UNIVERSITY OF ICELAND

Improved utilization, preservation, and quality of brown seaweed

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Thesis for the degree of Philosophiae Doctor

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Ágrip

Þrátt fyrir gífurlega aukningu í stórþörungaframleiðslu á síðustu áratugum eru þeir enn frekar vannýtt auðlind í Evrópu. Hins vegar hefur áhugi á stórþöþörungum aukist verulega í Evrópu undanfarin ár, og spáð hefur verið fyrir því að framleiðslan gæti aukist frá þeim 300 þúsund tonnum sem framleidd eru í dag yfir í 8 milljónir tonna árið 2030. Með aukinni framleiðslu stórþörunga er mikilvægt að fullnýta, varðveita og meðhöndla lífmassann á viðeigandi hátt til að hámarka gæði afurðarinnar. Því var markmið rannsókarinnar að kanna og bæta virðiskeðju valinna brúnþörunga, með áherslu á fullnýtingu hráefnis í mjölsvinnslu, varðveislu og geymsluþol á ræktuðum brúnþörungum, og meta nýtingu fjöllitrófsmyndgreiningartækni til að meta gæði stórþörunga innan iðnaðarins.

Til að auka nýtingu klóþangs (e. *Ascophyllum nodosum*) voru lagðar til breytingar á mjölsvinnslu (e. alternative processing) og þær metnar. Þar var lögð áhersla á að kanna árstíðabundinn breytileika hráefnis, og uppskölun á breyttum framleiðsluháttum á iðnaðarskala. Breytta vinnslan byggir á vatnsútdrætti lífvirkra efna úr lífmassanum eftir fínhökkun (e. microcutting) og aðskilnaði á vökva-, og þurrefnisfasa (e. liquid and solid phase) áður en þangmjölið er þurrkað. Metnar voru tvær mismunandi aðskilnaðaraðferðir á vökva-, og þurrefnafasa, eða fleytibúnaður (e. decanter) og skrúfupressa. Ásamt því var nýting diskaskilvindu og þurrkun á vökvanum metin. Niðurstöðurnar benda til þess að breytta vinnslan sé hagkvæmust frá júlí til október en henti verr í júní þegar þörungurinn er í blóma (á frjósemistímabili sínu), þá sérstaklega ef áætlað er að nýta vökvann vegna andoxunarvirkni hans. Ennfremur benda niðurstöðurnar til þess að þurrefnafasinn sem fæst úr breyttu vinnslunni gæti nýst áfram til framleiðslu á alginötum, þar sem alginatið helst í þurrefnafasanum eftir vökvaaðskilnaðinn. Við mat á aðskilnaðartækni á iðnaðarskala kom í ljós að fleytibúnaður gaf betri aðskilnað á vökva- og þurrefnafösunum en skúfupressan. Auk þess má þurrka vökvafasann með úðaþurrkara án þess að hitunin í þurrkuninni hafi teljandi áhrif á lífvirknieiginleika vökvans. Út frá niðurstöðunum var því ályktað að aðskilnaður með fleytingu ásamt úðaþurrkun á vökvafasanum gæti verið hagkvæmur kostur til að auka verðmæti lífmassans sem áætlaður er í alginatframleiðslu.

Ferskir stórþörungar brotna niður og skemmast aðeins nokkrum dögum eftir uppskeru ef þeir eru ekki varðveittir með réttum hætti. Í þessari rannsókn var

kannað hvort hægt væri að nýta sýrumeðhöndlun sem varðveisluaðferð fyrir ræktaðan marinkjarna (*Alaria esculenta*) og beltisþara (*Saccharina latissima*). Niðurstöðurnar sýndu að bæði mjólkursýra og sítrónusýra væru hentugar sýrur til að varðveita ræktaða lífmassann í að minnsta kosti 32 vikur. Lítill munur var á efnasamsetningu, sýrustigi og lit í gegnum geymslutímann og örverufjöldi var tiltöllulega stöðugur í gegnum geymsluna. Niðurstöður gefa þó til kynna að aðferðin sé ekki jafn hentug til að verðveita fjölfenól og andoxunareiginleika lífmassans (metið með DPPH), þar sem lækkun á þessum þáttum varð vegna sýringar á beltisþara. Ásamt því sýndu niðurstöður skynmats að sterkt salt-, og sýrubragð gæti haft áhrif á notkun sýrðu brúnþörunganna í matvæli. Út frá niðurstöðunum var því ályktað að sýrumeðhöndlun sé vel nothæf til að varðveita beltisþara og marinkjarna sem hráefni í matvæli eða fóðurgerð en væri ekki eins hentug til að varðveita andoxunareiginleika þeirra.

Í dag er gæðamat stórþörunga í iðnaðinum að mestu leyti byggt á efnagreiningum og sjónrænu mati starfsmanna. Aðrar aðferðir gætu auðveldað gæðamat afurða, eins og fjöllitrófsmyndgreiningartækni (MSI), sem er auðveld í notkun, eyðileggur ekki sýnið og gefur hraðar niðurstöður. Sýni af beltisþara og marinkjarna voru mynduð og efnagreind, og spálíkön byggð með línulegri aðhvarfsgreiningu (e. partial least square regression, PLSR) úr litrófsgögnunum miðað við niðurstöðunum úr hefðbundnu efnagreiningum. Niðurstöðurnar sýndu að líkönin gátu spáð áreiðanlega fyrir um grunnefnasamsetningu, sýrustig og fjölfenólinnihald í þangsýnunum. Þar, til viðbótar reyndust önnur líkön ásættanleg til greininga svo sem líkön fyrir joðinnihald og andoxunareiginleika (ORAC og DPPH). Út frá niðurstöðunum var ályktað að MSI gæti vel nýst til að spá fyrir um ýmsa gæðaþætti í stórþörungum, en að aukinn sýnafjöldi sé nauðsynlegur til að styrkja líkönin.

Niðurstöður núverandi rannsóknar sýna tækifæri í því að auka verðmæti stórþörunga með bættum framleiðsluferlum. Ennfremur benda niðurstöðurnar til þess að sýring gæti hentað vel sem varðveiðsluaðferð fyrir ræktaða brúnþörunga og að MSI gæti verið notuð til gæðamats á stórþörungum innan iðnaðarins.

Lykilorð:

Stórþörungar, þang, vinnsla, nýting hliðarafurða, varðveisluaðferðir, gæði, andoxunarvirkni, fjöllitrófsmyndgreiningartækni.

Abstract

Despite the significant increase in seaweed production in the last decades, seaweed remains a fairly underutilised resource in Europe. However, the interest in seaweed has been growing in Europe, with predictions indicating that seaweed production could potentially expand from the already harvested 300 thousand tons to 8 million tons in 2030. With the increased production of seaweed biomass, it is crucial to fully utilise the biomass, prioritise the stabilisation of the biomass, and use good manufacturing practices to ensure the quality and safety of the product. Therefore, the aim of the current study was to explore and enhance the studied seaweed value chains, focusing on full utilisation during seaweed meal processing, preservation of cultivated brown seaweed biomass, and assessment of the use of multispectral imaging (MSI) as a quality control technology within the seaweed industry.

Alternative processing was suggested to utilise *Ascophyllum nodosum* biomass to its full potential, where seasonal variation of the raw material and industrial-scale processing methods were evaluated. The process included water extraction with micro-cutting, and separating the liquid and solid phases before drying the seaweed meal. Two methods were assessed to separate the liquid and solid phases, or decanter and screw press. In addition, centrifugation and drying of the liquid phase were evaluated. The results indicate that the suggested alternative processing of seaweed meal from *A. nodosum* is feasible between July and October but less suitable when the seaweed is in its fertile period from May to June, especially if the liquid is intended for antioxidant purposes. Furthermore, the results suggest that the solids obtained from the alternative processing could still be used as a raw material in alginate production, as the alginate content remains in the solid stream during the liquid extraction process. Evaluation of separation technologies on an industrial scale for the alternative processing showed that a decanter is more suitable as the main separation method than using a screw press. Additionally, the obtained liquid could be dried with spray-drying technologies without affecting the bioactive properties of the extract. Therefore, it was concluded that the alternative process with a decanter as the main separation step and subsequent spray drying of the liquid stream could be a feasible option to add value to the biomass alongside the algal meal intended for alginate production.

Seaweed is a perishable material in its fresh state and deteriorates only a few days post-harvest if not preserved. In the present study, an assessment of acid preservation as a stabilisation method for cultivated *Alaria esculenta* and *Saccharina latissima* was performed. The results showed that lactic and citric acid are both suitable to preserve the cultivated seaweed biomass for at least 32 weeks. Minimal changes were observed in the proximate composition, pH and colour during storage, and microbial counts were relatively stable throughout the storage period. The method might, however, not be suitable for preserving phenolic compounds and antioxidant properties (assessed by DPPH), as a decrease in these parameters was observed with acid treatment in the *A. esculenta* samples. Based on sensorial results, the biomass had strong salt and acid flavour, which could affect the uses of the acid-preserved biomass in food products. Therefore, it was concluded that the method is applicable to stabilise the cultivated biomass as an ingredient in food and feed applications but might not be as suitable to preserve the antioxidant properties of the biomass.

Quality assessment within the seaweed industry is mainly based on chemical analysis and visual inspection of the employees. Alternative methods such as MSI could provide the seaweed industry with easy-to-use, fast and non-destructive quality assessment of their products. *A. esculenta* and *S. latissima* samples were analyzed and imaged, and predictive partial least square regression (PLSR) models were constructed from the spectral data and traditional analytical results. The results showed that the MSI technology effectively predicted proximate composition, pH and total phenolic content in the seaweed samples. In addition, other constructed models showed promising results, such as models made for iodine content and antioxidant properties (ORAC and DPPH). Based on the obtained results, it was concluded that MSI is a promising technology to predict multiple parameters in seaweed biomass, but a larger sample size is required to strengthen the models further.

The results of the present study show opportunities to increase the value of the harvested biomass by changing the production processes. Furthermore, the results suggest that acid preservation could be a valuable preservation method for cultivated seaweed biomass and that multispectral imaging techniques could be used as a quality control tool within the seaweed industry.

Keywords:

Macroalgae, seaweed, processing, side stream utilisation, preservation methods, quality, antioxidant activity, multispectral imaging.

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Contents

List of abbreviations

A. esculenta – *Alaria esculenta*

A. nodosum – *Ascohyllum nodosum*

As – Arsenic

Aw – Water activity

Cd – Cadmium

Da – Daltons

DPPH – 2,2-Diphenyl-1-picrylhydrazylradical

dw – Dry weight

F. vesiculosus – *Fucus vesiculosus*

GDA – General descriptive analysis

Hg – Mercury

HSI – Hyperspectral imaging

I – Iodine

IAs – Inorganic arsenic

IMTA – Integrated multi-trophic aquaculture

IR – Infrared

kDa – kilo Daltons

L. digitata – *Laminaria digitata*

MC – Metal chelating abilities

MSI – Multispectral imaging

nCDA - Normalized Canonical Discriminant Analysis

NIR – Near infrared

NMR – Nuclear magnetic resonance

ORAC – Oxygen radical absorbance capacity

PCA – Principal component analysis

pH – Potential of hydrogen

PLSR – Partial least square regression

R_c – Coefficient of determination of calibration

R_{cv} – Coefficient of determination of cross-validation

RGB – Red, green and blue

RMSEC – Root mean square error of calibration

RMSECV – Root mean square error of cross-validation

S. latissima – *Saccharina latissima*

sRGB image – Standard red, green and blue image

SVM – Support vector machine

TBARS – Thiobarbituric acid reactive substances

TC – Total carbohydrate content

TPC – Total phenolic content

TVB-N – Total volatile basic nitrogen

TVC – Total viable count

UV – Ultraviolet

VIS – Visible

ww – Wet weight

w/w – Weight/weight

List of Figures

List of tables

List of original papers

This thesis is based on the following original publications, which are referred to in the text by their Roman numerals:

I. Hrólfsdóttir, A.Þ., Arason, S., Sveinsdóttir H.I., Gudjónsdóttir, M. (2022). Added value of *Ascophyllum nodosum* side stream utilization during seaweed meal processing. Marine Drugs 20, 340. https://doi.org/10.3390/md20060340

II. Hrólfsdóttir, A.Þ., Arason, S., Sveinsdóttir H.I., Gudjónsdóttir, M. (2024). Alternative industrial processing of seaweed meal from *Ascophyllum nodosum*: Value adding, and product development potential of side-streams obtained with different liquid separation methods. Submitted to Food and Bioprocess Technology in December 2023.

III. Hrólfsdóttir A.Þ, Sveinsdóttir H.I, Arason S, Sæther M, Aasen I.M, Gudjónsdóttir M. 2024. Physicochemical- and bioactive properties of acid preserved *Alaria esculenta* and *Saccharina latissima* during storage. LWT Food Science and Technology, 116109. https://doi.org/10.1016/j.lwt.2024.116109.

