Processing of Seaweed and the Effects on Food Quality and Safety

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Processing of Seaweed

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PhD Thesis

by

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«Noen skrev en gang at planeten ikke burde hete Jorda. Den burde opplagt hete Havet.»
- Morten A. Strøknes

Preface

One of the most important lessons learned during my academic career is that communication is the key to science. Science is of no use if we do not communicate it in means that are understandable, or if we do not communicate it at all. The purpose of science writing is what you want your audience to understand [1]. So before writing the thesis, I had to ask: who is my audience?

My scientific work gives needed answers to questions from the seaweed industry. The scientific questions asked throughout the past four years have always been established based on the needs from the industry. Therefore, I want the results to be communicated towards a broad audience, so many have the chance to use the results, whether it be in their production, the development of the seaweed industry, regulations from authorities, or for other scientists. My vision of this thesis is to communicate the scientific results broadly.

I hope you find the thesis useful no matter who you are.

April 2023,

Kgs. Lyngby, Denmark

Cecilie Bay Wirenfeldt

Acknowledgement

To participate in the contribution of new knowledge for humankind to understand the different aspects of the world has been a lifelong dream of mine. During this journey, I understood that science is not a one-person achievement, it is teamwork and communities that strengthen it. Alone I can move a rock, but together we can literally move mountains. I wish to express my sincerest thanks to everyone involved in my PhD research.

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I'm forever grateful to my husband, Morten. It means the world to me to share nerdy passions and explore the universe with you. Thanks for our long discussions on physics, thermodynamics, mathematical modeling, and my lifelong subscription on Excel tips with you. You have taught me that the world is our oyster and time after time shown me that I can do things outside my comfort zone.

I owe my co-supervisors Aberham H. Feyissa, Grethe Hyldig, Jens J. Sloth, and Lisbeth Truelstrup Hansen great thanks for good fruitful discussions and teaching me skills and expertise within each of your respective fields from the first day I entered the doors of DTU.

My absolute greatest thanks go to my two main supervisors Susan Løvstad Holdt and Turid Rustad. You both were always there for me in good and tough times. Thanks to Susan for showing me the colorful world of seaweeds, opening endless opportunities, teaching me that everything is possible. You have been a great supervisor, colleague, and friend throughout the years. Thanks to Turid for keeping me grounded, and always having your door open for personal and professional talks, mentoring me with your half-a-century worth of knowledge and experience in academia. It has been a pleasure learning from you both for the past seven years!

Summary (English)

The potential of seaweed as a sustainable food source has gained increasing attention in recent years, driven by the fact that it can be cultivated in the sea, without taking up land areas or requiring freshwater. Additionally, seaweed can play a role in meeting the world's future need for food. However, as with most new food sources, seaweeds come with new challenges within post-harvest processing and ensuring food safety. This PhD project aims to study industrial post-harvest processes, such as drying, blanching, and washing of commercially available European seaweeds to ensure controlled, stable, and safe food products. The study has an industrial relevance, and the intention is to clarify good practices within each respective post-harvest process and species. The species in focus are sugar kelp, winged kelp, bladderwrack, sea lettuce, and dulse. The thesis is divided into four main chapters: vitamin C from seaweeds, blanching and washing of kelp, drying of bladder wrack and sea lettuce, and shelf-life of refrigerated sugar kelp.

Generalizing claims that seaweeds are rich in vitamins, are seen in both commercial promotion and scientific literature. Therefore, the vitamin C content of Northern European seaweed species are examined by a review and compared to commonly consumed foods, the recommended intake, and the possibility to claim Tance of vitamin C. Results showed that seaweed has lower vitamin C content (0.330-0.942 mg $(g\ dw)^{-1}$) than oysters, lettuce, potatoes, cucumber, broccoli, and rosehip (0.704-36.4 mg $(g\ dw)^{-1}$). This shows that seaweeds are not a rich source of vitamin C.

The presence of potential toxic elements such as arsenic, cadmium, and iodine as well as microbial load on sugar kelp and winged kelp are a matter of concern. The effects of washing and blanching on the levels of potential toxic elements are investigated, as well as their impact on desirable food qualities such as nutrient and bioactive content

and sensory properties such as color, texture, and odor. Blanching (45-80 °C for 30-120 s) can reduce the initial microbial load of 3.52-4.54 log (CFU g^{-1} wet weight) to 0.906-2.32 log (CFU g^{-1} wet weight). It also reduces the levels of potential toxic elements such as arsenic from 47.0-66.7 mg (kg dw)$^{-1}$ to 28.5-39.7 mg (kg dw)$^{-1}$, as well as iodine. For the first time a predictive model has been made that can estimate the iodine reduction in sugar kelp depending on the process parameters: time and temperature. It predicts, as an example, that a blanching process carried out at 60 °C for 2 minutes results in an iodine content between 500-750 mg (kg dw)$^{-1}$ or blanching at 45 °C for a duration of 8 minutes will achieve a maximum iodine content of 500 mg (kg dw)$^{-1}$.

Of the more positive quality nutrients and bioactive compounds, several are reduced due to blanching, including the amino acids with umami taste, magnesium, mannitol, vitamin B9, and vitamin C. Moreover, sensory, and physico-chemical properties are also altered due to blanching. A principal component analysis reveals that blanching increases the intensity of the odors: sweet, fresh sea, umami, sour, and rubber. Additionally, the color of the kelp is found to shift from brown to an intense green during blanching at 80 °C. In conclusion, it is recommended to blanch sugar kelp and winged kelp at minimum a temperature of 45 °C for a duration of 30 seconds.

Drying preserves seaweeds by removing water and lowering the water activity with the possibility to retain the food quality. However, several drying methods exist, which each have different energy and time consumptions as well as different impacts on the food quality. Three drying methods (convective air (52 °C), freeze drying (-20 to 20 °C at 20 Pa), and microwave-vacuum drying (-40 to 40 °C at 10 Pa) are explored on the seaweed species: bladder wrack and sea lettuce. Overall, the findings are not consistent for the two species. Microwave-vacuum drying does not reduce the bioactive compounds comparably to freeze drying in any of the species. However, as a result of convection drying, only free glutamic acid decreases for bladder wrack. For sea lettuce more valuable compounds are compromised because of convection drying (free aspartic acid, free glutamic acid, the pigment lutein, and polyunsaturated fatty acids). This means that microwave-vacuum drying leads to similar chemical quality as freeze drying. For both species, the three drying methods result in products which vary in the physico-chemical and sensory qualities analyzed (color, water activity, water absorption, and water holding capacity, appearance, odor, flavor, and texture).

Nonetheless, microwave-vacuum drying gives a product closer to freeze drying, compared to the product dried by convective air. This means that microwave-vacuum drying has the potential to replace freeze drying, if a high quality product is required, since it is faster.

The pH, water activity, and salt concentration of fresh sugar kelp promote the growth of microorganisms, which can cause food spoilage. Thus, shelf-life extension of sugar kelp is important to reach a safe and shelf stable product. Washing and blanching sugar kelp in either potable tap water or seawater are investigated to understand their influence on shelf life. The shelf life for refrigerated (2-3 °C) sugar kelp (untreated, washed (4-16 °C for 5 minutes), or blanched (76-80 °C for 2 minutes)) is 7-9 days with *Pseudomonas* spp. as the dominant spoilage bacteria, meaning it is not treatment dependent. To predict spoilage of sugar kelp it is recommended to control the aerobic viable count (AVC) on marine agar and kept below 7 log (CFU g^{-1} wet weight).

This research provides a foundation for best practice and innovation in the European seaweed industry and can help in choosing and developing post-harvest processes with food safety and quality in mind. In the future, the seaweed industry must prioritize developing end-products and establishing what the important food qualities in seaweeds are. This will be essential to be able to optimize the post-harvest processing methods for the future.

Resumé (Danish)

Potentialet for tang som en bæredygtig fødevarekilde har fået stigende opmærksomhed i de seneste år på grund af deres hurtigvoksende egenskaber og det faktum, at det kan dyrkes i havet uden at kræve land eller ferskvand. Derudover kan tang spille en rolle i at imødekomme verdens fremtidige øgende behov for mad. Som oftest med en ny fødekilde, følger der også nye udfordringer med tangdyrkning, såsom ved forarbejdningsmetoder og fødevaresikkerhed. Formålet med dette ph.d.-projekt er at studere industrielle forarbejdningsmetoder, såsom tørring, blanchering og vaskning af kommercielle relevante europæiske tangarter for at sikre kontrollerede, stabile og sikre fødevareprodukter. Ph.d.-projektet har industriel relevans, og hensigten er at afklare den bedste praksis inden for hver respektive forarbejdsningsmetode og art. De tangarter, der fokuseres på, er sukkertang, vingetang, blæretang, havsalat og søl. Afhandlingen er opdelt i fire hovedkapitler: vitamin C i tang, blanchering og vask af sukkertang, tørring af blæretang og søsalat, og holdbarhed af sukkertang.

Der ses, både i kommerciel promovering og videnskabelig litteratur, generaliserende påstande om, at tang er rige på vitaminer. Derfor undersøges indholdet af C-vitamin i de nævnte tangarter og de sammenlignes med almindelige kendte fødevarer, det anbefalede daglige indtag og lovgivningen for at anprise vitamin C. Resultaterne viser, at tang har lavere C-vitamin indhold (0,330-0,942 milligram pr. gram tørstof) end østers, salat, kartofler, agurk, broccoli, og hyben (0,704-36,4 milligram pr. gram tørstof). Dette viser, at tang ikke kan siges at være en rig kilde til C-vitamin.

Potentielle toksiske elementer som f.eks. arsen, kadmium og jod samt mikroorganismer på sukkertang og vingetang er en bekymring. Effekterne på de potentielle toksiske elementer, næringstoffer, og den sensoriske kvalitet undersøges i afhandlingen. Blanchering (45-80 °C i 30-120 sekunder) kan reducere antallet af

mikroorganismer fra 3,52-4,54 til 0,906-2,32 log CFU pr. gram. Blanchering reducerer også niveauerne af arsen fra 47,0-66,7 til 28,5-39,7 milligram pr. kilogram tørstof, samt jod.

For første gang er der blevet lavet en prediktiv model, der kan forudsige jodreduktionen i sukkertang afhængigt af procesparametrene: tid og temperatur. Den forudsiger, at en blancheringsproces udført ved 60 °C i 2 minutter resulterer i et jodindhold mellem 500-750 milligram pr. kilogram tørstof eller blanchering ved 45 °C i 8 minutter vil opnå et maksimalt jodindhold på 500 milligram pr. kilogram tørstof. Af de positive bioaktive forbindelser reduceres flere på grund af blancheringen, herunder aminosyrer med umamismag, magnesium, mannitol, vitamin B9 og vitamin C. Derudover ændres sensoriske og fysisk-kemiske egenskaber også.. En principal komponentanalyse viser, at blanchering øger intensiteten af lugtene: sød, frisk hav, umami, syrlig og gummi. Derudover at farven på tangen skifter fra brun til en intens grøn under blanchering ved 80 °C. Ud fra resultaterne anbefales det at blanchere sukker tang og vingetang ved mindst 45 °C i en varighed af 30 sekunder.

Tørring øger holdbarheden af tang ved at fjerne vand og sænke vandaktiviteten. Der findes dog flere tørringsmetoder, der hver har forskellige krav til energi- og tidsforbrug samt forskellig indflydelse på fødevarekvaliteten. Tre tørringsmetoder (konvektion (52 °C), frysetørring (-20 til 20 °C ved 20 Pa) og mikrobølge-vakuumtørring (-40 til 40 °C ved 10 Pa)) undersøges på tangarterne: blæretang og havsalat. Kvaliteten ændres ikke ensartigt for de to arter. Mikrobølge-vakuumtørring reducerer ikke de bioaktive forbindelser relativt til frysetørring i nogen af arterne. For blæretang falder kun frit glutaminsyre som et resultat af konvektionstørring. For havsalat kompromitteres flere bioaktive forbindelser på grund af konvektionstørringen (frit asparaginsyre, frit glutaminsyre, pigmentet lutein og flerumættede fedtsyrer). Dette betyder, at mikrobølge-vakuumtørring og frysetørring fører til en lignende kemisk kvalitet. For begge arter resulterer de tre tørringsmetoder i produkter, der varierer i de analyserede fysisk-kemiske og sensoriske kvaliteter (farve, vandaktivitet, vandoptagelse, vandholdningsevne, udseende, lugt, smag og tekstur). Altså giver mikrobølge-vakuumtørring et produkt, der er tættere på frysetørring, sammenlignet med produktet tørret ved konvektion. Dette betyder, at mikrobølge-vakuumtørring har potentiale til at erstatte frysetørring, hvis et produkt af høj kvalitet ønskes.

pH-værdien, vandaktiviteten og saltkoncentrationen af frisk sukkertang fremmer væksten af mikroorganismer, der kan forårsage fordærv. Derfor er det vigtigt at forstå og forlænge holdbarheden af sukkertang for at opnå et sikkert og stabilt produkt. Undersøgelser af vaskning og blanchering af sukkertang i henholdsvis drikkevand og havvand er blevet udført for at forstå deres indflydelse på holdbarheden. Holdbarheden for sukkertang i køleskab (2-3 °C) (ubehandlet, vasket (4-16 °C i 5 minutter) eller blancheret (76-80 °C i 2 minutter)) er 7-9 dage med *Pseudomonas* bakteriearterne som de dominerende fordærvende bakterier. Dette betyder, at holdbarheden ikke afhænger af behandlingsmetoden. For at forudsige fordævelse af sukkertang anbefales det at kontrollere det totale kimtal på ”marin agar”, og holde det under 7 log CFU pr. gram.

Forskningen bag afhandlingen danner grundlag for innovation i den europæiske tangindustri og kan hjælpe med at vælge og udvikle forarbejdningsmetoder med fødevaresikkerhed og kvalitet i fokus. I fremtiden skal tangindustrien prioritere udviklingen af slutprodukter og etablere, hvilke vigtige fødevarekvaliteter tang har. Dette er afgørende for at kunne optimere forarbejdningsmetoderne i fremtiden.

Dissemination

List of Publications

Paper 1	**CW Nielsen**, T Rustad & SL Holdt, Vitamin C from Seaweed: A Review Assessing Seaweed as Contributor to Daily Intake, *Foods* 2021, *10,* 198 [2]
Paper 2	**CW Nielsen**, SL Holdt, JJ Sloth, GS Marinho, M Sæther, J Funderud & T Rustad, Reducing the High Iodine Content of *Saccharina latissima* and Improving the Profile of Other Valuable Compounds by Water Blanching. *Foods* 2020, *9*. [3]
Paper 3	**CB Wirenfeldt**, MS Sletta, M Sæther, Ø Arlov, IM Aasen, H Sletta, PL Švarc, SL Holdt, SS Jacobsen, FL Aachmann & T Rustad, Blanching of two commercial Norwegian brown seaweeds – for reduction of iodine and other compounds of importance for food safety and quality, *in preparation for Food Chemistry*
Paper 4	**CB Wirenfeldt**, JS Sørensen, KJ Kreissig, G Hyldig, SL Holdt & LT Hansen, Post-harvest quality changes and shelf-life determination of washed and blanched sugar kelp (*Saccharina latissima*). *Front. Food. Sci. Technol.* 2022 *2:1030229.* [4]
Paper 5	**CB Wirenfeldt**, DB Hermund, AH Feyissa, G Hyldig & SL Holdt, Nutritional value, bioactive composition, physico-chemical and sensory properties of *Ulva* sp. and *Fucus vesiculosus* depending on post-harvest processing: a drying comparison study, *in preparation for Journal of Applied Phycology*

The name in **bold** is the author of the PhD thesis. I changed my name during the project period.

Conference presentations and posters

CW Nielsen*, SL Holdt, JJ Sloth, M Sæther, J Funderud & T Rustad, “Decreasing iodine content in *Saccharina latissima* by hydrothermal processing and determining the possible loss of nutritive and bioactive compounds”, 23rd International Seaweed Symposium, Jeju, Republic of Korea, May 2019 (Poster)

CW Nielsen*, SL Holdt, JJ Sloth, M Sæther, J Funderud & T Rustad, “Is blanching the solution for lowering iodine in sugar kelp and which quality consequences does it have to blanch sugar kelp?”, 9th Nordic Seaweed Conference, Grenaa, Denmark, October 2019

CW Nielsen*, JS Sørensen, KJ Kreissig, G Hyldig, LT Hansen & SL Holdt, “What is the shelf-life of *Saccharina latissima* stored at 2 °C and can pre-processing prolong it?”, Science Industrial Seaweed Symposium 2020, Virtual, October 2020

CB Wirenfeldt*, T Rustad & SL Holdt, “Are seaweeds rich in vitamin C? A systematic review of 92 species assessing the contribution to daily intake”, 3rd Seaweed for Health Conference, Virtual, May 2021

CB Wirenfeldt, MS Sletta, M Sæther, Ø Arlov, IM Aasen, H Sletta, SL Holdt, and T Rustad*, “Solutions to reduce iodine”, 6th SIG Seaweed Conference, Trondheim, Norway, November 2021

CB Wirenfeldt, DB Hermund*, “Optimized drying for preservation of bioactive compounds in *Ulva* sp. and *Fucus vesiculosus* – a comparison study of drying methods”, Seaweed four Health, Galicia, Spain, August 2022

CB Wirenfeldt, MS Sletta, M Sæther, Ø Arlov, IM Aasen, H Sletta, SL Holdt, and T Rustad*, “Kelp on the menu: reduction of the high iodine content in brown seaweeds”, 36th EFFoST International Conference, Dublin, Ireland, 2022 (Poster)

CB Wirenfeldt, DB Hermund, AH Feyissa, G Hyldig, and SL Holdt*, “Investigating the effect of microwave-vacuum drying, freeze drying and hot air drying of *Ulva* sp. and *Fucus vesiculosus*”, 24th International Seaweed Symposium, Hobart, Australia, February 2023

*presenting author

Other dissemination activities

Lecture on seaweed, Course at DTU: Producing new sustainable food ingredients, Spring 2020, 2021 and 2022.

"Vitamins in macroalgae – a perspective", Focus group presentation, Norwegian Seaweed Cluster, Virtual, March 2022

Co-arranging "Girls in Science 2022" about using seaweed as commodities, at DTU Food, October 2022

PhD seminar in Sensory and Consumer Science, The Danish Sensory Society and IDA Food Science, October 2022

Lecture: "Why do we need seaweed on the plate?", NaturNord, November 2022

Popular science

CB Wirenfeldt , "Danske forskere fjerner afgørende forhindring", Videnskab.dk, 30th November 2021, https://videnskab.dk/forskerzonen/krop-sundhed/tang-paa-tallerkenen-danske-forskere-fjerner-afgoerende-forhindring

CB Wirenfeldt, "Forskere fjerner barriere, der forhindrer tang i at komme på tallerkenen" , FoodWatch, 6th of January 2022, https://fodevarewatch.dk/Fodevarer/article13569630.ece

Abbreviations

ANOVA	analysis of variance
AVC	aerobic viable count
a_w	water activity
CFU	colony forming units
DPPH	2,2-diphenyl-1-picrylhydrazyl
dw	dry weight
EFSA	European Food Safety Authority
FAME	fatty acid methyl esters
FAO	Food and Agriculture Organization
GAE	gallic acid equivalents
LOQ	limit of quantification
n.a.	not available/not analyzed
PCA	principal component analysis
PC	principal component
PTE	potential toxic element
PUFA	polyunsaturated fatty acids
SD	standard deviation
SE	standard error
sp.	species
spp.	several species
TPC	total phenolic content
UL	upper intake level
UN	United Nations
WA	water absorption
WHC	water holding capacity
WHO	The World Health Organization
ww	wet weight

Table of Contents

List of Figures and Tables

Figures

Tables

1 Why seaweed?

I stand on the sandy beach on the Danish shore of Northern Jutland with the dunes behind me. I glimpse out on the glassy yet moving surface of the North Sea. When I am out struggling with the waves on my surfboard, all I see around me is this glassy blue surface on top of the sea. It is like a glassy lid keeping a secret. Although, I know the secret. Below me is a landscape just as, if not more, vivid, and beautiful as the barren dunes and the pine forests that I can scout up on land.

In the under-the-surface landscape are orcas, seals, squid, and blue mussels living. Nevertheless, most importantly to me and possibly also to the others, it is the home for algae. And why is that?

Let us focus a bit on the world's most productive forest. Surprisingly, it is not a big terrestrial forest. The most productive forest in the world is the kelp forest in the coastal waters of California [5]. At ideal conditions, the seaweed called giant kelp (*Macrocystis pyrifera*) can grow half a meter in just one day [6]. Seaweeds, and especially the type called kelp, are fast growing. By cultivating kelp species, we can get lots of biomass quickly. However, this is not the only reason seaweed has caught focus recently.

Seaweeds do, as the name implies, grow in the sea. When farming seaweed there is no need for land areas, nor for supplying the seaweed with freshwater, as terrestrial crops need. Moreover, they use nutrients from the sea to grow, which makes the surrounding waters cleaner [7], and they have a potential to sequestrate carbon [8,9]. Seaweed can support the marine ecosystems and provide habitats for other species [10]. This goes hand-in-hand with the United Nations' (UN) sustainable development goals: 12) Responsible Consumption and Production, 13) Climate Action, and 14) Life Below Water [10].

All the points above are reasons we need to have our eyes on macroalgae. Nevertheless, in my opinion the most important reason is: cultivating macroalgae can be done in the sea and hence does not take up any land area. Agriculture has a maximum capacity because of the limitation of arable land areas and freshwater disposals [8], there is not enough space to meet the future food demands. Seaweed aquaculture is one solution to meet the world's need for food. Seaweed is future food, thus can contribute to the UN sustainable development goal: 2) Zero Hunger.

Seaweed cultivation has been a practice in Asia for decades. However, the work in this thesis is established based on the wish from the young European seaweed industry to exploit the possibilities of seaweed as a sustainable food source. Seaweed farming is a relatively new sector in Europe, but it is a sector that grows rapidly [11].

The European seaweed industry is now at a point where it can cultivate some species commercially such as *Saccharina latissima*, *Alaria esculenta*, and to some extent *Palmaria palmata* [12–14]. The European seaweed industry is still young. Agriculture has been developed over 10,000 years, whereas European seaweed cultivation is relatively more recent and still in need of expansion and optimization [8]. This raises questions in this new sector about post-harvest processing abilities. This is where this thesis comes into the picture.

When we focus on new food sources, it comes with new challenges. How should we handle and treat the newly harvested seaweed? Two major points are important. One, the utilization: what is the end-product? How should we eat it? Second, the safety and stability of the newly harvested seaweed: what is the shelf-life? How should we process it to have a stable product? How do we ensure food safety? This second point is what this thesis focuses on. The main responsibility of any in the food sector is to ensure safe food for the consumers. This is also indeed important for any seaweed producer and retailer.

The overall aim of this PhD project was to study industrial post-harvest processes, such as drying, blanching, and washing of commercially available European seaweeds to ensure controlled, stable, and safe food products. Any of the work done as part of the thesis had an industrial relevance, with the intention to clarify the best practices within each respective post-harvest process or species.

2 Background

2.1 Seaweeds and their taxonomy

Algae range from being microscopic to several meters tall tree-like structures forming under-water sea forests. Algae are the vegetable livings of the oceans, yet they are not directly plants. They grow by photosynthesis, taking up carbon dioxide, and they take up nutrients from the water as terrestrial plants would from the soil. Their taxonomy difference is complex, and they do not have their own life's kingdom as, for example, fungi or animals have. In fact, they do not even belong to the same kingdoms.

The red and green algae belong to the kingdom of plants (*Plantae*), although from here they split into different taxonomical levels (Figure 1) [15,16]. These specific algae are the closest related to plants, yet they are not in the same family, class or even phylum of terrestrial plants. The brown algae are even more diverse. You could in fact argue that humans are just as closely related to the brown algae as the brown algae are to the green- and red algae. Therefore, you cannot really compare brown algae to the red- and green algae, it would be like comparing humans to potatoes [15].

What the red, green, and brown algae do share besides living under the surface of the sea is that they are commonly known as macroalgae or seaweed. Macroalgae means that they are macroscopic. Translated, it means algae that are large enough to be seen by the naked eye [17].

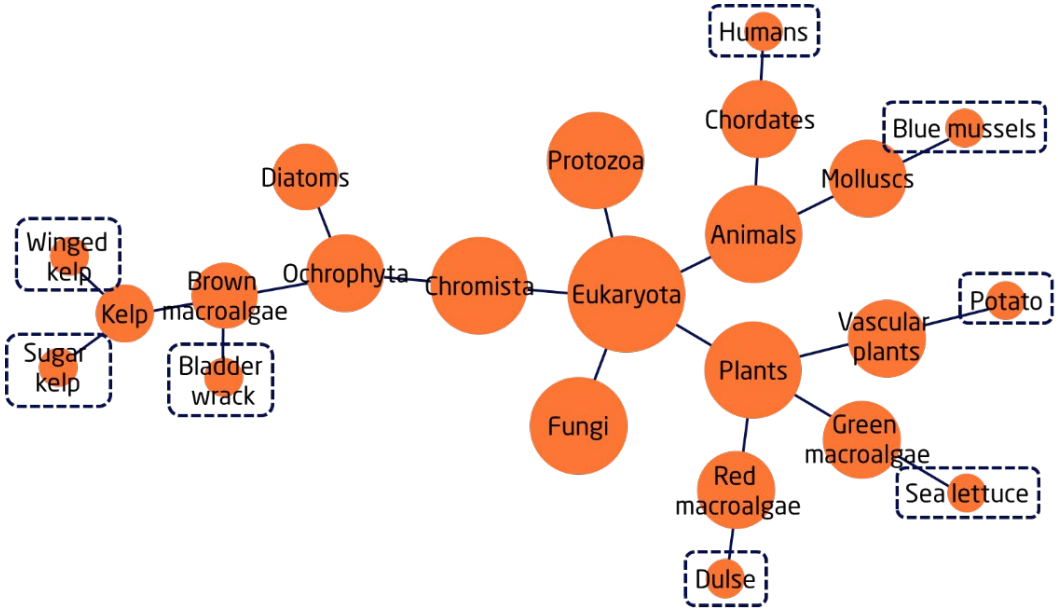

Figure 1: A depiction of a taxonomic hierarchy shown by a tree structure, with each circle representing a taxon and the lines denoting the hierarchical relationships between them. The length of the lines is not indicative of any particular significance. A taxonomical distance is seen for the brown macroalgae (to the left) and the other macroalgae (to the lower right), also compared to other species: humans, blue mussels, and potatoes (to the upper right).

2.2 Important European species for food

In the World's oceans, seas, coves, and fjords are over 10,000 seaweed species living [16]. It is estimated that 145 of these species are used for food, which is mainly included in the Asian cuisine in the countries: Japan, Korea, and China [18]. Seaweed is not as integrated into the Western diet, despite sporadic historical uses in coastal areas and for down streamed ingredients. However, the industry for producing seaweed for foods in Europe is developing and has increased by 150% in the last decade [9].

The research behind this thesis is based on seaweed species, which are commercially important and available in Europe or even more important in Denmark and Norway. By commercially important, it means that they are growing wild, being so abundant that it is sustainable for companies to build their business around them. This is the case for, e.g., bladderwrack (*Fucus vesiculosus*) and sea lettuce (*Ulva* sp.) [19]. Also, they can be commercially cultivated and harvested, which is the case for the two kelp species; sugar kelp (*Saccharina latissima*) and winged kelp (*Alaria esculenta*), and to some extent dulse (*Palmaria palmata*) [20–22].

In 2021, a scientific paper provided a complete description of the seaweed production in Europe [9]. They found a total of 225 seaweed producing companies in the following 13 European countries: Denmark, Estonia, Faroe Islands, France, Greenland, Iceland, Ireland, the Netherlands, Norway, Portugal, Spain, Sweden, and United Kingdom [9]. The species that by far are the most produced in Europe are the kelps (*Laminaria* spp.; 209,772 tons), followed by *Ascophyllum nodosum* with 82,476 tons, all from wild stocks. These species are mostly harvested for production of the gelling agent alginate, and in fact 25% of the World's alginate production is from Europe [9].

Nevertheless, the focus of this thesis is on the seaweeds with a less established industry around them: bladder wrack, dulse, sea lettuce, sugar kelp, and winged kelp. Pictures of the mentioned species can be found in Figure 2 and their taxonomy in Figure 1.

These species (Figure 2) do not reach as high production amount per year as the *Laminaria* spp. and *Ascophyllum nodosum* do. The production amount and the number of companies working with these newly relevant species in Europe can be found in Table 1.

Table 1: The production type, production amount and number of European companies producing the relevant species in focus of this thesis.

Seaweed	General name	Production type	Production per year (tons)	Number of companies
Alaria esculenta	Winged kelp	Cultivation	107	16
Fucus spp.	Bladder wrack	Wild stocks	*n.a.*	37
Palmaria palmata	Dulse	Wild stocks	455	35
Palmaria palmata	Dulse	Cultivation	*n.a.*	6
Saccharina latissima	Sugar kelp	Cultivation	376	26
Saccharina latissima	Sugar kelp	Wild stocks	*n.a.*	25
Ulva spp.	Sea lettuce	Wild stocks	217	38
Ulva spp.	Sea lettuce	Cultivation	50	10

The production (column 4) is the fresh weight produced in tons per year in Europe. The table is based on Araújo et al. (2021) [9]. *n.a.* – not available

The most produced is dulse > sugar kelp > sea lettuce > winged kelp > bladder wrack (not available). Together the production amount is around 1,205 tons per year, which is less than 0.5% of the total European production of *Laminaria* spp. and *Ascophyllum nodosum*. Setting these two sections of the seaweed industry into perspectives of one another demonstrates how small the newly introduced species are on the market and

indirectly how important research and development is for this section of the industry to grow.

However, the various seaweed species are processed in different ways depending on their end-product. It is not every process that is important for each species. With a whole food product as a target the main goal is safety. But safety is not necessarily an important aspect when processing the seaweeds for e.g., extracting ingredients, where the rest of the biomass is not utilized for food. This thesis focuses on seaweed as a whole food, and therefore investigates which processing methods are relevant for the newly introduced species.

Figure 2: Overview of the different seaweed species used in the thesis research with their binomial nomenclature and common names (Photos: Cecilie Bay Wirenfeldt).

2.3 Definition of (seaweed) food quality

Food is defined as any substance or product, whether processed or not, that is ingested by humans [23]. This is a very broad definition. Thus, food quality is also broadly defined, and includes various aspects. The aspects that are the most important depend on the food product in scope. This chapter will explain what food quality is and some of its various aspects.

Food quality has numerous dimensions, and it is difficult to define it to include all foods. It is influenced by various factors: safety, origin, nutrition, sensory, authenticity, convenience, functionality, aesthetics, and ethics [24]. The Food and Agriculture Organization (FAO) described food quality in relation to their 24th FAO Regional Conference for Europe and divided quality into three main groups (Figure 3), which also represents a prioritized order of importance [25].

Figure 3: The three groups of food quality and their prioritized order described by FAO [25].

The first group is safety: it is important that there is an absence of defects and adulteration. Second, the original quality is the minimum expected properties of the food such as sensory and nutritional composition. The last group includes the increase in appreciated characteristics by production methods, geographical origin, or sustainability measures that justify added value to the product.

Of the previously mentioned dimensions of food quality, this thesis focuses on four: chemical, microbial, physico-chemical and sensory quality (Figure 4). Any dimension can be part of any of the three groups shown in Figure 3. As an example, unwanted constituents can define the chemical quality of a food such as potential toxic elements (PTEs), which influence the safety for the consumer. However, it can at the same time

be defined by the original vitamin content in the food or the increased value by a processing technology method that produces desirable flavor compounds, and hence increases the sensory quality.

Since food quality is a very broad term and depends on the product in focus, processing can increase food quality in one dimension and, at the same time, decrease another dimension. Consequently, it is important to understand which food quality aspects are the most important for a product. This can be a challenging task when introducing new food products to the market. With seaweed being increasingly introduced to Western cuisine, it is difficult to state what quality is, because it generally is an unused, unexplored food source, which producers and consumers know little about. Therefore, it is crucial that the seaweed industry defines the quality of their product and establishes analytical reference methods to document quality parameters [26]. To simply answer, what defines seaweed quality?

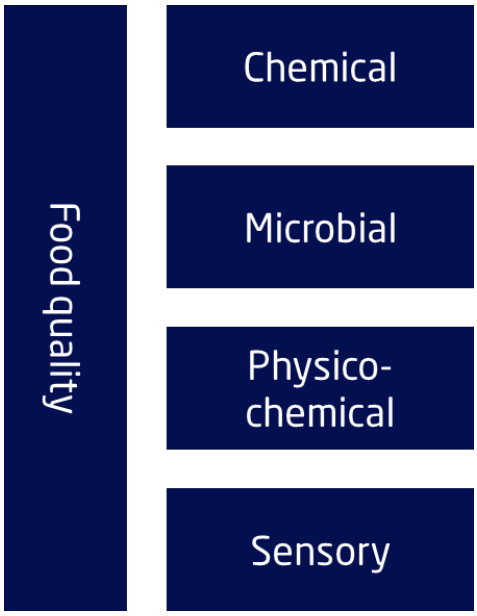

Figure 4: The four dimensions of food quality used throughout the thesis.

The seaweed producers need to have many stakeholders in mind. The primary goal for food authorities is to have safe food products for the consumers. However, the main drive of consumers is not safety, since they expect that this is in place. As professor Ole G. Mouritsen states: “Taste comes first”. Even though a food product is nutritious, few will consume it, if it is not tasty [27]. If seaweeds are intended for introduction into the Western cuisine, the taste should be in the industry's mind [18].

Several studies have reported on the various aspects of the food quality of seaweeds. Table 2 presents examples of the quality parameters, which have been used to describe the food quality of seaweeds. They are divided into the four quality dimensions: chemical, microbial, physico-chemical, and sensory qualities. The selected parameter depends on the seaweed species in focus, the intention of it as a food product and the post-harvest processing. Some of these quality measures were considered during the work of this thesis.

Table 2: Some recognized measures of food quality of seaweeds divided into the groups: safety, original quality, and increased value with references in brackets as examples.

Group	Chemical	Microbial	Physico-chemical	Sensory
1) Safety				
	Allergens [18]	*Bacillus* spp. [26]		
	Arsenic [28]	*E. coli* [28]		
	Cadmium [28]	Norovirus [28]		
	Environmental pollutants [28]	*Salmonella* [28]		
	Iodine [28]	*Vibrio* spp. [28]		
	Lead [18]			
2) Original quality or 3) Increased value				
	Amino acids [29]	Aerobic viable count [30]	Color space [26]	Appearance [18]
	Antioxidants [31]		Drip loss [30]	Aroma [30]
	Aromatic volatile compounds [32]		pH [33]	Color [30]
	Ash (minerals) [34]		Swelling ability [35]	Flavor [35]
	Dietary fibers [34]		Texture analyzer [26]	Odor [35]
	Hydrocolloids [19]		Water absorption [35]	Texture [18]
	Iodine [36]		Water activity [33]	Umami [27]
	Fatty acids [18]			
	Mannitol [34]			
	Minerals [37]			
	NaCl [38]			
	Organic acids [38]			
	Pigments [19]			
	Total phenols [39]			
	Total volatile basic nitrogen [40]			
	Vitamins [41]			
	Water content [42]			

3 Thesis structure, hypotheses, and experimental approach

3.1 The structure of this thesis

Based on the main aim, the work of this thesis research different processing methods on various seaweed species. The choice of processing method and species was based on industrial needs, which was discussed with companies to assist the industry to develop seaweed raw materials for food applications. Figure 5 illustrates the four main chapters of the thesis to which the papers mainly refer. In the next section of the chapter, the hypotheses for the PhD thesis are stated, which are answered in the conclusion. Moreover, in each chapter are stated specific research questions that are answered at the end of the chapter.

Chapter 4 discusses the vitamin C content of seaweeds from the relevant European species from the orders Laminariales, Fucales, Palmariales, and Ulvales. The data is extracted from Paper 1. The potential of the species to contribute to fulfilling daily dietary requirements is discussed as well as comparing the vitamin C content of European seaweeds to other foods. Vitamin C was reviewed and studied since it is interesting when studying seaweed processing, as the instable vitamin C can act as an indicator of the effect of processing.

Chapter 5 examines results from Paper 2, 3, and 4 and focus on the brown kelp species: winged kelp and sugar kelp. It discusses the impact of blanching, focusing on its effects on food safety, nutrient composition, and sensory characteristics. Iodine, and the reduction of it, is a major part of this chapter and a predictive model is given. The recommended blanching temperature and duration are also discussed.

Chapter 6 discusses the effects of three drying methods (convective air drying, freeze drying, and microwave-vacuum drying) on the quality of bladder wrack and sea lettuce. It investigates how chemical and physico-chemical properties, and sensory characteristics are changed after drying. The chapter is based on Paper 5, which is the first to investigate microwave-vacuum drying as a substitute for freeze drying for seaweeds.

Last, chapter 7 is based on Paper 4 and considers the bacterial deterioration process and off-odors that occur during the storage of sugar kelp. The dominant microorganisms are identified, and other pathogenic bacteria are discussed. Different off-odors detected during storage are also assessed.

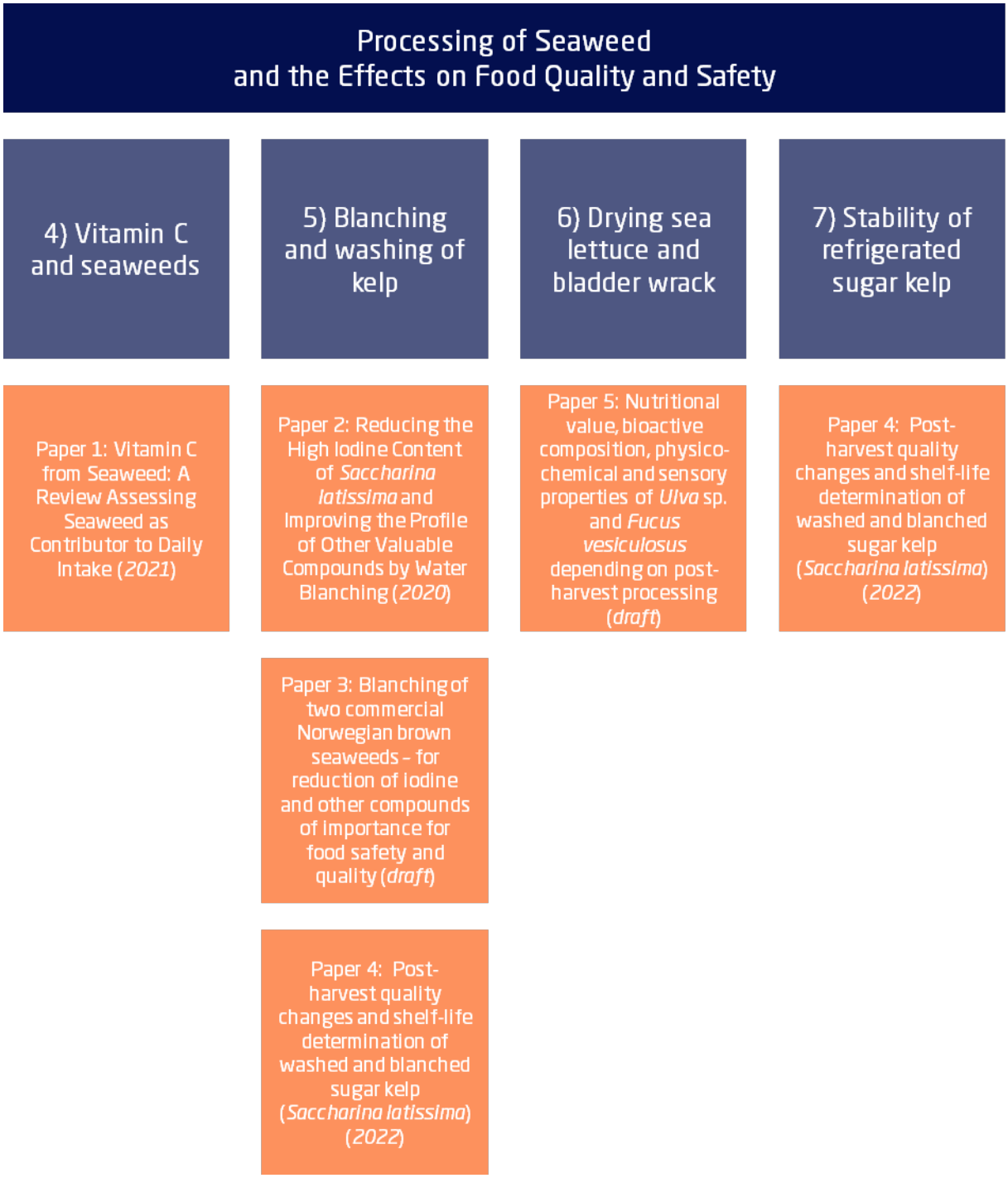

Figure 5: Overview of the papers developed (orange boxes) as part of this thesis work and their distribution into chapter 4-7 (blue boxes) of the thesis.

3.2 Hypotheses

The PhD thesis investigated the following hypotheses, divided by the chapters.

Chapter 4: Vitamin C and seaweeds.

- The seaweed species in focus (bladder wrack, sea lettuce, dulse, and kelp) cannot be claimed as a source of vitamin C according to food legislation (**H4.1**).
- They can, however, contribute to the daily human need for vitamin C (**H4.2**).

Chapter 5: Blanching and washing of kelp.

- Arsenic, cadmium, iodine, and microorganisms will pose as a health risk if untreated kelps are used for human consumption (**H5.1**).
- Blanching and washing will lower the health risk from consumption of kelps and decrease the concentration of the potential toxic elements to below the maximum allowed thresholds and upper intake level (**H5.2**).
- The blanching temperature and duration will reduce the iodine concentration in the kelps, and a correlation will be seen, which can be the base of a predictive model (**H5.3**).
- The proximate composition, other beneficial nutrients, and other dimensions of food quality such as sensory and physico-chemical properties in kelps are affected by blanching (**H5.4**).

Chapter 6: Drying sea lettuce and bladder wrack.

- Microwave-vacuum drying (-40 to 40 °C at 10 Pa) will result in the same quality (chemical compounds retained, physico-chemical properties, and sensory) as freeze drying (-20 to 20 °C at 20 Pa), however convective drying (52 °C) will not (**H6.1**).
- The drying method will not influence the two species tested differently (**H6.2**).

Chapter 7: Stability of refrigerated sugar kelp.

- The bacterial species present on the sugar kelp samples during refrigerated storage will differ and depend on the process treatments (**H7.1**).
- Untreated, washed, and blanched refrigerated sugar kelp provides a favorable environment for both spoilage and pathogenic bacteria proliferation (**H7.2**).

3.3 Overview of analyses

Several analyses have been performed to test the hypotheses. The individual methods used for analysis can be found in the respective papers. The methods used in each paper can be found in Table 3 and Table 4. Paper 1 was a review paper, thus, no analyses were conducted.

Table 3: The various chemical and sensory analysis behind the data in the different respective papers (Papers 2-5).

Analysis	Paper 2	Paper 3	Paper 4	Paper 5
Chemical				
Amino acids and protein	X	X	X	X
Ash	X	X	X	X
DPPH	X			X
Dry matter	X	X	X	X
Fatty acids	X			X
Iodine	X	X	X	
Minerals and PTEs		X		
Monosaccharides		X	X	
NaCl			X	
Organic acids			X	
Pigments			X	X
Total lipid content	X	X		X
Total phenolic content	X			X
Vitamin B9		X		
Vitamin C		X	X	
Sensory				
Descriptive profile			X	X

Table 4: The various microbial and physico-chemical analyses behind the data in the different respective papers (Papers 2-5).

Analysis	Paper 2	Paper 3	Paper 4	Paper 5
Microbial				
Aerobic viable counts		X	X	
Actinomycetes spp.			X	
DNA by 16S rRNA			X	
Pseudomonas spp.			X	
Respiration			X	
Shewanella spp.			X	
Yeast			X	
Physico-chemical				
Color			X	X
Drip loss			X	
pH			X	
Texture			X	
Water activity			X	X
Water absorption				X
Water holding capacity				X

3.4 Statistical method

This sub-chapter will explain the statistical method as an overall method description. First, the importance of replication and how average and standard deviation are calculated are explained. This approach has been used in all the experimental designs performed throughout any of the papers. Second, other used statistical methodologies are explained below. The analysis of variance (ANOVA) has been used in all the papers. Whereas principal component analysis (PCA) has been used in Paper 3-5.

3.4.1 The importance of replication

Sample replication is crucial when designing experiments. It involves repeating the treatment or the analysis independently multiple times, to improve the reliability and validity of the data obtained. When we have replicated samples, we can ensure that

the observed effect is not because of random variability or experimental error, but represents the true underlying relationship studied.

In this work, the replications are divided into two groups: treatment replication and analytical replication (Figure 6). Treatment replicates are, as the name implies, those that are divided when performing a treatment or process. They can consist of multiple individuals in a group or in some cases it is one individual. As an example, when performing blanching as a process on the seaweed samples, then each specific blanching step with the chosen blanching parameters is performed a minimum of three times independently, giving three treatment replicates. The analytical replicates are a sub sample randomly chosen from the treatment replicates for analysis. They typically consist of 1-3 sub samples, which depend on the analytical measurement performed. Other practices can validate the analyses, such as certified reference materials or internal reference materials, thus sometimes only one analytical replicate is used. If this is not the case, two or more analytical replicates can help validate if the method of analysis is consistent.

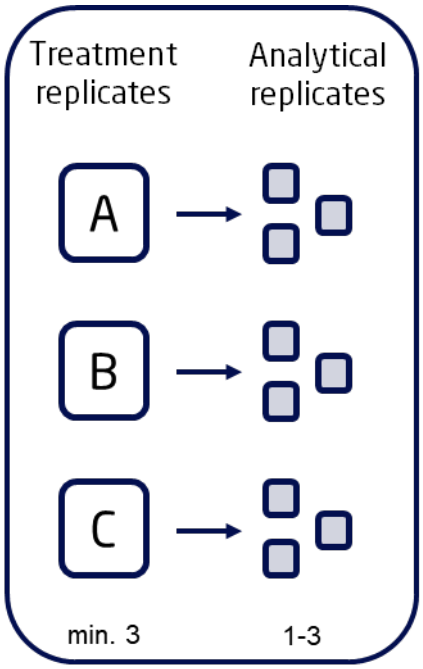

Figure 6: Overview of the two replicate types used: Treatment replicates are the same treatment conducted several times. The analytical replicates are replication of that treatment one to three times at the analysis level.

Replication also makes it possible to calculate statistics such as the average and standard deviation of the mean, which provide a measure of the precision or dispersion of the data and allow for more accurate interpretation of the results. Overall, the practice of replication is essential to generate robust and reliable scientific findings.

3.4.2 Analysis of variance

Analysis of variance (ANOVA) is used to compare groups to determine if there is a significant difference between them. In the experimental setup there are controlled explanatory variables. They are also called factors or independent variables. An example of two factors could be “pressure” and “temperature”. Each of the factors has two or more levels, e.g., three different temperatures and three different pressures.

The response variables, also called dependent variables, are what we measure in the experiment, as it is the result of the manipulation of the factors.

The ANOVA can be one to several directions or “ways”. One-way ANOVA is used when there is a single factor with three or more levels. If it was a single factor with just two levels, a t-test is used. With two or more factors, it is called a two-way or multi-way ANOVA (Figure 7), and they would each have two or more levels. Working with multi-way ANOVA also allows the interactions among the factors to be studied. It can be estimated whether a response to one factor depends on another factor [43].

Any ANOVA should also have replication integrated. This means that each experiment at each level is performed multiple times under the same conditions. This is a direct reference to the treatment replicates in Figure 6. A visualization of a two factors, three levels and three replicate experimental design is shown in Figure 7. Each box in the front (the x-axis and y-axis) represents a sample type. Thus, this study has 9 sample types, each replicated three times.

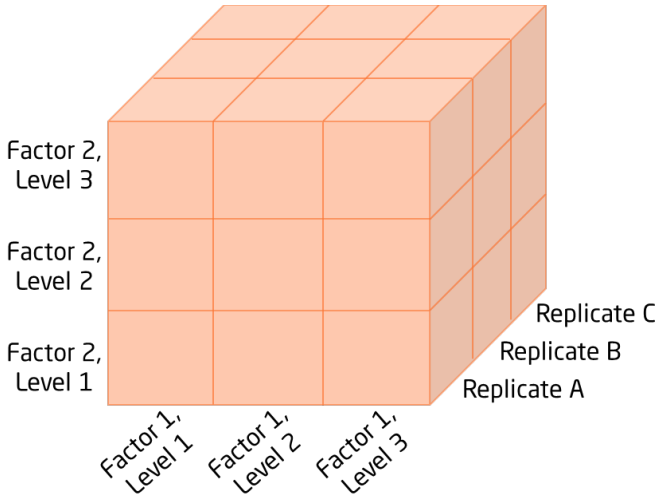

Figure 7: An overview of an experimental set up for a two-way ANOVA with two factors, each with three levels and all replicated three times (replicate A, B, and C). Such a system has three dimensions, whereas more factors would lead to more than three dimensions, which is not possible to visualize.

The assumptions to run an ANOVA is that the measurements are random, they follow a normal distribution, and they have equal variances. The ANOVA compares the means of the groups by comparing the within group variation with the overall variation, which is why it is called analysis of variance. Simplified, the means are significantly different if the group variation is smaller than the overall variation.

3.4.3 Principal component analysis

Principal component analysis (PCA) is a data exploration technique that summarizes and maps information in a dataset with multiple analytical response variables by reducing the dimensions in a dataset and identifying directions with maximal sample variation.

It is used to compute linear latent variables, known as components on standardized data. The resulting components are orthogonal (perpendicular) to each other [44]. The number of components that can be computed is equal to the number of analytical variables in the dataset. However, the goal is to explain as much variance as possible with as few components as possible [45].

To determine the number of necessary components, a scree plot can be used (Figure 8A) [44]. As a rule, 10% variance is applied as a cutoff since components that have low variance may reflect noise in the data. As an example, we see in Figure 8A that PC1 and PC2 explain over 10% of the variance each and 81% together. Thus, they should be used to understand the variance, whereas PC3 and above should not. It is generally considered that a total explained variance of 70% gives a good description of the data, while 90% gives an excellent description.

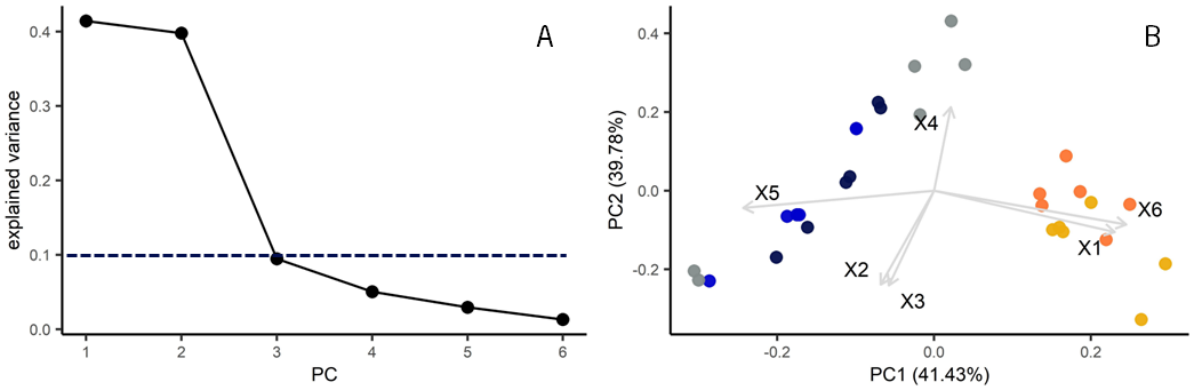

Figure 8: (A) A scree plot visualizing how much variance is explained by which principal component (PC). The blue dashed line is the threshold, giving that 10% variance is explained for the specific PC. (B) An example of a bi-plot with the PC scores of five different treatments (colors) and six variables (arrows). The percentages at the axes show how much variance is explained by each PC.

Principal component analysis can be illustrated using several types of plots. A bi-plot is a plot that combines the score plot and the loading plot of a PCA (Figure 8B). A score plot is a scatterplot that uses two score vectors (the axes) and includes a point for each sample object. A loading plot includes a vector (represented by an arrow) for

each response variable and demonstrates the similarities and influence of the response variables on the scores. Response variables closer to the central origin have smaller vectors (loadings) and therefore less impact on the samples (objects). In Figure 8B the orange and yellow objects are impacted by the response variables X1 and X6. The length of the vector is proportional to the contribution of the response variable. Additionally, the smaller the angle between two loadings, the stronger they correlate. As an example, X2 and X3 highly correlate, whereas X4 and X5 do not.

4 Vitamin C and seaweeds

This chapter is based on Paper 1 [2], which is a review assessing the vitamin C content in various seaweeds and the contribution of seaweed to the human need by gathering results from peer reviewed papers. Vitamin C, also known as L-ascorbic acid, is an essential nutrient that acts as an antioxidant, protecting DNA, proteins, and lipids from oxidative damage by sacrificing its electrons to scavenge free radicals [46]. Vitamin C is highly sensitive to various environmental factors, such as pH, temperature, light, and oxygen [47]. During seaweed processing or even storage, vitamin C is likely to oxidize, making it one of the most sensitive micronutrients [48,49]. Although, if it does not oxidize, other nutrients probably do not either [47]. This insight can be important when studying seaweed processing, as the instable vitamin C can act as an indicator of the effect of processing.

4.1 Aim and research questions of the vitamin C chapter

Claims about the richness of vitamins are often seen in commercial promotion, and even in scientific literature [18,50]. However, such claims are too generalizing, also considering the previous statement in section 2.1 that seaweeds are as different as humans and potatoes. It is interesting to investigate the truthfulness of the claim that seaweeds are rich in vitamins. Given that there are over 10,000 known seaweed species [16] and thirteen essential vitamins, the investigation began with an exploration of vitamin C.

Paper 1 provides an evaluation of the vitamin C content of seaweed and compares it to other food sources. It also investigates how processing influences the vitamin C

content of seaweeds. The review concludes by examining the potential of seaweed to meet human vitamin C requirements.

This chapter is an excerpt from Paper 1 and has the aim to investigate the vitamin C content of the selected Northern European seaweed species (sugar kelp, winged kelp, bladder wrack, sea lettuce, and dulse) as defined in section 2.2. Moreover, it is to compare their content to the vitamin C content of commonly consumed foods and the recommended intake established by FAO and WHO.

The objective of the chapter is to address the following research questions:

- Are the selected European seaweeds a rich source of vitamin C?
- Can the commercially relevant European seaweeds be claimed as a source of vitamin C?
- Which common foods are the vitamin C content in seaweeds comparable to?
- Can European seaweed contribute to the daily human need for vitamin C?

