Norwegian University of
Science and Technology

Variation of Iodine Content in Processed Cultivated *Saccharina latissima*

Alkaline Extraction and Quantification by ICP-
MS

Nicoline Korsvold

Chemical Engineering and Biotechnology
Submission date: June 2017
Supervisor: Turid Rustad, IBT

Norwegian University of Science and Technology
Department of Biotechnology and Food Science

Preface

This Master's thesis concludes five rewarding years studying Industrial Chemistry and Biotechnology at NTNU in Trondheim. It was written at the Department of Biotechnology and Food Science between January and June, 2017. Seaweed Energy Solutions provided the macroalgal material from their cultivation site on Frøya. My supervisor was Turid Rustad at NTNU, alongside my co-supervisor Maren Sæther at Seaweed Energy Solutions.

The project was undertaken at the request of Seaweed Energy Solutions, whom I approached with my interest for utilising macroalgae. My research question was formulated together with Maren Sæther and Turid Rustad.

I would like to thank Turid for the scientific and personal guidance during this process. Her faster-than-light emailing response time and supporting wisdom has made me certain that I was one of the lucky ones. I also wish to thank Maren and SES for their enthusiasm and a beautiful seaweed harvest day. Syverin Lierhagen at the Department of Chemistry has been vital to conduct the ICP-MS analyses, and I owe him thanks for providing time and help beyond what was required. Thank you to Trude Johansen and Siri Stavrum for helping me with laboratory equipment and methodology; you have made the work atmosphere enjoyable. The last-minute analyses had not been possible without the encouragement from Georg Kopplin - thank you. And finally, I wish to thank my mum for always believing in me and dragging me out of a writer's block.

I hope you enjoy your reading.

Nicoline Korsvold

Trondheim, June 2017

Abstract

Norway is an established seafood nation, and with the shift to a green economy, macroalgae present themselves as an exciting contributor. Concern has been expressed about the high accumulation of iodine by some brown macroalgal species.

The objective of this study was to develop a process for extracting and quantifying iodine content in rope cultured *Saccharina latissima* after air-drying, freeze-drying, rehydration and boiling. The samples were provided by Seaweed Energy Solutions from their cultivation site on Frøya.

Three methods were used to digest the biomass and extract iodine from the processed macroalgae: A microwave-assisted alkaline extraction, an oven alkaline extraction, and a nitric acid digestion. The digestion solutions were analysed by ICP-MS. The formation of volatile iodine species at acidic pH makes nitric acid digestion an unreliable method. The oven alkaline extraction was believed to give the most accurate results with the equipment available. Microwave-assisted alkaline extraction is faster, but has a higher demand for advanced laboratory equipment. According to the oven alkaline extraction, the total iodine content of *S. latissima* harvested in May 2016 and April 2017 was $3802 \pm 123\,\mathrm{mg\,kg^{-1}}$ and $2930 \pm 168\,\mathrm{mg\,kg^{-1}}$ on a dry weight basis, respectively.

The goal of preservation and cooking processes is to minimise the loss of beneficial compounds. Macroalgae are rich in polyphenols, which are potent antioxidants. Folin-Ciocalteu's total phenols method was used on acetonic extracts of freeze-dried, rehydrated and boiled macroalgal samples to assess any unwanted loss. Important parameters for the reliability and extraction efficiency were found to be accurate dry weight measurements, incubation time with Folin-Ciocalteu's reagent and storage time starting from sampling of processed macroalgae until spectrophotometric analyses. A standard was prepared with propyl gallate, a synthetic antioxidant with high antioxidant capacity. The phenolic content was found for freeze-dried, rehydrated and boiled *S. latissima* harvested in May 2016 to be 0.070 - 0.10 mg PGE/g DW. The values were not significantly different (ANOVA, $p < 0.05$).

The remaining water from rehydration and boiling dried *S. latissima* was evaluated for the extraction potential of fucoidans as a part of a biorefinery process. Fucoidans are a structurally diverse class of polysaccharides that are challenging to quantify directly. Instead, the remaining water was acid hydrolysed to release L-fucose. The boiling water had the highest content at $15.4 \pm 0.2\,\mathrm{mg\,L^{-1}}$ (5 g macroalgae per 500 mL deionised water), or $1.56 \pm 0.02\,\mathrm{mg\,g^{-1}}$ on a dry weight basis. The fucose concentration in the rehydration water was too low to give accurate measurements. Although negative from a biorefinery perspective, it is promising from a nutritional perspective that rehydration does not seem to influence the fucoidan content of the macroalgae.

Sammendrag

Sjømatsnasjonen Norge har gode forutsetninger for å basere en ny grønn økonomi på makroalger, som har vist seg å være en spennende bidragsyter. Enkelte brune makroalger akkumulerer høye konsentrasjoner av jod, som kan utgjøre en helserisiko.

Hensikten med denne masteroppgaven var å utvikle en metode for ekstrahering og kvantifisering av jodinnholdet i dyrket *Saccharina latissima* (sukkertare) etter frysetørking, rehydrering og koking. Prøvene kom fra dyrkningsanlegget til Seaweed Energy Solutions på Frøya.

Det ble bnyttet tre metoder for å bryte ned biomassen og ekstrahere jod fra den prosesserte makroalgen: Mikrobølgeovn-assistert basisk ekstrahering, basisk ekstrahering i varmeskap og ved salpetersyredekomponering. Jodinnholdet i den dekomponerte løsningen ble analysert ved ICP-MS. Ettersom jod danner flyktige forbindelser ved lav pH, er salpetersyredekomponering en usikker metode. Basisk ekstrahering i varmeskap ble vurdert til å gi de mest nøyaktige resultatene med det utstyret som var tilgjengelig. Mikrobølgeovn-assistert basisk ekstrahering er raskere, men stiller krav til kostbart utstyr. Jodinnholdet i frysetørket sukkertare fra mai 2016 og april 2017 ble bestemt til henholdsvis 3802 ± 123 mg kg^{-1} og 2930 ± 168 mg kg^{-1} på tørrvektsbasis etter basisk ekstrahering i varmeskap.

Konservering og tilberedning har som mål å minimere tap av sunne bestanddeler. Makroalger har rike forekomster av polyfenoler med gode antioksidantegenskaper. Folin-Ciocalteus totalfenolsmetode ble brukt til å bestemme polyfenolinnholdet i acetonekstrakter av frysetørkede, rehydrerte og kokte sukkertareprøver for å vurdere mulig tap. Ekstraksjonsevnen og påliteligheten til målingene ble påvirket av tørrvektsmålinger, inkubasjonstid med Folin-Ciocalteus reagens, samt tidsforløpet fra rehydreringen eller kokingen begynte til spektrofotometriske avlesninger. Den syntetiske antioksidanten propylgallat ble benyttet som standard. Polyfenolinnholdet i frysetørket, rehydrert og kokt sukkertare fra mai 2016 og april 2017 lå mellom 0.070 - 0.10 mg PGE/g på tørrvektsbasis. Forskjellene var ikke signifikante (ANOVA, $p < 0.05$).

Vannet som ble igjen etter rehydrering og koking kan inneholde vannløselig fucoidaner, som er et ettertraktet sluttprodukt ved bioraffinering. Fucoidaner er mangfoldige komplekse polysakkarider som det er vanskelig å kvantifisere. Derfor ble avfallsvannet syrehydrolysert for å frigjøre L-fukose. Avfallsvannet etter koking hadde det høyeste innholdet med 15.4 ± 0.2 g L^{-1} (5 g sukkertare per 500 mL deionisert vann), eller 1.56 ± 0.02 mg g^{-1} på tørrvektsbasis. Fukosekonsentrasjonen i avfallsvannet etter rehydrering var for lavt til å gi nøyaktige målinger. Dette er negativt for bioraffinering, men positivt for næringsinnholdet i rehydrert sukkertare.

Table of Contents

Preface i

Abstract iii

Sammendrag iv

Table of Contents vi

List of Tables vii

List of Figures xi

Abbreviations xii

1 Introduction **1**

1.1 Role of iodine in humans 2
1.2 Role of iodine in macroalgae 4
1.3 Iodine quantification 5
1.4 Iodine variability through the seaweed market value chain 7
1.5 Polyphenols 8
1.5.1 Phlorotannins 8
1.5.2 Fucoxanthin 9
1.5.3 Antioxidant activity of polyphenols 9
1.6 Fucoidan 10
1.6.1 Extraction of fucoidan 10
1.6.2 Bioactivity and potential use of fucoidans 11

2 Materials and methods **13**

2.1 Seaweed samples 13
2.2 Apparatus 13
2.3 Reagents 14
2.4 Processing 14

2.5 Partial digestion and extraction of iodine 15
2.5.1 Microwave-assisted alkaline extraction 15
2.5.2 Muffle furnace alkaline extraction 15
2.5.3 Nitric acid decomposition 16
2.5.4 Oven alkaline extraction by NIFES 16
2.6 ICP-MS determination of iodine 16
2.7 Phenolic extraction and Folin-Ciocalteu's method 17
2.8 Fucose quantification 17
2.9 Statistical analyses 18

3 Results and discussion **19**
3.1 Particle size distribution 19
3.2 Variation in dry weight of *S. latissima* 20
3.2.1 Rehydration 20
3.2.2 Boiling 20
3.3 Iodine contents 22
3.3.1 Microwave-assisted alkaline extraction 23
3.3.2 Oven alkaline extraction 26
3.3.3 Nitric acid digestion 29
3.3.4 Iodine quantification by NIFES 31
3.3.5 Literature value comparisons 31
3.4 Daily consumption of *S. latissima* 32
3.5 Polyphenols 33
3.5.1 Acetonic extraction 33
3.5.2 Homogeneity of the samples 35
3.5.3 Dry weight variation 36
3.5.4 Incubation time 36
3.6 Fucoidans 41

4 Conclusion **45**

5 Future research **47**

Bibliography **55**

A Harvest site data **I**

B Nitric acid decomposition temperature profile **III**

C Standard curves **V**
C.1 Fucose determination VI

Product declaration for *S. latissima*, 2016 **VIII**

NIFES iodine quantification of *S. latissima*, 2017 **XI**

List of Tables

1.1 Recommended daily intake of iodine for different age groups as defined by the World Health Organisation (WHO) and the Nordic Council of Ministers (NCM) (Meltzer et al., 2016). 3
1.2 Tolerable upper limit intake of iodine (μg d^{-1}). 4

2.1 ICP-MS equipment. 14
2.2 Sample uptake and flushing for ICP-MS. 16
2.3 ICP-MS gas flow rates. 17
2.4 Enzymatic L-fucose kit manual assay procedure. 18

3.1 The mean DW content of *S. latissima* after freeze-drying, rehydration (RH), boiling (B) and boiling after rehydration (RH-B). 21
3.2 Iodine contents of reference material undergoing MWA extraction. 25
3.3 Comparison of DW *S. latissima* iodine contents of present study with previous studies. 31
3.4 Estimated daily consumption of DW and FW *S. latissima* to comply with recommended daily intake (RDI, 150 μg d^{-1}) and the tolerable upper limit (UL, 600 μg d^{-1}). The iodine content was found by oven alkaline extraction of iodine from *S. latissima* harvested in May 2016. 32
3.5 Overview of variables during extraction and FC total phenols method. . . 33
3.6 Sample-to-solvent ratios during extraction with aqueous acetone (80:20 acetone:deionised water). 34
3.7 Phenols in *S. latissima* harvested in May 2016, treated by freeze-drying, rehydration and boiling. 40
3.8 Dry weight (DW), iodine, phenolic and L-fucose content of *S. latissima* harvested in May 2016 and April 2017. 43

List of Figures

1.1 Chemical structures of the thyroid hormones. 2
1.2 Iodine metabolism in *Laminaria* as postulated by Küpper et al. (2008). . . 5
1.3 A model of brown macroalgal cell wall as described by (Deniaud-Bouët et al., 2014). 6
1.4 Chemical structure of tetramethylammonium hydroxide (TMAH). 6
1.5 Seaweed market chain, starting with research and development, through cultivation and harvest, up until consumer usage of the processed end product. 7
1.6 Structure of phlorotannins. 8
1.7 Structure of fucoxanthin. 9
1.8 Phenoxyl radical stabilised by resonance delocalisation. 9
1.9 Structural excerpt of a fucose-containing sulfated polysaccharide. 11

2.1 Schematic flow chart summarising processing of *S. latissima* involving dehydration, rehydration and boiling. 15

3.1 Particle size distribution of *S. latissima* milled in a zircon ball mill. 19
3.2 Variation in dry weight content of *S. latissima* harvested in May 2016 and April 2017 during rehydration treatment. 21
3.3 Variation in dry weight content of *S. latissima* harvested in May 2016 and April 2017 during boiling treatment. 22
3.4 Iodine in *S. latissima* recovered by microwave-assisted alkaline extraction (MWA) and oven extraction (oven), compared to the iodine content given by the 2016 product declaration and the 2017 NIFES analysis. 23
3.5 Iodine contents of freeze dried and air dried *S. latissima* harvested in 2016 found by microwave-assisted alkaline extraction. 24
3.6 Attempted microwave assisted alkaline extraction of *S. latissima*, illustrating loss of product due to inappropriate vessels. 25
3.7 Iodine contents of freeze dried (FD) *S. latissima* harvested in May 2016, expressed on DW basis. 26

3.8 Iodine contents of freeze dried (FD) *S. latissima* harvested in May 2016, expressed on FW basis. 27
3.9 Iodine contents of freeze-dried *S. latissima* harvested in May 2016 and April 2017. 28
3.10 Iodine contents of freeze-dried *S. latissima* harvested in May 2016 and April 2017. 28
3.11 Iodine contents of freeze-dried *S. latissima* harvested in May 2016 and April 2017. 29
3.12 Iodine contents of freeze-dried *S. latissima* harvested in May 2016 and April 2017. 29
3.13 Iodine contents of freeze dried *S. latissima* harvested in May 2016, following nitric acid digestion. 30
3.14 Phenolic contents in acetonic extracts of freeze-dried *S. latissima* harvested in May 2016 and April 2017. 34
3.15 Structures of polar synthetic antioxidants. 35
3.16 Phenols in acetonic extracts of dehydrated *S. latissima* harvested in May 2016. 36
3.17 Phenols in acetonic extracts of freeze-dried (FD), rehydrated and sequentially boiled *S. latissima* harvested in May 2016. 37
3.18 Phenols in acetonic extracts of freeze-dried (FD), rehydrated and sequentially boiled *S. latissima* harvested in May 2016. 37
3.19 Phenols in acetonic extracts of rehydrated *S. latissima* harvested in May 2016. 38
3.20 Phenols in acetonic extracts of *S. latissima* harvested in May 2016. 39
3.21 Phenols in acetonic extracts of *S. latissima* harvested in April 2017. . . . 39
3.22 Phenols in *S. latissima* harvested in May 2016, treated by freeze-drying (FD), rehydration (1 h, 8 h, 24 h) and boiling (10 min, 20 min). 40
3.23 L-Fucose concentration (mg/L) in the waste water remaining following rehydration and boiling of freeze dried *S. latissima* harvested in May 2016. 41
3.24 L-Fucose content (mg/g DW) in the waste water remaining following rehydration and boiling of freeze dried *S. latissima* harvested in May 2016. 42

A.1 Average sea temperatures measured at the cultivation site on Tareskjæret, Frøya, Norway. I
A.2 Average salinity measurements from the cultivation site on Tareskjæret, Frøya, Norway. I

B.1 The UltraClave temperature profile during nitric acid decomposition of *S. latissima*. III

C.1 Propyl gallate standard curve, concentration range 0 to 0.4244 $mg\,L^{-1}$. . . V
C.2 Propyl gallate standard curve as the mean of standard curves prepared on 5 different days, each in triplicate. The concentration range was 0 to 0.220 $mg\,L^{-1}$. VI

C.3 Absorbance at 340 nm for reaction mixtures containing waste water collected from 1 h, 8 h, 24 h rehydration and 10+20 minutes pooled waste water. VI
C.4 Absorbance at 340 nm for reaction mixture containing the pooled waste water from 10 and 20 minutes boiling. VII

Abbreviations

Abs	Absorbance
AD	Air-dried
ANOVA	Analysis of variance
A. nodosum	*Ascophyllum nodosum*
DW	Dry weight
FCR	Folin-Ciocalteu's reagent
FCSP	Fucose-containing sulfated polysaccharide; fucoidans
FD	Freeze-dried
ICP-MS	Inductively coupled plasma - mass spectrometry
IIT	Iodine-induced hyperthyroidism
L. hyperborea	*Laminaria hyperborea*
PGE	Propyl gallate equivalents
PSD	Particle size distribution
ROS	Reactive oxygen species
SD	Standard deviation
SES	Seaweed Energy Solutions AS
S. latissima	*Saccharina latissima*; *Laminaria saccharina*; sugar kelp
T_3	Triiodothyronine
T_4	Thyroxine
TMAH	Tetramethyl ammoniumhydroxide
UL	Tolerable upper intake level
WW	Wet weight

Chapter 1

Introduction

Much of future human food supply is predicted to originate from the world's oceans. Throughout the past decades, use of marine macroalgae in food production has undergone a renaissance in the western world, as seaweeds offer potential solutions in the context of global climate change and environmental challenges. In comparison with terrestrial biomass, seaweeds are produced without demands on fertilisers, soil, fresh water or land area and with a higher productivity than any land plants (Bruhn et al., 2016). With its extensive coastline and existing know-how on processing of marine raw materials and related infrastructure, Norway is a prime candidate for developing a bioeconomy based on cultivation and processing of seaweeds.

Seaweed harvest in Norway is done primarily of *Laminaria digitata*, yielding 150 000 to 170 000 t yearly. Once an area has been harvested, it is left for 5 years to ensure regrowth of the kelp forest. *L. digitata* is used to extract alginate, with an annual value of 1-1.5 million NOK (Steen et al., 2016). With the exception of some traditional coastal usage of seaweeds as food and feed and imported Asian cuisines like sushi and wakame salad, seaweeds are not a mainstream food staple in western countries. Amongst local macroalgal species in Norway, *Saccharina latissima* (sugar kelp) stands out for its biomass yield, nutritional content, cultivation possibility and desirable flavour. Macroalgae may also be important sources of minerals, such as iodine, iron and calcium; ingredients which are often limiting in traditional food sources. However, the high iodine content may also pose a problem for utilisation as food. In addition, macroalgae contains many other bioactive compounds such as fucoxanthin, fucoidan, and vitamins.

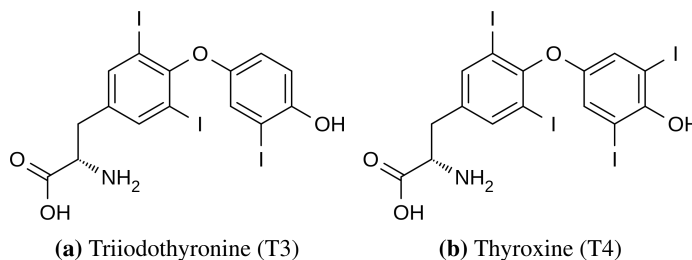
Sugar kelp forests give rise to a diverse and active ecosystem and serve as habitat for fish and other species. The macroalga can live for 3-5 years and grow up to 4-5 meters tall (Indergaard, 2010). In 2002, it was found that expected *S. latissima* habitats were instead populated by filamentous ephemeral macroalgae, attributed to increased temperature and eutrophication (Moy and Christie, 2012). Cultivation can be an approach to a sustainable source, and currently the largest rope cultivation site is run by Seaweed Energy Solutions

(SES) outside of Frøya. SES focuses on seaweed production for food and other high value markets, such as feed and cosmetics. Cultivation enables consistent quality and some predictability of nutritional content that will vary based on location and season. Lüning and Mortensen (2015) reported epiphytic fouling and high accumulation of iodine as limits to *S. latissima* cultivation aimed at the food industry. The presence of marine bacteria can speed up the algal biodegradation as the bacteria feed on inorganic and organic components. Proteins, carbohydrates and organic acids are released in the process, now available as nutrients to any bacterioplankton that may be present, constituting a microbial loop (Quartino et al., 2015). This, alongside the high water content, sets urgent requirement for preservation prior to transportation and storage. Primary treatment such as washing, freezing and dehydration can affect raw material characteristics and content of both beneficial as well as non-desirable components. To increase availability as food, heat treatment can be used. It is important to evaluate how different processing steps influence the composition of macroalgae including loss of both health beneficial and undesirable compounds.

It is a lack of knowledge on content and bioavailability of both health beneficial and risk components in seaweed. The aim of this thesis is to investigate how various processing methods influence the content of such components in the seaweed. The effect of drying, storage, rehydration and boiling on the changes in iodine contents of *Saccharina latissima* will be investigated. The total phenolic content will be analysed as a control of remaining nutrients, and the waste water from the rehydration of dried seaweed will be evaluated for biorefinery potential.