IV. Hrólfsdóttir, A.Þ., Sigurðardóttir, A.R., Sveinsdóttir H.I., Arason, S., Schultz, N., Einarsson, H., Gudjónsdóttir, M. 2024. Multispectral imaging techniques for evaluating physicochemical and antioxidant properties of *Alaria esculenta* and *Saccharina latissima.* Submitted to Algal Research in June 2024.

Declaration of contribution

Paper I: The PhD candidate participated in designing and conducting the experiment, analyzed the data, and wrote the paper in collaboration with the co-authors.

Paper II: The PhD candidate participated in designing and conducting the experiment, analyzed the data, and wrote the paper in collaboration with the co-authors.

Paper III: The PhD candidate participated in designing and conducting the experiment, analyzed the data, and wrote the paper in collaboration with the co-authors.

Paper IV: The PhD candidate participated in designing and conducting the experiment, analyzed the data, and wrote the paper in collaboration with the co-authors.

1 Introduction

Seaweed is a low trophic plant-like organism that is a highly underutilized resource in Europe (Blikra et al., 2021). The total production of algae reached 38 million tonnes in 2022. In Europe, the use and cultivation of seaweed are in their infancy compared to Asia, where approximately 97% of the total seaweed production worldwide is provided (FAO, 2021). Seaweed has, however, recently been gaining more interest within Europe due to its sustainable production practices and possibilities of various applications, including to feed the growing population (van den Burg et al., 2021; WHO, 2022). Seaweed is cultivated or harvested in about 50 countries, with approximately 300 species commercially utilised. Of these, 46% are brown algae species, and 54% are red algae species, while green seaweed species constitute less than 1% of the total worldwide production (Moreira et al., 2022). Brown seaweed species contribute to approximately 96% of the total cultivated and harvested seaweed biomass in Europe. Among these are the species *Alaria esculenta* (*A. esculenta*)*, Ascophyllum nodosum* (*A. nodosum*) and *Saccharina latissima (S. latissima)*. *A. nodosum* is obtained from wild sources while increasing interest has been in the cultivation of *A. esculenta* and *S. latissima* (FAO, 2021).

The seaweed industry in Europe is expected to grow substantially in the future, where the production could increase from the 300 thousand tons harvested in 2020 to 8 million tons in the future (Cai et al., 2021; Adrien Vincent et al., 2020). As the cultivation of seaweed increases emphasis must be set on optimising processing practices and safety of the produced seaweed. Some of the main bottlenecks within the seaweed cultivation sector in Europe include the lack of automation, proper preservation of the biomass, standardisation of quality control practices, and lack of regulations regarding seaweed for human consumption (García-Poza et al., 2020; Stévant & Rebours, 2021; WHO, 2022).

The harvesting period of cultivated brown seaweed species in Europe, including *S. latissima* and *A. esculenta*, is very short, or usually only 1-2 months annually, mainly due to biofouling of the biomass in the summer (Stévant, Marfaing, et al., 2017). The short harvesting window presents challenges for the producers to offer high-quality seaweed biomass all year around, which makes it necessary to rely on various preservation methods to ensure product stability and stable supply. The current preservation methods used to preserve the biomass include, e.g., drying, freezing, salting, and

fermenting (Blikra et al., 2021; Stévant et al., 2018). In northern regions, drying and freezing are not only expensive due to high energy costs but also require large instruments and infrastructures that would only be used a few months per year. Freezing and drying may also result in nutritional degradation and contribute to quality loss of the biomass if not performed properly (Blikra et al., 2021; Singh & Heldman, 2001; Stévant, Rebours, et al., 2017). Additionally, current quality control practices within the seaweed cultivation sector include mainly visual inspection of the biomass by employees and traditional chemical analysis (Monteiro et al., 2022). These methods are often costly, slow, and destroy the tested sample. The variability in the chemical composition of the seaweed biomass and the lack of legislation calls for standardised quality control practices and fast analytical methods for seaweed to provide consumers with detailed descriptions of the products.

Seaweed is used for many different applications, as a food source, in pharmaceutical products, and in hydrocolloid production (Buschmann et al., 2017; FAO & WHO, 2022). The hydrocolloid alginate is for example, extracted from the brown seaweed species *A. nodosum* and *Laminaria* species in Europe, while other species are used in other parts of the world, including *Macrocystis pyrifera* and *Lessonia trabeculate* (McHugh, 2003). A total of 236.820 tons of seaweed biomass were used to produce alginate in 2015, with *A. nodosum* accounting for approximately 6% of the overall output (Porse & Rudolph, 2017). However, in alginate production, large quantities of the biomass end up as leftover pulp, often discarded in landfills or occasionally used as fertilisers. This leaves opportunities for utilisation and valorisation of the biomass prior to alginate extraction.

As the seaweed industry expands in Europe, optimisation of the seaweed value chains and increasing utilisation is crucial to protect ocean resources, make the production more sustainable, and offer consumers high-quality seaweed biomass. Hence, there is a need to examine how the industry can utilise the biomass to its full potential, explore less energy-requiring processing methods, and explore how quality control of seaweed can be standardised. This study tried to increase knowledge on the above mentioned topics.

2 Literature review

2.1 Seaweed

Macroalgae, commonly referred to as seaweed, are diverse plant-like organisms known for their unique composition and properties. Seaweed is multicellular and photosynthetic organisms that rely on essential nutrients, including carbon dioxide (CO_2), light, and substrates to grow (Gupta & Abu-Ghannam, 2011b). Seaweed hence serves as a vital primary producer and ecosystem engineer, holding a pivotal position in coastal environments and habitats, spanning from kelp forests to coastal reefs (Harley et al., 2012). Seaweed does not only provide shelter and reproductive grounds for various marine animals but is, along with other algae, the foundation of the ocean food web, where they are a food source for fish and sea urchins (Baweja et al., 2016). Seaweeds live in the littoral zone of the ocean, both in the intertidal and subtidal areas, thriving at depths where only 0.1% of photosynthetic light is accessible (Gupta & Abu-Ghannam, 2011b).

There are over 10,000 species of seaweed known that can be classified into three main categories based on their pigmentation characteristic, i.e., ***brown seaweed*** (*Phaeophyceae*) that comprise kelps and perennials, ***red seaweed*** (*Rhodophyceae*), and ***green seaweed*** (*Chlorophyceae*) (Ferdouse et al., 2018; Holdt & Kraan, 2011a). The structure and chemical composition of seaweed varies not only between seaweed groups (i.e., brown, red, and green) but also between species. Furthermore, the composition of each species can vary throughout the year, and is also affected by numerous environmental factors, including salinity, temperature fluctuations, and light conditions (Marinho-Soriano et al., 2006). Seaweed is considered a good source of food since it generally contains a high carbohydrate content, and some species are relatively high in protein content (red and green seaweed), low in lipid content, and rich in vitamins and minerals (Holdt & Kraan, 2011a). However, seaweed also contains a wide range of bioactive compounds, such as polyphenols, carotenoids, tocopherols, sulphated polysaccharides (e.g., fucoidan), amino acids, and peptides, which have been reported to have multiple biological activities, including antioxidant, antifungal, antiviral, and antibacterial properties (Kumar et al., 2008; Qin, 2018). Therefore, the possibilities of using seaweed biomass in food and feed applications are quite diverse, currently ranging from lower-value products, such as fertilisers and feed enhancers, towards more high-end value products, such as food, nutraceutical, and cosmetic products (Blikra et al., 2021; Farghali et al., 2023; Holdt & Kraan, 2011a).

2.1.1 Brown seaweed

Brown algae (*Phaeophyceae*) is a large group of algae, mainly consisting of marine macroalgae or seaweed. Brown seaweed can then be divided into thirteen orders according to Bold and Wynne (1985), with approximately 2000 species within the 300 known genera (Davis et al., 2003; Silberfeld et al., 2014). The brown colour of the seaweed group is contributed by secondary metabolites, mainly from the presence of the carotenoid fucoxanthin, but in some cases, phaeophycean tannins also contribute to the seaweed colour (Haugan, 1994; Miyashita et al., 2020). Brown seaweed is dominant in the marine littoral zone, spanning from subpolar to the equatorial regions, and presents large morphological diversity. Unlike traditional land plants, which consist of leaves and stems, seaweed possesses a structure known as blades and stipes, which attach themselves to the bottom of the ocean by developing a holdfast instead of having roots (Gupta & Abu-Ghannam, 2011b). Brown seaweed can range from small (50-60 cm long) species up to large kelps species (20 meters long) (Baweja et al., 2016; Qin, 2018). The smaller seaweed plants mostly form filamentous tuft or cushion-like structures but can also grow closely to the substrate, where they form crust-like structures. The larger fleshy species, commonly known as wracks and kelps, are in the intertidal and upper subtidal zones. They have more diverse structures, which include unbranched or branched thalli that consist of different blades, stipes, and holdfasts (Kawai & Henry, 2016). Out of the three major seaweed groups (green-, brown-, and red seaweed), brown seaweed stands as the largest group in terms of produced biomass (Baweja et al., 2016). Only a few species out of the 2000 known are commonly used, including *Laminaria*, *Ascophyllum*, *Macrocystis*, *Saccharina*, *Undaria*, *Lessonia*, *Ecklonia,* and *Durvillaea* species. However, each different species is used for different applications (Ferdouse et al., 2018; Qin, 2018).

2.1.1.1 Ascophyllum nodosum

Ascophyllum nodosum (A. nodosum), commonly known as rockweed or knotted wrack, is a brown seaweed species that belongs to the order of *Fucales* within the family of *Fucaceae*. The structure of *A. nodosum* consists of a holdfast, a main thallus, and secondary branches (Figure 1) (Pereira et al., 2020). On the thallus and branches are air bladders, which support the thallus. The bladders look unevenly distributed through the plant but are generally closer to each other on the distal ends of the branches while being more distributed on the proximal end of the branches (Stengel & Dring, 1997). In the spring, receptacles grow out of the branches, which are the plant's

reproductive organs, which then fall off a few weeks later. *A. nodosum* grows in the North Atlantic in the intertidal zone on rocky and sheltered shores. It is widely distributed across the northern hemisphere, from Portugal in the eastern Atlantic Ocean up to the White Sea and to Norway (Doty et al., 1987). The plant can live up to 15 years and grow up to 4 meters in length but is usually 30-150 cm long (Pereira et al., 2020).

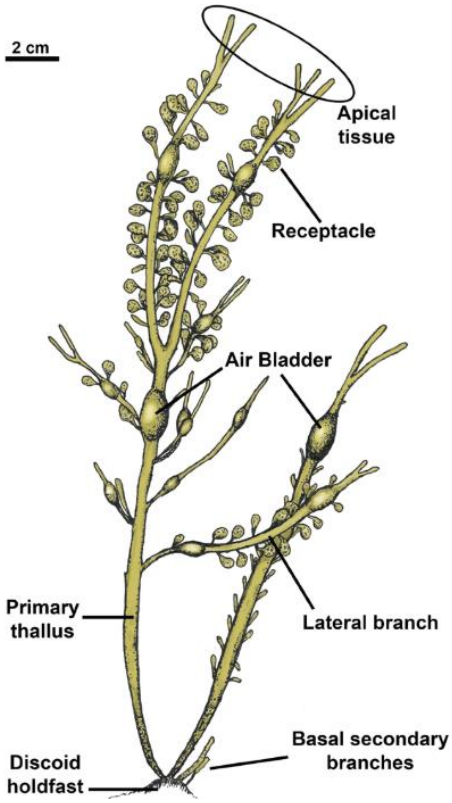

Figure 1 Structure of Ascophyllum nodosum (Pereira et al., 2020).

The chemical composition of *A. nodosum* has been explored extensively due to its commercial interest and current uses (Pereira et al., 2020). As for most seaweed species, the chemical composition of *A. nodosum* can vary depending on numerous factors, including environmental factors, such as salinity and temperature of the ocean, as well as available nutrients and light conditions (Baardseth, 1968; Marinho-Soriano et al., 2006). The water content of *A. nodosum* has been reported by multiple resources, indicating that the water content can account for 66-80% of the wet weight (ww) (Table 1) (Baardseth, 1968; Peinado et al., 2014; Tabassum et al., 2016). On a dry

weight basis (dw), the carbohydrate content is usually high and varies from 42-64% dw (Morrissey et al., 2001), where the polysaccharide alginate is the main contributor and can reach up to 30% of the dw (Shanmugam et al., 2018). The rest is in the form of fucoidan, mannitol, cellulose, and laminarin (MacArtain et al., 2007). The ash content has been reported to be in the range of 15-33% dw (Baardseth, 1968; Peinado et al., 2014; Tabassum et al., 2016), where sodium (Na), calcium (Ca), magnesium (Mg), and potassium (K) are the main contributors (Lorenzo et al., 2017; MacArtain et al., 2007). The protein content then ranges between 4.2-12% of the dw, and the lipid content is relatively low, or from 1.8% to 7% of the dw (Morrissey et al., 2001; Peinado et al., 2014). Furthermore, *A. nodosum* also produces secondary metabolites such as polyphenols and carotenoids (including fucoxanthin), which have been gaining more interest in the last decades due to their bioactive properties (Ahn et al., 2007; Wang et al., 2009).