4.2 Vitamin C in selected European species

Data from higher taxonomical levels (i.e., Fucales, Laminariales, Palmariales, and Ulvales) are used because of limited literature data on seaweed species level. The results, presented in Figure 9 by four box plots, reveal that the median vitamin C content of brown seaweeds (Fucales and Laminariales) and Palmariales is similar. In contrast, the green seaweed species (Ulvales) have a higher median, but also indicate greater variability.

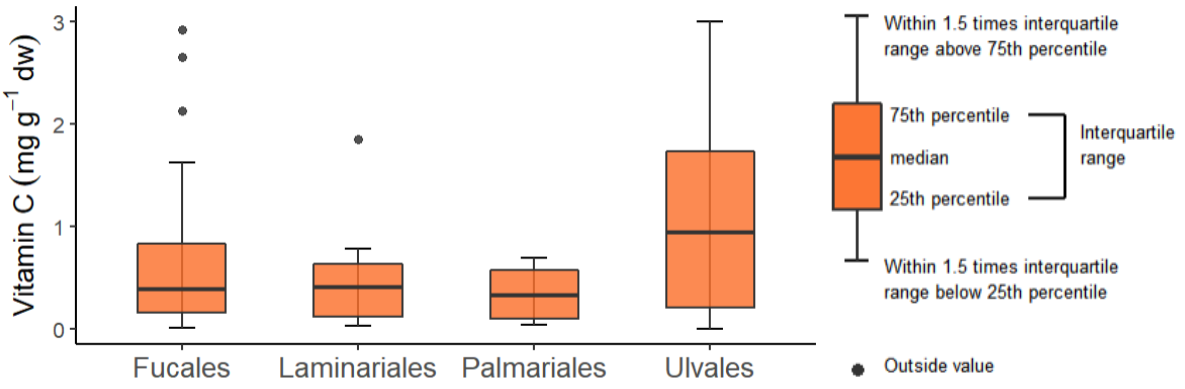

Figure 9: Boxplots of vitamin C content in mg (g dw)$^{-1}$ for the four different taxonomical orders: Fucales (brown), Laminariales (brown), Palmariales (red), and Ulvales (green) from Paper 1 [2]. The boxplot to the far right explains how to interpret the boxplots.

The question that arises is, why do Ulvales have a higher median of vitamin C? This can be explained by photosynthesis, and that photosynthesis depends on light exposure. Paciolla et al. (2019) established that photosynthesis leads to an increase in soluble carbohydrates, which serve as precursors to vitamin C [45]. To put it more simply, the amount of light shining on the plant or algae is influencing the concentration of vitamin C positively [46,51]. The fact that Ulvales species generally grow more at the sea surface, meaning they have a high exposure to sunlight, can most likely explain their higher vitamin C content.

The vitamin C content of the selected European seaweed species is now established. However, to understand if their content can be considered as a rich source, it remains to be determined whether their vitamin C content qualifies them. To address this question, a comparison is made to other foods, the recommended nutrient intake, and regulations on nutritional claims.

Table 5 displays the median vitamin C content of the selected seaweeds, oysters, and various plants. The second column is in dry weight, and the third is in wet weight, which represents foods as we eat them. To compare to common foods, some vegetables found in a European supermarket were selected. Moreover, oysters were chosen as they grow in the marine environment, and rosehip, as it has the highest vitamin C content among foods listed in the Danish Food Composition Database [52].

Table 5: The median of vitamin C content in selected seaweeds (by their taxonomic order), oysters, and vegetables. Given in both dried product (mg (g dw)$^{-1}$) and wet product (mg (100 g ww)$^{-1}$) calculated by assuming 85% water content in the seaweeds. The last column demonstrates the total number of observations (seaweed) or number of studies (other foods).

Order or common name	Median mg (g dw) $^{-1}$	Median mg (100 g ww) $^{-1}$	Total number of observations (n)
Palmariales	0.330	4.95	4
Fucales	0.383	5.75	27
Laminariales	0.404	6.06	14
Ulvales	0.942	14.1	19
Oyster	0.704	10.7	7
Lettuce, iceberg	1.17	5.52	17
Potato	1.29	26.4	49
Cucumber	2.81	10.4	52
Broccoli	10.5	117	28
Rosehip	36.4	840	17

First, comparing the seaweeds to rosehip can determine if they can be considered a rich source of vitamin C. As shown in Table 5, the vitamin C contents of the selected seaweeds are considerably lower than the 36.4 mg (g dw)$^{-1}$ found in rosehip, indicating that they are not abundant sources of vitamin C.

Based on the vitamin C content in Table 5, the selected seaweeds have less vitamin C than any of the other foods, except for oysters, on a dry weight basis (second column). However, considering raw seaweeds, they have similar levels of vitamin C to oysters, cucumber, and iceberg lettuce.

To meet the recommended daily intake of 45 mg of vitamin C, an average intake of 400 g wet portion of seaweed is required, as calculated in Paper 1 [2,53]. This is a significant and possibly unrealistic portion of seaweed, considering that Duarte, Bruhn, and Krause-Jensen (2021) established a target consumption in 2050 to be approximately 26.5 g wet portion [8]. Nonetheless, Ulvales can be considered a source of vitamin C according to European food regulations, as its median content of 14.1 mg per 100 g wet weight exceeds the minimum requirement of 12 mg per 100 g for such a claim [54,55].

4.3 Summary of the vitamin C chapter

It has been determined that the species belonging to the Laminariales, Fucales, Palmariales, and Ulvales orders have been found to have a low concentration of vitamin C. Nevertheless, these seaweeds, if consumed fresh, can to some extent contribute to fulfilling our daily dietary requirements of vitamin C.

To answer the research questions stated in the chapter:

- The selected European seaweeds are not considered a rich source of vitamin C compared to other foods, including rosehip, which has the highest vitamin C content among the foods listed.
- Ulvales have a higher median vitamin C content most likely due to their high exposure to sunlight.
- Ulvales can be considered a source of vitamin C according to European food regulations.
- The vitamin C content of the selected European seaweeds is comparable to oysters, cucumber, and iceberg lettuce on wet weight basis, but to meet the recommended daily intake of 45 mg of vitamin C, a significant portion of seaweed (400 g wet portion) is required.
- Although the vitamin C content of the selected European seaweeds is not high, if consumed fresh, consumption of them can contribute to the human daily need of vitamin C.

5 Blanching and washing of kelp

5.1 Reasons to wash and blanch food products

Washing is commonly used for vegetables as a post-harvest treatment to clean off any surface dirt, and furthermore it can reduce some pesticide and fertilizer residues, reduce the microbial load, and enhance the appearance of the product [56].

Blanching is a mild heat treatment often used for fruits and vegetables prior to freezing, drying, or canning. It can be performed by steam or hot water at temperatures of 50-95 °C for 1-2 minutes, but sometimes up to 40 minutes. The main reason to blanch is to denature enzymes. However, it also reduces the number of microorganisms, preserves color before drying, reduces pesticide residues on vegetable surfaces, changes texture, and can enhance nutritional value and flavor [57,58].

The word blanching is usually used when considering high temperatures and short durations (80-95 °C, 1-2 min) to the food item. However, the seaweed industry is using the word blanching even for temperatures down to 45 °C. Perhaps a term such as “warm water treatment” would be more descriptive. But to simplify, this thesis will use the word blanching for any temperature at or above 30 °C and at any process duration, this is even though it can be argued that it is a washing or a warm water treatment process.

Recently the seaweed industry and researchers have explored to utilize blanching or washing in their production to reduce the iodine content in especially kelp [59,60], but also the impact on other safety aspects [61]. The work behind this thesis including Papers 2-4 focus on how blanching and washing influence sugar kelp and winged kelp on the various food quality aspects including the retention of nutrients and other bioactive compounds.

5.2 Aim and research questions of the washing and blanching chapter

The aim of this chapter is to describe the influence of washing and blanching on the quality of sugar- and winged kelp. Also, to illustrate how washing and blanching can help obtain safer seaweed products with a focus on reducing the content of potential chemical hazards such as iodine and arsenic.

Some scientists view the term "heavy metals" as arbitrary, as it lacks a precise definition [62,63], and propose the use of the term "potentially toxic elements" (PTEs) to cover a wider range of elements, thus include metals (e.g., magnesium, sodium), metalloids (e.g., arsenic, silicon), and non-metals (e.g., bromine, iodine) [64]. We know that excessive exposure to some elements, e.g., sodium or iodine, can be toxic to humans. Therefore, within food science, the definition “potentially toxic elements” aligns better and will be used in the thesis.

The chapter also describes a predictive model for the iodine concentration in sugar kelp depending on process parameters. It will furthermore address the retention of nutrients and other beneficial compounds. And it answers questions on the influence of washing and blanching on the microbial load. The chapter builds upon the findings of Papers 2-4.

The objective of the chapter is to address the following research questions:

- If any, which components pose as a potential health risk if kelps are used for human consumption?
- In what ways can blanching enhance the safety of kelp intended for human consumption?
- How does the blanching temperature and duration affect the iodine concentration?
- How is the proximate composition and other beneficial nutrients affected by blanching?
- What are the effects of blanching on other dimensions of food quality in kelp?
- Based on the research outcomes, which blanching or washing parameters are recommended for use by the industry in the processing of kelp species in the future?

5.3 Safety aspects of blanched kelp

In the background chapter the three groups of food quality were stated (Figure 3) with safety as the most important group. Washing and blanching can increase the food safety of seaweed products within three of the dimensions: physical, chemical, and microbial (Figure 4). The two processes can wash away unwanted physical hazards, such as sand, stones, and small crustaceans on the product. The processes can also, as in vegetable production, reduce the microbial load (Paper 2 and 3) [4]. Seaweed accumulates PTE's such as arsenic, cadmium, as well as high amounts of sodium and iodine. Arsenic, iodine, and sodium can be reduced by washing and blanching (Paper 2, 3 and 4) [3,4], whereas cadmium increase after the processes (Paper 3). It is important to understand the fate of the various PTE's and microorganisms during processing to enable a correct safety evaluation of the seaweed products.

5.3.1 Iodine from kelp – a possible health concern

Iodine is a micronutrient essential for the synthesis of thyroid hormones (T3 and T4) and maintaining normal thyroid function [65]. Thyroid hormones play a key role in regulating energy, lipid and protein metabolism, cellular oxidation, and thermoregulation [66]. Iodine deficiency has been a global concern, which in 1994 led the World Health Organization (WHO) to recommend a universal salt iodization to increase the iodine status in the general population [67]. Contradictory, it is also possible to suffer from thyroid dysfunction by an excess of iodine intake [65,66]. Because of the risk of excess iodine intake, a tolerable upper intake level (UL) of 600 µg day^{-1} has been established for adults by the European Food Safety Authority (EFSA) [68]. Seaweed may represent a new dietary iodine source and high iodine levels have been reported in some seaweed species, and consequently the intake of some seaweed products may cause excessive iodine exposure [69].

Seaweeds, and in particular kelp species from the order Laminariales, are well known to contain high levels of iodine [37,59,70]. The iodine content in kelp is influenced by various factors, including geographical origin, environmental factors (such as season and water salinity), as well as intrinsic factors such as the part of seaweed used, and their age [71,72]. Küpper et al. (1998) found that kelp species use iodine, in the form of iodide, as an osmolyte, photo inhibitor and inorganic antioxidant [73,74], which may

explain the high iodine levels in the seaweeds. Table 6 shows the iodine content from unprocessed Norwegian and Danish cultivated kelp at different locations and harvest times from Papers 2-4, as well as results generated during the project SusKelpFood [75]. All results are consistent with data from peer reviewed literature [37,59,69,70].

Table 6: Iodine content (mean ± SD) in cultivated unprocessed sugar kelp and winged kelp divided into cultivation location and harvest time of the year.

	Iodine content (mg (kg dw)$^{-1}$)	Source
Sugar kelp		
Frøya, Norway		
April, 2018	4,610 ± 274	Paper 2 [3]
May, 2020	4,820 ± 331	Paper 3
Herøy, Norway		
May, 2022	5,740 ± 540	[75]
Isefjord, Denmark		
May, 2020	2,000 ± 331	Paper 4 [4]
Winged kelp		
Frøya, Norway		
May, 2020	682 ± 95	Paper 3
Værlandet, Norway		
April, 2022	2,140 ± 169	[75]

All iodine contents are based on three to five samples (n=3-5).

Marine foods are known to be high in iodine, however, other marine foods do not contain levels as high as the kelp species. For example, raw lobster contains 29.2 mg (kg dw)$^{-1}$ and raw cod 13.1 mg (kg dw)$^{-1}$ [52], which is less than 5% of any of the levels reported in Table 6. In other words, kelp species can easily pose as a health concern by just consuming two grams wet weight of fresh sugar kelp, because it will lead to an exposure above the UL (Paper 2) [3]. Aakre et al. (2021) found that consumption of one portion of 31 out of 40 commercially available wholefood seaweed products would lead to a higher exposure of iodine than the UL. It is therefore recommended that the seaweed industry controls the iodine content and find ways to reduce the iodine content before introducing the seaweed products on the market [69,71,76].

5.3.2 Iodine reduction in kelp including a predictive model for sugar kelp

A recent EFSA report on the dietary exposure to iodine from seaweed consumption confirmed that brown seaweeds, hereunder kelp, have the highest mean iodine levels compared to red and green seaweed species. They recommend that a continuing monitoring of iodine should be conducted and include both raw seaweeds and processed products [71]. It is therefore crucial to understand how iodine is reduced in the kelp species. A major focus of the work behind this thesis has been the reduction of iodine by blanching (Paper 2, 3, and 4). This section will later present a predictive model for the iodine concentration in sugar kelp.

A scatter plot featuring all collected data from Norway is presented in Figure 10, with Danish sugar kelp excluded from the plot. This exclusion is due to the notably lower initial iodine content in Danish kelp, as shown in Table 6.

According to Paper 2, the effect blanching time has on iodine, is lower at higher temperatures (> 60 °C) compared to lower temperatures (30-45 °C), meaning the iodine reduction is highly dependent on temperature in the beginning of the process treatment (as illustrated in Figure 10). Furthermore, Paper 3 reveals that the seaweed-to-water ratio is a critical factor to consider, as illustrated by the observed reduction in iodine when the concentration of seaweed is increased from 50 kg m^{-3} to 500 kg m^{-3}, as shown in Figure 1 of Paper 3. Additionally, the type of water used in blanching, (seawater or tap water), has shown to be equally effective in reducing the iodine levels in both Norwegian and Danish sugar kelp, as shown in both Paper 3 and Paper 4 [4].

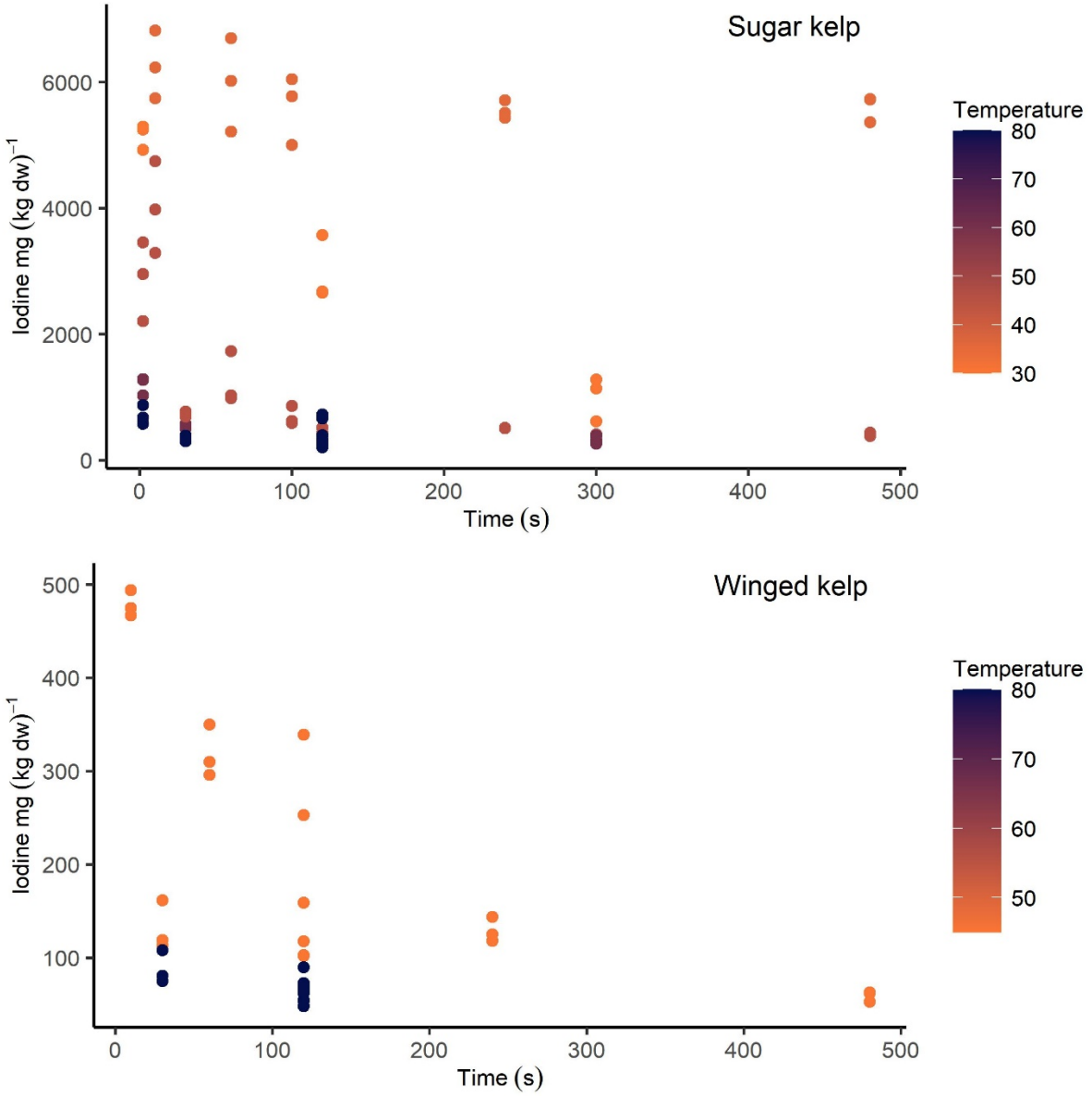

Figure 10: Iodine levels in Norwegian cultivated sugar kelp (top) and winged kelp (bottom) following blanching at various temperatures (colors) and blanching duration times. Data was collected from several studies with a total of 92 and 36 data points for sugar kelp and winged kelp, respectively. (Paper 2-3, [3,75]).

A predictive model for iodine reduction is constructed based on data collected from Paper 2 and 3 on Norwegian kelp [3,4], in order to provide information on how the iodine level is affected at a range of temperatures and durations. This was to see the effect between the temperatures (30, 45, 60, and 80 °C) and blanching duration times (2, 30, 120, and 300 s) from Paper 2.

Exponential regression was found to be the most suitable model for the sugar kelp data. For winged kelp, insufficient amount of data was available to construct a reliable model. The equation of the model for iodine content in sugar kelp can be found in

Equation 1, and the coefficients and regression diagnostics are shown in Table 7. The model is further illustrated in a contour plot in Figure 11. Other regressions such as linear and polynomial regression were also tested but did not provide promising fits according to the model diagnostics compared to the exponential regression.

Equation 1: Prediction of the iodine concentration in blanched sugar kelp by exponential regression in the temperature range 30-80 °C, and blanching durations ranging from 2 to 480 seconds.

$$I_{sugar\ kelp} = e^{a_0 - a_1 T + a_2 t - a_3 T t}$$

The model can predict the iodine concentration in blanched sugar kelp, where $I_{sugar\ kelp}$ is the iodine concentration in mg (kg dw)$^{-1}$ after blanching at T, temperature (°C) for t, time duration (s). The model is subject to certain limitations including that the sugar kelp has an initial iodine concentration of about 4,000-7,000 mg (kg dw)$^{-1}$, a kelp-to-water ratio between 15-50 kg m^{-3}, use of either seawater or tap water as the blanching medium, blanching water temperatures between 30-80 °C, and blanching durations ranging from 2 to 480 seconds. However, the model does not account for possible come-up times, which is the case that a decrease in water temperature occurs due to the addition of seaweed.

Table 7: Coefficients and regression diagnostics of the exponential predictive model for iodine content in blanched sugar kelp

Coefficients of the model	Estimated values
a_0	9.14
a_1	$3.63 \cdot 10^{-2}$
a_2	$3.79 \cdot 10^{-3}$
a_3	$1.43 \cdot 10^{-4}$
F-value	48.4
p-value	$< 2.2 \cdot 10^{-16}$
Residual SE	2.06
R^2	0.623

When investigating the coefficients, the temperature coefficient a_1 was shown to have the largest effect on the iodine concentration; ten times bigger than the coefficient for time (a_2). This means that temperature is the most significant variable for reducing the iodine concentration within the limitations of the model.

The statistical significance of the model is evidenced by the low p-value. Additionally, the F-value of the model implies that the overall variance of the model is 48.4 times higher than the within-group variance, further supporting the validity of the model.

The R squared (R^2) shows that 62.3% of the variance in iodine concentration can be explained by the predictors (temperature and time), while 37.7% of the variance is depending on other unaccounted factors (presumably location, initial iodine content, growing depth, and time of harvest). While the R^2 is not optimal, increasing the number of data points within the model's limitations may improve it. Another suggestion is to include studies on the effect of the kelp-to-water ratio and the influence it has on the iodine concentration and incorporate the ratio as a predictor variable in the model.

The residual standard error (SE) measures the average distance between observed values and the regression line, indicating that the observed values of the model deviate from the regression line by an average of 2.06 mg $(kg\ dw)^{-1}$. It is essential to recognize that the predicted numbers are only estimates, and there is uncertainty associated with the model.

The contour plot (Figure 11) illustrates the influence, which the predictor variables “temperature” and “blanching duration time” have on the iodine concentration in blanched sugar kelp and can be used to make an approximate estimation of the concentration. For instance, if the blanching is conducted at 60 °C for 120 seconds, the predicted iodine concentration would be 500-750 mg $(kg\ dw)^{-1}$. If the aim is to achieve a maximum level of 500 mg $(kg\ dw)^{-1}$ at the lowest possible temperature, then the blanching should be conducted at approximately 45 °C for 480 seconds (8 minutes).

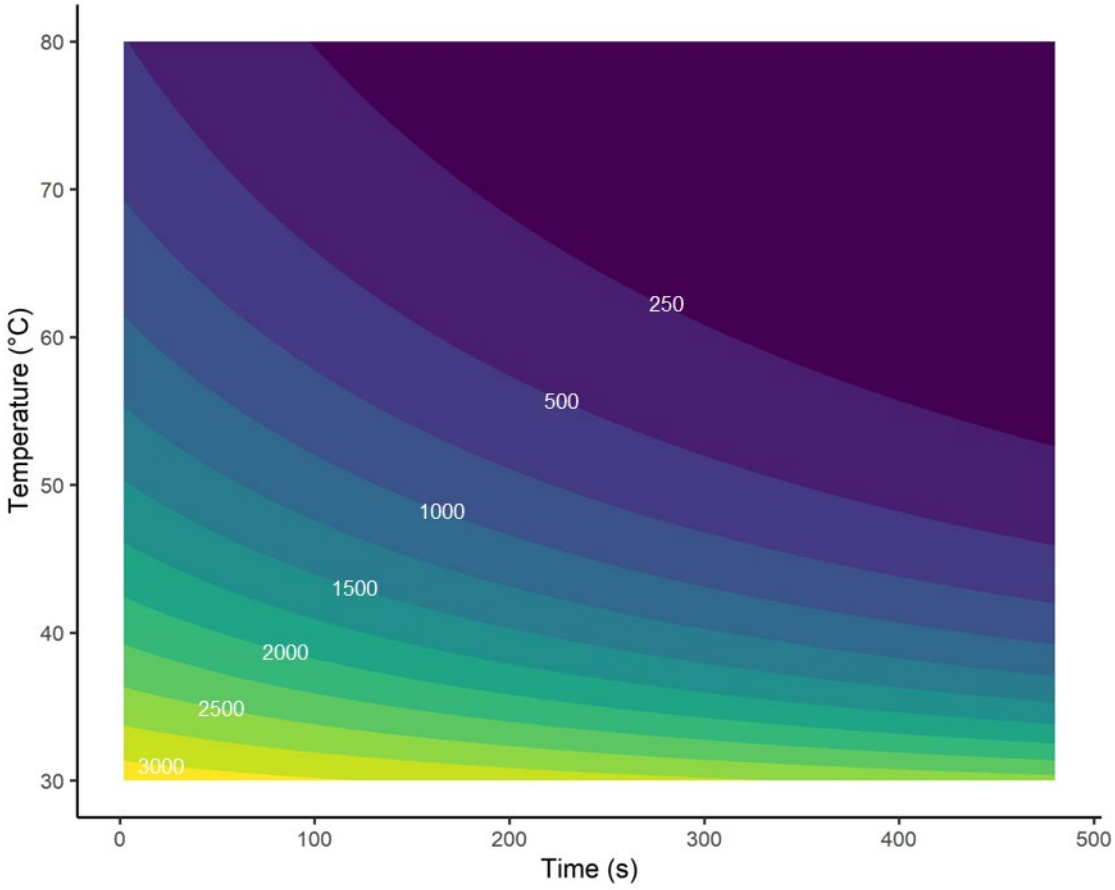

Figure 11: A contour plot of the predictive model for the end concentration of iodine (white numbers) in mg (kg dw)$^{-1}$ for sugar kelp with time in seconds and temperature (°C) as predictor variables. The initial iodine concentration in the sugar kelp should be 4,000-7,000 mg (kg dw)$^{-1}$, and blanched in a kelp-to-water ratio between 15-50 kg m^{-3}.

5.3.3 Two potential chemical hazards: arsenic and cadmium

Banach et al. (2020) reviewed and prioritized the food safety hazards in seaweeds and identified both arsenic and cadmium to be two major chemical hazards [28]. Based on a study on PTEs in different seaweed products, Desideri et al. (2016) recommended more monitoring of the concentrations of total arsenic (As) and cadmium (Cd) in seaweed products [77]. Paper 3 examines how blanching and washing influence these two PTEs and discusses if the concentrations pose any health concerns to consumers. A subset of the results from Paper 3 and its supplementary material is found in Table 8.

Table 8: The content (mean ± SD) of total arsenic, inorganic arsenic, and cadmium in fresh and blanched winged or sugar kelp and the maximum allowed levels in feed or food.

	Total arsenic mg (kg dw) $^{-1}$	Inorganic arsenic mg (kg dw) $^{-1}$	Cadmium mg (kg dw) $^{-1}$
Maximum allowed level	40[A]	2.0[A]	3.0[B]
Winged kelp			
Fresh	47.9 ± 2.7	<0.033	2.09 ± 0.05
Blanched	▼28.5 ± 5.0	<0.033	2.45 ± 0.35
Sugar kelp			
Fresh	66.7 ± 13.8	0.0553 ± 0.0146	0.838 ± 0.085
Blanched in tap water	58.9 ± 5.2	▲0.123 ± 0.048	▲1.35 ± 0.16
Blanched in seawater	▼39.7-39.9	*n.a.*	1.08 ± 0.04

The blanching conditions are between 45-80 °C for 30-120 seconds. The specific conditions can be found in Paper 3 and its Table 1. [A]Based on EU Directive 2002/32/EC on animal feed [78]. [B]Based on Commission Regulation (EC) 1881/2006 in foodstuffs [79]. ▼Decrease relative to the content in the fresh kelp. ▲Increase relative to the content in the fresh kelp. *n.a.* not analyzed.

Stévant et al. (2018) found an increase of Cd in sugar kelp soaked in fresh water for 22 hours (16 °C). However, two hours of hyper-saline bath treatments (0.5-2.0 M) reduced the Cd content significantly [59], indicating that seawater blanching might reduce Cd. Bruhn et al. (2019) found an increase of total arsenic in sugar kelp boiled for 15 minutes. [80]. Recently, after the experimental work in Paper 3 was finalized, two other studies were published on the influence of hydro-processing on cadmium and arsenic in kelp [61,81]. Blikra et al. (2021) found a significant reduction of total arsenic in sugar kelp after boiling [61] and Trigo et al. (2023) found that blanching at 45 and 80 °C reduced total arsenic content, but increased cadmium in sugar kelp [81]. However, to the best of our knowledge there has until now been no study reported on the influence of hydro-processing on cadmium, total arsenic, and inorganic arsenic in winged kelp.

In the present work, the total arsenic content in winged kelp was reduced to levels below the maximum level by blanching. However, blanching of sugar kelp in tap water did not reduce the total arsenic content similarly, in agreement with the results reported by Trigo et al. (2023). Seawater blanching was found to be more effective in reducing the total arsenic content. Since the total arsenic content can vary between different kelp species and is not always reduced to safe levels, monitoring and documentation of total arsenic levels in kelp production for both winged kelp and sugar kelp is important, as emphasized by Banach et al. (2020) [28]. Additionally, it would be

beneficial to identify or develop methods for reducing total arsenic content in both kelp species to ensure safety for consumers.

The content of inorganic arsenic in winged kelp is not of safety concern due to very low levels in fresh kelp (Table 9). Blanching of sugar kelp in tap water increased the concentration of inorganic arsenic, most likely because of the decrease in dry matter content (Paper 3). Nevertheless, it was only 6% of the maximum level in the EU legislation [78]. Based on these results, the industry should not worry about the inorganic arsenic contents of winged kelp and sugar kelp.

All cadmium levels were below the maximum levels. Blanching of sugar kelp in tap water increased the content, as also reported in the study by Stévant et al. (2018) [59]. No increase occurred due to seawater blanching, which was as expected. It is relevant to further study the effects of seawater blanching on both species, as the lower cadmium concentration the better. It is noteworthy to mention that Desideri et al. (2016) found levels up to 7.2 mg (kg dw)$^{-1}$ in another brown kelp *Laminaria digitata* [77]. Therefore, it is important to continue monitoring the cadmium content of kelp intended for consumption.

5.3.4 Microbial safety

There are several methods that can investigate microbial food safety. A simple method to determine microbial safety is to count (enumerate) the bacterial colonies by plating out a sample on an agar plate containing a substrate for the bacteria to grow. When enumerating the total counts, it is also called aerobic viable count (AVC). Seaweed is not explicitly mentioned in European regulations on food microbiological criteria. However, France has established standards for dried seaweed products, which include limits on mesophilic aerobic bacteria (such as *Bacillus*, *Pseudomonas*, and *Enterobacteriaceae*), and fecal coliforms, as well as a requirement for the absence of *Salmonella* in 25 g of dried product [82]. Kreissig et al. (2023) mentions that currently no effort is made to establish microbiological standards tailored to seaweed food safety [82]. In vegetable products, a threshold value of 7 log (CFU g^{-1}) is often used, which gives a maximum acceptable contamination value and an acceptable food quality [4,39,83]. This threshold was confirmed in Paper 4 by following the bacterial

growth during storage [4]. Chapter 7 provides more information on the stability of sugar kelp during storage.

Obviously, this threshold value should not be used on fermented kelp, but only on fresh and otherwise processed kelp. This is because bacteria are not always the cause of spoilage or are hazardous. Fermented foods have a high AVC, but the purpose of the fermentation bacteria is to keep the product safe.

Vegetative bacterial cells are heat sensitive [84]. Thus, blanching can inactivate microorganisms. Table 10 is a collection of the AVC from Paper 3 and Paper 4. It demonstrates the AVC in unprocessed (fresh) sugar and winged kelp and kelp processed by washing and blanching. The washing and blanching were performed in either tap water or filtrated seawater. The blanching water type had the same effects on the AVC, which is why they are merged in the table.

Table 9: Aerobic viable counts (AVC) before and after washing and blanching (mean ± SD). Descending accordingly to the AVC. All harvested in May 2020 with the blanching and washing processes performed in a ratio of 50 g seaweed per liter of water.

Kelp	Country	Processing	AVC (log (CFU g^{-1}))	Source
Sugar kelp	Denmark	Fresh	4.54 ± 0.29	Paper 4 [4]
Winged kelp	Norway	Fresh	3.87 ± 0.02	Paper 3
Sugar kelp	Norway	Fresh	3.52 ± 0.20	Paper 3
Sugar kelp	Denmark	4-16 °C, 300 s	3.95-4.03	Paper 4 [4]
Sugar kelp	Norway	45-80 °C, 30-120 s	1.80-2.32▼	Paper 3
Winged kelp	Norway	45-80 °C, 30-120 s	1.50-2.40▼	Paper 3
Sugar kelp	Denmark	76-80 °C, 120 s	0.906-1.77▼	Paper 4 [4]

▼Decrease relative to the AVC in the fresh kelp.

All samples are below the threshold of 7 log (CFU g^{-1}), which indicates that they are all a safe, unspoiled food product when considering total enumeration. This is, of course, with the assumption that the seaweeds do not contain any pathogens (disease-causing microorganisms). The initial AVC in fresh kelp depends on the time of harvest and location as demonstrated by the countries.

Even though the AVCs in the table are all below the threshold value, the industry might have an interest in inactivating bacteria present on their product to delay spoilage. One assumption was that the bacteria would be diluted or washed away independent of the

processing temperature. The study from Paper 4 found that washing at 4-16 °C for five minutes did not significantly reduce the AVC. Thus, the bacteria are not simply washed away. On the other hand, washing or blanching at as low as 45 °C for just 30 seconds will decrease the AVC drastically. This suggests that the bacteria present on the kelp are inactivated because of the elevated temperatures. These findings are corresponding to a previous study on Norwegian winged and sugar kelp by Blikra et al. (2019), who investigated blanching at 60-95 °C [26].

The maximum water temperatures in which the kelp grows are 15-20 °C, considering that the farmers deploy the kelp in fall in Northern Europe at latitudes of N55-65°, where they will grow until harvest in spring. This means that the microorganisms present are most likely psychrotolerant (grows down to -1 °C with optimum temperatures 20-30 °C), with a limited content of mesophiles. Mesophiles can grow between 10 to 45 °C with an optimum between 30 and 40 °C [84]. This can explain why we don't see a reduction in the microbial load for processing temperatures of 4-16 °C, but that we see from ≥45 °C. When blanching at 45 °C, it is above the optimum cardinal temperature of the psychrotolerant bacteria. This might lead to an irreversible denaturation of enzymes and proteins in the bacteria, thus damaging cell function [84].

A knowledge gap exists in the temperature range of 17-44 °C regarding the significant impact of temperature on bacterial inactivation in kelp. It is recommended that the industry investigate the influence of temperatures below 45 °C on bacterial inactivation if they plan to wash kelp at such temperatures. However, it is likely that the industry should avoid washing or blanching kelp below 45 °C, based on the maximum growth temperature of 45 °C for mesophiles.

5.4 Positive quality aspects of blanched kelp

The previous chapter discussed the most important aspect of food quality, namely safety. However, food quality is also about the original quality and increasing the value by e.g. processing. This section will discuss how blanching changes the proximate composition, how other valuable compounds are retained or decreased, and how the sensory and physico-chemical properties are altered.

5.4.1 Proximate composition and other valuable compounds

The proximate composition of the kelp species varies depending on the blanching procedure applied. Table 11 illustrates the water, ash, carbohydrates, fat, protein, and salt content after different blanching procedures based on Papers 2-4. The results in Table 11 are given in % dry weight (dw) and can be used for nutrient labeling of dry kelp products. However, it is important to remember that biological material will vary due to time of harvest, location, age of the kelp, etc. [29,85].

Paper 2 discusses in depth the true retention factors and mass balances of sugar kelp occurring during blanching in tap water (Table 3 and Figure 2 of Paper 2) to establish if there is a product loss due to blanching [3]. Paper 3 includes a TreeMap (Figure 3), which illustrates the proximate difference between fresh and blanched winged and sugar kelp. Interestingly, the proximate composition of winged kelp and sugar kelp after 80 °C blanching at 120 seconds became almost identical.

Seawater blanching is in particular interesting, as the composition is not affected as much as when blanching in tap water. To the best of our knowledge, only our work in Papers 3 and 4 has investigated the blanching of kelp using seawater in a scientific context. The effect of seawater blanching is discussed in detail in Paper 3. The Paper concludes that blanching kelp using membrane filtered UV-treated seawater at 80 °C for 120 seconds have promising industrial applications as it effectively reduces iodine and arsenic, inactivates microorganisms, and retains important carbohydrates.

The effect of blanching on other valuable compounds, such as selected minerals, vitamins, amino acids, and polyunsaturated fatty acids (PUFAs) are gathered from Papers 2-4 in Table 12.

Where possible, Table 12 also gives the daily recommended nutrient intake of compounds established by the Nordic Nutrition Recommendations (2012) [86]. If considering the previous estimation of a realistic intake of 2.65 g dw day^{-1} in 2050 [8], which is approximately equivalent to 26.5 g ww, then consuming fresh kelp will contribute less than 10% of the recommended intake of calcium, folate, magnesium, and vitamin C (ascorbic acid) (Paper 3) [2]. Since the two vitamins ascorbic acid and folate are heat labile, their concentration decreases when blanched. This is not the case for magnesium in sugar kelp and calcium in both kelps. They most likely increase

due to the loss of other compounds during blanching. Consuming 2.65 g dw of blanched kelp will therefore provide a higher portion of those two minerals.

Table 10: The average proximate and salt composition of fresh and blanched cultivated kelp species from Denmark and Norway. The blanching duration was 2 minutes for all cases.

Proximate	Fresh	Blanched tap water, 45 °C	Blanched tap water, 80 °C	Blanched, seawater, 80 °C
Sugar kelp				
Water (% ww)	88.1-91.3	94.8-95.7	91.2-95.6	90.2-90.4
Ash (% dw)	25.7-50.1	10.8-13.3▼	10.4-12.5▼	35.7-41.9
Carbohydrates (% dw)	39.3-41.8	68.3-68.7▲	63.7-68.7▲	*n.a.*
Fat (% dw)	3.87-5.80	4.15-10.2	5.30-8.70	*n.a.*
Protein (% dw)	7.64-7.90	12.3-14.2▲	13.4-15.3▲	7.17
Salt (% dw)	10.3-13.2	2.55▼	1.23-2.84▼	22.3-27.7▲
Winged kelp				
Water (% ww)	89.0	90.1	92.8	89.2
Ash (% dw)	40.8	16.4▼	12.9▼	31.8▼
Carbohydrates (% dw)	47.1	*n.a.*	67.0	*n.a.*
Fat (% dw)	3.64	*n.a.*	6.67▲	*n.a.*
Protein (% dw)	8.99	12.4▲	13.7▲	8.83
Salt (% dw)	19.3	2.83▼	2.07▼	22.1

The numbers are gathered from Paper 2, 3 and 4 and given as the minimum average and maximum average from the different papers [42], [43]. In the case only one number is given, and not a range, it is because only one paper investigated that proximate. *n.a.* not available. ▼Decrease relative to the content in the fresh kelp. ▲Increase relative to the content in the fresh kelp.

Table 11: The average content in fresh and blanched kelp of other valuable compounds found in sugar kelp and winged kelp compared to the recommended intake established by the Nordic Nutrition Recommendations 2012 [86].

Compound	Recommended intake	Unit	Fresh	Blanched in tap water			
				45 °C		80 °C	
Sugar kelp				30 s	120 s	30 s	120 s
Ascorbic acid	75 mg day^{-1}	mg (100 g ww)$^{-1}$	3.09-8.73	*n.a.*	*n.a.*	*n.a.*	<LOQ▼
Aspartic acid (free)	*n.a.*	mg (g dw)$^{-1}$	1.68-2.62	*n.a.*	*n.a.*	*n.a.*	LOQ-0.783▼
Aspartic acid (total)	*n.a.*	mg (g dw)$^{-1}$	12.5	16.1▲	18.2▲	20.1▲	22.0▲
Calcium	800 mg day^{-1}	mg (g dw)$^{-1}$	9.10	12.4▲	14.2▲	14.1▲	13.4▲
Folate	300-400 µg day^{-1}	µg (100 g ww)$^{-1}$	18.3	*n.a.*	*n.a.*	*n.a.*	3.97▼
Glutamic acid (free)	*n.a.*	mg (g dw)$^{-1}$	0.977-3.49	*n.a.*	*n.a.*	*n.a.*	LOQ-1.00▼
Glutamic acid (total)	*n.a.*	mg (g dw)$^{-1}$	13.8	16.7▲	18.1▲	20.3▲	21.8▲
Magnesium	280-350 mg day^{-1}	mg (g dw)$^{-1}$	11.9	7.43▼	7.92▼	8.14▼	8.73▼
Mannitol	*n.a.*	% dw	7.66-16.6	*n.a.*	*n.a.*	*n.a.*	0.675▼
PUFA	*n.a.*	% FAME	51.5	57.6▲	*n.a.*	*n.a.*	67.2*▲
TPC	*n.a.*	µg GAE (mg extract)$^{-1}$	5.66	29.8▲	*n.a.*	*n.a.*	54.4*▲
Winged kelp							
Ascorbic acid	75 mg day^{-1}	mg (100 g ww)$^{-1}$	4.27	0.573▼	*n.a.*	*n.a.*	<LOQ▼
Aspartic acid (free)	*n.a.*	mg (g dw)$^{-1}$	3.05	*n.a.*	*n.a.*	*n.a.*	0.427▼
Calcium	800 mg day^{-1}	mg (g dw)$^{-1}$	10.2	11.6	13.5▲	13.2▲	14.5▲
Folate	300-400 µg day^{-1}	µg (100 g ww)$^{-1}$	113	*n.a.*	*n.a.*	*n.a.*	17.0▼
Glutamic acid (free)	*n.a.*	mg (g dw)$^{-1}$	3.41	*n.a.*	*n.a.*	*n.a.*	1.08▼
Magnesium	280-350 mg day^{-1}	mg (g dw)$^{-1}$	8.47	7.07	8.25	8.68	8.55
Mannitol	*n.a.*	% dw	2.75	*n.a.*	*n.a.*	*n.a.*	*n.a.*

The numbers are gathered from Paper 2, 3, and 4 and given as the minimum average and maximum average from the different papers [42], [43]. In the case only one number is given, and not a range, it is because only one paper investigated that compound. The recommended intake is for adults. *n.a.* not available. ▼Decrease relative to the content in the fresh kelp. ▲Increase relative to the content in the fresh kelp. *Blanching duration was 300 seconds.

5.4.2 Sensory and physico-chemical properties

So far, this chapter has discussed chemical and microbial dimensions of quality changes occurring due to washing and blanching. However, this section will discuss the sensory and physico-chemical quality changes. In Paper 4 the effects on quality from blanching (76-80 °C for 2 minutes) and washing (4-16 °C for 5 minutes) were assessed by physico-chemical measurements such as color (Lightness, red-green, and yellow-blue), texture (firmness), drip loss, and water content and a sensory panel performed a descriptive profile analysis. The individual analytical results can be found in Paper 4, Figure 2-3, and Table 3-4. Nevertheless, a compilation of these results is examined by a principal component analysis (PCA) and illustrated by a bi-plot in Figure 12. The scree plot (not shown) indicated that PC1 and PC2 were enough to explain the variance, with a total variance explained of 69%.

The plot shows a division of the treatments into two groups demonstrated by the dashed blue line. This means blanching leads to changes relative to the untreated product, however washing does not. The analysis also shows that the water type used for blanching is not relevant when considering the physico-chemical and the sensory descriptions measured. It is important to note that the panel did not taste the samples. It is known from Table 11 that the seawater blanched samples consist of higher NaCl concentrations, but it is not possible for humans to detect an odor from NaCl. As the panel did not assess the taste, it is not possible to draw a direct conclusion regarding whether samples blanched in seawater have a distinct taste in comparison to those blanched in tap water. However, it is likely that they differ in terms of salty taste. Further investigation of the taste differences is required since taste significantly impacts consumer preferences and utilization possibilities.

Odors such as sweet, fresh sea, umami, sour, and rubber describe the blanched kelp more than the washed and untreated sugar kelp, which, according to the PCA, had less odor. Interestingly, the only other study found, which conducted sensory analysis on hydro processed sugar kelp found that the processed (95 °C for 15 min.) and untreated kelp would have the same intensity of “sea smell” [80]. The color measurements also showed that untreated and washed kelp had a redder color on the red-green scale (a* CIELab). This color difference can clearly be seen in Figure 13 on the sugar kelp pictures (A and B) and on the microscopic pictures (C and D).

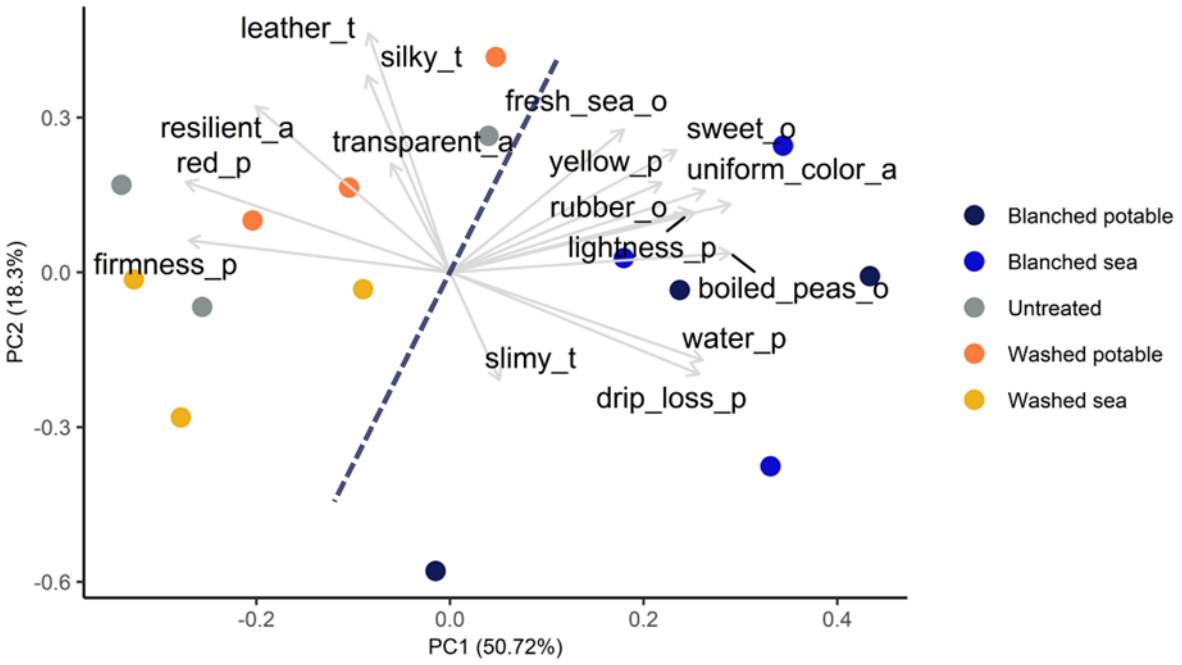

Figure 12: Bi-plot showing the difference and correlation of sensory (appearance (a), odor (o) and texture-touch (t)) and physico-chemical properties (p) of sugar kelp treated by blanching (76-80 °C for 2 minutes) and washing (4-16 °C for 5 minutes) in sea water or potable water (colors).

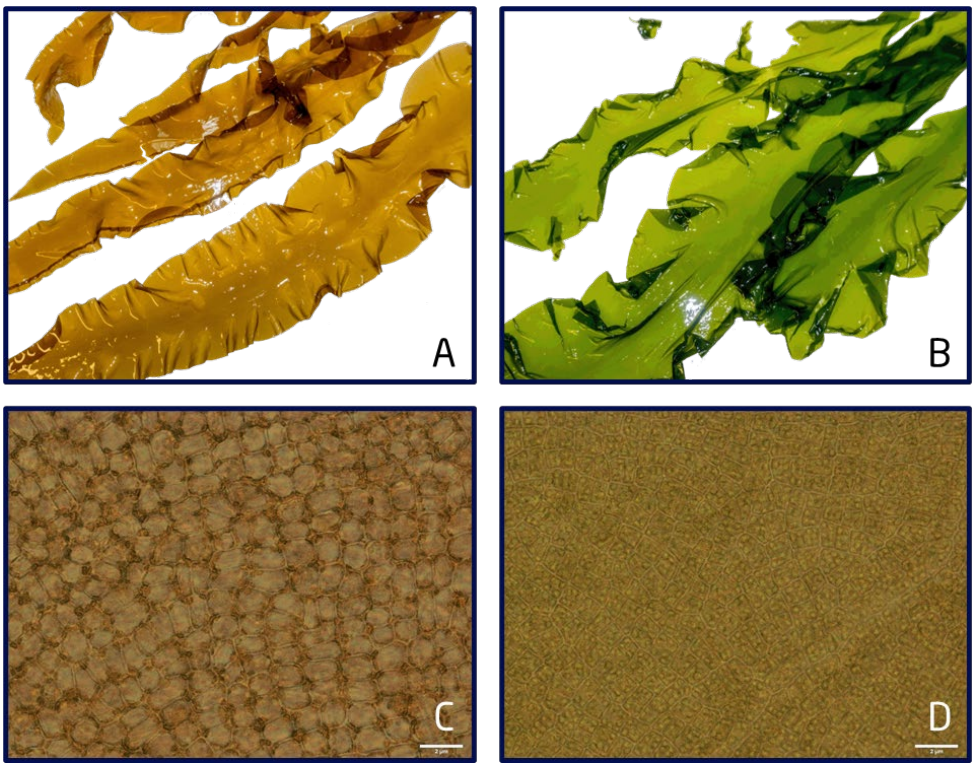

Figure 13: Pictures of (A) untreated sugar kelp and (B) blanched sugar kelp (80 °C for 2 minutes). Microscopic picture at cellular levels of (C) untreated sugar kelp, and (D) blanched sugar kelp (80 °C for 2 minutes). The bars below the C and D letter represents 2 mm.

5.5 Summary of the washing and blanching chapter

Ensuring food safety is a top priority in food quality assessment. Blanching influences the kelp species in terms of all quality aspects, which is summarized below.

To answer the research questions stated in the chapter:

- The high iodine content in kelp can lead to an excess intake and possibly cause thyroid dysfunction, leading to iodine from kelp posing as a health risk.
- The total arsenic content can be considered high in both sugar kelp and winged kelp when comparing to the maximum level in animal feed, thus should be monitored. Inorganic arsenic does not pose as a health risk in the two species.
- Blanching is an effective way to enhance the safety of commercial kelp intended for human consumption by reducing iodine levels, total arsenic, and inactivating bacteria.
- The iodine concentration is affected by blanching temperature and duration, and the reduction of it in sugar kelp can be predicted by the provided exponential regression.
- Blanching affects the proximate composition and concentration of other nutrients in kelp, with blanched kelp providing a higher portion of calcium but decreasing the concentration of magnesium (in sugar kelp), mannitol and heat labile vitamins like ascorbic acid and folate.
- Blanching can change the sensory characteristics of kelp, with blanched kelp having different odors and color compared to washed and untreated kelp.
- Based on the research outcomes, it is recommended that the industry blanches kelp at temperatures of minimum 45 °C for 30 seconds based on the maximum growth temperature of mesophiles and the iodine reduction.
- Seawater blanching should be investigated further as it does not affect the proximate composition as much as blanching in tap water, but still reduces iodine and arsenic, and inactivates microorganisms.

6 Drying sea lettuce and bladder wrack

6.1 The relationship between water and drying

Drying of foods is an important preservation method that has been used for centuries to extend the shelf-life of food products. Drying of foods, also known as dewatering and dehydration, is a process where water is removed. Water is dominating in almost all food raw materials. It influences the physical properties, how the food behaves during processing, microbial growth, chemical reactions, stability, taste, and the phase transitions [87]. Therefore, the presence of water can lead to decomposition of the food. Drying leads to a food product with low moisture content and water activity (a_w), which reduces the potential for most chemical reactions that cause off-flavors and discoloration. A low water activity also inhibits the growth of mold and bacteria and even destroys some microorganisms [88]. By drying a food, it can also enhance the quality of the food with regards to taste, digestibility, color, and flavor.

Moisture removal depends on the external factors: temperature, humidity, and pressure [89]. The various drying methods are based on the change in these factors. These can be adjusted in numerous ways forming the basis of different drying methods.

Phase changes are essential when discussing removal of moisture. Water can exist in different phases, such as solid (ice), liquid (water), and gas (vapor). Figure 14 demonstrates the phase diagram of pure water. Here the different phase transitions and how temperature and pressure relate are seen. For example, at 1 atm (101,325 Pa) and 100 °C, water vaporizes or vapor condensates. And, for example, at 1 atm 0 °C, water freezes or ice melts. At the triple point, the ice, water, and vapor coexist in a thermodynamic equilibrium. Below the triple point, ice can sublimate into vapor, or vapor can deposit into ice.

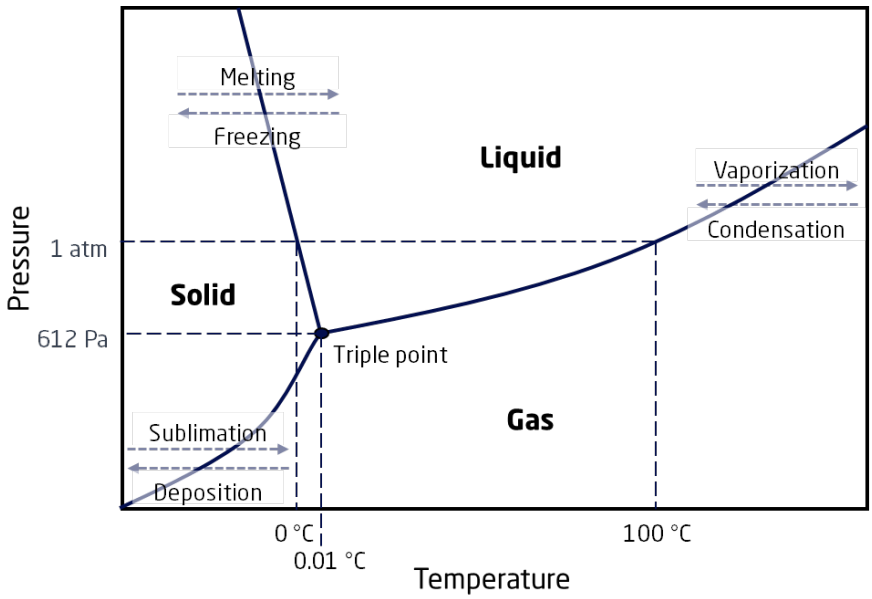

Figure 14: Phase diagram of water for the solid, liquid, and gas states and the phase transitions.

For high water content foods, e.g., seaweeds, the phase behavior is similar to pure water. However, the drier the food becomes, the less it behaves as water. This is because water in foods is not chemically pure, as water-soluble compounds, e.g., salts and sugars, are dissolved in it. When drying, the solids become concentrated, which affects the phase behavior of the remaining mixture of solids and water, leading the solids to control the physical state and phase transitions [87].