1.1 Role of iodine in humans

Iodine is an essential element required in trace amounts for the biosynthesis of the thyroid hormones triiodothyronine (T_3) and thyroxine (T_4). Structures are included in Figure 1.1 The hormones are involved in growth, bone formation, metabolism and brain development.



(a) Triiodothyronine (T3) **(b)** Thyroxine (T4)

Figure 1.1: Chemical structures of the thyroid hormones.

Iodine deficiency disorders (IDD) affects 15 % of the world population, and is the main preventable source of mental retardation (Markou et al., 2001). Europe is the region with the most occurrences of iodine deficiency (de Benoist et al., 2004). The recommended daily intake (RDI) of iodine for different age groups is included in Table 1.1. The RDI

for adults is set to 150 µg d^{-1}, which is set 50 µg higher than the average requirement to counter the effect of goitrogenic compounds and dietary variations. The increased iodine requirement during pregnancy is due to the higher biosynthesis of thyroid hormones and excretion of iodine through the kidneys (Meltzer et al., 2016).

The foodstuffs contributing to iodine in Norway are mainly seafood and dairy products, as cattle feed is mandatorily supplemented with iodine (Dahl et al., 2004). Supplements, iodised salt and seaweed were not included. Dairy sources of iodine have a seasonal variation, being 20% lower during summer. A study found that the average iodine consumption in a day was 176 µg d^{-1} for men, 136 µg d^{-1} for women, and 100-120 µg d^{-1} for children (Dahl et al., 2004). Table salt can be supplemented with up to 5 µg g^{-1}, but industrial salt used in food production is not fortified. A report by the Council of Nutrition in Norway advised national authorities to take measures towards ensuring adequate iodine consumption, especially adult women, lactating women and children (Meltzer et al., 2016). The

Table 1.1: Recommended daily intake of iodine for different age groups as defined by the World Health Organisation (WHO) and the Nordic Council of Ministers (NCM) (Meltzer et al., 2016).

Population	**NCM** (µg d^{-1})	**WHO** (µg d^{-1})
Adults and children $\geq$ 10 yr	150	150
Pregnant	175	250
Lactating	200	250
Children 6-11 months	50	90
Children 12-23 months	70	90
Children 2-5 yr	90	90
Children 6-9 yr	120	120

response to an increase in iodine consumption is dependant on the preliminary intake. In a population with iodine deficiency, the thyroid gland may have formed nodules that will be triggered by a sudden increase in iodine consumption, leading to iodine-induced hyperthyroidism (IIH) (de Benoist et al., 2004). Tolerable upper intake level (UL) of iodine intake is summarised in Table 1.2. UL is the highest level of daily nutrient intake that is likely to pose no risk of adverse health effects in almost all individuals (Institute of Medicine and Food and Nutrition Board, 2001). The difference in recommendations reflect the median iodine intake in the regional areas.

As a response to excessive iodine intake, the thyroid halts thyroid hormone production. This is known as the Wolff-Chaikoff effect, and may last up to 24 hours (Teas et al., 2004). The thyroid hormone production will resume given that the thyroid is healthy. Patients with Graves' disease or other autoimmune thyroid diseases have glands that are unable to escape the Wolff-Chaikoff effect, which could cause hypothyroiditis (Eng et al., 1999).

Goitrogenic compounds that can have antithyroid effects may contribute to endemic goiter, usually only after prolonged exposure and limited iodine intake. The goitrogens are found in the chemical groups such as sulfurated organics (thiocyanate, goitrin and disulphides), flavonoids (polyphenols), polyhydroxyphenols and phenol derivatives, pyridines, phtalate esters and metabolites, lithium, and others. Cruciferous vegetables such as cab-

bage, kale, cauliflower, broccoli and rapeseeds contain glucosinolates. Cyanogenic glucosides are found especially in cassava, lima beans, linseed and sweet potatoes. The metabolites of glucosinolates and cyanogenic glucosides compete with iodine for uptake by the thyroid (Eastman and Zimmermann, 2009). Iodine from seaweed can prevent the anti-nutritional effect of goitrogenic compounds. Likewise, the goitrogenic compounds can assist in preventing hyperthyroidism from a diet rich in iodine (Yeh et al., 2014). Traditional Asian cuisine often combines seaweed with vegetables high in goitrogens, like cabbage, bok choi and soy (Zava and Zava, 2011). Thiocyanate is an example of a goitrogenic compound inhibiting iodine transport. Smokers are specifically exposed, and can increase iodine consumption to reverse the effect (Erdogan, 2003; Teas et al., 2004).

Table 1.2: Tolerable upper limit intake of iodine ($\mu g\, d^{-1}$).

Population	**Japan**[a]	**US**[b]	**Europe**[c]
1-3 yr	250-350	200	200
4-6 yr	350-500	300	250
7-10 yr	500	300	300
11-14 yr	500-1200	300	450
15-17 yr	2000	900	500
Adults	3000	1100	600
Pregnancy	2000	1100	600

[a] Minister of Health Labour and Welfare Japan (2015)
[b] Institute of Medicine and Food and Nutrition Board (2001)
[c] European Food Safety Authority (2006)

1.2 Role of iodine in macroalgae

Kelps like *S. latissima* belong to the Phaeophyceae, commonly referred to as brown algae. The genus *Saccharina* alongside *Laminaria* have species that can accumulate iodine in the magnitude 3-10 $g\, kg^{-1}$ dry weight (Lüning and Mortensen, 2015). Küpper et al. (2008) suggested that iodine functions as an inorganic antioxidant protecting against oxidative stress. Upon exposure to UV radiation, *S. latissima* will emit iodine alongside other reactive organic halogens (Laturnus et al., 2010). A model of iodine metabolism in *Laminaria* is included in Figure 1.2. As ozone is scavanged by iodide, aerosol particles are formed. These particles form cloud nuclei, and are central to the global iodine cycle (Leblanc et al., 2006).

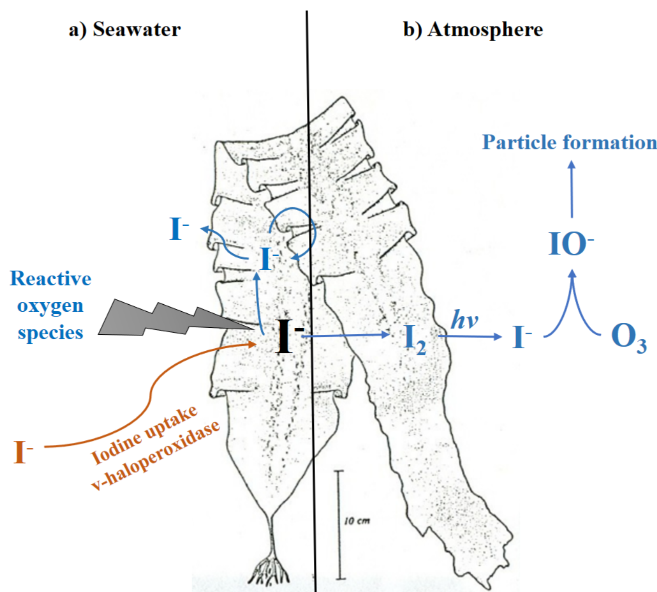


Figure 1.2: Iodine metabolism in *Laminaria* as postulated by Küpper et al. (2008). The left side a) is to illustrate submerged and unstressed kelp using vanadium haloperoxidase (orange) to accumulate iodine as iodide. When exposed to reactive oxygen species (blue), iodide is released as an antioxidant and is recycled or released into the seawater. When *Laminaria* is exposed to air and oxidative stress in b), the ozone-scavenging reactivity of iodide lead to aerosol particle bursts (Figure adapted from Küpper et al. (2008)).

Iodine was located in the extracellular matrix in the peripheral tissue of *Laminaria hyperborea* (Küpper et al., 2008). The only detectable form was as iodide in noncovalent association with organic molecules such as carbohydrates, polyphenols, or proteins. A model of the extracellular matrix from the brown macroalgae order Fucales is shown in Figure 1.3. Here the iodide association with other polymers was undetermined, but Küpper et al. (2008) found that a number of biomolecules could associate with iodide, including polysaccharides, phlorotannins and proteins.

In addition to scavenging of reactive oxygen species, iodine could be used to combat bacterial biofilms. The biofilms release oligoguluronates which trigger iodide release in *Laminaria* (Küpper et al., 2008).

1.3 Iodine quantification

The quantification of iodine in a biological sample follows 2-3 steps. 1) Complete decomposition of the biological matrix; 2) reduction of all iodine species to iodide; and 3) iodide analysis. The second step is achieved in some decomposition methods, otherwise it must be done separately. The method of decomposition must be chosen according to the matrix and analysis procedure. Often acid digestion procedures are used for total elements determinations, but in the case of iodine there is a risk of loss in form of volatile iodine

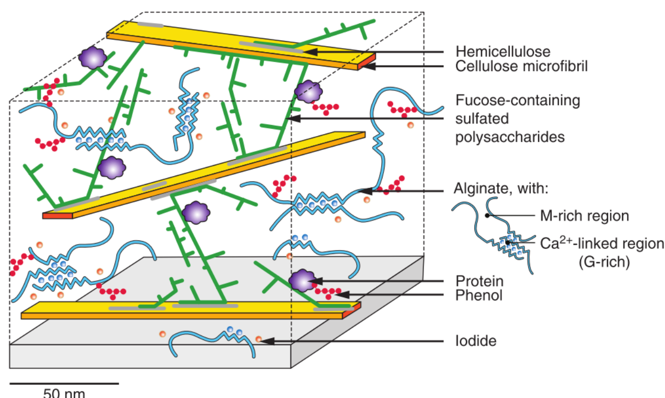


Figure 1.3: A model of brown macroalgal cell wall as described by (Deniaud-Bouët et al., 2014).

compounds. Due to this, alkaline digestions are reported to be advantageous in literature (Wifladt et al., 1989). Some alkaline extraction methods ensure that all iodine is reduced to iodide, such as dry alkaline incineration using KOH in a muffle furnace. This method is time consuming, but an alternative using microwave-assisted alkaline extraction has been developed (Gamallo-Lorenzo et al., 2005). Tetramethylammonium hydroxide (TMAH, Figure 1.4) is used to dissolve the sample material under microwave radiation. Decomposition is achieved as the product becomes a homogeneous solution with a low organic content, achievable by high temperatures. Sufficiently small particle size was found to be essential for efficient extraction of insoluble iodine species from algae (Fecher et al., 1998). The microwaves exert a changing electromagnetic field on the samples, leading to dipole rotation and ionic migration causing friction heat (Bradshaw et al., 1998). TMAH is an alkaline solution with pH 13.4-14.7 (Nóbrega et al., 2006). It is often used for alkaline partial digestion or extraction when iodine volatility would otherwise be an issue (Oliveira et al., 2010).

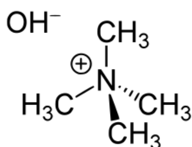
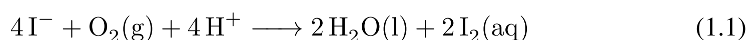


Figure 1.4: Chemical structure of tetramethylammonium hydroxide (TMAH).

The go-to traditional iodine analysis is the Sandell-Kolthoff method, published in 1934 (Sandell and Kolthoff, 1934). It requires iodine in the form of iodide as a catalyst on the arsenic(III)-cerium(IV) reaction. The Sandell-Kolthoff method is a colorimetric approach, using a spectrophotometer that is available in most laboratories.

Inductively coupled plasma-mass spectrometry (ICP-MS) has become the new standard for iodine analysis, chosen for speed, accuracy, high throughput and possibility to combine with other elemental quantifications (Haldimann et al., 1998). The quantity or relative isotopes is measured as ions formed in an inductively coupled argon-plasma. The

sample is transported as a liquid alongside the argon carrier gas to the plasma where the analytes are transferred to positively charged ions. The ions are extracted to high vacuum through an interface, upon which it is focused in an ionic lens system, separated by mass/charge ratio (m/Z), and finally detected and measured in a detector (Nødland, 2014). Spectral interferences occur when the argon plasma gas, water, organic solvent and the sample matrix form polyatomic ion species, and are the main limitation of ICP-MS. Some of these ion species may have the same m/Z ratio as an analyte ion, leading to an overestimation of the analyte quantity (Thermo Scientific, 2008). Interferences from memory effects arise when the analyte remains in the nebuliser and is a known issue when analysing iodine in a low pH solution. Volatile molecular ion is formed as shown in Equation (1.1), but the effect can be prevented by the addition of ammonia in the sample solution (Larsen and Ludwigsen, 1997). Non-volatile NH_4I is formed instead.



$$4\,I^- + O_2(g) + 4\,H^+ \longrightarrow 2\,H_2O(l) + 2\,I_2(aq) \tag{1.1}$$

This is another argument for employing alkaline sample extraction when analysing iodine by use of ICP-MS.

1.4 Iodine variability through the seaweed market value chain

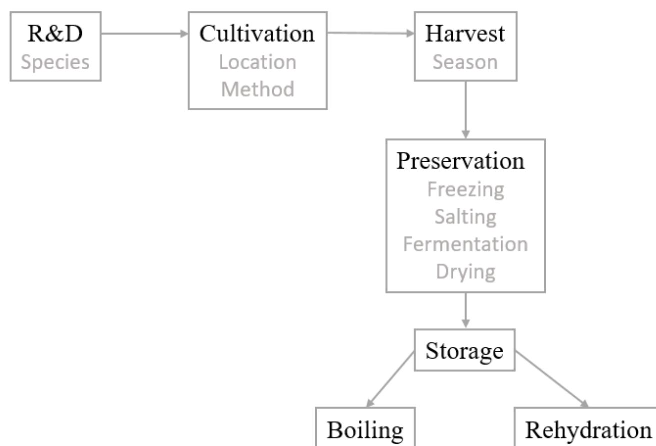


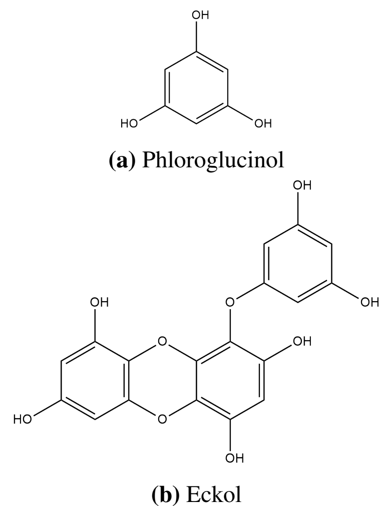
Figure 1.5: Seaweed market chain, starting with research and development, through cultivation and harvest, up until consumer usage of the processed end product.

Iodine levels vary throughout the market value chain (Figure 1.5). Deciding on which species to cultivate is the first iodine determining step as different species accumulate at different levels. Commercially available processed seaweeds were found to contain iodine in the range from $16\,\mu g\,g^{-1}$ in *Porphyra tenera* to $8165\,\mu g\,g^{-1}$ in *Laminaria digitata*. The genus *Laminaria* can contain more than ten times as much iodine as *Alaria*, another brown

macroalgae genus (Teas et al., 2004). The location of the cultivation site will affect the iodine content through varying latitude, salinity, tidal amplitude and temperature (Schiener et al., 2014; Nitschke and Stengel, 2014). Choosing whether to grow the seaweed as a rope culture, tank culture with high or low turnover of seawater can affect the iodine content, going down to 380 $mg\,kg^{-1}$ DW for tank cultured *S. latissima* with low turnover of seawater (Lüning and Mortensen, 2015). Harvesting date was found to influence iodine concentration in *S. latissima*, ranging from 39 $mg\,kg^{-1}$ in October to 4855 $mg\,kg^{-1}$ in August (Schiener et al., 2014). Fresh seaweed has a high water content and undergoes fast microbial deterioration, presenting a challenge for preservation and packaging to increase shelf life (Stévant et al., 2017b). Freezing and drying are common alternatives, but salting and fermenting may present themselves as exciting ways to preserve and change the flavour profile. Packaging material and whether or not the product is in vacuum, as well as storage time can release more or less volatile iodine species, especially in humid conditions (Larsen and Ludwigsen, 1997; Teas et al., 2004). Lastly, the seaweed product reaches the consumer who will commonly consume it dried, rehydrated or boiled. This step can have great influence on the iodine concentration, reported by Nitschke and Stengel (2016) for one red, green and brown macroalgae.

1.5 Polyphenols

Preservation of foodstuffs aim to preserve the the nutritional profile and bioactive compounds over time. Polyphenols are a class of antioxidants richly present in macroalgae. Main bioactive phenolic compounds in brown macroalgae include phlorotannins, bromophenols, meroditerpenoids, colpols and carotenoids like fucoxanthin (Robledo and Freile-Pelegriín, 2013; Haugan and Liaaen-Jensen, 1994; Sathya et al., 2013; Shang et al., 2011). These compounds can have, amongst others, anti-diabetic and anti-carcinogenic properties (Nwosu et al., 2011; Maeda et al., 2009; Xia et al., 2013).



(a) Phloroglucinol

(b) Eckol

Figure 1.6: Structure of phlorotannins.

1.5.1 Phlorotannins

Tannins are compounds that precipitate proteins (Adamczyk et al., 2012). Phlorotannins are oligomers or polymers of phloroglucinol linked by ether linkages, C-C bonds or diaryl ether bonds (Ph-O-Ph), and are found exclusively in brown algae (Figure 1.6). They are located within intracellular compartments in the outer cortical layer. The compounds have an absorption maxima between 190 to 240 nm, and are assumed to protect the macroalgae from UV radiation. Due to this and their high antioxidant activity, phlorotannins are used in cosmetics with claimed anti-aging, skin whitening and UV protection properties (Jeon et al., 2009).

1.5.2 Fucoxanthin

Fucoxanthin plays a role in brown algae photosynthesis and photoprotection, and has been reported to be the main carotenoid of freshly sampled *F. serratus* and *S. latissima* (Haugan and Liaaen-Jensen, 1994; Shang et al., 2011). Total fucoxanthin content is dependant on species, geographical location and seasonal variation (Haugan and Liaaen-Jensen, 1989; Gerasimenko and Logvinov, 2016; Schiener et al., 2014). The chemical structure is shown in Figure 1.7. Notable features are the allenic bond, which is not present in β-carotene or astaxanthin, and the functional groups epoxy, hydroxyl, carbonyl and carboxyl groups. The allenic bond is thought to give rise to the high antioxidant activity (Miller et al., 1996; Sachindra et al., 2007). The biosynthetic pathway of fucoxanthin by macroalgae is not completely known, but a hypothesis has been presented by Mikami and Hosokawa (2013). Understanding the biosynthesis is important for the development of cost efficient production by biotechnological methods. Macroalgae are the predominant source of natural fucoxanthin, but some microalgal strains have been reported to have up to 100 times higher fucoxanthin content (Guo et al., 2016) and may be important contributors in the future.

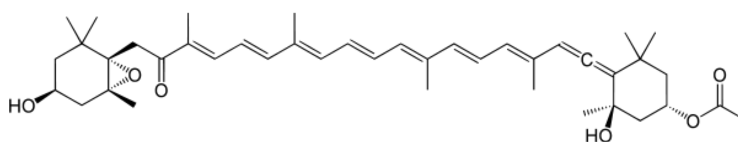


Figure 1.7: Structure of fucoxanthin.

Fucoxanthin is reported to have anti-obesity, anti-tumor, anti-diabetic, antioxidant and anti-inflammatory activities (Maeda et al., 2009; Xia et al., 2013; Tan and Hou, 2014; Takahashi et al., 2015), making it highly relevant as a nutraceutical.

1.5.3 Antioxidant activity of polyphenols

When polyphenols are near reactive oxygen species (ROS), phenoxyl radical species are formed. These radicals are stabilised by resonance delocalisation of the unpaired electron to the ortho and para positions of the ring (Figure 1.8), as well as by hydrogen bonding with adjacent hydroxyl groups (Sathya et al., 2013). The reducing power of the polyphenol is higher the more hydroxylated and conjugated the compound is (Prior et al., 2005).

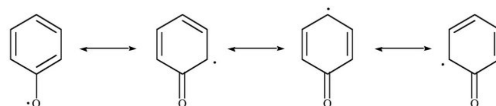
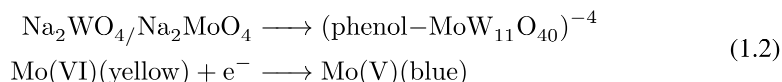


Figure 1.8: Phenoxyl radical stabilised by resonance delocalisation.

