding silver carbonate (successively 100 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 (GraceSmart 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 the CEVA 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 sample 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 colourimetrically 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 of distilled water and 100 μL of 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 (BioTek Instruments, USA), with phloroglucinol used as the standard reference (Sigma–Aldrich, 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 60/40 ethanol/water solvent for 2 h in ice bath protected from light (1% *w*/*v* 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 × 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.

Surface colour analysis

The surface colour of seaweed samples was analyzed by a computerised 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 colour 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 colour difference (ΔE) using Eq. (1), where L^*_0, a^*_0 and b^*_0 are colour coordinates of the samples before treatment.

$$\Delta E = \sqrt{\left(L^* - L_0^*\right)^2 + \left(a^* - a_0^*\right)^2 + \left(b^* - b_0^*\right)^2} \quad (1)$$

Statistical analysis

All statistical analyses were performed on R (version 3.4.1, R Development Core Team 2017) including functions from nlme (Pinheiro et al. 2017) and multcomp packages (Hothorn et al. 2008). Raw data were pre-processed for descriptive statistics and the results expressed as mean ± standard error ($n = 3$). For each species, differences among treatments (between-subject variable) over time (within-subject variable) in the nutrient content of the samples and their levels of potentially toxic elements were analyzed by repeated measures analysis of variance (RM ANOVA, R function lme) at $p < 0.05$. A Tukey's honest significant difference (HSD) test (R function glht) was used for post hoc comparisons of significant results from RM ANOVA.

Results

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 tenfold 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 1-week interval in 2015 (end of May, beginning of June). *Alaria esculenta* and *S. latissima* exceeded the recommended French limits for Cd and I content approximately by factors 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

	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

Values are given as mean ± standard error ($n = 3$)

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

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 DW losses measured in both species following soaking in fresh water (i.e. FW and WW) were significantly greater than those observed in control groups (SW, RM ANOVA, $p < 0.05$). 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. 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.

An increase in the relative Cd content of *A. esculenta* samples was observed during FW soaking treatment (Fig. 2) from 2.0 ± 0.1 to 2.9 ± 0.2 mg kg^{-1} DW while levels remained stable in SW. However, the overall difference in Cd levels among FW- and SW-treated samples over time was not detected significant (RM ANOVA; interaction effect time × treatment, $p = 0.06$). 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).

Table 2 summarises variations in the relative composition in bioactive compounds in both kelps, after 22 h of soaking treatments, as well as changes in surface colour (ΔE). Both fresh water soaking treatments (FW and WW) resulted in lower mineral and higher total 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

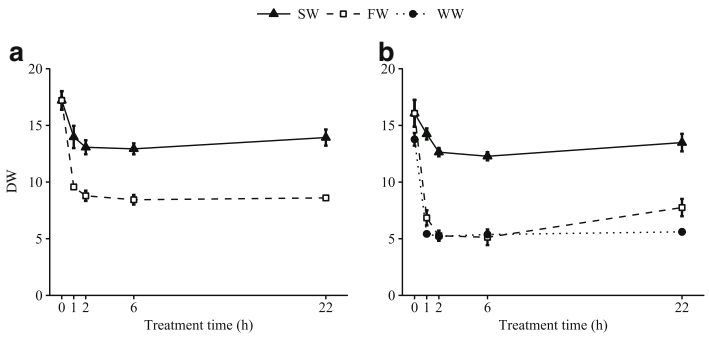

Fig. 1 Variations in dry weight of *A. esculenta* (**a**) and *S. latissima* (**b**) during soaking treatments in seawater (SW), fresh water (FW) and warm fresh water (WW). Values are given as mean ± standard error ($n = 3$)

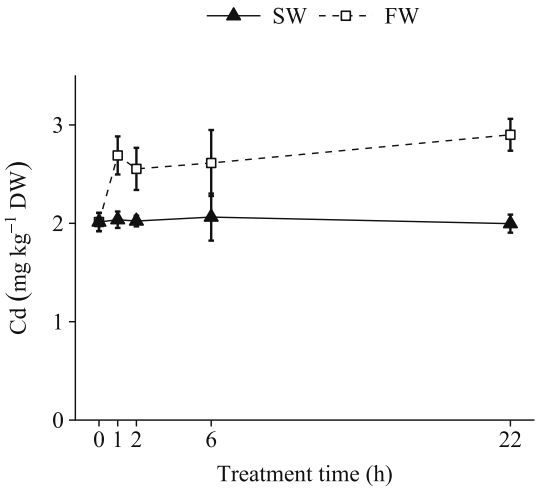

Fig. 2 Variations in Cd content in *A. esculenta* during soaking treatments in seawater (SW) and fresh water (FW). Values are given as mean ± standard error ($n = 3$)

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 of soaking in FW, although not statistically significant, while the levels of this pigment decreased in *S. latissima* following FW and WW treatments.