2.1.1.2 Alaria esculenta

Alaria esculenta (*A. esculenta*), commonly known as winged kelp or Atlantic wakame, is a brown seaweed species that belongs to the order Laminariales and the family of *Alariaceae* (Kraan et al., 2000). *A. esculenta* grows mainly between the lower eulittoral, sublittoral fringe and the upper infralittoral zone, preferably at depths ranging from 0-8 meters, but can grow at more depths (up to 35 meters) (Tyler-Walters, 2008). The plant consists of a blade, a stipe, and a holdfast. The stipe is short to medium-sized, containing stipe-derived sporophylls. The blade (lamina) is long and thin with an obvious midrib (Figure 2) (Ronowicz et al., 2022). The plant can reach up to 4 meters in length, and the monthly growth can be up to 20 cm. *A. esculenta* is present in both the North Atlantic and North Pacific Oceans, and can e.g., be found at the coastlines of Norway, Britain, Iceland, Netherlands, Ireland, and Svalbard. Its life span is estimated to be 5-10 years, but the plants can live up to 7 years in Norway, although they usually live for 4-5 years around Ireland (Tyler-Walters, 2008). *A. esculenta* is one of Europe's most cultivated seaweed species today (FAO, 2021). In recent years, the chemical composition of both cultivated and wild-harvested biomass from *A. esculenta* has been reported extensively due to an increased interest in the cultivation of the species. According to published data on *A. esculenta*, the plant normally contains a water content of 83% to 88% ww (Table 1), a high carbohydrate content, ranging from 65% to 78% of the dw, consisting mainly of alginate (20-35% of dw), mannitol (10.5% dw), and glucose (8.5% dw), but may also contain fucose in smaller quantities (around 1% dw) (Schiener et al., 2015). *A. esculenta* also contains a high ash content (approx. 20-30%), where sodium, potassium, chloride, and calcium are

dominant minerals (Nøkling-Eide et al., 2023; Schiener et al., 2015; Stévant, Marfaing, et al., 2017). The protein content can range from 9.4-12% dw (Mæhre et al., 2014; Schiener et al., 2017; Stévant, Marfaing, et al., 2017), while the lipid content is usually low (approx. 1.3% dw) (Mæhre et al., 2014). Furthermore, *A. esculenta* has also been reported to contain polyphenols, polysaccharides (laminarin, fucoidan), and fucoxanthin (Afonso et al., 2021; Einarsdóttir et al., 2022), which have been reported to exhibit bioactivity properties, including antioxidant properties (Holdt & Kraan, 2011a).

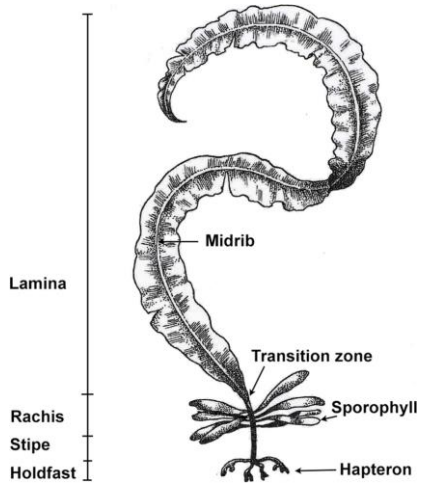

Figure 2 Structure of *Alaria esculenta*. Image retrieved from Kraan (2020).

2.1.1.3 Saccharina latissima

Saccharina latissima (*S. latissima*), commonly known as sugar kelp, is a brown seaweed species belonging to the order of the *Laminariales* and the family of *Laminariaceae* (Peteiro & Freire, 2013). The plant can reach up to 5 meters in length but is usually 1.5 to 2 meters long. The plant of *S. latissima* contains a holdfast and a thallus (consisting of a stipe and a blade) (Figure 3). The stipe can vary in size, from short to long, but the blade is usually 20 to 30 cm in width. *S. latissima* is typically found in the sublittoral rocky seabed but can grow from the upper sublittoral zone to a depth where only 1% of sunlight is accessible. The optimal depth range for growth of *S. latissima* is, however, estimated to be between 5-6 meters, and the optimal temperature range is between 5 and 15°C (Kerrison et al., 2015). *S. latissima* naturally grows in Europe in areas from Portugal to Spitsbergen, but also in the North Pacific and North-west Atlantic oceans. *S. latissima* forests are important for ocean ecosystems since they provide food, shelter, and an area for a nursery for

multiple species (including fish and macrophytes) (Moy & Christie, 2012). The chemical composition of the plant includes high water content, ranging from 82-88% ww, and the ash content ranges from 24-39% dw (Table 1) (Schiener et al., 2015; Stévant, Marfaing). The carbohydrate content is generally high, ranging from 51.7% to 74.5 % of the dw, where alginate is the main contributor (21-32% of the dw), along with mannitol (up to 17-23% dw). Other carbohydrates are glucose (5% dw), and fucose (less than 1% of dw) (Schiener et al., 2015; Stévant, Marfaing, et al., 2017). The protein content is approximately 10% of the dw, and the lipid content is low. Like other brown seaweeds, *S. latissima* contains the carotenoid fucoxanthin and polyphenols, which both have been shown to exhibit some biological activities (Afonso et al., 2021; Wang et al., 2009).

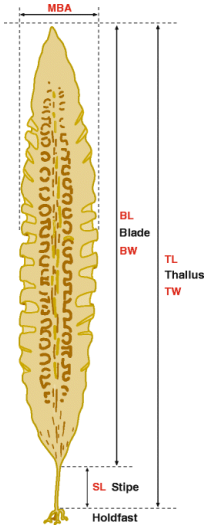

Figure 3 Structure of *Saccharina latissima.* Image retrieved from Peteiro and Freire (2013).

The variation in chemical composition between the three studied brown seaweed species can be seen in Table 1. This variation in chemical composition affects the potential processing and stability of the biomass, as well as bioactive activities, which is crucial for improved utilisation and valorisation of the biomass.

Table 1 Typical chemical composition of *A. nodosum*, *A. esculenta* and *S. latissima*.

Seaweed specie	Water (% ww)	Protein (% dw)	Lipid (% dw)	Carbohydrate (% dw)	Ash (% dw)
A. nodosum	66-80[b]	4.2-12[a,d]	1.8-7[b,d]	42-64[b]	15-33[a,c,d]
A. esculenta	83-88[e]	9.4-12[e,g,h]	1.3[h]	65-78[e]	20-30[e,f,g]
S. latissima	82-88[e,g]	10-15.2[a,g]	1.5[a]	51-75[e,g]	24-40[a,e,g]

Data gathered from a (Samarasinghe et al., 2021), b (Morrissey et al., 2001), c (Tabassum et al., 2016), d (Peinado et al., 2014), e (Schiener et al., 2015), f (Nøkling-Eide et al., 2023), g (Stévant, Marfaing, et al., 2017), h (Mæhre et al., 2014).

2.2 Bioactive compounds in brown seaweed

Despite being exposed to challenging environmental conditions such as changes in oxygen and light levels, which can lead to the generation of free radicals and other potent oxidising agents, wild seaweed biomass rarely undergoes photodynamic damage. Therefore, it can be suggested that seaweed possesses the ability to generate essential compounds with antioxidant properties as protection against external factors, such as stress, pollution, or ultraviolet (UV) radiation (Gupta & Abu-Ghannam, 2011a). Multiple compounds have been identified in brown seaweed as possible contributors to the defence mechanism, including polysaccharides, polyphenols, and peptides. Furthermore, other biological activities have been identified in association with diverse compounds in brow seaweeds, e.g., antimicrobial-, antiviral-, antitumor-, and anticoagulant activities (Gupta & Abu-Ghannam, 2011a; Holdt & Kraan, 2011a).

2.2.1 Polysaccharides

Seaweed polysaccharides are long polymers of monosaccharides linked with glycosidic bonds (Damodaran et al., 2007). They are primarily located in the cell walls, where their major role is to provide the seaweed with flexibility and strength to resist harsh environmental factors and maintain the cell's ionic equilibrium. They are also found in the form of storage polysaccharides, which serve as a food reserve for the plant (Gupta & Abu-Ghannam, 2011a; Synytsya et al., 2015). Multiple different polysaccharides are found in brown seaweed, including alginate, fucoidan, and laminarin (Gupta & Abu-Ghannam, 2011a; Holdt & Kraan, 2011a).

2.2.1.1 Alginate

Alginate, or alginic acid, is a linear polysaccharide made of two uronic acids, β-D-mannuronic (M) and α-L-guluronic acid (G), which are linked with 1,4-glycosidic bonds (Damodaran et al., 2007; Li et al., 2021). Alginates are primarily located in the cell wall, although they also exist as intracellular material in brow seaweed (Ye et al., 2023). Alginate is a hydrocolloid, and its gel-forming abilities are linked to the amount of G and M blocks in their structure, whereas alginates with higher amounts of G-blocks produce gels with higher strength. Furthermore, alginates form a strong gel under specific conditions involving proper pH and cations concentrations (Mg^{2+}, Na^{+}, Ca^{2+}) (Damodaran et al., 2007). Additionally, alginates have been proven to possess multiple bioactive properties, including antibacterial-, antioxidant-, antidiabetic-, and antibiotic properties, which makes them beneficial in different applications, such as in pharma-, and nutraceutical industries (Ye et al., 2023).

2.2.1.2 Laminarin

Laminarin is a storage polysaccharide located in the cell vacuoles. It consists of 20-25 glucose units of (1,3)-β-d-glucan with β-(1,6) branching (Kadam et al., 2015). They have two major forms, i.e., a G-chain structure (glucose residue on end) and a M-chain structure (mannitol residue on end). Most laminarins are resistant to hydrolysis in the gastrointestinal tract (GIT) due to their complex structure, which is stabilised by inner-chain hydrogen bonds, and are, as a result, considered dietary fibres (Gupta & Abu-Ghannam, 2011a). The chemical structure and ratio of the M-and G-chains vary with seaweed species and external environmental factors. Laminarin has been proven to possess multiple bioactive properties, including antibacterial, wound-healing-, antioxidant-, and anti-tumour activities, and their activities are dependent on their structure and ratio of M-and G- chains (Zargarzadeh et al., 2020). Laminarins have, hence, promising potential for applications in the functional food and biomedicine sectors (Li et al., 2021).

2.2.1.3 Fucoidan

The polysaccharide fucoidan is a branched sulphate ester, primarily composed of l-fucose 4-sulphate building blocks with attached sulphate groups (Gupta & Abu-Ghannam, 2011a; Wijesinghe & Jeon, 2011). It can also contain some other monosaccharides, including glucose, mannose, galactose, and uronic acid, as well as other compounds, such as protein and acetyl groups, but the structure varies between seaweed species (Li et al., 2021). Fucoidan is found in the cell wall and as an intracellular material in brown seaweed. The

molecular weight of fucoidans has been reported within the range of 100 kDa to 1600 kDa (Gupta & Abu-Ghannam, 2011a). Fucoidan has gained increased interest due to its multiple bioactive properties, but the biological activity that each fucoidan structure exhibits greatly depends on its degree of sulphation. It has been reported to exhibit antioxidant-, antitumor-, anti-inflammatory-, anticancer-, anticoagulant-, and antibacterial activities (Li et al., 2021; Wijesinghe & Jeon, 2011). Currently, fucoidan has several different applications, but it is commonly used as functional food ingredients, as dietary supplements, in cosmetic products and in aquaculture feed supplements (Okolie et al., 2017).

2.2.2 Polyphenols

Polyphenols are diverse groups of compounds constructed of two or more aromatic hydrocarbon rings with one or more hydroxyl groups (-OH). They are secondary metabolites in plants and algae and serve as protection against predators and UV radiation (Manach et al., 2004). Polyphenols in algae are derived from phloroglucinol units (1,3,5-trihydroxylbenzene). The main polyphenolic compounds in seaweed are phlorotannins, phenolic terpenoids, flavonoids, and bromophenols. Phlorotannins are the main phenolic compound in brown seaweed species, but other above-mentioned phenols can be found in larger quantities in green and red seaweed (Cotas et al., 2020).