The antioxidant activity of polyphenols can be measured in a number of ways, among them ABTS, DPPH and Folin-Ciocalteu total phenolic assay (FC). The latter is a colorimetric method used to estimate the phenolic content of a sample. Folin-Ciocalteu's

reagent (FCR) is a yellow solution containing Mo(IV) in a complex that can be reduced in an electron-transfer reaction to Mo(V) (Equation (1.2)).



$$\begin{aligned} \mathrm{Na_2WO_{4/}Na_2MoO_4} &\longrightarrow \mathrm{(phenol{-}MoW_{11}O_{40})^{-4}} \\ \mathrm{Mo(VI)(yellow)} + \mathrm{e^-} &\longrightarrow \mathrm{Mo(V)(blue)} \end{aligned} \tag{1.2}$$

Phenols form phenolate anions under alkaline conditions ($pK_a > 9$) that can react to form a blue complex with FCR. Phenolic compounds are not the only possible reducing agents. Other compounds could contribute to the formation of the blue complex, hence it is more accurately an assay of the reducing capacity of the sample. It is assumed that the antioxidant capacity is equal to the reducing capacity. Some interfering substances include sugars, aromatic amines, sulfur dioxide and uric acid, all causing elevated phenolic conentrations (Prior et al., 2005).

1.6 Fucoidan

Fucoidans are structurally diverse polysaccharides that also go under the broader term fucose-containing sulfated polysaccharides (FCSP) and sulfated galactofucans (SGF). Fucoidans, FCSP and SGF are often used interchangeably. Fucoidans are also classified as fucans, alongside glycorunogalactofucans and xylofucoglycuronans (Pádua et al., 2015). Other sulfated polysaccharides are carrageenans and agarans, native to red algae. Fucoidans are located in the extracellular matrix of brown macroalgae, and appear to be more abundant in intertidal species exposed to drying. So far, fucoidans have been used in food supplements and cosmetics. Recent studies indicate that fucoidans exhibit beneficial bioactivity, and an effort is being made to standardise extraction and purification protocols.

The fucoidan structures are characterised by L-fucose 4-sulfate as the main component in branched structures mostly $\alpha(1 \rightarrow 2)$-linked shown in Figure 1.9a. Acid hydrolysis will also release D-xylose, D-galactose and uronic acid (Davis et al., 2003). A suggested structure for fucoidan isolated from *S. latissima* is shown in Figure 1.9b. A thorough structural characterisation was performed by Bilan et al. (2010), where they found other types of sulfated polysaccharide molecules highlighting the diversity of fucoidan structures. The fucoidan structure and composition is dependent on the species, extraction method, harvest location and season, and can vary depending on where in the alga it is located.

1.6.1 Extraction of fucoidan

Different methods have been used to pretreat, extract, and purify fucoidans from the macroalgae. Often the macroalgae is pretreated by drying and milling to increase the surface-area-to-volume ratio. Low molecular components like phenols can be extracted with methanol, chloroform and water. Proteolytic enzymes are used to remove proteins. Formaldehyde functions as a polymerising agent to precipitate phenols as they are linked and fixed. An ethanolic solution with high ethanol-to-water ratio will remove mannitol and chlorophyll without co-extracting fucoidans. These solvents can be used successively (Ale and Meyer, 2013).

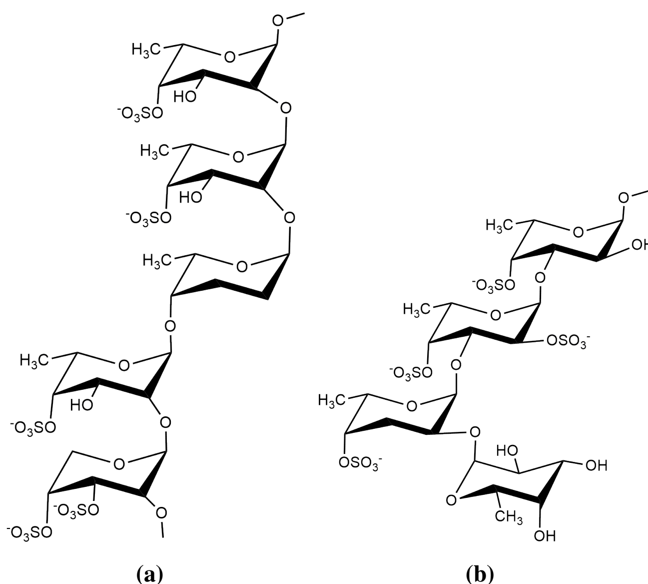


Figure 1.9: (a) Structural excerpt of a fucose-containing sulfated polysaccharide. The main monomer is L-fucose in $\alpha(1 \rightarrow 2)$ linkages (Davis et al., 2003); (b) Fucoidan isolated from *S. latissima*. It has a poly-α-$(1 \rightarrow 3)$-fucopyranoside backbone sulfated at C-4 or C-2 (Ale et al., 2011).

Hot water treatment or repeated hot acid extraction are common extraction methods. Adjusting the pH can increase the yield by releasing polysaccharides as protons or hydroxide ions interfere with the hydrogen bonds. After repeated acid extraction, the fractions are pooled and neutralised to prevent further hydrolysis. Extracting under acidic conditions has the added benefit of precipitating alginate as alginic acid. Alginate is an anionic polysaccharide consisting of mannuronate (M) and guluronate (G) monomers. GG-blocks form structural pockets where divalent cations can ionically bind, leading to cross-linking and gelling. Ca^{2+} in solution is used for this purpose and can be exploited to purify a crude fucoidan solution. Because of the sulfate ester groups linked to the carbohydrate backbone, fucoidans are anionic polyelectrolytes with a negative charge even at low pH, and so anion-exchange chromatography (AEC) can be used to separate fucoidans from other compounds. It can however be challenging to increase the resolution sufficiently to isolate homogeneous fucoidan fractions (Bilan et al., 2010).

1.6.2 Bioactivity and potential use of fucoidans

Fucoidans have gained considerable interest as bioactive components that may be anti-carcinogenic, immunomodulatory, anti-inflammatory, antiviral, antithrombotic, anticoagulant and antioxidant (Ale et al., 2011). Fucoidans are often fractionated to smaller molecular weights during the extraction. For use in drug applications where fucoidans generally have a too high molecular weight, the low molecular weight substructures of fucoidans

(LMWF) are interesting.

Due to the large variety of extraction methods that have been used, definite conclusions about fucoidan bioactivity is a challenge, as the resulting extract may have varying degree of purity and different structures (Ale et al., 2011).

Chapter 2

Materials and methods

2.1 Seaweed samples

The brown macroalgae *Saccharina latissima* was provided by Seaweed Energy Solution AS (SES). It was cultivated on Tareskjæret, Frøya (63°42′N 8°52′E). Sea temperature and salinity on the harvest site is included in Figures A.1 and A.2. The harvest from the 24th of May 2016 was washed in seawater and air-dried in a drying container heated by 3 hot-air ovens (9 kW each), maintaining a temperature of 30-35°C for approximately 20 hours. The same harvest was also freeze-dried by Freeze Dry Foods in Germany. Product data sheet analyses were performed by an accredited analytical laboratory (Kystlab preBIO). The dehydrated seaweed was stored in sealed plastic bags. Fresh *S. latissima* was harvested from the same cultivation site on the 25th of April 2017 and transported to the laboratory in zip-lock bags with seawater. It was frozen on the day of harvest and freeze-dried over 72 hours. The dehydrated seaweed was stored in zip-lock bags in the dark at room temperature. The importance of sufficiently small particles ($<$ 300 µL) for the alkaline extraction dictated that all seaweed samples were pulverised in a vibrating zircon ball mill (6700 rpm, 3x60 sec) prior to further processing. The dry weight (DW) was found gravimetrically as the remaining biomass of freeze dried macroalgae, or as the remaining biomass of rehydrated or boiled macroalgae dried at 105 °C for 24 h.

2.2 Apparatus

Martin Christ Alpha 1-4 LDplus was used to freeze dry samples. Dried seaweed samples were milled in a mortar and ground by Precellys 24 using 1.4 mm zirconium oxide beads. Sorvall RC5B Plus centrifuge equipped with rotor SLA-1500 was used when separating rehydration water from the seaweed, and a bench-top Eppendorf centrifuge 5804R equipped with rotor A-4-44 was used in between phenol extraction steps. The ground seaweed samples were extracted using either a Matsui microwave (MAT SMS17) with output effect of 650-700 W set to ”High” intensity in clear glass vials with screw top (22ml, Sigma-

Aldrich), or a Termaks laboratory oven in round-bottomed tubes (13ml, 100x16mm, PP, Supelco). The elemental measurements were made using Thermo Scientific ELEMENT 2 HR-ICP-MS, equipped with SC2 DX auto-sampler. The ICP-MS specifications are listed in Table 2.1.

Table 2.1: ICP-MS equipment.

Nebuliser	PFA-ST with volume range 50 to 700 µL min^{-1}
Spray chamber	Quarts baffled micro cyclonic, with dual gas inlet type ESI-ES-3452-111-11
Cooling	PC^{3x} - Peltier cooling and heated inlet system
Torch	Quarts Demountable with o-rings
Injector	Quarts 2.5 mm with o-rings, ES-1024-0250
Sample cone	Aluminium ES-3000-18032
Skimmer cone	Aluminium type X-skimmer ES-3000-1805 X
RF-power (W)	1350

Nitric acid decompositions were done using Milestone UltraClave microwave and PFA vessels (18 mL). Spectrophotometer Pharmacia Biotech Ultrospec 2000 was used for colorimetric analyses. The particle size distribution was analysed by Mastersizer 3000 laser diffraction particle size analyser.

2.3 Reagents

Tetramethylammonium hydroxide (TMAH) 25%, 2 N Folin-Ciocalteu's reagent and 97% propyl gallate were purchased from Sigma-Aldrich (Steinheim, Germany). Anhydrous sodium carbonate $\geq$ 99.0% was purchased from Merck Millipore (Darmstadt, Germany). Ultrapure, HPLC grade methanol, AnalaR NORMAPUR methanol and AnalaR NORMAPUR acetone was purchased from VWR International. L-Fucose Assay Kit was purchased from Megazyme (USA). Deionised MilliQ water was used throughout the experiments.

2.4 Processing

The dehydrated and pulverised seaweed was subjected to sequential processing as illustrated in Figure 2.1. The seaweed was rehydrated by soaking in deionised water (5 g DW per 500 mL) at room temperature under continuous stirring by a magnetic stirrer. For each sampling, the suspended powdered seaweed was centrifuged for 20 min (5000xg, 4 $°C$). The remaining rehydrated seaweed after taking samples at 1, 8 and 24 h was boiled in deionised water (5 g wet weight (WW) per 500 mL) at 100 $°C$. Samples for analyses were taken after 10 and 20 min. Another approach where the dehydrated samples were directly boiled was also implemented. The rehydration water was removed for each sampling step and replaced by deionised water up to 500 mL. All steps were done in duplicates. The samples were stored at 4 $°C$ for 1-7 days until analyses could be made.

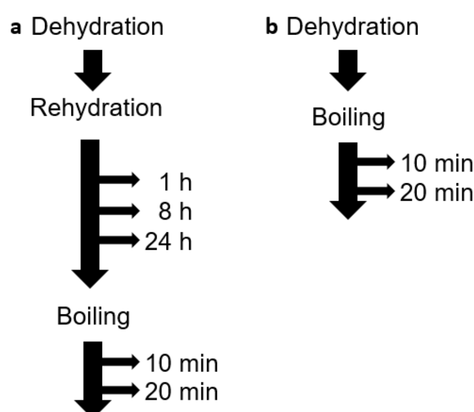


Figure 2.1: Schematic flow chart summarising processing of *S. latissima* involving dehydration, rehydration and boiling. a) Illustrates the longest sequential processing; b) illustrates direct boiling of dehydrated samples.

2.5 Partial digestion and extraction of iodine

2.5.1 Microwave-assisted alkaline extraction

The microwave-assisted alkaline extraction was performed using tetramethylammonium hydroxide (TMAH) 25% as described by Gamallo-Lorenzo et al. (2005) and Romarís–Hortas et al. (2011), with modifications. Reference material (0.030-0.050 g), freeze-dried seaweed (0.100 g) or rehydrated seaweed (1.000 g) were weighed into clear glass vials and wetted in deionised water (5 mL). 25% TMAH (5 mL) was pipetted to the decomposition solution and the tubes were vortexed to ensure full suspension. Blanks were prepared of deionised water (5 mL) and 25% TMAH (5 mL) and mixed. The vials were closed with screw caps. The microwave oven was operated at maximum power (700 W for two 5.5 min steps. The alkaline extracts were cooled and transferred to centrifuge tubes (15 mL, polystyrene) and centrifuged (6000 rpm, 10 min). The supernatant was separated by decantation and diluted to 10 mL prior to ICP-MS analysis.

2.5.2 Muffle furnace alkaline extraction

Alkaline decomposition in a muffle furnace was adapted from a method by Fecher et al. (1998). Milled freeze dried seaweed samples (0.250 g) were wetted with deionised water (4 mL) in round-bottomed tubes. Alternatively, rehydrated seaweed samples (1 g) were wetted with deionised water (3 mL). Deionised water was used for blanks (1 mL). TMAH (1 mL) was added, and the tubes were incubated at 90 $^{\circ}C$ for 1 h. The tubes were vortexed and left at 90 $^{\circ}C$ for an additional 2 h. The extracted samples were diluted with deionised water to a total volume of 10 mL, and allowed to settle over night. The supernatant was diluted 1:5 in deionised water prior to ICP-MS analysis.

2.5.3 Nitric acid decomposition

Total decomposition by nitric acid was performed using a Milestone UltraClave. The temperature profile is included in Figure B.1. Freeze-dried and milled *S. latissima* (0.150 g) were decomposed in HNO_3 (50 % (v/v), 5 mL). The decomposed solution was diluted to 50 mL prior to analysis by ICP-MS.

2.5.4 Oven alkaline extraction by NIFES

The alkaline extraction of iodine was done with modifications of NIFES method no. 198. The adaptions for macroalgal samples are not yet accredited. Freeze-dried seaweed samples were homogenised in a kitchen blender. The samples (0.20 to 0.25 g) were wetted with deionised water (5 mL) and added alginate lyase (200 µL). The alginate lyase helps break down starches. The mixture was allowed to stand over-night in a rotator. TMAH (1000 µL) was added, and the solution was heated at 90 $°C$ for 3 h. The extracted samples were cooled down and diluted to a total volume of 25 mL. The supernatant was diluted 1:5000 in deionised water prior to ICP-MS analysis. Tellurium was used as an internal standard, as well as a standard addition procedure to correct for matrix interferences that would otherwise give systematic errors.

2.6 ICP-MS determination of iodine

Flushing and sample uptake procedures involve increasing of pump speed during flushing of the valve and loop with flushing solution from flush station 2 prior to sample uptake or filling of the sample loop. Key parameters are included in Table 2.2.

Table 2.2: Sample uptake and flushing for ICP-MS.

Sample uptake (sec)	3
Sample aspirating speed (mL/sec)	0.84
Sample loop (µL)	500
Washing loop programmed (sec)	5
Flushing sampling probe in flush station (sec)	2
Read delay (sec)	20

In addition to an ordinary setup for sample gas, the sample gas lines were split, and methane was used alongside argon (10 % methane in argon, corresponding to 0.04 % in the sample gas). The splitting of the sample gas allows for optimisation of both the nebuliser gas flow (PFA-ST 0.7-0.8 L/min), and the gas flow in the plasma (1.2-1.4 mL/min). This lowers the RSD with 50 %. The sample gas line 1 was connected to the nebuliser, and the sample gas line 2 was connected to a T-connection between the spray chamber and the injector. The addition of methane gas to the sample gas lowers occurance of oxides, and increases the sensitivity of selenium (Se) and arsenic (As). The gas flow rates are included in Table 2.3.

Table 2.3: ICP-MS gas flow rates.

Argon and methane gas	Gas flows ($L\,min^{-1}$)
Cool gas	15.5
Auxiliary gas	1.1
Sample gas 1 (nebuliser)	0.75
Sample gas 2 (T-connection)	0.55

2.7 Phenolic extraction and Folin-Ciocalteu's method

Seaweed extracts were prepared prior to Folin-Ciocalteu's method of total phenolic contents, adapted from Stévant et al. (2017b). Dried milled seaweed samples (0.250 g) rehydrated seaweed samples (1.000 g) were suspended in acetone/water (80/20, v/v, 10 mL) and incubated for 1 h in darkness. The supernatant was collected following 10 minute centrifugation (5000 g, 4 $°C$), and the extraction was repeated on the remaining pellet. The pellet was re-suspended by shaking and vortexing. The pooled supernatants were kept at 4 $°C$ and analysed on the day of preparation. Extraction was performed in duplicate or triplicate. Seaweed extract (200 µL), distilled water (3.3 mL) and Folin-Ciocalteu's reagent (FCR) were vortexed in a test tube and incubated for 3 min at room temperature. Na_2CO_3 (29%, 100 µL) was added and mixed by vortexing. The test tubes were incubated for 1 h in darkness at room temperature. Absorbance at 760 nm was measured with distilled water as blank. Propyl gallate was used to prepare a standard curve with linear regression equation on the form $y = ax + b$. All analyses were performed in triplicate, with Folin-Ciocalteu reducing capacity expressed as mg propyl gallate equivalents per g dry weight seaweed sample (mg PGE/g DW), calculated based on on the standard curve and Equation (2.1),

$$\text{mg PGE/g DW} = \frac{Abs - b}{a} \cdot V \cdot \frac{1}{DW} \tag{2.1}$$

where Abs is the measured absorbance at 760 nm, a is the slope of the standard curve, b is the intersect of the standard curve with the y-axis, V is the sample volume used during incubation with FCR, and DW is the estimated dry weight of the sample.

2.8 Fucose quantification

Fucoidans are a class of complex polysaccharides. Exact quantification is challenging due to the structural variety, but L-fucose monomers is a common denominator. The rehydration water (5 g seaweed per 500 mL deionised water) from Section 2.4 was acid hydrolysed at pH 1-2 in a water bath at 100 $°C$ for 1 hour, then cooled down and neutralised to pH 7-8. An enzymatic L-fucose kit from Megazyme was used to measure the L-fucose concentration. The manual assay procedure provided in the kit was adjusted to Table 2.4. The cuvettes were covered with parafilm and inverted to mix. Absorption values in the UV range at 340 nm were read in 30 minute intervals until they plateaued.

The absorbance difference (A_2-A_1) was found for both blank and sample. The absorbance difference of the blank was subtracted from the absorbance difference of the

Table 2.4: Enzymatic L-fucose kit manual assay procedure. Samples were of the *S. latissima* rehydration water sampled at 1, 8 and 24 h, as well as the pooled boiling water sampled after 10 and 20 min.

Sample	Blank	1	2	3	4	
Treatment time	-	1 h RH	8 h RH	24 h RH	10+20 min B	(min)
Sample concentration	-	1	1	1	1	(mg/mL)
Sample volume	-	1000	1000	1000	1000	(µL)
Dist. water	2160	1010	1010	1010	1010	(µL)
Buffer	400	400	400	400	400	(µL)
$NADP^+$	50	50	50	50	50	(µL)
3 min, read Abs (A_1)						
Enzyme	10	10	10	10	10	(µL)
10 min, read Abs (A_2)						

sample to obtain Δ $A_{\text{L-fucose}}$. The concentration of L-fucose was calculated by using Equation (2.2),

$$c = \frac{V \cdot MW}{\epsilon \cdot d \cdot v} \cdot \Delta A_{\text{L-fucose}} \tag{2.2}$$

where V is the final volume (mL), MW is the molecular weight of L-fucose (164.16 g mol^{-1}), ϵ is the extinction coefficient of NADH at 340 nm (6300 mol^{-1} cm), d is the light path through the cuvette (cm) and v is the sample volume (mL).

2.9 Statistical analyses

Microsoft Excel was used for data processing and statistical analysis. Values were given as the arithmetic mean $\pm$ standard deviation (SD). Analysis of variance (ANOVA) at $p < 0.05$ was used to find significant differences. Student's t-test with Bonferroni correction was performed for post-hoc comparisons of significant ANOVA results.

Chapter 3

Results and discussion

3.1 Particle size distribution

When extracting insoluble iodine from macroalgal samples, the particle size is an important factor. This was evaluated by (Fecher et al., 1998), who concluded that the particle size should be smaller than 300 µm in diameter for optimum iodine recovery during sample extraction with TMAH.

Dehydrated *S. latissima* was milled by a zircon ball mill. The particle size distribution (PSD) in diameter was estimated by laser diffraction. The PSD is shown in Figure 3.1, where 90 % of the particles was below 424 ± 10 µm, 50 % was below 159 ± 1 µm, and 10 % was below 28.00 ± 0.05 µm.