6.2 Aim and research questions of the drying chapter

With an increasing market demand for European seaweeds comes a need for fast and controlled drying methods [19]. While drying of foods has been studied widely, the studies on the effect of drying on seaweed quality are limited. Before this thesis work, few studies have been conducted on the drying of *Fucus vesiculosus* (bladder wrack) and *Ulva* sp. (sea lettuce). For sea lettuce, the studies are focusing on sun, convective, freeze, and vacuum drying [19,90,91]. For bladder wrack, the studies are mainly on convective and freeze drying [19,42,92], they mainly focus on the chemical quality, some physico-chemical properties, and technical aspects.

This study is the first to investigate microwave-vacuum drying as a substitute for freeze drying for seaweeds. With their high moisture content (68-87%), seaweeds degrade fast if taken out from their natural habitat [34,93]. Drying is a method to preserve seaweeds, and to ensure a safe product for consumers and retain the quality.

The aim of this chapter is to understand how three different drying methods (convective air drying, freeze drying, and microwave-vacuum drying) influence the quality of bladder wrack and sea lettuce. These two seaweeds were chosen due to their availability and industrially relevance for the seaweed industry [94,95].

The chapter addresses the findings of Papers 5, which contains a detailed description of the pretreatment of the seaweeds prior to drying.

The objective of the chapter is to address the following research questions:

- Which drying method has the fastest drying rate?
- Does microwave-vacuum drying result in the same quality as freeze drying?
- Does convective drying lead to a different product than the other drying methods?
- Are valuable chemical compounds influenced by the different drying methods?
- Are the physico-chemical properties altered differently between the three drying methods?
- How is sensory quality affected by the drying methods?
- Does freeze drying live up to expectations as the best overall drying method for food?
- Does the drying method influence the two diverse species differently?
- Based on the findings, which drying method is recommended for the industry to use on the two species in the future?

6.3 The concepts of the three drying methods in focus

Convective drying, or air drying, uses air to remove the moisture from the surface of the food. The air drying can be by natural convection or forced convection using a fan. The efficiency of convective drying depends on the air temperature, airflow speed, and humidity of the drying air [56]. The temperature of the air should be high enough to create a difference in vapor pressure between the water of the food and the drying air,

and the airflow rate should be sufficient to remove the vapor from the food surface [96]. As an example, the vapor pressure of water at 50 °C is 122 mbar, at 20 °C it is 23.4 mbar, and at -14 °C it is 1.82 mbar [97]. Vapor pressure of a liquid is the pressure exerted by its molecules when they are in the gaseous state. Increasing the temperature and airflow often leads to efficient energy transfer. But increasing these too much can cause damage to the food and thereby alter the quality by case hardening, and loss of nutrients and flavor [98].

Freeze drying is based on sublimation and is therefore carried out at low temperature, and reduced pressure. It is also called lyophilization. The product is first frozen to around -20 to -60 °C, then placed in a chamber where low pressure is applied (13-27 Pa) [99]. During drying, the temperature increases with time and ends at room temperature. Freeze drying keeps the structure of the food, so the product does not collapse, which gives a porous structure. It is believed to produce the highest food quality compared to all drying methods because it keeps the structure and has limited loss of flavor and nutritional value [98].

Microwave-vacuum drying uses microwave radiation to generate heat while absolute pressure is kept constant. The process can be both above and below the triple point of water. If the first step of the process is freezing the product, and thereafter conducting the drying at vacuum, the concept of microwave-vacuum drying is close to freeze drying. However, applying microwave radiation provides a fast energy transfer compared to the freeze drying. When freeze drying, the product dries from the outside and in. Energy must diffuse into the product by conduction from shelves in the freeze drying chamber, and later in the drying stage, it must also travel through the dried outer part of the product. Whereas the microwaves provide energy for the initial location of the water in the product, and the water diffuses from the inner location as vapor and out to the surface of the product. This gives a faster drying rate compared to freeze drying [96].

The drying parameters applied in Paper 5 are found in Table 13.

Table 12: Drying parameters from Paper 5 for freeze drying, convection drying, and microwave-vacuum drying.

Drying	Drying temperature (°C)	Pressure (Pa)	Drying time bladder wrack (hr)	Drying time sea lettuce (hr)
Freeze drying	-20 to 20	20	24	24
Convection	52	$10.1 \cdot 10^4$*	1.13	1.17
Microwave-vacuum	-40 to 40	10	3	4

*$10.1 \cdot 10^4$ Pa is equal to 1 atm.

That microwave vacuum drying is indeed a faster drying method than freeze drying is shown in Table 12. Different equipment within the same drying type has different efficiency, which affects the drying time, but the variation will be minimal compared to the in between drying types.

It is now established that the three different drying methods lead to different drying times. Convection drying, by forcing 52 °C air onto the product, gave the fastest drying. It did not have any pressure changes in the drying system, which means it was most likely the method with the lowest energy consumption. However, whether the convection drying would alter the product is yet to be understood in section 6.4.

6.4 How will the quality of seaweeds change during drying?

6.4.1 Chemical changes by three different drying methods

Badmus et al. (2019) investigated the composition of five different brown seaweeds after various drying methods and concluded that low-temperature drying techniques such as freeze-drying and oven-drying at 40 °C yielded higher concentrations of nutritionally important chemicals and higher antioxidant activities. They suggested that microwave drying could be an alternative, and should be investigated further [100]. In this thesis work the impact of the drying method on the chemical composition of the two seaweed species: bladder wrack and sea lettuce, was evaluated, and the proximate composition was reported in Table 1 of Paper 5 and the bioactive compounds in Table 4. For both species, the proximate composition varied depending on the drying method, with sea lettuce being more susceptible to changes than bladder wrack. Table 13 presents the concentration of other valuable compounds, with some

of the concentrations differing depending on the drying method, relative to the freeze-dried products.

Table 13: The average content of different valuable compounds found in bladder wrack and sea lettuce after the three different drying methods: convection drying, freeze drying, and microwave-vacuum drying.

Compound	Units	Convection drying	Freeze drying	Microwave-vacuum drying
Bladder wrack				
Aspartic acid (free)	mg (g dw)$^{-1}$	0.121±0.005	0.0936±0.0117	0.149±0.025▲
Aspartic acid (total)	mg (g dw)$^{-1}$	1.57±0.37	1.53±0.13	2.07±0.16
Beta-carotene	µg (g dw)$^{-1}$	24.0±3.4	22.5±2.8	26.2±1.0
EAA ratio	%	36.6±5.8	35.3±1.0	37.8±0.4
Fucoxanthin	µg (g dw)$^{-1}$	209±21▲	117±4	228±2▲
Glutamic acid (free)	mg (g dw)$^{-1}$	0.115±0.004▼	0.195±0.031	0.148±0.026
Glutamic acid (total)	mg (g dw)$^{-1}$	1.99±0.07	1.82±0.15	2.12±0.09▲
PUFA	% FAME	23.8±0.6	24.8±0.7	23.8±0.5
TPC	µg GAE (g dw)$^{-1}$	20.3±3.0	22.8±1.0	24.3±4.9
Sea lettuce				
Aspartic acid (free)	mg (g dw)$^{-1}$	0.095±0.045▼	0.260±0.013	0.207±0.016
Aspartic acid (total)	mg (g dw)$^{-1}$	5.06±0.21	4.87±0.23	4.45±0.29
Beta-carotene	µg (g dw)$^{-1}$	20.3±1.9	28.8±3.9	25.0±3.7
EAA ratio	%	47.1±0.7	46.8±0.7	47.3±1.6
Glutamic acid (free)	mg (g dw)$^{-1}$	0.082±0.032▼	0.279±0.035	0.289±0.023
Glutamic acid (total)	mg (g dw)$^{-1}$	4.91±0.22	4.81±0.12	4.61±0.21
Lutein	µg (g dw)$^{-1}$	11.1±0.78▼	22.0±1.9	19.7±3.9
PUFA	% FAME	2.53±0.37▼	4.59±0.52	5.84±1.61

The numbers are gathered from Paper 5 and given as the average and standard deviation. ▼Decrease relative to the content in the freeze dried seaweed. ▲Increase relative to the content in the freeze dried seaweed.

In bladder wrack, only free glutamic acid decreased due to convection drying, while other compounds remained unchanged or increased. Microwave-vacuum drying resulted in an increase in free aspartic acid, fucoxanthin, and total glutamic acid. Therefore, it can be concluded that freeze-drying is not necessarily the best overall drying method for retaining valuable compounds, as microwave-vacuum drying

appears to retain more of these. Freeze-drying apparently may lead to oxidation of carotenoids such as fucoxanthin [19], possible due to the longer drying time, which does not occur with the other two drying methods. Silva et al. (2019) conducted freeze-drying and oven drying of bladder wrack at various temperatures and found that freeze drying retained total phenolic compounds (TPC) better than oven-drying [19]. However, our study did not observe the same trend.

Sea lettuce was affected differently from bladder wrack. Freeze-drying and microwave-vacuum drying yielding similar results, while convection drying resulted in a significant decrease in free aspartic acid, free glutamic acid, the pigment lutein, and polyunsaturated fatty acids (PUFA). This suggests that convection drying of sea lettuce reduces the chemical quality with respect to these compounds.

6.4.2 Physico-chemical and sensory changes due to drying

This section focuses on how the three drying processes affect the sensory and physico-chemical properties of the two seaweed species. The main results are presented in Paper 5. To investigate the results further, the physico-chemical and sensory results were combined by principal component analysis (PCA).

Figure 15 and Figure 16 are pictures of the product after each drying method, along with a visualization of the average colors and their CIELab color results. By a qualitatively visual inspection, it can be concluded for both species that the differently dried products do not appear similar, which is confirmed by the various physico-chemical and sensory results. Drying by convection gave visually different products for both species. In the case of sea lettuce, there was no difference in color between the products obtained from microwave-vacuum drying and freeze drying. This finding was consistent with the results of the chemical quality parameters.

The PCAs are presented in Figure 17 as bi-plots. The scree plots (not shown) indicated that the first two principal components (PC1 and PC2) together explained 62% and 68% of the variance for bladder wrack and sea lettuce, respectively.

The physico-chemical analyses are color (Lightness, red-green, and yellow-blue), water activity (aW), water absorption (WA), and water holding capacity (WHC). The sensory analysis was performed by a panel, who did a descriptive profile analysis.

They assessed appearance (a), odor (o), flavor (f), and texture (t). The methods of the analyses can be found in Paper 5.

Figure 15: Pictures of bladder wrack after freeze drying, convection drying, and microwave vacuum drying. Below the pictures are the color analysis results. The letters in brackets denote significant difference between drying methods (ANOVA; p > 0.05)

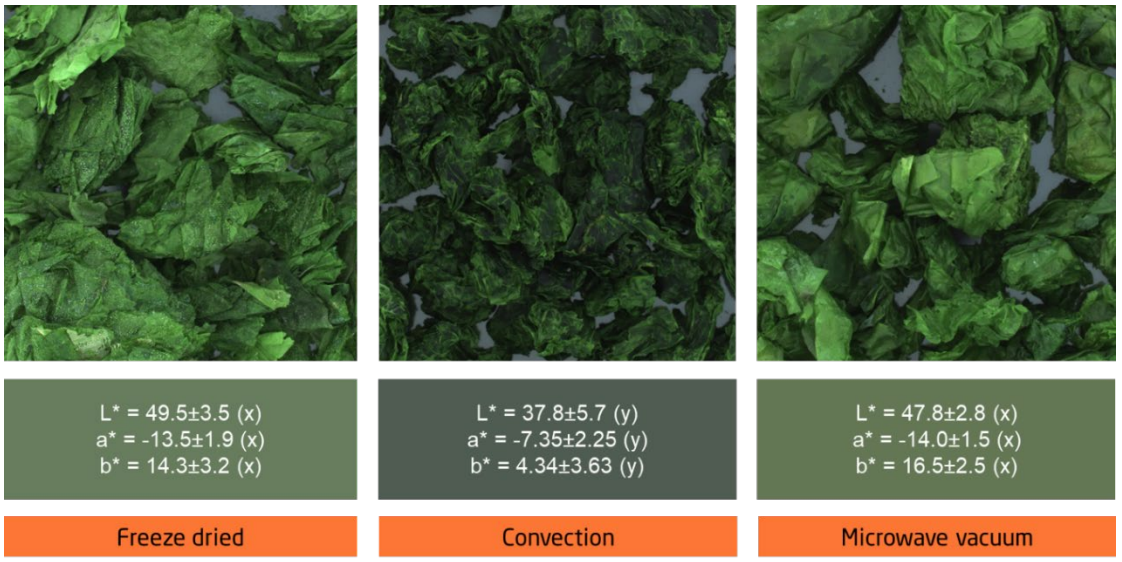

Figure 16: Pictures of sea lettuce after freeze drying, convection drying, and microwave vacuum drying. Below the pictures are the color analysis results. The letters in brackets denote significant difference between drying methods (ANOVA; p > 0.05)

For both species, none of the drying methods overlap in the bi-plots, meaning they vary in the physico-chemical and sensory qualities analyzed.

For bladder wrack (top) the convective drying lead to a higher water activity, water holding capacity, water absorption and appeared curlier. The microwave-vacuum drying retained slightly the flavors of seaweed, umami, and hay. Freeze drying also

gave a more yellow (b*), light (L*), and uniform color. This is also indicated by the significant differences given in Figure 15. Moreover, freeze drying had a somewhat more astringent and clotted texture with bitter and greener flavors.

For sea lettuce (bottom), the convective drying lead to higher values of texture such as crispy, astringent, and firm. It also appeared more viscous and curlier. It had a different color compared to the other two drying methods, as shown in Figure 16, which is in consistency with Uribe et al. (2019), who found convective drying at 70 °C to alter the color significantly [90]. The freeze drying led to retention of many of the flavors and odors: sea, sweet, seaweed, fresh fish, umami, and salty. An earlier study found a decrease in the volatile compounds of *Ulva* sp. when drying by convection at 60 °C [101]. This agrees with the lower intensity of odor and flavor attributes found for convective drying in this thesis study. The samples from microwave-vacuum drying did not follow any of the loadings when studying the PC1 and PC2. This is because its sensory and physico-chemical properties lie in between the two other drying methods.

Thus, in terms of sensory and physico-chemical properties, the convection, freeze drying and microwave-vacuum drying are not resulting in the same product. Overall, it can be established that the convection drying leads to a crispier and curlier product, whereas freeze drying retains many of the flavors.

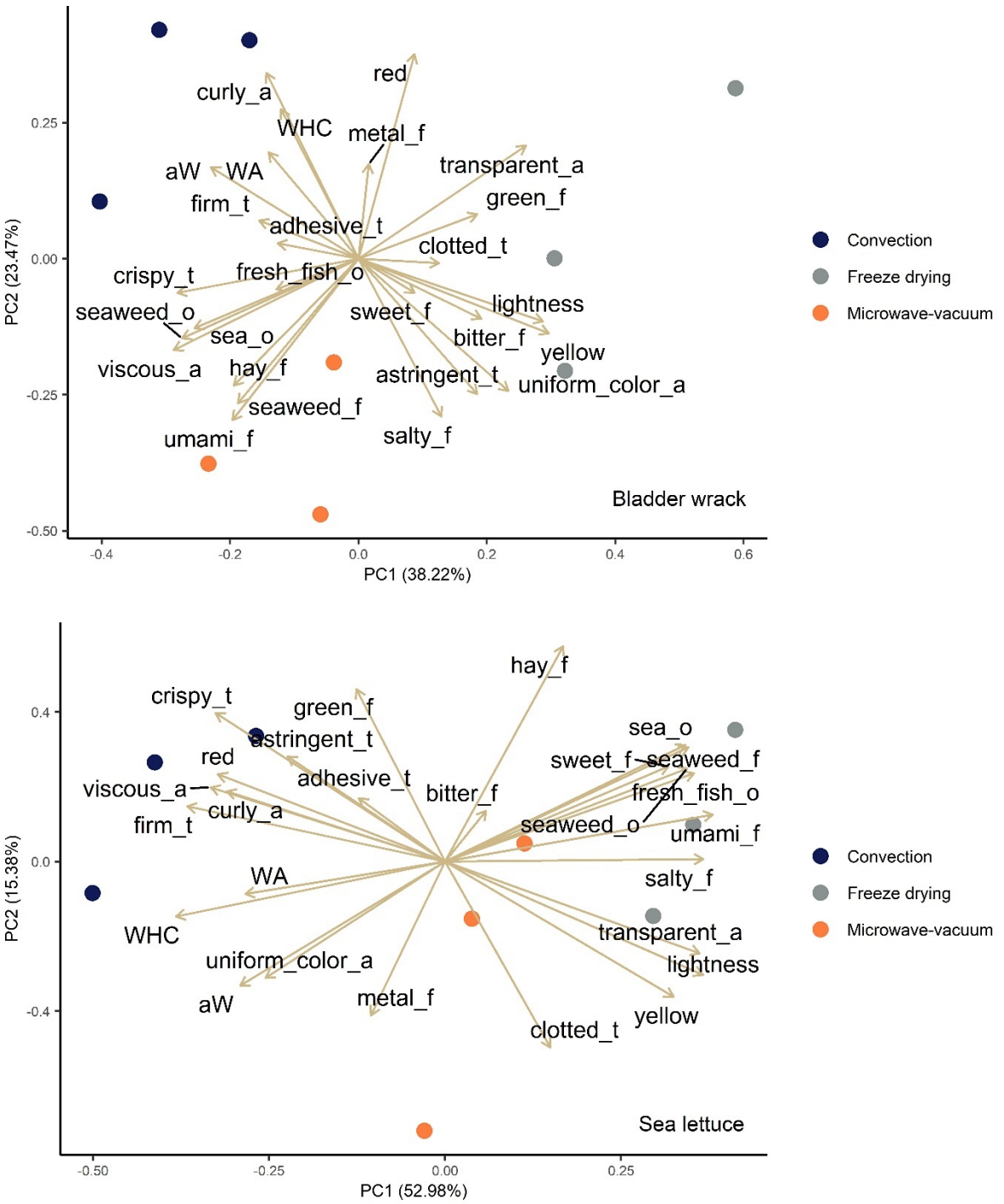

Figure 17: Bi-plot illustrating the PCA showing the difference and correlation of sensory and physico-chemical properties for bladder wrack (top) and sea lettuce (bottom) dried by convective drying, freeze drying and microwave-vacuum drying.

6.5 Summary of the drying chapter

The three different drying methods (convective drying, freeze drying, and microwave-vacuum drying) did alter the two seaweed species (sea lettuce and bladder wrack) differently, which will be elaborated by answering the research questions stated in the chapter:

- Microwave-vacuum drying was faster than freeze drying. But convection drying, using forced air at 52 °C onto the product, gave the fastest drying rate among the three drying methods explored.
- Microwave-vacuum drying did not result in the same quality as freeze-drying. For bladder wrack microwave-vacuum drying retained more valuable compounds, such as free aspartic acid, fucoxanthin, and total glutamic acid. Their physico-chemical qualities also differed. For sea lettuce the qualities were similar between the two drying methods.
- Convection drying resulted in distinct products compared to the other drying methods, possibly due to the higher temperature employed. Sea lettuce had reduced levels of free aspartic acid, free glutamic acid, lutein pigment, and PUFA, while bladder wrack only showed a decrease in free glutamic acid. Both species also showed changes in physico-chemical properties. It also led to a darker appearance and case hardening, rendering the product more crispy, firm, viscous, and curly in texture, compared to the other dried products.
- For both bladder wrack and sea lettuce, the proximate composition varied depending on the drying method, with sea lettuce being more susceptible to changes than bladder wrack.
- When deciding on an appropriate drying method for a specific species, it should be acknowledged that the choice of drying, and the chosen external factors do affect the species differently. Therefore, the identification of an optimal drying method should be based on the species in question.

7 Stability of refrigerated sugar kelp

7.1 State of the art and shelf-stability

In foods, there are several factors that influence stability and shelf-life. These include intrinsic factors (nutrient content, water activity, pH, and presence of enzymes), and extrinsic factors that are influenced by the environmental conditions (temperature, humidity, atmosphere type, air flow) [102].

Research on shelf-life extension and post-harvest quality changes of sugar kelp is still in its early stages. Only a few studies have been conducted to understand the microbial influence on sugar kelp as a food product [26,103], with one of them being a master thesis. Other studies have investigated the shelf-life of different seaweeds such as *Gracilaria* spp., *Ulva rigida*, and *Palmaria palmata* with shelf-lives ranging from 3-14 days depending on species, storage conditions, and process treatments [30,33,104].

There is a lack of understanding how different post-harvest treatments, such as blanching and washing, affect the quality of sugar kelp [105]. Liot et al. (1993) found that washing *Ulva rigida* and *Palmaria palmata* in tap water would decrease their shelf-life from 7 days to 3 days. Overall, further research is needed to understand how different post-harvest treatments can delay microbial spoilage and potentially extend the seaweed product's shelf-life.

7.2 Aim and research questions of the stability chapter

In Paper 4 a shelf-life of 7-9 days was established for refrigerated (2-3 °C) sugar kelp independent of treatment (washing or blanching in either tap water or seawater). The

temperature was chosen as it is what seafood in general is stored at. Quality changes were assessed by sensory evaluation, physical changes (texture, drip loss, and color), chemical composition, and microbiology. A single-compound quality index for spoilage was determined to be the quantification of aerobic viable count (AVC) with a shelf-life limiting threshold of 7 log (CFU g^{-1}). The study in Paper 4 describes the above-mentioned parameters. However, some quantitative microbial data and qualitative sensory assessment of off-odors are not described in detail in the paper. Therefore, the aim of this chapter is to give an outline of bacterial deterioration of sugar kelp, and the off-odors evolving during storage.

The objective of the chapter is to address the following research questions:

- What bacterial species were present during storage of untreated, washed, and blanched sugar kelp?
- Which bacterial classes were dominant in the refrigerated samples at the time of spoilage?
- What other microbial risks should be considered by the industry?
- What are the off-odors associated with sugar kelp spoilage?

7.3 Bacterial deterioration of sugar kelp

The spoilage of seafood is a common issue, which can cause economic and environmental losses and risks to public health. A range of bacterial species have been identified as responsible for seafood spoilage, including *Pseudomonas* spp., *Alteromonas nigrifaciens*, *Shewanella putrefaciens*, *Brochothrix* spp., *Photobacterium phosphoreum*, and *Aeromonas* spp. [106]. Seaweeds have an epiphytic bacterial community on their surface, which can form a biofilm that contributes to the health and defense of the seaweed against harmful pathogens. However, seaweeds are still susceptible to contamination by pathogenic bacteria, such as *Salmonella* and *E. coli*, which can pose risks to human health [82]. In addition to these, other foodborne pathogens such as norovirus, *E. coli*, *Vibrio* spp., and *Bacillus* spp. have been reported in fresh or processed seaweed [26,107–109], highlighting the need for further investigations to understand what occurs during processing of the seaweed. Microbial food spoilage results from microbial activity by molds, yeasts, or bacteria, which makes

the product unsuitable for consumption [102]. In Paper 4, changes in several parameters were observed during deterioration, with the count of microbial colonies being the primary parameter used to quantify shelf-life. Bacterial growth and loss of flavors were found to be the main causes of shelf-life determination in fresh, washed, and blanched kelp [4].

The food matrix in which microorganisms live has a significant influence, particularly in terms of pH, water activity (a_w), and concentration of components [84]. In our study, fresh untreated and washed kelp were shown to have high water activity (>0.978), pH ranging from 6.21 to 8.67, and salt concentration from 0.09 to 2.19% ww, as well as the presence of mannitol and free amino acids. These factors promote the growth of microorganisms on kelp, leading to spoilage.

To mitigate bacterial deterioration in washed and blanched kelp, the industry should aim to control AVC and keep levels below 7 log (CFU g^{-1}) on marine agar (Paper 4) [4]. Marine agar was employed to determine the total colony forming units (CFU), i.e., AVC, while other media can identify specific bacterial types. Our study focused on *Pseudomonas* spp., *Shewanella* spp. (which produce hydrogen sulfide), *Actinomycetes* spp., and yeast (Table 14).

Actinomycetes spp. are a group of Gram-positive bacteria that are found in soil, water, and the marine environment. They produce secondary metabolites prolifically and are recognized as sources of earthy, woody, musty, potato-bin-like, hay-like, fishy, and grassy flavors at low threshold levels [110,111]. *Actinomycetes* spp. were detected in all treatment types at levels below 2.50 log (CFU g^{-1}). However, in samples washed with potable water, the microbial count increased to 3-4 log (CFU g^{-1}). This increase could be attributed to introducing microorganisms from the potable water source. The presence of yeast was sporadic and did not reveal any pattern.

Shewanella spp. are marine bacteria that contribute to the spoilage of protein-rich foods with high pH, such as marine fish and chicken. They can lead to unpleasant sensory changes due to their production of volatile sulfides [112]. Despite sugar kelp being less protein-rich than fish and chicken, our study detected *Shewanella* spp. in four out of five kelp treatments, ranging from 2 to 5 log (CFU g^{-1}), except for the treatment blanched in potable water. The low appearance of *Shewanella* spp. in the

potable water treatment can be attributed to its significantly higher pH (7.70-8.67) and lower salt concentration (0.03-0.09% ww) compared to the other treatments.

Table 14: Quantification range of the colony forming units (log (CFU g^{-1})) of *Actinomycetes* spp., *Pseudomonas* spp., *Shewanella* spp., and yeast on sugar kelp untreated, washed, and blanched.

log (CFU g^{-1})		*Actinomycetes* spp.	*Pseudomonas* spp.	*Shewanella* spp.	Yeast
Fresh (Day 1-6)	Un	0.3-2.3	3.0-5.1	0.0-2.4	1.4-2.7
	WS	0.9-1.6	3.1-5.2	0.0-3.3	0.0-1.6
	WP	0.8-1.7	3.1-4.5	0.0-3.5	1.1-1.6
	BS	0.0-1.2	0.3-4.7	0.0-2.4	0.3-0.7
	BP	0.3-1.8	0.0-2.8	0.0-0.0	0.0-0.3
Time of spoilage (Day 7-9)	Un	1.9-2.1	5.1	2.2-4.4	2.9
	WS	0.4	5.6	4.3	2.2
	WP	3.2	5.7	5.2	3.3
	BS	0.8	6.3	2.8	1.6
	BP	0.0	2.4	0.4	0.0
Spoiled (Day 10-17)	Un	1.0	5.8-7.7	3.2	3.1
	WS	0.3-0.8	6.1-8.0	0.0-3.1	1.7-2.1
	WP	1.3-4.1	5.9-8.8	2.8-4.2	3.8-5.2
	BS	0.0	6.4-8.3	2.3	1.6-2.7
	BP	0.7-1.0	7.1-8.8	0.0-1.4	0.0-0.7

Un = Untreated, WS = washed for 5 min in 4.0 °C UV-treated seawater with a PSU of 35, WP = washed in potable tap water at a temperature of 16 °C for 5 minutes, BS = blanched by submerging in hot UV-treated seawater (80 °C) for 2 minutes followed by rapid cooling for 3 min in UV-treated seawater (4.2 °C), BP = blanched by submerging in hot potable water (76 °C) for 2 minutes followed by rapid cooling for 3 min in potable water (16 °C). All, but the untreated samples, were transferred to a tray after the treatment to drip off excess water for 5 minutes.

During aerobic storage, spoilage in fresh foods is mostly caused by *Pseudomonas* spp. [102]. *Pseudomonas* species are psychrotolerant, meaning they can grow at temperatures below 7 °C, making them spoilage microorganisms for refrigerated foods. These bacteria can utilize non-carbohydrate sources for growth, including amino acids, peptides, and lipids. Because they have fewer growth requirements for available organic growth factors such as specific amino acids and vitamins, they often outcompete other bacteria [113]. This was also observed in refrigerated sugar kelp, where *Pseudomonas* spp. rapidly increased over time, as shown in Figure 5B of Paper 4.

At the time of spoilage (day 7-9) for sugar kelp diverse bacterial communities were observed between different treatments, as depicted in Figure 7B of Paper 4.

Gammaproteobacteria became the dominant class in the spoiled macroalgae, with noticeable variation within the bacterial families between treatments. For instance, the taxonomic family *Pseudomonadaceae* dominated in sugar kelp blanched in potable water, while *Pseudoalteromonadaceae* dominated in sugar kelp blanched in seawater. The untreated and washed samples contained a substantial proportion of bacteria within the family *Psychromonadaceae*. The temperature used for blanching probably accounts for the observed differences, since microorganisms growing at lower temperatures have increased unsaturated fatty acids in their membrane lipids to remain fluid at low temperatures, leading to a decrease in their melting point and a breakdown of the cell wall at low temperatures [84], which could explain why the bacteria within the family *Psychromonadaceae* are not dominant in the blanched samples, since they probably are inactivated during the elevated temperature treatments.

The above-mentioned microorganisms are important to understand for the sake of food spoilage, but not necessarily food safety. Some microorganisms do not spoil the food, but they pose a risk to food safety. Examples of food borne pathogens include *Vibrio parahaemolyticus*, *Vibrio vulnificus*, *Vibrio cholera*, *Listeria monocytogenes*, *Clostridium botulinum*, and *Salmonella* spp. *L. monocytogenes* can grow under aerobic or anaerobic conditions and at refrigerated temperatures, and has been reported in unprocessed or processed fish, including frozen fish [106]. *C. botulinum* type E can grow and produce toxins at temperatures as low as <3.3 °C and 5% salt and is considered the most prevalent in seafood. Pathogenic *Vibrio* spp. thrive in marine environments at temperatures between 15-35 °C, but never below 10 °C [114]. Although they do not survive heat treatment, freezing, or low pH, *Vibrio* spp. can pose a health risk when fresh kelp is consumed as fresh or refrigerated foods [105]. The presence of *Vibrio* spp. in seafood products depends on temperature, as they are more prevalent in warmer seawater [28], hence harvesting the seaweeds at locations or seasons with lower temperatures will decrease the chances of *Vibrio* spp. on the harvested seaweeds. Future research should aim to better understand the relationship between pathogenic bacteria and kelp or seaweed, especially when used for food consumption after refrigeration.

7.4 Development of off-odors in raw sugar kelp when refrigerated

The changes in sensory attributes of raw sugar kelp stored at 2.8 °C are discussed in detail in Paper 4 [4]. The PCA bi-plot in Paper 4 reveals a decrease in positive attributes with storage time, such as the odors fresh sea, sweet, fresh sour, and boiled peas. Although the sugar kelp did not entirely lose its odor, the bi-plot indicates that it did.

In the paper it is described that the panel detected off-odors, including vinegar, acetic acid, old flower water, fermented, rotten, old hay, chlorine, and sulfuric after the established shelf-life of 7-9 days. Unfortunately, these off-odors were not included in the descriptive study. The panel established the vocabulary of the products by training with two-day-old, refrigerated sugar kelp and sugar kelp kept at room temperature for two days to increase the chances of off-odors.

However, these off-odors were not detected in the subsequent sensory descriptive sessions, and hence were not quantified. It is advisable to include these negative odor attributes in future descriptive profiling of raw kelp to comprehend the changes with storage time. The off-odors found in Paper 4 can be used directly in any future sensory profiling of raw kelp and its shelf-life.

7.5 Summary of the stability chapter

In conclusion, this chapter summarizes the bacterial deterioration process of sugar kelp and the off-odors that develop during storage, which are important factors to consider when evaluating the shelf-life of refrigerated sugar kelp, and other kelp species for human consumption.

To answer the research questions stated in the chapter:

- *Actinomycetes* spp., *Pseudomonas* spp., *Shewanella* spp., and yeast were detected in all treatment types.
- At the time of spoilage (AVC > 7 log (CFU g^{-1})), *Gammaproteobacteria* became the dominant class in the refrigerated samples. *Pseudomonas* spp. were dominant when evaluating plate counts.

- Besides the microorganisms mentioned above, the industry should consider other microbial risks, such as *Vibrio* spp., *Listeria monocytogenes*, *Clostridium botulinum*, and *Salmonella* spp.
- Off-odors detected in raw sugar kelp include vinegar, old flower water, fermented, rotten, old hay, chlorine, and sulfuric. These odor attributes should be included in future sensory profiling of raw kelp to better understand changes with storage time.
- Other off-odors related to spoilage can be earthy, woody, musty, potato-bin-like, and fishy produced by *Actinomycetes* spp.

8 Conclusions and recommendations

This PhD thesis addressed the challenges of post-harvest processing in the emerging European seaweed industry, which is rapidly expanding to meet the demand for sustainable food sources. As seaweed cultivation is a relatively new sector in Europe, there is a need to investigate and optimize post-harvest processing to ensure safety and stability of the newly harvested seaweed. This thesis specifically focused on how the processes ensured safe and stable seaweed products regarding food quality.

The thesis investigated the vitamin C content by reviewing selected Northern European seaweed species within the orders Laminariales, Fucales, Palmariales, and Ulvales, and comparing their content to commonly consumed foods and the recommended intake based on Paper 1. We showed that the vitamin C content of brown and red seaweeds is similar, while the green seaweed species have a higher median but also greater variability. This means that according to European food regulations, Ulvales can be considered a source of vitamin C, however, the other species cannot (rejecting and accepting **H4.1**). It is important to note that a significant portion (400 g wet portion) of seaweed is required to reach the recommended daily intake. However, seaweeds do contain some vitamin C, which can contribute to the human daily need (accepting **H4.2**). Caution should be taken when claiming that seaweeds are rich in vitamins, since there are a vast variety of seaweed species, each with a diverse chemical composition, possibly including varying amounts of the 13 essential vitamins.

Potential toxic elements in sugar kelp and winged kelp are of concern for the food authorities and seaweed producers. An extensive part of the thesis examined how washing and blanching would affect the potential toxic elements, but also if the processes would compromise the desired food qualities such as nutrients and sensory qualities. This was based on Paper 2, 3 and 4. The industry should be aware that

unprocessed sugar kelp and winged kelp can cause a health risk in terms of iodine and total arsenic when compared to the official upper intake levels and EU directives on animal feed and foodstuffs. However, the concentration of inorganic arsenic is not posing as a risk (accepting and rejecting **H5.1**). Cadmium concentrations in unprocessed kelp were found to be below the maximum allowed thresholds, and is not per say a health risk, however, blanching can increase the cadmium concentrations, which the industry should be aware of (rejecting **H5.2**). Total arsenic was found to be reduced in some cases of blanching, although not always below the maximum allowed thresholds, thus total arsenic should be monitored in the kelp even after processing (rejecting **H5.2**).

Blanching was demonstrated to be an ideal process to reduce the iodine content in the two kelp species to levels below 600 mg $(kg\ dw)^{-1}$ even at low temperatures (45 °C) for short durations (10 seconds to 5 minutes) with either seawater or potable tab water (accepting **H5.2**). However, it is important to consider the seaweed to water ratio, as a higher ratio can lead to reduced efficiency of the blanching process. During the PhD study sufficient data was collected to make a predictive model for the iodine reduction of sugar kelp using exponential regression. The iodine content after blanching can be estimated by the blanching temperature and duration (accepting **H5.3**). Based on the maximum growth temperature of mesophile bacteria, it is recommended that blanching is performed at a minimum temperature of 45 °C for a duration of 30 seconds, to eliminate bacteria on the kelps (accepting **H5.2**).

In addition to its impact on the concentrations of the potential toxic elements in kelp, blanching also affects other quality aspects, including the reduction of ascorbic acid (vitamin C), folate (vitamin B9), mannitol, magnesium, and the free amino acids known to possess umami flavor (glutamic acid and aspartic acid) and an increase and calcium concentration (accepting **H5.4**). This is simplified as the temperature and blanching water type has an influence on how big an effect these changes are, which warrants further investigation in future studies. From a sensory point of view, blanching kelp will change the odors, taste, and color (accepting **H5.4**).

Furthermore, the effects of different drying methods on the quality of two commercially relevant seaweed species, bladder wrack and sea lettuce, were studied based on Paper 5. Convective air drying (52 °C), freeze drying (-20 to 20 °C at 20 Pa), and

microwave-vacuum drying (-40 to 40 °C at 10 Pa) were compared for their impact on the physico-chemical properties, sensory quality, and chemical composition of the seaweeds. It was found that microwave-vacuum drying is a promising alternative to freeze drying, as it resulted in similar quality and nutrient retention while having a faster drying rate (accepting **H6.1**). Convective air drying led to more changes in the sensory quality and higher degradation of valuable compounds compared to the other methods (accepting **H6.1**). It is worth mentioning that the optimal drying method for seaweeds highly depends on the specific species in focus, as the drying methods influenced the two species differently (rejecting **H6.2**). These findings gave valuable insights into the impact of drying methods on seaweed quality and can improve the development of more efficient seaweed processing practices for the future. It is important that the seaweed industry establishes and target specific quality measures prior to processing the seaweed, so some key indicators of high quality seaweed are recognized.

Post-harvest handling and shelf-life extension of sugar kelp is in its early stages, and there is a lack of understanding the microbial flora of the kelp, and how post-harvest treatments can delay microbial spoilage and extend the product's shelf-life. The water activities (>0.978), pH (6.21 to 8.67), and salt concentrations (0.09 to 2.19% ww) in sugar kelp (untreated, washed, or blanched) promote the growth of both spoilage and pathogenic bacteria (accepting **H7.2**). In the thesis work a shelf life for refrigerated (2-3 °C) sugar kelp was found to be 7-9 days. *Pseudomonas* spp. were the dominant spoilage bacteria during 2-3 °C refrigeration of untreated, washed (4-16 °C for 5 minutes), and blanched (76-80 °C for 2 minutes) sugar kelp. Other species were also identified. *Actinomycetes* spp. were found to be present in all treatment types, but highest in samples washed with potable water. While *Shewanella* spp. were found in four of the five treatments, but not in the sugar kelp blanched in potable water (accepting **H7.1**). To control the bacterial growth before, after, or during processing, quantifying the aerobic viable count (AVC) on marina agar is recommended with a spoilage threshold level of 7 log (CFU g^{-1}). This does not count for pathogenic bacteria such as *Vibrio* spp., *Listeria monocytogenes*, *Clostridium botulinum*, and *Salmonella* spp., whose presence should be controlled.

The research in this PhD thesis provides crucial insights that can help the emerging European seaweed industry to move forward in its journey towards bringing seaweed to the tables of consumers. The findings of this research can also help the industry to

understand the safety, original quality, and increased value of their products and to meet the diverse demands of consumers. By addressing the challenges of post-harvest processing and ensuring food safety and stability of the European seaweeds, the industry can establish as reliable and provide safe sources of food. Overall, the thesis is a significant contribution to the growth and development of the European seaweed industry and provides a solid foundation for further research and innovation.

9 Future perspectives

Moving forward, the seaweed industry must prioritize the possible end-products from seaweeds and answer, how should we eat seaweed? When this is answered, the industry can define what the important seaweed qualities are from a food perspective. Understanding the end-of-life for seaweed as a food product will facilitate the optimization of post-harvest processes. This needs to be answered to define what good quality of each seaweed species intended for human consumption is. Is it intended to be used as an ingredient to bind water in food products, or to be a source of iodine, or will it be used to give another depth in taste and flavor? Without knowing the end-of-life it is difficult to decide the most optimal processing method, as some constituents should be retained to live up to the intended function, but other properties are less important.

Since seaweeds are one of the few marine plant-based food sources, they have a place on the future dinner table. But to achieve further establishment of seaweed as a food, the industry must develop a market and create products that are appealing to consumers. Therefore, product development and consumer studies are necessary to understand what types of seaweed products are most appealing to consumers. The priority, however, must always be the safety of the consumers. The establishment of a market and a clear definition of the end-product facilitate the evaluation of the characteristics analyzed, thereby enabling the determination of their positive or negative impact.

Moreover, to achieve longer shelf-life without resorting to drying methods or using washing and blanching for other species than sugar kelp, the industry should explore other methods, such as pasteurization, sterilization, acid-stabilization, fermentation, or freezing. Fermentation or acidification methods may be particularly promising for

creating stable and longer shelf-life seaweed products that closely resemble fresh, newly harvested seaweed and at the same time utilize minimum amount of energy and utilities.

Overall, the seaweed industry must focus on developing marketable products that fit consumer preferences while ensuring food safety and stability. And thereby determine what qualities from the seaweeds are important from a food perspective. Through interdisciplinary collaboration between scientists, food technologists, and the industry, we can expand the potential of seaweed as a sustainable food source and create a more diverse and resilient food system.

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Paper 1

Vitamin C from seaweed: A review assessing seaweed as contributor to daily intake

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foods

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Review

Vitamin C from Seaweed: A Review Assessing Seaweed as Contributor to Daily Intake

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Abstract: Seaweeds are indiscriminately said to contain significant amounts of vitamin C, but seaweeds are a diverse group, which may limit the ability to generalize. Several studies have been performed on vitamin C in seaweed, and this review covers these findings, and concludes on how much vitamin C is found in seaweeds. A systematic review of vitamin C in 92 seaweed species was conducted followed by analyzing the 132 data entries. The average vitamin C content was 0.773 mg g^{-1} seaweed in dry weight with a 90th percentile of 2.06 mg g^{-1} dry weight. The vitamin C content was evaluated based on taxonomical categories of green, brown and red seaweeds (Chlorophyta (phylum), Phaeophyceae (class), and Rhodophyta (phylum)), and no significant differences were found between them. The vitamin C content was compared to other food sources, and this showed that seaweeds can contribute to the daily vitamin C intake, but are not a rich source. Moreover, seasonal variations, analytical methods, and processing impacts were also evaluated.

Keywords: ascorbic acid; macroalgae; comparison; food; quality; consumption; processing; recommended nutrient intake; dietary reference intake; seasonal variation; analyses; taxonomy

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1. Introduction

Humans are unable to synthesize vitamin C (chemically: ascorbic acid, ascorbate). Humans rely on an adequate supply of vitamin C from their diet and it is therefore considered an essential micronutrient [1]. Vitamin C is fully absorbed and distributed in the human body, with the highest concentrations found in the brain, eye, and adrenal gland [2]. It is involved in collagen synthesis, iron metabolism, tissue growth, and vascular functions, as well as biosynthesis of carnitine, and antioxidant reactions such and inhibiting lipid peroxidation [1–3]. Vitamin C is a reductant, meaning it functions as an electron donor and when donating two electrons, it oxidizes into dehydroascorbic acid [1,4]. The vitamin is known for the prevention of scurvy, but may also be able to prevent cardiovascular diseases and some cancer forms [1,2,5]. The Recommended Nutrient Intake (RNI) established by FAO/WHO for vitamin C is 45 mg day^{-1} for adults, which is the amount required to half saturate the body [1]. The Recommended Dietary Allowance (RDA) or Population Reference Intake (PRI) is defined as the average daily level sufficient to meet the nutrient requirements in nearly all healthy individuals. The RDAs established by the Institute for Medicine (U.S.) are 75 and 90 mg day^{-1} for women and men, respectively [6], whereas the PRIs set by EFSA are 95 and 110 mg day^{-1} for women and men, respectively [7]. The Institute for Medicine (2000) describes that smokers dispose of lower vitamin C, even when on a vitamin C rich diet, therefore it is recommended in the United States that the RDA for smokers is increased by 35 mg day^{-1} [6,8].

The Tolerable Upper Intake Level (UL) is not established by the European Food Safety Authorities (EFSA) [9], although the National Institute of Health (USA, NIH) has

established a UL for adults of 2000 mg day^{-1} [8], as intakes in that ratio have been shown to produce unpleasant diarrhea and abdominal cramps [1,6,8].

Oxidation of ascorbic acid into dehydroascorbic acid occurs easily due to exposure to high pH, high temperatures, light, oxygen, enzymes as well as exposure to the metals Fe^{3+}, Ag^{+}, and Cu^{2+} [3]. As dehydroascorbic acid also has corresponding biological importance as ascorbic acid it is important to include both compounds in the analysis of the total vitamin C content of the food [3].

Seaweeds are a common part of the diet in some Asian countries, but they are not traditional in the Western diet. There is an increasing commercial demand for seaweed products due to consumer focus on health and functional foods [10,11]. Some seaweeds contain several ingredients and bioactive compounds, that are of commercial interest such as hydrocolloids, minerals, and polyphenols [12,13].

Several peer-reviewed papers state that seaweeds are also a rich source for vitamin C [4,10,14–16] and vitamin C content has been analyzed for various species. More specifically stating that the highest content is found in brown and green algae [17,18] with concentrations of 0.5–3.0 mg g^{-1} dry weight (dw) and red algae containing between 0.1 and 0.8 mg g^{-1} dw [15]. Moreover, Munda (1987) stated that some species have sufficient amounts to cover the recommended daily intake for adults [4]. The statements that seaweeds do in fact contain a significant amount of vitamin C is interesting to investigate. First of all, the term "seaweeds" is very broad and diverse. It is not straight forward to conclude on such a broad term, when it consists of more than 10,000 species divided into green, red, and brown seaweed [19]. The chemical composition of seaweeds are therefore very different in regard to carbohydrates for cell walls and storage, amino acid profile, minerals and pigments, and most likely also when it comes to vitamins [13,20,21]. Secondly, to the best of the authors' knowledge, an overall picture of the vitamin C content in seaweeds has not yet been established in the literature. It is important to clarify which seaweeds, if any, are rich in vitamin C. A method to establish whether seaweed does in fact contain high contents of vitamin C is to compare the content to the dietary reference intakes such as RNI and other food sources. Examples of foods considered rich in vitamin C are citrus fruits, guava, kiwi, mango, and some berry types [1,22].

The review aims to create a collection of peer-reviewed studies and clarify the vitamin C content of various seaweed species. The data entries from the collection are assessed, evaluated by statistical analysis, and compared with metadata. The influence of processing on the vitamin C content is highlighted, and the method of analysis will be discussed in brief. Lastly, concluding whether seaweed can contribute as a vitamin C source at all or is a rich source of vitamin C compared to other foods.

2. Materials and Methods

The work is a systematic review with a meta-analysis performed on the data collected. A thorough search of several studies related to vitamin C in seaweed were identified and collected. Statistical techniques were applied to the data collected to examine and interpret the pooled data to understand the general picture of the vitamin C content in seaweed.

2.1. Literature Search

Relevant literature was collected in the period of August to November 2020 from the following databases; Scopus, Web of Science, and the internal university library database DTU Findit. The following keywords and combinations thereof were used: "Vitamin C OR Ascorbic acid", "Vitamin C content OR Ascorbic acid content" and "seaweed OR macroalgae". The titles and abstracts were assessed individually for their relevance. If the literature was not available online, the local university library requested and collected scans of the papers.

The sources were collected in Mendeley (Elsevier) and duplicates were removed. The initial criteria for inclusion were; peer-reviewed journals, books, or reports written in English, Norwegian, Danish, or German language. All sources but one (Norwegian) were

written in English. Moreover, a criterion for inclusion was that the unit of vitamin C should be expressed in dry weight (dw). Although, in the case where the unit was given in wet weight (ww) or 100 g edible portion, the water content should also be given or achieved from contacting the authors, so a calculation to dry weight was possible.

As the taxonomical names of the seaweed species are updated regularly, the species names were updated to the current official name found in AlgaeBase [19].

2.2. Data Collection and Meta-Analysis

For the literature review of vitamin C, 34 studies on seaweed were found relevant with a total of 132 inputs. The means and standard deviations from the papers were collected. Some metadata categories were chosen for the review tables; taxonomical order, harvest method, collection site, season, year of harvest, sample treatment, replicates (n), and analytical method. In the situations where the research focus of the paper was to study the effect of season or processing, the means from their analyses were kept apart and entered individually. These metadata categories are provided, so an individual assessment of the relevance and reliability of each data entry can be assessed by the reader. In the case of missing metadata, the study was still included in the review. If the unit was not indicated for vitamin C content, the study was excluded from the review. In the analysis of taxonomical categories as well as the comparison to other food sources, all data were included. It is important to be critical to this approach, as some processing might have influenced the vitamin C content, but it was not possible to make a valid objective decision of excluding specific data points, so all data with the correct unit was included. Even though the replicates were given from some of the different studies, each entry in the review tables weighted $n = 1$, when evaluating the data.

It was not possible to conclude on each individual species, as only one or few entries from each species were found in the literature. Therefore, the species were divided into taxonomical categories of green (G), brown (B), and red (R) seaweeds (Chlorophyta (phylum), Phaeophyceae (class), and Rhodophyta (phylum)) as well as taxonomical orders. This was to be able to make a representative overview of their specific vitamin C content.

For data analysis, visualization, and statistics the software program R [23] was used. Statistical analyses were boxplots with mean, median, standard deviations, minimum, and maximum. Moreover in the cases where statistically significant differences were interesting a one-way ANOVA with a Tukey post hoc test was applied. Means were considered significantly different when $p < 0.05$.

3. Results and Discussion

3.1. Taxonomical Analysis

Vitamin C content is shown in Tables 1–3 for a total of 92 species (Phaeophyceae; 36, Rhodophyta; 33 and Chlorophyta; 23). The vitamin C content is given in mg g^{-1} dw. This unit was chosen, as the sample treatment before analysis were different among the papers.

Table 1. Vitamin C in Phaeophyceae (Class). Species arranged by orders.

Species	Origin				Sample Treatment	*n*	Analytical Method	Vitamin C mg g^{-1} * ± SD	Reference
	Wild/ Cultivated	Collection Site	Season	Year					
Dictyotales									
Dictyota dichotoma	Wild	Piran, Slovenia	January–November	1984	Vacuum-dried at 30 °C	8	Spectrophotometrically	3.79 ± 0.44	[4]
Padina gymnospora	Wild	Tanjung Tuan, Malaysia	-	2008 ***	Washed in running water	-	Titration	0.085	[24]
Padina gymnospora	Wild	Hurghada, Egypt	April–June	2019 ***	Washed with tap and distilled water, air-dried	1	Chromatography	0.006	[25]
Padina pavonica	Wild	Piran, Slovenia	October–November	1984	Vacuum-dried at 30 °C	2	Spectrophotometrically	0.58 ± 0.26	[4]
Padina tetrastromatica	Wild	Visakhapatnam, India	Yearly	1996–1997	Washed in fresh water and freeze-dried	12	Spectrophotometrically	0.525	[26]
Ectocarpales									
Ectocarpus siliculosus	Wild	Piran, Slovenia	January–June	1984	Vacuum-dried at 30 °C	5	Spectrophotometrically	2.52 ± 0.58	[4]
Mesogloia vermiculata	Wild	Piran, Slovenia	April	1984	Vacuum-dried at 30 °C	1	Spectrophotometrically	3.10	[4]
Scytosiphon lomentaria	Wild	Piran, Slovenia	January–May	1984	Vacuum-dried at 30 °C	4	Spectrophotometrically	2.00 ± 0.26	[4]
Fucales									
Ascophyllum nodosum	Wild	-	-	1920	-	-	-	0.55–1.65	[27]
Ascophyllum nodosum	-	-	-	-	-	-	-	0.082	[28]
Carpodesmia crinita	Wild	Piran, Slovenia	March–November	1984	Vacuum-dried at 30 °C	5	Spectrophotometrically	1.62 ± 0.28	[4]
Cystoseira compressa	Wild	Piran, Slovenia	January–November	1984	Vacuum-dried at 30 °C	9	Spectrophotometrically	2.13 ± 0.28	[4]
Durvillaea antarctica	Wild	Santa Ana, Chile	October–December	2012	Washed in deionized water and dried at 20 °C	3	Chromatography	0.348	[29]

Table 1. *Cont.*

Species	Origin				Sample Treatment	*n*	Analytical Method	Vitamin C mg g^{-1} * ± SD	Reference
	Wild/ Cultivated	Collection Site	Season	Year					
Fucus vesiculosus	Wild	Chincoteague Island, USA	May	-	Freeze-dried	-	Chromatography	0.517 ± 0.078	[30]
Fucus vesiculosus	Wild	Chincoteague Island, USA	August	-	Freeze-dried	-	Chromatography	0.409 ± 0.101	[30]
Fucus virsoides	Wild	Piran, Slovenia	January–November	1984	Vacuum-dried at 30 °C	9	Spectrophoto-metrically	2.66 ± 0.45	[4]
Himanthalia elongata	Wild	Galicia, Spain	-	2010	Fresh	2	Chromatography	2.92 ± 0.37 **	[31]
Himanthalia elongata	Bought	Galicia, Spain	-	2014 ***	Dehydrated	6	Chromatography	0.207 ± 0.09	[32]
Himanthalia elongata	Wild	Galicia, Spain	December	2015	Dried < 38 °C	3	Chromatography	0.692 ± 0.053	[33]
Polycladia myrica	Wild	Hurghada, Egypt	April–June	2019 ***	Washed with tap and distilled water, air-dried	1	Chromatography	0.008	[25]
Sargassum baccularia	Wild	Tanjung Tuan, Malaysia	-	2008 ***	Washed in running water	-	Titration	0.224	[24]
Sargassum cervicorne	Wild	Tanjung Tuan, Malaysia	-	2008 ***	Washed in running water	-	Titration	0.254	[24]
Sargassum hemiphyllum	Wild	Tung Ping Chau, Hong Kong	December	1995	Washed then sun-dried for 4 days	3	Titration	0.519 ± 0.035	[18]
Sargassum hemiphyllum	Wild	Tung Ping Chau, Hong Kong	December	1995	Washed then oven dried for 15 h at 60 °C	3	Titration	0.977 ± 0.121	[18]
Sargassum hemiphyllum	Wild	Tung Ping Chau, Hong Kong	December	1995	Washed then freeze-dried	3	Titration	1.53 ± 0.12	[18]
Sargassum latifolium	Wild	Hurghada, Egypt	April–June	2019 ***	Washed with tap and distilled water, air-dried	1	Chromatography	0.007	[25]
Sargassum mcclurei	Wild	Nha Trang, Vietnam	June	2003	-	1	-	0.657 **	[34]

Table 1. *Cont.*

Species	Origin				Sample Treatment	*n*	Analytical Method	Vitamin C mg g^{-1} * ± SD	Reference
	Wild/ Cultivated	Collection Site	Season	Year					
Sargassum muticum	Wild	Bourgneuf Bay, France	-	-	Fresh	-	Chromatography	0.560	[35]
Sargassum muticum	Wild	Hurghada, Egypt	April–June	2019 ***	Washed with tap and distilled water, air-dried	1	Chromatography	0.012	[25]
Sargassum polycystum	-	Kota Kinabalu, Malaysia	-	2009 ***	Washed with distilled water	3	Titration	0.383 ± 0.000 **	[36]
Sargassum spp.	Wild	Hurghada, Egypt	April–June	2019 ***	Washed with tap and distilled water, air-dried	1	Chromatography	0.004	[25]
Sargassum tenerrimum	Wild	Visakhapatnam, India	Yearly	1996–1997	Washed in fresh water and freeze-dried	11	Spectrophoto-metrically	0.280	[26]
Sargassum vulgare	Wild	Visakhapatnam, India	Yearly	1996–1997	Washed in fresh water and freeze-dried	1	Spectrophoto-metrically	0.300	[26]
Turbinaria conoides	Wild	Tanjung Tuan, Malaysia	-	2008 ***	Washed in running water	-	Titration	0.112	[24]
Turbinaria spp.	Wild	Hurghada, Egypt	April–June	2019 ***	Washed with tap and distilled water, air-dried	1	Chromatography	0.008	[25]
Laminariales									
Alaria spp.	-	-	-	-	-	-	-	0.0221–0.497	[37]
Eisenia arborea	Wild	Bahía Asunción, Mexico	March–December	-	Sun-dried	10	Chromatography	0.344 ± 0.06	[38]
Laminaria digitata	-	-	-	-	-	-	-	0.355	[28]
Laminaria ochroleuca	Wild	Galicia, Spain	December	2015	Dried < 38 °C	3	Chromatography	0.785 ± 0.092	[33]
Laminaria spp.	Wild	Redondela, Spain	February	2011	Fresh	6	Chromatography	nd	[39]
Laminaria spp.	Wild	Galicia, Spain	-	2010	Fresh	2	Chromatography	0.096 ± 0.004 **	[31]
Saccharina latissima	Cultivated	Damariscotta Bay, USA	Early May	2017	Washed in running water	3	Titration	0.611 ± 0.074	[40]

Table 1. *Cont.*

Species	Origin				Sample Treatment	*n*	Analytical Method	Vitamin C mg g^{-1} * ± SD	Reference
	Wild/ Cultivated	Collection Site	Season	Year					
Saccharina latissima	Cultivated	Damariscotta Bay, USA	May and June	2017	Washed in running water and dried in various ways	16	Titration	0.104 ± 0.016 **	[40]
Undaria pinnatifida	Wild	Redondela, Spain	February	2011	Fresh	6	Chromatography	0.118 ± 0.022	[39]
Undaria pinnatifida	Wild	Redondela, Spain	February	2011	Boiling 20 min.	6	Chromatography	nd	[39]
Undaria pinnatifida	-	-	-	-	-	-	-	1.85	[28]
Undaria pinnatifida	Wild	Galicia, Spain	December	2015	Dried < 38 °C	3	Chromatography	0.693 ± 0.090	[33]
Wakame	Bought	-	-	2008 ***	-	-	Titration	0.030	[24]
Sphacelariales									
Halopteris scopari	Wild	Piran, Slovenia	January–November	1984	Vacuum-dried at 30 °C	9	Spectrophotometrically	2.04 ± 0.48	[4]

* mg ascorbic acid per g dry weight; ** mean of given numbers on various drying methods with no significant differences; *** year of publication, year of harvest not given; nd, not detected

Table 2. Vitamin C in Rhodophyta (phylum). Species arranged by orders.