The total colour variation (ΔE) of algal thalli was recorded in order to monitor changes in appearance throughout treatments. The ΔE parameter reflects the variation in each of the three chromatic coordinates (L^*, a^* and b^*) during treatment

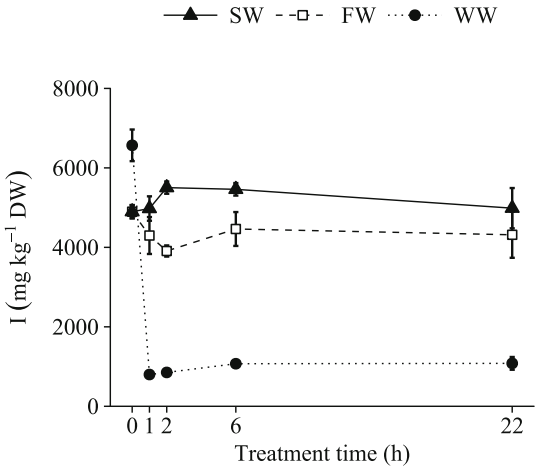

Fig. 3 Variations in I content of *S. latissima* during soaking treatments in seawater (SW), fresh water (FW) and hot fresh water (WW). Values are given as mean ± standard error ($n = 3$)

as compared to initial values measured at t_0. In both *A. esculenta* and *S. latissima*, relatively similar and moderate colour variations were observed between SW- and FW-treated samples after 22 h of soaking. Large variations in surface colour 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).

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 (not significant) along with significant increase in minerals (Na and ash, Table 3) in each group, upon 2 h of soaking treatments. A steady reduction of Cd was observed during soaking in 2.0 M NaCl (Fig. 4) resulting in significantly lower levels compared to initial values (Table 3), 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 three times the initial level measured in the samples. Large variations in surface colour were observed from samples soaked in hypersaline solutions as compared to the control treatment at 0.5 M NaCl.

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 of 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

Table 2 Chemical composition of the seaweed biomass prior to (t_0), and after 22 h of soaking treatments as well as variation in the surface colour of the samples (ΔE). Concentrations are expressed in g (100 g)$^{-1}$ DW, except for the fucoxanthin content expressed in mg kg^{-1} DW and the dimensionless ΔE