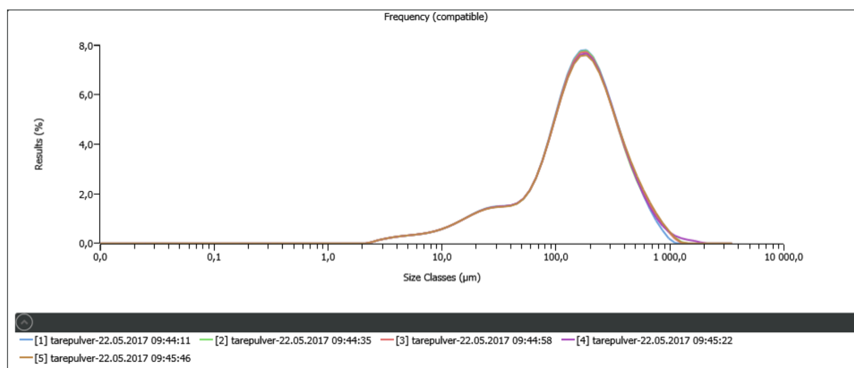


Figure 3.1: Particle size distribution of *S. latissima* milled in a zircon ball mill. The y-axis shows results (%) and x-axis shows the size classes (µm). The maxima is at 424 µm.

The method has major limitations, but can give an indication. Ideally, the refractive index (RI) of the particles should be known. Instead, the RI of saw dust was used for approximation. Laser diffraction problems arise with the assumed uneven surface of de-

hydrated and milled seaweed, as laser diffraction gives the best result on perfect spheres. Furthermore, the milled seaweed has excellent swelling capacity, seen by evaluating the DW content in Section 3.2, changing the shape of the particles from the moment they begin rehydration. Because of these limitations, an alternative approach to controlling the particle size should be set up by sieving the milled seaweed using a mesh with 300 µm pore size.

3.2 Variation in dry weight of *S. latissima*

In the final steps of the seaweed market value chain in Figure 1.5, the macroalgae undergoes preservation, and cooking by the consumer, i.e. rehydration or boiling. These steps change the dry weight (DW) content. During rehydration, the particles will swell as they bind water. At the same time, some water-soluble components can seep into the water, representing a loss of DW. To quantify iodine or polyphenols on a DW basis, accurate DW measurements are essential.

The DW content in *S. latissima* harvested in May 2016 was given in the product declaration (see Appendix) as 89.7 % water content, equivalent to 10.3 % DW. The method for determining the water content was not stated. The DW content in the harvest from April 2017 was 9.1 ± 1.2 % found by weighing frozen *S. latissima* prior to and after freeze-drying.

In this study, *S. latissima* was processed as shown in Figure 2.1. Each step will lead to a different DW content and must be measured individually. The results are presented in Sections 3.2.1 to 3.2.2.

3.2.1 Rehydration

Samples of rehydrated *S. latissima* were taken after 1 h, 8 h and 24 h. The variation of DW content is given for the 2016 and 2017 harvests in Figure 3.2.

Rehydration resulted in the highest decrease of DW content after the first hour, where it was 6.5 ± 0.1 % and 3.8 ± 0.3 % for 2016 and 2017, respectively. The DW found by freeze-drying the 2017 harvest was not significantly lower than the content given for the 2016 harvest, but it was significantly lower following 1 h rehydration. The cause of discrepancy is unclear, but both harvests had similar DW contents after 8 h and 24 h rehydration.

3.2.2 Boiling

Samples of boiled *S. latissima* were taken after 10 min and 20 min. The variation of DW content is given for the 2016 and 2017 harvests in Figure 3.3.

Directly treating freeze-dried seaweed with boiling led to a significant ($p < 0.05$) reduction of DW, down to 4.801 ± 0.006 % and 5 ± 2 %.

The loss of DW reflects release of compounds such as carbohydrates, proteins and minerals, and binding of more water. Because the sample decomposition in Section 2.5.1 was sensitive to particle size, the freeze-dried seaweed was milled prior to rehydration and boiling. This could affect the water binding capacity as well as the release of compounds,

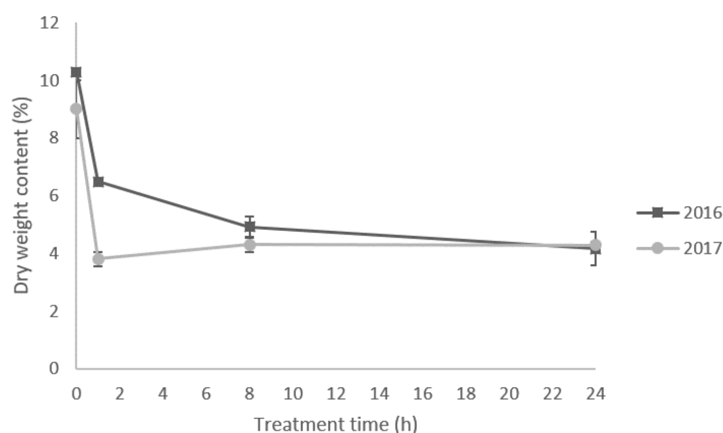


Figure 3.2: Variation in dry weight content of *S. latissima* harvested in May 2016 (■) and April 2017 (●) during rehydration treatment. Values are given as the mean ± SD (n=2 at t_0 and n=3 for the other sampling points).

effectively decreasing the DW contents more than if the seaweed had not been milled. It does indeed appear to be the case by comparing with the results from Stévant et al. (2017b), where they soaked fresh intact *S. latissima* for 22 h. The DW content was reduced from 16.1 % to 13.5 %, evaluated not to be significant. Because the DW content is dependent on treatment time and temperature, it is important to have accurate measurements in order to state the analyte contents on a DW basis. This could otherwise be a major source of error.

The mean DW contents of all treatments and sampling times are included in Table 3.1. For the occasions when DW samples were not taken at the same time as the samples for iodine and phenolic content analyses, the DW content was estimated to be equal to the average values.

Table 3.1: The mean DW content of *S. latissima* after freeze-drying, rehydration (RH), boiling (B) and boiling after rehydration (RH-B). The number of parallels is given as n.

Treatment	2016		2017	
	DW (%)	**n**	**DW (%)**	**n**
FD	10.3	-	9.1 ± 1.2	2
1 h RH	6.5 ± 0.1	3	3.8 ± 0.3	3
8 h RH	4.9 ± 0.4	3	4.3 ± 0.3	3
24 h RH	5.2 ± 1.1	7	4.3 ± 0.1	3
10 min B	6.4 ± 2.7	3	6.2 ± 0.3	3
20 min B	4.801 ± 0.006	2	5.0 ± 2.4	3
10 min RH-B	2.83 ± 0.01	3		
20 min RH-B	2.8 ± 0.1	3		

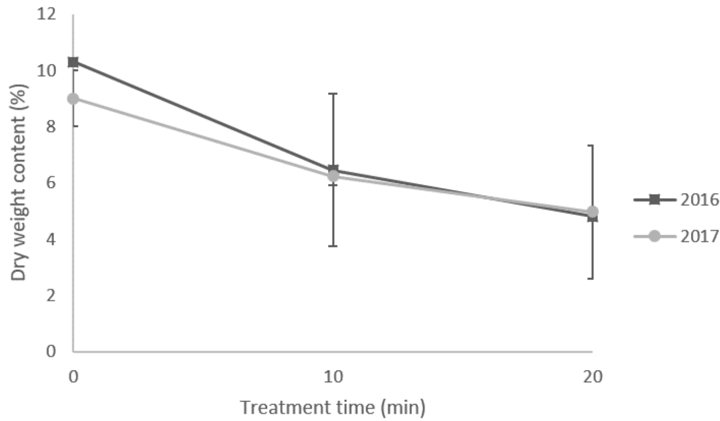


Figure 3.3: Variation in dry weight content of *S. latissima* harvested in May 2016 (■) and April 2017 (•) during boiling treatment. Values are given as the mean ± SD (n=2 at t_0 and n=3 for the other sampling points).

DW measurement of algae is not trivial; as the tissue is very hygroscopic, and the dehydrated samples can absorb air moisture even from a desiccator. Alginate can make out 30 % of the dry biomass of *S. latissima*, and is thus one of the main components behind the water-binding capacity (Manns et al., 2017). When the rehydrated and boiled samples are placed in a laboratory oven at 110 $°C$ to dry, some chemical changes and loss by volatilisation can occur. Although elevated to temperatures above the boiling point, some water molecules could remain bound to the hydrophilic molecules (Stein-Taylor and Phycological Society of America., 1973). The challenges of measuring accurate DW content are reflected in the uncertainties in Table 3.1. The values are within the same range as found by Stévant et al. (2017a) for fresh *S. latissima* soaked in fresh water and hot fresh water.

3.3 Iodine contents

Dried algae products can have a very high iodine content, which limits their culinary use to small portions as supplements or seasoning. Cooking processes such as rehydration and boiling can reduce the iodine content, allowing for larger portions without exceeding the iodine UL.

ICP-MS is often used for elemental iodine analyses. Before running the sample through the ICP-MS, the biomass must be fully or partially digested and the iodine extracted into the solvent. Three sample preparation methods were used in this study: A nitric acid digestion, microwave-assisted alkaline extraction and oven alkaline extraction. The iodine recovery from freeze-dried *S. latissima* was assessed by comparison to the product declaration for *S. latissima* harvested in May 2016, and to the analysis done by NIFES for the April 2017 harvest in Figure 3.4. One-way ANOVA was significant ($p < 0.05$), and pairwise t-test with Bonferroni correction revealed that the product declaration was not

significantly different from MWA or oven alkaline extraction results, but MWA extraction was significantly different ($p < 0.0125$) from both oven extractions samples. The oven extractions were also significantly different from each other.

The iodine content of freeze-dried *S. latissima* was quantified by ICP-MS following microwave-assisted alkaline extraction (Section 2.5.1) and oven alkaline extraction (Section 2.5.2), and compared in Figure 3.4.

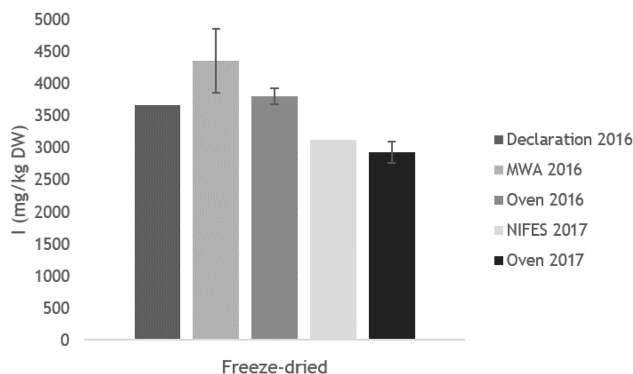


Figure 3.4: Iodine in *S. latissima* recovered by microwave-assisted alkaline extraction (MWA) and oven extraction (oven), compared to the iodine content given by the 2016 product declaration and the 2017 NIFES analysis. Values are the mean $\pm$ SD. The uncertainties were not given for the product declaration or the NIFES analysis.

The digestion and extraction methods are discussed separately in the following sections. Table 3.8 lists all iodine concentrations, as well as dry weight content, polyphenols and fucoidan.

3.3.1 Microwave-assisted alkaline extraction

The first method that was set up for quantifying iodine, was microwave-assisted alkaline extraction using TMAH. It was used on samples of *S. latissima* harvested in May 2016. The samples were air-dried (AD) or freeze-dried (FD). The freeze-dried samples were then rehydrated for up to 24 h, and subsequently boiled for up to 20 min. Performing a MWA extraction proved to be challenging with the available equipment, although a domestic microwave oven had been successfully employed by Gamallo-Lorenzo et al. (2005). Some trial and error was explored leading up to the iodine content result of air-dried and freeze-dried *S.latissima* obtained by ICP-MS, seen in Figure 3.5. The glass vessels were frequently overpressurised, which occurs when the contents boil. When this happens in vessels that are not gas-tight, the sample solution is lost both by volatilisation and overflowing. Metallic test tube racks cannot be used in microwave ovens, so a plastic rack was used instead. At one point the rack melted, trapping one of the glass vessels so the content could not be recovered. TMAH is highly toxic, and so the microwave oven was set up under a fume hood. It was a challenge to clean the spills that inevitably occurred as the extraction solution spilled outside of the vessels inside the microwave oven.

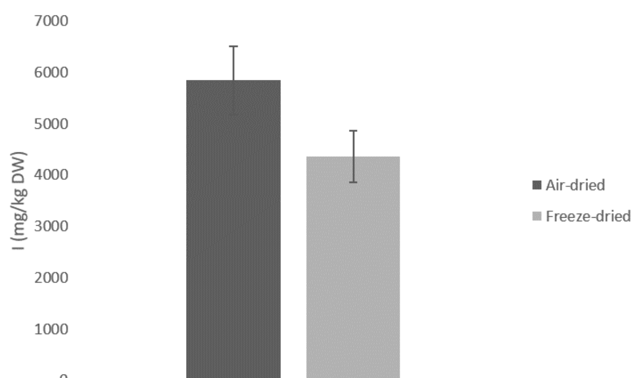


Figure 3.5: Iodine contents of freeze dried and air dried *S. latissima* harvested in 2016 found by microwave-assisted alkaline extraction. Values are the mean ± SD (n=2).

5844 ± 665 mg kg^{-1} was recovered from air-dried seaweed, and 4357 ± 498 mg kg^{-1} was recovered from freeze-dried seaweed. Comparing it to the iodine content in the product declaration of 3670 mg kg^{-1}, roughly 159 % and 119 % was recovered from AD and FD, respectively. To understand the higher yield, a discussion of the method underlying the product declaration iodine value is necessary. The data in the product declaration was found for frozen fresh macroalgae by use of method EPA 200.7/ 200.8, which is intended for the determination of trace elements in waters and wastes by ICP-MS. Iodine is not included on the list of validated elements, nor any other volatile organo-mercury compounds. The method solubilises the sample material with nitric acid. In these conditions, iodine readily forms volatile species. The problem was discussed by Badocco et al. (2017). HNO_3 decomposes at high temperatures to form NOx-gases, which remain in solution as long as it is closed and under pressure. They can reduce IO_3^- and IO_4^- to I_2. It is the formation of NOx-gases that drives the volatilisation of iodine, and so diluted nitric acid solutions will form less I_2 than concentrated HNO_3. Another driving factor is the low solubility of I_2 in water, which will shift the equilibrium to form more I_2. The recovery of iodine samples treated with concentrated HNO_3 in their study was of 32 to 67 %. Based on this, it is not all surprising that MWA extraction with TMAH had a higher yield of iodine. The principle of TMAH digestion/extraction will be discussed in Section 3.3.2.

Instead of assessing the iodine recovery by comparing to the product declaration that used a method that is prone to errors when used on iodine quantification, the iodine recovery could be evaluated by treating a certified reference material with an identical MWA alkaline extraction. The reference material should have a comparable matrix to the sample being analysed. This was not available, but the method was set up none the less using a certified reference material of human hair. Due to the higher reactivity of the reference material with the solvent solution, the same sample:solution (w:v) ratio as for macroalgal extraction could not be used. The attempts led to the overflowing of the sample solution and major loss of product. Instead, a range of sample:solution (w:v) ratios were tested (Table 3.2). The problem with using small quantities of the reference material, is the risk of not having a homogeneous selection. The vessel volume was not large enough to con-

tain a sufficient amount of reference material to be homogeneous. There is no difference in extraction principle when using different sample:solution ratio. The high SD for the reference material extracted as 3 $\mu g\,mL^{-1}$ is a testimony of the challenges with the domestic microwave oven. Using 4 $\mu g\,mL^{-1}$ had the yield closest to the certified value and the smallest SD, but the recovery was at 195.8 %. Overall, the iodine contents were higher than the certified value. This result, alongside the difficulties with boiling, shows that it is methodically challenging to get a reliable iodine quantity using a domestic microwave oven.

Table 3.2: Iodine contents of reference material undergoing MWA extraction. Different quantities have been used to find an appropriate sample:solvent (w:v) ratio. Values are given as the mean ± SD (n=3). Differences were not significant (ANOVA, $p > 0.05$).

3 $\mu g\,mL^{-1}$ ($\mu g\,g^{-1}$)	4 $\mu g\,mL^{-1}$ ($\mu g\,g^{-1}$)	5 $\mu g\,mL^{-1}$ ($\mu g\,g^{-1}$)	Certified reference value ($\mu g\,g^{-1}$)
6 ± 6	1.88 ± 0.03	3.9 ± 0.6	0.96

UltraClaves are laboratory microwave ovens constructed to avoid the issues present with a domestic microwave oven. First, teflon or quarts vessels should be used as these materials allow microwaves to pass through without absorbing their energy. In the present setup, glass vials were employed. Glass is virtually transparent to microwaves, but the cap was unable to withstand the pressure as the extraction solution boiled. This led to a substantial loss of product (Figure 3.6). In an UltraClave, the teflon vessels are kept at equal temperature by being lowered into a load typically made up of water, sulfuric acid (H_2SO_4) and hydrogen peroxide (H_2O_2). The issue of boiling is avoided by increasing the pressure so that the vapour pressure never overcomes the atmospheric pressure. The high pressure enables sufficiently high temperatures, which alongside the solvent digests the sample (Nødland, 2014).

Figure 3.6: Attempted microwave assisted alkaline extraction of *S. latissima*, illustrating loss of product due to inappropriate vessels.

The MWA extraction was used on freeze-dried *S. latissima* that had been rehydrated and subsequently boiled, according to the treatment flow chart in Figure 2.1a. During the sampling, the DW content was only determined for 24 h rehydration and the boiling treatments. The 1 h and 8 h rehydration sample DW was approximated as the mean DW content from Table 3.1. The result of the ICP-MS analyses of the MWA extractions is shown in Figure 3.7. After 1 h rehydration, the iodine content was 1710 ± 288 $mg\,kg^{-1}$ on a DW basis, which is 39.3 % of the initial freeze-dried iodine content. Further rehydration treatment at room temperature leads to no significant change of iodine content. Once the sample has been rehydrated for 24 h, it is boiled with samples

taken at 10 min and 20 min. The increase of temperature causes a significant reduction of iodine to 17.8 % and 10.6 % of the freeze-dried iodine content. Because the DW content

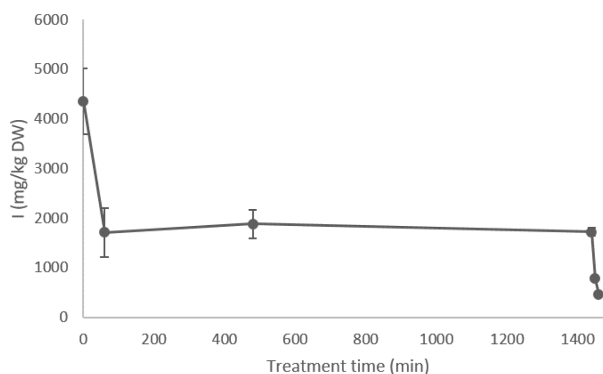


Figure 3.7: Iodine contents of freeze dried (FD) *S. latissima* harvested in May 2016, expressed on DW basis. The samples were rehydrated and subsequently boiled, underwent MWA extraction, and were analysed by ICP-MS. The extraction was done in duplicate, apart from the 24 h and 10 min boiling samples, which were not repeated. Values are given as the mean ± SD.

varies throughout the treatment time, the iodine content was also quantified on FW basis in Figure 3.8. This is to verify that the iodine loss is not only due to DW loss. It still shows that the main loss of iodine occurs during the initial first hour of rehydration, where it drops by 75 %. The iodine content was not significantly reduced after 8 h or 24 h rehydration. Boiling led to a significant iodine loss. The lowest iodine content was $13.0 \pm 0.2\,\mu g\,g^{-1}$ on a FW basis.

Microwave-assisted alkaline extraction has the benefit of being very fast: where traditional oven methods spend hours, the iodine is extracted within minutes. Using a domestic microwave oven, however, was much more time consuming due to toxic spills of TMAH that had to be disposed of carefully. The method was used on processed macroalgae, showing that iodine is extracted by deionised water in a temperature-dependent manner. This is valuable information when assessing the best preservation and cooking method of *S. latissima* with respect to the iodine content.