Species	Origin				Sample Treatment	*n*	Analytical Method	Vitamin C mg g^{-1} * ± SD	Reference
	Wild/ Cultivated	Collection Site	Season	Year					
Bangiales									
Nori	Bought	-	-	2008 ***	Washed in running water, freeze-dried	-	Titration	0.390	[24]
Pyropia acanthophora	Wild	Central West Coast, India	July	2013	Washed in seawater, shade dried	5	Chromatography	0.042 ± 0.019	[41]
Pyropia columbina	Wild	Brighton, New Zealand and Dunedin, New Zealand	June–October	1986	Washed with seawater and oven-dried at 30 °C	7	Chromatography	2.62 ± 0.68	[42]
Porphyra spp.	Wild	Galicia, Spain	December	2014	Dried < 38 °C	3	Chromatography	0.712 ± 0.102	[33]
Porphyra umbilicalis	-	-	-	-	-	-	-	1.61	[28]
Porphyra umbilicalis	Wild	Galicia, Spain	-	2010	Fresh	2	Chromatography	1.05 ± 0.27 **	[31]
Ceramiales									
Centroceras clavulatum	Wild	Visakhapatnam, India	Yearly	1996–1997	Washed in fresh water and freeze-dried	1	Spectrophoto-metrically	0.345	[26]
Ceramium ciliatum	Wild	Piran, Slovenia	March–June	1984	Vacuum-dried at 30 °C	4	Spectrophoto-metrically	3.19 ± 0.51	[4]
Halopithys incurva	Wild	Piran, Slovenia	January–November	1984	Vacuum-dried at 30 °C	9	Spectrophoto-metrically	1.13 ± 0.24	[4]
Laurencia obtusa	Wild	Khanh Hoa, Vietnam	July	2003	-	1	-	0.252**	[34]
Nitophyllum punctatum	Wild	Piran, Slovenia	April	1984	Vacuum-dried at 30 °C	1	Spectrophoto-metrically	2.62	[4]
Corallinales									
Amphiroa fragilissima	Wild	Visakhapatnam, India	Yearly	1996–1997	Washed in fresh water and freeze-dried	11	Spectrophoto-metrically	0.285	[26]

Table 2. *Cont.*

Species	Origin				Sample Treatment	*n*	Analytical Method	Vitamin C mg g^{-1} * ± SD	Reference
	Wild/ Cultivated	Collection Site	Season	Year					
Jania rubens	Wild	Piran, Slovenia	July	1984	Vacuum-dried at 30 °C	1	Spectrophoto-metrically	0.436	[4]
Jania rubens	Wild	Visakhapatnam, India	Yearly	1996–1997	Washed in fresh water and freeze-dried	1	Spectrophoto-metrically	0.310	[26]
Erythropeltales									
Pyrophyllon subtumens	Wild	Brighton, New Zealand	June–October	1986	Washed with seawater and oven-dried at 30 °C	4	Chromatography	2.32 ± 0.33	[42]
Gelidiales									
Gelidiella acerosa	Wild	Khanh Hoa, Vietnam	July	2003	-	1	-	0.522 **	[34]
Gelidium pusillum	Wild	Visakhapatnam, India	Yearly	1996–1997	Washed in fresh water and freeze-dried	2	Spectrophoto-metrically	0.150	[26]
Millerella myrioclada	Wild	Visakhapatnam, India	Yearly	1996–1997	Washed in fresh water and freeze-dried	1	Spectrophoto-metrically	0.185	[26]
Pterocladia heteroplatos	Wild	Visakhapatnam, India	Yearly	1996–1997	Washed in fresh water and freeze-dried	11	Spectrophoto-metrically	0.175	[26]
Gigartinales									
Callophyllis variegata	Wild	Santa Ana, Chile	October–December	2012	Washed in deionized water and dried at 20 °C	3	Chromatography	0.011	[29]
Chondrus crispus	Wild	Galicia, Spain	December	2014	Dried < 38 °C	3	Chromatography	0.538 ± 0.055	[33]

Table 2. *Cont.*

Species	Origin				Sample Treatment	*n*	Analytical Method	Vitamin C mg g^{-1} * ± SD	Reference
	Wild/ Cultivated	Collection Site	Season	Year					
Eucheuma denticulatum	Cultivated	Sulawesi, Indonesia	February	2016	Washed with distilled water and dried	2	Titration	0.035 ± 0.006	[43]
Eucheuma denticulatum	Wild	O'ahu, USA	February	2002	Washed in filtered seawater and dried at 60 °C in an air oven	1	Chromatography	2.0	[44]
Hypnea musciformis	Wild	Visakhapatnam, India	Yearly	1996–1997	Washed in fresh water and freeze-dried	9	Spectrophoto-metrically	0.370	[26]
Hypnea valentiae	Wild	Nha Trang, Vietnam	July	2003	-	1	-	0.438 **	[34]
Kappaphycus alvarezii	Cultivated	Bangi Sabah, Malaysia	-	2009 ***	Washed with distilled water	3	Titration	0.395 ± 0.000 **	[36]
Kappaphycus alvarezii	Cultivated	Popayato, Indonesia	-	2020 ***	Washed in distilled water and dried	2	Titration	0.033 ± 0.001	[45]
Kappaphycus alvarezii	Wild	Khanh Hoa, Vietnam	May	2003	-	1	-	0.551 **	[34]
Kappaphycus alvarezii	Cultivated	Sulawesi, Indonesia	February	2016	Washed with distilled water and dried	2	Titration	0.036 ± 0.006	[43]
Kappaphycus alvarezii	-	West Coast, India	-	2005	Dried for 6 h at 50 °C	3	Chromatography	0.107 ± 0.30	[46]
Kappaphycus striatum	Cultivated	Sulawesi, Indonesia	February	2016	Washed with distilled water and dried	2	Titration	0.035 ± 0.006	[43]
Sphaerococcus coronopifolius	Wild	Marmara, Turkey	June	2009	Washed in tap water and dried at room temperature	4	Titration	0.78 ± 0.07	[47]

Table 2. *Cont.*

Species	Origin				Sample Treatment	*n*	Analytical Method	Vitamin C mg g^{-1} * ± SD	Reference
	Wild/ Cultivated	Collection Site	Season	Year					
Gracilariales									
Crassiphycus changii	Wild	Tanjung Tuan, Malaysia	-	2008 ***	Washed in running water	-	Titration	0.285	[24]
Crassiphycus changii	Cultivated	Kedah, Malaysia	-	2000 ***	Washed in running water	3	Titration	0.285 **	[48]
Crassiphycus changii	Wild	Santubong, Malaysia	-	2017 ***	Washed with distilled water and freeze-dried	3	Chromatography	0.025 ± 0.002	[49]
Gracilaria corticata	Wild	Visakhapatnam, India	Yearly	1996–1997	Washed in fresh water and freeze-dried	12	Spectrophoto-metrically	0.100	[26]
Gracilaria gracilis	Wild	Marmara, Turkey	June	2009	Washed in tap water and dried at room temperature	4	Titration	0.24 ± 0.01	[47]
Gracilaria tenuistipitata	Wild	Nha Trang, Vietnam	May	2003	-	1	-	0.502 **	[34]
Hydropuntia edulis	Wild	Thondi, India	-	2015 ***	Washed in fresh water, shade dried 28 °C	2	Chromatography	5.01 ± 0.40	[50]
Nemaliales									
Liagora albicans	Wild	Visakhapatnam, India	Yearly	1996–1997	Washed in fresh water and freeze-dried	1	Spectrophoto-metrically	0.155	[26]
Palmariales									
Dulse	Bought	-	-	2008 ***	Dried	-	Titration	0.120	[24]
Palmaria palmata	-	-	-	-	-	-	-	0.69	[28]
Palmaria palmata	Wild	Galicia, Spain	-	2010	Fresh	2	Chromatography	0.039 ± 0.001 **	[31]
Palmaria palmata	Wild	Bretagne, France	December	2014	Dried < 38 °C	3	Chromatography	0.538 ± 0.055	[33]

* mg ascorbic acid per g dry weight; ** calculated from wet weight to dry weight based on given proximate composition; *** year of publication, year of harvest not given; nd, not detected.

Table 3. Vitamin C in Chlorophyta (phylum). Species arranged by orders.

Species	Origin				Sample Treatment	*n*	Analytical Method	C Vitamin mg g^{-1} * ± SD	Reference
	Wild/ Cultivated	Collection Site	Season	Year					
Bryopsidales									
Bryopsis pennata	Wild	Visakhapatnam, India	Yearly	1996–1997	Washed in fresh water and freeze-dried	4	Spectrophoto-metrically	0.250	[26]
Caulerpa lentillifera	Wild	Tanjung Tuan, Malaysia	-	2008 ***	Washed in running water	-	Titration	0.274	[24]
Caulerpa lentillifera	Wild	Amphor BanLam, Thailand	March	2014 ***	Washed in running water	3	Titration	0.013 **	[51]
Caulerpa lentillifera	-	Semporna, Malaysia	-	2009 ***	Washed with distilled water	3	Titration	0.389 ± 0.000 **	[36]
Caulerpa racemosa	Wild	Tanjung Tuan, Malaysia	-	2008 ***	Washed in running water	-	Titration	0.225	[24]
Caulerpa racemosa	Wild	Visakhapatnam, India	Yearly	1996–1997	Washed in fresh water and freeze-dried	9	Spectrophoto-metrically	0.275	[26]
Caulerpa racemosa	Wild	Khanh Hoa, Vietnam	July	2003	-	1	-	0.912 **	[34]
Caulerpa sertularioides	Wild	Visakhapatnam, India	Yearly	1996–1997	Washed in fresh water and freeze-dried	4	Spectrophoto-metrically	0.375	[26]
Caulerpa taxifolia	Wild	Visakhapatnam, India	Yearly	1996–1997	Washed in fresh water and freeze-dried	7	Spectrophoto-metrically	0.390	[26]
Codium tomentosum	Wild	Marmara, Turkey	June	2009	Washed in tap water and dried at room temperature	4	Titration	1.38 ± 0.19	[47]
Codium vermilara	Wild	Piran, Slovenia	July	1984	Vacuum-dried at 30 °C	1	Spectrophoto-metrically	1.00	[4]

Table 3. *Cont.*

Species	Origin				Sample Treatment	*n*	Analytical Method	C Vitamin mg g^{-1} * ± SD	Reference
	Wild/ Cultivated	Collection Site	Season	Year					
Cladophorales									
Chaetomorpha antennina	Wild	Visakhapatnam, India	Yearly	1996–1997	Washed in fresh water and freeze-dried	9	Spectrophoto-metrically	0.490	[26]
Chaetomorpha brachygona	Wild	Visakhapatnam, India	Yearly	1996–1997	Washed in fresh water and freeze-dried	8	Spectrophoto-metrically	0.225	[26]
Cladophora rupestris	Wild	Piran, Slovenia	November	1984	Vacuum-dried at 30 °C	1	Spectrophoto-metrically	1.06	[4]
Cladophora socialis	Wild	Visakhapatnam, India	Yearly	1996–1997	Washed in fresh water and freeze-dried	2	Spectrophoto-metrically	0.340	[26]
Cladophora spp.	Wild	Visakhapatnam, India	Yearly	1996–1997	Washed in fresh water and freeze-dried	1	Spectrophoto-metrically	0.675	[26]
Ulotrichales									
Acrosiphonia orientalis	Wild	Visakhapatnam, India	Yearly	1996–1997	Washed in fresh water and freeze-dried	12	Spectrophoto-metrically	0.500	[26]
Gayralia oxysperma	Wild	Hawai'i, USA	October	2001	Washed in filtered seawater and dried at 60 °C in an air oven	1	Chromatography	1.3	[44]
Monostroma nitidum	Wild	Nha Trang, Vietnam	May	2003	-	1	-	0.495 **	[34]
Ulvales									
Ulva compressa	Wild	Visakhapatnam, India	Yearly	1996–1997	Washed in fresh water and freeze-dried	6	Spectrophoto-metrically	0.310	[26]

Table 3. *Cont.*

Species	Origin				Sample Treatment	*n*	Analytical Method	C Vitamin mg g^{-1} * ± SD	Reference
	Wild/ Cultivated	Collection Site	Season	Year					
Ulva flexuosa	Wild	O'ahu, USA	January	2002	Washed in filtered seawater and dried at 60 °C in an air oven	1	Chromatography	3.0	[44]
Ulva intestinalis	Wild	Muğla, Turkey	August	2013	Washed in fresh water, frozen, thawed, dried at 40 °C for 24 hrs	3	Chromatography	0.028 ± 0.001	[52]
Ulva intestinalis	Wild	Muğla, Turkey	November	2013	Washed in fresh water, frozen, thawed, dried at 40 °C for 24 hrs	3	Chromatography	0.034 ± 0.000	[52]
Ulva intestinalis	Wild	Muğla, Turkey	January	2014	Washed in fresh water, frozen, thawed, dried at 40 °C for 24 hrs	3	Chromatography	0.026 ± 0.000	[52]
Ulva intestinalis	Wild	Muğla, Turkey	April	2014	Washed in fresh water, frozen, thawed, dried at 40 °C for 24 hrs	3	Chromatography	1.47 ± 0.02	[52]
Ulva lactuca	Wild	O'ahu, USA	January	2002	Washed in filtered seawater and dried at 60 °C in an air oven	1	Chromatography	2.2	[44]
Ulva lactuca	Wild	Visakhapatnam, India	Yearly	1996–1997	Washed in fresh water and freeze-dried	12	Spectrophoto-metrically	0.155	[26]
Ulva reticulata	Wild	Nha Trang, Vietnam	March	2003	-	1	-	0.971 **	[34]
Ulva reticulata	Wild	Pattani Bay, Thailand	May	2014 ***	Washed in running water	3	Titration	0.00 **	[51]

Table 3. *Cont.*

Species	Origin				Sample Treatment	*n*	Analytical Method	C Vitamin mg g^{-1} * ± SD	Reference
	Wild/ Cultivated	Collection Site	Season	Year					
Ulva rigida	Wild	Marmara, Turkey	June	2009	Washed in tap water and dried at room temperature	4	Titration	2.05 ± 0.33	[47]
Ulva rigida	Wild	Northern Adriatic	January–November	1984	Vacuum-dried at 30 °C	9	Spectrophotometrically	2.00 ± 0.52	[4]
Ulva rigida	Wild	Northwest Iberian coast, Spain	-	2010 ***	-	3	Chromatography	0.942	[53]
Ulva spp.	Wild	Piran, Slovenia	January–May	1984	Vacuum-dried at 30 °C	4	Spectrophotometrically	2.04 ± 0.34	[4]
Ulva spp.	Wild	Piran, Slovenia	October–November	1984	Vacuum-dried at 30 °C	2	Spectrophotometrically	1.23 ± 0.35	[4]
Ulva spp.	Wild	Visakhapatnam, India	Yearly	1996–1997	Washed in fresh water and freeze-dried	1	Spectrophotometrically	0.420	[26]
Ulva spp.	Wild	Locquirec, France	June–September	1982	Rinsed with seawater	5	-	0.247 ± 0.278 **	[54]
Ulva spp.	-	-	-	-	-	-	-	1.25	[28]
Ulva spp.	Wild	Galicia, Spain	December	2015	Dried < 38 °C	3	Chromatography	0.746 ± 0.136	[33]

* mg ascorbic acid per g dry weight; ** calculated from wet weight to dry weight based on given proximate composition; *** year of publication, year of harvest not given nd, not detected.

The average content of vitamin C in seaweed from the reviewed studies is 0.773 mg g^{-1} dw. Boxplots for each taxonomical category are seen in Figure 1. The mean for each category is Chlorophyta; 0.781, Phaeophyceae; 0.815, and Rhodophyta; 0.720 mg g^{-1} dw. The range, mean and median of the three categories are not varying considerably, thus no significant differences were found between the categories (one-way ANOVA; $p = 0.882$, F = 0.126). These results are not taking any of the metadata into consideration. The ranges found in this review for each category were broad, and for Rhodophyta the maximum content found was 5.01 mg g^{-1} dw.

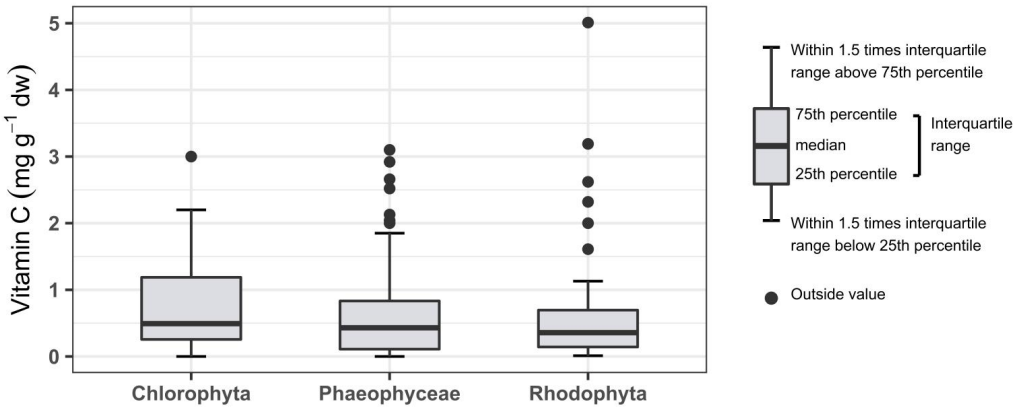

Figure 1. Data analysis of vitamin C content (mg g^{-1} dw) represented in boxplots and statistical output for the three categories; Chlorophyta (phylum), Phaeophyceae (class), and Rhodophyta (phylum).

The five species with the highest content of vitamin C (above 3.00 mg g^{-1} dw) were *Hydropuntia edulis* (R) > *Dictyota dichotoma* (B) > *Ceramium ciliatum* (R) > *Mesogloia vermiculata* (B) > *Ulva flexuosa* (G) and the 90th percentile of the data entries contained 2.06 mg g^{-1} dw. Their content is comparable to the amount found in peas. Common for the five species is that only one study is published for each of the species, meaning their reliability is not powerful. Data for each individual species are scarce, which is why the seaweed species were divided into the presented categories of green, brown, and red seaweeds. In addition, the entries showed a large variation among species and therefore a broad picture. Looking into the taxonomical order instead of the species, a more reliable and specific estimate of vitamin C content can be achieved.

In Figures 2–4, a boxplot for each represented order is shown. No significant differences were found between the orders of Chlorophyta. It can however be seen that seaweeds within the order *Ulvales* (G) have a wide range reaching up to 3.00 mg g^{-1} dw. This indicates that *Ulvales* are richer in vitamin C compared to other green seaweeds. For the orders within the class Phaeophyceae, a statistically significant difference was found (one-way ANOVA; $p = 0.005$, F = 4.334) with a Tukey post-hoc test showing the differences. The statistical results can be found in Figure 3. The order *Ectocarpales* (B) had a high mean of 2.54 mg g^{-1} dw, but all seaweeds within this order was from the same study. To confirm if *Ectocarpales* are high in vitamin C, other studies should look into species within this order. The orders *Fucales* (B) and *Laminariales* (B) have the lowest content of vitamin C within the Phaeophyceae. This is interesting as some of these brown species (*Alaria esculenta*, *Ascophyllum nodosum*, *Fucus vesiculosus*, *Laminaria* spp., *Saccharina* spp., *Sargassum* spp., and *Undaria pinnatifida*) are of commercial interest [10,11,37,55–58] and probably useful to avoid claiming they are rich in vitamin C. For Rhodophyta no significance was found between orders, although a broad range was seen for the *Ceramiales* (R). It is worth mentioning that the meta-analysis only considers the taxonomical orders, all other metadata that can influence the vitamin C content such as processing are not included in this analysis.

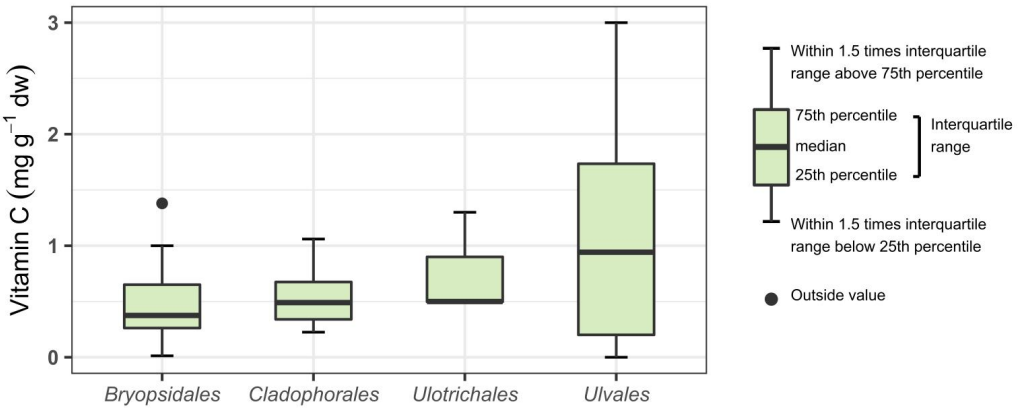

Figure 2. Data analysis of vitamin C content (mg g^{-1} dw) represented in boxplots and statistical output for some orders of the phylum Chlorophyta.

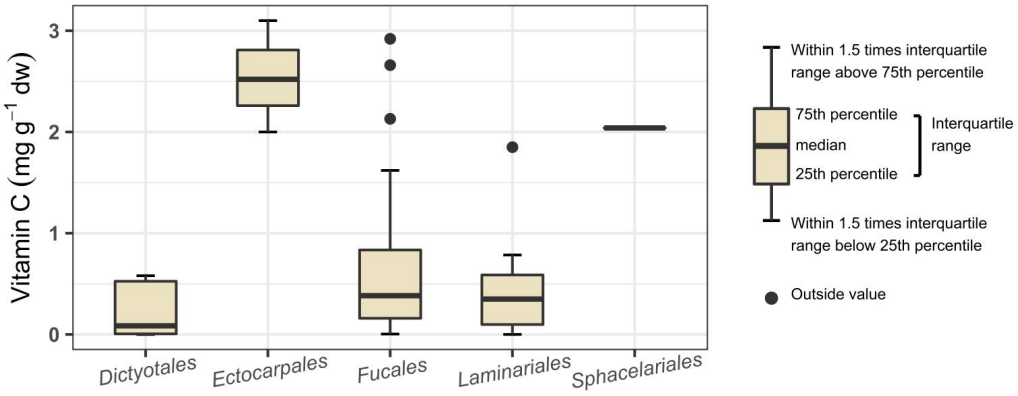

Figure 3. Data analysis of vitamin C (mg g^{-1} dw) content represented in boxplots and statistical output for some orders of the class Phaeophyceae. The letters "a" and "b" indicate statistically significant differences between orders.

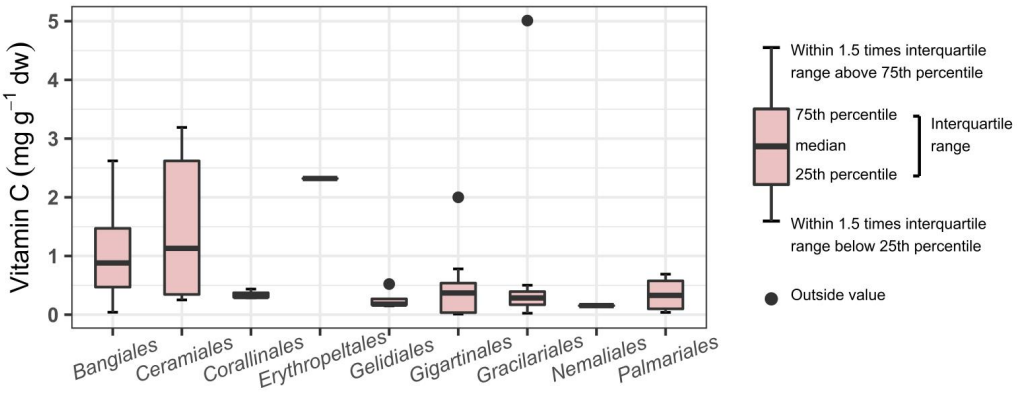

Figure 4. Data analysis of vitamin C content (mg g^{-1} dw) represented in boxplots and statistical output for some orders of the phylum Rhodophyta.

3.2. Comparison to other Foods and RNI

Vitamin C is known to be abundant in rose hips, black and red currants, strawberries, parsley, oranges, and grapefruit [1,59]. In Table 4 the content of vitamin C can be seen for various terrestrial fruits regarded as foods, and the content of seaweeds found in this review. The amount needed to reach the recommended nutrient intake (RNI) is also given as a method to compare the foods and seaweeds. The dietary reference intake RNI is chosen

to compare to, as it is an established value set by FAO/WHO on a global evaluation. Other types of dietary reference intakes exist, and those set by EFSA and the Institute of Medicine are all higher and gender-based. This means, that more food production is needed to reach the levels. It is understood by the authors that the RNI is created to consider the entire diet, but it is simply used for comparison between foods.

Table 4. Vitamin C content found in other food sources as well as these reviewed data categorized on different levels of the taxonomy. Moreover, the amount that is assumed necessary to consume to meet the RNI. All the contents from other foods are calculated based on [22]. The list is made in descending order with seaweeds shaded in the color of the seaweed categories.

Food	mg Vitamin C g^{-1} dw	g ww to Meet RNI; 45 mg Day^{-1}
Rosehip	36.4	5.35
Parsley	20.8	14.6
Broccoli	10.1	40.1
Black currant	8.66	24.9
Strawberry	6.67	67.5
Grapefruit	4.08	95.1
Ectocarpales *	2.54	118
Peas	2.11	105
90th percentile seaweed *	2.06	146
Potato	1.29	170
Iceberg lettuce	1.17	818
Dictyotales *	0.997	301
Chlorophyta *	0.781	384
Average seaweed *	0.773	388
Rhodophyta *	0.720	417
Fucales *	0.686	437
Laminariales *	0.496	605

* the amount needed of macroalgae (g ww) calculated based on the assumption of a moisture content of 85% ww. Brown, Phaeophyceae; grey, seaweed in general; green, Chlorophyta; red, Rhodophyta.

Rosehip has a high vitamin C content compared to other foods, and to meet the RNI, less than 6 g of rosehip is needed, whereas for seaweed about 400 g is needed based on wet weight. This is half the amount compared to iceberg lettuce, which shows that seaweeds are a better source of vitamin C compared to iceberg lettuce. Although species within the division Rhodophyta and the orders of *Fucales* (B) and *Laminariales* (B) on average contain less than the average of all seaweed species and thereby more than 400 g ww is needed to be consumed to achieve the RNI. It was mentioned that consuming 2–3 g day^{-1} of vitamin C can cause diarrhea [1], but to reach that more than 5 kg ww seaweed should be consumed. It can be concluded based on the reviewed literature that in general seaweeds are not an abundant source of vitamin C for food consumption. Although some can contribute to the daily intake and assist to achieve the RNI, whereas others only have a minimal contribution. Moreover, these results also indicate, that stating either that seaweeds have a fairly high content of vitamin C, or that they have a low content is difficult. "Seaweed" is undiscriminating, and is a category of a large variety of macroalgal species, but the species variation can be significant and therefore conclusions should be made on the level of e.g., taxonomical divisions, order, or species and not on "seaweed".

3.3. Seasonal Variation

Three studies looked into the seasonal variation of vitamin C for the species of brown; *Saccharina latissima*, *Fucus vesiculosus*, and green; *Ulva intestinalis* and *Enteromorpha* spp. [4,30,40,52]. They all found that the highest content of vitamin C was around April–May, with all seaweeds collected in the Northern Hemisphere. It points towards those seasonal fluctuations of vitamin C that occur in seaweed species. Škrovánková (2011) suggests that seaweeds growing closer to the water surface level will contain higher levels of vitamin C than seaweeds harvested from

deep waters [17]. This may be due to the higher antioxidant level needed for the seaweed when exposed to high levels of sun, which fits the results seen for seasonal variation.

3.4. Analytical Method for Vitamin C

Various analytical methods exist to analyze the vitamin C content in food. The reviewed studies can be divided into three categories. Spectrophotometric methods by reducing cupric ions [4,26], titration with 2,6-dichlorophenolindophenol (Titration) [18,24,36,40,43,45,47,48,51], and chromatography such as High Performance Liquid Chromatography (HPLC) [25,29–33,35,38,39,41,42,44,46,49,50,52,53]. Five papers did not mention the analytical method used [27,28,34,37,54].

It is worth to mention that indophenol titration is the official AOAC Method 967.21 for juices [60,61], although many studies on vitamin C in foodstuff are performed by chromatographic methods [3], which is the AOAC First Action Official Method 2012.22 for infant formula and nutritional formulas [62]. No studies referred to the AOAC 984.26-1985, Vitamin C (Total) in Food-Semiautomated Fluor [63].

Quantification of vitamin C by different analytical methods with the same samples was not performed by any study, and it was therefore not possible to conclude the effect of the analytical method. In the case where two or more studies had analyzed the same species with different analytical methods, no specific trend was found such as one analysis always quantifying a higher content. The results, therefore, indicate that even though one analysis might be over- or underestimating, other factors such as biological variations, season, harvest site, sample treatment or other unknown factors can influence the result as well.

3.5. Processing and the Influence on Vitamin C

Vitamin C is somewhat easily degraded, and in nutrient stability studies in foods, it is assumed that if vitamin C is well retained, then other nutrients will be just as well retained if not better. The degradation of vitamin C depends on moisture-, oxygen, light, and metal ion catalysis as well as temperature and pH [5,64]. An analysis of the metadata of processing was performed in this present review (data not shown). No trend for washing methods or drying methods was observed. Therefore it was not possible to conclude the effect of processing on vitamin C in seaweed based on the overall reviewed literature.

A few studies looked into the degradation of vitamin C [18,39,40]. Both Sappati et al. (2019) and Chan et al. (1997) found that sun drying and oven drying at temperatures between 30 and 70 °C had a significant, negative influence on the vitamin C content in *Saccharina latissima* (B) and *Sargassum hemiphyllum* (B), respectively. Vegetable blanching and boiling can be performed to reduce microbial load and inactivate enzymes, but it is known that it also compromises quality compounds such as vitamin C [65,66]. Amorim et al. (2012) studied the influence of 20 min. boiling on *Undaria pinnatifida* (B) and found the reduction of vitamin C to be below the detection limit [39]. Amorim-Carrilho et al. (2014) studied different processing methods on *Himanthalia elongata* (B). They found that 15 min boiling in 100 °C water, rehydration in water for 10 min, and steaming for 40 min reduced the vitamin C content below the detectable limit [32].

Friedlander (1989) found that seven months of storage decreased the initial value of ascorbic acid in *Pyrophyllon subtumens* (R) and *Pyropia columbina* (R) to 15% and 34%, respectively [42]. Some vegetables can lose up to 70% of the initial content during storage [19]. Balan et al. (2016) suggested that a matrix with a fibrous texture and low water content would preserve ascorbic acid better during storage [66], which could be an interesting hypothesis for dried seaweed. Friedlander (1989) also found that drying at 30 °C for 4 h did not affect the ascorbic acid content, moreover did washing or toasting for 15 s of nori sheets not influence the ascorbic acid content.

Although no studies looked into the effect of cutting the seaweed biomass, a study on rose hips showed that cutting would lead to a decrease in vitamin C content, which might also be the case for seaweeds [64].

4. Conclusions

Seaweeds are not a rich source of vitamin C, but when consumed they feed into the daily intake. To reach the Recommended Nutrient Intake approximately 400 g ww of seaweed should be consumed per day, which in contrast to rosehip is 5.35 g ww.

The vitamin C content can vary, due to biological, seasonal, locational, and treatment variations. Moreover, evaluating and generalizing seaweeds can be difficult, the nutritional quality should be evaluated based on e.g., taxonomical category, order, or species. The mean content in seaweed is 0.773 mg g^{-1} dw with a 90 percentile of 2.06 mg g^{-1} dw. A study of the taxonomical orders of the species indicated that the green seaweeds *Ulvales* contained up to 3.00 mg g^{-1}. Whereas, brown species within the orders *Fucales* and *Laminarales* had low amounts of vitamin C.

It was found that drying, boiling and long storage time lead to a decrease in vitamin C in seaweed, as it is easily oxidized.

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Paper 2

Reducing the high iodine content of *Saccharina latissima* and improving the profile of other valuable compounds by water blanching

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Reducing the High Iodine Content of *Saccharina latissima* and Improving the Profile of Other Valuable Compounds by Water Blanching

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Abstract: *Saccharina latissima* contains high amounts of iodine in comparison to other seaweeds. The present study aimed to decrease the iodine content of *S. latissima* (sugar kelp) by water blanching and freezing to avoid an excess intake of iodine by consumption of sugar kelp. Various blanching conditions were investigated (temperature; 30, 45, 60 and 80 °C, and duration; 2, 30, 120 and 300 s). Some conditions resulted in a significant decrease in iodine content (≥45 °C and ≥30 s). Non-processed *S. latissima* contained on average 4605 mg iodine kg^{-1} dw^{-1} which significantly decreased following the treatments. The lowest content obtained was 293 mg iodine kg^{-1}·dw^{-1} by water blanching at 80 °C for 120 s. The study also investigated if other valuable compounds were affected during the processing conditions. No significant changes were observed for total lipid and protein, but significant changes were seen for ash. A significant loss of two non-essential amino acids (glutamic acid and alanine) due to the blanching process was found. This also resulted in a protein quality increase as the essential amino acid to total amino acid ratio changed from 42.01 ± 0.59% in fresh seaweed to 48.0 ± 1.2% in blanched seaweed. Moreover, the proportion of eicosapentaenoic acid, α-linolenic acid, polyunsaturated fatty acids, and omega-3 fatty acids (%FAME), and the omega-3 to omega-6 fatty acids ratio was significantly higher in the samples blanched at 60 °C for 300 s compared to the fresh and samples blanched at 45 °C for 30 s. The total phenolic content (TPC) and the radical scavenging activity were significantly higher in treated samples. The results indicate that the processing did not compromise the valuable compounds in focus in this study for *S. latissima*; they did, however, result in biomass with an improved profile of health beneficial compounds.

Keywords: sugar kelp (*Saccharina latissima*); seaweed; blanching; freezing; iodine; nutrients; bioactives; antioxidant activity

1. Introduction

Seaweed as a food source is currently in focus in Europe due to its potential as an environmentally friendly and nutritious food source. It grows in seawater, does not take up any land areas and does not need any freshwater supply. Moreover, seaweeds contain highly valuable bioactive compounds [1], which are of interest from a nutritional point of view. *Saccharina latissima* (Linnaeus) is a brown macroalga commonly known as sugar kelp, which is successfully cultivated in Europe and is commercially available. Valuable compounds specifically for *S. latissima* are minerals, essential amino

acids, polyunsaturated fatty acids, phenolic compounds, antioxidants, etc. [2–5]. Kelps, in general, contain high amounts of the trace element iodine [6] and contents as high as 6500 mg·kg^{-1}·dw^{-1} have been found in European sugar kelp [7]. Marine foods are considered rich in iodine and contain up to 30 mg·kg^{-1}·dw^{-1} [8]. It is clear that the iodine content of sugar kelp is extremely high.

Due to the possibilities of both insufficient and excess intake of iodine, dietary values for recommended intake (RI) (150 µg iodine day^{-1} for adults) and upper intake level (UL) (600 µg iodine day^{-1} for adults) have been established to provide guidance to consumers by both the Nordic Council of Ministers [9] and the European Food Safety Authorities (EFSA) [10,11]. No maximum levels for iodine in food (including seaweed) have been established in European Food Regulation. However, some member states, e.g., France, have published a recommended maximum level of 2000 mg iodine kg^{-1} dry seaweed product [12].

It is in the manufacturer's interest, as well as their responsibility, to ensure that their food products are safe and comply with food legislation (Council Directive (EC) 178/2002; [13]). The high iodine content of sugar kelp can, even from low consumption, lead to an intake of iodine above the upper level and hence, this is seen as a possible market barrier for the trade of sugar kelp. Consequently, the European seaweed industry demands procedures that can reduce the iodine content of their final products.

Previous studies have investigated how to reduce iodine in sugar kelp, e.g., by water soaking (low temperatures) or boiling [7,14]. Recently, Stévant et al. (2018) [7] found that soaking in water at 32 °C for 1–6 h would reduce the iodine content significantly by 84%–88%. Another study by Lüning and Mortensen (2015) [14] found a significant iodine reduction of 33% and 75% for sugar kelp boiled for 2 and 20 min, respectively.

The aim of this study was to investigate the possible iodine reduction by processing. Moreover, to investigate if the various processing conditions compromised other valuable compounds, the nutritional value of the final product was determined. The processing conditions investigated were water blanching at short processing times (2, 30, 120 and 300 s) at various water temperatures (30, 45, 60 and 80 °C) or by freezing followed by thawing. Moreover were the protein by sum of amino acids, amino acid profile, total lipid, fatty acid profile, ash, total phenolic content, and antioxidant capacity quantified to evaluate the possible quality compromise of *S. latissima* due to processing. In addition, the true retention factors were calculated in order to show not only the proximate composition, but also link to losses of biomass during processing. Lastly, a brief assessment of the iodine content compared to the recommended intake (RI) and upper intake levels (UL) was conducted.

2. Materials and Methods

2.1. Chemicals

All chemicals were of analytical grade unless otherwise stated. More specifically, tetra-methyl-ammonium-hydroxide (TMAH) 25%, sodium hydroxide, o-phtaldialdehyde and butylated hydroxytoluene were purchased from Sigma-Aldrich (Steinheim, Germany). Toluene, hydrogen chloride, methanol, chloroform, sodium chloride, and 20% boron trifluoride were purchased from Merck (Darmstadt, Germany). n-Heptane was purchased from VWR (Radnor, Pennsylvania, USA). The purified C23:0 was purchased from Nu Chek Prep (Elysian, MN, USA).

2.2. Raw Material

Saccharina latissima was harvested at Seaweed Energy Solution's cultivation site at Frøya, Norway (N63° 42.279′ E8° 52.232′). Approximately 1 m long blades were harvested on 23 April 2018. After harvest, the sugar kelp was stored in aerated tanks with flow-through seawater at 7–8 °C for 2–3 days until processing was carried out. Stem and holdfast were kept, but fouling organisms were removed by hand.

2.3. Water Blanching and Freezing

All treatments were performed on samples (n = 3) of 150 ± 20.0 g wet weight (*ww*) whole thallus sugar kelp. Water blanching was conducted in a JBN12 (Grant Instruments Ltd., England) water bath in 5 L tap water with the following variables: temperature 30, 45, 60 and 80 °C and processing durations of 2, 30, 120 and 300 s. The water bath was cleaned and the water renewed prior to each treatment process including between treatment replicates. After blanching, the sugar kelp was drained by keeping it vertical for 5–10 s, then placed in a zip lock plastic bag and cooled in ice water for 3 min.

Freezing was conducted in a −20 °C freezing room (Schneider Electric, Rueil-Malmaison, France) for 8 h and thawing was done at 5 °C overnight (freeze-thawed). The freeze-thaw caused drip water to appear, which was drained by keeping it vertical for 5–10 s.

From the harvest batch were three replicates of approximately 150 g fresh sugar kelp collected, drained and stored until analyses. These samples are referred to as "fresh sugar kelp".

2.4. Sample Preparation for Chemical Analysis

Prior to analysis, the sugar kelp samples were cut into 3 × 3 cm pieces and gently mixed. Approximately 20% of the material was used for water and ash analysis. The rest was freeze dried (Alpha 1-4 LDplus, Martin Christ, Germany) at −40 °C, and then homogenized by milling (MM 400, Retsch, Germany) to particle sizes of <300 µm.

2.5. Dry Matter and Ash

Dry matter content (DM) was determined gravimetrically by vaporizing water at 105 °C for 20–24 h in an oven (Termaks AS, Bergen, Norway) until stable weight [15]. Ash content was determined gravimetrically by ignition in a muffle furnace (Nabertherm, Lilienthal, Germany) at 600 °C for 15–20 h [16]. Both analyses were performed in duplicates.

2.6. Iodine

Inductively Coupled Plasma Mass Spectrometry (ICP-MS) was used for the quantification of the total iodine content in the sugar kelp samples. The samples were prepared according to EN17050:2017 [17]. Briefly, 0.15–0.20 g of freeze-dried milled homogenized powder was weighed into tubes (Kimax®). Subsequently, 5 mL Milli-Q® water and 1 mL 25% tetra-methyl-ammonium-hydroxide (TMAH) were added. The tubes were then sealed and placed in a preheated oven at 90 ± 3.0 °C for 3 h followed by cooling and diluting to a final volume of 20 mL with Milli-Q® water. To remove coarse particles, the samples were centrifuged at 10,000× g for 20 min. Prior to analysis, the supernatant was filtered through a 0.45 µm filter and samples were diluted 50 times. Sample extracts were stored in metal free plastic tubes for a maximum of 5 days prior to ICP-MS analysis. The iodine quantification (n = 1) was performed by ICP-MS (Finnigan ELEMENT-2, Thermo Fisher, Waltham, MA, USA) combined with an SC2 DX auto sampler and a prepFAST auto dilution system (Elemental Scientific, Omaha, NE, USA). The parameter settings were 15.5 L·min^{-1} coolant gas, 1.1 L·min^{-1} auxiliary gas, and 0.75 L·min^{-1} nebulizer gas. Isotopes monitored were 127I and 185Re for internal standard. The limit of quantification (LOQ) for iodine was 37 µg·g^{-1}. The certified reference material (NIST 3232, Kelp powder) was analyzed together with the samples and the obtained results complied well with the certified value (recovery 96.8%, n = 2).

2.7. Amino Acid Hydrolysis and Calculation of Protein Content

Briefly, 50 mg sample was hydrolyzed in 1 mL 6 M HCl at 105 °C for 22 h (n = 2). Prior to quantification, the samples were neutralized by NaOH and HCl to pH 7.0 ± 0.5 and filtered through a Whatman glass microfiber filter (GF/C) using suction. The samples were diluted 1:100 with distilled water followed by a 0.22 µm filtration. Then the amino acids were quantified by High-Performance Liquid Chromatography (HPLC) (Dionex UltiMate® 3000 HPLC+ focused, Dionex UltiMate® 3000 Autosampler, Dionex RF

Fluorescence Detector, Thermo Scientific, USA) including precolumn derivatization of the amino acids with o-phtaldialdehyde and Nova-Pak® column (C18, 4 µm). Tryptophan was destroyed in acid hydrolysis, thus not detected. The chromatographic peaks for glycine and arginine gathered in one, therefore an average of their molar masses was used to calculate their content.

The total protein content was calculated by summing the total moles of amino acids as recommended by Angell et al. (2016) [18] and FAO (2003) [19] and then subtracting the water mass (18 g H_2O mol^{-1} amino acid), which was integrated during disruption of peptide bonds in the acid hydrolysis [20].

2.8. Determination of Total Lipid Content

The gravimetric method described by Bligh and Dyer (1959) [21] was used to quantify total lipid content. Briefly, a mixture of demineralized water, methanol and chloroform (0.8:2:1 mL) was added to 30 mg freeze-dried sample followed by homogenizing with 1 mL chloroform (20 s) and then 1 mL demineralized water (20 s). The mixture was centrifuged thoroughly at 4 °C. The chloroform phase (0.5 mL) was added to a pre-weighed glass container and vaporized overnight in a fume hood. The following day the container was weighed again. The total lipid content was calculated by the following Equation:

$$\text{tatal lipid}(\%) = \frac{1v \cdot C \cdot 100}{Cv \cdot m} \quad (1)$$

where l_v is the lipid weighed after vaporization in mg, c is the added chloroform (2 mL), c_v is the vaporized chloroform (0.5 mL), and m is the mass of the weighed sample before extraction.

2.9. Carbohydrates by Difference

Estimation of the total carbohydrate content was done by "total carbohydrate by difference" [19], which includes fibers:

$$\text{carbohydrates} = 100 - (\text{weightinggram [protein} + \text{lipid} + \text{water ash]in 100 g of food}) \quad (2)$$

2.10. Fatty Acids

Direct methylation of fatty acids were performed according to [22]. Approximately, 100 mg of sample was mixed with 1 mL 1.0 M NaOH in methanol, 1 mL toluene, and 0.1 mL 2% (*w*/*v*) C23:0 in *n*-heptane and sonicated for 10 min, followed by 100 °C water bath for 2 min and cooling in cold water. Next, 2 mL boron trifluoride (20% solution) were added and boiled and cooled as earlier described. Lastly, 2 mL 6.8 M saturated sodium chloride solution along with 1 mL heptane with 0.01% butylated hydroxytoluene were added and shaken. The heptane phase was transferred to a GC vial and FAMEs were analyzed by GC (HP 5890A, Agilent Technologies, Palo Alto, CA, USA) according to the American Oil Chemists' Society (AOCS) [23]. For separation, DB127-7012 column (10 m × ID 0.1 mm × 0.1 µm film thickness, Agilent Technologies, Palo Alto, CA, USA) was used. Injection volume was 0.2 µL in split mode (1:50). The initial temperature of the GC-oven was 160 °C. The temperature was set to increase gradually as follows: 160–200 °C (10.6 °C min^{-1}), 200 °C kept for 0.3 min, 200–220 °C (10.6 °C min^{-1}), 220 °C kept for 1 min, 220–240 °C (10.6 °C min^{-1}) and kept at 240 °C for 3.8 min. The determination was conducted in duplicates. Fatty acids were identified by comparison of retention times with those from a mixture of known fatty acid standards. Results were given in area %.

2.11. Extraction for Antioxidant Analyses and Total Phenolic Content

The extraction of antioxidants was executed according to [24] with some modifications. Briefly, 0.2 g sugar kelp sample was weighed, and 5 mL methanol was added, and the samples were placed in a sonicator for 30 min. Thereafter, the samples were centrifuged (2164× g for 10 min) and the supernatant was collected. The pellets were resuspended and extractions repeated twice. The solvent

was evaporated under nitrogen flow. When the extracts were completely dry, they were stored in the freezer (−18 °C). Prior to the analyses, the dried powders were dissolved in 1 mL methanol.

2.12. Total Phenolic Content (TPC)

The procedure was carried out according to [25]. Extracts (100 µL) were mixed with Folin–Ciocalteu reagent (0.75 mL, 10% *v*/*v*). After 5 min, 0.75 mL sodium carbonate (7.5% *w*/*v*) was added to the mixture, which was then incubated at room temperature in darkness for 90 min. The absorbance was measured at 725 nm. The measured absorbance was converted into gallic acid equivalents by a standard curve of gallic acid in the range of 7.8–250 µg mL^{-1}.

2.13. DPPH Radical Scavenging Activity

The radical scavenging activity was performed according to the method described by Yang, Guo and Yuan (2008) [26] with modifications. Briefly, 100 µL of methanolic extracts were added to a microplate followed by 100 µL 0.1 mM DPPH soluted in methanol and mixed followed by incubation for 30 min in darkness at room temperature. The absorbance was measured at 517 nm in a microplate reader (Synergy 2 BioTek, Winooski, VT, USA). Triplicate measurements were performed and butylated hydroxytoluene (BHT) was included in the assay as a positive control since a concentration of 0.91 mM of BHT is giving approximately 70% inhibition. A sample blank was made with DPPH but without extract solution (Ab) and a sample control was made without DPPH but with extract/fraction solution (A0). Results are expressed as IC50, i.e., the concentration of extract needed to obtain 50% inhibition. The % DPPH radical scavenging activity was calculated as follows:

$$\text{DPPH radical scavenging activity} = \left(1 - \frac{\text{As} - \text{A0}}{\text{Ab}}\right) \times 100 \quad (3)$$

2.14. Mass Balances and True Retentions

All samples were weighed before and after treatment with one decimal accuracy. Before weighing, the samples were drained by keeping them vertical for 5–10 s. The true retention (TR) of a compound is the proportion of a particular nutrient that remains after processing relative to the original content of that specific nutrient. True retentions were calculated based on the proximate composition before and after processing and were calculated as suggested by [27]:

$$\text{TR} = \frac{\text{g retasined nutrient} \cdot \text{g total product post treatment}}{\text{g original nutrient} \cdot \text{g total product prior treatment}} \quad (4)$$

In cases where a replicate of a specific nutrient concentration was missing due to analytical mistakes, the missing replicate was interpolated from the other analytical replicates by taking an average of the known replicates for that specific treatment.

2.15. Statistical Analysis

The results are given as mean ± standard deviation. The statistical analyses were carried out in the software SPSS Statistics 24 (IBM Corp., Armonk, NY, USA). The test run to define the statistically significant difference between the means of the groups (fresh, freeze-thawed, and blanched material) was a one-way ANOVA with Tukey's post-hoc test. A one-way PERMANOVA was used to test the effect of processing on total phenolic content, and radical scavenging activity (PERMANOVA package in PRIMER+; [28]; type III sum of squares and unrestricted permutation (9999) on raw data; $\alpha = 0.05$) with a posteriori analysis (pairwise test). Means were considered statistically significantly different when levels of $p < 0.05$ were obtained

3. Results and Discussion

3.1. Iodine Content of Sugar Kelp

Fresh Norwegian sugar kelp harvested in April 2018 contained 4605 ± 274 mg iodine·kg^{-1}·dw^{-1}, which is comparable to other European cultivated sugar kelp (3460–6568 mg·kg^{-1}·dw^{-1}) [6,7,29,30]. The process of freeze-thawing sugar kelp did not decrease the iodine content significantly (one-way ANOVA; F = 117, df = 15, $p < 0.001$) (Table 1). However, water blanching decreased the iodine content significantly for all blanching treatments except when treated at 30 °C for 2 s. All blanching treatments, except 30 °C below 120 s and 45 °C at 2 s, sufficiently reduced the iodine content below the maximum level of 2000 mg·kg^{-1}·dw^{-1} as recommended by ANSES (2018) [12] in seaweed products. The iodine content in the blanched sugar kelp approached a constant level for various treatments with an average content of 328 ± 19 mg·kg^{-1}·dw^{-1} (Figure 1). Similarly, Stévant et al., (2018) [7] also reported that a constant level was achieved when subjecting *S. latissima* to warm water at 32 °C for 1 h. The most efficient treatment in this present study reduced the iodine content to 12% relative to the initial iodine content in fresh sugar kelp.

For a better perspective of the iodine content and safe intake of sugar kelp, the recommended intake (RI) and upper intake levels (UL) for adults are used [9–11]. If considering the only dietary source of iodine for an adult was from sugar kelp, then to reach the RI and UL 0.35 g or 1.4 g of fresh non-processed sugar kelp should be consumed, respectively. In the case of blanched sugar kelp then 9.2 or 37 g of sugar kelp could be consumed for RI and UL, respectively. A risk assessment considering other sources of iodine in a daily diet should be taken into consideration when evaluating the potential risk of sugar kelp consumption, but overall, this study proves that a reduction of iodine in sugar kelp can be obtained.

The iodine content reached a constant level at 120 s for the treatments at 45 °C and 60 °C, thus the treatments at those temperatures with a longer processing time (300 s) did not undergo further chemical analysis. The 30 °C treatments and the freeze-thawed treatments were also not further investigated, as the treatments did not reduce the iodine content as satisfactorily as the others.

Table 1. Iodine content in fresh, freeze-thawed and water-blanched *Saccharina latissima* expressed in mg·kg^{-1}·dw^{-1}. Results are mean ± standard deviation (n = 3).

Time	Temperature/Treatment	Iodine (mg·kg^{-1}·dw^{-1})
N/A	Fresh	4605 ± 274 ^{ab}
N/A	Freeze-thawed	4057 ± 419 ^{b}
2 s	30 °C	5157 ± 201 ^{a}
	45 °C	2873 ± 627 ^{c}
	60 °C	1198 ± 146 ^{d}
	80 °C	711 ± 151 ^{de}
30 s	45 °C	667 ± 120 ^{de*}
	60 °C	472 ± 121 ^{de}
	80 °C	343 ± 41 ^{e}
120 s	30 °C	2973 ± 523 ^{c}
	45 °C	346 ± 35 ^{e}
	60 °C	334 ± 55 ^{e}
	80 °C	293 ± 90 ^{e}
300 s	30 °C	1014 ± 349 ^{de}
	45 °C	388 ± 23 ^{de}
	60 °C	321 ± 68 ^{e}

N/A designates not applicable. (*) indicates two replicates (n = 2). Letters (a–e) denote significant differences between treatments by one-way ANOVA and Tukey's post-hoc test.