	A. esculenta (May 2015)			*S. latissima* (May 2015)			*S. latissima* (June 2015)	
	t_0 [1]	SW[1] $t = 22$ h	FW $t = 22$ h	t_0 [1]	SW[1] $t = 22$ h	FW $t = 22$ h	t_0	WW $t = 22$ h
Dry weight (%)	17.2 ± 0.8 ^{c}	13.9 ± 0.7 ^{b}	8.6 ± 0.1 ^{a}	16.1 ± 1.2 ^{c}	13.5 ± 0.8 ^{bc}	7.7 ± 0.8 ^{a}	13.8 ± 0.6 ^{bc}	5.6 ± 0.2 ^{a}
Minerals								
Ash	24.2 ± 1.4 ^{ac}	27.0 ± 1.6 ^{bc}	13.4 ± 0.8 ^{a}	26.2 ± 2.6 ^{b}	30.0 ± 2.1 ^{b}	16.7 ± 0.2 ^{a}	30.5 ± 1.1 ^{b}	15.5 ± 0.2 ^{a}
Na	3.92 ± 0.23 ^{b}	5.21 ± 0.20 ^{a}	0.93 ± 0.03 ^{c}	3.6 ± 0.2 ^{b}	4.3 ± 0.2 ^{c}	1.2 ± 0.1 ^{a}	3.85 ± 0.04 ^{bc}	0.97 ± 0.02 ^{a}
K	4.2 ± 0.3 ^{b}	4.4 ± 0.5 ^{b}	2.1 ± 0.3 ^{a}	6.5 ± 1.1 ^{b}	7.2 ± 0.8 ^{b}	2.7 ± 0.1 ^{a}	8.17 ± 0.42 ^{b}	1.86 ± 0.03 ^{a}
Carbohydrates								
Total carbohydrates	40.7 ± 1.5 ^{ab}	37.7 ± 1.5 ^{a}	45.5 ± 1.5 ^{b}	46.1 ± 2.63 ^{ac}	40.0 ± 1.0 ^{ab}	49.6 ± 2.1 ^{c}	39.6 ± 1.0 ^{a}	47.1 ± 1.8 ^{bc}
Alginate	19.9 ± 0.5 ^{a}	18.6 ± 0.4 ^{a}	26.7 ± 0.9 ^{b}	21.5 ± 0.5 ^{a}	23.1 ± 1.5 ^{a}	38.2 ± 0.8 ^{b}	21.0 ± 0.5 ^{a}	41.1 ± 0.9 ^{b}
Mannitol	10.5 ± 0.4 ^{b}	10.4 ± 0.3 ^{b}	6.6 ± 0.7 ^{a}	17.6 ± 1.2 ^{b}	12.3 ± 2.2 ^{b}	3.1 ± 0.8 ^{a}	14.8 ± 1.1 ^{b}	n.d.
Glucose	8.5 ± 1.9 ^{a}	7.5 ± 1.4 ^{a}	10.1 ± 1.8 ^{a}	5.0 ± 2.0 ^{a}	2.8 ± 0.6 ^{a}	4.3 ± 0.4 ^{a}	1.9 ± 0.4 ^{a}	1.3 ± 0.1 ^{a}
Fucose	1.25 ± 0.03 ^{a}	0.98 ± 0.04 ^{a}	1.76 ± 0.14 ^{b}	0.76 ± 0.03 ^{a}	0.89 ± 0.07 ^{a}	1.50 ± 0.00 ^{b}	0.89 ± 0.07 ^{a}	1.75 ± 0.03 ^{c}
Proteins	10.5 ± 0.2 ^{a}	9.9 ± 0.1 ^{a}	12.7 ± 0.2 ^{b}	10.6 ± 0.3 ^{a}	11.6 ± 0.2 ^{a}	12.7 ± 0.7 ^{bc}	11.3 ± 0.3 ^{ab}	12.6 ± 0.2 ^{c}
Polyphenols	3.43 ± 0.08 ^{bc}	2.55 ± 0.09 ^{a}	2.93 ± 0.29 ^{ab}	0.69 ± 0.04 ^{c}	0.49 ± 0.04 ^{b}	0.22 ± 0.01 ^{a}	0.44 ± 0.02 ^{b}	0.26 ± 0.01 ^{a}
Fucoxanthin	871 ± 53 ^{a}	829 ± 45 ^{a}	1052 ± 114 ^{a}	431 ± 19 ^{bc}	360 ± 27 ^{b}	343 ± 24 ^{b}	526 ± 27 ^{c}	201 ± 34 ^{a}
ΔE	–	11 ± 3	8 ± 2	–	6 ± 2	7 ± 1	–	27 ± 1

Values are given as mean ± standard error ($n = 3$). For each species, different superscript letters in the same row indicate significant differences (RM ANOVA, Tukey HSD, $p < 0.05$)

n.d. non detected

[1] Data published in Stévant et al. (2017)

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 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 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 3 Total variation in dry weight, Cd, ash, Na and surface colour (ΔE) analyzed after 2 h of soaking treatments in 0.5, 1.0 and 2.0 M NaCl

	A. esculenta (May 2016)			
	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 ^{ab}	10.9 ± 0.1 ^{ab}	14.4 ± 0.2 ^{ab}	15.4 ± 0.2 ^{b}
Cd (mg kg^{-1} DW)	1.55 ± 0.20 ^{b}	1.46 ± 0.05 ^{ab}	1.48 ± 0.07 ^{ab}	0.92 ± 0.02 ^{a}
Ash (g (100 g)$^{-1}$ DW)	29.3 ± 1.7 ^{a}	35.0 ± 1.0 ^{ab}	39.9 ± 0.5 ^{bc}	45.1 ± 0.7 ^{c}
Na (g (100 g)$^{-1}$ DW)	4.69 ± 0.18 ^{a}	6.36 ± 0.01 ^{ab}	9.44 ± 0.05 ^{bc}	12.72 ± 0.13 ^{c}
ΔE	–	9 ± 3	18 ± 2	19 ± 9

Values are given as mean ± standard error ($n = 3$). Different superscript letters in the same row indicate significant differences (RM ANOVA, Tukey HSD, $p < 0.05$)