3.3.2 Oven alkaline extraction

To avoid the issues of improper vessels and microwave equipment, a method using an oven at $90\,°C$ was set up. Round-bottom polypropylene tubes were used as they give a higher surface area for contact between the sample and solvent. ICP-MS was used to quantify iodine in the sample solutions. Iodine in freeze-dried *S. latissima* was $3802 \pm 123\,mg\,kg^{-1}$ and $2930 \pm 168\,mg\,kg^{-1}$ for the May 2016 and April 2017 harvests, respectively. This could reflect a seasonal variation. It also indicates that little iodine is lost during storage following freeze-drying. Compared to the product declaration for the 2016 harvest and the NIFES analysis for the 2017 harvest, the iodine recovery was 103 % and 94 %, respectively. The oven extraction method behaved very nicely, with no spills or loss of extraction

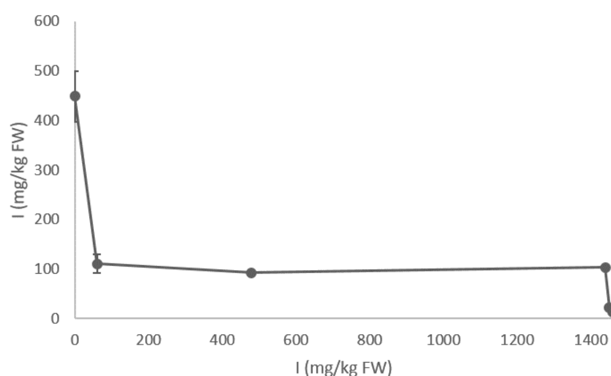


Figure 3.8: Iodine contents of freeze dried (FD) *S. latissima* harvested in May 2016, expressed on FW basis. The samples were rehydrated and subsequently boiled, underwent MWA extraction, and were analysed by ICP-MS. The extraction was done in duplicate, apart from the 24 h and 10 min boiling samples, which were not repeated. Values are given as the mean ± SD.

parallels.

The MWA extraction and oven extraction with TMAH differ in that the oven extraction uses a temperature below the boiling point (90 $°C$) and a longer treatment time (3 h vs 11 min). Oven alkaline extraction gave a more reliable result in this study because the issue of boiling was avoided. The literature is inconsistent with the terms digestion, decomposition, extraction and solubilisation when using TMAH procedures (Nóbrega et al., 2006). If it caused a complete digestion of the macroalgal samples, the digestion solution would look homogeneous and completely solubilised. Instead, a large portion of the sample remained as a slurry sedimented at the bottom of the polypropylene tube. Although the sample was not completely digested, the iodine yield was in accordance with the accredited analytical laboratory values. This, alongside the importance of sufficiently small particles, leads to the conclusion that TMAH partially dissolves the macroalgae and extracts iodine. This is a different mechanism than complete acid digestion.

Iodine was not extracted from a certified reference material with the oven alkaline extraction as for the MWA extraction. As well as assessing the method by comparing the yield to the accredited analytical laboratories, the reliability could have been verified by completely decomposing the biomass by wet digestion or burning under oxygen (Fecher et al., 1998). Wet digestion can be done by using a high-pressure asher technique. As discussed, TMAH is not a total digestion method, but an approach to *extract* or *liberate* iodine from the biological macromolecules it is bound to. Less than 50 % of macroalgal iodine is in water-soluble form, so it is important that the bound species are transferred into the liquid phase. Iodine species in macroalgae was discussed in Section 1.2, and in depth by Küpper et al. (2008).

The oven alkaline extraction method was used on both the 2016 and 2017 harvest. The freeze-dried samples were either rehydrated or boiled. The result for freeze-drying and boiling *S. latissima* is shown on DW basis in Figure 3.9 and on FW basis in Figure 3.10. The iodine is mainly lost during the first 10 min, where it drops to 24.0 % and 48.7 %

of the initial iodine content for the 2016 and 2017 harvests, respectively. Further boiling treatment did not significantly decrease the iodine content. The 2016 and 2017 harvests did not behave significantly different during boiling treatment (ANOVA, p¿0.0.05). The

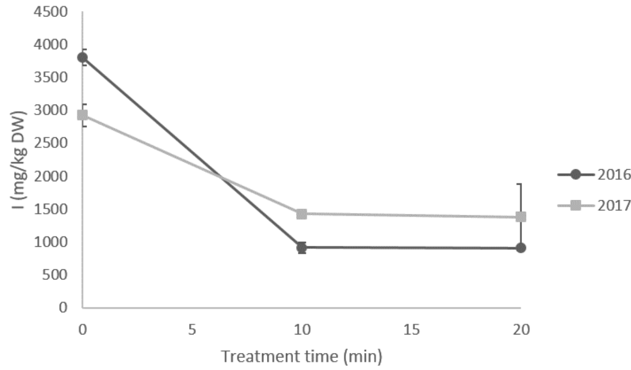


Figure 3.9: Iodine contents of freeze-dried *S. latissima* harvested in May 2016 (●) and April 2017 (■). The seaweed was treated by boiling in deionised water. The extraction was done in duplicate. Values are given as the mean ± SD (n=2).

iodine content is given on FW basis as well as DW basis. The iodine content reduction is not solely due to the loss of DW content, as apparent from Figure 3.10.

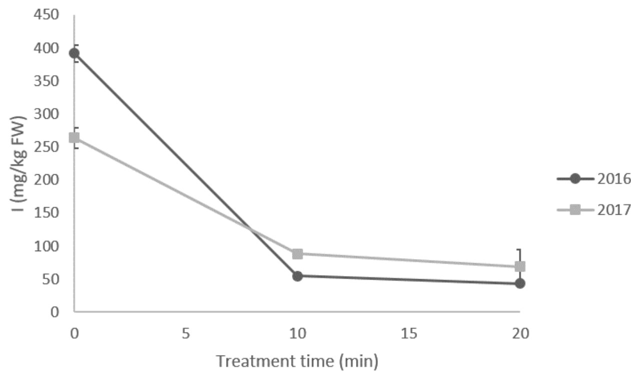


Figure 3.10: Iodine contents of freeze-dried *S. latissima* harvested in May 2016 (●) and April 2017 (■). The seaweed was treated by boiling in deionised water. Values are given as the mean ± SD (n=2).

The result for freeze-drying and rehydrating *S. latissima* is shown on DW basis in Figure 3.11 and on FW basis in Figure 3.12. After 1 h rehydration, the May 2016 harvest had 44.9 % remaining iodine, whereas the April 2017 harvest actually had 1.6 % more. The increase is not significant (p¡0.05), and is perhaps caused by the challenges of DW measurements that were discussed in Section 3.2.

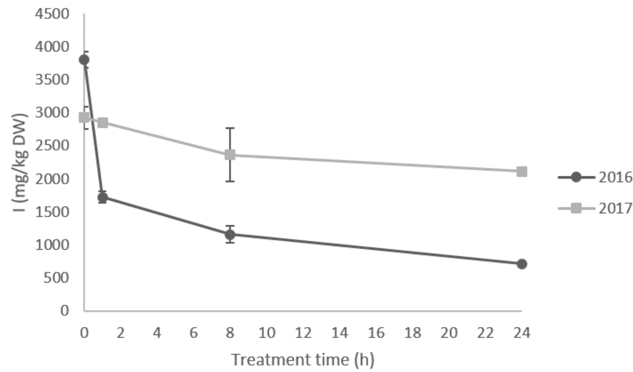


Figure 3.11: Iodine contents of freeze-dried *S. latissima* harvested in May 2016 (•) and April 2017 ($+blacksquare$). The seaweed was treated by rehydration in deionised water at room temperature. Values are given as the mean ± SD (n=2).

Interestingly, the 2016 and 2017 iodine content after 1 h rehydration on FW basis in Figure 3.12 are very similar at $111 \pm 6\,\mathrm{mg\,kg^{-1}}$ and $113 \pm 1\,\mathrm{mg\,kg^{-1}}$. This reaffirms that the differences on DW basis were caused by an underlying issue with the DW content measurements.

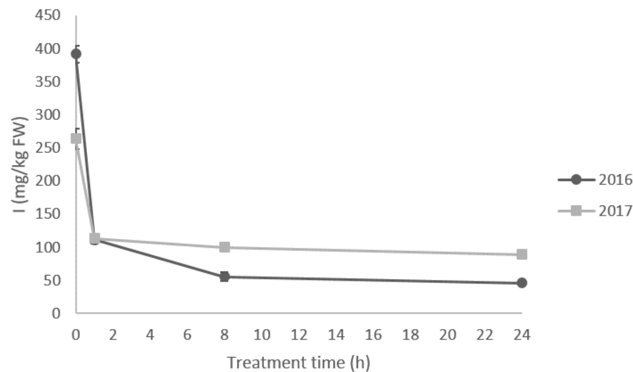


Figure 3.12: Iodine contents of freeze-dried *S. latissima* harvested in May 2016 (•) and April 2017 (■). Values are calculated back to fresh weight. The seaweed was treated by rehydration in deionised water at room temperature. Values are given as the mean ± SD (n=2).

3.3.3 Nitric acid digestion

A standard routine nitric acid digestion in an UltraClave was done for comparison (Figure 3.13). This method yielded $45\,662 \pm 3463\,\mathrm{mg\,kg^{-1}}$, more than ten times as much as the iodine content provided in the product declaration. This striking difference in yields

highlights the importance of choosing a digestion/extraction method appropriate for the biomass and element of interest.

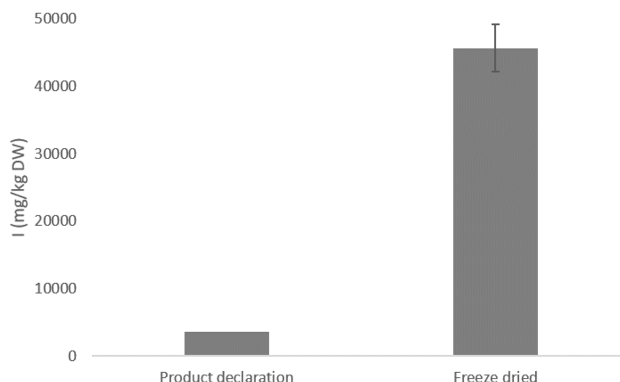


Figure 3.13: Iodine contents of freeze dried *S. latissima* harvested in May 2016, following nitric acid digestion. The result is compared with the product declaration.

Iodine is known to be susceptible to memory interference during analysis by ICP-MS. It occurs when isotopes of elements in a previous sample contribute to the signals measured in a new sample as they may build up in the plasma torch and spray chamber. Niedobová et al. (2005) examined how acidic pH led to a higher iodine signal than in an alkaline conclusion due to the volatilisation of iodine. I_2 and HI cause transport interference by aerosol enrichment. When TMAH was used as the extraction solvent, the alkaline conditions prevented the volatilisation, and the iodine recovery was more accurate. It is suspicious that the nitric acid digestion led to a tenfold overestimation of iodine content, and so it is natural to question whether another error could have been made. On the same ICP-MS run, another set of samples unrelated to this study was analysed, also yielding unreasonably high iodine contents.

Unlike the alkaline extraction methods, the nitric acid digestion in the UltraClave is a complete digestion method. The high temperature and oxidative agent HNO_3 will oxidate organic biomass in an exotherm reaction, whilst the high pressure in the UltraClave prevents boiling. The temperature profile is included in the appendix. The step-wise temperature increase is to control the exotherm reaction speed and temperature (Nødland, 2014).

As previously discussed, an alkaline extraction is preferred to avoid iodine memory and adhesion effects during the ICP-MS analysis, resulting in an overestimation of the iodine content. It can be possible to use nitric acid digestion when analysing iodine if additional steps are taken to stabilise iodine in nonvolatile compounds. One such method could be the addition of 3 % ammonia solution to the digest (Julshamn et al., 2001). Larsen and Ludwigsen (1997) did a similar approach by adding perchloric acid to the digest, also achieving stable nonvolatile iodine compounds.

3.3.4 Iodine quantification by NIFES

The iodine content of *S. latissima* harvested in April 2017 was analysed by NIFES, an accredited analytical laboratory, by their method no. 198. It is based on Julshamn et al. (2001), and is validated for iodine concentrations within 0.4 to 5.0 mg kg^{-1} DW. The proof of analysis is included in the appendix. They found 284 mg kg^{-1} on a fresh weight basis. The analysis was performed 4 times with very even results. Assuming a DW content of 9.1 % as found for the April 2017 harvest in Section 3.2, it is equivalent to 3121 mg kg^{-1} on a DW basis. Compared with their result, the oven alkaline extraction done in this study had a recovery of 93.88 %. The main difference in the two methods was the addition of alginate lyase, which specifically breaks down the glycosidic bond linking the monomers mannuronate and guluronate in different sequences in alginate polysaccharides. The cleavage is a β-elimination reaction, forming 4-deoxy-L-erythro-hex-4-enepyranosyluronate at the nonreducinig end (Kim et al., 2011). The enzymatic partial digestion likely assists in releasing some iodine species that would otherwise be bound to intact macromolecules. There is some indication that iodine-alginate complexes do form, but mainly around a pH of 1, and is avoided during alkaline extraction (Atwood et al., 1985).

3.3.5 Literature value comparisons

Iodine contents of *S. latissima* have been quantified by several studies, some examining the seasonal variation like Schiener et al. (2014), some comparing different cultivation methods like Lüning and Mortensen (2015). A selection of iodine values reported in existing literature is included in Table 3.3. The range of variation makes it challenging to predict the iodine content. This is a challenge both from a culinary perspective, when seaweed products are labelled with nutritional content, and from a biorefinery perspective, when certain compounds are more desirable than others undergo seasonal variations etc.

Table 3.3: Comparison of DW *S. latissima* iodine contents of present study with previous studies.

Study	Note	Location	Season	I (mg kg^{-1})
Stévant et al. (2017a)	cultured	CEVA, France	May	4898 ± 166
	cultured	CEVA, France	June	6568 ± 398
Schiener et al. (2014)	wild	Scotland	May	3193
	wild	Scotland	July	3499
Lüning et al. (2015)	TCHT[a]	Lysefjord, Norway	June	1655
	cultured	Faroe Islands	August	2700
Nitschke et al. (2015)	part: blade	Ireland	October	3341 ± 265
	part: stipe	Ireland	October	5149 ± 1595
This study	cultured	Frøya, Norway	April	2930 ± 168
	cultured	Frøya, Norway	May	3802 ± 123

[a] Tank culture with high turnover of seawater.

In this study, the iodine content was higher in the May 2016 harvest than the April 2017 harvest. The intention was not to study the seasonal variation. If it was, the samples should have been taken from the same year due to slightly different growth conditions. However,

iodine appears to accumulate as the summer progresses, and so the higher content in the 2017 harvest could reflect the seasonal variation.

3.4 Daily consumption of *S. latissima*

The daily intake of *S. latissima* required to reach the RDI and UL of iodine for adults by European recommendations (Table 1.2) was estimated in Table 3.4. Freeze-dried *S. latissima* fulfils the requirement with as little as 0.04 g d^{-1}. This limits consumption for individuals with thyroid dysfunctions, although a healthy individual may very well tolerate more. As the Wolff-Chaikoff effect is active for about 24 hours (Section 1.1), one larger *S. latissima* meal per week may not cause adverse health effects. Better yet if the meal is abundant in goitrogenic vegetables to limit iodine uptake by the thyroid (Teas et al., 2004; Yeh et al., 2014). Individuals with pre-existing thyroid disease, elderly, fetuses and neonates or with other risk factors should be aware that the chances of developing IIH can increase when consuming iodine above the RDI (Leung and Braverman, 2013).

Table 3.4: Estimated daily consumption of DW and FW *S. latissima* to comply with recommended daily intake (RDI, 150 µg d^{-1}) and the tolerable upper limit (UL, 600 µg d^{-1}). The iodine content was found by oven alkaline extraction of iodine from *S. latissima* harvested in May 2016.

Oven 2016	DW (g/day)		FW (g/day)	
Treatment	RDI	UL	RDI	UL
FD	0.04	0.16	0.38	1.53
10 min B	0.16	0.66	2.73	10.94
20 min B	0.17	0.66	3.44	13.77
1 h RH	0.09	0.35	1.35	5.40
8 h RH	0.13	0.53	2.69	10.76
24 h RH	1.13	0.52	3.25	12.99

Romarís–Hortas et al. (2011) examined the in vitro bioavailability of edible seaweed given as iodine dialysability. They found that kombu (*Laminaria ocholueca Laminaria sacharina*) had a dialysability of 17.0 ± 1.9 %. Combet et al. (2014) found that encapsulated *Ascophyllum nodosum* containing 712 mg kg^{-1} was bioavailable at 33 % in vivo. Assuming that the bioavailability of *S. latissima* is within this range, consuming 1 g of dried seaweed containing 3802 mg kg^{-1} could provide 646 to 1256 µg of bioavailable iodine.

Given the recommendations to increase iodine consumption from the Norwegian National Council of Nutrition (Meltzer et al., 2016) and the results of supplementing iodine-insufficient women with *A. nodosum* capsules by Combet et al. (2014), *S. latissima* supplementation can be a part of a healthy, balanced diet to ensure sufficient iodine. Individuals without thyroid risk factors can safely consume 10 g d^{-1} of FW *S. latissima* that has been boiled for 10 min without exceeding the UL of iodine. Cooking may also increase the antioxidant activity of the polyphenols. There is reason to be careful with dried *S. latissima*, as only 0.16 g d^{-1} is enough to reach the UL. More studies are needed on the bioavailability

of seaweed iodine after preservation and cooking, as well as the influence of goitrogenic compounds in order to safely assess the maximum intake on a daily, weekly, or monthly basis.

The German Federal Institute for Risk Assessment advised against allowing sales of dried algae products with an iodine content exceeding 20 mg kg^{-1}, based on an iodine UL of 500 µg d^{-1} (Bundesinstitut für Risikobewertung, 2007). They chose a lower UL than stated by European Food Safety Authority (2006). This was adapted because Germany has a high occurrance of iodine deficiency amongst the older generation, and the Federal Institute of Risk Assessment meant that a lower UL was necessary to avoide excessive iodine intake for this group. They further recommended that algae products marketed within the EU should include labelling of iodine content and maximum daily portion to comply with the RDI. Dried *S. latissima* exceeds the iodine threshold at 2930 to 5844 mg kg^{-1}.

Due to the variation during cooking treatments of iodine content in dried algae products, the French Food Safety Agency recommend that the iodine content can be up to 2000 mg kg^{-1} (Agence Française de Sécurité Sanitaire des Aliments, 2009).

3.5 Polyphenols

For the determination of total phenols by Folin-Ciocalteu's method, different parameters were used and found to influence the results and reliability. An overview of sample treatment is included in Table 3.5. The parameters and results are discussed separately in the following sections.

Table 3.5: Overview of variables during extraction and FC total phenols method. Treatments are air-dried (AD), freeze-dried (FD), rehydration (RH), boiling after rehydration (RH-B) and boiling after FD (B). The dates given in start-end indicate time from the treatment began until the extraction and analyses were made. The freeze-dried sample-to-solvent ratio (FD:solvent) is given when the FD samples were analysed. Number of extraction parallels is given as n, incubation time with FCR as Rx, and propyl gallate standard curve concentration range as PG.

Treatment	Start-end	FD:solvent (g:mL)	n	Rx. (min)	PG (mg/mL)
AD, FD	Same day	0.250:10	2	30	0-0.4244
FD	Same day	0.250:10	2	30	0-0.220
1 h RH, 8 h RH	21.02-22.02	FD not analysed	2	30	0-0.220
24 h RH, RH-B	22.02-23.02	FD not analysed	2	30	0-0.220
All RH, B	28.02-06.03	FD not analysed	2	60	0-0.220
FD, all RH, B, waste water	20.04-21.04	0.250:10	3	60	0-0.220
2017: FD, all RH, B	02.05-07.05	0.050:10	2	60	0-0.220

3.5.1 Acetonic extraction

Dried, rehydrated or boiled *S. latissima* was milled and extracted in aqueous acetone (80:20 acetone:deionised water) as described in Section 2.7. Different DW content of

Table 3.6: Sample-to-solvent ratios during extraction with aqueous acetone (80:20 acetone:deionised water).

Treatment	2016 Sample (g)	2017 Sample (g)	Solvent (mL)
FD	0.250	0.050	10
1 h RH	0.065	0.380	10
8 h RH	0.049	0.043	10
24 h RH	0.052	0.043	10
10 min B	0.064	0.062	10
20 min B	0.048	0.050	10
10 min RH-B	0.028	n.a.	10
20 min RH-B	0.028	n.a.	10

each sample led to varying sample-to-solvent ratio. Based on the mean DW contents in Table 3.1, the ratios are evaluated in Table 3.6.