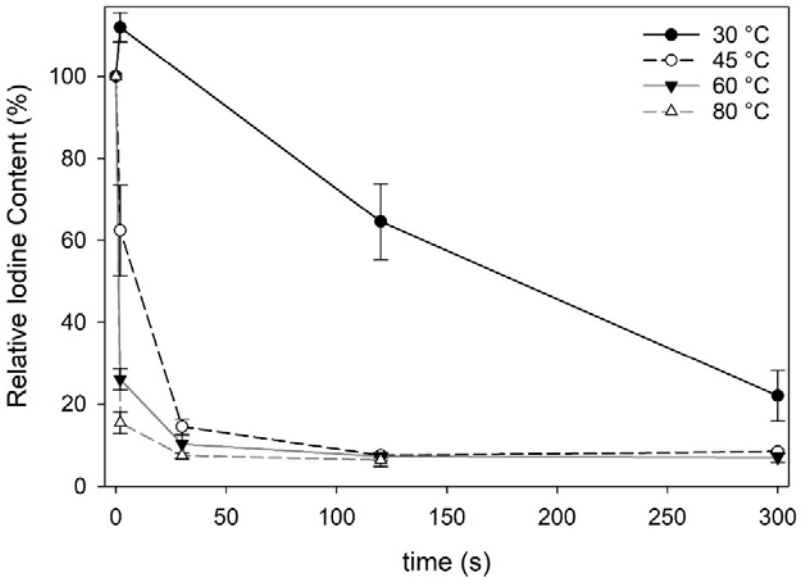

Figure 1. Iodine content in water blanched *Saccharina latissima* relative to fresh *S latissima* expressed in %. Each data point represent the mean iodine content with standard deviations (n = 3).

3.2. Proximate Composition

The proximate composition (n = 3) for the selected treatments can be found in Table 2. Ash content of fresh sugar kelp was 44.51 ± 0.86% dw. The content of ash varied significantly for all water-blanched samples (one-way ANOVA; F = 79, df = 10, $p < 0.001$). The ash content for water-blanched samples was between 9.1 ± 1.6% dw and 26.3 ± 1.5% dw. Protein content in fresh sugar kelp was 7.9 ± 2.5% dw, and in the blanched samples it was 9.8 ± 3.0% dw to 15.3 ± 2.6% dw. No significant differences were found for protein content between any of the samples (one-way ANOVA; F = 2.4, df = 10, $p = 0.064$). The lipid content was 5.8 ± 2.6% dw in fresh *S. latissima* and varied for the blanched samples between 6.9 ± 0.8% dw to 10.2 ± 0.6% dw with no significant differences (one-way ANOVA; F = 1.8, df = 10, $p = 0.132$). Carbohydrates were calculated from the other proximates. As the ash content showed significant difference between treatments, the carbohydrates also showed significant variations (one-way ANOVA; F = 14, df = 10, $p < 0.001$).

Table 2. Proximate composition of fresh and water-blanched *Saccharina latissima.* Data are expressed as means ± SD and represent three process replications (n = 3). Water is given in % ww, whereas ash, protein (total amino acids), fat, and calculated carbohydrates are given in % dw.

Component	Fresh	45 °C			60 °C			80 °C		
		2 s	30 s	120 s	2 s	30 s	120 s	2 s	30 s	120 s
Water	90.68 ± 0.30 ^{a}	93.42 ± 0.77 ^{b}	94.79 ± 0.47 ^{c}	95.70 ± 0.20 ^{c}	94.49 ± 0.46 ^{bc}	95.45 ± 0.20 ^{c}	95.44 ± 0.28 ^{c}	95.23 ± 0.20 ^{c}	95.36 ± 0.03 ^{*c}	95.64 ± 0.14 ^{c}
Ash	44.51 ± 0.86 ^{a}	26.3 ± 1.5 ^{b}	18.4 ± 1.7 ^{cd}	10.8 ± 2.5 ^{ef}	20.5 ± 3.2 ^{bc}	12.3 ± 2.8 ^{def}	9.1 ± 1.6 ^{f}	17.2 ± 1.7 ^{cde}	11.7 ± 1.2 ^{*def}	11.2 ± 1.4 ^{def}
Protein	7.9 ± 2.5 ^{a}	11.8 ± 2.4 ^{a}	10.5 ± 1.4 ^{*a}	12.3 ± 1.0 ^{a}	10.2 ± 3.0^{a}	9.8 ± 3.0 ^{a}	13.6 ± 1.8 ^{a}	12.6 ± 2.3 ^{a}	13.6 ± 2.3 ^{a}	15.3 ± 2.6 ^{a}
Fat	5.8 ± 2.6 ^{a}	6.9 ± 0.8 ^{a}	7.9 **	10.2 ± 0.6 ^{*a}	9.1 ± 1.5^{a}	8.6 ± 4.0 ^{*a}	9.0 ± 1.7 ^{a}	9.7 ± 0.7 ^{*a}	9.1 ± 1.5 ^{a}	8.7 ± 1.3 ^{*a}
Carbohydrates	41.8 ± 4.7 ^{a}	55.0 ± 0.3 ^{abc}	65.2 **	68.7 ± 3.9 ^{cd}	60.1 ± 5.0^{bcd}	65.3 ± 4.6 ^{*cd}	68.3 ± 1.4 ^{cd}	60.2 ± 1.8 ^{*cd}	64.9 ± 0.6 ^{*cd}	63.7 ± 1.4 ^{*cd}

(*) Included only duplicates (n = 2), (**) indicated one replicate (n = 1). Means with different letters (a–f) within each row are significantly different ($p \leq 0.05$).

3.3. Retention of Nutrients

The proximate composition is given for samples after each individual treatment, but does not take the potential loss of biomass into consideration due to processing. From the proximate composition, it cannot be concluded that there is a loss or gain due to treatment, therefore the true retention factors were calculated relative to fresh sugar kelp (Table 3). In Figure 2 the concentrations of each individual proximate and the true retention factors are seen. This together defines the mass balances of the treatments, which can indicate if there is a loss of each individual proximate. The total loss of ash, protein, lipid, and carbohydrate all together (total proximate) for each individual treatment are given relative to the fresh *S. latissima*. Fresh sugar kelp had a total proximate composition of 9.3 g 100 g^{-1} ww and the retained total amount of each treatment varied from 4.3 to 6.6 g 100 g^{-1} ww. The treatment that had the least loss (29%) of proximate was 45 °C at 2 s, whereas the others had a loss that ranged from 41% to 54%.

Table 3. True retention factors post processing relative to the fresh sugar kelp. The retention factors are presented in means ± SD (n = 3).

Component	45 °C			60 °C			80 °C		
	2 s	**30 s**	**120 s**	**2 s**	**30 s**	**120 s**	**2 s**	**30 s**	**120 s**
Water	0.87 ± 0.03 ^{a}	0.99 ± 0.06 ^{a}	0.93 ± 0.13 ^{a}	0.83 ± 0.12 ^{a}	0.83 ± 0.04 ^{a}	0.87 ± 0.03 ^{a}	0.74 ± 0.05 ^{a}	0.81 ± 0.04 ^{a}	0.86 ± 0.13 ^{a}
Ash	0.39 ± 0.07 ^{b}	0.22 ± 0.02 ^{c}	0.09 ± 0.02 ^{d}	0.013 ± 0.005 ^{d}	0.005 ± 0.001 ^{d}	0.004 ± 0.000 ^{d}	0.007 ± 0.001 ^{d}	0.005 ± 0.001 ^{d}	0.004 ± 0.001 ^{d}
Protein	0.89 ± 0.22 ^{ab}	0.69 ± 0.05 ^{ab}	0.63 ± 0.11 ^{ab}	0.65 ± 0.28 ^{ab}	0.48 ± 0.16 ^{b}	0.70 ± 0.11 ^{ab}	0.57 ± 0.07 ^{ab}	0.67 ± 0.11 ^{ab}	0.73 ± 0.09 ^{ab}
Fat	0.73 ± 0.11 ^{a}	0.74 ± 0.06 ^{a}	0.69 ± 0.07 ^{a}	0.79 ± 0.23 ^{a}	0.60 ± 0.26 ^{a}	0.79 ± 0.23 ^{a}	0.60 ± 0.26 ^{a}	0.65 ± 0.14 ^{a}	0.62 ± 0.03 ^{a}
Carbohydrates	0.72 ± 0.12 ^{ab}	0.79 ± 0.10 ^{ab}	0.65 ± 0.08 ^{b}	0.89 ± 0.16 ^{ab}	0.74 ± 0.01 ^{ab}	0.74 ± 0.01 ^{ab}	0.66 ± 0.08 ^{b}	0.71 ± 0.04 ^{ab}	0.69 ± 0.10 ^{ab}

Means with different letters (a–f) within each row are significantly different ($p \leq 0.05$). The factors are relative to the fresh sugar kelp, which had a factor of 1.0 and statistical letter (a). Statistical descriptions: water (one-way ANOVA; F = 3.6, df = 10, p = 0.006), ash (one-way ANOVA; F = 297, df = 10, $p < 0.001$), protein (one-way ANOVA; F = 2.2, df = 10, p = 0.060), fat (one-way ANOVA; F = 1.3, df = 10, p = 0.287), and carbohydrates (one-way ANOVA; F = 2.7, df = 10, p = 0.025).

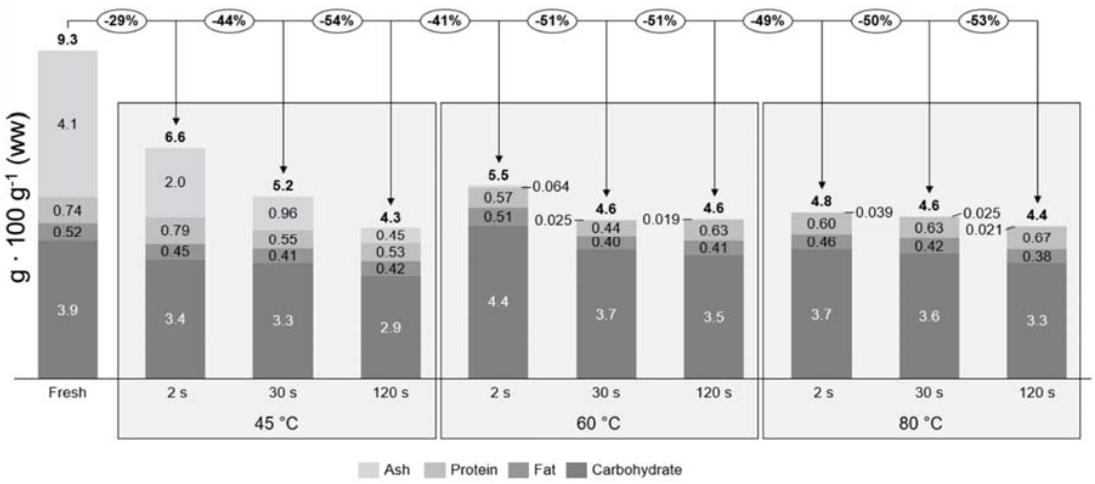

Figure 2. Mass balances for the proximate composition relative to the fresh sugar kelp for each blanching treatment. The percentages in circles describe the total loss of the proximate composition (excluding water). The concentration of each proximate is described by the bar diagram and the bold number above the bars are the total proximate composition relative to wet weight.

To the best of the authors' knowledge, no earlier study has explored the potential loss of proximate composition in *S. latissima*, due to water blanching. Therefore, comparisons to peer-reviewed studies on vegetables were performed. Water blanching (60, 150 and 180 s) of bell peppers, peas, and potatoes led to a significant protein loss of between 8% and 24% [31,32]. In this current study a significant difference in protein was only found for 60 °C at 30 s with a retention of 0.48 ± 0.16 (Tukey's post-hoc test; $p = 0.047$). In all other cases, no significant differences were found due to high standard deviations (Tukey's post-hoc test; $p > 0.154$). The true retention factor of protein was on average 0.67, meaning a total protein loss of 33%, which was higher than that found for vegetables. The high standard deviations are most likely due to the method of treating the product after blanching. The sugar kelp surface consists of mucus and seawater, which was probably interfering with the blanching water during treatment. If the mucus and seawater were washed away during blanching it would be replaced by blanching water on the surface. By shaking the sugar kelp consistently, it was expected that the blanching water would be removed, but some of the blanching water would stay on the product surface and interfere when weighing the samples, creating high standard deviations. The true retention factors and the mass balances indicated a significant loss for the ash content (one-way ANOVA; F = 297, df = 10, $p < 0.001$). *Saccharina latissima* is rich in minerals and trace elements such as Na, K, Mg, and Fe [33]. This significant loss of ash is probably not only due to the loss of iodine, but also other minerals and trace elements. If these minerals are located on the surface of the sugar kelp, they could dissolve into the water when blanched. Moreover, the relatively high processing temperature and low salinity of the blanching water could create a shock to the cells, leading to cell bursts and protein and minerals leaking from the cells.

3.4. Amino Acid Composition

The amino acid composition was quantified for selected water blanching treatments and the fresh sugar kelp sample. Two of the amino acids (glutamic acid and alanine) had a significant loss due to treatments when compared to the fresh sugar kelp (Figure 3). Fresh sugar kelp contained significantly higher amounts of glutamic acid (173 mg·g^{-1} protein; one-way ANOVA; F = 6.4, df = 10, $p < 0.001$) when compared to the treated samples, although not when compared to the 45 °C at 2 and 30 s (Tukey's post-hoc test; $p > 0.395$). This meant that the treatments with higher temperatures and process times had a significant loss of glutamic acid. On average, the significantly different samples had an average content of 146 mg glutamic acid g^{-1} protein. For alanine, the fresh sample was significantly different to the treated samples, meaning that there was a significant loss due to processing (one-way ANOVA; F = 52, df = 10, $p < 0.001$). The content in fresh sugar kelp was 178 mg alanine·g^{-1} protein and the average of the treated samples were 128 mg alanine·g^{-1} protein. The entire amino acid profile can be found in the data repository.

No significant changes were found for the essential amino acids. The essential amino acid to total amino acid ratio (EAA ratio) increased, since there was a significant loss of the non-essential alanine and glutamic acid. Fresh sugar kelp had an EAA ratio of 42.01 ± 0.59% EAA, and this was comparable to studies from Denmark and the Faroe Islands [2,34]. Whereas, the blanched samples on average had a ratio of 48.0 ± 1.2%, and were significantly different from the fresh sample (one-way ANOVA; F = 7.9, df = 10, $p < 0.001$).

The limiting amino acid for all samples was histidine, which is also seen in other studies [2,34]. The amino acid score (not considering digestion) was on average above 100% (108 ± 12%), with no significant differences between neither sample (one-way ANOVA; F = 1.1, df = 10, $p = 0.388$). Summing up, the blanching treatment did not compromise the amino acid quality but actually increased it as two non-essential amino acids had a significant loss.

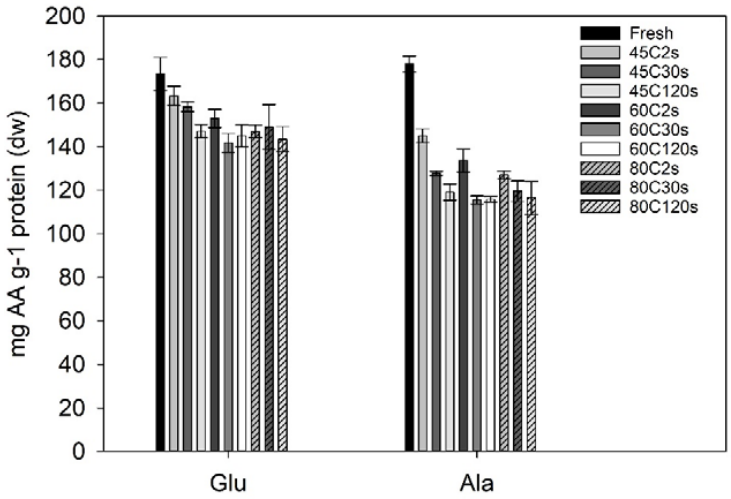

Figure 3. Glutamic acid and alanine in *Saccharina latissima* after different treatments given in mg amino acid (AA) g^{-1} of protein. Error bars represent the standard deviation (n = 3). A one-way ANOVA indicated a significant difference between the fresh sugar kelp sample compared to the treated sugar kelp for both glutamic acid (Glu) and alanine (Ala).

3.5. Fatty Acid Composition

The fatty acid (FA) profile was quantified by direct methylation and given in % FAME for fresh sugar kelp and the samples blanched at 45 °C and 60 °C for 30 s and 300 s, respectively. The complete FA profile (% FAME) can be found in the data repository. The quality of FAs can be explained by the content of the individual fatty acids, which the human body cannot synthesize—α-linolenic acid (ALA) and linoleic acid (LA), but also the two fatty acids docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). Moreover, the total amount of polyunsaturated fatty acids (PUFA) and omega-3 fatty acids (n-3) as well as the ratio of n-3 FA to omega-6 fatty acids (n-6) indicate the quality of the lipid fraction. The 60 °C 300 s blanched samples presented a higher proportion of EPA, ALA, PUFA, and n-3, and a higher n-3/n-6 ratio compared to the fresh and 45 °C 30 s blanched samples (Table 4). The increased proportion results from the reduction of other fatty acids, namely unsaturated and monounsaturated, during the processing. Overall, this results in a biomass with an improved profile of health-beneficial fatty acids. No significant difference was found for LA, while DHA, which was present in the sugar kelp in very low amounts, seem to be degraded during processing.

Table 4. Fatty acid composition of fresh and blanched sugar kelp expressed in % FAME. Data are expressed as means ± SD and represents three process replications (n = 3). The fatty acids are given in % FAME although the ratio (n-3/n-6) is without unit.

Fatty Acids	**Fresh**	**45 °C**	**60 °C**
		30 s	**300 s**
18:2 (n-6) (LA)	4.96 ± 0.12 ^{ab}	5.50 ± 0.23 ^{a}	4.87 ± 0.16 ^{b}
18:3 (n-3) (ALA)	15.2 ± 1.5 ^{a}	18.1 ± 2.1 ^{ab}	22.63 ± 0.45 ^{b}
20:5 (n-3) (EPA)	12.18 ± 0.82 ^{a}	13.2 ± 1.0 ^{a}	17.38 ± 0.16 ^{b}
22:6 (n-3) (DHA)	0.36 ± 0.02 ^{a}	0.15 ± 0.08 ^{b}	0.00 ± 0.00 ^{c}
n-3	29.0 ± 2.4 ^{a}	32.2 ± 3.0 ^{a}	41.20 ± 0.71 ^{b}
n-6	22.51 ± 0.91 ^{a}	25.37 ± 0.61 ^{b}	26.00 ± 0.03 ^{b}
n-3/n-6	1.29 ± 0.08 ^{a}	1.27 ± 0.09 ^{a}	1.59 ± 0.03 ^{b}
PUFA	51.5 ± 3.1 ^{a}	57.6 ± 3.6 ^{a}	67.19 ± 0.67 ^{b}

(a–e) denote significant difference between sample treatments. From the top linoleicacid (LA), α-linolenic acid (ALA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), total omega-3 fatty acids (n-3), total omega-6 fatty acids (n-6), the omega-3 to omega-6 fatty acids ratio (n-3/n-6), and total polyunsaturated fatty acids (PUFA).

3.6. Antioxidant Activity and Total Phenolic Content

Processing had a significant effect on the amount of methanolic extract obtained (one-way PERMANOVA; F = 20.5, df = 3, $p = 0.002$), with significantly higher amounts extracted from the fresh samples compared to the water-blanched samples (7.2–11.1% dw; Figure 4). This demonstrates that blanching sugar kelp will result in a significant amount of compounds being transferred to the water phase or degraded during processing.

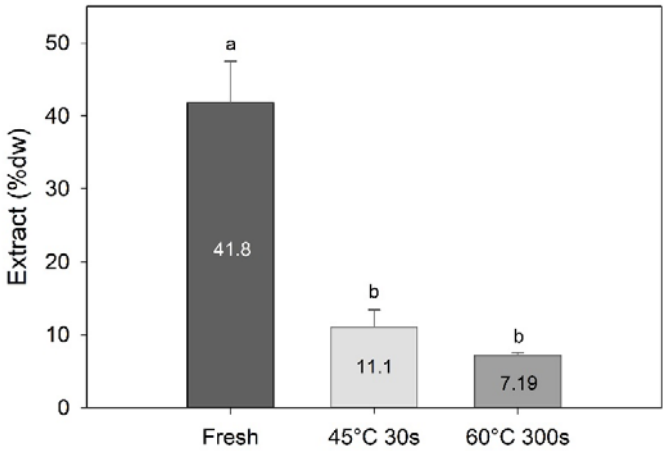

Figure 4. Amount of methanolic extracts (% dw) of sugar kelp for fresh and two different blanching treatments. Different letters represent a significant difference ($p < 0.05$) between treatments. Data are mean ± SD; $n = 3$.

Blanching had a significant effect on total phenolic content (TPC) (one-way PERMANOVA; F = 26.0, df = 3, $p = 0.0011$; Figure 5). TPC was higher in the 60 °C 300 s treated sample ($p = 0.013$) compared to the fresh sample. On the other hand, there was no significant difference in TPC between fresh and 45 °C 30 s blanched samples ($p = 0.07$). TPC found in the present study for fresh is within the range of the values reported for *S. latissima* harvested at different seasons (0.84–2.41 mg·GAE/g sugar kelp [5]). On the other hand, TPC values obtained for the blanched samples are above those reported in the same study.

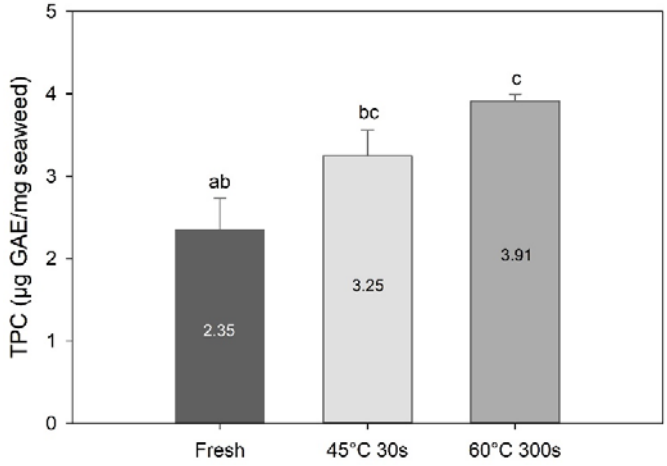

Figure 5. Total phenolic content of fresh and selected blanched samples of *Saccharina latissima* expressed in gallic acid equivalents per mg of freeze-dried samples. Data are mean ± SD; $n = 3$. Different letters represent a significant difference between treatments ($p < 0.05$).

TPC results expressed in gallic acid equivalents per mg of extract revealed an even greater effect of processing on the TPC. Blanching significantly increased the content of TPC as compared to the fresh

sugar kelp (one-way PERMANOVA; F = 392, df = 3, $p < 0.01$, Figure 6). The highest TPC was found in the 60 °C 300 s blanched samples ($p < 0.013$), followed by the 45 °C 30 s blanched samples, and then fresh samples ($p = 0.96$). These results suggest that the extraction of other compounds during water blanching may have resulted in concentration of phenolic compounds in the processed sugar kelp.

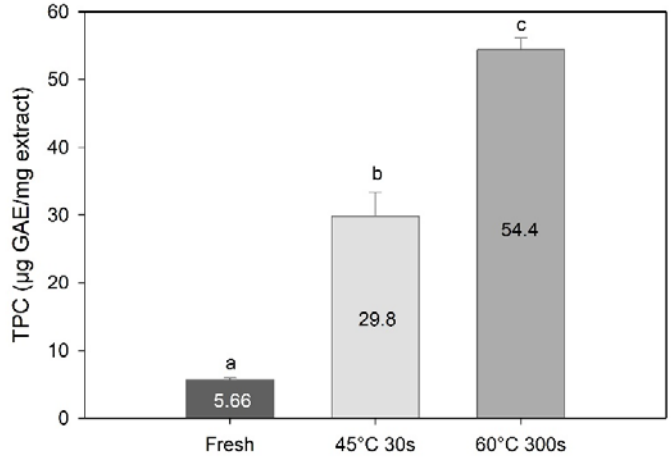

Figure 6. Total phenolic content expressed in gallic acid equivalents per mg of methanolic extract of *Saccharina latissima* from fresh and two types of blanching. Data are mean ± SD; $n = 3$. Different letters represent a significant difference ($p < 0.05$) between treatments.

DPPH radical scavenging activity revealed a concentration dependency and increased with increasing concentrations of algal extract (data not shown). Processing increased the radical scavenging activity significantly (F = 13.5, df = 3, $p = 0.0053$, Figure 7). These results suggest that compounds with high radical scavenging activity are retained and up-concentrated in the sugar kelp during water blanching. This correlates well with the current results; TPC have been identified as a major component contributing to radical scavenging activity of seaweed [5,35–38].

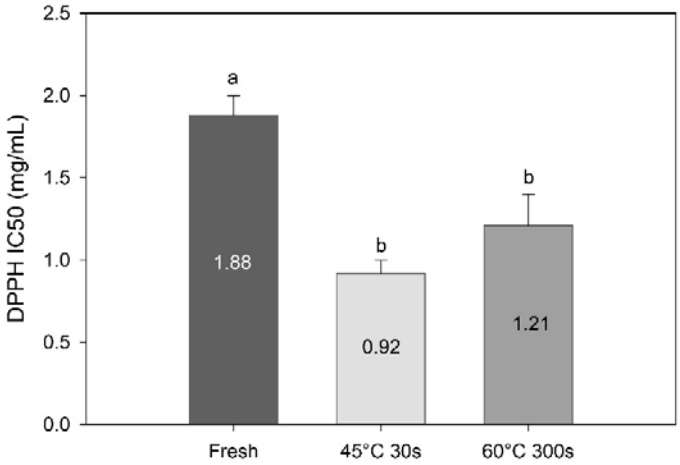

Figure 7. DPPH radical scavenging activity (IC50; mg/mL) of methanolic extracts of fresh and blanched *Saccharina latissima*. Different letters represent a significant difference ($p < 0.05$) between treatments. Data are mean ± SD; $n = 3$.

4. Conclusions

This study showed that water blanching is a promising approach for reducing the iodine content in Norwegian-cultivated *Saccharina latissima*. Up to 88% reduction was obtained by blanching at optimized conditions (≥45 °C and ≥30 s. Considering the recommended intake and upper intake level reported by the Nordic Nutrition Recommendations (2012)). If sugar kelp was the only source of

dietary iodine, a maximum 9.2 g or 37 g, respectively of blanched sugar kelp can be consumed daily to avoid exceeding these recommendations. However, freeze-thawing did not decrease the iodine content of sugar kelp. These are important findings for the food-producing industry that is using seaweed as a raw material and is responsible for consumer safety. In terms of processing effects on other nutritionally valuable compounds, the treatment that had the least loss (29%) of total proximate composition was 45 °C at 2 s, whereas the other treatments had a loss that ranged from 41% to 54%. More specifically, a significant loss of ash occurred, which is comparable with the degree of loss of iodine together with other minerals. Water blanching also caused a significant loss of two amino acids (glutamic acid and alanine), which led to a higher EAA/AA ratio. Moreover, water blanching resulted in biomass with an improved composition of health beneficial compounds, namely PUFA and phenolic compounds, and antioxidant activity.

In perspective, other valuable compounds with antioxidant activity found in sugar kelp such as the pigment fucoxanthin and carbohydrates could have been interesting to study. Moreover, the change in texture, color, and taste (e.g., umami) due to blanching is also interesting and worth further study.

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Paper 3

Blanching of two commercial Norwegian brown seaweeds – for reduction of iodine and other compounds of importance for food safety and quality

CB Wirenfeldt, MS Sletta, M Sæther, Ø Arlov, IM Aasen, H Sletta, PL Švarc, SL Holdt, SS Jacobsen, FL Aachmann & T Rustad, *In preparation for Food Chemistry*

Blanching of two commercial Norwegian brown seaweeds – for reduction of iodine and other compounds of importance for food safety and quality

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Highlights

1. Blanching of *A. esculenta* and *S. latissima* reduced the iodine contents by 80-95%.
2. Blanching at 80 °C gave the highest loss of iodine.
3. Reuse of blanching water impacted iodine reduction in *S. latissima*.
4. Blanching in seawater reduced iodine but retained minerals and fucoidan.
5. Free amino acids, vitamin C and folate were compromised due to 80 °C blanching.

Abstract

The two commercially relevant kelp species, *Alaria esculenta* and *Saccharina latissima,* are both accumulators of iodine to an extent that can pose as a health concern if consumed. Water blanching is used industrially to reduce the iodine content. This study aimed to optimize the blanching conditions to reduce energy consumption and environmental impact by investigating temperature, duration, blanching media, ratio, and recycling of water. Also, to understand if other compounds were lost during blanching. This study proved that the iodine content could be reduced by any of the blanching treatments to 85-95% (*S. latissima*) and 80-92% (*A. esculenta*) of the initial content. Seawater blanching retained more valuable compounds, such as fucoidan and magnesium, while effectively removing iodine. However, free amino acids, vitamin C and folate were lost regardless of blanching treatment. These findings are relevant for industrial scale up and to understand the compromises that occur when reducing iodine by blanching.

Keywords

Iodine ; Arsenic ; Cadmium ; *Saccharina latissima* ; *Alaria esculenta* ; Vitamin ; Nutrient retention ; Chemical composition ; Laminariales

1 Introduction

The cultivation of seaweed and their use as foods have increased in the last decade in several European countries. The subtidal cold-water brown kelps *Alaria esculenta* (winged kelp) and *Saccharina latissima* (sugar kelp) are currently cultivated along the Norwegian coast. The long coastline of Norway with cold and clean waters is ideal for kelp cultivation. Over the last ten years, interest in kelp cultivation in Norway has grown, as evidenced by increased research activity, number of cultivation sites, companies involved, and production output (Stévant, 2019). In 2021, the number of cultivation licenses was 520, compared to 54 in 2014 (Directory of Fisheries, 2022).

Kelp is the most effective accumulator of iodine of any living species (Küpper et al., 2008). Iodine is an essential trace element, and the recommended intake for adults is 150 µg day^{-1} (FAO/WHO Expert Consultation, 2001) with a European specified upper intake level of 600 µg day^{-1} (EFSA, 2018). However, the high iodine content in kelp may pose a risk of overconsumption for the consumers when included in the diet (Banach et al., 2020).

Sugar kelp has the highest iodine content of the two kelps in this study, ranging from 3,124 to 6,568 mg $(kg\ dw)^{-1}$ (Kreissig et al., 2021; Nielsen et al., 2020; Stévant, Marfaing, et al., 2018), whereas winged kelp has concentrations from 213 to 670 mg $(kg\ dw)^{-1}$ (Kreissig et al., 2021; Nitschke & Stengel, 2016; Stévant, Marfaing, et al., 2018). The iodine content of kelp varies depending on the species and the external iodine concentration of the seawater and environmental stressors (Küpper et al., 1998). By consuming just 2 g of fresh, non-processed sugar kelp, the consumer would exceed the upper intake level (Nielsen et al., 2020).

Other hazards from the consumption of kelp are the potentially toxic elements such as arsenic and cadmium (Banach et al., 2020). The levels of these compounds generally correlate to their abundance in the seawater. Brown algae take up inorganic arsenic, presumably because it resembles the phosphate ion, and converts it to organic compounds, which are predominantly arsenosugars (EFSA, 2009). Total arsenic is the most commonly reported form of arsenic, but only the inorganic forms are considered toxic. Kelp species have demonstrated to have a low inorganic arsenic to total arsenic ratio (Kim et al., 2020). Cadmium and other divalent cationic

potential toxic elements (Pb^{2+}, Cu^{2+}, Ba^{2+}) can accumulate in the biomass by binding to alginate through replacement of calcium in cross-linking junction zones (Haug & Smidsrød, 1965).

Blanching is a common vegetable processing method that is usually used prior to freezing, drying or canning to increase product quality by inactivating endogenous enzymes, decreasing microbial load, and removing toxic constituents (Hamid et al., 2020; Xiao et al., 2017). In traditional hot water blanching, the product is submerged into 70 to 100 °C water for several minutes, followed by draining and cooling. Blanching is well known to cause nutrient loss from the product, particularly for water-soluble nutrients such as carbohydrates, proteins, minerals, and vitamins that are not bound in macromolecular or cellular structures. (Xiao et al., 2017).

To the best of the authors' knowledge, no studies on blanching of fresh winged kelp have been published to date. However, (Nitschke & Stengel, 2016) showed that rehydrating and boiling in deionized water for 20 minutes reduced the iodine content of winged kelp from 670 to 165 mg $(kg\ dw)^{-1}$. Several studies have shown that blanching or boiling for 1-15 minutes is effective at reducing the iodine content of sugar kelp (Blikra et al., 2022; Bruhn et al., 2019; Nielsen et al., 2020; Nitschke & Stengel, 2016). However, further research on the blanching conditions to remove more iodine from the kelp or applying other more sustainable methods, such as using lower temperature, shorter blanching duration, reusing water and using seawater as a blanching media is needed. For the health of the consumers, iodine reduction to levels even lower than what has already been accomplished, would be beneficial.

The aim of this study was to optimize the blanching conditions of the two commercial kelp species; sugar kelp and winged kelp with the main objective to explore different conditions to maximize iodine reduction in the kelp. Conditions tested were blanching temperature and duration, fresh or seawater, seaweed to water ratio, water re-use, and cut or whole seaweed. Specifically, it was expected that the blanching procedure on sugar kelp here chosen as standard conditions would reduce the iodine by 80-90% as shown in previous studies (Nielsen et al., 2020). For winged kelp, with the lower iodine content, our question was whether iodine would be reduced in the same relative ratio as for sugar kelp, or if the resulting content in sugar kelp (200-300 mg

(kg dw)$^{-1}$) is a minimum level. Moreover, to save costs, it would be important to assess whether the same iodine reduction as in fresh water could be obtained, despite the higher ion strength in seawater. Cutting the kelp prior to blanching could be expected to decrease the iodine even more than the standard conditions. However, increasing the seaweed to water ratio, lowering the temperature or the blanching duration, as well as reusing blanching water would lead to a less efficient blanching in terms of iodine reduction when compared to the standard blanching.

Other objectives were to understand the effect of blanching on other potential health hazards such as potential toxic elements, microbial load, and on the nutrient composition (lipids, total and free amino acids, monosaccharides, vitamin C and folate). The results from this work lead to recommendations in the conclusion that will assist kelp processors in understanding the best ways to reduce the iodine level of their product and ensuring quality during a conventional industrial blanching technique.

2 Materials and Methods

2.1 Raw material

Commercial cultivated winged kelp and sugar kelp were harvested from the sea farm of Seaweed Solutions AS outside Frøya, Norway (N63° 42.279' E8° 52.232') in May 2020 with a salinity of 33-34 ppt. After harvest the seaweed were stored in tanks in membrane filtered, UV-treated seawater (8 °C). The experiments were conducted on the same harvest batch over a span of two to three days, starting on the day of harvest. Stipes and holdfast were kept. The seaweeds had no visible fouling organisms. Winged kelp varied in length from 15 cm to 150 cm with an average of 67 cm (n = 10). For sugar kelp it varied from 10 cm to 100 cm with an average of 52 cm (n = 10). Water used for the experiments was either fresh water (FW) or filtered, UV-treated seawater (SW).

2.2 Blanching methods

A complete overview of the experimental design is given in Table 1. The blanching was carried out in a water bath (20 L). Standard conditions were selected based on the data from preliminary experiments and set as a reference for these experiments.

The variables were whole seaweed heat treated at 80 ˚C for 120 s, using fresh water for both blanching and cooling (FW, FW), at a ratio of 50 g wet seaweed per liter of water (50 g/L) without reusing the water (Rep1).

In each of the experiments (Exp.), one or more of the standard variables were replaced to evaluate its effect. Exp. 1 explored temperatures (45 and 80 ˚C) and durations (30 and 120 s). Exp. 2 investigated the ratio of seaweed to water, while in Exp. 3, the seaweed was cut mechanically into pieces of 1-10 cm in width/length prior to blanching. Exp. 3 was only performed for sugar kelp. In Exp. 4 the blanching and cooling liquid varied between fresh water (FW) and membrane filtered, UV-treated seawater (SW). The final experiment, Exp. 5, examined the effect of blanching multiple times in the same water, thus reusing the water.

Table 1: Summary of the different blanching conditions and the abbreviations of the experiments.

Set-up	Abbreviation for condition	Seaweed to water ratio (g ww) L^{-1}	Blade	Blanching liquid, cooling liquid	Temperature (˚C), duration (s)	Rep*
Standard	Standard	50	whole	FW, FW	80, 120	1
Exp. 1	45 ˚C, 30 s 45 ˚C, 120 s 80 ˚C, 30 s	50	whole	FW, FW	**45, 30** **45, 120** **80, 30**	1
Exp. 2	500 g/L	**500**	whole	FW, FW	80, 120	1
Exp. 3	Cut	50	**cut**	FW, FW	80, 120	1
Exp. 4	FW, SW SW, SW SW, FW	50	whole	**FW, SW** **SW, SW** **SW, FW**	80, 120	1
Exp. 5	Rep X**	50	whole	FW, FW	80, 120	**10**

Conditions deviating from Standard are in **bold**. The experiments 500 g/L and Rep X were performed in duplicates (n = 2) for winged kelp, all other experiments were performed in triplicates (n = 3). *Number of repetitions of blanching in the same water. **X denotes number of times the blanching water has been reused (Rep10 equals 10 sequential blanching).

The blanching was carried out as follows: Fresh seaweed stored in filtered seawater were collected and put on a grate to drip for one minute. The seaweed was moved around while dripping to ensure as much excess water as possible was removed. After dripping, 500 ± 10 g (2,500 g in Exp. 2) of seaweed were weighed for

blanching. The seaweed was dipped in the water bath containing 10 L (5 L in Exp. 2) pre-heated water and blanched for 30 or 120 s according to the experimental setup. After blanching, the seaweed was taken from the water bath and directly transferred into a cooling bath. After one minute of cooling, the dripping procedure was repeated, and the blanched seaweed was weighed. The water bath was rinsed between each blanching. A thermometer was used to ensure correct blanching water temperature.

2.3 Chemical analysis

2.3.1. Sample preparation

The frozen seaweed samples collected for chemical analyses (except vitamin analyses) were dried in a freeze dryer (Alpha 1-4 LD plus, Christ, Osterode am Harz, Germany) at -60 ˚C and 0.1 mbar vacuum, before being coarsely ground using a food processor. The samples for mineral analyses were further milled (MM 400, Retsch, Haan, Germany, Settings: 30 Hz for 40 s). For vitamin analysis the frozen seaweed at -80 ˚C was homogenized by a coffee grinder (Rommelsbacher EGK 200) using liquid nitrogen to keep it frozen.

2.3.2 Dry matter and ash content

The dry matter and ash content were determined gravimetrically on fresh and blanched seaweed (n = 2) according to (AOAC Method 950.46 Official methods of analysis, 1990).

2.3.3 Iodine, inorganic arsenic and other minerals and trace elements

The minerals and trace elements analyzed were: sodium (Na), magnesium (Mg), phosphorus (P), sulfur (S), potassium (K), calcium (Ca), iron (Fe), cobalt (Co), zinc (Zn), arsenic (As), selenium (Se), cadmium (Cd), barium (Ba), chlorine (Cl), bromine (Br), inorganic arsenic (iAs) and iodine (I). For Cl, Br and I analyses, samples were prepared for ICP-MS in accordance with NS-EN 15111_2007 by making a solution with tetramethyl-ammonium-hydroxide (TMAH) and placing it in a heated bath (70 °C) over night. For the remaining elements, sample preparation for ICP-MS was done in accordance with NS-EN 15763_2009 making a solution with HNO_3 and placing it in an UltraWAVE microwave oven (Milestone S.r.l., Sorisole, Italy) at 250 °C for 10 min. The prepared samples were analyzed on an 8800 Triple

Quadropole ICP-MS (ICPQQQ) with SPS 4 auto sampler (Agilent Technologies, Santa Clara, USA). Samples were quantified by use of standards from Inorganic Ventures (JRC Plankton BCR-414) and with 115In as internal standard. Inorganic arsenic was quantified in a selection of the samples based on methodology published by the National Institute of Standards and Technology (Yu et al., 2006), using KI/I_2 to oxidize all inorganic As to As (V).

2.3.4 Lipid content

The gravimetric method described by (Bligh & Dyer, 1959) was used to quantify total lipid content. For lipid extraction, 0.5 g sample was homogenized with a mixture of distilled water, methanol and chloroform in the ratio 13.5:15:15 mL. To separate the mixture into phases, it was centrifuged at 13,200 g for 10 min. Further, 5 ml chloroform phase was transferred to a pre-weighed glass tube and evaporated on a heating block (60 ˚C) with supply of N_2-gas. Tubes were cooled in a desiccator and weighed. Total lipid content was calculated using the following equation:

$$Total\ lipid(\%) = \frac{m_{lipid} \cdot c \cdot 100}{c_v \cdot m_{initial}} \qquad \text{Equation 1}$$

Where m_{lipid} is the weight of the lipid after evaporation (g), c is the added chloroform (mL), c_v is the evaporated chloroform (mL), and $m_{initial}$ is the initial weight of the sample (g).

2.3.5 Free and total amino acids

Protein extracts were prepared as described by (Stévant, Indergård, et al., 2018); agitating 50 mg dried sample in 5 mL distilled water for one hour, followed by centrifugation (4 ˚C, 10,000 g, 20 min) (n = 2). Protein precipitation was performed as described by (Osnes & Mohr, 1985). The quantification process is described by (Nielsen et al., 2020).

The total amino acids (excluding tryptophan) extraction and quantification followed the exact procedure with the same equipment as described by (Nielsen et al., 2020) (n = 2). The chromatographic peaks of glycine and arginine merge in one, therefore an average of their molar masses was used for calculation. The total protein content was calculated by summing the amino acids and then subtracting the water (18 g H_2O (mol amino acid)$^{-1}$), which was incorporated during acid hydrolysis (FAO, 2003).

2.3.6 Monosaccharides

Freeze-dried blanching water and kelp were milled (3 mm stainless steel balls, Retsch 400 MM, 45 min, 30 Hz) and desiccated overnight. 10-50 mg sample was hydrolyzed with sulfuric acid (12 M, 0.5 mL) at 30 °C for 60 min, diluted to 2 M sulfuric acid with ion free water and hydrolyzed at 100 °C for 4 hours. Then 6 mL of ion free water was added, and the samples were centrifuged. Sulphate was precipitated by adding 850 µL 0.15 M $Ba(OH)_2$ to an aliquot (180 µL) of the supernatant. After centrifugation, the sample was diluted 10 times with ion free water.

High-performance anion-exchange chromatography with pulsed amperometric detection (HPAE-PAD) was performed on a Dionex ICS 5000+ (Thermo Scientific) with a 4x250 mm CarboPac SA10 main column and 4x50 mm SA10 guard. 25 µL sample was injected and eluted by a gradient with flow rate 1.2 mL min^{-1} at 28 °C. The gradient consisted of the mobile phases: 0-10 min 1 mM NaOH, 10-17 min 40 mM NaOH and 400 mM NaOAc, 15-25 min 100 mM NaOH. Post column addition of 0.4 M NaOH, 0.3 mL min^{-1} from a LC-20Ai pump was used to give a concentration of 80 mM NaOH during detection.

Mannitol, fucose, arabinose, galactose, rhamnose, glucose, xylose, and mannose standards were used for calibration curves (0.1-12.5 mg L^{-1}). Factors correcting for the degradation of released monosaccharides during hydrolysis were previously determined as the ratio of peak areas for 5 mg L^{-1} standard before and after hydrolysis and can be found in Appendix B. For calculation to dry weight the monosaccharides were corrected for the addition of water when glycosidic linkages were hydrolyzed.

2.3.7 Water-soluble vitamins (Vitamin C and folate)

Vitamin C was quantified by the HPLC-UV method previously described by (Wirenfeldt et al., 2022).

Folate was analyzed by LC-MS/MS method using a single-enzyme extraction step as described by (Ložnjak Švarc et al., 2020). Folate vitamers (tetrahydrofolate, 5-methyltetrahydrofolate, formyl forms and folic acid) were determined and expressed

as folic acid equivalents, and their sum was reported as total folate content (Ložnjak Švarc et al., 2020).

2.4 Microbial counts

Total aerobic viable count was determined as colony forming units (CFU) by the (NMKL Method 184, 2006) on Compact Dry TC plates (Labolytic, Trondheim, Norway). Briefly, 5 g of seaweed (n = 1) and 45 g of peptone saline (1 g peptone and 8.5 g NaCl in 1 L deionized water, autoclaved) were mixed for one minute in a stomacher bag. One mL from either the stomacher bag or a tenfold dilution series was inoculated on the Compact Dry TC and incubated at room temperature for 3-4 days and colony forming units (CFU) were counted and reported as log CFU per g wet seaweed.

2.4 Data presentation and statistics

All data is presented as mean ± standard deviation unless otherwise stated. One way Analysis of Variance (ANOVA) was conducted in the software R Studio with the treatments as factors (R-Core-Team, 2022). Homogeneity of variance across groups was confirmed by Levene's test. The pair-wise comparison information was acquired using a Tukey Honest Significant Difference (HSD). Pearson correlation was performed to find correlation between minerals and trace elements. Correlation was assumed when above 0.75. Principal Components Analysis (PCA) was performed on a standardized and scaled data matrix for minerals and trace elements.

3 Results and discussion

3.1 Iodine reduction, blanching water recycling, and allowable seaweed consumption levels

Currently, no regulations exist on the maximum iodine content allowed in food. Only the French Food Safety Agency has recommended a maximum content of 2,000 mg iodine kg^{-1} (AFSSA, 2009). The initial iodine content in fresh, unprocessed seaweed was 4,818 ± 331 and 682 ± 95 mg $(kg\ dw)^{-1}$ in sugar kelp and winged kelp, respectively, meaning that the level in sugar kelp was more than twice the allowable content.

By using any of the blanching conditions explained in Table 1, the iodine was reduced to levels of 228-741 for sugar kelp and 52.1-134 mg (kg dw)$^{-1}$ for winged kelp. Thus, the contents were reduced by 85-95% and 80-92% for sugar kelp and winged kelp, respectively. The different blanching conditions led to significantly different levels of iodine, as illustrated in Figure 1 (ANOVA; $p > 0.001$, $F = 36.4$). These are all below the AFFSA recommendations.

Figure 1 shows that the maximum iodine reduction was achieved by treatments at 80 °C. For both species, all the 80 °C treatments reached similar levels of iodine content after blanching, except for sugar kelp blanched by reusing the water (Rep 5 and 10) and the increased seaweed to water ratio (500 g L^{-1}). Nielsen et al., (2020) showed that blanching at 80 °C reduced iodine to the same levels as in this study, but that blanching duration did not have an influence at this temperature. This was further supported by the current study. The results also showed that using membrane filtered, UV-treated seawater (SW, FW and SW, SW) or cutting the sugar kelp (Cut) had the same effect on iodine reduction as the standard conditions. Using seawater for blanching instead of fresh water has great potential, as it is easily accessible, has a lower cost, and is more environmentally sustainable for companies at the shore or working offshore.

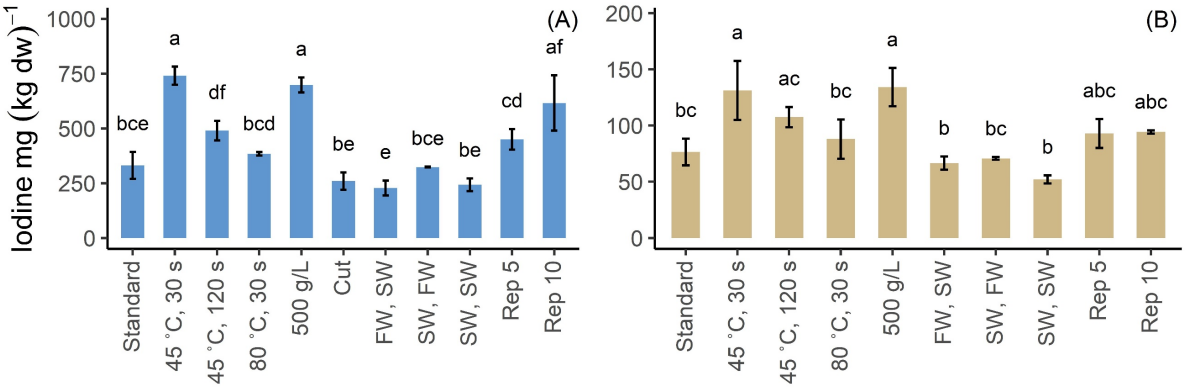

Figure 1: Iodine levels (mg (kg dw)$^{-1}$) in sugar kelp (A) and winged kelp (B) after blanching by different blanching conditions. Means are presented by the bars, and standard deviation by error bars. Significant difference (ANOVA; $p < 0.05$) is illustrated by lower-case letters (a-e) within each sub-graph.

Increasing the seaweed to water ratio from 50 to 500 g L^{-1} resulted in less effective iodine reduction for both species. In addition, water temperature dropped about 20 °C when adding this larger proportion of seaweed, which probably reduced the blanching efficiency as the kelp took longer to reach the target temperature. Another

explanation to this lower iodine efflux with increased seaweed to water ratio could also be an iodine saturation in the water because equilibrium between water and kelp is reached when adding a high portion of kelp. This is linked to the reuse of water for treatment, which is discussed later. Therefore, it is important for the industry to consider the seaweed to water ratio in their processing plants as well as a possible temperature drop when adding the kelp to the water.

Reusing blanching water can reduce costs, water consumption, and carbon emissions. It was possible to reuse the water at least 10 times for winged kelp at the standard conditions without a negative impact on iodine reduction in the biomass. However, the blanching effect was substantially affected by the repetitions for sugar kelp with the concentration of 50 g kelp to 1 L blanching water. This means that the industry should consider a lower reduction of iodine in their sugar kelp if reusing the blanching water. The different iodine reduction seen between the two species when reusing blanching water could contribute to the suggestion that the iodine efflux is depending on the concentration in the blanching water. It seems that the iodine equilibrium between kelp and water is reached when repeating the blanching of sugar kelp 10 times, since the iodine content does not reach the same level as after one blanching (Figure 1).

By modelling with an assumption of linearity, it was possible to predict when the blanching water in standard conditions should be changed in the blanching system. We established a threshold, based on EFSA regulations on upper intake levels (EFSA, 2018) that 600 µg iodine g^{-1} dry kelp was the maximum acceptable level in the final blanched products. The prediction was that in 1 m^3 of fresh water 0.479 tons of wet sugar kelp or 12.9 tons of winged kelp can be blanched under the standard conditions, giving the last portion blanched a final iodine concentration of 600 µg iodine kg^{-1} dry kelp. However, the iodine efflux might not follow a linear trend, and it is recommended to study this further.

Using the same threshold as above, a daily maximum consumption level for sugar kelp and winged kelp was calculated. To not exceed the iodine recommendation of 600 µg day^{-1} by consuming kelp, a maximum of 0.125 g dry (1.44 g ww) sugar kelp or 0.879 g dry (8.02 g ww) winged kelp can be consumed daily of the fresh, unprocessed kelp. Average consumption maximum for the blanched samples varied

337 from 0.810-2.63 g dry (9.29-48.3 g ww) for sugar kelp and 4.47-11.5 g dry (51.1-109
338 g ww) for winged kelp. The calculated consumption possibilities can be found in
339 Appendix C for each blanching process.

340 **3.2 Other minerals and trace elements released due to blanching**

341 Besides iodine (I), fifteen other minerals and trace elements were analyzed. This
342 was to assess their correlation during blanching and the possible effect of the
343 blanching conditions on their presence in the two kelp species.

344 The bi-plots in Figure 2 illustrate that for both species, the fresh kelp varied from the
345 blanched kelp. Potassium (K) and I were the only two elements that were reduced by
346 all the blanching treatments, indicating that the major parts are not bound to
347 insoluble biomass components, and that there was no influx of these ions from
348 seawater, when seawater was used for cooling. There was observed a positive linear
349 correlation between K and I for both species, with a Pearson's correlation coefficient
350 higher than 0.9 (Appendix D). Usually, 80-90% of iodine in kelp is mainly stored as
351 the inorganic form iodide (I^-) (Blikra et al., 2022). This correlation is most likely
352 because the anion iodide needs a positive counter cation like K^+ to efflux
353 simultaneously, and K is the dominating cation in the biomass, constituting 14% of
354 the dry weight and 29% of the ash for sugar kelp.

355 Most treatments, not considering "SW, SW" and "FW, SW", which will be discussed
356 later, also had a reduction of arsenic (As), bromine (Br), chlorine (Cl), magnesium
357 (Mg), sodium (Na), phosphorus (P), and sulfur (S). The concentrations of minerals
358 and trace elements for each treatment can be found in Appendix A. Pearson's
359 correlation (Appendix D) showed that cadmium (Cd) and iron (Fe) did not correlate
360 with any other minerals or trace elements in both species. This is also partly
361 illustrated in the bi-plots (Figure 2), as the loadings (arrows) of these specific
362 minerals are not in the same dimensions as the others.