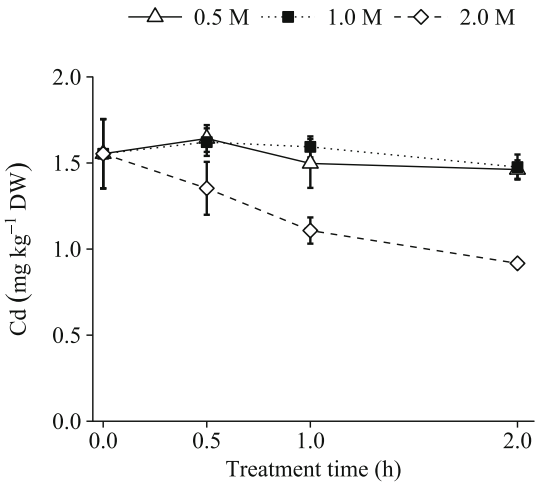

Fig. 4 Variations in Cd content in *A. esculenta* during soaking treatments in 0.5, 1.0 and 2.0 M NaCl solutions. Values are given as mean ± standard error ($n = 3$)

Discussion

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

Despite a number of studies reporting on seaweeds' bioactive compounds and their associated nutritional benefits (MacArtain et al. 2007; Holdt and Kraan 2011; Déléris et al. 2016; Wells et al. 2017), relatively high levels of potentially undesirable elements, 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). *Alaria 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.

Alaria esculenta accumulated approximately 10 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 by Davis et al. (2003) emphasises 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 (100 g)$^{-1}$ DW alginate and 1.25, 0.76 g (100 g)$^{-1}$ 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

Table 4 Daily dose of potentially toxic elements 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)

[c] From $BMDL_{01}$ (lower bound, EFSA CONTAM Panel 2009)

cancer as a result of environmental and occupational exposure (Järup 2002). However, none of these clinical manifestations has been reported related to seaweed consumption.

High concentrations of I were found in *S. latissima*, more than 30 times higher than in *A. esculenta*. These levels reflect results reported in previous studies showing kelp species, including *S. latissima*, to be among 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 although large intraspecific variations (420 to 4000 mg I kg^{-1}) are reported depending on the origin of the biomass and growth conditions (Lüning and Mortensen 2015). 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 classes, 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 (Teas et al. 2004; Mæhre et al. 2014).

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 alterations, 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 treatments of both species was also likely due to the diffusion of minerals out of the thalli. 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 2 h of bath treatment in 1.0 M NaCl. Although no effect on the Cd content after treatment at this concentration was achieved in the present 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 (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 (100 g)$^{-1}$ 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 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 fresh water was reported to greatly reduce the I level of *S. latissima* (by 70%, Lüning and Mortensen 2015) and other edible seaweed species (Nitschke and Stengel 2016). A review by Zava and Zava (2011) also reports on cooking processes to remove I from konbu (*Saccharina japonica*). 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 substances measured in this study, i.e. minerals, mannitol and polyphenols, and severely compromised the nutrient content of the biomass. 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 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. These changes (i.e. lighter and greener colour) can be attributed to the leaching of fucoxanthin leading to the greening of the material as the chlorophylls become more exposed. Similar colour changes following blanching of *H. elongata* were observed by Cox et al. (2011) who considered the final product to be visually more attractive.

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-kg adult. 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 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 one to two 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 of *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; Crawford et al. 2010; Zava and Zava 2011; Leung and Braverman 2014). Several studies reported increase of serum levels of thyroid-stimulating hormone (TSH) following a long-term daily ingestion of konbu (*S. japonica*) or konbu 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 two billion people are estimated to have inadequate I intakes even though salt iodisation 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; Rose et al. 2007; Besada et al. 2009). 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 on 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 95th 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 (from consuming *S. latissima* and *A. esculenta*, respectively), corresponding to 0.01 μg iAs per kg body weight per day for a 70-kg person, from both kelp species, this would correspond to a 7% increased exposure relatively to the lower limit 95th percentile dietary exposure of 0.14 μg day^{-1}. The intake from kelp would hence add to the current 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 kelp species should not be considered as a potential source of increased exposure to iAs.

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 (Holdt and Kraan 2011; Wells et al. 2017). However, these edible kelps also contained either Cd or 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 of dried kelp, as used in previous studies (Phaneuf et al. 1999; Desideri et al. 2016) appears rather unrealistic in the perspective of a broader consumption in Europe. Although seaweeds receive increasing consumer acceptance, they are 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 kelps. 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' nutrient content. Although a reduced nutrient content 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 substances (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 commercialised 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.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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