A study by Rezaei et al. (2013) found 150 % higher yield of polyphenolic compounds using a 1:20 sample-to-solvent ratio compared to a 1:10 ratio. More accurate results could have been achieved by reducing the amount of freeze-dried samples to correspond to the average DW content of the rehydrated and boiled samples, in this case about 0.050 g. Evaluating the phenolic content in freeze-dried *S. latissima* from the 2016 and 2017 harvests in Figure 3.14 gave significantly different results. Although the sample-to-solvent ratio had been adjusted to 0.050:10, the 2017 harvest had a lower phenolic content yield. This is more likely due to seasonal variation than the extraction efficiency. Polyphenols start accumulating at the beginning of algal growth, and are most abundant between May and July. *S. latissima* has the least polyphenols in March (Schiener et al., 2014). An optimisation of the most efficient sample-to-solvent ratio should be done under identical conditions.

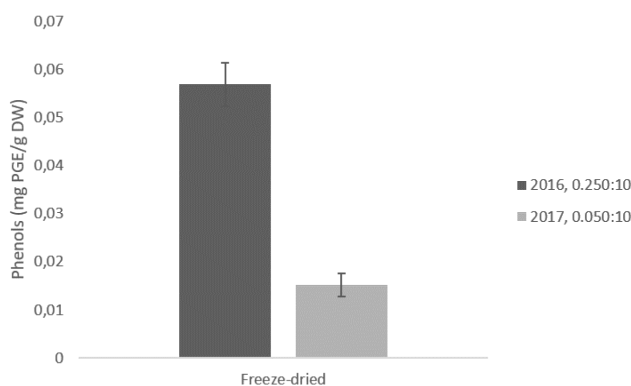


Figure 3.14: Phenolic contents in acetonic extracts of freeze-dried *S. latissima* harvested in May 2016 and April 2017. Sample-to-solvent ratio 0.250:10 and 0.050:10 (g:mL) were used for the 2016 and 2017 harvests, respectively.

A polyphenolic content of 0.057 ± 0.005 mg PGE/g DW from the May 2016 harvest appears somewhat low. Another study found $0.23 - 0.68$ g/100 g using gallic acid as the standard, depending on the season (Schiener et al., 2014). For comparison with other studies, the same standard should be used. Usually, Folin-Ciocalteu total phenols is given in gallic acid equivalents (mg GAE/g extract). The structures of gallic acid and propyl gallate respectively are shown in Figure 3.15. Both are polar synthetic antioxidants. Propyl gallate has a higher radical scavenging activity than gallic acid (Alamed et al., 2009; Majid et al., 1991). A unit conversion from PGE to GAE is possible by preparing a calibration curve for both and relating the linear regression slopes.

(a) Gallic acid

(b) Propyl gallate

Figure 3.15: Structures of polar synthetic antioxidants.

During the extraction incubation time, the milled seaweed accumulated at the bottom of the vessel. The yield could have been improved by using an orbital shaker to increase the particle surface-area exposed to the solvent at any time. Allowing for longer incubation times and repeating extraction more than twice are also factors that could improve the yield.

Rajauria et al. (2013) used aqueous methanolic solvent to prepare crude extracts of seaweed for Folin-Ciocalteu total phenols analysis. The highest yield was obtained with a 60 % solution. Increased or lower methanol concentration had lower yields. The dielectric constant of 60 % methanol at 40 $°C$ is 47.52 (by linear extrapolation ($R^2 = 0.9985$) of 60 % methanol dielectric constants as a function of temperature from Albright and Gosting (1946)). Considering the different yields with different solvent concentrations, it is clear that the polarity of the solvent must be optimised for the compound that is being extracted. The 80 % aqueous acetone solvent at 25 $°C$ has a dielectric constant of 29.62 (Åkerlöf, 1932), which may not be optimal for phenolic extractions. Acetone has however been shown to inhibit interactions between phenolic groups and protein carboxyl groups (Werner, 2013).

3.5.2 Homogeneity of the samples

Figure 3.16 shows the yield from air-dried and freeze-dried *S. latissima* harvested May 2016. The first and second extract of air-dried *S. latissima* had $0.0205 \pm 0.0009\,\mathrm{mg\,g^{-1}}$ and $0.092 \pm 0.004\,\mathrm{mg\,g^{-1}}$ phenols, respectively. The extracts of the freeze-dried samples had $0.052 \pm 0.002\,\mathrm{mg\,g^{-1}}$ and $0.063 \pm 0.002\,\mathrm{mg\,g^{-1}}$ each.

The extraction duplicates were significantly different ($p < 0.05$), highlighting the consequence of not ensuring a homogeneous selection of the dehydrated samples. The phenolic content will vary with the part of macroalgae being analysed. For such heterogeneous

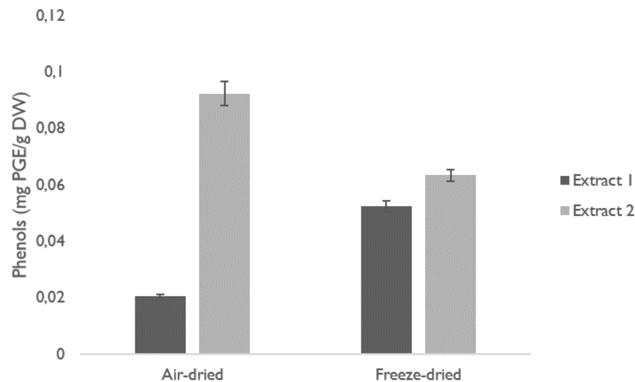


Figure 3.16: Phenols in acetonic extracts of dehydrated *S. latissima* harvested in May 2016. Values are the means of triplicate measurements of duplicate extractions ± SD.

samples, three approaches are possible: i) divide the macroalgae into the parts likely to have different phenolic content, i.e. stem and blade; ii) homogenise the seaweed by milling a sufficient amount and take a subsample; or iii) analyse one whole macroalga (Poppiti and Sellers, 1994). The second approach is the simplest and was used for subsequent phenolic analyses.

The standard curve was prepared with propyl gallate (0 to 0.4244 mg mL^{-1}, Figure C.1), which gave absorbance readings > 1. The sample readings, however, were within the linear range of the standard curve. The concentration range was adapted to give absorbance readings < 1 for the other analyses (Figure C.2).

3.5.3 Dry weight variation

The rehydration and boiling of rehydrated *S. latissima* resulted in samples with varying DW content, as discussed in Section 3.2. When finding phenolic content on a DW basis, Equation (2.1) is used. It is clear that inaccuracies in DW estimation can have a large influence on the reliability of the determination of the phenolic content.

Figure 3.17 shows the phenolic content of *S. latissima* harvested in May 2016 treated with freeze-drying, rehydration, and boiling of the rehydrated samples.

Accurate dry weight measurements were not taken during the rehydration and boiling treatment, but are based on mean DW contents in Table 3.1.

3.5.4 Incubation time

During the Folin-Ciocalteu method, the samples are incubated in the dark for 30 min prior to absorbance readings. As seen in Figure 3.17, the second extract parallel had consistently higher values than the first. This could indicate insufficient incubation time as the reaction was still ongoing after 30 min. Other than allowing for longer reaction time, the temperature could also be adjusted from room temperature to 45 $°C$, as done by Stévant et al. (2017a).

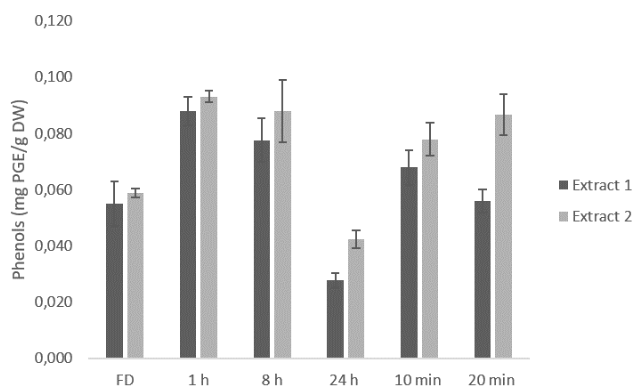


Figure 3.17: Phenols in acetonic extracts of freeze-dried (FD), rehydrated and sequentially boiled *S. latissima* harvested in May 2016. Values are the means of triplicate measurements of duplicate extractions ± SD.

To get a clearer view of the treatment time dependency, the mean value of the extraction parallels was evaluated in Figures 3.18 and 3.19.

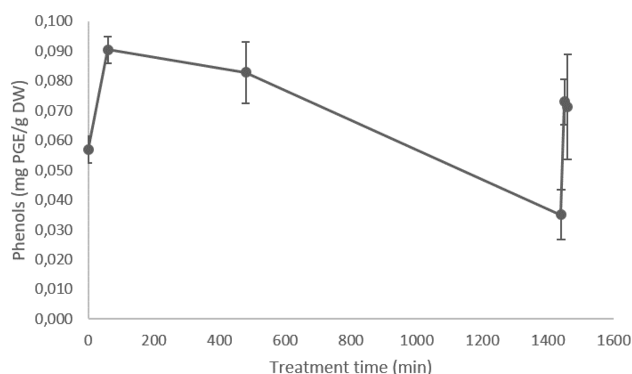


Figure 3.18: Phenols in acetonic extracts of freeze-dried (FD), rehydrated and sequentially boiled *S. latissima* harvested in May 2016. Values are the means of triplicate measurements of duplicate extractions ± SD.

The rehydration and boiling led to higher yields. During rehydration, the dried seaweed simultaneously absorbs water, swells, and loses soluble components (Lee et al., 2006). As it is unlikely that rehydration and boiling does not extract polyphenols into the waste water, the result must instead reflect the acetonic extraction method. The increased yield following 1 h rehydration could be the result of increased extractability as swelling further disrupts the macroalgal tissue.

Increased phenols is also seen when the rehydrated samples are boiled, suggesting that the sample:solvent ratio may not be the only reason. Interestingly, Amorim-Carrilho

et al. (2014) found a higher content of phloroglucinol and fucoxanthin in dried *Himantalia elongata* that had been rehydrated for 10 min or boiled for 15 min. It is not clear whether they took the different DW contents of the rehydrated and boiled samples into consideration or not. Another study by Cox et al. (2012) evaluated the rehydration kinetics of *H. elongata*, as well as the phenolic content. They reported a rapid decrease of total phenolic content within the first 10 min of rehydration at temperatures between 20 to 40 $°C$. At the same time, the DPPH radical scavenging activity, a much-used colorimetric assay of antioxidant capacity, increased. As discussed, Folin-Ciocalteu's total phenolic assay is a method more accurately indicating the antioxidant activity of the sample rather than a quantitative method. As such, although polyphenols are partly extracted during rehydration and boiling, the antioxidant activity of the remaining polyphenol extracted in acetone increases.

Between 1 h and 24 h, the phenols decreased in a linear trend ($R^2 = 0.8898$) before a rapid increase during boiling to just below the activity at 8 h.

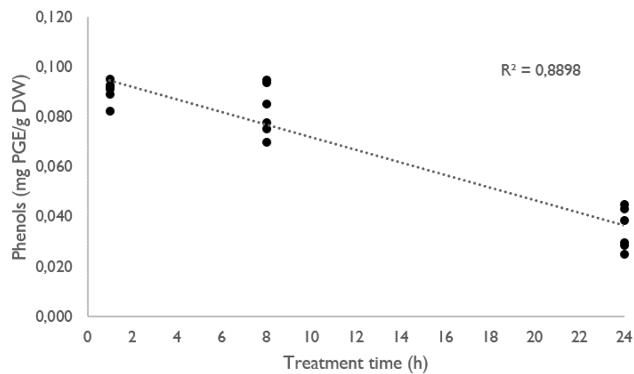


Figure 3.19: Phenols in acetonic extracts of rehydrated *S. latissima* harvested in May 2016. The values are from both duplicate extractions with triplicate measurements ± SD.

Figure 3.20 shows the phenolic content of *S. latissima* harvested in May 2016, where the freeze-dried samples are either rehydrated or boiled (Figure 2.1, b). The incubation time was increased to 1 h. Although the rehydration up to 24 h underwent the same treatment as in Figure 3.17, the results are significantly different ($p < 0.05$). The lack of reproducibility reflects the importance of doing accurate DW measurements for each sampling point.

The phenolic content of *S. latissima* harvested in April 2017 was evaluated in Figure 3.21. The analysis was done 5 days after the harvest, which was too long to get reliable results. The samples had deteriorated. The freeze-dried sample, however, was preserved and reliable, containing 0.015 ± 0.002 mg PGE/g DW. It is 52 % less than the harvest from May 2016, and could reflect the seasonal variation.

For the final phenolic content analysis of *S. latissima* harvested in May 2016, the parameters evaluated previously were optimised: the FC incubation time was 1 h; DW measurements were done for each sample; and the rehydration and boiling treatment was done

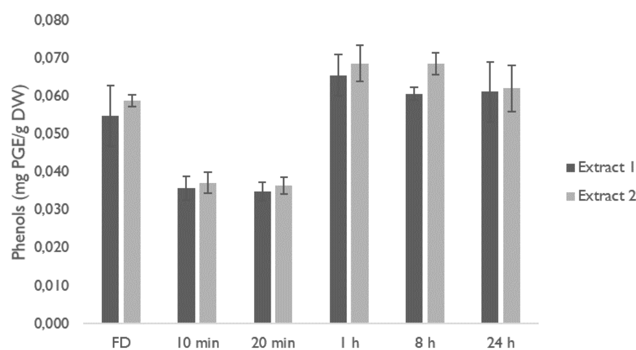


Figure 3.20: Phenols in acetonic extracts of *S. latissima* harvested in May 2016. The seaweed was freeze-dried and then rehydrated (1 h, 8 h, 24 h) or boiled (10 min, 20 min). Values are given as the mean ± SD.

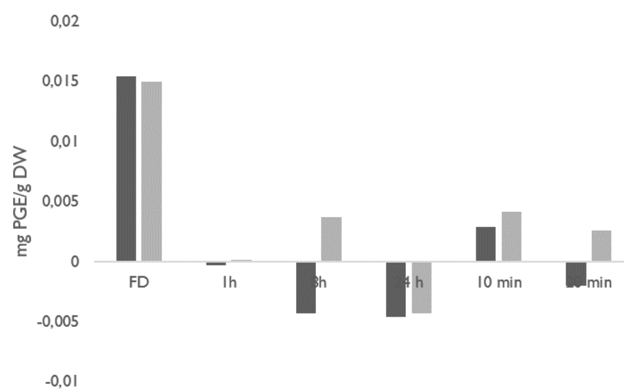


Figure 3.21: Phenols in acetonic extracts of *S. latissima* harvested in April 2017. The seaweed was freeze-dried and then rehydrated (1 h, 8 h, 24 h) or boiled (10 min, 20 min). The values are from both duplicate extractions with triplicate measurements ± SD.

on the day of the extraction and analysis to limit degradation during storage. The extractions were prepared in triplicate. Figure 3.22 shows the phenolic content of the acetonic extracts of freeze-dried, rehydrated and boiled *S. latissima*.

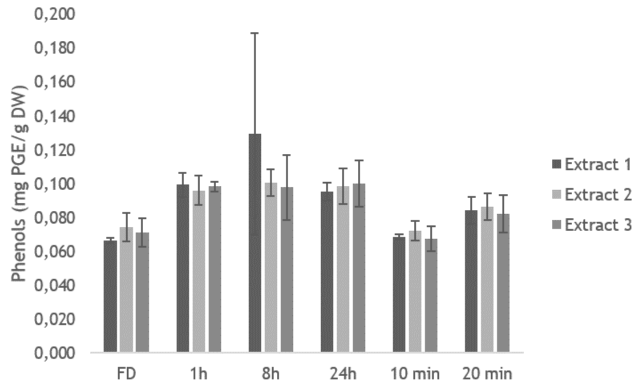


Figure 3.22: Phenols in *S. latissima* harvested in May 2016, treated by freeze-drying (FD), rehydration (1 h, 8 h, 24 h) and boiling (10 min, 20 min). Extracts and absorbance readings were done in triplicate. Values are the means ± SD.

The mean phenolic content of the three extracts is included in Table 3.7. The phenolic contents after rehydration and boiling treatments were not significantly different ($p > 0.05$). This indicates that rehydration or boiling are processing steps that can reduce the iodine content without decreasing the beneficial phenolic content of *S. latissima*.

Table 3.7: Phenols in *S. latissima* harvested in May 2016, treated by freeze-drying (FD), rehydration (1 h, 8 h, 24 h) and boiling (10 min, 20 min). Extracts and absorbance readings were done in triplicate. Values are the means ± SD.

Treatment	Phenols (mg PGE/g DW)
FD	0.071 ± 0.007
1 h RH	0.098 ± 0.006
8 h RH	0.10 ± 0.01
24 h RH	0.098 ± 0.009
10 min B	0.070 ± 0.005
20 min B	0.084 ± 0.008

3.6 Fucoidans

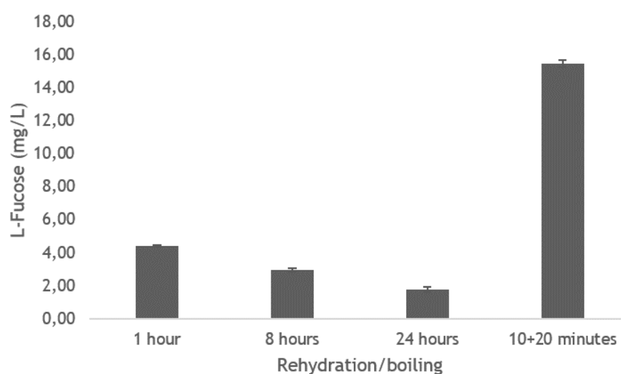


Figure 3.23: L-Fucose concentration (mg/L) in the waste water remaining following rehydration and boiling of freeze dried *S. latissima* harvested in May 2016.

Rehydration and boiling will extract water soluble compounds from the seaweed. The waste water remaining following rehydration of dried *S. latissima* may be a potential source of fucoidan. In order to assess this hypothesis, the L-fucose content of the waste water (5 g DW per 500 mL deionised water) was quantified and included in Figure 3.23. The absorption values are included in the appendix. The seaweed was rehydrated and boiled separately.

The 1 h extraction yielded $4.41 \pm 0.04\,\mathrm{mg\,L^{-1}}$. The next extracted fraction had $2.96 \pm 0.08\,\mathrm{g\,L^{-1}}$, and finally $1.7 \pm 0.2\,\mathrm{g\,L^{-1}}$ after 24 h. The pooled boiling water sampled after 10 and 20 min had the highest L-fucose concentration with $15.4 \pm 0.2\,\mathrm{g\,L^{-1}}$. In order to obtain sufficiently accurate results, the absorbance difference A_1-A_2 must be higher than 0.100. This was only achieved for the boiling treatment. It would have been interesting to analyse the pooled rehydration extracts as the rehydration water was replaced with fresh deionised water in between sampling points. The concentration can be calculated to mg L-fucoidan per g DW seaweed for each fraction and summarised. Doing so in Figure 3.24 reveals that the 10+20 min boiling extracts contain 169.5 % more L-fucose than rehydrating at room temperature for 24 h.

Although low yields are discouraging for biorefinery purposes, it is positive that not all of the fucoidan is lost during rehydration from a nutritional perspective. The potential of extracting water-soluble fucoidan is confirmed, but the extraction protocol must be optimised. Elevated temperatures are a good place to start.

A summary of the DW content, iodine, polyphenols and fucoidan results is given in Table 3.8.

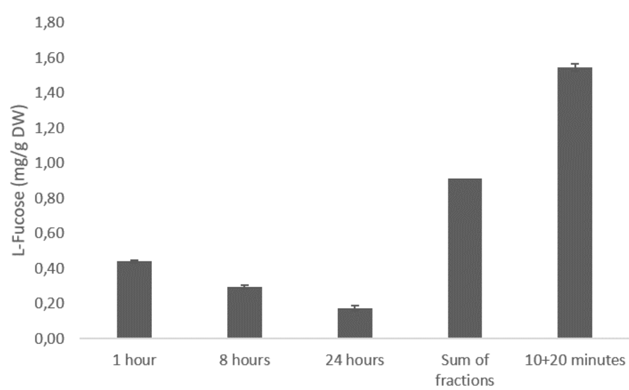


Figure 3.24: L-Fucose content (mg/g DW) in the waste water remaining following rehydration and boiling of freeze dried *S. latissima* harvested in May 2016.

Table 3.8: Dry weight (DW), iodine, phenolic and L-fucose content of *S. latissima* harvested in May 2016 and April 2017. The samples were air-dried (AD) or freeze-dried (FD), then rehydrated (RH), boiled following rehydration (R-B), or boiled following freeze-drying (B). Iodine contents were found by alkaline extraction in an oven or by microwave-assisted extraction (MWA). The phenols were determined on two separate laboratory days for the 2016 harvest, hence the two columns. The L-fucose content was found for rehydration or boiling waste water (5 g macroalgae per 500 mL deionised water). Values are the means ± SD.