Phlorotannins are phenolic compounds found only in brown algae. They consist of polymerised phloroglucinol units (1,3,5-trihydroxybenzene) (Gómez & Huovinen, 2020; Li et al., 2011) and are found in vesicles called physodes (an organelle of the seaweed cell) (Gupta & Abu-Ghannam, 2011a). The total phlorotannin content of seaweed can vary significantly between seaweed species, where species such as *A. nodosum* and *Fucus vesiculosus (F. vesiculosus*) have been reported to have high phlorotannin concentrations compared to other brown seaweed species such as *A. esculenta* and *S. latissima (Wang et al., 2009)*. Additionally, external factors, e.g., seasonality and location of the collected biomass, can also affect the phlorotannin content of seaweed (Meng et al., 2021). Phlorotannins are known to play important biological roles throughout all stages of algal development. They also serve a vital role in protecting against oxidative stress within the algal cell and provide protection against various external factors, including light, desiccation, nutrient availability, UV radiation, and herbivore attacks (Meng et al., 2021).

Phlorotannins exist in various structures and can be classified into four classes based on their linkages (Figure 4): fucols (contain phenyl linkage), eckols (contain dibenzodioxin linkage), phloroethols and fuhalols (containing

ether linkage), and fucophloroethols (containing both ether and phenyl linkages). Furthermore, their molecular size can vary and has been recorded from 126 Da to 650 kDa but are most commonly in the range from 10 to 100 kDa (Hermund, 2018; Li et al., 2011).

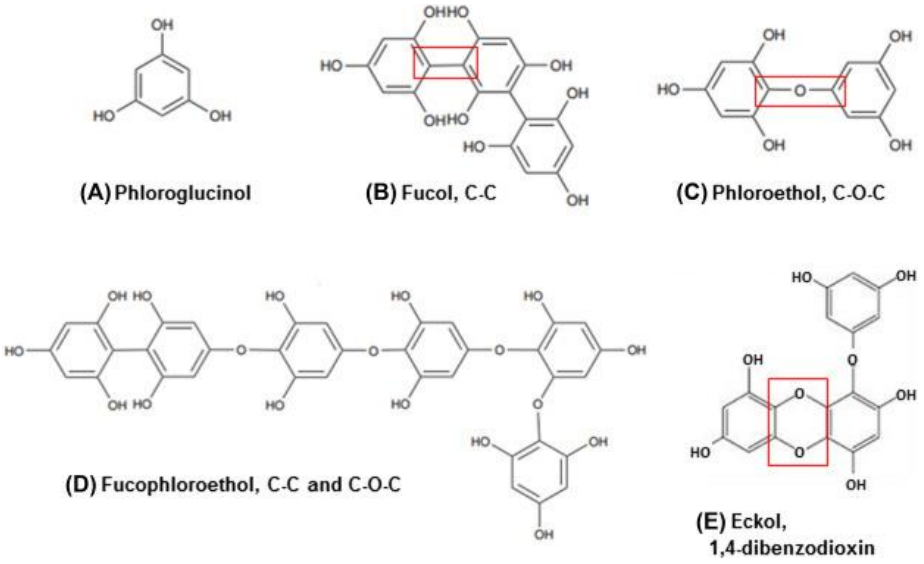

Figure 4 Different structures of phlorotannins. Figure retrieved from Hermund (2018).

2.2.2.1 Antioxidant activity of phlorotannins

Phlorotannins have been identified as biologically active substances, as they exhibit antioxidant-, enzyme inhibitory-, antibacterial-, and anticancer activities (Li et al., 2011). The interest in phlorotannins has increased significantly due to their potential as antioxidants, but multiple studies have examined the antioxidant capacities of different phenolic compounds derived from different seaweed species (Qin, 2018). The antioxidant mechanism varies from one phenolic compound to another and is based on the molecular structure, oligomerisation, and molecular weight of the phlorotannins (Hermund et al., 2018; Wang et al., 2009). For instance, higher antioxidant activity is generally observed in low-molecular-weight phlorotannins, and increased polymerisation may decrease the activity (Hermund et al., 2018).

Phlorotannins are prone to engaging in efficient electron donation reactions due to their electron-rich chemical structure and are hence considered strong antioxidant agents (Shrestha et al., 2021). Phlorotannins have been reported as radical scavengers, ferrous ion chelators, superoxide scavengers, and peroxyl radical scavengers, where the phenol rings within the phlorotannin structure exhibit multifunctional antioxidant capacities (Figure 5) (Ahn et al., 2007; Gupta & Abu-Ghannam, 2011b; Wang et al., 2009).

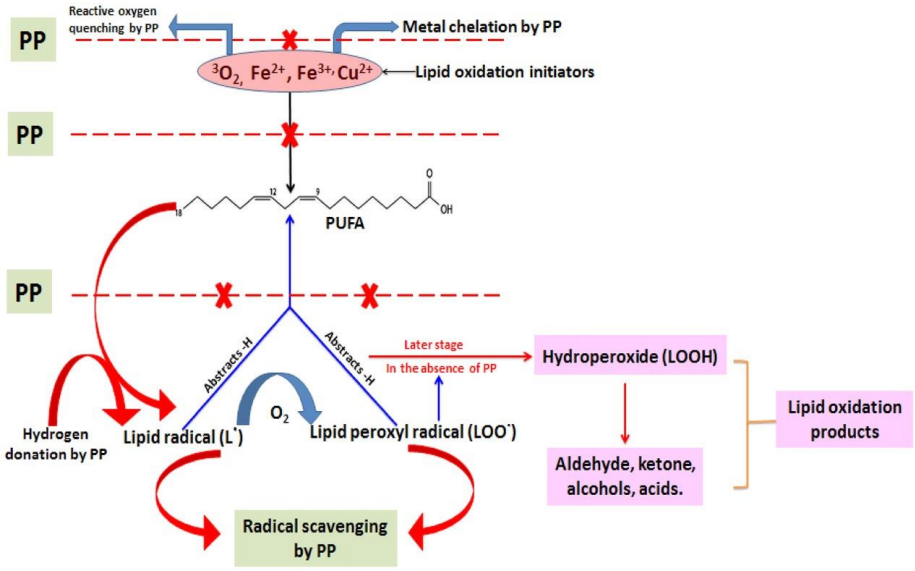

Figure 5 Image showing role of Phlorotannins (PP) in inhibiting oxidation of polyunsaturated fatty acid (PUFA). Image retrieved from Maqsood et al. (2014).

2.3 Seaweed value chain – from harvesting to processing

2.3.1 Seaweed production and the UN-SDGs

Food production touches all the United Nations' Sustainability Development Goals (UN-SDGs) either directly or indirectly. Two of the 17 UN-SDGs are especially relevant to seaweed production: UN-SDG 12 Responsible Consumption and Production and UN-SDG 14 Life below water. Overall, these UN-SDGs aim to promote environmentally sustainable production and consumption practices and protect the ocean's resources. Seaweed has been identified as an exceptional raw material with one of the highest potential to achieve sustainable growth, including advances in the food, environmental and gender dimensions of the SDGs (UNCTAD, 2024).

2.3.2 Global seaweed production

Globally, the total production of seaweed has increased drastically, with an increase of over 60-fold between 1950 and 2019 (Figure 6). During this period, the total algae production (including micro and macroalgae) expanded from roughly half a million tons to 35.82 million tons wet weight (ww) (Gerhard et al., 2023). The production continues to increase, with a total production of 37.8 million tonnes ww in 2022 (FAO, 2024). This development is primarily attributed to the rise in seaweed aquaculture, but the total seaweed production in 2019 accounts for 99.8% (35.76 tons ww) of the total production. In these years, the majority of the biomass was cultivated, or 97% in 2019, while only a small fraction (3%) was harvested from the wild (FAO, 2021).

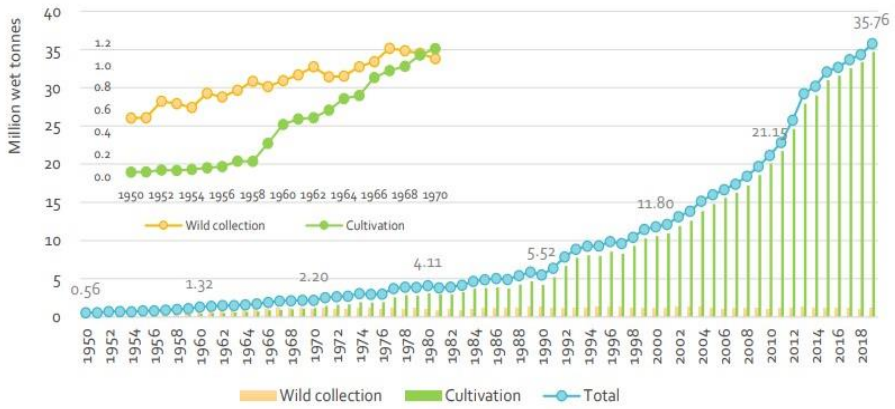

Figure 6 Status and trends in global seaweed production from 1950 to 2019 (retrieved from (FAO, 2021).

The Asian continent contributes to 97% of the global seaweed production, whereas China, Indonesia, Philippines, Korea, and Japan are the main producers. Interestingly, around 99% of their production in Asia is derived through cultivation, leaving less than 1% originating from wild sources. In contrast, Europe's contribution was only 0.8% (roughly 287 thousand tons) of the world's production in 2019. Moreover, most of the harvested biomass in Europe, specifically 96%, was obtained from wild sources (Cai et al., 2021).

European oceans offer ideal conditions for scaling up the seaweed industry. The suitability arises from the nutrient-rich and cold oceanic environments. An estimation of the potential growth within the European seaweed industry has been outlined in the report "Hidden Champion of the Ocean: Seaweed as a Growth Engine for a Sustainable European Future", published by Seaweed for Europe (Adrien Vincent et al., 2020). According to the report, remarkable growth could take place between 2020 and 2030, where the seaweed

production within the European seaweed industry could increase over 26 fold or rise from the harvested roughly 300 thousand tons in 2019 (ww) to up to 8 million tons (Adrien Vincent et al., 2020).

2.3.3 Wild seaweed harvesting

Harvesting of wild seaweed biomass is an ancient practice in regions such as Europe and Asia (Radulovich et al., 2015), where the population in coastal communities mainly used it as a food source. Later, the industry grew due to discoveries of industrial uses for seaweed biomass, and higher demand for biomass led to more effective harvesting and processing techniques. Today, seaweed is either harvested manually or mechanically by boats and is then processed (e.g., dried, blanched, refrigerated or frozen) (Delaney et al., 2016; Mac Monagail et al., 2017). Even though over 10.000 seaweed species have been identified (Ferdouse et al., 2018; Holdt & Kraan, 2011a) only 48 species were commercially used in 2019, including 36 wild-harvested species. In Europe, three main species are harvested from the wild, *Laminaria digitata* (*L. digitata*), *Laminaria hypoborea,* and *A. nodosum,* which were mostly harvested by five European countries, i.e., France, Ireland, United Kingdom, Norway, and Iceland (Cai et al., 2021; FAO, 2021).

2.3.3.1 Wild harvesting of Ascophyllum nodosum

Several methods are used to harvest *A. nodosum,* such as manual biomass collection, where the plants are either cut by hand or using a sickle or mechanical harvesting with specifically made vessels. Currently, mechanical methods are the most common method of harvesting *A. nodosum*. Most harvesters use mechanical methods, such as hydraulically driven Aquamarine harvesters or flat-bottom vessels containing rotating cutters with a fitted pipe. The seaweed is collected in a net located on the vessel and once the net is full, it is thrown overboard, where it floats till collected by another boat, which transports the biomass to land (McHugh, 2003). *A. nodosum* is the most harvested seaweed in Iceland today (FAO, 2021). Most of the harvest is performed by a company located in the west of Iceland, Thorverk hf., which has an annual harvesting reaching up to 15.000 tons. The company harvests *A. nodosum*, whereas mechanical harvesting is performed by hydraulically driven Aquamarine harvesters (Aquamarine, Ontario, Canada). Reciprocating knives, located on the head (front) of the harvester, cut the plant a few centimetres above the holdfast, allowing it to regrow. The cut biomass is transferred by a conveyor system through the aquamarine harvesters, where it is collected in nets, which, when full, are allowed to float till collected by another boat and transported to the dock.

2.3.4 Seaweed cultivation

There has been a rapid growth in seaweed cultivation in the last 50-70 years in a few countries, especially in Asia, while other regions are still adapting to a changing industry. Nevertheless, the increase in production and demand for seaweed biomass is a good indicator of the possibilities involved in the global expansion of the industry (Radulovich et al., 2015). Algae production (mainly from seaweed) currently contributes to approximately 30% ww of the total aquaculture worldwide. Global seaweed production is mainly sustained by aquaculture, where around 27 species are cultivated. However, in Europe, only a few species are commercially cultivated, including the brown seaweed species *S. latissima*, *A. esculenta,* and the *Undaria species (FAO, 2021)*.