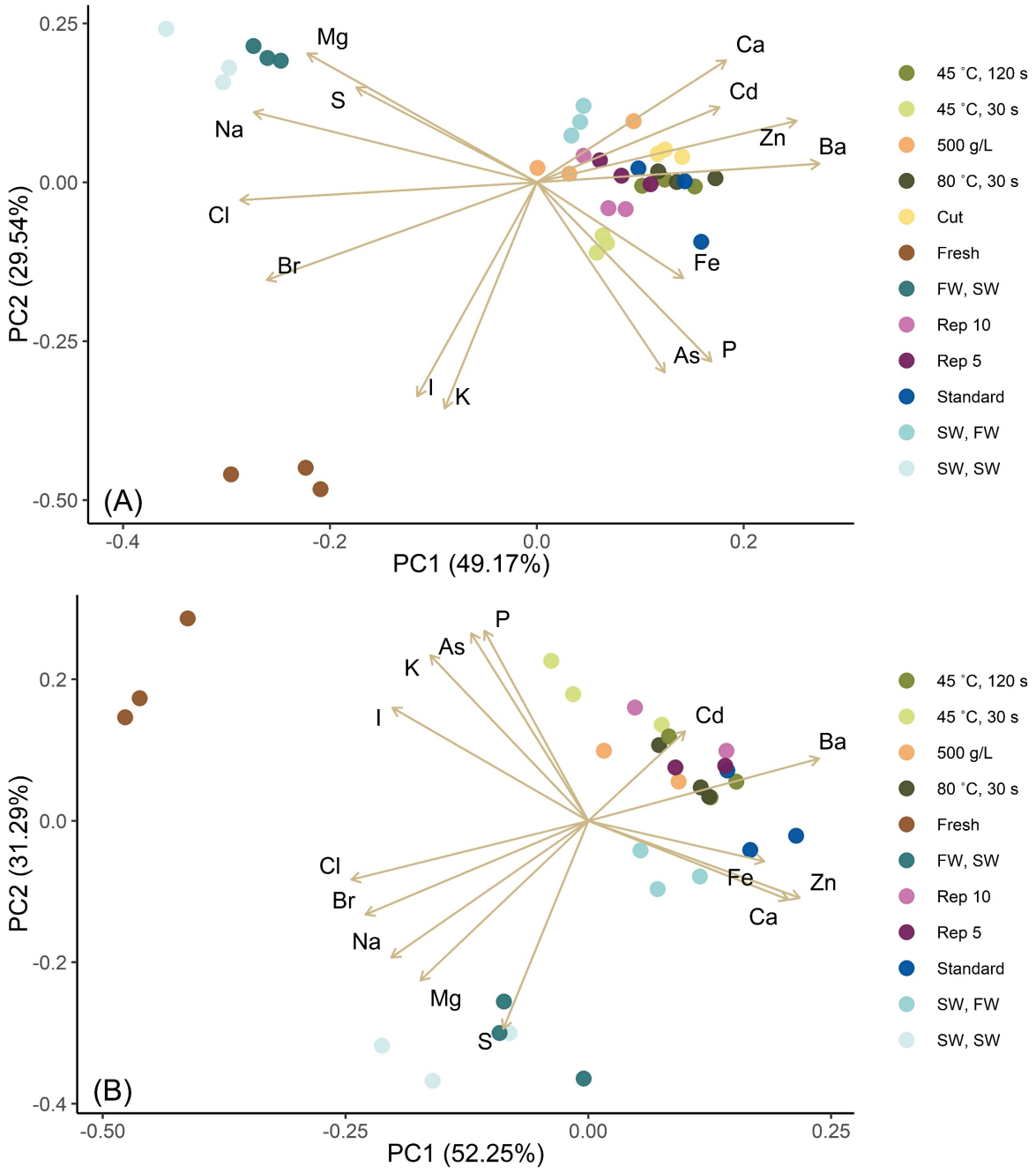

Figure 2: Bi-plot with PCA scores and loadings for minerals and trace element composition of sugar kelp (A) and winged kelp (B) blanched by different blanching conditions (colors). Fresh kelps differ from any of the blanched kelps. FW, SW and SW, SW also differ from the other blanched kelps. Each data point indicates one treatment replicate, with a total of n = 3 replicates for each treatment type, but for (B) 500 g/L, Rep 10, and Rep 5 only n = 2 treatment replicates. A total of 78.71% (A) and 83.54% (B) of the variance is explained by PC1 and PC2.

Interestingly, we also see for both species that the treatments “SW, SW” and “FW, SW” have a different mineral and trace metals profile than the other treatments. It is noteworthy that the two halogens Cl and Br are retained in the kelp with “SW, SW” and “FW, SW” blanching, when these two methods are in fact some of the most

efficient to reduce the other halogen, iodine (Figure 1). The data points of these two treatments ("SW, SW" and "FW, SW") followed the loadings of Br, Cl, Mg, Na, and S. Seawater contains different iodine, where Cl^- and Na^+ dominates, but SO_4^{2-}, Br^-, Mg^{2+}, Ca^{2+}, and K^+ are also present (Wright & Colling, 1995). These ions probably efflux from the kelp cells when cooling down in fresh water "SW, FW", but flow in again to reach equilibrium when cooling in seawater "FW, SW".

The two treatments also acted differently when investigating the ash content. It showed a considerable reduction from the fresh kelp (sugar kelp: 50.1±5.3% dw; winged kelp: 40.8±2.1% dw) to most of the blanched kelp (sugar kelp: 11.5-17.8% dw; winged kelp: 12.9-18.7% dw). The kelp cooled in seawater ("SW, SW" and "FW, SW") had an ash content lower than the fresh kelp, but higher than the ones cooled in tap water (sugar kelp: 39.9-41.9% dw; winged kelp: 28.1-31.8% dw).

The retention of the inorganic ions in kelp where seawater was used for cooling can be explained by the different concentration gradients. When alive, the kelp cells accumulate ions (e.g. halogens) or osmoprotectants (e.g. mannitol and free amino acids) to achieve equilibrium (Stiger-Pouvreau et al., 2016). Tap water contains low amounts of ions and molecules, blanching with tap water led to a concentration difference, resulting in the release of salt and small organic molecules into the blanching water. Since seawater contains high concentrations of inorganic ions (Wright & Colling, 1995) the concentration gradient was lower, and less ions were lost. The dry matter analysis showed that a significant reduction occurred in the kelp for the treatments in tap water (Tukey HSD; $p < 2.81\ 10^{-3}$) and an increase of minerals and trace elements in the water after blanching (Appendix A).

3.3 The possibility to lower the potential toxic hazards: arsenic, inorganic arsenic, and cadmium by various blanching conditions.

No regulation on maximum levels of total arsenic allowed in food products exists, however there are for animal feed. The maximum allowance of total arsenic is 40 mg kg^{-1} in seaweed meal for feed with 12% moisture content (EFSA, 2019), which is equivalent to 44.8 mg $(kg\ dw)^{-1}$. For winged kelp, total arsenic was significantly reduced by the blanching conditions (ANOVA; $p < 0.001$, $F = 15.6$). The initial concentration was 47.9 ± 2.7 mg $(kg\ dw)^{-1}$ and the average of the blanched winged

kelp was 28.5 ± 5.0 mg (kg dw) $^{-1}$, which meant the winged kelp was below the maximum allowance after any blanching condition.

For sugar kelp the blanching conditions “500 g/L”, “FW, SW” and “SW, SW” led to a significant reduction of total arsenic (39.8 ± 0.1 mg (kg dw) $^{-1}$) compared to fresh sugar kelp (66.7 ± 13.8 mg (kg dw) $^{-1}$) (ANOVA; $p > 0.001$, $F = 16.5$). The other blanching methods did not differ from the content in fresh sugar kelp and had an average of 58.1 ± 6.1 mg (kg dw) $^{-1}$. This is problematic, as the levels are above the maximum allowance in feed products. However, Blikra et al. (2021) successfully reduced total arsenic in sugar kelp when blanching at 92-99 °C for 15 min. in a ratio of 102 g kelp L^{-1} fresh water. The initial level was 62.7 ± 4.3 mg (kg dw) $^{-1}$ and ended with 36.0 ± 3.1 mg (kg dw) $^{-1}$.

The current study suggests that cooling with seawater after blanching successfully reduced the total arsenic levels. Moreover, the study by Blikra et al. (2021) suggests that elevated blanching temperatures or longer blanching duration in fresh water could also reduce the total arsenic content. These are interesting observations, which would be industrially relevant to research further.

A recent legislative focus has been to monitor inorganic arsenic instead, rather than organic arsenic, as organic arsenic is thought to be less toxic. Inorganic arsenic (arsenate and arsenite) is known to be carcinogenic (EFSA, 2009). Still, it is worth to mention that no information on the toxicity of organic arsenolipids and arsenosugars exists (EFSA, 2009; Sá Monteiro et al., 2019), but that they are present in the two kelp species (Pétursdóttir et al., 2019). Therefore, we cannot conclude whether the total arsenic content affects the food safety or not.

The Norwegian regulation on animal feed has set a maximum allowance of inorganic arsenic in feed based on macroalgae to be 2,000 ng g^{-1} (Norsk Lovtidend, 2020). Neither the fresh nor blanched winged kelp used in the present study had inorganic arsenic levels above the LOQ of 33 ng (g dw)$^{-1}$. However, other studies detected levels of 220 ng (g dw)$^{-1}$ inorganic arsenic in French winged kelp (Stévant, Marfaing, et al., 2018). Fresh sugar kelp had 55.3 ± 14.6 ng (g dw)$^{-1}$ and blanched by standard conditions had 123 ± 48 ng (g dw)$^{-1}$. Because of the large standard variations, no significant differences were found by the ANOVA analysis. One sample of the blanching involving seawater was analyzed. The blanching FW, SW had

68.5 ng (g dw)$^{-1}$ and SW, FW had 97.8 ng (g dw)$^{-1}$, and SW, SW was below the LOQ. It is important to mention all samples analyzed are a maximum 7% of the Norwegian regulation.

Cadmium content was not reduced by blanching in any of the species. Divalent cations bind to alginate, and due to the reduction of other compounds, the content in fact increased in some cases. Fresh sugar kelp contained 0.838 ± 0.085 mg (kg dw)$^{-1}$ and the average in blanched sugar kelp was 1.30 ± 0.20 mg (kg dw)$^{-1}$ (ANOVA; $p > 0.001$, $F = 5.63$). Winged kelp contained more cadmium with levels of 2.09 ± 0.05 mg (kg dw)$^{-1}$ in fresh kelp and averagely 2.45 ± 0.35 mg (kg dw)$^{-1}$ in blanched with no significant differences (ANOVA; $p = 0.103$, $F = 1.94$). These values are within the same range as reported by other sources (Blikra et al., 2021; Sá Monteiro et al., 2019; Stévant, Marfaing, et al., 2018). Blikra et al. (2021) did not find a reduction when boiling the kelp. Legislative threshold value of cadmium in seaweed used as food supplement is 3 mg (kg dw)$^{-1}$ (Commission Regulation (EU) No 1881/2006, 2006). In adults, average exposure to cadmium in Europe is already close to the tolerable weekly intake (TWI) with main sources being rice, grains, and vegetables. However, Sá Monteiro et al. (2019) reported that these concentrations of cadmium would, with a serving size of 5 g freeze dried seaweed, contribute to only 1.2–3.5% of the TWI, which is inconsequential compared to other sources. However, if the serving size increases, kelp could be a source of undesirable cadmium intakes.

3.4 Proximate composition of fresh and blanched biomass

The proximate composition for fresh kelp and blanched at standard condition in tap water is illustrated in Figure 3. The initial composition Figure 3 (A) and (C) was different between the two species. Sugar kelp had a higher ash content, which leads to a difference in calculated carbohydrates, since the protein and lipid contents were similar. Interestingly is that after blanching by standard conditions Figure 3 (B) and (D), the two kelps had similar proximate composition. Even though sugar kelp initially contained more total ash, they reach the same concentration after blanching.

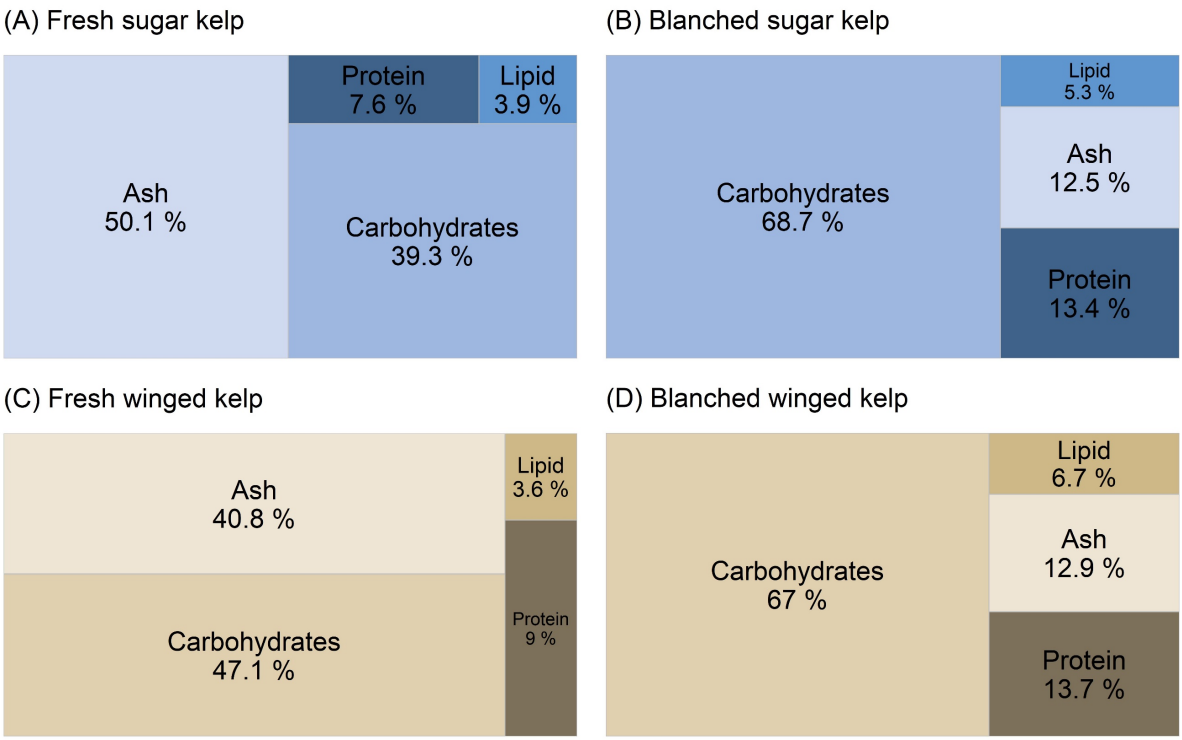

Figure 3: The proximate composition for fresh and blanched sugar kelp and winged kelp. The subgraphs are fresh (A) and blanched (B) sugar kelp, and fresh (C) and blanched (D) winged kelp. The blanched seaweeds were treated by the standard conditions. The data is given in % dry weight. All data is presented as the average in % of dw for the respective proximate. The protein content is measured by sum of the total amino acids using the molecular weight subtracted weight of water removed by formation of peptide bonds. The carbohydrates are calculated carbohydrates by subtracting the other proximates from 100%.

Overall, there was a substantial difference for dry matter. For winged kelp, the samples, which only used tap water for blanching and cooling, had a lower dry matter content (7.20-9.92% ww) compared to the fresh kelp (11.0% ww). Whereas the samples that were washed and/or cooled with seawater, had a similar dry matter content (10.8-13.3% ww) compared to the fresh winged kelp. For sugar kelp the fresh, “500 g/L”, and the ones cooled in seawater (“SW, SW” and “FW, SW”) had a higher dry matter content (8.05-9.60% ww) compared to the rest (4.47-6.68% ww).

For both species, the protein content in the “SW, SW” blanched kelp kept the same concentration as the fresh kelp, while those blanched or cooled in tap water all had significantly higher protein content than the fresh kelp (ANOVA; $p < 0.001$). This can be explained by the loss of other components when using tap water, and that most of the protein is insoluble (only free amino acids, peptides and soluble protein is lost). More specifically, the protein content in fresh and “SW, SW” blanched was 7.12-7.64

and 8.83-8.99% dw in sugar and winged kelp, respectively. In the other blanched kelps, it was 12.0-14.3 and 11.5-13.7% dw in sugar and winged kelp, respectively.

3.5 The effect of blanching on carbohydrate content

The monosaccharide content were analyzed to determine which carbohydrates were affected by the various blanching methods. In the fresh untreated sugar kelp was found 7.66 ± 6.33% dw mannitol, 0.933 ± 0.180% dw fucose, and 3.64 ± 1.03% dw glucose, and fresh untreated winged kelp had 2.75 ± 0.10% dw mannitol, 1.02 ± 0.07% dw fucose, and 1.98 ± 0.25% dw glucose.

Haug & Jensen (1954) found the mannitol content was fluctuating as a result of season and place of harvest, and they found a concentration of 8% in April in Norwegian wild winged kelp. Wirenfeldt et al. (2022) found a content of 16.6% dw in Danish cultivated sugar kelp from April. Table 2 shows the results of fucose, glucose, and mannitol. The other monosaccharides can be found in the supplementary material (appendix A).

Table 2: The fucose, glucose and mannitol in the blanching water after blanching. Fucose represents the total fucoidan content, and glucose the laminarin content.

	Fucose mg l^{-1}	Glucose mg l^{-1}	Mannitol mg l^{-1}
Sugar kelp			
Standard	26.6 ± 2.6	1.88 ± 0.61	140 ± 6
45 ˚C, 30 s	6.59 ± 0.69	0.926 ± 0.123	11.9 ± 9.5
Cut	90.5 ± 6.7	2.68 ± 0.81	42.9 ± 29.2
SW, SW	0.933 ± 0.077	0.337 ± 0.089	9.32 ± 1.31
Rep 5	128 ± 81	48.5 ± 61.3	764 ± 644
Rep 10	296 ± 127	155 ± 127	2,009 ± 1,156
Winged kelp			
Standard	6.37*	0.666*	112*

Mannitol is a sugar alcohol found in the cytoplasm of brown algae cells that serves as an energy storage compound as well as a regulator of osmotic pressure (Stiger-Pouvreau et al., 2016). Mannitol is easily released from seaweed into the blanching water due to its small molecular size. This is indicated by the increase of mannitol in

505 the blanching water after five (Rep 5) and ten (Rep 10) reuses of the blanching water
506 as compared to the Standard blanching. Using seawater (SW, SW) for blanching
507 reduces the driving force for mannitol release and less mannitol is found in the
508 blanching. However, cutting and other forms of mechanical processing can open
509 tissue structures and promote mannitol release. Cutting the kelp (Cut) exposes the
510 structure of the kelp, resulting in greater loss into the blanching water and overall
511 biomass reduction.

512 Fucose is a measure of the sulfated polysaccharide fucoidan and may also contain
513 minor amounts of galactose, mannose, xylose, uronic acids and glucose (Stiger-
514 Pouvreau et al., 2016). It is located within the cell walls and intercellularly in brown
515 algae, and it is believed their function is to prevent them from drying out (Jacobsen
516 et al., 2019). The various blanching treatments influence how much fucose leaks into
517 the blanching water. Interestingly, minor amounts of fucose got extracted when
518 blanching in seawater (SW, SW) as compared to tap water blanching, which had
519 more extracted. It is noteworthy that fucoidan is sulfated, and the retention of fucose
520 is in consistency with the data on sulfur, which is also retained in the seawater
521 blanched sugar kelp (Figure 2). Moreover, cutting the kelp (Cut) made it easier for
522 polymeric fucoidan to be released, which increased the amounts of fucose found in
523 the blanching water. This makes sense as fucoidan has a high molecular weight but
524 is easily soluble in water. We also found that elevated temperature and time
525 (Standard) had higher concentrations in the blanching water compared to the “45 ˚C,
526 30 s” blanching. This is in consistency with the work by Ferreira et al. (2019), who
527 studied distilled water extraction of fucose in *Fucus vesiculosus* and found that
528 elevation of temperature would extract more fucose (Ferreira et al., 2019).

529 Laminarin is a low molecular weight polysaccharide that is used in kelp to store
530 energy. Laminarin is a linear β-(1→3)-glucan backbone having infrequent β-(1→6)-
531 branches and mannitol-substituents at the reducing end with a molecular weight of
532 approximately 5 kDa (Kadam et al., 2015). We assume that the glucose composition
533 represents the release of laminarin during blanching. Haug and Jensen (1954) found
534 that the laminarin content in kelp would be absent or in very small amounts during
535 spring. Laminarin are found to only partial solute at room temperature but fully
536 dissolve at 50 °C (Birgersson et al., 2023). This was also indicated by this present
537 study, where we observed a lower release of laminarin into blanching water for 45

°C, 30 s treatment as compared to the standard blanching. To sum up, using seawater for blanching will retain more of the carbohydrates in the kelp.

3.6 Loss of nutrients and flavor compounds.

Kelps have a high content of the free amino acids; glutamic- and aspartic acid, which are known to contribute to the taste of umami (Mouritsen et al., 2012). Moving towards are more plant-based diet, a plant-based umami source is of high interest for the food producers. Table 3 shows the content of glutamic- and aspartic acid in fresh kelp and kelp blanched by standard conditions. For both species a noteworthy loss of the two free “umami” amino acids occurs due to blanching at standard conditions. Hamid et al. (2020) studied the effect of 100 °C blanching on the same amino acids in the kelp *Undaria pinnatifida* and found that they would drastically decrease within 20 seconds.

In addition, the changes in the content of vitamin C and folate during blanching were studied as they are known as water-soluble vitamins labile to various processing conditions (Delchier et al., 2016; Nielsen et al., 2021). The vitamin C content in fresh and kelp blanched by standard conditions is found in Table 3. Furthermore, the treatment 45 °C, 30 s was analyzed, and the vitamin C content was lowered to 0.573 ± 0.034 mg $(100\ g\ ww)^{-1}$ (not shown in the table) compared to the fresh kelp. The fresh sugar kelp had a lower content compared to Danish cultivated sugar kelp, which contained 8.73 mg vitamin C $(100\ g\ ww)^{-1}$ (Wirenfeldt et al., 2022). The loss of vitamin C during heat processing is in consistency with previous studies (Nielsen et al., 2021). However, this proves that even short time of blanching at 45-80 °C will lower the vitamin C content considerably. The recommended vitamin C intake is 45 mg day^{-1} (FAO/WHO Expert Consultation, 2001), which means that blanched kelp would not contribute to the vitamin C intake.

Folate is a generic term for a group of vitamers found mainly in leafy vegetables, legumes, offal, certain fruits, and cereals (Delchier et al., 2016). Total folate content found in fresh winged kelp (113 ± 37 µg $(100\ g\ ww)^{-1}$) indicates that this kelp could be considered as a good folate source taking into account recommendations of 330 µg day^{-1} for adults (EFSA, 2014). On the other side, a considerably lower folate content was observed in sugar kelp (18.3 ± 1.7 µg $(100\ g\ ww)^{-1}$). The most abundant folate vitamer in both kelps was 5-methyltetrahydrofolate (≥90%) (Appendix A),

followed by formyl forms, which agrees with the study from Rodríguez-Bernaldo De Quirós et al. (2004) who studied folate in other types of seaweed. Folate is known as a vitamin sensitive to processing because of oxidation, leaching into surrounding liquid or thermal instability of vitamers (Delchier et al., 2016). To our knowledge, this is the first study that considered changes in the folate content during processing of kelp and showed that under studied conditions (80 °C, 120 s), the loss of folate is significant. The loss of total folate was 78% and 85% after blanching of sugar kelp and winged kelp, respectively. Delchier et al., (2013) did not observe such a loss after industrial blanching of spinach at 90-95 °C during up to 120 s. However, the differences in laboratory vs. industrial conditions, as well as between food groups (vegetables vs. kelp) contribute to these discrepancies. Due to the loss of folate after blanching, winged kelp processed by studied conditions does not contribute considerably to the daily intake of folate.

Table 3: The content of the valuable nutrients: free aspartic acid, free glutamic acid, vitamin C and total folate in the two kelp species before and after blanching.

	Free aspartic acid	Free glutamic acid	Total vitamin C	Total folate
	mg (g dw)$^{-1}$	mg (g dw)$^{-1}$	mg (100 g ww)$^{-1}$	µg (100 g ww)$^{-1}$
Sugar kelp				
Fresh	1.68 ± 0.49	1.16 ± 0.40	3.09 ± 0.35	18.3 ± 1.7
Standard blanched	0.261 ± 0.105	0.334 ± 0.169	<LOQ	3.97 ± 0.12
Winged kelp				
Fresh	1.02 ± 0.35	1.14 ± 0.12	4.27 ± 0.39	113 ± 37
Standard blanched	0.142 ± 0.094	0.359 ± 0.137	<LOQ	17.0 ± 12.9

LOQ for vitamin C was 2 mg (100 g ww)$^{-1}$

Standard conditions are leading to a loss of the quality compounds such as free amino acids and heat labile vitamins. It is important that the industry is aware, that when blanching to reduce iodine, these nutrients are also lost.

3.7 The decrease of the microbial load

The initial aerobic viable count (AVC) on fresh, unprocessed sugar kelp was 3.52 ± 0.20 log CFU g^{-1}. This decreased significantly (ANOVA; p = 0.001; F = 8.34) to 1.80-

2.32 log CFU g^{-1} for any of the blanching methods analyzed (“Standard”, “45 ˚C, 30 s”, “45 ˚C, 120 s”, “80 ˚C, 30 s”, “SW, SW”). Similar results were found for fresh winged kelp, which had 3.87 ± 0.02 log CFU g^{-1} and decreased significantly (ANOVA; p = 0.017; F = 5.05) to 1.50-2.40 log CFU g^{-1} for the methods analyzed (“Standard”, “45 ˚C, 30 s”, “45 ˚C, 120 s”, “80 ˚C, 30 s”). The filtrated seawater had 0.85 log CFU mL^{-1}, and no detected AVC in any heated blanching water.

A recent study on Danish cultivated sugar kelp found initial AVC between 4.0–4.5 log CFU g^{-1}, whereas blanching (80 °C 120 s) lowered the AVC to 0.9-1.8 log CFU g^{-1} on marine agar (Wirenfeldt et al., 2022). These findings are comparable to this study, however (Blikra et al., 2019) found 1.1-2.0 log CFU g^{-1} in both raw and heat-treated sugar and winged kelp, also on marine agar. Obviously, the initial AVC depends on the environment where the seaweeds grow. Overall, ≥45 °C treatments will reduce the AVC.

The AVC was decreased to below 2.40 log CFU g^{-1}, when blanched under any conditions. These results show that the industry can blanch at 45 °C and still reduce potential microbial hazards. Wirenfeldt & Sørensen et al. (2022) suggest using 7 log CFU g^{-1} as a threshold of shelf-life and the AVC found in this study are all below that threshold.

5 Conclusion

This study proved that the iodine content could pose as less of a health concern as it was reduced by all the blanching treatments. This also means, that it is possible to consume more kelp before reaching the upper daily intake of iodine.

One of the key findings was that blanching in membrane filtered UV-treated seawater at the standard conditions (80 °C for 120 s) was as efficient as the same conditions in tap water. Besides iodine, blanching and cooling in tap water at a ratio of 50 g kelp per liter will lead to a loss of bromine, chlorine, magnesium, sodium, and potassium. However, cooling in membrane filtered, UV-treated seawater will have a decrease of arsenic, potassium, and phosphorus.

The industry should be aware that increasing the seaweed to water ratio will lower the blanching efficiency on the iodine content. Considering blanching in 1 m^3 water,

0.479 tons or 12.0 tons of wet sugar kelp or winged kelp can be processed without any of the kelp exceeding a final iodine concentration of 600 µg iodine kg^{-1} dw.

Blanched winged kelp was below the maximum allowance of arsenic in feed products, whereas most of the blanched sugar kelp were above, which can be problematic. However, the inorganic arsenic content was for all maximum 7% of the Norwegian regulation on the maximum allowed content in feed products. It is important to note that more research is needed to understand the health concerns from the organic arsenic compounds.

The cadmium content was below a European legislative threshold value. We expect that cadmium is not a potential health hazard from kelp until portion sizes increase remarkably above 5 g dw.

Seawater blanching retains the carbohydrates: mannitol, laminarin, and fucoidan due to reduced driving force, while cutting supports mannitol and fucoidan loss. It was also found that temperature and time also increase the release of carbohydrates into blanching water. The industry should be mindful that blanching at standard conditions (80 °C for 120 s) will compromise the free amino acids and therefore lead to a change in product taste. Moreover, the standard blanching will also compromise the vitamin C and folate content.

Overall, the results suggest that blanching with membrane filtered UV-treated seawater at 80 °C for 120 seconds has potential for industrial application since it reduces iodine, arsenic, and inactivate microorganisms, and retains valuable carbohydrates. The effect of this blanching conditions on folate, vitamin C, and free amino acids are still to be investigated and is recommended for future studies.

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Supplementary material

Appendix A: Full dataset in Excel.
Appendix B: Monosaccharides correction factors.
Appendix C: Daily consumption that will lead to intake of 600 microgram iodine.
Appendix D: Pearson's correlation of minerals and trace elements.
Appendix E: Mineral and trace element bar diagrams for winged kelp.
Appendix F: Mineral and trace element bar diagrams for sugar kelp

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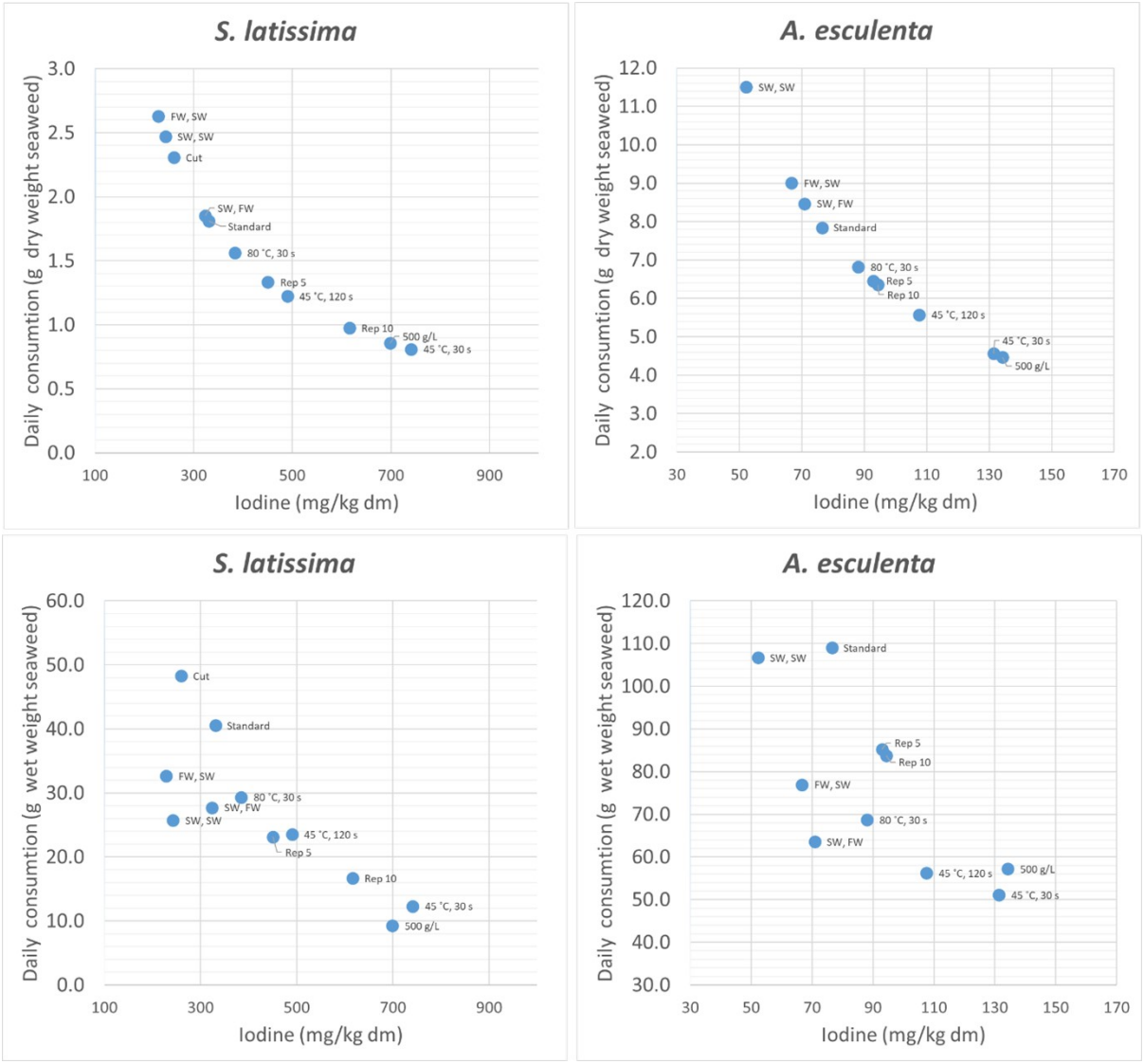
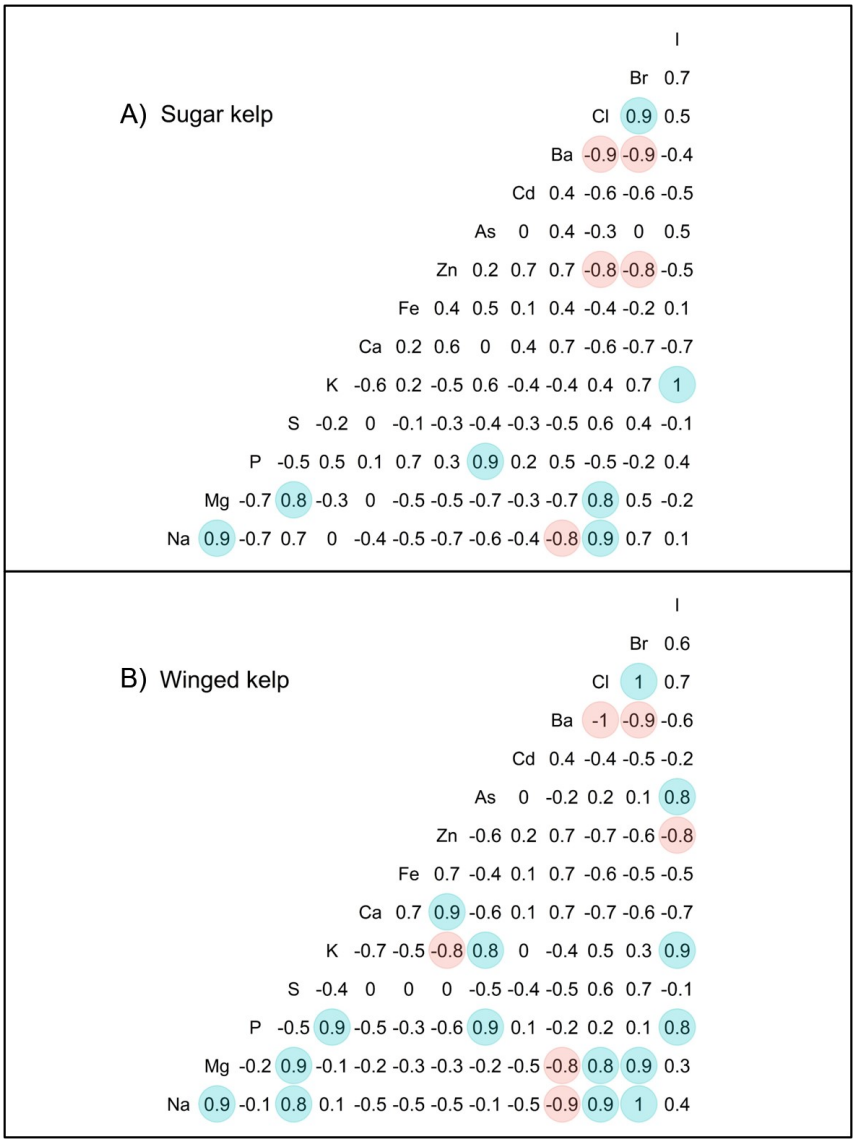
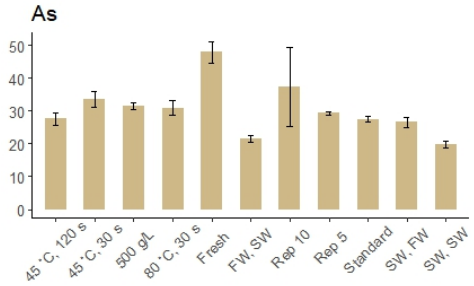
Supplementary material

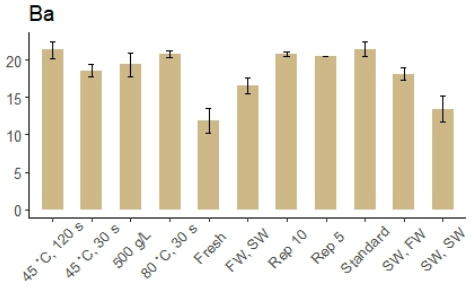
Appendix C: Daily consumption that will lead to intake of 600 microgram iodine for after the kelp has been exposed to the different blanching treatments.

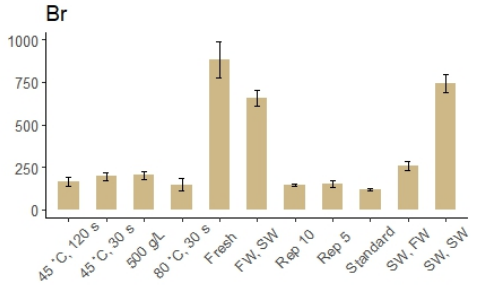


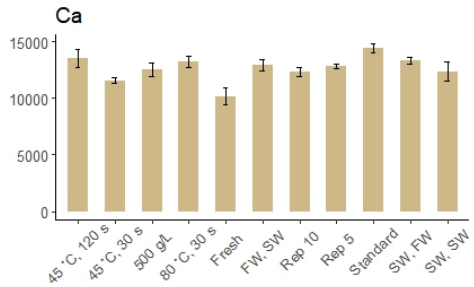
Appendix D: Pearson's correlation of minerals and trace elements. The blue circles indicate a high positive correlation (≥ 0.8) and the read circles a high negative correlation (≤ -0.8).

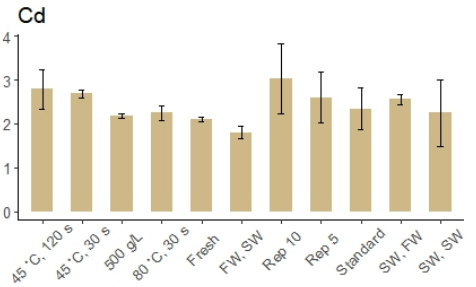


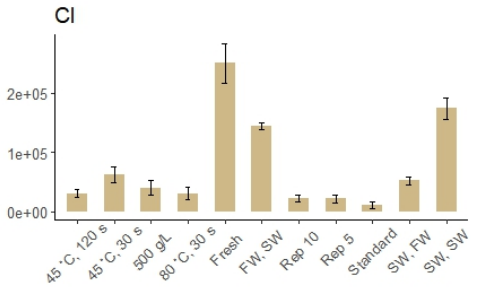


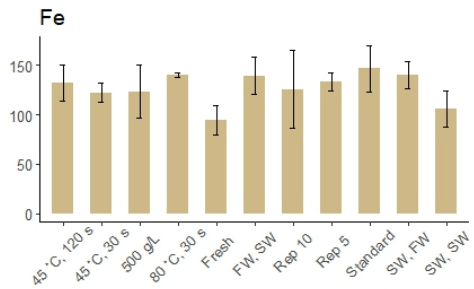


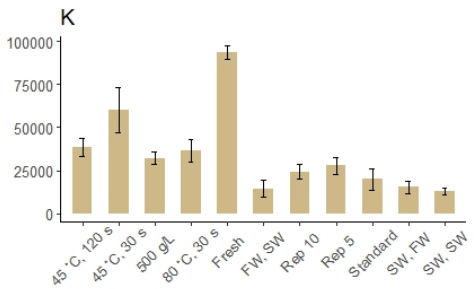


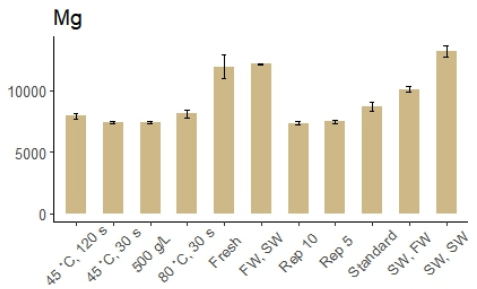


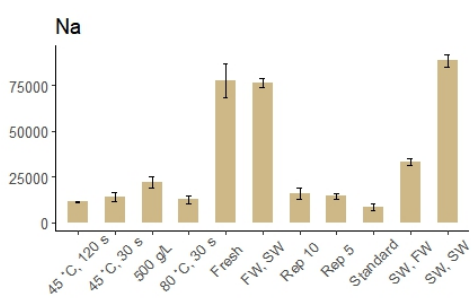


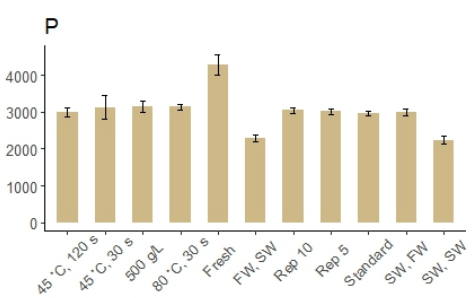


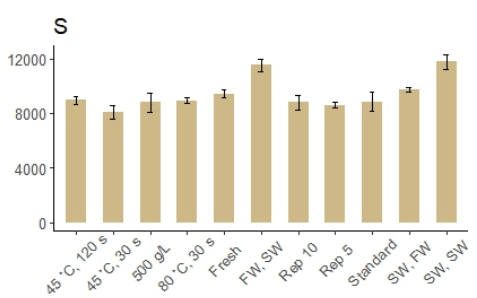


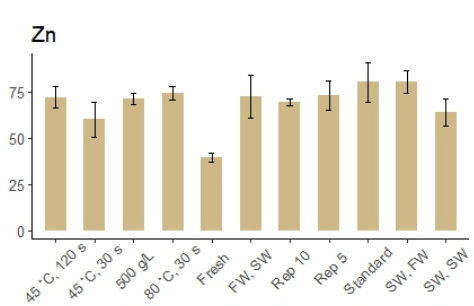




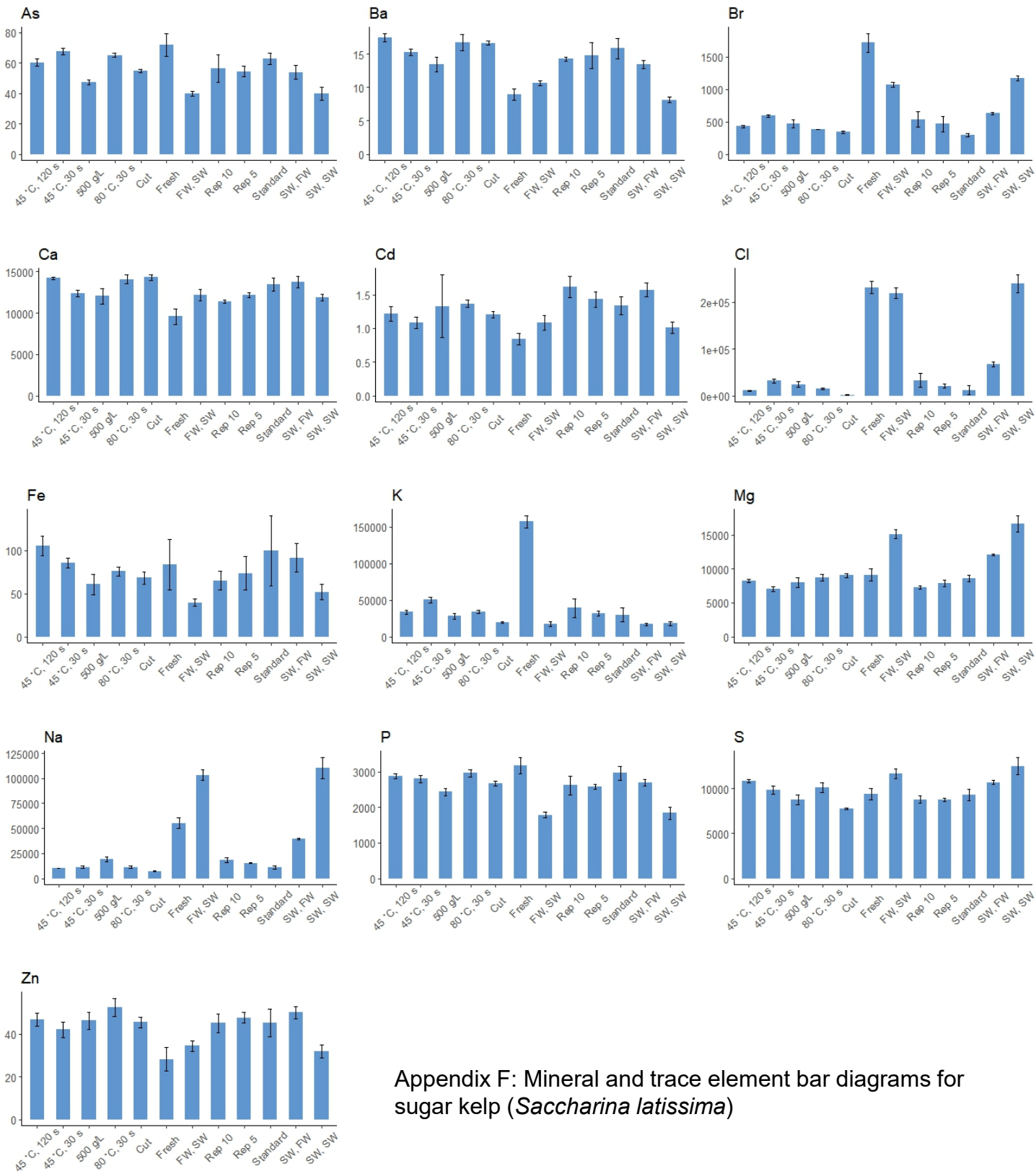








Appendix E: Mineral and trace element bar diagrams for winged kelp (*Alaria esculenta*)



Appendix F: Mineral and trace element bar diagrams for sugar kelp (*Saccharina latissima*)

Paper 4

Post-harvest quality changes and shelf-life determination of washed and blanched sugar kelp (*Saccharina latissima*)

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Post-harvest quality changes and shelf-life determination of washed and blanched sugar kelp (*Saccharina latissima*)

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Sugar kelp (*Saccharina latissima*) is a native European brown macroalga with the potential to become a vital part of the green transition of the food industry. Knowledge of the sugar kelp shelf-life is essential to designing the food supply chain to ensure safe and high-quality food. Establishing a single-compound quality index (SCQI) of freshness would be useful for the industry. However, information is currently lacking on how different post-harvest treatments affect the shelf-life of sugar kelp, even though it is important knowledge for manufacturers, authorities and consumers. The objective of this study was to establish the shelf-life of refrigerated sugar kelp following five post-harvest treatments and evaluate the effect of these treatments on changes in quality attributes (sensory, microbial, chemical and physical) during storage to select the SCQI. The post-harvest treatments included washing in sea water, washing in potable water, blanching for 2 min in sea water or potable water and untreated sugar kelp. Based on sensory analysis, the refrigerated (+ 2.8°C) shelf-lives for sugar kelp from all treatments were seven to 9 days. The end of the sensory shelf-life correlated with the development of >7 log (CFU g^{-1}) aerobic viable counts, suggesting this attribute can be used as a SCQI to evaluate the shelf-life of sugar kelp. The microbiota was dominated by putative spoilage organisms from the *Pseudoalteromonadaceae* and *Psychromonadaceae* families. Untreated and washed sugar kelp continued to respire and consume carbohydrates up to 5 days post-harvest, indicating respiration rates may be used to determine freshness of non-blanched kelp. Favorable organoleptic properties, e.g., sweetness and umami, decreased during storage and coincided with a reduction in water-soluble mannitol and free glutamic acid. Both blanching treatments changed texture and color and reduced iodine and vitamin C contents while retaining components such as fucoxanthin, chlorophyll a and β-carotene. This study provides crucial documentation of quality changes during the post-harvest storage period of sugar kelp, including information about sugar kelp spoilage and nutrient changes, which would facilitate the development of best practices for manufacturers using sugar kelp in their production of food.

KEYWORDS

sensory, microbiota, chemical composition, nutrients, physicochemical, processing, best practice, storage trial

1 Introduction

Macroalgal production in Europe is limited, as it only contributes to 0.8% or 0.3 million metric tons of the global output (Cai et al., 2021). Wild harvest currently dominates European macroalgal production with just 3.9% of the produced biomass originating from macroalgal farms (Cai et al., 2021). The low share of cultivated macroalgae in Europe is unusual compared to the global dominance of cultivated macroalgae with an estimated total cultivated market share of 97.0% of the total production (Cai et al., 2021). Sugar kelp (*Saccharina latissima*) is a brown macroalga native to Europe, which grows from the Iberian Peninsula in the South (Peteiro et al., 2016) to Tromsø in the North (Matsson et al., 2019). It has been identified as a cultivable species with the potential to increase its annual production rapidly (Olafsen et al., 2012). Sugar kelp is thought to represent the future of European macroalgal production because of its many potential uses in human consumption (Mahadevan, 2015), food additives (Bixler and Porse, 2011), animal feed (Rajauria, 2015) and biofuels (Marquez et al., 2015).

Several studies imply the importance of extending the shelf-life of sugar kelp (Sappati et al., 2017; Akomea-Frempong et al., 2021b, 2021a; Skonberg et al., 2021). However, only a few studies have conducted storage trials to determine sugar kelp's shelf-life or post-harvest quality changes. Løvdal et al. (2021) reviewed the microbial food safety of macroalgae and recommended further research into the impact of processing technologies on pathogenic microorganisms and product shelf-life. To our knowledge, data on the shelf-life of sugar kelp has so far only been reported in a master thesis (Nayyar, 2016), while there are several published reports on the shelf-life of fresh or lightly processed products made from green and red macroalgae (Liot et al., 1993; Paull and Chen, 2008; Nayyar and Skonberg, 2019). However, direct comparison of shelf-lives of different macroalgae should be done with caution or not at all. The reason for the caution is the extreme taxonomical differences between brown, red and green algae, i.e., green macroalgae (*Chlorophyta*) and red macroalgae (*Rhodophyta*) belong to the *Viridiplantae* and *Biliphyta* subkingdoms, respectively, located within the *Plantae* kingdom. In contrast, brown macroalgae (*Phaeophyceae*) belong to the *Ochrophyta* phylum in the *Chromista* kingdom (Ruggiero et al., 2015).

Shelf-life is defined as the period after harvest, where the sugar kelp under the given storage conditions remains safe to consume and unspoiled, i.e., it retains desirable qualities in terms of sensory, chemical, physical, microbiological and functional characteristics (Man, 2016). Storage at temperatures above freezing will cause the sugar kelp to spoil over time due to non-microbial and microbial changes, which can coincide. Since knowledge about shelf-lives is limited for macroalgae, inspiration for determination of post-harvest quality changes may be taken from research on vegetables. For example, respiration rate has been shown to be associated with the shelf-life of horticultural products, leading to its use as an indicator of deterioration rate and freshness. Harvested products are still alive, and the cells will be metabolic active and respire (Watada and Qi, 1999). The shelf-life of plant products can generally be correlated to the CO_2 production rate (Robinson et al., 1975). Another method to determine the shelf-life of horticultural products uses the total viable count of microbes. A maximum acceptable contamination level of 7.7 log (CFU g^{-1}) has been proposed (Corbo et al., 2006). However, establishing an inaccurate or too conservative shelf-life can lead to increased food waste (Man, 2016). It is believed that the level of food waste for macroalgae is high and in the same range as fruits and vegetables, as a single annual harvest characterizes both commodities. Here large biomasses with a relatively short shelf-life become available over a short period. Taken together, this has been estimated to lead to 22–49% losses during the post-harvest stage of the value chain (FAO, 2022).

Suitable post-harvest treatments have not been thoroughly investigated for sugar kelp. Food manufacturers and authorities need to understand how different post-harvest treatments changes the quality of sugar kelp. Washing of the sugar kelp is commonly applied as a first step post-harvest process to remove contaminants, e.g., sand, mud, stones, and small crustaceans (Liot et al., 1993). Blanching is a common food processing method used for multiple purposes. Nielsen et al. (2020) showed that blanching of sugar kelp in potable water decreases the iodine content, leading to compliance with the recommended iodine threshold level (2,000 mg kg^{-1} (ANSES, 2018)) while retaining the desirable total phenolic content (TPC) and the radical scavenging activity. Blanching can also remove undesirable substances and microorganisms (Dagostin, 2016) or be used industrially as a pre-treatment before a fermentation process (Bruhn et al., 2019). Suitable post-harvest treatments with control of temperature and respiration rates can delay microbial spoilage and potentially extend the product's shelf-life (ICMSF, 2011). Potable water is a costly resource, which is limited in some coastal regions. The macroalgal industry is therefore looking at ways to reduce potable water utilization, including the use of sea water during post-harvest treatments. However, more information is needed on whether this can be done without negative quality changes. In addition, there is a knowledge gap on potential post-harvest treatment-induced changes in other quality parameters, such as vitamin C, organic acids, fucoxanthin, chlorophyll a and beta-carotene.

The objective of this study was to determine the sensory shelf-life of sugar kelp and evaluate post-harvest quality parameters to establish a single-compound quality index for spoilage. In addition, the study aimed to investigate if potable and sea water usage during sugar kelp washing and blanching affected the quality and shelf-life. A single batch of freshly harvested sugar kelp was processed, analyzed and evaluated to achieve these aims. First, we studied the sensory, physical, chemical, and microbial changes in a 16-days storage trial. Second, we identified potential single-compound quality indices and studied their treatment-related quality changes during refrigerated storage of sugar kelp from the different treatment methods. This study will aid food manufacturers and food authorities working with sugar kelp to establish best practices for post-harvest treatments and aid in shelf-life determination for the industry to provide safe and high-quality sugar kelp.

2 Material and methods

2.1 Harvest of sugar kelp, processing, packing and storage

Sugar kelp (*Saccharina latissima*) was harvested from the cultivation site of the company Dansk Tang Aps in Isefjord South of Rørvig, Denmark (N55°56′ E11°46′) during the commercial harvest in May 2020. The salinity at the site was 22 PSU. A total of 16 kg (400 individual blades) of sugar kelp were harvested by hand-cutting the blades just above the growth zone, leaving the holdfast and growth zone of the blade for re-growth. The blades were approximately 80 cm long. The sugar kelp was gently packed in food-grade plastic containers and transported by car from the harvest site to our laboratories in Kgs. Lyngby, Denmark (110 km). During the transportation and storage, the temperature was logged every 30 min with four loggers (TinyTag Plus, Gemini Data Loggers Ltd., Chichester, United Kingdom) and remained at an average of 17 ± 5 C during the 2.5 h of transportation. Upon arrival at the laboratory, the sugar kelp was placed in a cold room (2.8 ± 0.4°C) until the sugar kelp was processed later the same day (less than 12 h after harvest).

A storage trial with five treatments was carried out by randomly dividing the harvested sugar kelp into five sub-samples. The treatments included Untreated (Un), packed directly from the plastic containers. Washed in potable water (WP), comprised of washing the sugar kelp for 5 min in 16°C potable tap water (Lyngby-Taarbæk Forsyning A/S, Denmark) followed by transfer to a tray for drip-drying for 5 min. Washed in sea water (WS), comprised of washing for 5 min in 4.0°C UV-treated sea water with a PSU of 35 (DTU Aqua, Kgs. Lyngby, Denmark), followed by transfer to a tray for drip-drying for 5 min. Blanched in potable water (BP), where the sugar kelp was submerged for 2 min in hot potable water (76°C), followed by rapid cooling for 3 min in potable water (16°C) and drip-drying for 5 min. Blanched in sea water (BS), where the sugar kelp was submerged for 2 min in hot (80°C) treated sea water followed by rapid cooling in chilled treated sea water (4.2°C) for 3 min and drip-drying for 5 min. All treatments were carried out in the ratio of 50 g sugar kelp to 1 L water. Aliquots of approximately 45–55 g (1-2 individuals) were placed in plastic trays (71–51A hvid/PS, Færch Plast, Holstebro, Denmark). The tray and sugar kelp were placed in thick (70 µm) polyethene (PE) plastic bags with a high permeability of >6 g m^{-2} d^{-1} for water vapor, >3,000 cm^3 m^{-2} d^{-1} atm^{-1} for O_2 and >14,000 cm^3 m^{-2} d^{-1} atm^{-1} for CO_2 (H902, Topiplast A/S, Greve, Denmark) and sealed. However, the samples (five bags from each treatment) intended for the determination of respiration rates were packed in multiple barrier (MB) bags made from a 117 ± 6 µm laminate film with low gas permeability of 0.45 cm^3 m^{-2} d^{-1} atm^{-1} for O_2 and 1.8 cm^3 m^{-2} d^{-1} atm^{-1} for CO_2 (NEN 40 HOB/LLPDE 75, Amcore, Horsens, Denmark) and sealed. All packaged samples were stored at an average product temperature of 2.8 ± 0.4°C during the storage trial.