Harvest date	Treatment	DW (%)	I (MWA) (mg/kg DW)	I (oven) (mg/kg DW)	Phenols[a] (mg PGE/g DW)	L-Fucose (mg/g DW)
2016	AD	10.30	5844 ± 665	n.a	0.06 ± 0.04	n.a
	FD	10.30	4357 ± 498	3802 ± 123	0.071 ± 0.007	n.a
	1 h RH	6.5 ± 0.1	1710 ± 288	1709 ± 86	0.098 ± 0.006	0.441 ± 0.004
	8 h RH	4.9 ± 0.4	1881 ± 82	1138 ± 128	0.10 ± 0.01	0.296 ± 0.008
	24 h RH	4.2 ± 0.6	1722 [b]	1154 ± 24	0.098 ± 0.009	0.17 ± 0.02
	10 min R-B	2.83 ± 0.01	777 [b]	n.a	0.073 ± 0.008	n.a
	20 min R-B	2.8 ± 0.1	462 ± 7	n.a	0.07 ± 0.02	n.a
	10 min B	6 ± 3	n.a	914 ± 82	0.070 ± 0.005	n.a
	20 min B	4.801 ± 0.006	n.a	907 ± 20	0.084 ± 0.008	n.a
	10+20 min B	n.a	n.a	n.a	n.a	1.54 ± 0.02
2017	FD	9 ± 1	n.a	2930 ± 168	0.015 ± 0.002	n.a
	1 h RH	3.8 ± 0.3	n.a	2975 ± 16	0.000 ± 0.008	n.a
	8 h RH	4.3 ± 0.3	n.a	2484 ± 367	0.000 ± 0.007	n.a
	24 h RH	4.3 ± 0.1	n.a	2079 ± 39	−0.004 ± 0.005	n.a
	10 min B	6.2 ± 0.3	n.a	1425 ± 50	0.004 ± 0.003	n.a
	20 min B	5 ± 2	n.a	1381 ± 505	0.000 ± 0.008	n.a

[a] The phenolic content in *S. latissima* harvested in 2017 is inaccurate because the samples had decomposed.
[b] No SD as overpressurisation led to loss of sample duplicate.

Chapter 4

Conclusion

For this thesis, iodine contents of *S. latissima* (harvested May 2016 and April 2016) were investigated after dehydration by freeze-drying and air-drying, as well as rehydration and boiling treatment. Three methods of macroalgal decomposition and iodine extraction were employed: microwave-assisted TMAH extraction, oven TMAH extraction and UltraClave nitric acid digestion. The methods highlight some of the issues linked to iodine quantification. The formation of volatile iodine species in acidic solutions can lead to an underestimation of iodine, as well as an overestimation due to memory effects in the ICP-MS. The iodine contents were compared with either the product declaration for the 2016 macroalgae harvest, or with an iodine quantification performed on the 2017 harvest by NIFES.

Oven alkaline extraction with TMAH gave the most reliable result with the laboratory equipment that was available. Freeze-dried *S. latissima* harvested May 2016 and April 2017 had $3802 \pm 123\,\mathrm{mg\,kg^{-1}}$ and $2930 \pm 168\,\mathrm{mg\,kg^{-1}}$ on a DW basis, respectively. Rehydration reduced the iodine content mainly during the first hour. Boiling reduced the iodine content mainly within the first ten minutes. The DW content was reduced during rehydration and boiling treatments, but the iodine loss did not correspond with the DW loss, which signifies that the water treatment extracts iodine from the matrix.

The iodine RDI is satisfied with the consumption of $0.04\,\mathrm{g\,d^{-1}}$ freeze-dried *S. latissima* based on the May 2016 harvest analysed by alkaline oven extraction and ICP-MS. No more than $0.16\,\mathrm{g\,d^{-1}}$ can be consumed to stay within the iodine UL. In its freeze-dried form, *S. latissima* can contain 190 times as much iodine as the recommended maximum for dried algae products given by the German Federal Institute for Risk Assessment (Bundesinstitut für Risikobewertung, 2007). The French Food Safety Agency are less conservative, and recognise that the iodine content can be reduced by cooking the macroalgae, recommending that the iodine content does not exceed $2000\,\mathrm{mg\,kg^{-1}}$. Boiling freeze-dried *S. latissima* for 10 minutes can decrease the iodine content so that $10\,\mathrm{g\,d^{-1}}$ on a FW basis can be consumed without exceeding the UL. In light of the report from the Council of Nutrition in Norway, advising that measures towards ensuring adequate iodine consumption must be taken, *S. latissima* could be a healthy supplement in small amounts.

Polyphenols are a class of antioxidants richly present in macroalgae. Phlorotannins

and fucoxanthin are polyphenols that may have anti-diabetic, anti-carcinogenic, and anti-inflammatory activities (Maeda et al., 2009; Xia et al., 2013; Tan and Hou, 2014; Takahashi et al., 2015). During preservation and cooking, the aim should be to preserve the polyphenols so that their possible health benefits are retained. This study extracted polyphenols with aqueous acetone (80:20 v/v acetone:deionised water) and used Folin-Ciocalteu's method to quantify the polyphenolic content in mg propyl gallate equivalents (PGE) per g DW. The most important parameters for reliable results were found to be the incubation time with Folin-Ciocalteu's reagent, and accurate DW measurements for each sampling point. It was also crucial that the acetonic extracts were prepared and analysed within 1 day of the rehydration and boiling treatment. Otherwise, the macroalgae will decompose. The rehydration and boiling treatments did not significantly reduce the polyphenol content. It could indeed be that the antioxidant activity of polyphenols is initially increased by rehydration and heat treatment. This indicates that 10 min boiling of *S. latissima* is advisable prior to consumption both to reduce the iodine content and increase the antioxdiant activity of the polyphenols.

As well as having a nutritional potential, *S. latissima* could be used as marine biomass in a biorefinery setup. The aim of biorefinery is to sequentially extract compounds with a higher market value than the biomass feed, producing as little waste as possible. Fucoidans are a diverse class of sulfated polysaccharides unique to brown algae that may be anti-carcinogenic, immunomodulatory, anti-inflammatory, antiviral, antithrombotic and anticoagulant (Ale et al., 2011). This study evaluated the potential of extracting fucoidans with deionised water at room temperature and by boiling. As fucoidans are challenging to quantify as whole structures, acid hydrolysis was used to free L-fucose, which could be quantified spectrophotometrically. Pooled supernatants after 10 min and 20 min boiling had the highest L-fucose content, confirming the possibility of extracting fucoidans with deionised water at elevated temperatures. This can have value for developing biorefinery protocols for macroalgae, where water-soluble compounds such as fucoidans are extracted prior to compounds requiring harsher conditions.

Chapter 5

Future research

In this project, different methods for quantifying iodine were explored. Oven extraction using TMAH gave a 93.88 % recovery compared to the results from NIFES, an accredited analytical laboratory. The variation of iodine as *S. latissima* is freeze-dried, rehydrated and boiled was examined. The quantification method relied on sufficiently small particle sizes to enable total iodine extraction, and as such the macroalgae had to be milled prior to rehydration and boiling. It would be valuable to develop a method that does not destroy the macroalgal tissue, effectively changing the rehydration kinetics. Furthermore, the method cannot be used on fresh macroalgae because of the small particle size requirement. Quantification of iodine in fresh macroalgae is necessary to evaluate the recommended portion size to comply with iodine UL. To achieve this, the fresh biomass must be broken down, for example by enzymatic digestion of cellulose and alginate using cellulase and alginate lyase. Given by how much the iodine quantification varies depending on which decomposition method is employed, a standardised macroalgae decomposition method should be defined.

The main loss of iodine occurred within the first hour of rehydration or the first ten minutes of boiling. It could be that the iodine is primarily lost within even shorter treatment times, which should be examined to empirically determine iodine loss kinetics.

There are several drying methods, and this step requires more attention as it represents a huge energy input in the seaweed market value chain. Once the iodine can be quantified for fresh macroalgae, an evaluation of the best drying method with respect to reduction of iodine can be made. Other preservation methods like salting and fermentation can change the flavour profile for a different end product. These preservation methods are likely to influence the iodine content, but there is little or no research on how salting or fermentation possibly reduces the iodine content.

The in vivo bioavailability of macroalgal iodine species must be studied. It would be interesting to examine how goitrogenic foods regulate iodine uptake by the thyroid and based on that develop recipes including both. Risk-assessment of he upper intake level should be further evaluated for healthy individuals.

How does rehydration and boiling affect the quality of the macroalgal product? More

knowledge on the nutritional content and their bioavailability. The taste and smell. Mechanical and textural properties can affect the consumer, as well as any change in colour or appearance. Evaluate whole structures. Investigate shorter treatment times, as the main iodine loss occurs within the first hour or the first 10 min of boiling.

This study only briefly touched upon biorefinery and fucoidans. This is a field that is likely to be central to the Norwegian bioeconomy, but it requires the development of macroalgal biorefinery protocols. The variation of the chemical composition in macroalgae is a challenge. A systematic mapping of the seasonal and geographical variations would help in making decisions on when and where to harvest macroalgae for biorefinery.

Bibliography

Adamczyk, B., Salminen, J. P., Smolander, A., and Kitunen, V. (2012). Precipitation of proteins by tannins: Effects of concentration, protein/tannin ratio and pH. *International Journal of Food Science and Technology*, 47(4):875–878.

Agence Française de Sécurité Sanitaire des Aliments (2009). Avis de l'Agence Française de Sécurité Sanitaire des Aliments relatif à la teneur maximale en arsenic inorganique recommandée pour les algues laminaires et aux modalités de consommation de ces algues compte tenu de leur teneur élevée en iode. Saisine n° 2.

Åkerlöf, G. (1932). Dielectric constants of some organic solvent-water mixtures at varioius temperatures. *Journal of the American Chemical Society*, 54(11):4125–4139.

Alamed, J., Chaiyasit, W., McClements, D. J., and Decker, E. A. (2009). Relationships between free radical scavenging and antioxidant activity in foods. *Journal of Agricultural and Food Chemistry*, 57(7):2969–2976.

Albright, P. S. and Gosting, L. J. (1946). Dielectric Constants of the Methanol-Water System from 5 to 55°C. *Journal of the American Chemical Society*, 68(6):1061–1063.

Ale, M. T. and Meyer, A. S. (2013). Fucoidans from brown seaweeds: an update on structures, extraction techniques and use of enzymes as tools for structural elucidation. *RSC Advances*, 3(22):8131–8141.

Ale, M. T., Mikkelsen, J. D., and Meyer, A. S. (2011). Important determinants for fucoidan bioactivity: A critical review of structure-function relations and extraction methods for fucose-containing sulfated polysaccharides from brown seaweeds. *Marine Drugs*, 9(10):2106–2130.

Amorim-Carrilho, K., Lage-Yusty, M. A., and López-Hernández, J. (2014). Variation of bioactive compounds in dried seaweed Himanthalia elongata subjected to different culinary processes. *CyTA - Journal of Food*, 12(4):336–339.

Atwood, J. L., Davies, J. E. D., and Osa, T. (1985). *Clathrate Compounds, Molecular Inclusion Phenomena, and Cyclodextrins : Proceedings of the Third International Sym-*

posium on Clathrate Compounds and Molecular Inclusion Phenomena and the Second International Symposium on Cyclodextrins, Tokyo, Japan, July 2. Springer Netherlands.

Badocco, D., Romanini, F., Di Marco, V., Mondin, A., and Pastore, P. (2017). Formation of volatile iodine compounds under hot concentrated acid conditions (nitric acid or aqua regia) and in diluted acid solutions with or without thiocyanate.

Bilan, M. I., Grachev, A. A., Shashkov, A. S., Kelly, M., Sanderson, C. J., Nifantiev, N. E., and Usov, A. I. (2010). Further studies on the composition and structure of a fucoidan preparation from the brown alga Saccharina latissima. *Carbohydrate Research*, 345:2038–2047.

Bradshaw, S. M., Wyk, E. J. V., and Swardt, J. B. D. (1998). Microwave heating principles and the application to the regeneration of granular activated carbon. *The Journal of the South African Institute of Mining and Metallurgy*, (August):201–212.

Bruhn, A., Tørring, D., Thomsen, M., Canal-Vergés, P., Nielsen, M., Rasmussen, M., Eybye, K., Larsen, M., Balsby, T., and Petersen, J. (2016). Impact of environmental conditions on biomass yield, quality, and bio-mitigation capacity of Saccharina latissima. *Aquaculture Environment Interactions*, 8:619–636.

Bundesinstitut für Risikobewertung (2007). Gesundheitliche Risiken durch zu hohen Jodgehalt in getrockneten Algen. Technical Report 026, Bundesinstitut für Risikobewertung.

Combet, E., Ma, Z. F., Cousins, F., Thompson, B., and Lean, M. E. J. (2014). Low-level seaweed supplementation improves iodine status in iodine-insufficient women. *British Journal of Nutrition*, 112(05):753–761.

Cox, S., Gupta, S., and Abu-Ghannam, N. (2012). Effect of different rehydration temperatures on the moisture, content of phenolic compounds, antioxidant capacity and textural properties of edible Irish brown seaweed. *LWT - Food Science and Technology*, 47(2):300–307.

Dahl, L., Johansson, L., Julshamn, K., and Meltzer, H. M. (2004). The iodine content of Norwegian foods and diets. *Public health nutrition*, 7(4):569–576.

Davis, T. A., Volesky, B., and Mucci, A. (2003). A review of the biochemistry of heavy metal biosorption by brown algae. *Water Research*, 37(18):4311–4330.

de Benoist, B., Andersson, M., Egli, I., Takkouche, B., and Allen, H. (2004). Iodine status worldwide. *WHO Global Database on Iodine Deficiency. Geneca: World Health Organization*, pages 1–12.

Deniaud-Bouët, E., Kervarec, N., Michel, G., Tonon, T., Kloareg, B., and Hervé, C. (2014). Chemical and enzymatic fractionation of cell walls from Fucales: Insights into the structure of the extracellular matrix of brown algae. *Annals of Botany*, 114(6):1203–1216.

Eastman, C. J. and Zimmermann, M. (2009). *The Iodine Deficiency Disorders*. Number 2.

Eng, P. H. K., Cardona, G. R., Fang, S. L., Previti, M., Alex, S., Carrasco, N., Chin, W. W., and Braverman, L. E. (1999). Escape from the acute Wolff-Chaikoff effect is associated with a decrease in thyroid sodium/iodide symporter messenger ribonucleic acid and protein. *Endocrinology*, 140(8):3404–3410.

Erdogan, M. F. (2003). Thiocyanate overload and thyroid disease. *BioFactors*, 19(3-4):107–111.

European Food Safety Authority (2006). *Tolerable Upper Intake Levels for Vitamins and Minerals*. Number February.

Fecher, P. A., Goldmann, I., and Nagengast, A. (1998). Determination of iodine in food samples by inductively coupled plasma mass spectrometry after alkaline extraction. *Journal of Analytical Atomic Spectrometry*, 13(9):977–982.

Gamallo-Lorenzo, D., Barciela-Alonso, M. D. C., Moreda-Piñeiro, A., Bermejo-Barrera, A., and Bermejo-Barrera, P. (2005). Microwave-assisted alkaline digestion combined with microwave-assisted distillation for the determination of iodide and total iodine in edible seaweed by catalytic spectrophotometry. *Analytica Chimica Acta*, 542(2):287–295.

Gerasimenko, N. and Logvinov, S. (2016). Seasonal Composition of Lipids, Fatty Acids Pigments in the Brown Alga Sargassum pallidum: The Potential for Health. *Open Journal of Marine Science*, 06(04):498–523.

Guo, B., Liu, B., Yang, B., Sun, P., Lu, X., Liu, J., and Chen, F. (2016). Screening of diatom strains and characterization of Cyclotella cryptica as a potential fucoxanthin producer. *Marine Drugs*, 14(7):125.

Haldimann, M., Zimmerli, B., Als, C., and Gerber, H. (1998). Direct determination of urinary iodine by inductively coupled plasma mass spectrometry using isotope dilution with iodine-129. *Clinical Chemistry*, 44(4):817–824.

Haugan, J. A. and Liaaen-Jensen, S. (1989). Improved isolation procedure for fucoxanthin. *Phytochemistry*, 28(10):2797–2798.

Haugan, J. A. and Liaaen-Jensen, S. (1994). Algal carotenoids 54. Carotenoids of brown algae (Phaeophyceae). *Biochemical Systematics and Ecology*, 22(1):31–41.

Indergaard, M. (2010). *Tang og tare-i hovedsak norske brunalger: Forekomster, forskning og anvendelse*.

Institute of Medicine and Food and Nutrition Board (2001). *Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc*. National Academies Press, Washington, D.C.

Jeon, Y.-J., Wijesinghe, W. A. J. P., and Kim, S.-K. (2009). Properties of Brown Algae and Its Industrial Applications. *Marine Cosmeceuticals*, pages 305–318.

Julshamn, K., Dahl, L., and Eckhoff, K. (2001). Determination of iodine in seafood by inductively coupled plasma/mass spectrometry. *Journal of AOAC International*, 84(6):1976–1983.

Kim, H. S., Lee, C.-G., and Lee, E. Y. (2011). Alginate lyase: Structure, property, and application. *Biotechnology and Bioprocess Engineering*, 16(5):843–851.

Küpper, F. C., Carpenter, L. J., McFiggans, G. B., Palmer, C. J., Waite, T. J., Boneberg, E.-M., Woitsch, S., Weiller, M., Abela, R., Grolimund, D., Potin, P., Butler, A., Luther, G. W., Kroneck, P. M. H., Meyer-Klaucke, W., Feiters, M. C., and Feiters, M. C. (2008). Iodide accumulation provides kelp with an inorganic antioxidant impacting atmospheric chemistry. *Proceedings of the National Academy of Sciences of the United States of America*, 105(19):6954–8.

Larsen, E. H. and Ludwigsen, M. B. (1997). Determination of iodine in food-related certified reference materials using wet ashing and detection by inductively coupled plasma mass spectrometry. *Journal of Analytical Atomic Spectrometry*, 12(4):435–439.

Laturnus, F., Svensson, T., and Wiencke, C. (2010). Release of reactive organic halogens by the brown macroalga Saccharina latissima after exposure to ultraviolet radiation. *Polar Research*, 29(3):379–384.

Leblanc, C., Colin, C., Cosse, A., Delage, L., La Barre, S., Morin, P., Fiévet, B., Voiseux, C., Ambroise, Y., Verhaeghe, E., Amouroux, D., Donard, O., Tessier, E., and Potin, P. (2006). Iodine transfers in the coastal marine environment: the key role of brown algae and of their vanadium-dependent haloperoxidases. *Biochimie*, 88(11):1773–1785.

Lee, K. T., Farid, M., and Nguang, S. K. (2006). The mathematical modelling of the rehydration characteristics of fruits. *Journal of Food Engineering*, 72(1):16–23.

Leung, A. M. and Braverman, L. E. (2013). Consequences of excess iodine. *Nature Reviews Endocrinology*, 10(3):136–142.

Lüning, K. and Mortensen, L. (2015). European aquaculture of sugar kelp (Saccharina latissima) for food industries: Iodine content and epiphytic animals as major problems. *Botanica Marina*, 58(6):449–455.

Maeda, H., Hosokawa, M., Sashima, T., Murakami-Funayama, K., and Miyashita, K. (2009). Anti-obesity and anti-diabetic effects of fucoxanthin on diet-induced obesity conditions in a murine model. *Molecular Medicine Reports*, 2(6):897–902.

Majid, S., Khanduja, K. L., Gandhi, R. K., Kapur, S., and Sharma, R. R. (1991). Influence of ellagic acid on antioxidant defense system and lipid peroxidation in mice. *Biochemical Pharmacology*, 42(7):1441–1445.

Manns, D., Nielsen, M. M., Bruhn, A., Saake, B., and Meyer, A. S. (2017). Compositional variations of brown seaweeds Laminaria digitata and Saccharina latissima in Danish waters. *Journal of Applied Phycology*, 29:1439–1506.

Markou, K., Georgopoulos, N., Kyriazopoulou, V., and Vagenakis, A. (2001). Iodine-Induced Hypothyroidism. *Thyroid*, 11(5):501–510.

Meltzer, H. M., Torheim, L. E., Brantsæter, A. L., Madar, A., Abel, M. H., and Dahl, L. (2016). Risiko for jodmangel i Norge: Identifisering av et akutt behov. Technical report, Nasjonalt råd for ernæring.

Mikami, K. and Hosokawa, M. (2013). Biosynthetic pathway and health benefits of fucoxanthin, an algae-specific xanthophyll in brown seaweeds.