The seaweed cultivation process is similar in all scenarios, including breeding and seeding, deployment, farming, and harvesting (Radulovich et al., 2015). The cultivation techniques can, however, differ. Many techniques have been developed to cultivate seaweed, including different locations, installations, and equipment. The cultivation can take place onshore, offshore, near shore, or as part of integrated multi-trophic aquaculture (IMTA) systems. Onshore cultivation of seaweed is performed in closed systems (ponds, lagoons, tanks, or raceways) either indoors (with artificial lights) or outdoors (with sunlight as a light source) with constant water flow. Off-shore cultivation takes place in the ocean, either near- or off-shore, where many different systems have been developed, including flat-tidal farms, ring cultivation, floating cultivation, and IMTA systems. The harvesting can be performed by hand, by cutting or by boat harvesting (which still requires manual cutting) (García-Poza et al., 2020) in a similar manner as described earlier.

2.3.4.1 Cultivation of Alaria esculenta and Saccharina latissima

A. esculenta and *S. latissima* are the most cultivated seaweed species in Europe. The first step in the cultivation process is the breeding and seeding process (Figure 7), where spores are collected and attached to thin ropes and grow into seedlings. The seedlings are then deployed by wrapping the thin ropes on thicker ropes that can be deployed in the ocean on their floating cultivation system. The seaweed is most often farmed from October to April/May, when they are harvested. The harvesting is performed by ship, where the ropes are lifted from the ocean with a crane, and the seaweed is manually cut from the ropes into plastic tubs, which are then transported to land on another vessel for processing. Upon arrival on land, the seaweed is

processed (generally frozen or dried), packed, and then distributed by trucks to the market.

Figure 7 Seaweed cultivation process of *Alaria esculenta* and *Saccharina latissima*.

2.3.5 Post-harvest handling and processing

Seaweed is considered a perishable material in the fresh state, and the biomass deteriorates in few days after harvest. Therefore, good post-harvest handling- and processing practices are important to ensure the stability and safety of the seaweed produced (Gupta et al., 2011; Santhoshkumar et al., 2023). Various methods are used within the industry to expand the shelf life of seaweed biomass, where methods such as drying, refrigeration, freezing, fermentation, and dry salting/brining are currently used (Blikra et al., 2021). Other methods, such as blanching or soaking, are also commonly used to reduce unwanted contaminants and compounds in the biomass before other processing methods (Nielsen et al., 2020).

2.3.5.1 Drying

Drying is a method commonly used to reduce water content through heating and mass transfer (Santhoshkumar et al., 2023). Due to seaweed's high water content, it is considered a perishable material. Drying is hence often used to reduce the water content from 70-95% ww down to 12-35% or even lower, depending on the method used (Gupta et al., 2011; Radulovich et al., 2015; Santhoshkumar et al., 2023). This also reduces water activity (A_w) and inhibits microbial growth on the biomass. With drying, the shelf life of the seaweed is extended, desirable qualities are conserved, and the storage volume and weight of the biomass are reduced significantly (Gupta et al., 2011). The method also allows the producer to offer the customers a quality product for extended periods (Moses et al., 2014). Several factors need to be considered when drying seaweed, including the drying method and duration, the physical structure of the biomass, and the effect the drying process has on the nutritional quality of the biomass (Singh & Heldman, 2001; Stévant, Rebours, et al., 2017). Different methods have been used for drying seaweed, including sun-drying, solar-drying, or drying within processing facilities.

Sun-drying is performed by spreading seaweed on surfaces such as nets or coconut leaves by placing the products on racks, where they are allowed to dry for a few days or until the biomass reaches a water content of 30-35% (Gupta et al., 2011; Radulovich et al., 2015). Sun-drying is a cheap method but has its disadvantages. The method requires constant care (e.g., turning of the biomass) to ensure even drying, and it takes several days to dry the biomass fully. Lastly, sun-drying exposes the biomass to potential contamination and the possibility of damage caused by weather conditions if it is not covered properly (Radulovich et al., 2015). However, mechanical solar-drying systems have been gaining more interest as a drying method for seaweed. These systems use solar energy as an energy source to heat up the mechanical structures, allowing a more enhanced and reliable drying process. However, solar drying systems are considered environmentally friendly and economically viable for developing countries and other regions where access to energy is costly and restricted (Fudholi et al., 2015).

Sun-drying or solar-drying methods cannot be applied in countries with unstable weather conditions and little sunlight, such as Ireland, Norway, and Iceland. Hence, industrial drying methods are generally used to dry seaweed and other food products in these regions. Industrial drying of seaweed is performed by using tunnel dryers or ovens/cabinets, where the heat input is from external sources and forced air. Many advantages are to using industrial drying methods, including complete control of the process, high volumes of biomass can be dried, the drying process takes a relatively short time, and the water content of the biomass can reach lower levels than with sun-drying or below 20% (Radulovich et al., 2015). However, there are also some disadvantages to using industrial drying methods, which mainly include the intensive energy usage and high capital cost (Moses et al., 2014). This makes it especially challenging for seaweed cultivators, who face short harvesting periods and high energy costs in most European countries, to establish such processing facilities.

Industrial drying methods are currently used to dry *A. nodosum* in Iceland due to easy access to green and cheap energy as well as unstable weather conditions discouraging solar drying. The current seaweed drying process is as follows (Figure 8): post-harvest, the seaweed is transported to the processing facilities, where an excavator is used to shovel the biomass into processing equipment. The biomass is then chopped into smaller pieces and dried on a conveyor belt dryer with pre-heated air at a maximum temperature of 85°C. Heat exchangers heat up the air using geothermal hot water from

boreholes. Following drying, the seaweed is ground, sieved, weighted and packed in sacks (Hallsson, 1964; Thorverk, 2021).

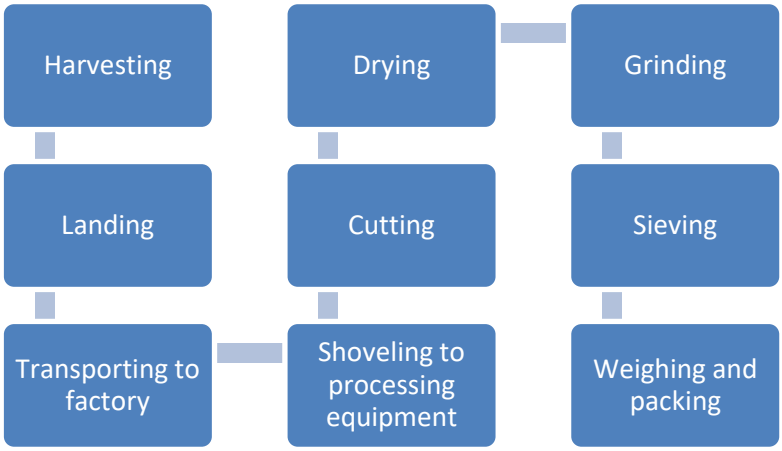

Figure 8 Overview of the seaweed meal production at Thorverk hf.

2.3.5.2 Freezing

Freezing is a commonly used method worldwide in the industry to preserve food products, and it generally preserves the texture, taste, and nutritional values of food in an efficient way when performed correctly (Obluchinskaya & Daurtseva, 2020). Ice crystal formation starts when the temperature is below 0°C, making water continuously more unavailable to participate in reactions with decreasing temperature. Hence, by freezing, microbial activity and growth reduce significantly, along with a reduction of other degradation reactions that normally occur at higher temperatures. However, freezing practices (e.g., freezing rate) and a low and stable storage temperature are important to ensure high product quality. Suitable freezing methods depend mainly on the product characteristics. Some products require quick freezing, where smaller ice crystal formation occurs to minimise damage to structural and textural properties, while other products have structures that are not as suitable for quick freezing (Singh & Heldman, 2001).

Freezing seaweed is a common method for preserving the biomass. Seaweed is either frozen pre- or post-blanching, but blanching is often performed on biomass if intended for human consumption (Løvdal et al., 2021). Freezing, like other processing methods, can have both advantages and disadvantages. Freezing infrastructures are commonly available at low costs compared to other processing infrastructures. However, running such facilities is often energy-demanding and may be expensive. Additionally, by freezing

the seaweed biomass, it can be preserved for a long period, but only if the correct freezing, storage and thawing protocols are applied (Blikra et al., 2021). There can, however, be substantial loss of mass due to drip loss and nutritional loss when the biomass is thawed, both affected by the chosen freezing and thawing methods. Generally, quick freezing retains the dry matter content better than slow freezing (Sund et al., 2024).

2.3.5.3 Acidification

Acidification of food products can be performed either by natural acidification (fermentation) or with artificial acidification (addition of acids). The preservation method is based on the principle of preventing food spoilage by producing a medium with a low pH, unsuitable for the growth of pathogenic microorganisms (Coban, 2020; Dauthy, 1995). A nearly neutral pH (pH of 7) is the optimal pH for most microbial cells to maintain their chemical equilibrium. With changes in the pH and other external chemical factors, alternation can occur in the cell membrane units, such as phospholipids and proteins, which can impact the membrane permeability, resulting in leakage of internal cell metabolites. Furthermore, organic acids can affect intracellular activities, e.g., protein synthesis and DNA replication, by penetrating the cell, which leads to cell death (Coban, 2020).

Natural acidification or fermentation is an ancient and commonly used method in the food industry to preserve perishable food products, such as vegetables and fruits (Bruhn et al., 2019; Dauthy, 1995). The fermentation principle is based on the process of microbial cultures (most often lactic acid bacteria (LAB)), converting available carbohydrates to ethanol, carbon dioxide, and acids (e.g., lactic-, propionic-, or citric acid). The fermentation process lowers the pH of the surroundings and hence controls the growth of pathogenic microorganisms (Caplice & Fitzgerald, 1999). Fermentation of seaweed can increase the shelf life of the biomass for long periods if the pH is reduced sufficiently (Bruhn et al., 2019), but it can also alter the sensory properties of the final product, such as flavour, aroma, and texture (Caplice & Fitzgerald, 1999). When the process has been optimised with regard to microbial cultures, pH, and unwanted microbial growth, the method can be used on an industrial scale, where large volumes can be produced (Blikra et al., 2021).

Artificial acidification, or the addition of organic acids to food products, is used to reduce microbial growth of pathogenic bacteria by reducing the pH below 4.6 (CFR, 1979; Dauthy, 1995; Theron & Lues, 2010). Organic acids have been used for a long time as a food additive where the main organic acids used include citric acid, lactic acid, benzoic acid, formic acid, acetic acid, and

propionic acid (Anyasi et al., 2017). They are applied both pre- and postharvest to extend the shelf life of perishable food products. The purposes of acid applications vary between products and include using organic acids as antioxidants, acidulants, pH adjusters, nutrients, and flavour agents. Just like with natural acidification, the acid treatment of foods with organic acids not only affects the shelf life of the product but can also affect sensory properties, e.g., taste, texture and odour (Theron & Lues, 2010). Reports of artificial acidification of seaweed biomass intended for food applications are limited, leaving a gap within the literature on the matter.

2.4 Seaweed applications

Throughout history, coastal communities have harvested various seaweed species for different purposes. Initially, seaweed served the domestic needs of the population and was particularly used as a food source. Over time, their potential for various industrial applications came to light when different species were identified, as well as the properties of multiple compounds within the biomass. The earliest documented commercial uses of seaweed in Europe, particularly in Norway and France, date to the 17th century, when seaweed biomass was used in glass manufacturing, and then later, kelp biomass was used in iodine manufacturing. With chemical substitutes arising after World War II, iodine production from seaweed declined, which led to that the extraction of alginates became a more viable option for seaweed producers (Delaney et al., 2016). Today, seaweed is used in multiple applications, including in food-, agriculture-, bioplastics-, nutraceutical-, pharmaceutical-, and cosmetic industries (Buschmann et al., 2017; FAO & WHO, 2022).

2.4.1 Seaweed in food

From a dietary perspective, seaweed is an outstanding nutritional source, as it contains high carbohydrate content, is rich in vitamins, minerals, and bioactive compounds, and has a low lipid content (Gupta & Abu-Ghannam, 2011b; Holdt & Kraan, 2011a). It has been consumed since ancient times and is currently incorporated into the human diet in three main ways either consumed directly as food (e.g., fresh, canned, dried, or salted), in the form of thickening agents (alginate, carrageenan, and agar), or as food supplements (Buschmann et al., 2017; WHO, 2022). The majority, or 80% of the harvested seaweed biomass, is used as a direct or indirect food source (WHO, 2022). Seaweed has traditionally served as a food source in Asia for hundreds of years, with its use in Japan dating back to the fourth century and in China from the sixth century (McHugh, 2003).