2.2 Sampling plan

In order to determine the shelf-life of sugar kelp and post-harvest quality changes, a storage trial was designed following the ISO standard (ISO, 2015). A descriptive profile analysis (Section 2.3) was used to determine a sensory shelf-life, with the harvest as the starting point and a test period of 16 days. The test period was chosen based on the reported estimated shelf-life of six to 12 days (Nayyar, 2016). The test steps (sampling days) were set to the following intervals on days: 1, 3, 5, 7, 9, 13 and 16. On each sampling day, 45 bags were randomly selected from the five treatments, nine from each, and analyzed as described in Figure 1A. Three biological replicates were evaluated in all cases, with technical replicates ranging from one to three depending on the analysis (Figure 1A). Five biological replicates were used for gas analysis for the respiration rates of the harvested and processed sugar kelps (Figure 1B).

2.3 Sensory evaluation

The sensory evaluation was performed as a descriptive profile analysis. The first session was used to set up the vocabulary, and the next session was used to train the judges in using the scale for the 13 attributes (Table 1). On each day of analysis, 15 bags of sugar kelp were evaluated by an internal panel consisting of five judges. Each bag was given a random three-digit code and placed on cooling plates with moist clean linens on top to avoid changing sensory scores during the session. Samples were presented under artificial daylight (6500 K, L 36W 965 Lumilux De Luxe, Osram, Germany). The individual

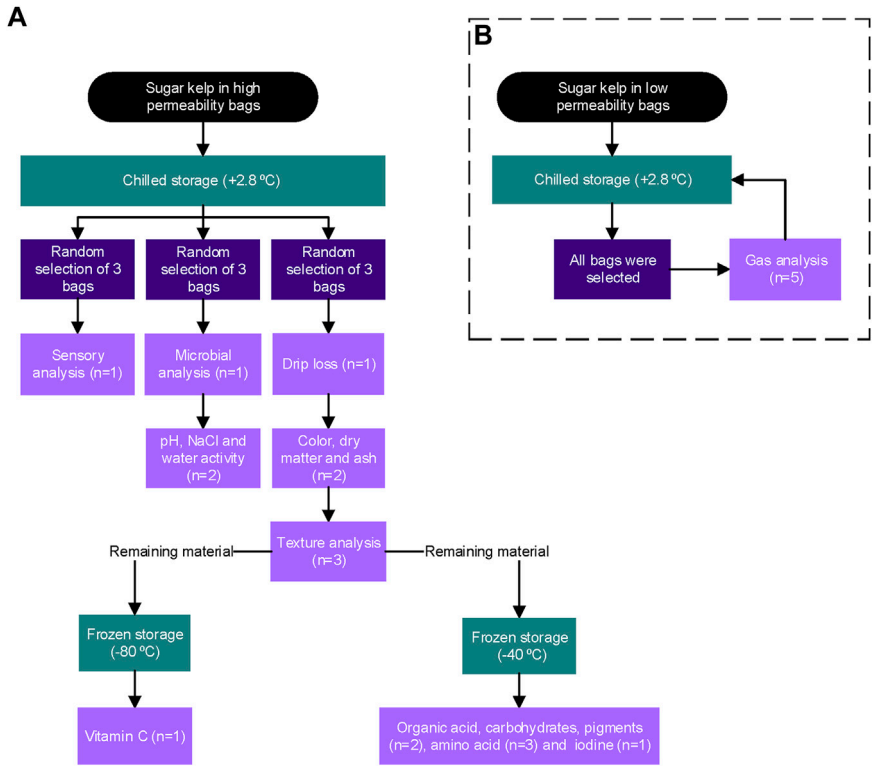


FIGURE 1
Overview of the sensory, microbial, and physicochemical analyses performed on stored sugar kelp on each sampling day. **(A)** Sugar kelp packed in high permeability polyethylene bags were analyzed for all variables except respiration. Nine bags were randomly withdrawn from each treatment and analyzed. **(B)** Sugar kelp packaged in low permeability multiple barrier bags were analyzed for respiration rates, all bags were selected, the gas composition was analyzed, and the bags were placed in the chilled storage room again. The number of technical replicates is indicated in the boxes (n) for each variable.

judge assessed samples individually, and the judges came to a consensus on the intensity of the attributes under the guidance of the panel leader. Off-odors and new characteristics were also noted down. The attributes were evaluated on a 15 cm unstructured line scale with two anchors placed 1.5 cm from each end. All attributes had the scale anchor words "little" (left side) and "a lot" (right side), apart from the texture attribute "leather", which had the anchor words "leather" and "parchment".

2.4 Physical changes during storage

Drip loss, color, and texture were the parameters analyzed to determine physical changes during storage. Drip loss was measured by gravity draining of liquid in each bag (n = 3) for 1 min and calculated as the percentage loss of the total weight (Guldager et al., 1998). Color was measured by a Chroma meter (CR-200, Konica Minolta, Tokyo, Japan) recording the CIE L* a* b* color scale. From each bag (n = 3), two pieces (Ø = 8 cm) of sugar kelp were cut out. The two pieces were measured three times each by the Chroma meter. The samples were put on a white surface (L* = 91.5, a* = -4.26, b* = 3.33). Texture analyses were performed at three sites of the sugar kelp blade, i.e., one site close to the harvest cut, one at the middle of the blade and the last site at the top section of the blade. The texture analysis was run on TA. XTplus Texture Analyser (Stable Micro Systems, Surrey, United Kingdom) equipped with a 5 kg load cell, with a Warner Bratzler Blade Set with "V" slot blade (HDP/WBV) running at a speed of 5 mm s^{-1}. The texture curve was recorded with a resolution of 500 points s^{-1} and analyzed by the instrumental software (Texture Exponent, Version 6.1.15, Stable Micro System, Surry, England). From the time-force graphs, the firmness (g) and toughness (g s^{-1}) were calculated by the instrument's software. The remaining sample material in the three bags from each treatment was frozen at −40 C and freeze-dried (Christ beta one to eight, Merck) for further analyses. However, for vitamin C analysis, an aliquot was frozen and ground with liquid nitrogen before analysis.

The content of water, ash, and NaCl, as well as the pH level and water activity (a_w) in the sugar kelp samples, were analyzed on days 0 and 16 of the storage experiment. The water (100—dry

TABLE 1 Sensory attributes used for the descriptive profile analysis of the seaweed samples together with the corresponding label used for the PCA bi-plot and the description of the attributes.

Sensory attribute	Label	Description
Visual appearance		
Transparency	V-Trans	How transparent the sample appeared from looking down on the sample in the tray
Resilience	V-Resil	Resilience as related to 3D structure, high resilience meaning the sample would fill out the tray in all directions
Uniform color	V-Unifo	Samples of sugar kelp were scored less uniform, if more areas of different colors were seen
Odor		
Sweet	O-Sweet	A sugar-sweet caramel-like odor
Fresh sea	O-FrSea	The odor from a breezy sea
Rubber	O-Rubbe	Pepper like, rubbery, flounder fish odor
Beach-cast	O-Beach	Odor of sea shore, warm summer day or hay-like
Sourish	O-Sour	A fresh, green sour towards a lime-like fruity odor
Boiled peas	O-BoPea	Green sweet or sickly sweet
Umami	O-Umami	The round broth-like, meaty odor
Metal	O-Metal	Metallic odor
Texture (touch)		
Leather	T-Leath	The touch of a robust leather-like not fragile sample, opposite to crackly, thinner touch
Silky	T-Silky	The feeling of a silky smooth touch
Slimy	T-Slimy	The feeling of slimy mass on the fingers after touching the sample

matter (DM) (%)) and ash concentration were determined gravimetrically according to AOAC 938.08. a_w was measured using a water activity meter (Aqua Lab model 4TE, Decagon devices Inc., Pullman, US). pH was measured by placing a PHC805 universal electrode probe (HQ411D Benchtop Meter, HACH Company, Loveland, United States) in the sample solution consisting of 5 g of minced sugar kelp mixed with 25 ml of distilled water and stirred for 1 h. NaCl concentration was quantified by automated potentiometric titration (785 DMP Titrino, Metrohm, Hesisau, Switzerland) of a sugar kelp-distilled water mix (1:5).

2.5 Respiration rate of sugar kelp

Five bags of sugar kelp for each treatment were packed in low gas permeability bags (see Section 2.1) and evaluated based on changes in the headspace gas composition to determine the respiration rate. The bags represented a closed system. CO_2 and O_2 concentrations were determined using a gas analyzer (Checkmate3, MOCON Dansensor®, Ringsted, Denmark) twice daily. The respiration rates were calculated by Eq. 1, 2, and the respiration quotient (RQ) was the ratio of CO_2 produced to O_2 consumed (Fonseca et al., 2002).

$$O_2\ respiration\ rate = \frac{([O_2]_{Time1} - [O_2]_{Time2}) \cdot free\ volume}{100 \times mass\ of\ product \cdot (Time2 - Time1)}. \quad (1)$$

$$CO_2\ respiration\ rate = \frac{([CO_2]_{Time2} - [CO_2]_{Time1}) \cdot free\ volume}{100 \times mass\ of\ product \cdot (Time2 - Time1)}. \quad (2)$$

The free volume was measured by submerging the entire bag at the end of the storage trial in water to determine the volume displacement in mL.

2.6 Culture-dependent microbial changes during storage

Triplicate bags were used on each sampling day to enumerate bacterial concentrations. Each bag's sugar kelp was aseptically cut into smaller pieces using a sterile blade. A random sample of 15.0 g was mixed with 135.0 g of chilled physiological saline (0.85% NaCl) with 0.1% peptone (PSP) (NMKL, 2006). The mixture was homogenized for 60 s in a Stomacher 400 (Seward Medical, London, United Kingdom). Further 10-fold dilutions with PSP were performed as required. Aerobic viable counts (AVC) were determined by spread plating on Marine agar (MA, PanReac AppliChem GmbH, Darmstadt, Germany) and enumeration after incubation for 7 days at 15 C. The lower temperature and longer incubation time regime was chosen over standard incubation conditions to allow the detection of psychrotrophic and psychrophilic microorganisms (Broekaert et al., 2011). Presumptive *Pseudomonas* spp. was determined by spread plating on *Pseudomonas* agar (CM0559, Oxoid, Basingstoke, United Kingdom) with CFC selective supplement (SR0103, Oxoid, Basingstoke, United Kingdom) and incubation for 2 days at 25°C. H_2S-producing *Shewanella*

spp. was determined as black colonies by pour plating in Iron Agar Lyngby (CM0964, Oxoid, Basingstoke, United Kingdom) with L-cysteine hydrochloride and incubation for 3 days at 25 C (NMKL, 2006). *Actinomycetes* were enumerated by spread plating on *Actinomycete* Isolation Agar (17117, Sigma-Aldrich, Merch, Darmstadt, Germany) after incubation for 2 days at 37 C. Yeast was determined by spread plating on Oxytetracycline Glucose Yeast Extract agar (CM0545, Oxoid, Basingstoke, United Kingdom) with oxytetracycline supplement (SR0073A, Oxoid, Basingstoke, United Kingdom) and incubated for 3 days at 25 C.

The composition of the dominant culture-dependent microbiota was determined by isolation of 12–14 colonies from MA plates from each treatment on the day of spoilage (day 9) as determined by sensory evaluation. Selection, handling and identification of the bacteria were performed as described in Sørensen et al. (2020), except DNA from the isolates was extracted using a boiling method. Briefly, 1 mL from an overnight culture was centrifuged (5,000 × *g*) to harvest cells. The resulting pellet was resuspended in TE buffer (1×) and boiled for 10 min to lyse cells (Fachmann et al., 2017). This crude DNA extract was subsequently used in Sanger sequencing of the 16S rRNA gene as detailed in Sørensen et al. (2020).

2.7 Culture-independent microbial changes during storage

Culture-independent analysis of the microbiota of the fresh sugar kelp (day 1) and spoiled sugar kelp (day 13) were conducted using DNA extracts prepared from the stomacher bags using the protocol described in Sørensen et al. (2021). Briefly, the V3-V4 region of the 16S rRNA gene was targeted in an amplicon sequencing (Illumina, 2021). The resulting sequences were analyzed using the QIIME2 pipeline (Bolyen et al., 2019) with the SILVA 138.1 SSU Ref NR 99 database (Quast et al., 2013), following filtration and trimming of the reads with the amplicon region primers using RESCRIPt (Robeson et al., 2020). Reads were deposited at the NIH NCBI Sequence Read Archive with the accession number PRJNA788340.

2.8 Chemical changes during storage

Water-soluble carbohydrates and organic acids were determined by preparing 0.2 g of homogenized freeze-dried sugar kelp mixed with 12 ml of 5 mM sulphuric acid. The samples were mixed by vortexing, followed by removal of impurities by centrifugation at 5,000 *g* for 5 min and filtration of the supernatant through a 0.22 µm syringe filter (Labsolute, Th. Geyer GmbH & Co. KG, Germany). Construction of standard curves and the HPLC protocol were carried out as described in Sørensen et al. (2021).

To determine the free glutamic and aspartic acid levels, approximately 50 mg of freeze-dried sugar kelp was vortexed with 1 ml 5% trichloroacetic acid and kept at 5 C overnight. On the following day, the samples were centrifuged for 5 min at 5,000 × *g*. Derivatization and chromatography were performed as described by Bak et al. (2019).

Pigments (fucoxanthin, chlorophyll a and beta-carotene) were determined by HPLC on methanolic extracts. The procedure followed the protocol by Safafar et al. (2015), with three technical replicates on each sample. The extracts were run on an HPLC-DAD system (Agilent 1,100 Liquid Chromatograph) equipped with a Zorbax Eclipse C8 column 150 mm × 46 mm x 3.5 µm (Phenomenex Inc. CA, United States). The mobile phase consisted of 75% methanol and 25% 0.028 M tert-butyl acetoacetate at a flow rate of 0.9 ml min^{-1} and an acquisition time of 40 min. A standard pigment mix (DHI, Hørsholm, Denmark) was used to produce a standard curve. The detection of the pigments was at 440 nm.

Vitamin C was determined by adding aliquots of 1.4 g sugar kelp frozen in liquid nitrogen to 5 ml of 2% metaphosphoric acid with 20 mM Tris (2-carboxyethyl)-phosphine hydrochloride. The samples were shaken for 2 min (Multi Rex, Heidolph Instruments, Germany) and rested for 13 min. Five millilitres of Milli-Q* (Merck, NJ, United States) water were added and sample mixed by inverting five times followed by 10 min centrifugation at 3,000 *g* and 4 C. The supernatant was filtered on a 0.2 µm filter. Vitamin C was quantified by UPLC with a C18 Acquity BEH column (s100 mm × 2.1 mm x 1.7 µm, Waters, MA, United States) at 25 C and detected at 265 nm. The mobile phase consisted of a gradient comprised of two eluents. Eluent 1: A filtered buffer made of 600 mg dodecyl trimethylammonium chloride, 10 ml acetonitrile and 100 ml of 0.5 M acetate buffer (pH 5.4) filled with Milli-Q* water with a total volume of 1 L. Eluent 2: Acetonitrile:Milli-Q* in a ratio of 1:1. The injection intervals were 5 min with a flow rate of 0.35 ml min^{-1}. The gradient consisted of the following steps: 0–2 min: 100% eluent 1; 2–3 min: 50% eluent 1 and 50% eluent 2; 3–5 min: 100% eluent 1. L-ascorbic acid in concentrations of 2, 4, 6, 8 and 10 µg ml^{-1} were used to construct a standard curve. An in-house reference (broccoli, *Brassica oleracea* var. italica) was included in each analytical run. The limit of detection was 1 mg 100 g^{-1} ww.

The iodine content was analyzed as described by Jerše et al. (2021) according to the CEN standard (EN 17050:2017).

2.9 Statistical analyses

The data software R (R-Core-Team, 2020) was used for data analyses and statistics. The following packages were used:

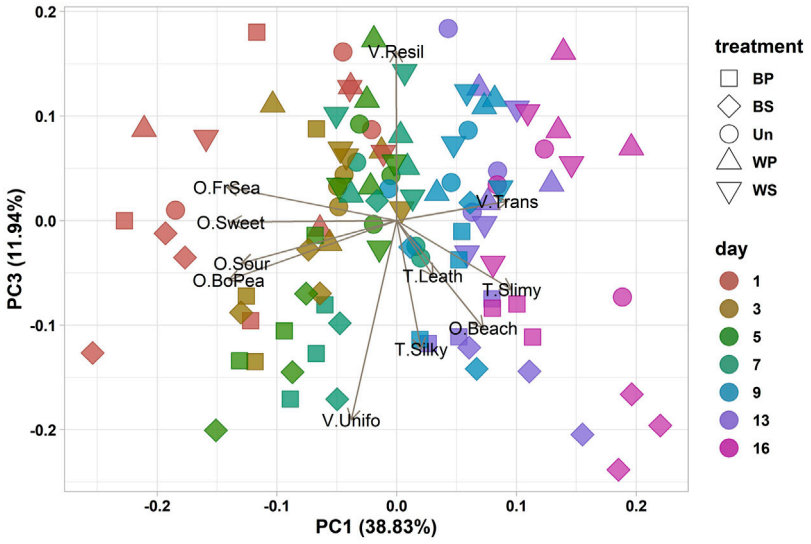


FIGURE 2
Principal Component Analysis bi-plot with PC scores for component 1 and 3 and loadings of the variables with the labels from the descriptive profile analysis (Table 1). Each data point represent the consensus judgement of the five sensory evaluators of each sample, the form of the data point represent the treatment (blanched in potable water (BP), blanched in sea water (BS), untreated (Un), washed in potable water (WP) and washed in sea water (WS)) and the color of the data point represents the storage period from day 1 to day 16.

"stats", "GGally" (Schloerke et al., 2021), "dplyr" (Wickham et al., 2021), "car" (Fox and Weisberg, 2019), and "multcompView" (Graves et al., 2019). A two-way analysis of variance (ANOVA) was used with the two factors (storage time and treatment) to test for differences among treatments and storage time and potential interactions. A Levene's test was used to test the homogeneity of variance. In case of significant differences within or among factors, a Tukey's post hoc test was performed to find the significant differences between samples at the 5% level ($p \leq 0.05$). The sensory descriptive profile analysis data were first analyzed by calculating Pearson's correlation coefficients of the sensory attributes. The attributes were assumed fully correlating when the specific coefficient was >0.90. Then a principal component analysis (PCA) was performed on standardized data with 105 observations and 11 non-correlating attributes. Three principal components explaining 70% of the variance were chosen, as they had an eigenvalue above one as indicated by a Screen-plot. The PCA is illustrated by a bi-plot, including the PC scores and loadings. Beta diversity was calculated with Bray-Curtis dissimilarity distances (Sørensen, 1948). The alpha diversities were determined by species richness (DeSantis et al., 2006) and Shannon entropy (Shannon, 1948) and analyzed with ANOVA and Kruskal–Wallis pairwise analysis (Kruskal and Wallis, 1952), respectively. The linear regressions were calculated in GraphPad Prims 9.3.1 (GraphPad Software Inc., CA, United States).

3 Results

3.1 Changes in the organoleptic and physical properties

3.1.1 Determination of shelf-life based on sensory evaluation

Based on the descriptive profile analysis as well as the appearance of off-odors, the shelf-life of sugar kelp from all treatments was determined to seven to 9 days when stored at +2.8°C. On day nine, off-odors were noted in a minimum of two out of three bags from all treatments. The off-odors were described as vinegar, acetic acid, old flower water, fermented, rotten, old hay, chlorine, or sulfuric. A Pearson's correlation analysis showed that some attributes correlated ($\rho > 0.90$) (data not shown), i.e., the terms *Umami* and *Boiled Peas* as well as *Fresh Sea* and *Rubber* correlated with each other. In the principal component analysis (PCA) of results from the sensory evaluation, the correlating attributes were regarded as redundant and only data for *Boiled Peas* and *Fresh Sea* were used for the PCA. The PCA bi-plot showed two notable patterns in the scores and loadings; a correlation between PC1 (explaining 38.8% of variation in the data) and storage time and PC3 (11.9%) and treatments (Figure 2). Following the PC1 of the PCA bi-plot, sugar kelp stored for 7 days or less was in the II and III quadrants (Figure 2), while spoiled sugar kelp (>7°days) was in the I and IV quadrants. The positive odor attributes, *Fresh Sea*, *Sweet*, *Sourish*, and *Boiled Peas*, correlated with the fresh sugar kelp (PC1 < 0). In

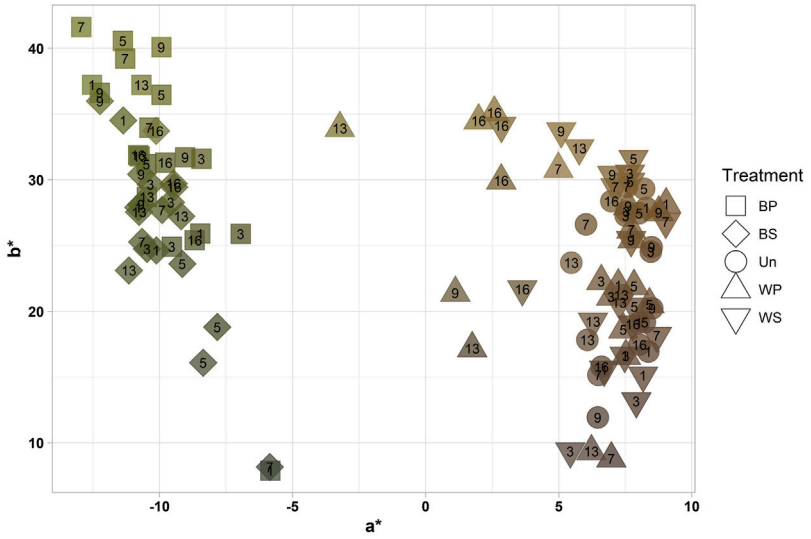


FIGURE 3
CIE L* a* b* color measurements showing the relationship between a* and b* of each sample measurement of untreated, washed and blanched sugar kelp during refrigerated storage (2.8°C) for 16 days. The treatments were blanched in potable water (BP), blanched in sea water (BS), untreated (Un), washed in potable water (WP) and washed in sea water (WS) with the storage days (1–16) indicated by the number within the data points. The color of the data points visualizes the actual CIE L* a* b* color. For a* < 0 the blanched samples dominate.

TABLE 2 Changes in vitamin C, pigments and iodine content of untreated, washed and blanched sugar kelp during refrigerated storage (2.8°C) for day 1 and 13.

		Pigments			
Treatment	**Iodine (μg g^{-1} DM)**	**β-carotene (μg g^{-1} DM)**	**Chlorophyll a (μg g^{-1} DM)**	**Fucoxanthin (μg g^{-1} DM)**	**Vitamin C (mg 100 g^{-1} ww)**
Untreated (Un)					
Day 1	2,002 ± 331^{A}	29.0 ± 20.9^{A}	1,158 ± 611^{A}	885 ± 355^{A}	8.73 ± 2.31^{BC}
Day 13	1,986 ± 145^{A}	16.1 ± 5.4^{A}	726 ± 472^{A}	513 ± 304^{A}	3.82 ± 0.46^{C}
Washed in potable water (WP)					
Day 1	1,547 ± 270^{B}	16.4 ± 2.3^{A}	713 ± 334^{A}	502 ± 275^{A}	16.4 ± 1.3^{AB}
Day 13	1,487 ± 542^{B}	30.5 ± 8.1^{A}	797 ± 104^{A}	467 ± 45^{A}	2.06 ± 0.75^{C}
Washed in sea water (WS)					
Day 1	1,303 ± 287^{B}	29.0 ± 6.7^{A}	670 ± 334^{A}	478 ± 139^{A}	19.4 ± 7.4^{A}
Day 13	1,349 ± 473^{B}	25.3 ± 5.8^{A}	795 ± 154^{A}	642 ± 183^{A}	2.97 ± 1.49^{C}
Blanched in potable water (BP)					
Day 1	195 ± 33^{C}	20.0 ± 1.9^{A}	540 ± 24^{A}	514 ± 49^{A}	<0.30
Day 13	239 ± 23^{C}	13.6 ± 1.2^{A}	496 ± 111^{A}	453 ± 101^{A}	<0.30
Blanched in sea water (BS)					
Day 1	200 ± 22^{C}	39.4 ± 13.0^{A}	1,037 ± 292^{A}	1,189 ± 272^{B}	0.638
Day 13	171 ± 27^{C}	17.9 ± 3.2^{A}	1,139 ± 607^{A}	1,192 ± 406^{B}	<0.30

Results are expressed as average ± standard deviations. $^{A–C}$ Capital letters indicate significant ($p < 0.05$, two-way ANOVA) differences between samples within the same column. ww is the abbreviation for wet weight. DM, is the abbreviation for dry matter.

TABLE 3 Texture properties and drip loss of untreated, washed and blanched sugar kelp during refrigerated storage (2.8°C) for day 1, 9 and 13.

	Texture		
Treatment	**Firmness (g)**	**Toughness (g s^{-1})**	**Drip loss (% ww)**
Untreated (Un)			
Day 1	1,635 ± 145^{C}	1891 ± 290^{A}	0.3 ± 0.2^{A}
Day 9	1,590 ± 498^{C}	1965 ± 762^{A}	0.5 ± 1.3^{A}
Day 13	1,667 ± 45^{C}	1822 ± 96^{A}	5.0 ± 2.3^{ABCD}
Washed in potable water (WP)			
Day 1	1,438 ± 109^{AC}	1,687 ± 228^{A}	2.0 ± 0.5^{AB}
Day 9	1,274 ± 309^{AC}	1,663 ± 363^{A}	5.5 ± 0.8^{ABCD}
Day 13	1,532 ± 146^{AC}	1829 ± 301^{A}	13.6 ± 6.6^{D}
Washed in sea water (WS)			
Day 1	1709 ± 346^{C}	2,183 ± 285^{A}	0.9 ± 1.4^{A}
Day 9	1,390 ± 591^{C}	1,698 ± 1034^{A}	4.5 ± 3.4^{ABCD}
Day 13	1,547 ± 180^{C}	1753 ± 162^{A}	2.2 ± 1.0^{AB}
Blanched in potable water (BP)			
Day 1	938 ± 242^{B}	1,551 ± 367^{A}	7.4 ± 0.6^{ABCD}
Day 9	1,119 ± 38^{B}	1,692 ± 204^{A}	12.0 ± 2.9^{CD}
Day 13	1,024 ± 72^{B}	1,605 ± 254^{A}	10.2 ± 3.4^{BCD}
Blanched in sea water (BS)			
Day 1	1,139 ± 208^{AB}	1972 ± 350^{A}	9.2 ± 3.1^{ABCD}
Day 9	1,134 ± 170^{AB}	1822 ± 429^{A}	11.5 ± 2.9^{CD}
Day 13	999 ± 290^{AB}	1,387 ± 167^{A}	7.0 ± 5.7^{ABCD}

Results are expressed as average ± standard deviations. $^{A–D}$ Capital letters indicate significant ($p < 0.05$, two-way ANOVA) differences between samples within the same column. The unit % ww is the abbreviation for percentage in wet weight.

contrast, the negative attributes, *Transparency*, *Slimy* and *Beach-cast*, correlated with spoiled sugar kelp (PC1 > 0). The panel did not identify the *Beach-cast* odor until day 13, and only for the samples blanched in sea water.

3.1.2 Changes in color, pigments and texture

The color of the sugar kelp was analyzed both physically and chemically. The results of the physical measures in CIE L* a* b* color space are shown in Figure 3. Two groups appeared, where blanching gave a green hue with an average a* = −10.4. In contrast, washing did not affect the color, which remained at an average a* = 6.47. The blanched samples did not change color over time, while the variability of the color of spoiled washed sugar kelp (days 13 and 16) increased without forming a second cluster.

No significant differences ($p > 0.05$) were found for the content of chlorophyll a (807 ± 362 μg g^{-1}) or β-carotene (23.7 ± 10.7 μg g^{-1}), regardless of the treatment or length of storage (Table 2). There was no significant difference in concentration on day 1 compared to day 13 for any treatments for the pigment fucoxanthin. A significantly higher fucoxanthin content ($p < 0.001$, F = 8.95) was found in sugar kelp blanched in sea water (1,189–1,192 μg g^{-1}) in comparison with sugar kelp from all other treatments and the untreated control (453–885 μg g^{-1}).

The textural property, firmness (i.e., the sampling point at which the sugar kelp ruptured) was significantly higher ($p < 0.001$, F = 10.2) for the untreated and washed sugar kelp, followed by sugar kelp blanched in sea water and lastly the sugar kelp blanched in potable water (Table 3). The order of firmness for the different treatments agreed with observations for the textural attributes *Leather* and *Silky* in the PCA biplot from the sensory analysis (Figure 2). Storage time did not influence firmness; toughness was also unchanged among treatments and storage time (Table 3).

3.1.3 Changes in water, water activity, ash, drip loss, pH and NaCl

The treatments influenced the water activity, water and ash content of the sugar kelp, where blanching in potable water led to significantly higher ($p < 0.05$) contents of water, higher water activity and lower ash content. Washing of sugar kelp in potable water also resulted in reduction in ash content (Table 4). None of the parameters were affected by the storage period. In contrast, NaCl content depended significantly on both storage time ($p = 0.003$, F = 9.55) and treatment ($p < 0.001$, F = 280) with interactions observed among the independent factors ($p < 0.001$, F = 7.97) (Table 4). The lowest NaCl content was found in the sugar kelp blanched

TABLE 4 Physicochemical properties and factors affecting microbial growth of untreated, washed and blanched sugar kelp during 2.8°C refrigerated storage at day 1, 9 and 13.

Treatment	Water (% ww)	Water activity (a_w)	Ash (% ww)	NaCl (% ww)	pH
Untreated (Un)					
Day 1	88.1 ± 2.4^{A}	0.985 ± 0.002^{BC}	3.06 ± 0.24^{B}	1.22 ± 0.17^{BC}	6.21 ± 0.08^{B}
Day 13	86.9 ± 5.5^{A}	0.981 ± 0.001^{BCa}	2.83 ± 0.28^{B}	1.75 ± 0.10^{AD}	5.76 ± 0.09^{D}
Washed in potable water (WP)					
Day 1	88.3 ± 2.1^{A}	0.986 ± 0.002^{B}	2.04 ± 0.12^{C}	1.18 ± 0.28^{BC}	6.22 ± 0.12^{B}
Day 13	89.4 ± 1.2^{A}	0.984 ± 0.003^{Ba}	2.60 ± 0.58^{C}	0.88 ± 0.05^{C}	5.60 ± 0.11^{D}
Washed in sea water (WS)					
Day 1	87.8 ± 2.2^{A}	0.982 ± 0.000^{AC}	3.21 ± 0.16^{AB}	1.46 ± 0.34^{BD}	6.33 ± 0.06^{B}
Day 13	88.3 ± 1.6^{A}	0.979 ± 0.001^{ACa}	3.34 ± 0.66^{AB}	1.97 ± 0.15^{AD}	5.87 ± 0.30^{D}
Blanched in potable water (BP)					
Day 1	91.2 ± 1.5^{B}	0.996 ± 0.002^{D}	0.916 ± 0.101^{D}	0.09 ± 0.13^{E}	8.67 ± 0.17^{E}
Day 13	91.1 ± 2.0^{B}	0.995 ± 0.001^{Da}	0.816 ± 0.193^{D}	0.03 ± 0.01^{E}	7.70 ± 0.07^{A}
Blanched in sea water (BS)					
Day 1	90.2 ± 2.8^{A}	0.978 ± 0.002^{A}	3.50 ± 0.22^{A}	2.19 ± 0.25^{A}	7.59 ± 0.20^{A}
Day 13	87.9 ± 4.0^{A}	0.979 ± 0.004^{Aa}	3.60 ± 0.11^{A}	2.85 ± 0.09^{F}	6.76 ± 0.30^{C}

Results are expressed as average ± standard deviations. [a] Data from day 9. [A–F] Capital letters indicate significant ($p < 0.05$, two-way ANOVA) differences between samples within the same column. The unit % ww is the abbreviation for percentage in wet weight.

in potable water (0.09% ww), and the highest in the sugar kelp blanched in sea water (2.19% ww). Regarding the storage period, the untreated sugar kelp, and the sugar kelp blanched in sea water, showed significant increases in NaCl ($p = 0.027$ (Un), $p = 0.033$ (BS)) over time. Initial pH values differed significantly ($p < 0.001$, F = 368) between 6.2–6.3 and 7.6–8.7 for untreated/washed and blanched sugar kelp, respectively, and decreased significantly ($p < 0.001$, F = 207) over time for all treatments to final values of 5.6–5.9 and 6.8–7.7 for the unheated and heated samples, respectively (Table 4).

The observed drip losses of untreated or washed sugar kelp were minimal within the sensory shelf-life, ranging from 0.3 to 5.5% (Table 3). After spoilage, the sugar kelp washed in potable water reached a significantly ($p < 0.05$) higher drip loss of 13.6% relative to day 1. The blanching treatments resulted in a higher drip loss with an average of 9.6% during the storage period of 13 days.

3.1.4 Respiration rates and quotient

The untreated and washed sugar kelp respired during the early storage period (Figure 4A). The change in respiration rates initially followed a linear decrease during the first 5 days of storage (linear regression, $p < 0.001$, F value of 28.79–60.69). The sugar kelp ceased to respire (intercept at the x-axis) between days 4 and 5. In contrast, the blanched sugar kelp exhibited no significant ($p > 0.05$, F value of 0.03–0.04) respiration indicating that endogenous enzymes of the sugar kelp, and not the commensal microorganisms, were responsible for the respiration. During the initial 6 days of storage, the average respiration quotient (RQ) for untreated, washed in potable or sea water (n = 15) was 0.97 ± 0.33 SD for sugar kelp.

3.2 Microbial changes during storage

3.2.1 Culture-dependent

The untreated and washed sugar kelp had initial AVC counts between 4.0–4.5 log(CFU g^{-1}), whereas blanching lowered AVC counts to 0.9–1.8 log(CFU g^{-1}) (Figure 5A). Microbial communities grew to AVC counts of 7.2–7.9 log(CFU g^{-1}) after 7 days in all samples, apart from in samples of sugar kelp blanched in potable water, which contained lower levels of 3.3–5.7 log(CFU g^{-1}) (Figure 5A). Based on the selective media used in the study, presumptive *Pseudomonas* spp. dominated the microbiota in sugar kelp blanched in potable water throughout the entire storage period (Figure 5B). As for the four other treatments, presumptive *Pseudomonas* spp. increased to levels of 5–6.3 log(CFU g^{-1}) after 7 days, thus remaining at 1.6–2.3 log below AVC counts. H_2S-producing bacteria, *Actinomycetes* and yeast showed no or limited growth during the storage period with initial levels of 0–2.5 log(CFU g^{-1}) and maximum levels of 5.2 log(CFU g^{-1}) (data not shown).

3.2.2 Culture in-dependent

Amplicon sequences of DNA extracted from all 30 fresh and spoiled macroalgal samples passed the quality control

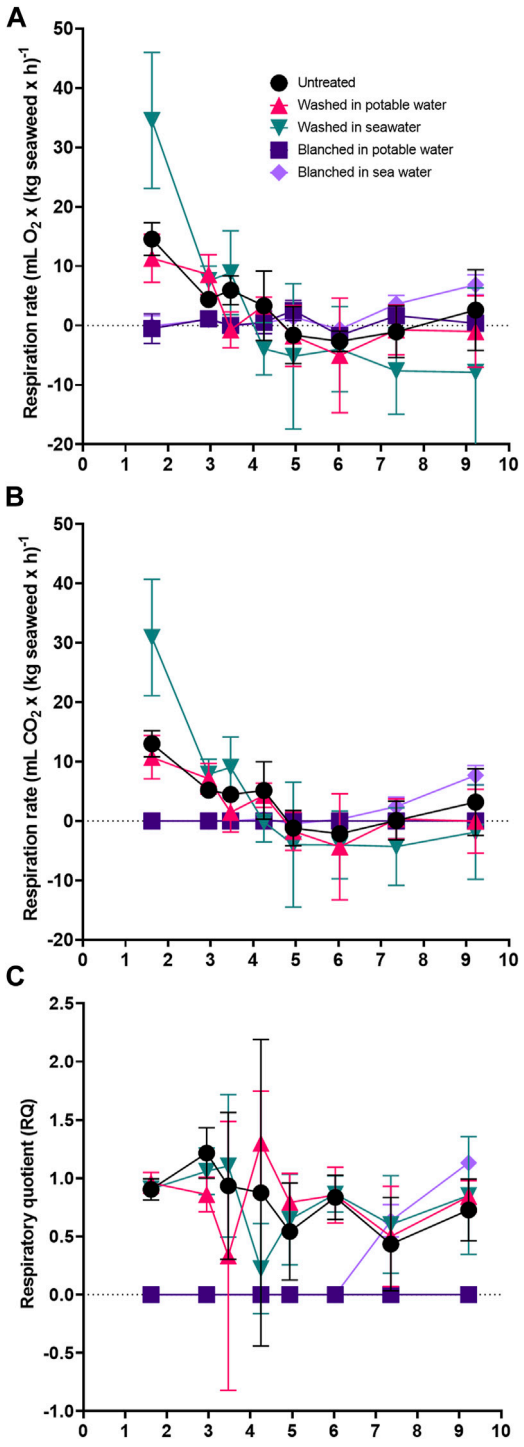


FIGURE 4
Respiration during refrigerated storage of untreated, washed and blanched sugar kelp during packaged refrigerated storage at 2.8°C for day 1–10. **(A)** Oxygen respiration rate. **(B)** Carbon dioxide respiration rate. **(C)** Respiratory quotient.

and were subjected to further bioinformatic analysis. Based on the rarefaction curves, the minimum sampling depth was set to 7,000 reads per sample, with a maximum sampling depth of 327,435 reads. All rarefaction curves levelled off at the minimum sampling depth (data not shown). A PCA plot of the beta-diversity, determined by the Bray-Curtis dissimilarity, shows how the phylogenetic beta-diversity of bacterial communities changed from being highly similar in fresh (day 1) samples (0.30–0.44) to being different among treatments on day 13 with separate clusters formed by WP, BP and one common cluster for BS, Un and WS (Figure 6A). The Bray-Curtis dissimilarity matrix (Figure 6B) showed the microbiota changed during storage, resulting in high dissimilarity (0.75–0.99) between the fresh and spoiled (day 13) samples. In addition, the spoilage microbiota depended on the type of treatment, except for the similarity (0.30) of the microbiota found on spoiled untreated sugar kelp and sugar kelp washed in sea water (Figure 6B). The spoilage microbiota on sugar kelp blanched in sea water was also similar (0.60–0.69) to the untreated and sugar kelp washed in sea water.

ASVs in fresh sugar kelp samples belonged to a diverse mixture of bacterial classes, including *Gammaproteobacteria*, *Alphaproteobacteria*, *Bacteroidia* and *Planctomycetes*. In contrast, families in *Gammaproteobacteria* came to dominate the spoiled macroalgae, with notable differences among the treatments, e.g., *Pseudomonadaceae* dominated sugar kelp blanched in potable water as opposed to *Pseudoalteromonadaceae* in sugar kelp blanched in sea water (Figure 6B). Untreated and washed spoiled sugar kelp contained a large proportion of *Psychromonadaceae*, while the presence of other families depended on the treatment. Calculation of the Bray-Curtis dissimilarity to compare the culture-independent and -dependent identification methods (number of families determined by amplicon sequencing and isolates of colonies) resulted in dissimilarity of 0.35, indicating that the spoilage microbiota results were comparable. Common for all treatments was a reduction of the observed ASVs from levels of 93–210 in fresh sugar kelp to 27–91 in spoiled sugar kelp (Table 5). Blanching in sea water resulted in the lowest ASVs levels. The Shannon entropy decreased in the spoiled samples (2.6–3.5) compared to the fresh samples (4.6–5.4).

3.3 Chemical changes during storage

The carbohydrate fucoidan comprised between 28 and 43% of the DM content in the untreated or washed sugar kelp (Table 6). This content remained unchanged during the storage time. However, blanching in potable water significantly diminished ($p < 0.001$, F = 51.3), the fucoidan concentration to levels of 4.77–11.0% DM. Interestingly, blanching in sea water increased the content to 46.5–54.3%

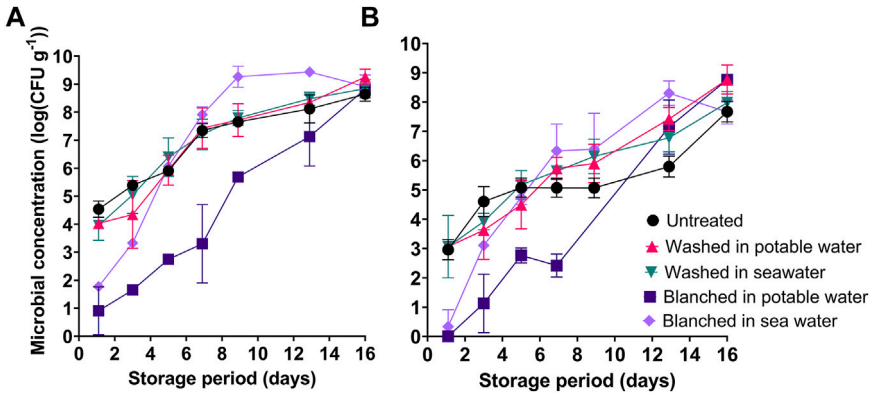
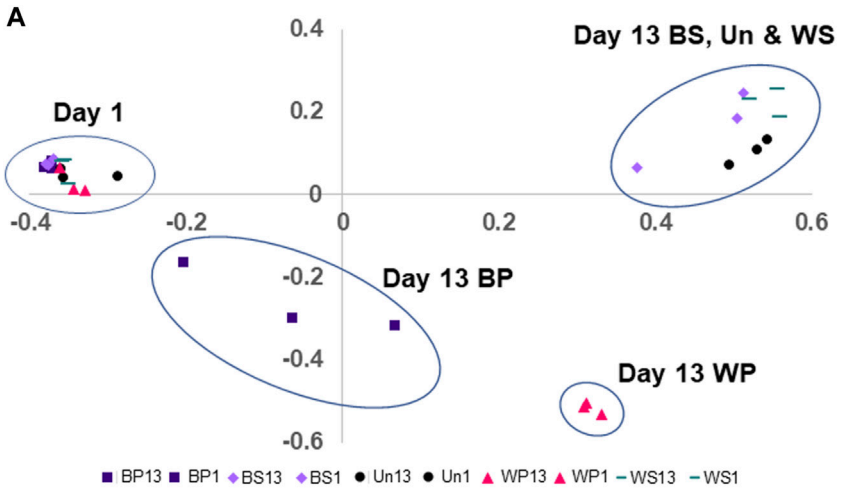


FIGURE 5
Microbial load in untreated, washed and blanched sugar kelp during refrigerated storage at 2.8°C. **(A)** Aerobic viable counts (AVC) on Marine agar; **(B)** *Pseudomonas* spp. counts on CFC agar.



B

	Day 1					Day 13				
	Un	WP	WS	BP	BS	Un	WP	WS	BP	BS
Day 1										
Untreated	0.22									
Washed in potable water	0.44	0.23								
Washed in sea water	0.44	0.43	0.26							
Blanched in potable water	0.41	0.42	0.39	0.16						
Blanched in sea water	0.43	0.44	0.32	0.30	0.20					
Day 13										
Untreated	0.95	0.97	0.96	0.97	0.97	0.17				
Washed in potable water	0.98	0.99	0.98	0.99	0.99	0.79	0.29			
Washed in sea water	0.97	0.98	0.98	0.99	0.99	0.30	0.89	0.15		
Blanched in potable water	0.80	0.75	0.80	1.00	0.82	0.97	0.97	0.98	0.39	
Blanched in sea water	0.99	0.99	0.99	0.81	1.00	0.69	0.92	0.60	0.99	0.24

FIGURE 6
(A) Shows the Bray–Curtis Principal Component Analysis of fresh and spoiled microbiota, circles have no statistical properties. **(B)** The Bray-Curtis dissimilarity matrix of bacterial communities found in fresh and spoiled untreated (Un), washed in potable water (WP), washed in sea water (WS), blanched in potable water (WP), or blanched in sea water (WS) sugar kelp. Blue color indicate low dissimilarity and red color indicate high dissimilarity.

TABLE 5 Bacterial species richness as measured by the number of amplicon sequence variants (ASVs) and Shannon entropy in samples of untreated, washed and blanched samples of sugar kelp stored at 2.8°C for day 1 and 13.

Treatment	Species richness (n of ASV)	Shannon entropy
Untreated (Un)		
Day 1	210 ± 44^{C}	5.4 ± 0.2^{A}
Day 13	91 ±8^{AB}	3.5 ± 0.4^{BC}
Washed in potable water (WP)		
Day 1	196 ± 24^{C}	4.6 ± 0.6^{DE}
Day 13	52 ±2^{AB}	2.6 ± 0.2^{CFG}
Washed in sea water (WS)		
Day 1	117 ± 17^{A}	4.7 ± 0.2^{DF}
Day 13	70 ± 18^{AB}	2.9 ± 0.1^{EG}
Blanched in potable water (BP)		
Day 1	107 ± 23^{A}	5.0 ± 0.1^{G}
Day 13	73 ± 30^{AB}	3.4 ± 0.8^{C}
Blanched in sea water (BS)		
Day 1	93 ± 26^{AB}	4.6 ± 0.1^{F}
Day 13	27 ± 16^{B}	2.6 ± 0.2^{CF}

Results are expressed as average ± standard deviations. $^{A–G}$ Capital letters indicate significant ($p < 0.05$, two-way ANOVA) differences between samples within the same column.

DM. The simple sugars, glucose and maltose, were detected in some of the untreated or washed samples, but not in any of the blanched samples (data not shown). The content of the sugar alcohol mannitol was unaffected by storage time but significantly reduced ($p < 0.001$, F = 79.3) in blanched sugar kelp from both treatments.

Free glutamic and aspartic acids were detected in the untreated or washed sugar kelp but not in blanched sugar kelp (Table 6). Initial levels of the amino acids underwent a significant decrease ($p < 0.001$, F > 25.8) during the storage period. Citric acid was the only organic acid, aside from Vitamin C, detected. Similarly to the two amino acids, it was only detected in the untreated samples and some of the washed samples, while being below the detection limit in all blanched samples. Levels of citric acid remained constant during storage. Both blanching treatments degraded vitamin C to a level that was below the detection limit (Table 2). In contrast, vitamin C occurred in levels of 8.7 mg 100 g^{-1} ww for untreated and 16–19 mg 100 g^{-1} ww for washed sugar kelp, respectively. The content of vitamin C decreased significantly ($p < 0.001$, F = 59.4) over the storage time for non-blanched sugar kelp. Iodine showed no changes during storage, and the total iodine for untreated sugar kelp was 2,002 µg g^{-1} DM on day 1, which was significantly higher ($p < 0.001$, F = 66.9) than levels found in washed (1,303–1,547 µg g^{-1} DM) or blanched (195–200 µg g^{-1} DM) sugar kelp (Table 2).

4 Discussion

To the best of our knowledge, the present study represents the to-date most comprehensive investigation of the shelf-life of sugar kelp during refrigerated storage. A shelf-life was established to be 7–9 days for fresh washed or lightly heat-treated sugar kelp stored at 2.8 ± 0.4°C. The short shelf-life is in agreement with previous shelf-life studies of other brown, green, and red macroalgae during refrigerated storage at 2 to 7°C. This indicates that macroalgae are highly perishable food products with sensory shelf-lives between 3–14°days depending on species and washing treatment (Nayyar, 2016; Nayyar and Skonberg, 2019). To compare shelf-lives from different studies and at different storage temperatures, all shelf-lives were transformed using the relative rate of spoilage square-root model (RRS) (Dalgaard, 2002). Nayyar (2016) reported the shelf-life for sugar kelp to be 12°days when stored at 1.1°C. Our predicted shelf-life for sugar kelp at the same temperature would be 9–12°days. A study of thawed and chilled (refreshed) *Undaria pinnatifida* (wakame) stored at 10°C had a shelf-life of 2–3°days based on the overall acceptability score (Choi et al., 2012). Using the RRS, our sugar kelp was predicted to have a shelf-life of 3.7°days if stored at 10°C. Interestingly, Nayyar (2016) studied another brown algal and found a significantly faster deterioration for winged kelp (*Alaria esculenta*). In addition to species variation, a seasonal variation was observed with a faster deterioration during the winter compared to the summer season (Nayyar, 2016). The difference between species and season highlights the variations and the need for further studies of the shelf-life of different macroalgae, seasons and cultivated or wild collected kelp. Two studies disagreed with the general acceptance of kelp as a highly perishable food product (Perry et al., 2019; López-Pérez et al., 2020). Lightly salted (a_w of 0.96, 30–50 g salt kg^{-1}) winged kelp stored at 5°C was found to have a 6-week shelf-life based on consumer acceptance (Perry et al., 2019). However, the development or changes of sensory properties during the storage period were not described. López-Pérez et al. (2020) found the sensory shelf-life of the raw untreated brown macroalga *Laminaria ochroleuca* to be under 60°days at a storage temperature of 5°C. However, the sensory properties of the sugar kelp were not tested between day one and day 60, during which the microbial concentration increased from initial levels of 5 log(CFU g^{-1}) to 8 log(CFU g^{-1}) within the first 40°days of storage.

The respiration of plants and macroalgae acts as a metabolic process to maintain cell viability and could be used as a freshness indicator. The non-blanched sugar kelp showed active respiration in the initial storage phase (Figure 4A). The respiration rate of sugar kelp in the present study dropped in washed and untreated treatments to zero within 5 days of storage, while the blanching inhibited the respiration immediately after treatment, and it remained zero for the first 6 days of storage (Figures 4A,B). From day 7 of storage, we

TABLE 6 Content of water soluble carbohydrates, mannitol, free amino acids and citric acid in untreated, washed and blanched sugar kelp during refrigerated storage for day 1, 7 and 13.

	Carbohydrates	Sugar alcohols	Free amino acids		Organic acids
Treatment	Fucoidan (% of DM)	Mannitol (% of DM)	Glutamic acid (mg g^{-1} DM)	Aspartic acid (mg g^{-1} DM)	Citric acid (mg g^{-1} of DM)
Untreated (Un)					
Day 1	29.4 ± 1.8^{A}	16.6 ± 2.8^{A}	0.977 ± 0.295^{BC}	2.62 ± 0.82^{BDE}	3.44 ± 1.26
Day 7	34.6 ± 10.6^{A}	18.0 ± 4.5^{A}	0.556 ± 0.123^{AB}	1.42 ± 0.15^{CDE}	4.28 ± 0.61
Day 13	39.9 ± 18.1^{A}	12.8 ± 2.9	0.315 ± 0.186^{A}	0.779 ± 0.853^{C}	4.55 ± 0.84
Washed in potable water (WP)					
Day 1	28.4 ± 3.4^{A}	15.6 ± 1.8^{A}	1.17 ± 0.51^{C}	5.89 ± 2.92^{A}	2.58^{b}
Day 7	35.2 ± 15.9^{A}	15.9 ± 0.6^{A}	0.977 ± 0.514^{BC}	2.20 ± 1.41^{BCDE}	4.49^{a}
Day 13	27.7 ± 5.1^{A}	13.8 ± 4.3^{A}	0.558 ± 0.231^{AB}	1.40 ± 0.24^{CDE}	5.36^{a}
Washed in sea water (WS)					
Day 1	42.7 ± 11.0^{A}	13.2 ± 0.7^{A}	1.20 ± 0.31^{C}	3.19 ± 1.19^{B}	3.04^{a}
Day 7	32.8 ± 3.6^{A}	15.1 ± 1.5^{A}	0.579 ± 0.161^{AB}	1.08 ± 0.23^{CD}	4.11 ± 1.45
Day 13	37.8 ± 8.0^{A}	11.6 ± 3.5^{A}	0.651 ± 0.223^{AB}	2.88 ± 1.28^{BE}	4.75 ± 1.38
Blanched in potable water (BP)					
Day 1	11.0 ± 4.6^{B}	0.675 ± 0.192^{B}	n.d	n.d	n.d
Day 7	4.77 ± 1.74^{B}	0.799^{b}	n.a	n.a	n.d
Day 13	10.3^{a}	0.973 ± 0.197^{B}	n.a	n.a	n.d
Blanched in sea water (BS)					
Day 1	54.3 ± 5.5^{C}	9.91^{a}	n.d	n.d	n.d
Day 7	50.4 ± 5.6^{C}	n.d	n.a	n.a	n.d
Day 13	46.5 ± 4.2^{C}	n.d	n.a	n.a	n.d

Results are expressed as average ± standard deviations. n.a. not analyzed. $^{A–E}$ Capital letters indicate significant ($p < 0.05$, two-way ANOVA) differences between samples within the same column. ^{a} n = 2 (others below limit of quantification). ^{b} n = 1 (others below limit of quantification). DM, is the abbreviation for dry matter.

observed increased respiration for blanched sugar kelp in sea water. The increased respiration seems to be correlated with increased AVC levels. Interestingly, the lack of respiration in sugar kelp blanched in potable water and low levels of AVC indicate modest microbiological activity throughout the storage period.

The average respiration quotient (RQ) was 0.97 during the initial 6 days of storage for untreated, and sugar kelp washed in potable or washed in sea water. If the metabolic substrates were carbohydrates, then the RQ would be assumed to be equal to 1.0. The RQ would be lower in lipid-driven metabolic pathways and higher when consuming amino acids (Fonseca et al., 2002). An RQ of 0.97 in sugar kelp indicates that carbohydrates are the primary energy source. The value was within the range of previously reported RQ values for algae. Literature RQ values were between 0.6 and 1.2, with no differences among species for three macroalgae: *Ulva* sp. (*Chlorophyta*), *Pterocladia capillacea* Bornet (*Rhodophyta*) and *Sargassum* sp. (*Ochrophyta*) (Carvalho and Eyre, 2011). Similar RQ values have also been observed in harvested fruit and vegetables, with a reported range of 0.7–1.3 (Kader, 1987).