Miller, N. J., Sampson, J., Candeias, L. P., Bramley, P. M., and Rice-Evans, C. A. (1996). Antioxidant activities of carotenes and xanthophylls. *FEBS Letters*, 384(3):240–242.

Minister of Health Labour and Welfare Japan (2015). Overview of Dietary Reference Intakes for Japanese. Technical Report 103.

Moy, F. E. and Christie, H. (2012). Large-scale shift from sugar kelp (Saccharina latissima) to ephemeral algae along the south and west coast of Norway. *Marine Biology Research*, 8(4):309–321.

Niedobová, E., MacHát, J., Kanický, V., and Otruba, V. (2005). Determination of iodine in enriched chlorella by ICP-OES in the VUV region. *Microchimica Acta*, 150(2):103–107.

Nitschke, U. and Stengel, D. B. (2014). Iodine contributes to osmotic acclimatisation in the kelp Laminaria digitata (Phaeophyceae). *Planta*, 239(2):521–530.

Nitschke, U. and Stengel, D. B. (2016). Quantification of iodine loss in edible Irish seaweeds during processing. *Journal of Applied Phycology*, 28(6):3527–3533.

Nóbrega, J. A., Santos, M. C., de Sousa, R. A., Cadore, S., Barnes, R. M., and Tatro, M. (2006). Sample preparation in alkaline media. *Spectrochimica Acta - Part B Atomic Spectroscopy*, 61(5):465–495.

Nødland, L. K. (2014). *Utvikling og validering av metode for bestemmelse av jod i urin fra mor og barn ved hjelp av trippel kvadrupol induktivt koplet plasma massespektrometri (ICP-MS-QQQ)*. PhD thesis, Norges miljø- og biovitenskapelige universitet.

Nwosu, F., Morris, J., Lund, V. A., Stewart, D., Ross, H. A., and McDougall, G. J. (2011). Anti-proliferative and potential anti-diabetic effects of phenolic-rich extracts from edible marine algae. *Food Chemistry*, 126(3):1006–1012.

Oliveira, A. A., Trevizan, L. C., and Nóbrega, J. A. (2010). Iodine Determination by Inductively Coupled Plasma Spectrometry. *Applied Spectroscopy Reviews*, 45(6):447–473.

Pádua, D., Rocha, E., Gargiulo, D., and Ramos, A. A. (2015). Bioactive compounds from brown seaweeds: Phloroglucinol, fucoxanthin and fucoidan as promising therapeutic agents against breast cancer.

Poppiti, J. A. and Sellers, C. (1994). *Practical Techniques for Laboratory Analysis*. Lewis Publishers.

Prior, R. L., Wu, X., and Schaich, K. (2005). Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements.

Quartino, M. L., Vazquez, S. C., Latorre, G. E., and Mac Cormack, W. P. (2015). Possible role of bacteria in the degradation of macro algae Desmarestia anceps Montagne (Phaeophyceae) in Antarctic marine waters. *Revista Argentina de Microbiología*, 47(3):274–276.

Rajauria, G., Jaiswal, A. K., Abu-Gannam, N., and Gupta, S. (2013). Antimicrobial, antioxidant and free radical-scavenging capacity of brown seaweed himanthalia elongata from western coast of Ireland. *Journal of Food Biochemistry*, 37(3):322–335.

Rezaei, S., Rezaei, K., Haghighi, M., and Labbafi, M. (2013). Solvent and solvent to sample ratio as main parameters in the microwave-assisted extraction of polyphenolic compounds from apple pomace. *Food Science and Biotechnology*, 22(5):1–6.

Robledo, D. and Freile-Pelegriín, Y. (2013). Bioactive Phenolic Compounds from Algae In: Bioactive Compounds from Marine Foods: Plant and Animal Sources, First Edition. Edited by Blanca Hernández-Ledesma and Miguel Herrero. In Hernández-Ledesma, B. and Herrero, M., editors, *Bioactive Compounds from Marine Foods: Plant and Animal Sources*, chapter 6, pages 113–129. Wiley, Yucatán, Mexico, 1st edition.

Romarís–Hortas, V., García-Sartal, C., Barciela-Alonso, M. d. C., Domínguez-González, R., Moreda-Piñeiro, A., and Bermejo-Barrera, P. (2011). Bioavailability study using an in-vitro method of iodine and bromine in edible seaweed. *Food Chemistry*, 124(4):1747–1752.

Sachindra, N. M., Sato, E., Maeda, H., Hosokawa, M., Niwano, Y., Kohno, M., and Miyashita, K. (2007). Radical scavenging and singlet oxygen quenching activity of marine carotenoid fucoxanthin and its metabolites. *Journal of Agricultural and Food Chemistry*, 55(21):8516–8522.

Sandell, E. and Kolthoff, I. (1934). Chronometric catalytic method for the determination of micro quantities of iodine. *Journal of the American Chemical Society*, 56(6):1426.

Sathya, R., Kanaga, N., Sankar, P., and Jeeva, S. (2013). Antioxidant properties of phlorotannins from brown seaweed Cystoseira trinodis (Forsskål) C. Agardh. *Arabian Journal of Chemistry*.

Schiener, P., Black, K. D., Stanley, M. S., and Green, D. H. (2014). The seasonal variation in the chemical composition of the kelp species Laminaria digitata, Laminaria hyperborea, Saccharina latissima and Alaria esculenta. *Journal of Applied Phycology*, 27(1):363–373.

Shang, Y. F., Kim, S. M., Lee, W. J., and Um, B. H. (2011). Pressurized liquid method for fucoxanthin extraction from Eisenia bicyclis (Kjellman) Setchell. *Journal of Bioscience and Bioengineering*, 111(2):237–241.

Steen, H., Bodvin, T., and Moy, F. (2016). God gjenvekst av stortare etter prøvehøsting i Nordland. *Havforskningsrapporten*, pages 63–65.

Stein-Taylor, J. R. and Phycological Society of America. (1973). *Handbook of phycological methods.* University Press.

Stévant, P., Marfaing, H., Fleurence, J., Rustad, T., Sandbakken, I., and Chapman, A. (2017a). Cadmium, iodine and inorganic arsenic levels in edible seaweeds sugar kelp (Saccharina latissima) and winged kielp (Alaria esculenta): status and possible remediation processes. *Pending manuscript*.

Stévant, P., Marfaing, H., Rustad, T., Sandbakken, I., Fleurence, J., and Chapman, A. (2017b). Nutritional value of the kelps Alaria esculenta and Saccharina latissima and effects of short-term storage on biomass quality.

Takahashi, K., Hosokawa, M., Kasajima, H., Hatanaka, K., Kudo, K., Shimoyama, N., and Miyashita, K. (2015). Anticancer effects of fucoxanthin and fucoxanthinol on colorectal cancer cell lines and colorectal cancer tissues. *Oncology Letters*, 10(3):1463–1467.

Tan, C. P. and Hou, Y. H. (2014). First evidence for the anti-inflammatory activity of fucoxanthin in high-fat-diet-induced obesity in mice and the antioxidant functions in PC12 cells. *Inflammation*, 37(2):443–450.

Teas, J., Pino, S., Critchley, A., and Braverman, L. E. (2004). Variability of Iodine Content in Common Commercially Available Edible Seaweeds. *Thyroid*, 14(10):836–841.

Thermo Scientific (2008). High Performance Thermo Scientific ELEMENT 2/XR High Performance High Resolution ICP-MS Thermo Scientific ELEMENT 2/XR.

Werner, T. (2013). *Presence of phenolic compounds in acetone extracts from Saccharina latissima and their antibacterial and ferrous ion-chelating activities*. PhD thesis, Chalmers University of Technology.

Wifladt, A. M., Lund, W., and Bye, R. (1989). Determination of iodine in seaweed and table salt by an indirect atomic-absorption method. *Talanta*, 36(3):395–399.

Xia, S., Wang, K., Wan, L., Li, A., Hu, Q., and Zhang, C. (2013). Production, characterization, and antioxidant activity of fucoxanthin from the marine diatom odontella aurita. *Marine Drugs*, 11(7):2667–2681.

Yeh, T. S., Hung, N. H., and Lin, T. C. (2014). Analysis of iodine content in seaweed by GC-ECD and estimation of iodine intake. *Journal of Food and Drug Analysis*, 22(2):189–196.

Zava, T. T. and Zava, D. T. (2011). Assessment of Japanese iodine intake based on seaweed consumption in Japan: A literature-based analysis. *Thyroid Research*, 4(1):14.

Appendix A

Harvest site data

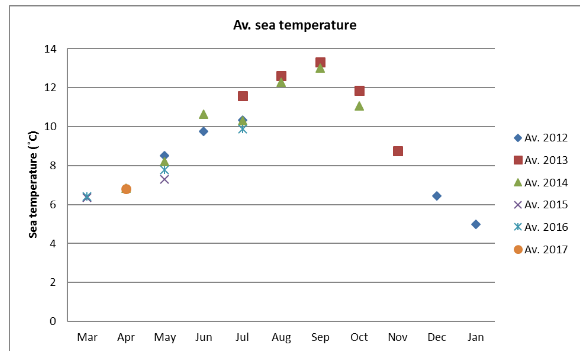


Figure A.1: Average sea temperatures measured at the cultivation site on Tareskjæret, Frøya, Norway.

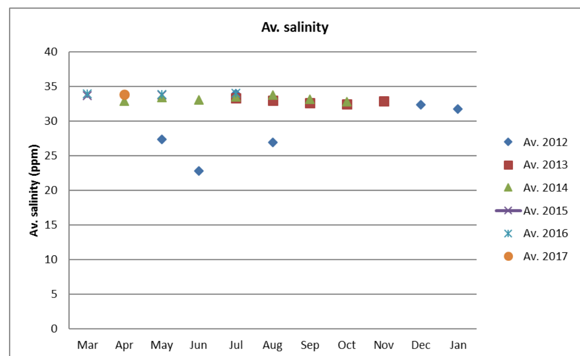


Figure A.2: Average salinity measurements from the cultivation site on Tareskjæret, Frøya, Norway.

Appendix B

Nitric acid decomposition temperature profile

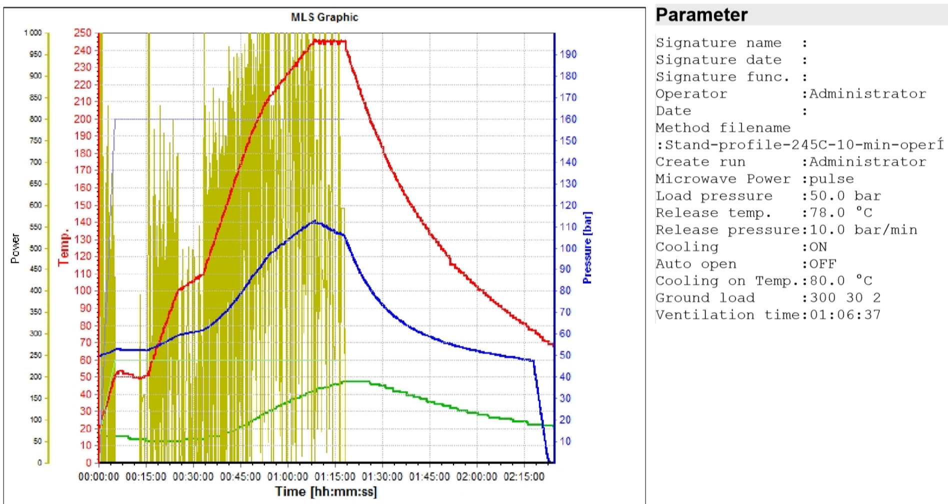


Figure B.1: The UltraClave temperature profile during nitric acid decomposition of *S. latissima*.

Appendix C

Standard curves

The propyl gallate standard curves were used to calculate the phenolic content in mg PGE/g DW. The absorbance values should be in the linear range and between 0-1 to comply with Beer's law. The first standard curve in Figure C.1 had too high absorbance values, and so the PG concentration range was adapted. Figure C.2 uses values obtained by preparation of standard curves on 5 different days, each in triplicate. It was used to calculate the phenolic content of all the acetonic *S. latissima* extracts.

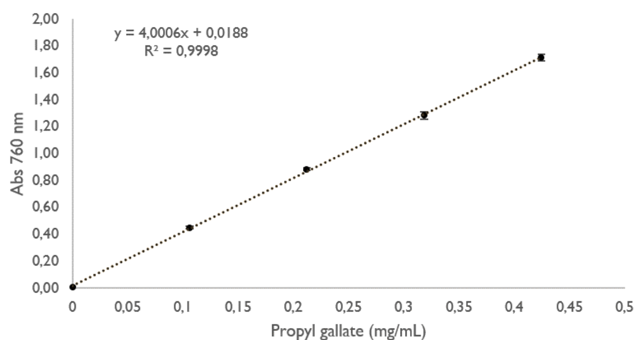


Figure C.1: Propyl gallate standard curve, concentration range 0 to 0.4244 mg L^{-1}.

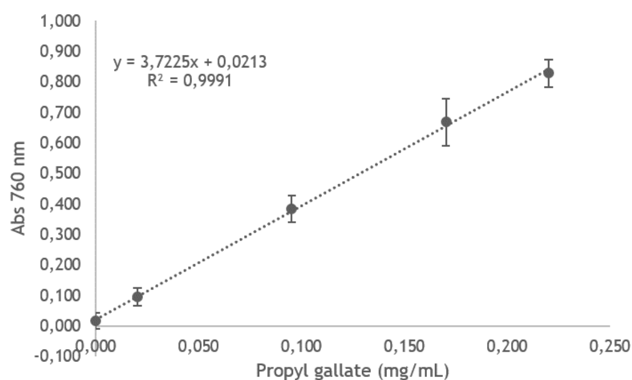


Figure C.2: Propyl gallate standard curve as the mean of standard curves prepared on 5 different days, each in triplicate. The concentration range was 0 to 0.220 mg L^{-1}.

C.1 Fucose determination

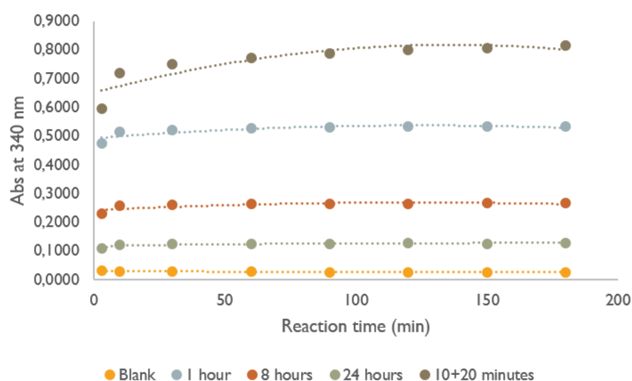


Figure C.3: Absorbance at 340 nm for reaction mixtures containing waste water collected from 1 h, 8 h, 24 h rehydration and 10+20 minutes pooled waste water. The first measurement was done prior to addition of enzyme (L-fucose dehydrogenase) and is A_1 according to the assay procedure provided by Megazyme. The final measurement was done when readings reached a plateau.

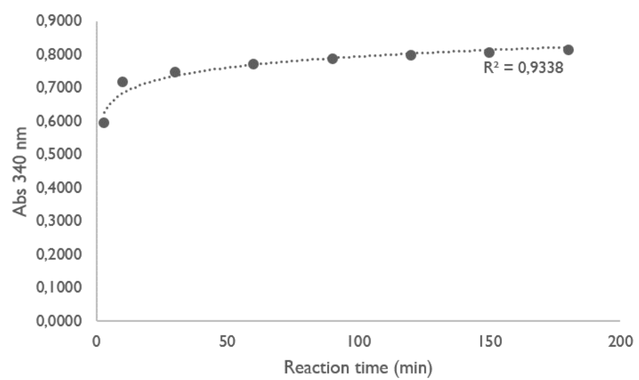


Figure C.4: Absorbance at 340 nm for reaction mixture containing the pooled waste water from 10 and 20 minutes boiling.

Seaweed Energy Solutions AS
Bynesveien 48
7018 Trondheim
Norway
www.seaweedenergysolutions.com

Issue date: 26.09.2016
No. of pages: 3

Raw material specification: *Saccharina latissima* (Sugar kelp)

Species: *Saccharina latissima*, whole plant

Origin: Cultivated and processed at Frøya, Norway

Nutritional values

Energy MJ/Kg dw	12.6
Energy kcal/100 g dw	301
Water content % (frozen product)	89.7

Main components (g/100 g dw)

Ash	37.9
Protein	13.2
Fat	2.9
Saturated fatty acids	1.4
Monounsaturated fatty acids	0.8
Polyunsaturated fatty acids	0.6
Total Carbohydrates	55.3
Dietary fiber	46.6
Salt (NaCl)	14.6

Minerals (mg/kg dw)

Macrominerals

Sodium (Na)	52000
Magnesium (Mg)	7500
Phosphorus (P)	1700
Sulfur (S)	7300
Potassium (K)	84000
Calcium (Ca)	10000

Trace elements

Lead (Pb)	0.11
Cadmium (Cd)	1.0
Mercury (Hg)	<1
Arsenic (As)	54
Nickel (Ni)	0.24
Chromium (Cr)	0.59
Iron (Fe)	39
Zinc (Zn)	27
Copper (Cu)	1.2
Selenium (Se)	<0.1
Iodine (I)	3670

Vitamins (mg/100 g dw)

Vitamin A (Retinol)	<1.17
Vitamin B1 (Thiamine)	<0.49
Vitamin B2 (Riboflavin)	<0.49
Vitamin B3 (Niacin)	1.84
Vitamin B6 (Pyridoxine)	0.17
Vitamin B9 (Folate)	0.29
Vitamin B12 (Cobalamin)	0.09
Vitamin C	<9.71
Vitamin E	<3.88

Amino acids (g/100 g dw)

Aspartic acid (Asp, D)	1.46
Glutamic acid (Glu, E)	1.55
Glycine (Gly, G)	0.68
Histidine (His, H)	<0.19
Arginine (Arg, R)	0.68
Isoleucine (Ile, I)	0.49
Leucine (Leu, L)	0.97
Lysine (Lys, K)	0.58
Methionine (Met, M)	0.29
Phenylalanine (Phe, F)	0.58
Proline (Pro, P)	0.58
Serine (Ser, S)	0.68
Threonine (Thr, T)	0.58
Tyrosine (Tyr, Y)	0.39
Valine (Val, V)	0.68
Alanine (Ala, A)	1.07
Cysteine (Cys, C)	<0.19

Analysis performed by Kystlab preBIO, Frøya, Norway (accredited analytical laboratory)

Microbiology (cfu/g dw)

Total Viable Count 30°C	<100
Enterobacteriaceae	<10
Coliform count	<10
Escherichia coli	<10
Coagulase-positive staphylococci	<10
Lactic acid bacteria	<100
Yeast and molds	<10
Presumptiv Bacillus cereus	<100
Anaerobic bacteria, suphite-reducing	<10
Clostridium perfringens	<10
Enterococcus	<10
Listeria monocytogenes	Not detected in 25 g
Salmonella bacteria	Not detected in 25 g

Analysis performed by Kystlab preBIO, Frøya, Norway (accredited analytical laboratory)

X

ERSTATTER

ANALYSEBEVIS

Oppdragsgiver:
NTNU Norges teknisk-naturvitenskapelige universitet
Realfagbygget
7491 Trondheim

Kontakt person: Nicoline Korsvold

PrøveID: 2017-682/1 **Rapportdato**: 19.06.2017 **Side 1 av 1**

Prøvemateriale: Tang
Prøvemerking: Saccharina latissima
Mottatt dato: 23.05.2017

Analysedato	Analyse	Parameter	Resultat	Benevnelse
16.06.2017	198	Jod	280	mg/kg

Metodikk:
198 - Jod bestemmelse med induktivt koblet plasma- massespektrofotometri (ICP-MS) etter våtoppslutning i mikrobølgeovn.

Analysebeviset er elektronisk godkjent av Marita Eide Kristoffersen

Resultatene er oppgitt i våtvekt, hvis annet ikke er spesifisert. Måleusikkerhet kan oppgis på forespørsel.
Analysebeviset kan ikke gjengis i utdrag, uten skriftlig godkjenning fra Nasjonalt institutt for ernærings- og sjømatforskning (NIFES).

Postadresse
NIFES
Postboks 2029 NORDNES
5817 BERGEN
NORWAY

Besøksadresse
NIFES
Strandgaten 229
N-5004 BERGEN
NORWAY

Varelevering
NIFES
Nordnesboder 2
5005 BERGEN
NORWAY

Telefon: 55 905100
Telefaks: 55 905299

Internet: www.nifes.no
E-post: postmottak@nifes.no
Org.no. NO 979529783