Most of the harvested seaweed biomass, or 48%, is used directly for human consumption, while 32% is consumed through processed foods. However, using seaweed as a food source remains rare in Europe, even though historical records show that seaweed was used for direct human consumption by previous generations in certain parts of Europe (Mouritsen et al., 2013; WHO, 2022). Consumer acceptance due to unfamiliar sensory attributes of seaweed (e.g., their appearance, chewy and elastic texture and intense smell and flavour) is considered one of the major obstacles in reintroducing seaweed into European cuisine (Figueroa et al., 2023; Mouritsen et al., 2013). Despite these challenges, the global popularity of traditional Asian dishes that contain seaweed, including sushi and ramen, has increased (Blikra et al., 2021). Several seaweed-based food products have emerged on the market in the last few years, including pasta, mayonnaise, liquor, burgers, and seasoning mixes (van den Burg et al., 2019). Additionally, Scandinavian and other European chefs have innovatively added seaweeds into fusion dishes to enhance their sensory attributes (Mouritsen, 2012).

Seaweed is used to produce three hydrocolloids, i.e., agar and carrageenan, which are extracted from red seaweed species, and alginates extracted from brown seaweed species. The common method used to extract alginate from the seaweed biomass involves pre-treatments of the seaweed biomass, including treatment with acid, extractions with alkaline, precipitation, bleaching and drying (Garcia-Vaquero et al., 2017) These hydrocolloids are then used as thickening and stabilising agents in multiple products, including food-, pharmaceutical-, and cosmetic products, as well as in textile printing (McHugh, 2003).

2.4.2 Other uses of seaweed biomass

The rest of the harvested seaweed biomass, or approximately 20% of the total worldwide production, is used for other non-food purposes, such as in agriculture as animal feed, fertiliser, or bio-stimulant formulations, and the pharmaceutical and cosmetic industries (WHO, 2022).

2.4.2.1 Seaweed as feed

Historically, livestock such as horses, sheep, and cattle in coastal regions in Europe consumed seaweed, especially large brown seaweed species that drifted ashore (McHugh, 2003). Seaweed is utilised as animal feed primarily in two forms, as fodder or as processed meal, with the latter being more common (WHO, 2022). Today, as seaweed serves as a sustainable and rich source of both macro- and micronutrients, dried seaweed meal is produced from, for

example, *A. nodosum* and *L. digitata* in Norway, Iceland, and France and is used for livestock (McHugh, 2003; Morais et al., 2020). Additionally, in some countries, such as the United States, seaweed is used in animal nutrition for livestock and household pets (McHugh, 2003). Furthermore, multiple studies have been conducted to evaluate the possible uses of seaweed and seaweed-derived substances as feed or feed enhancers, for example, in fish aquaculture (Ergün et al., 2009; Güroy et al., 2011; Valente et al., 2006), to reduce methane production of ruminants (Brooke et al., 2020; Kinley et al., 2020), and to improve the gut health of poultry (Dierick et al., 2010; Gahan et al., 2009; McDonnell et al., 2010). Overall, the use of seaweed meal as feed in agriculture is considered promising for future development (Morais et al., 2020; WHO, 2022).

2.4.2.2 Seaweed as fertiliser/bio-stimulant

Seaweed has been used for a long time as a natural fertiliser to improve soil health and the growth of plants, particularly among coastal farmers, who have easy access to the resource. Generally, seaweed is applied to soil in two main ways, either in the form of dried seaweed meal or as a seaweed extract (often referred to as a bio-stimulant) (Nedumaran, 2017). The latter application has increased over the last decade as novel processing techniques have emerged. These liquid extracts can be produced by using red, green, and brown seaweed species. Commonly used brown seaweed species include *A. nodosum*, *L. digitata* and *F. vesiculosus* (Ali et al., 2021). The application of seaweed extracts has been linked to numerous benefits for plant growth and health, including stimulation of seed germination, improved root deployment, increased frost resistance, enhanced nutrient absorption, as well as protection against pests such as fungi and bacteria. Additionally, higher crop yields and improved plant health have been observed in some cases (Ali et al., 2021; Raghunandan et al., 2019).

2.4.2.3 Seaweed in cosmetics products

Diverse bioactive compounds are found in seaweed that can exhibit various bioactive properties, including antioxidant, antiviral, antibacterial and antifungal properties. Seaweeds are thus commonly utilised as ingredients in multiple pharmaceutical and cosmetic products (Jesumani et al., 2019; Kumar et al., 2008; Qin, 2018).

Cosmetic products can be described as substances designed to improve or change the appearance and conditions of the skin and/or hair. Due to consumer preference for using natural products, the cosmetic industry has

been exploring ways to reduce the use of synthetic chemicals and swift towards using ingredients of a natural origin (Jesumani et al., 2019). Compounds of interest to the cosmetic industry include both primary and secondary metabolites, such as phenolic compounds, peptides, carotenoids, and carbohydrates. These compounds are used for multiple purposes, including as an active ingredient for their antioxidant or antibacterial properties. Furthermore, hydrocolloids extracted from seaweed are used as thickening and gelling agents, but cosmetic products constitute nearly 40% of the world's hydrocolloid market (Kumar et al., 2008; Qin, 2018; Salehi et al., 2019).

2.5 Quality control in seaweed processing

As the cultivation and utilisation of seaweed are expected to increase significantly in the future, it is important to consider good production practices and the safety of the biomass prior to use for human consumption. As seaweed contains a high water content, it is a perishable product, meaning that microbial decomposition can occur rapidly post-harvesting (Blikra et al., 2021), highlighting the importance of good manufacturing production processes and efficient quality control practices.

Multiple factors can influence its safety, including the seaweed species and its physiology, seasonality, the environment it is grown in, and production practices (harvesting methods and processing). The main hazards that have been identified in the seaweed value chain can be classified into three main groups, *chemical hazards* (heavy metals, iodine, allergens, biotoxins, radionuclides, pesticide residues, and persistent organic pollutants), *microbial hazards* (presence of pathogenic microbes such as *Bacillus*, *Salmonella* pathogenic *Escherichia coli*, *Vibrio, Listeria,* and *Staphylococcus aureus)*, and *physical hazards* (e.g., shells from small crustaceans and muscles and small stones). Currently, regulations are lacking regarding the safety of seaweed intended for human consumption (Banach et al., 2020; FAO & WHO, 2022). However, a few reports have recently been published as guidelines on how to ensure safety and good production practices by the Norwegian Seaweed Association (Hogstad et al., 2023; Norwegian Seaweed Association, 2021), as well as guidelines for heavy metal content in algae published by French authorities (ANSES, 2018, 2020).

The composition of seaweed biomass varies not only between species but also within the same species (Schiener et al., 2015; Tabassum et al., 2016). As a result, the quality of the harvested biomass within each harvest and between harvesting periods may differ. Furthermore, multiple other external

factors, such as temperature, pH, location, and light, can influence the composition and quality of the biomass (Marinho-Soriano et al., 2006; Peteiro & Freire, 2013). The main methods used in quality control within seaweed processing include traditional chemical analysis and visual inspection. However, most traditional chemical analyses are expensive, time-consuming, and destroy the analysed sample (Monteiro et al., 2022). The variation in the biomass, lack of standard quality control practices, and cons of traditional analysis highlight the importance of exploring new options for quality control of seaweed intended for human consumption.

2.5.1 Spectroscopic methods in quality control

The use of spectroscopic methods has increased as a quality control tool within the food industry to determine the chemical composition and adulteration of various food products (Franca & Nollet, 2017). These methods are non-destructive and hence allow repeated sample measurements, are cost-effective, and provide quick real-time analysis (El-Mesery et al., 2019; Liu et al., 2015).

2.5.1.1 Vibrational spectroscopy

Spectroscopy is a scientific technique based on the interactions of electromagnetic radiation with matter (Sathyanarayana, 2015). Numerous spectroscopic methods are available to address a variety of analytical challenges. Methods applied differ depending on the analytical sample and analysis aim (molecular vs. atomic spectroscopy), the type of interactions between the electromagnetic radiation and the matter being observed (absorption, emission, or diffraction), and which region of the electromagnetic spectrum is being used. Spectroscopic methods are highly informative and widely employed in food analysis laboratories for quantitative and qualitative analysis. The most used methods include techniques based on the absorption and emission of radiation in the ultraviolet (UV), visible (VIS), and infrared (IR) radio frequency ranges (Figure 9) (Penner, 2017).

The UV-VIS spectrum spans wavelengths from approximately 200 to 780 nm (Power et al., 2019). UV-VIS spectroscopy is based on electronic transitions, meaning that the changes in energy are due to changes in the valence electrons in the molecules (Sathyanarayana, 2015). UV-VIS spectroscopy is used in food analysis for qualitative and quantitative sample characterisation, e.g., using fluorophores (Power et al., 2019). UV-VIS methods are commonly used to identify multiple compounds (phenolic compounds, astaxanthin, melamine, and collagen, as well as oxidation

products (peroxide value, anisidine value, TBARS) within food products and more (Karoui, 2018; Power et al., 2019).

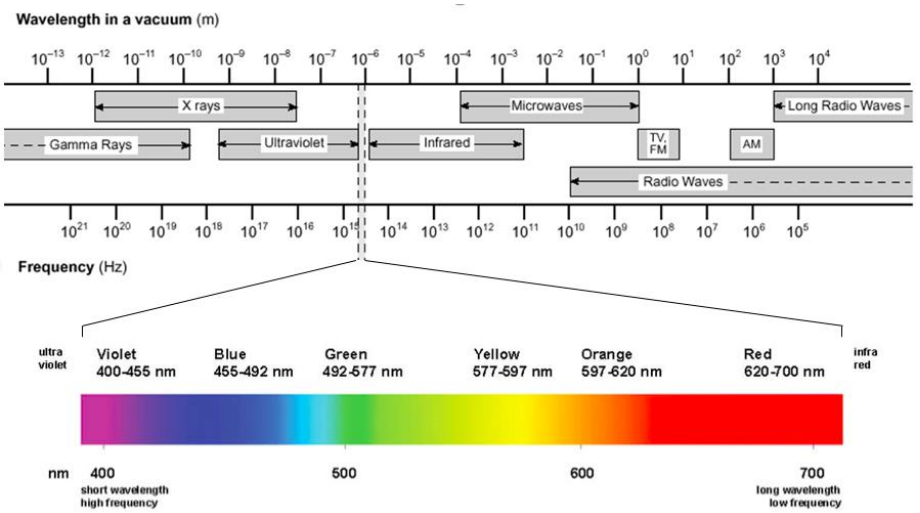

Figure 9 The electromagnetic spectrum, highlighting the visual (VIS) spectrum (Libretexts).

The infrared (IR) spectrum spans wavelengths from 780 nm to 1 mm. It is a vibrational spectroscopic method, meaning that the changes in energy are due to changes in the vibrational levels of the molecules. The IR region can be divided into three sections: the near-infrared (NIR) (wavelengths between 780 nm to 2500 nm), mid-infrared (2500 nm to 50 µm) and far infrared (FIR) (50 µm to 1 mm) (Sathyanarayana, 2015). NIR spectroscopy is a fingerprinting analytical technique that provides chemical and structural information of the chemical compounds from the electromagnetic absorption at selected wavelengths. This information can then be used to identify, confirm, and quantify specific compounds of interest (Indrayanto & Rohman, 2020). NIR has, thus, been widely used as a standard analytical method to analyse multiple parameters in food processing, including chemical composition, sensory properties, and physical properties, by building predictive models with traditional reference methods (Osborne, 2006).

2.5.1.2 Multispectral imaging (MSI)

Conventional sensing techniques, including imaging and spectroscopy, have been used as non-destructive methods to assess food quality in the last decades. However, conventional imaging techniques cannot collect spectral data, and spectroscopic methods usually only cover small areas of a product at a time (Qin et al., 2013). Spectral imaging, such as Multispectral imaging

(MSI) and Hyperspectral imaging (HSI), are non-destructive and novel technologies based on imaging systems that capture images while simultaneously obtaining spectroscopic data at various wavelengths (Boelt et al., 2018; Liu et al., 2014).

MSI systems typically capture images across 3 to 20 discrete spectral bands or wavelengths from the UV, VIS and NIR spectral ranges, while HSI includes a continuous range of wavelengths (Su & Sun, 2018). In contrast, traditional RGB (red, green, and blue) imaging technologies are limited to capture images within the visual spectrum (Figure 10). Using MSI involves rapid image capture, followed by image processing by using known algorithms to analyse the images and extract the spectral data (Qin et al., 2013). The extracted spectral and imaging data can then be used to build predictive models capable of estimating various quality parameters (Ni et al., 2014; Osborne, 2006; Qin et al., 2013). The technology has been used to assess the quality of seed (ElMasry et al., 2019), various plant foods (Su & Sun, 2018), as well as to assess freshness and quality within the fish industry (Jayasundara et al., 2020; Khoshnoudi-Nia & Moosavi-Nasab, 2019a, 2019b). The use of MSI or HSI in the seaweed industry is still in its infancy. However, some studies have evaluated the use of HSI and MSI, such as to map the density and distribution of seaweed biomass (Borges et al., 2023; Rossiter et al., 2020), and for evaluating microbial growth of cultivated seaweed (Lytou et al., 2022).