The decrease in the beta- and alpha-diversity between fresh and spoiled sugar kelp (Tables 5 and Figures 6, 7) are similar to those observed in seafood products (Chaillou et al., 2015; Kuuliala et al., 2018; Sørensen et al., 2020). Bacterial isolates from the spoiled sugar kelp belonged predominantly to *Pseudoalteromonadaceae, Psychromonadaceae* and *Pseudomonadaceae* (Figure 6) and agreed with the 16S rRNA amplicon sequence data. Sørensen et al. (2020) showed the same agreement between 16S rRNA amplicon sequencing and identification of bacterial isolates in Atlantic cod. *Psychromonadaceae*, *Marinomonadaceae* and *Pseudoalteromonadaceae* dominated the microbiota of spoiled untreated sugar kelp. The first two families also came to dominate in an unsuccessful natural fermentation of sugar kelp from Greenland (Sørensen et al., 2021), indicating a potential role of these bacteria in spoilage of macroalgae. In a study of the bacterial communities on six different macroalgae, the same families were also identified in fresh and stored macroalgae, however, they were not dominating the microbiota (Picon et al., 2021). The application of the RSS model to the shelf-life studies of *S. latissima* (Nayyar, 2016;

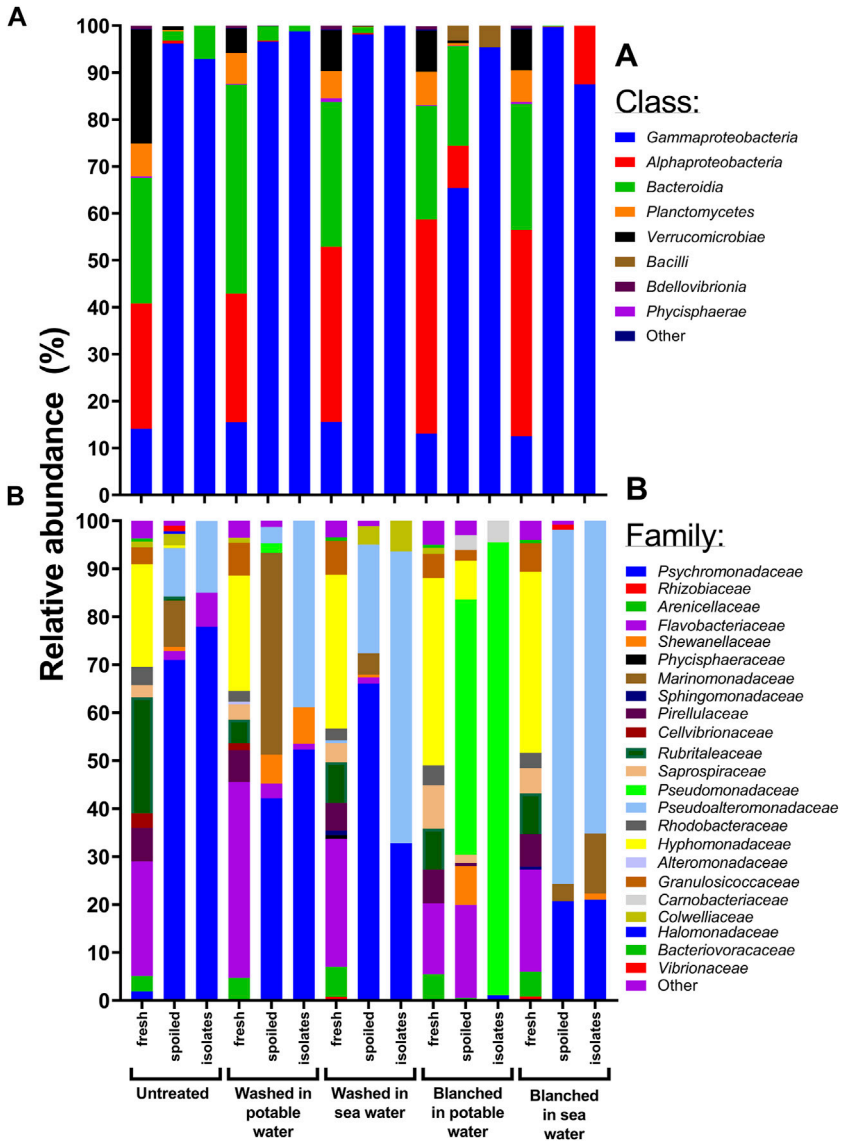


FIGURE 7
Composition of bacterial communities in untreated, washed and blanched sugar kelp as determined after 1 (fresh) and 13 days of storage (spoiled) at 2.8°C. Fresh and spoiled relative abundance are based on amplicon sequencing variants, while isolate abundance values are based on identified representative isolates from day 9. **(A)** Shows the composition of bacterial taxonomic classes. **(B)** Is more detailed with the composition of bacterial taxonomic families.

our study) and *U. pinnatifida* (Choi et al., 2012) indicates that the shelf-life may be limited by spoilage caused by the growth of psychrotrophic (T_{min} = −9°C) microorganisms (Ratkowsky et al., 1982). The composition of the sequenced microbiota in the spoiled sugar kelp included members of *Pseudoalteromonadaceae, Psychromonadaceae* and *Pseudomonadaceae,* all of which are predominantly psychrotrophic bacteria. Future research should determine the spoilage potential for the isolated microorganisms (Figure 6), and thereby elucidate which of the bacteria are responsible for the spoilage characteristics described in the sensory analysis (Figure 2).

The search to identify single-compound quality indices (SCQI) uncovered that the following parameters were time-dependent (Table 7) and, therefore, potential SCQIs: pH, drip loss, NaCl, free glutamic- and aspartic acid, vitamin C and microbial load. Diving into the potential parameters of pH, NaCl, free glutamic- and aspartic acid, and vitamin C, it was observed that although the parameters were storage dependent (Table 7), no common level could be established to determine the shelf-life. To exemplify, the

TABLE 7 Summary of parameter dependency on storage time or post-harvest treatment. "x" marks whether the factor was dependent on storage time and/or varied due to treatment (untreated, washed or blanched in potable or sea water).

Factor	Time dependent	Treatment dependent
Sensory		
Positive odors	x	
Beach-Cast odor	x	x
Transparency	x	
Slimy	x	x
Silky		x
Resilient and uniform color		x
Physical		
Drip loss	x	x
Color		x
Texture		x
Ash		x
Water content		x
Water activity		x
pH	x	x
NaCl	x	x
Microbial		
Respiration rate	x	x
Microbial load	x	x
Species community dissimilarity	x	x
Species richness	x	x
Shannon entropy	x	x
Chemical		
Fucoidan		x
Mannitol		x
Citric acid		
Glutamic acid	x	x
Aspartic acid	x	x
Vitamin C	x	x
Fucoxanthin		x
Chlorophyll a		
β-carotene		
Iodine		x

pH dropped by 0.45–0.97 units during the 13 days of storage (Table 4), but the initial starting points (after 1 day of storage) were not identical, and the difference in the range of pH values, from 6.21 to 8.67, was larger than the drop. The drawback of having an SCQI depending on changes instead of a set level is the need to establish different SCQIs for each treatment process. A microbial SCQI has been proposed for both protein-based seafood and terrestrial vegetables, with spoilage occurring when the bacterial concentration exceeds 7 to 7.7 log(CFU g^{-1}) (Corbo et al., 2006; ICMSF, 2011). Using 7 log(CFU g^{-1}) as an SCQI, the microbial shelf-life would be 7 days for all treatments, except for sugar kelp blanched in potable water (Figure 5A). In the case of sugar kelp blanched in potable water, the microbial shelf-life would be 13 days, which would be a significant overestimation compared to the sensory shelf-life. However, it is possible that the use of MA, with its high salt and mineral content, to quantify bacteria may have led to an underestimation of the number of microorganisms in samples of sugar kelp with almost no NaCl present (0.1%) after blanching in potable water. Based on the evaluation of the parameters in Table 7, a threshold of shelf-life SCQI of 7 log(CFU g^{-1}) determined on MA (15°C, 7 d) is suggested.

Previous studies of the microbial quality, i.e., the AVC, of macroalgae have mainly used agar substrates without high salt or mineral concentrations, such as Plate Count Agar (PCA), 3M Petrifilm AVC and tryptic soy agar (TSA) (Choi et al., 2012; Nayyar and Skonberg, 2019; Perry et al., 2019). PCA has, however, been reported to underestimate marine-associated bacterial concentration compared to MA by approximately 2 log(CFU g^{-1}) (Broekaert et al., 2011; del Olmo et al., 2020). Besides the use of non-optimal agar substrates, the choice of incubation temperature may also lead to the use of temperatures close to T_{max} for psychrotrophic bacteria or above, e.g., 25–37°C (Liot et al., 1993; Choi et al., 2012; Nayyar and Skonberg, 2019; Perry et al., 2019; del Olmo et al., 2020; López-Pérez et al., 2020). In future studies, it is recommended that the AVC be determined on MA incubated for 7 days at 15°C during storage trials with refrigerated macroalgae unless the salt content has been reduced by treatments such as blanching in potable water. It is known that the microbiota is highly dependent on the incubation temperature (Dalgaard and Jørgensen, 2000).

The choice of treatment affects the final product. Interestingly, the sensory analysis showed a decrease in the odor attributes "sweetness" and "umami", while the chemical indicators aspartic and glutamic acid also experienced a reduction during storage. The concentration of the sugar alcohol mannitol decreased by 11–23% in untreated or washed sugar kelp during the storage trial. Mannitol is used as an industrial sweetener and is estimated to be half as sweet as sucrose (Schiweck et al., 2012), which might explain the decrease in the sensory score. Umami flavor is only associated with two amino acids in humans: aspartic and glutamic acid (Chandrashekar et al., 2006). Both amino acids were significantly reduced during storage (Table 6). Blanching removed both free amino acids and almost all the water-soluble mannitol (Table 6). Previously, it has been shown that blanching decreases the content of amino acids in sugar kelp and the calculated carbohydrates (Nielsen et al., 2020). Our finding showed that blanching totally removed the free amino acids and not necessarily the ones incorporated in protein. The bioactive water-soluble fucoidan concentration tended to be related to the level of NaCl in the process water and the sugar kelp (Table 6). The correlation between NaCl and fucoidan levels might be due to the affinity of fucoidan for NaCl. During extraction of fucoidan, it has been shown that the molarity of

NaCl strongly affects the eluted fucoidan, where the higher the NaCl concentration in the washing or blanching water, the less fucoidan is eluted from the sugar kelp (Zayed et al., 2016). The fucoidan affinity for NaCl could explain the finding of 54.3% DM fucoidan in the sugar kelp blanched in sea water compared to 11.0% in the sugar kelp blanched in potable water.

The initial levels of vitamin C in untreated or washed sugar kelp ranged from 8.7 to 19.4 mg 100 g^{-1}. To compare our finding with concentrations found in the literature, the unit was recalculated to mg g^{-1} DM, giving a range of 0.73–1.59 mg g^{-1} DM. The concentrations were higher in our study compared to reports of washed or dried sugar kelp (Sappati et al., 2019) but within a similar range to other macroalgae (Nielsen et al., 2021). During storage, the vitamin C concentration decreased significantly (Table 2), likely due to oxidation to protect degradation of other constituents (Spínola et al., 2014). No other studies of vitamin C during storage have been conducted with sugar kelp or other macroalgae. However, the same decreasing trend has been described in storage experiments with broccoli, green beans, peas, and spinach (Balan et al., 2016). Vitamin C has been proposed as an SCQI in fresh vegetables and freshness indicator of frozen products (Favell, 1998). However, vitamin C is very sensitive to heat, light and oxygen. Thus, blanching of sugar kelp resulted in completely removal of vitamin C. The same result was observed in boiled (for 15–20 min) macroalgae *U. pinnatifida* and *H. elongata*, which decreased the vitamin C content below detection limit (Amorim et al., 2012; Amorim-Carrilho et al., 2014; Nielsen et al., 2021). In addition to sensitivity to processing, vitamin C fluctuates between seasonality, location, and storage (Nielsen et al., 2021).

Washing in potable water increased the drip loss within the shelf-life to 5.5% (Table 3), which was similar to drip losses found in *U. rigida* (Sánchez-García et al., 2021), *Palmaria palmata* and *Gracilaria tikvahiae* (Nayyar and Skonberg, 2019). Higher drip losses were observed in blanched sugar kelp, which could be due to the short drip-drying treatment before packaging or blanching-induced changes to the sugar kelp tissue. More research would be required to understand and quantify sugar kelp's drip-loss level to ensure the best quality and correct labelling of the product. The treatment and packaging atmosphere might be the primary factors to investigate for future research.

The findings and results presented in this article aim to assist food manufacturers and authorities in the establishment of a best practice for chilled storage of sugar kelp. All tested treatments resulted in a shelf-life of 7–9 days at 2.8 ± 0.4°C. The microbiota associated with the sugar kelp became dominated by Gram-negative spoilage bacteria at the end of shelf-life. Among the possible SCQIs investigated in this study, the recommended SCQI would be a threshold AVC of seven log (CFU g^{-1}) determined on MA (15°C, 7 days). Future research is needed to establish a chemical SCQI, preferably a compound whose development correlates to the activity of spoilage organisms and hence development of sensory spoilage characteristics. Additional post-harvest washing of the kelp does not prolong the shelf-life and the type of wash water has no effect on the quality of the chilled kelp. The use of blanching reduced the iodine content to more tolerable levels. Moreover, blanching in potable water lowered the NaCl content to undetectable levels. For kelp industries wanted a low iodine and sodium product, the best practice would be a 2 min blanching in potable water at 80 C followed by quick cooling. The negative effect of blanching is the reduction of vitamin C content to below the detection limit, which indicates that other valuable compounds might decrease due to blanching as well including the "umami amino acids", aspartic and glutamic.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.ncbi.nlm.nih.gov/bioproject/PRJNA788340.

Author contributions

CW: Conceptualization, Methodology, Investigation, Formal analysis, Writing—original draft, Writing—review and editing, Funding acquisition. JS: Conceptualization, Methodology, Investigation, Formal analysis, Writing—original draft, Writing—review and editing. KK: Conceptualization, Investigation, Funding acquisition, Writing—Review and Editing. GH: Supervision, Methodology, Writing—review and editing. SH: Funding acquisition, Supervision, Writing—Review and Editing. LH: Funding acquisition, Supervision, Writing—Review and Editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Paper 5

Nutritional value, bioactive composition, physico-chemical and sensory properties of *Ulva* sp. and *Fucus vesiculosus* depending on post-harvest processing: a drying comparison study

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Nutritional value, bioactive composition, physico-chemical and sensory properties of *Ulva* sp. and *Fucus vesiculosus* depending on post-harvest processing: a drying comparison study

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Abstract

Drying is an important post-harvest process to preserve seaweed as they are highly susceptible to spoilage due to their high moisture content. Drying can be performed in multiple ways by changing the temperature, pressure, air flow, and humidity. Therefore, the choice of drying method can affect the quality of the product in terms of sensory, chemical, and physico-chemical properties. Seaweeds contain nutrients and bioactive compounds, which include protein, lipids, carbohydrates, vitamins, and minerals, among other. The compounds also impact properties such as texture, taste, odor, and appearance. However, there is currently limited knowledge about how different drying methods affect the quality of seaweed products. In the paper we demonstrate how convective drying (52 °C), microwave-vacuum drying (-40 to 40 °C at 10 Pa), and freeze drying (-20 to 20 °C at 20 Pa) influence the food quality of *Fucus vesiculosus* and *Ulva* sp. by investigating physico-chemical properties such as water holding capacity, water absorption, and color, the changes in some of the chemical compounds such as macronutrients, fatty acids, amino acids, antioxidants, and pigments, as well as the taste, odor, appearance, and texture within sensory attributes. This study found that different drying methods have a species-dependent influence on the quality of seaweed, with *Ulva* sp. showing more similar quality products using microwave-vacuum and freeze-drying methods, while the drying method for *F. vesiculosus* should be selected based on the desired food quality due to significant variations between the drying methods.

1. Introduction

Seaweed is used as food for several purposes such as extracted ingredients (gelling agents), supporting the flavor (umami), nutritional composition (protein, minerals) or as a crispy snack as it is. Holdt & Kraan (2011) summarizes the nutritional composition and other interesting bioactive compounds of brown, red, and green seaweeds, including protein and amino acids, lipid and fatty acids, polysaccharides and dietary fibers, vitamins, and minerals. Some of these compounds are also important regarding the physico-chemical properties, e.g., water holding capacity and texture, which are related to the sensory properties of the seaweed.

The protein content varies between seaweed species. In brown seaweed, the protein content is generally low (5-24% dw), compared to red and green seaweed, which have a protein content between 10-47% dw (Mohamed et al., 2012). The protein content of seaweed varies greatly with season and harvest location. Hence, for *Fucus* sp. the protein content ranges between 1.4-17% dw, while for *Ulva* sp. it has a range of 4-44% dw (Holdt & Kraan, 2011). The proteins found in seaweed are found to contain all the essential amino acids as well as aspartic acid and glutamic acid, which are associated with umami flavor (Yamaguchi, 1991). Brown seaweed (*Durvillaea antarctica*) has been shown to have three times more umami flavor compared to green seaweed (*Ulva* sp.), related to high amount of aspartic acid and glutamic acid (Figueroa et al., 2022). Processing of seaweed can reduce the free amino acids and thereby change the flavor (Wirenfeldt et al., 2022).

The lipid content of seaweed is generally low (0.5-3.1% dw for *Fucus* sp. and 0.3-1.6% dw for *Ulva* sp. (Holdt & Kraan, 2011)). However, the composition of fatty acids of most seaweed species is rich in some of the same long-chain polyunsaturated omega-3 fatty acids associated with fish and seafood, especially EPA (C20:5, n-3). Seaweed can therefore be a great source of supply of EPA for the maintenance of health (Murata & Nakazoe, 2001). However, EPA containing foods are compromising a long shelf-life due to EPA's high susceptibility to oxidative degradation during processing and storage (Arab-Tehrany et al., 2012).

Phlorotannins are the major group of phenolic compounds of brown seaweed. Phlorotannins constitute an extremely heterogeneous group of molecules (structure and polymerization degree heterogeneity) providing a wide range of potential

biological activity, e.g., antioxidant, anti-coagulant and anti-enzymatic (Karthik et al., 2016). *Fucus* sp. are especially rich in phlorotannins (approximately 14% dw) (Holdt & Kraan, 2011), which have evidently antioxidant activity (Hermund et al., 2018), and potential candidates for the development of unique natural antioxidants for further industrial applications as functional foods (Li et al., 2009). Moreover, other bioactive compounds from seaweed are the pigments carotenoids, which possess functional properties and have been associated with antioxidant activity (Stahl & Sies, 2003). Of the different types of carotenoids, green seaweed species include β-carotene, lutein, and some xanthophylls, whilst brown seaweed species are associated with β-carotene and fucoxanthin (Haugan & Liaaen-Jensen, 1994). Fucoxanthin is the dominant carotenoid in brown seaweed ranging from 172 to 720 mg kg^{-1} dw, with maximal concentration in *Fucus serratus* (Holdt & Kraan, 2011).

Drying is the oldest method of preserving food, and even today, it remains a critically important and widely used process operation for long-term storage by removing water to extend the shelf-life of food products. The process results in a food product with a low moisture content and low water activity, reducing the possibility of chemical reactions that lead to off-flavors and discoloration, hinders enzymatic activity and the growth of microorganisms, and may even eliminate bacteria (Claussen et al., 2007). Seaweeds are highly susceptible to spoilage (Wirenfeldt et al., 2022) due to their high water content ranging from 73 to 94% (Holdt & Kraan, 2011). Therefore, drying is an essential process to achieve shelf-stable seaweed products. Although several drying methods are available, some compromise quality for a fast drying rate. The choice of drying method will have varying effects on the quality of the product, particularly in terms of chemical, sensory, and physico-chemical properties.

Convective air drying is a process that utilizes either natural or forced convection of air to remove surface moisture from food. The efficiency of the drying process is mainly affected by air temperature, airflow rate, and humidity levels. Although higher temperature and airflow can increase energy transfer, they can also damage the quality of food by causing case hardening, nutrient loss, and flavor deterioration. Thus, there are other alternative methods available to achieve better quality. Freeze-drying is a superior method that maintains the quality of the food. This method relies on sublimation and operates at low-temperature, reduced-pressure conditions. The product is first frozen to -20 to -60 °C and then placed in a low-pressure chamber.

Through freeze-drying, the structure of the food is persevered leading to a porous structure, and minimal loss of flavor, and nutrient content. Thus, due to its ability to retain food quality, freeze-drying is widely regarded as the preferred method for drying of high-quality food (Mujumdar, 2014). However, it is an energy-intensive, and time-consuming method. As an alternative, microwave-vacuum drying uses microwave radiation and low pressure to dry products faster than freeze-drying. Unlike freeze-drying, which dries from the external part of the food to the internal, microwave-vacuum drying generates energy within the food matrix (volumetric or in-out), it particularly is heated where the water is present, which causes the water to diffuse out to the surface as vapor. As a result of its fast-drying rate, microwave-vacuum drying presents an attractive alternative to freeze-drying (Scaman et al., 2014).

F. vesiculosus and *Ulva* sp. are interesting from a food perspective due to their unique composition of essential nutrients and bioactive compounds (Burtin P, 2003). The two seaweed species *Ulva* sp. and *Fucus vesiculosus* are growing as wild populations in the Danish inner waters and are of industrial interest. Consequently, it is critical to characterize how post-harvest processing affects the food quality such as the nutrients and bioactive compounds.

The aim of this study was to investigate the effect of microwave-vacuum drying, freeze-drying and convection drying of the two species *Ulva* sp. and *Fucus vesiculosus*. The quality of the dried seaweeds was compared with regards to color, sensory (flavor, texture, appearance, and taste), moisture, minerals, protein, amino acid and fatty acid profiling, pigments, antioxidant, water holding and binding capacity. The assessment resulted in scientific insight into the drying methods and their effect on the seaweed food quality.

2. Materials and Methods

2.1. Seaweed material and experimental design

Two seaweed species were harvested from wild populations in October 2020 in Danish inner waters. The brown seaweed species *Fucus vesiculosus* was harvested in Juelsminde, Denmark (N55°70’ E10°03’) and the green seaweed *Ulva* sp. was harvested in Isefjord, Denmark (N55°56’ E11°46’). All seaweed material was frozen at

-40 ºC and kept below -20 ºC until further processing. Each batch of drying was 1.5 kg wet weight.

The convection drying was carried out at an industrial setup. The product was thawed, and the excess water was removed. The product had air flow directly to it from beneath (52 °C, 11% relative humidity). *Ulva* sp. was dried for 70 minutes and *F. vesiculosus* for 68 minutes. For freeze drying the product was frozen and the initial drying temperature of the product was -20 °C with the pressure in the chamber at 20 Pa, and the end of drying, the product reached a final temperature of 20 °C. Microwave-vacuum drying was performed in a rotary drum with a pressure of 10 Pa. The initial temperature of the product was -40 ºC and reached a final temperature of 40 ºC. All drying methods were done three times, resulting in three treatment replicates.

2.2. Physico-chemical properties of *Ulva* sp. and *F. vesiculosus*

The physico-chemical properties investigated of the two seaweed species were the water activity (a_w), color, water absorption and water holding capacity.

2.2.1. Water activity

a_w was measured by using a water activity meter (Aqua Lab model 4TE, Decagon devices Inc., Pullman, US).

2.2.2. Color measurement

Color was measured by a Chroma meter (CR-200, Konica Minolta, Japan) recording the CIE L* a* b* color scale. Approximately 5 grams of samples were added to a petri dish with a white surface (L* = 89.0, a* = -4.01, b* = 4.27) underneath. The samples were measured at five random locations for each sample.

2.2.3. Water absorption and water holding capacity

Water absorption was quantified by mixing ground dry samples (0.2-0.3 g) with 10 mL distilled water by vortex mixing in a 15 mL centrifuge tube. The analysis was performed in duplicates. The tubes were incubated at room temperature overnight (22 hours). Water absorption was calculated by decanting excess water and using the equation:

$$Water\ absorption = \frac{(m_{SW} + m_p - m_0)}{m_0}$$

Where m_{sw} is the mass of the seaweed and the water that has been absorbed by the seaweed, m_p is the mass of the particles lost when decanting excess water, and m_0 is the initial mass of the ground seaweed samples.

Water holding capacity was determined by centrifugation by applying the tubes containing the swelled seaweed to 3,000 g for 20 min, then decanting excess water, and using the equation:

$$Water\ holding\ capacity = \frac{(m_c - m_0)}{m_0}$$

Where m_c is the mass of seaweed after centrifugation and decanting.

2.2.4. Qualitative image analysis

Imaging was recorded with a VideometerLab2 device (Videometer A/S, Denmark). The camera was calibrated by three plates: a white for reflectance correction, a dark for background correction and a dotted plate for pixel position calibration.

2.3. Chemical composition of *Ulva* sp. and *F. vesiculosus*

All chemical analyses were carried out in duplicate (n = 2) unless otherwise stated, and results were reported as means ± standard deviation (SD). The moisture content and ash concentration were determined gravimetrically, according to (AOAC 938.08, 1990).

2.3.1. Pigment composition

Methanolic extracts were obtained as described in Safafar et al. (2015). The extracts were analyzed for pigments on a HPLC using Agilent 1,100 Liquid Chromatograph with diode array detector (DAD) (Agilent Technologies, Santa Clara, CA, United States). Separation was carried out on a Zorbax Eclipse C8 column 150 mm × 46 mm × 3.5 µm (Phenomenex Inc., Santa Clara, CA United States) at 60 °C. The mobile phase was a mixture of 75% methanol + 25% of 0.028 M tertiary butyl ammonium acetate in water and methanol at a flow rate of 0.9 mL min^{-1} with a total acquisition time of 40 min. DHI pigment standard mix (DHI LAB Products, Horsholm, Denmark) was used for the identification of peaks. Detection of chlorophylls and carotenoids was done at 660 nm and 440 nm, respectively, and for internal standard (BHT) at 280 nm. Pigments are reported as mg g^{-1} of the extract.

2.3.2. Amino acids and protein content

To quantify total amino acids, 30 mg of the dried sample were hydrolyzed with 6 M HCl at 110 °C for 18 h. To measure the free amino acids, dried samples weighing around 50 mg were mixed vigorously with 1 ml of 5% trichloroacetic acid and left overnight at a temperature of 5 °C. The next day, the samples were centrifuged at 5,000 g for 5 minutes. The process of derivatization and chromatography for both total and free amino acids was carried out in accordance with the methodology outlined by Bak et al. (2019).

The calculation of the total protein content followed the approach recommended by Angell et al. (2016), which involved adding up the total moles of amino acids and subtracting the mass of water (18 g H_2O mol^{-1} amino acid) that was released during the acid hydrolysis (Diniz et al., 2011). To access the quality of the amino acids the EAA ratio was determined as the sum of EAA divided by the total AA found in the sample.

2.3.3. Fat content and fatty acid composition

Lipid phase extraction and fat quantification were performed on the dry seaweed powder according to the method by Bligh & Dyer (1959) with minor corrections. Briefly, to 2 g of dried homogenized samples were added 10 mL distilled water, 30 mL methanol, and 15 mL chloroforms and homogenized for 30 s, followed by 30 s homogenization with addition of 15 mL chloroform, and then a 30 s homogenization with 15 mL distilled water. The mixture was centrifuged at 2,800 rpm for 10 min. Afterwards, the water and methanol phases were discarded. A known amount of the chloroform phase was added to a glass container and left for vaporization overnight in a fume hood. The following day the container was weighed, with the remaining content representing the fat content of the sample.

The extraction and quantification of the fatty acid methyl esters (FAME) were performed as described by Jacobsen et al. (2022).

2.3.4. Calculation of carbohydrates

The total carbohydrate content was determined using the "carbohydrate by difference" method. Specifically, the calculation was performed as follows:

$$total\ carbohydrates = 100 - (\%\ protein + lipid + ash\ in\ dw)$$

2.4. Antioxidant capacity of *F. vesiculosus*

In brief, methanolic extracts were obtained by weighing approximately 10 mg of the dry *Fucus vesiculosus* powder in a centrifugation tube and adding 10 mL of methanol.

The content of potentially antioxidant phenolic compounds was estimated by determining the total phenolic content (TPC) on the methanolic extracts. The methodology was modified from Farvin & Jacobsen (2013) and carried out as follows: the methanolic extract was diluted x10 prior to analysis. To 100 µL of diluted extract 0.75 mL Folin Ciolcalteu phenol reagent (10% v/v in water) was added and mixed. After 5 min, 0.75 mL sodium-carbonate solution (7.5% Na_2CO_3 w/v in water) was added and mixed. The reaction was incubated for 90 min at room temperature (dark). The absorbance was measured at 725 nm by a UV-vis spectrophotometer (Shimadzu UV mini−1240, Duisburg, Germany). Gallic-acid (2,3,4-trihydrobenzoic acid) was used for quantification (calibration curve: 0–250 µg mL^{-1}). The results are expressed in gallic acid equivalents (GAE) (µg GAE g^{-1} dw). Analysis was carried out in triplicates (n = 3).

The radical scavenging capacity of the methanolic extracts was quantified using 2,2-diphenyl-1-picrylhydrazyl (DPPH), by applying the method described by Yang et al. (2008) modified for use in a 96-well microplate. 100 µL extract solution (8 different dilutions of the extract) and 100 µL 0.1 mM DPPH solution (in 96% ethanol) were mixed in the microtiter plate. Reaction mixtures were incubated for 30 min at room temperature in the dark. The absorbance was measured at 517 nm using a microplate reader (BioTek Eon, BioTek Instruments Inc., Winooski, VT, USA) and Gen5 2.09 data analysis software. BHT was included as a positive control (63% inhibition in a concentration of 0.2 mg mL^{-1}). EC50 values were calculated (efficient concentration to obtain 50% inhibition) by linear regression (Y=50) and expressed as mg dw mL^{-1}. The analysis was carried out in triplicates (n = 3).

2.5. Sensory analysis of *Ulva* sp. and *F. vesiculosus*

The sensory characterization of the different sensory attributes was performed with an objective sensory panel at DTU Food in a sensory lab that fulfills the international standards and guidelines for the design and construction of sensory assessment

rooms (ISO 8589, 2007; NMKL Procedure No. 6, 2016). The assessors in the sensory panel were tested and trained according to ISO 8586 (2012) and ISO 13299 (2016).

The first sessions were used to develop a vocabulary to describe the sensory characteristics describing the attributes appearance, smell, taste, and texture of the samples. Furthermore, the panel was trained to measure the intensity of each attribute on an unstructured 15 cm line scale with anchor point at 1.5 cm and 13.5 cm. The dried seaweed samples were served in petri dishes. All samples were served in random order and the assessors were served peeled cucumber and water to clean their mouths between samples.

The final vocabulary was: Appearance: Thickness, Crumpled, Transparency, Uniform color. Odor: Sea, Seaweed, Green/hay, Fresh fish. Flavor: Seaweed, Sweet, Umami, Salty, Metal, Bitter, Green. Texture: Crispy, Firm, Clotted, Astringency, Adhesiveness.

2.6. Statistics and data treatment

Data analyses and statistics were performed using the R software (R-Core-Team, 2022). Analysis of variance (ANOVA) was conducted to assess differences between the three drying methods. Homogeneity of variance was tested using Levene's test. In the event of significant differences, a Tukey's post hoc test was carried out to identify significant differences between samples at a 5% level of significance ($p \leq 0.05$). Principal component analysis (PCA) was performed on standardized data and resulted in a score plot. Moreover, EC50 values were determined and EAA ratios calculated.

3. Results and discussion

3.1. Macronutrients

The compositions of macronutrients were quantified for the two types of seaweeds after convection drying (CD), freeze drying (FD) and microwave vacuum drying (MVD) (Table 1). Drying of *Fucus vesiculosus* led to a final water content between 8.4-11% wet weight (ww) and no significant differences between the drying methods (ANOVA; $p = 0.053$). However, for *Ulva* sp. FD led to a significantly lower water content compared to the others (ANOVA; $p < 0.001$). This could be explained by freeze drying preventing case hardening of *Ulva* sp. (Mujumdar, 2014), which is confirmed by the

sensory panel rating the firmness and crispiness higher for the convective dried *Ulva* sp. (Figure 2).

Table 1: Composition of water (% ww) and the other macronutrients (ash, protein (based on amino acids), fat, and carbohydrates) per dry weight (% dw) for F. vesiculosus and Ulva sp. dried by convection (CD), freeze drying (FD) or microwave-vacuum drying (MVD)

Species	Drying method	Water (% ww)	Ash (% dw)	Protein (% dw)	Fat (% dw)	Carbohydrates (% dw)
F. vesiculosus	CD	11±0.8^{a}	15±0.7^{b}	1.1±0.1^{a}	4.1±0.2^{a}	80
	FD	8.9±1.4^{a}	19±2.5^{a}	1.2±0.1^{a}	3.5±0.3^{b}	76
	MVD	8.4±0.8^{a}	17±0.4^{a,b}	1.6±0.1^{a}	4.1±0.1^{a}	77
Ulva sp.	CD	11±1.0^{y}	15±0.9^{z}	6.1±0.1^{x}	1.5±0.2^{x}	77
	FD	5.1±1.4^{x}	26±0.8^{x}	5.8±0.2^{x}	1.7±0.1^{x}	67
	MVD	11±0.6^{y}	20±1.6^{y}	5.4±0.1^{y}	2.2±0.1^{y}	72

All data is represented by the average ± standard deviation. The superscripted letters represent significant differences between the drying methods.

The fat content of *Ulva* sp. was significantly higher when using MVD compared to the other drying methods (ANOVA; $p < 0.001$). Moreover, the fat content of *F. vesiculosus* was significantly higher when using CD and MVD (ANOVA; $p = 0.035$). Microwaves are a well-known assisted extraction technology for increasing the extraction yield of lipids (Zhou et al., 2022). Microwaves break the cells and make the lipids more accessible for extraction resulting in a higher yield, as indicated by the higher fat content when using MVD of *Ulva* sp. compared to FD, which is a much gentler drying technique and might leave some fat embedded in the cell structure after lipid extraction. The higher fat content of *F. vesiculosus* when using both CD and MVD indicates that CD has the same cell breaking effect as MVD however only on *F. vesiculosus* and not on *Ulva* sp.

Protein content of *F. vesiculosus* was not significantly affected by the different drying methods ($p = 0.17$). The protein content of *Ulva* sp. was significantly lower ($p = 0.0017$) when using MVD compared to CD and FD, indicating that microwaves might affect the amino acids as the quantification of the protein content was based on these. In a previous study by (Xiang et al., 2020) the effect of microwaves on protein structure

and browning reactions was discussed. The heat from microwaves could cause amino acids to react with reducing sugars forming Maillard products. If this is the case, this will result in a decrease in the amino acids extracted and thereby the protein content of *Ulva* sp. dried by MVD.

The protein contents in both *F. vesiculosus* and *Ulva* sp. are very low compared to other studies. *Ulva* sp. usually have a protein content between 10 and 25% dw (Fleurence, 1999), but the protein content in seaweed varies with season (Bak et al., 2019). Juul et al. (2022) reported a total amino acid (TAA) content of freeze dried *Ulva* sp. of 9.3% of the dry matter. A review found that the protein content of *F. vesiculosus* would vary from 1-11% dw and that fat would be 1.2-4% dw (Catarino et al., 2018). Carbohydrate content was calculated based on the content of ash, water, protein, and fat. The reported content of carbohydrates varies between 15 and 65% (dw) for *Ulva* sp., and 62-66% of *Fucus* sp. (Rioux & Turgeon, 2015). This is less than what was found in the present study, however calculations are based on the protein contents, which were lower than what is usually found.

3.2. Physico-chemical properties

Food materials with water activity ≤ 0.25 are considered dry, powdery, and chemically stable, except for lipid oxidation. They have a lack of molecular mobility, which hinders biological processes, making them highly food safe (Fennema et al., 1996). All the samples but freeze dried *Ulva* sp. were on the border of this threshold. The water activity of the final dried products followed the water content also in terms of the statistics. Also, here the freeze dried *Ulva* sp. reached significantly lower water activity compared to the others (ANOVA; $p < 0.001$). This is well below the threshold of 0.25 and suggests that freeze drying of *Ulva* sp. is very efficient and might remove some of the bound water in this seaweed.

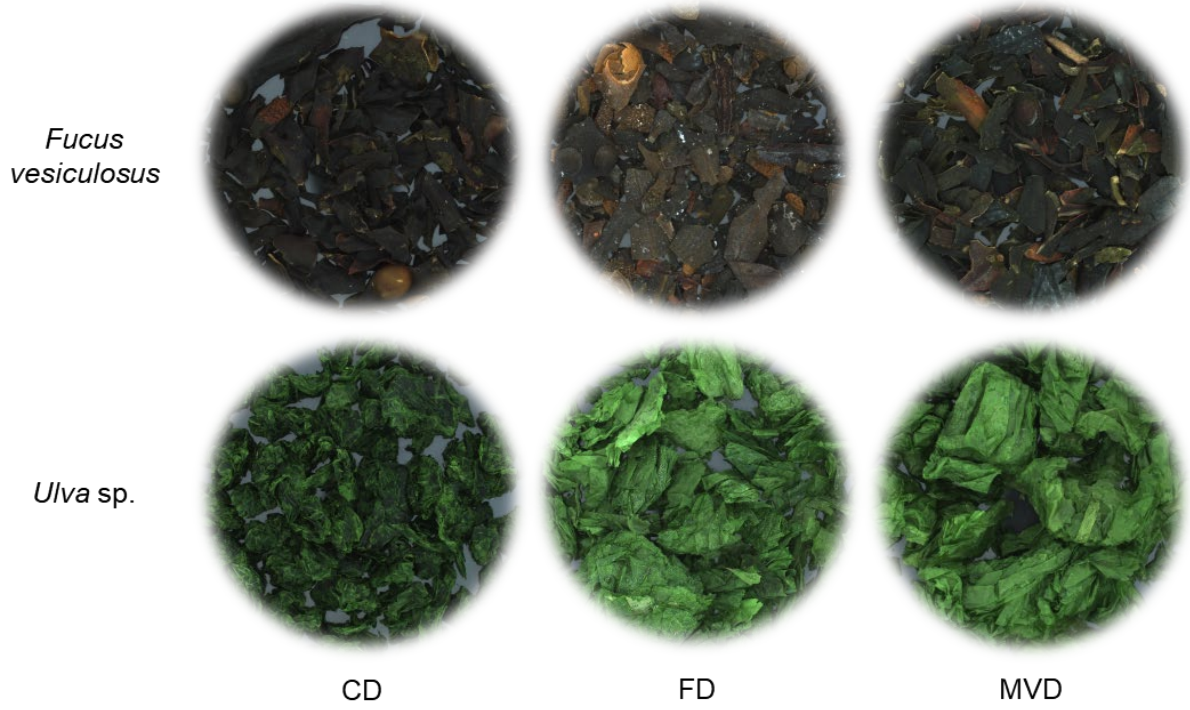
For *F. vesiculosus,* the water holding capacity (ANOVA; $p = 0.27$) and water absorption (ANOVA; $p = 0.18$) did not differ between the drying methods. Interestingly, *Ulva* sp. could absorb water 10-12 times its weight and hold 6.0-8.0 times its weight. These numbers are supported by Jannat-Alipour et al. (2019), who found the water holding capacity to be 9.5 for 60 °C convection dried *Ulva intestinalis* and utilized this property for surimi products.

Table 2: Physio-chemical properties (water activity, water holding capacity and water absorption) of F. vesiculosus and Ulva sp. dried by convection (CD), freeze drying (FD) or microwave-vacuum drying (MVD)

Species	Drying method	Water activity	Water holding capacity (g water/g sample)	Water absorption (g water/g sample)
F. vesiculosus	CD	0.31±0.05^{a}	5.4±1.5^{a}	6.9±0.3^{a}
	FD	0.18±0.08^{a}	4.6±0.6^{a}	6.3±0.7^{a}
	MVD	0.23±0.02^{a}	4.5±0.7^{a}	6.6±0.7^{a}
Ulva sp.	CD	0.24±0.04^{y}	8.0±0.7^{y}	12±1^{x}
	FD	0.069±0.031^{x}	6.0±1.9^{x}	10±3^{x}
	MVD	0.27±0.03^{y}	6.9±0.5^{xy}	11±1^{x}

All data is represented by the average ± standard deviation. The superscript letters represent significant differences between the drying methods.

The visual appearance of seaweed products after three different drying methods was qualitatively evaluated by examining all replicates (n=3) of each drying method. The products were photographed, and the resulting images are presented in Figure 1. Differences in color between the products were observed, with convective drying resulting in a darker product for both species. This is backed up by color measurements in Table 3, which showed that for *F. vesiculosus*, the lightness (L*) was significantly different, with convective drying resulting in the darkest color, followed by the microwave-vacuum drying (ANOVA; $p < 0.001$). For *Ulva* sp., convective drying resulted in a significantly darker product (ANOVA; $p < 0.001$), whereas the other measured did not differ. The observed color differences were likely due to temperature, with convective drying at 60 °C causing color changes and product shrinkage. Silva et al. (2019) found that convective drying at 60 °C would lead to color changes for *F. vesiculosus* whereas 25 and 40 °C would not. The convection drying also lead to case hardening which can be seen on the photographs in Figure 1, this was also backed up by the sensory results (Figure 2).



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370 *Figure 1: Qualitatively visual inspection (using Videometer) of F. vesiculosus and Ulva*
371 *sp. after drying by convection (CD), freeze drying (FD) or microwave-vacuum drying*
372 *(MVD)*

373

Table 3. Color (L, a* and b*) of F. vesiculosus and Ulva sp. after drying by convection (CD), freeze drying (FD) or microwave-vacuum drying (MVD)*

		L*	**a***	**b***
F. vesiculosus	CD	30.8±2.4^{c}	-1.74±0.27^{a}	-0.36±1.23^{c}
	FD	40.5±4.8^{a}	-1.83±1.03^{a}	5.77±3.23^{a}
	MVD	34.7±1.89^{b}	-2.84±0.69^{b}	3.17±1.86^{b}
Ulva sp.	CD	37.8±5.7^{y}	-7.35±2.25^{y}	4.34±3.63^{y}
	FD	49.5±3.5^{x}	-13.5±1.9^{x}	14.3±3.2^{x}
	MVD	47.87±2.8^{x}	-14.0±1.5^{x}	16.5±2.5^{x}

L*= dark to light (0-100), a*=green (-) to red (+), b*=blue (-) to yellow (+). All data is represented by the average ± standard deviation. The superscript letters represent significant differences between the drying methods.

3.3. Sensory differences

The radar charts in Figure 2 show the results from the sensory assessment. The attributes for *F. vesiculosus* (A) show a similar pattern for the three different drying methods. The firmness and crispiness ranked less for the FD compared to the other two dried *F. vesiculosus*. For *Ulva* sp. (B) however, the patterns were not similar. In terms of texture the CD dried *Ulva* sp. showed to be crispier and firmer, which was also seen by the qualitatively visual inspection (Figure 1) showing a case hardening. The odors: seaweed, sea, and fresh fish were stronger in the FD, possibly explained by the retention of flavor compounds due to the lower drying temperature.

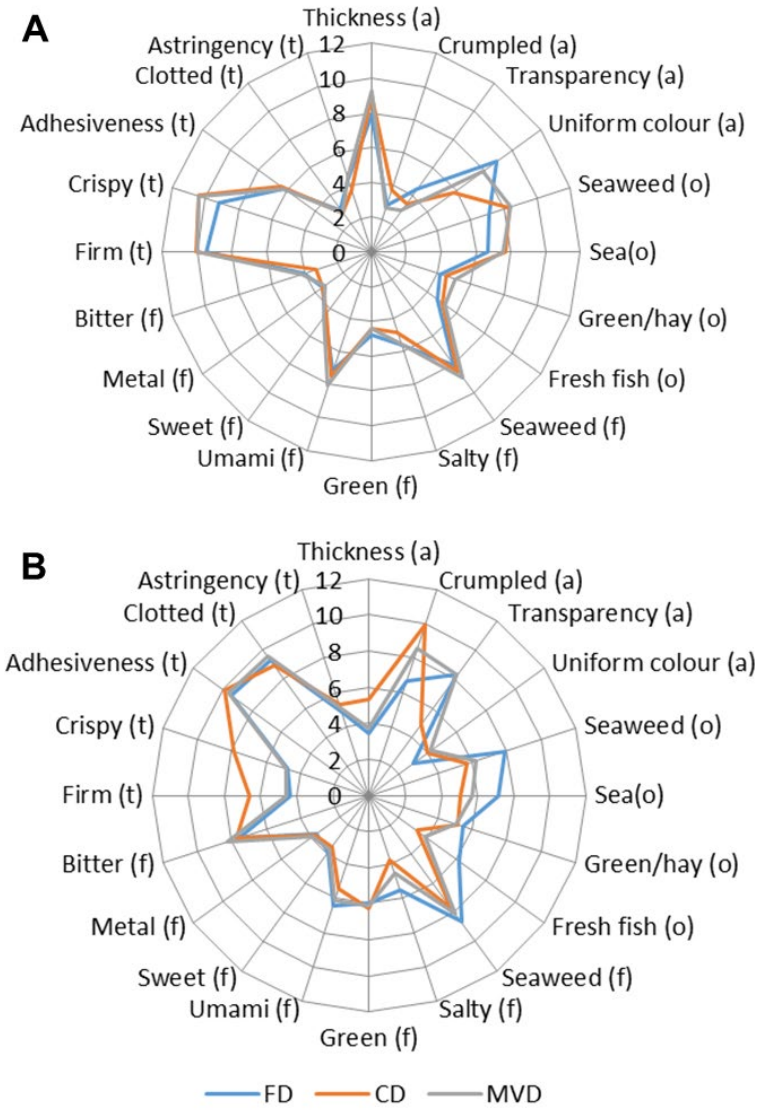

Figure 2. Intensity of the appearance (a), odor (o), flavor (f) and texture (t) measured by sensory profile (scaling 0 to 12) of F. vesiculosus (A) and Ulva sp. (B) after drying by convection (CD), freeze drying (FD) or microwave-vacuum drying (MVD)

3.4. Changes in bioactive compounds

Once the changes in macronutrients, physico-chemical and sensory differences for *F. vesiculosus* and *Ulva* sp. after drying by convection (CD), freeze drying (FD) or microwave-vacuum drying (MVD) were evaluated, the changes in the bioactive compounds were studied. Table 4 shows the composition of the following bioactive compounds in *F. vesiculosus* and *Ulva* sp.; Omega-3 and -6 (18:2 n-6, 20:5 n-3), EAA ratio, pigments, and antioxidant capacity (only *F. vesiculosus*).

The most abundant essential amino acids (EAA) found in the two seaweed types were phenylalanine, leucine and tryptophane (only *Ulva* sp.). *Ulva* sp. had in general 2-5 times higher content of EAAs compared to *F. vesiculosus* (data not shown). The essential amino acid ratio also revealed to be higher for *Ulva* sp. (46.8-47.3%), compared to *F. vesiculosus* (35.3-37.8%). The EAA ratios were comparable to animal-based proteins (whey 43%, milk 39%, casein 34%, and egg 32%), and higher than plant-based protein isolates such as oat (21%), lupin (21%), and wheat (22%) (Gorissen et al., 2018).

Free aspartic and glutamic acid are associated with the taste of umami. In *F. vesiculosus* it was found that the sum of these were 0.274 mg (g dw)$^{-1}$ with no significant difference among the three drying methods ($p = 0.19$). This fits with the results from the sensory panel. For *Ulva* sp. the sum of the two free amino acids was significantly different (ANOVA; $p = 0.0012$), with the CD treated samples (0.177 ± 0.078 mg (g dw)$^{-1}$) being lower than the two others (0.496-0.539 mg (g dw)$^{-1}$)), which the sensory panel also detected.

Whereas *F. vesiculosus* contained both eicosapentaenoic acid (22:5, n-3) (EPA) (4.5-4.9% of the total lipids) and linolenic acid (18:2, n-6) (LA) (7.4-8.2% of the total lipids), only LA was found in *Ulva* sp. (1.7-3.4% of the total lipids). The results are in correlation with the review by Catarino et al. (2018), showing a LA content of 7.5-10.0% of total lipids and an EPA content of 3.7-7.5% in *F. vesiculosus*. For *F. vesiculosus*, the EPA content was not affected by CD or MVD compared to FD, however LA (18:2, n-6) content reduced significantly when CD or MVD were applied ($p < 0.05$). For *Ulva* sp. MVD increased the LA content significantly ($p < 0.05$) compared to FD with the content in CD did not differing from either FD or MVD.

Different types of carotenoids were found in the seaweeds. Beta-carotene was found in both with similar concentrations (20.3-28.8 µg g^{-1} dw), whereas other types of carotenoids were specific for the species. *F. vesiculosus* showed high content of fucoxanthin, a xanthophyll associated with brown seaweed. Moreover, *Ulva* sp. contained lutein (11.1-22.0 µg g^{-1} dw). CD significantly decreased the content of beta-caroten of *Ulva* sp. (from 28.8 to 20.3 µg g^{-1} dw) compared to FD, where MVD to a higher extend preserved this pigment (25.0 µg g^{-1} dw). A similar trend was found for lutein in *Ulva* sp. On the other hand, both beta-carotene and fucoxanthin were highest

in the samples dried by CD and MVD compared to FD, however only significantly for fucoxanthin. Uribe et al. (2019) described the effect of different drying methods (freeze-, vacuum-, solar-, and convective drying) on the quality of *Ulva* sp. (color, pigments, amino acids, and fatty acids among other). Color was not affected by any drying method and total flavonoid content (TFC), total carotenoids and antioxidant capacity (DPPH and ORAC) were higher in convective drying, which conflicts with our finding since only *F. vesiculosus* showed this.

The total phenolic content and DPPH radical scavenging capacity of F. vesiculosus was not affected by the drying. Silva et al. (2019) found that air-drying increased extraction of pigments but was negative for extraction of phenolic compounds.

Table 4: Composition of the most abundant bioactive compounds in Ulva sp. and F. vesiculosus; Omega-3 and -6 (18:2 n-6, 20:5 n-3), EAA score, pigments, and antioxidant capacity (only F. vesiculosus)

		Fatty acid, omega 3 and 6 (% of total FA)		Essential amino acid ratio (%)	Carotenoids (µg/g dw)		Antioxidant capacity	
		Linolenic acid (18:2, n-6)	**Eicosapentaenoic acid (20:5, n-3)**	**EAA ratio**	**Fucoxanthin**	**Beta-carotene**	**TPC (µg GAE (g dw)$^{-1}$)**	**DPPH radical scavenging EC50 (mg mL^{-1})**
F. vesiculosus	**CD**	7.6±0.1 ^{a}	4.9±0.4 ^{a}	36.6±5.8	209.1±20.8 ^{a}	24.0±3.4 ^{a}	20.3±3.0 ^{a}	0.7±0.0 ^{a}
	FD	8.2±0.3 ^{b}	4.5±0.2 ^{a}	35.3±1.0	117.2±4.0 ^{b}	22.5±2.8 ^{a}	22.8±1.0 ^{a}	0.4±0.0 ^{a}
	MVD	7.4±0.2 ^{a}	4.9±0.2 ^{a}	37.8±0.4	228.0±1.7 ^{a}	26.2±1.0 ^{a}	24.3±4.9 ^{a}	0.3±0.0 ^{a}
					Lutein	**Beta-carotene**		
Ulva sp.	**CD**	1.7±0.2 ^{xy}	*nd*	47.1±0.7	11.1±0.8 ^{x}	20.3±1.9 ^{x}		
	FD	2.7±0.8 ^{x}	*nd*	46.8±0.7	22.0±1.9 ^{y}	28.8±3.9 ^{y}		
	MVD	3.4±0.3 ^{y}	*nd*	47.3±1.6	19.7±3.9 ^{y}	25.0±3.7 ^{xy}		

The superscript letters indicate statistically significance (ANOVA; $p < 0.05$) within column and seaweed species. *nd* = not detected

Summary

All, in all *Ulva* sp. and *F. vesiculosus* were affected differently by the drying methods, indicating that differentiation in the drying method between seaweed species is necessary to obtain the optimal quality of the final product regarding sensory, nutritional, physico-chemical properties and bioactive compounds. To summarize how the different drying methods influenced the final quality of the two seaweed species, principal component analyses are visualized in score plots in Figure 3.

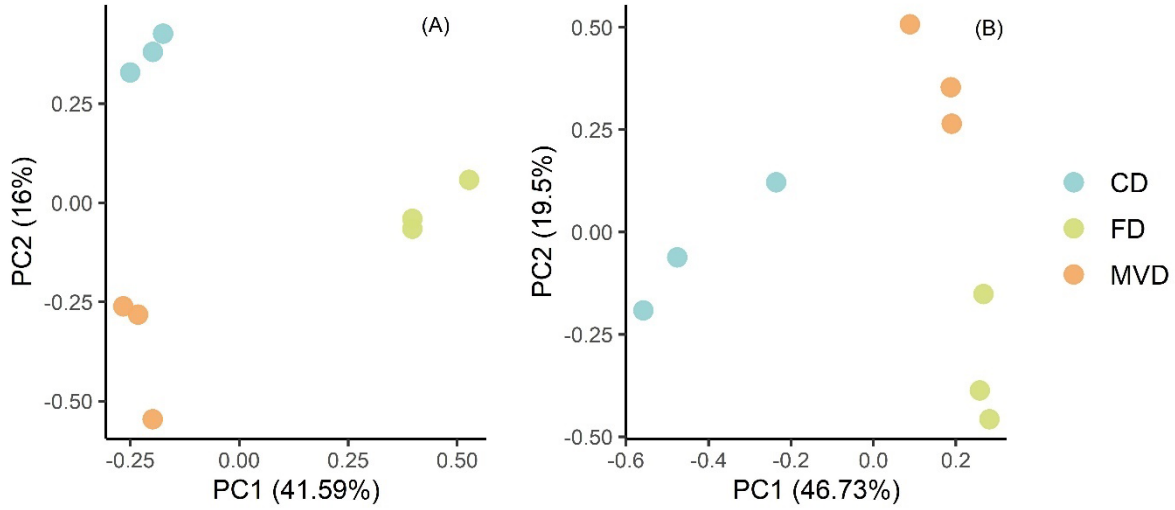

Figure 3: PCA score plots based on all variables for F. vesiculosus (A), and Ulva sp. (B). The colors represent the different drying methods. CD: convective drying, FD: freeze drying, and MVD: microwave-vacuum drying.

In the PCA score plot, it was observed that the quality of *F. vesiculosus* was affected differently by the three drying methods. The between-groups variance was larger than the within-group variance, as evidenced by both PC1 and PC2. In contrast, for *Ulva* sp., the differences in between-groups variances were not as pronounced as the within-group variance, especially for MVD and FD, indicating that these methods resulted in more similar products in terms of quality. On the other hand, CD differed from both MVD and FD. Therefore, it can be concluded that drying methods have a species-dependent influence on the quality. For *Ulva* sp., FD and MVD are similar and can be chosen based on factors such as energy consumption, while for *F. vesiculosus*, the selection of a drying method should be based on the desired food quality due to significant variations between the drying methods.

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