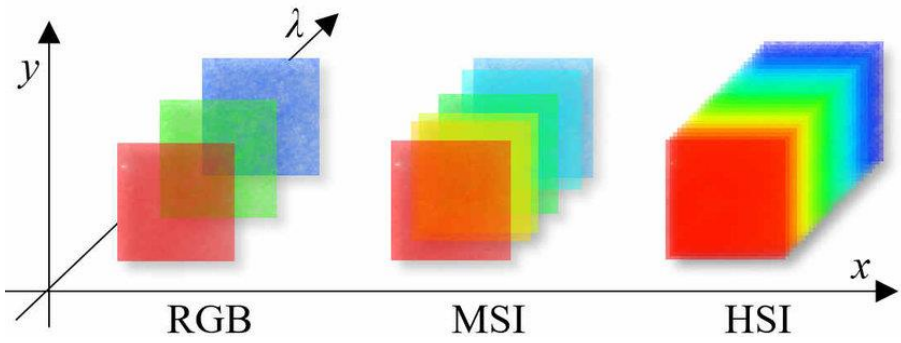

Figure 10 Visual comparison between RGB, MSI-, and HSI imaging methods. Image retrieved from Gevaux (2019).

3 Objectives

The objectives of the present study were to investigate and improve the current seaweed value chains with an emphasis on better utilisation and stabilisation of the biomass, as well as evaluating the reliability of multispectral imaging to predict and monitor the quality and safety of seaweed products. The objectives were attained by:

- Evaluating if side-product processing within *A. nodosum* seaweed meal production was feasible and sustainable, how it would affect the final seaweed meal product, and what kind of processing methods are most suitable for such processing changes (**Paper I and II**).
- Investigating the physicochemical properties and stability of *Alaria esculenta* and *Saccharina latissima* treated with two different acids, lactic acid and citric acid, during room temperature storage (**Paper III**).
- Assessing the possibilities of using multispectral imaging (MSI) as a quality and safety monitoring technique within seaweed production to ensure high quality of the final products (**Paper IV**).

4 Materials and methods

4.1 Experimental design

The study is divided into four papers, with the aim of exploring the seaweed value chain with an emphasis on better utilisation, preservation, storage, and safety of the harvested seaweed biomass. The study overview is presented in Figure 11

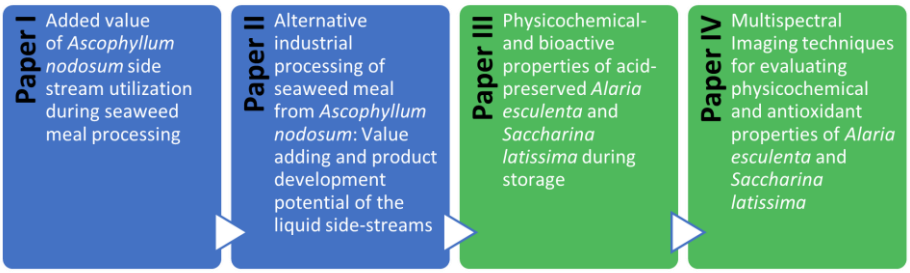

Figure 11 Flowchart of the study design. The blue-colored squares show work performed regarding full utilisation of *A. nodosum*, while the green-colored squares show work performed on acid preservation and quality assessment using *A. esculenta* and *S. latissima*.

4.1.1 Experimental design for Paper I

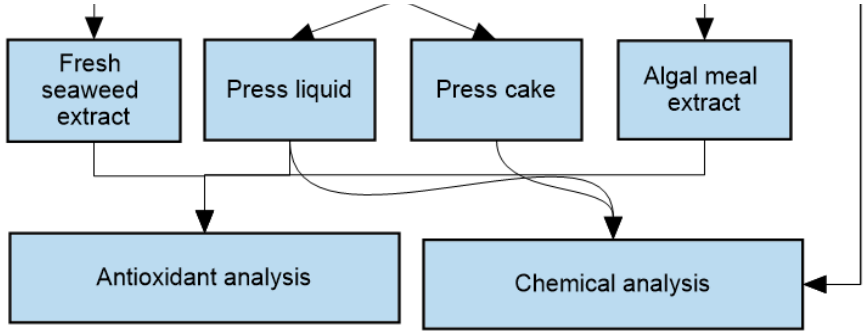

Figure 12).

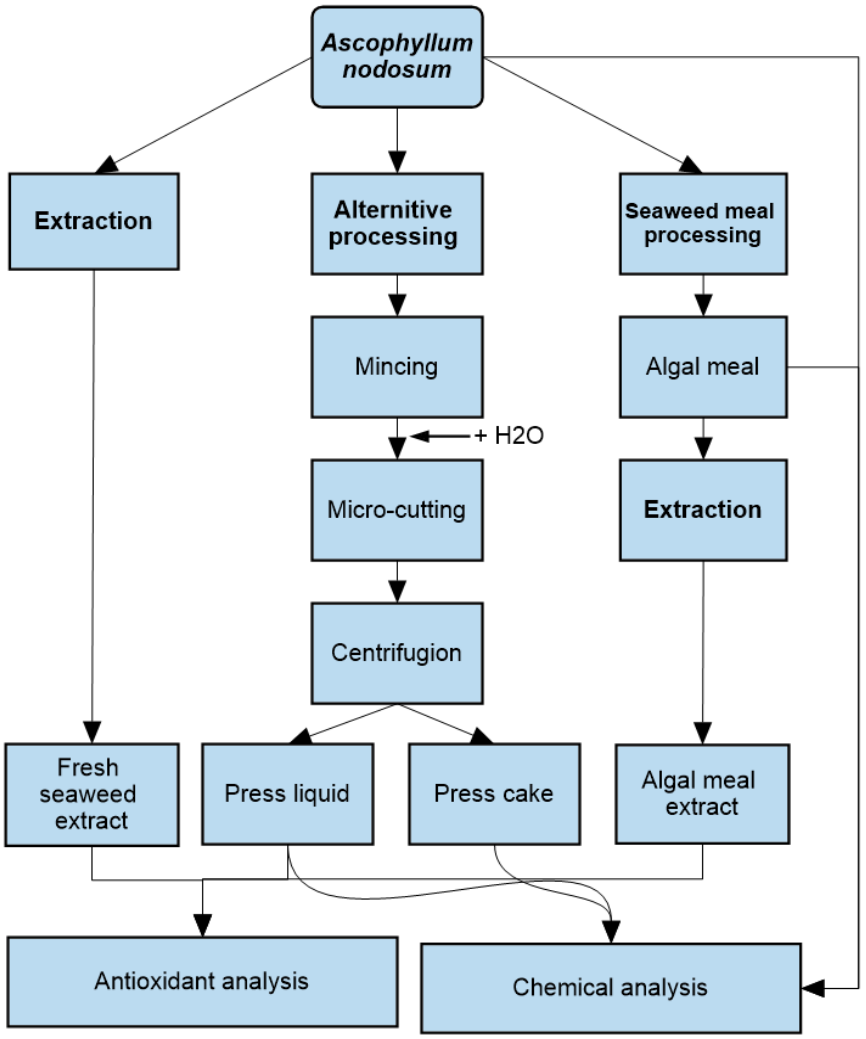

Figure 12 Overview of the experimental design for **Paper I**.

4.1.2 Experimental design for Paper II

The study aimed to evaluate the possibilities of producing a side product (a targeted seaweed extract) alongside the current commercial seaweed meal processing and what processing changes would be required for such an addition (Figure 13). *A. nodosum* was mechanically harvested by Thorverk hf. in August 2022, and approximately 300 kg of fresh biomass was collected. The biomass was transferred to the processing facilities and minced with a Wolfking C.250 Universal grinder (Koncept Tech ApS, Slagelse, Denmark). Water and seaweed were then blended in a ratio of 1/1 w/w, micro-cut in a

Stephan micro cutter (Stephan Machinery GmbH, Germany), and collected. Liquid separation of the micro-cut biomass was performed with two industrial methods, i.e., with a ***screw press*** (Bibun, Japan) and a ***decanter*** (GEA Westfalia Separator AG, Oelde, Germany). The resulting liquid extracts and solids were collected for analysis. A portion of the liquid extracts was further processed and was either centrifuged with an ASE 20-03-077 ***disk centrifuge*** (GEA, Düsseldorf, Germany) or dried. Two different drying methods were applied, i.e., ***freeze-drying*** with a Genesis 25 SQ EL freeze dryer (SP industry, Philadelphia, USA) and ***spray drying*** with a Mobile minor spray dryer (GEA Niro, Copenhagen, Denmark). Analysis of chemical properties was performed on the fresh seaweed biomass, the micro-cut blend, the liquid and solid phases after the first stage separation (screw press or decanter), on the liquid extracts and sediment post disc-centrifuging, and the dried (freeze-dried and spray dried) decanter liquids. Antioxidant properties were evaluated in the liquid extracts obtained, both before and after centrifugation, and on the differently dried decanter extracts.

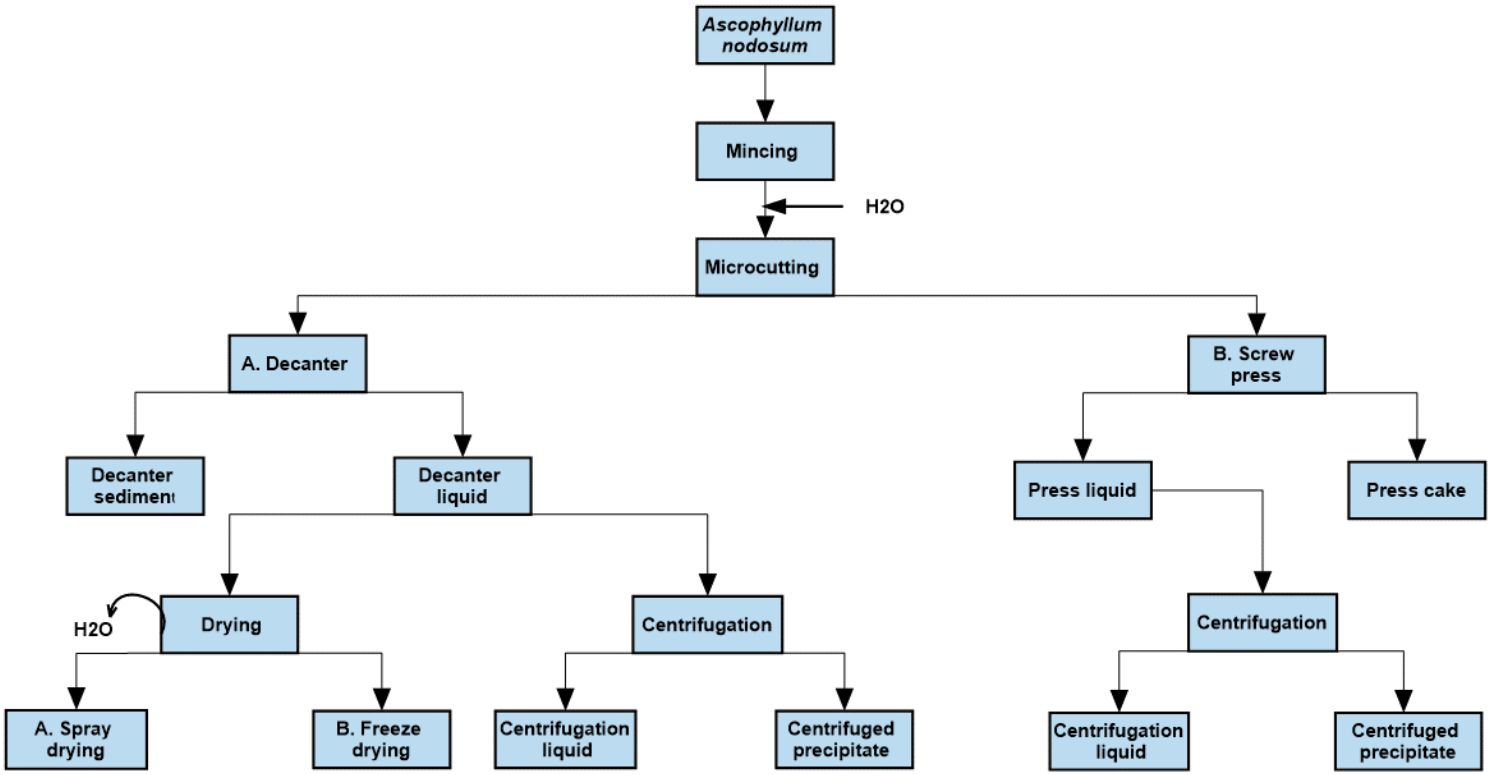

Figure 13 Overview of possible alternative processing at industrial scale (**Paper II**).

4.1.3 Experimental design for Paper III

The objective of **Paper III** was to assess the physicochemical- and bioactive properties, efficiency, and storage stability of acid treated *A. esculenta* and *S. latissima* during 32 weeks of room-temperature storage, and to evaluate if different acids (lactic- and citric acid) provide suitable preservation condition for the biomass. *A. esculenta* and *S. latissima* were cultivated by Seaweed Solutions AS as follows. Seedlings were deployed in January 2022 at Frøya, Måsskjæra (N63'44, E8'53N) in Norway, and the biomass was harvested on May 30th, 2022. The biomass was transported to SINTEF in Trondheim, Norway, where the biomass was minced and either frozen in 1 kg bags or preserved with acid (either lactic acid or citric acid) and vacuum packed (Figure 14). Physicochemical-, trace minerals-, and bioactive analyses were performed on the frozen biomass as control at the beginning of the trials, and analyses on the acid treated biomass were performed at approximately 4, 16, and 32 weeks after the acid treatment. Sensory evaluation was performed on the biomass at the beginning of the trial to evaluate the potential for biomass in food product development.

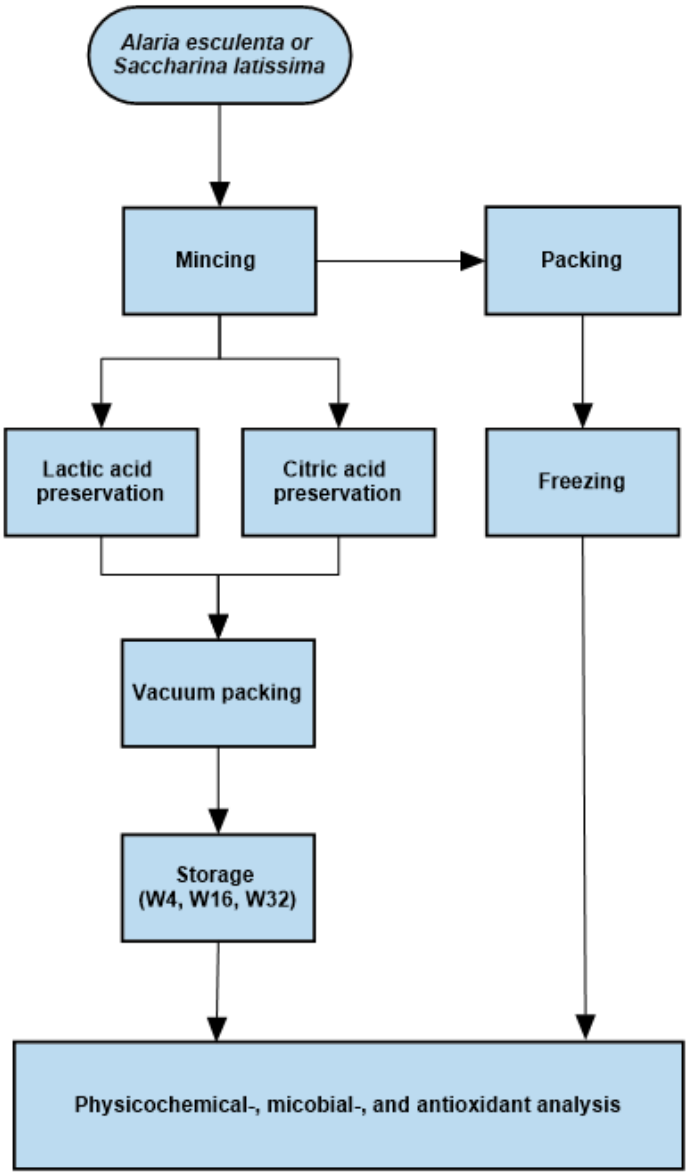

Figure 14 Overview of the experimental design for **Paper III**.

4.1.4 Experimental design for paper IV

The study's objective was to assess if multispectral imaging (MSI) is suitable for monitoring quality parameters of seaweed in a fast, non-destructive manner (Figure 15). *A. esculenta and S. latissima* were cultivated by Seaweed Solutions AS, in Frøya, Måsskjæra, Norway in 2022 and 2023. The seedlings were deployed in the ocean and allowed to grow till harvested. *Alaria esculenta* and *S. latissima* harvested in 2022 were harvested on the 30th of May, and *A. esculenta* harvested in 2023 on May 7th. For both harvesting years, the biomass was transported to the processing facilities the day after harvest, where it was minced with a meat grinder to approximately 5 mm particle size. The minced biomass was further processed using citric or lactic acid, fermentation, or blanching (processing is described in detail in the Supplementary materials in **Paper IV**). The following samples were used in the study:

- Frozen controls *S. latissima* and *A. esculenta* from 2022
- Lactic acid and citric acid treated samples of S. latissima and A. esculenta from 2022, where sampling was performed for 32 weeks, at week 4, week 16 and week 32.
- Frozen controls of *A. esculenta* harvested in 2023.
- Lactic acid and citric acid treated *A. esculenta* harvested in 2023.
- Fermented *A. esculenta* harvested in 2023, fermented by using either Flora Italia inoculum (a blend of *Pediococcus acidilactici, Lactobacillus curvatus, Staphylococcus xylosus*, and *Lactobacillus Sakei* cultures) or Vege inoculum (a blend of *Lactobacillus rhamnosus* and *Propionibacterium freudenreichii subsp. shermanii*).
- Blanched/soaked lactic acid treated and frozen-thawed *A. esculenta* harvested in 2023.

Reference analysis, including traditional assessment of the proximate composition, iodine content, colour, pH, and texture, was performed on the different treated biomasses and images taken with the multispectral imaging equipment Videometer Lab 4 (Videometer A/S, Herlev, Denmark) for each sample. Normalised Canonical Discriminant Analysis (nCDA) and support vector machine (SVM) with VideometerLab software and Unscrambler v11.0 (AspenTechnology, United States), respectively, were used to evaluate the efficiency of the image analysis from the MSI to differentiate between the

species *A. esculenta* and *S. latissima*. Partial least square regression (PLSR) prediction models were constructed between the spectral MSI data obtained from the Videometer Lab 4 MSI instrument and the traditional reference methods with random cross-validation using the Unscrambler v11.0 (AspenTechnology, United States). The strength and accuracy of the prediction models were evaluated based on the model coefficients of determination (R^2) and root mean square errors of calibration (R_C^2, RMSEC) and cross-validation (R_{CV}^2, RMSECV), respectively. Pearson's correlation analysis was, furthermore, performed to identify potential correlations between the physicochemical, antioxidant, and sensory parameters, and Principal Component Analysis (PCA) was performed on the spectral data by using Unscrambler (v 11.0, AspenTech, Massachusetts, United States) to identify similarities and variations between the studied seaweed samples.

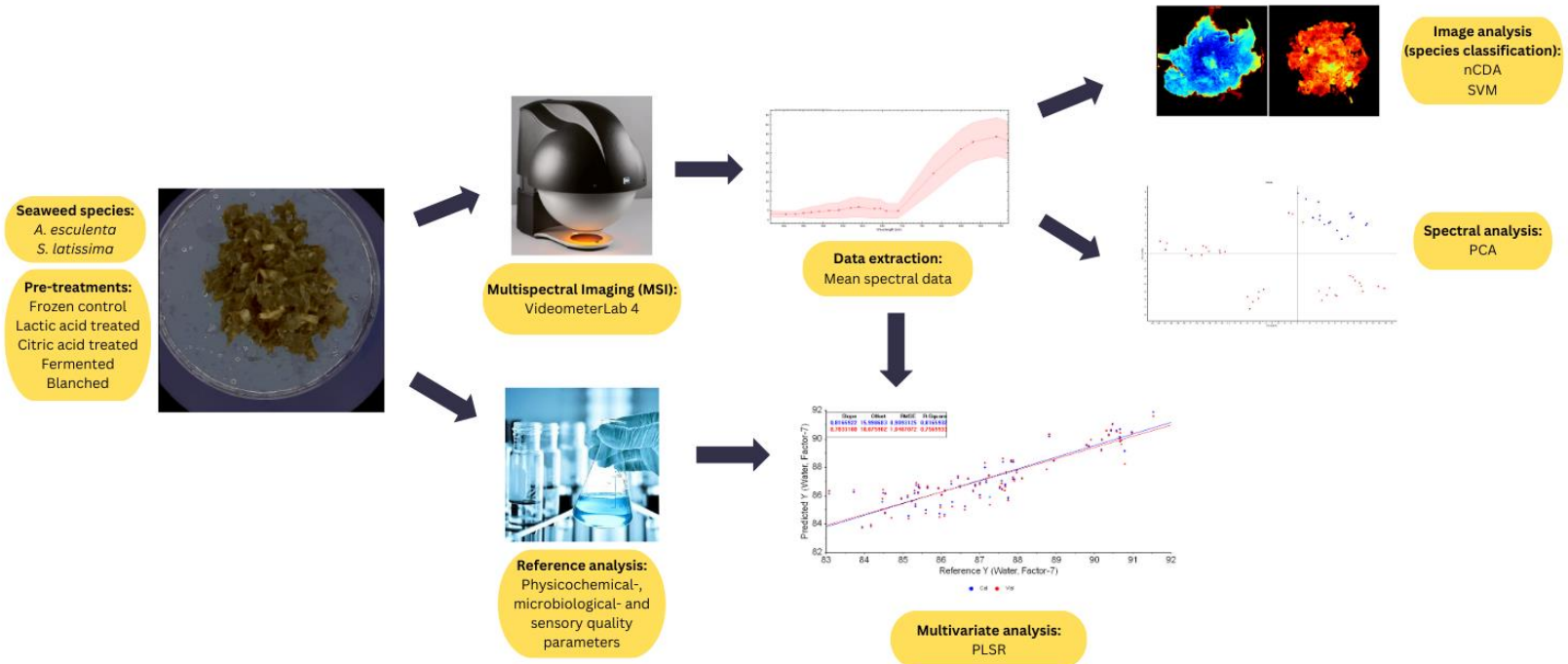

Figure 15 Overview of experimental design for **Paper IV**.

4.2 Analytical methods

Various analytical methods were used in the study to assess the different physical-, chemical-, and bioactive properties of the seaweed biomass and liquid extracts obtained during processing. An overview of the methods used in the study is represented in Table 2. Each analytical method and reasoning for their importance to the project are described in further detail in the following sections, as well as in **Papers I-IV**.

Table 2 Overview of the analytical methods used in Papers I-IV.

Factor investigated	I	II	III	IV
Proximate composition	X	X	X	X
Contaminants (Hg, As, Pb, Cd)	X	X	X	X
Iodine (I)	X	X	X	X
Total phenolic content (TPC)	X	X	X	X
Antioxidant properties (MC, ORAC, DPPH)	X	X	X	X
Total volatile basic nitrogen (TVB-N)			X	X
Total viable counts (TVC)			X	X
Color			X	X
pH			X	X
Water activity (A_w)			X	X
Multispectral imaging				X
Sensory evaluation (GDA)			X	X

4.2.1 Chemical composition

4.2.1.1 Proximate composition

All applied methods described below are well-known standard methods applied in food research to assess the proximate composition of food products. Analysis of the proximate composition was considered necessary not only to assess the seasonal variations of the biomass but also to monitor changes in the chemical composition during processing and storage trials in the study. The chemical

composition assessments also laid the foundations for the mass balances set up for the studied processes.

The water content was analysed according to ISO 6496:1999 (1999). The protein content was determined using either the Kjeldahl or Dumas method. The Kjeldahl method was performed as described by ISO 5983-1:2005 (2005) and ISO 5983-2:2009 (2009) and Dumas method was performed with the method as described by ISO 16634-1:2008 (E) method (ISO, 2008). The total lipid content was determined according to the OCS Official Method Ba-3-38 (AOCS, 2009b) by using Soxhlet extractions. The ash content was analysed according to ISO 5984:2002 (2002). Determination of salt content was performed by using the Volhard titration method (AOAC, 1990). The total carbohydrate content (TC) was then calculated from the results. (further details of measurements are described in **Paper I-IV**).

4.2.1.2 Trace minerals

Since brown seaweed species are known to accumulate both contaminants such as arsenic and cadmium as well as micronutrients such as iodine from their environment, it was considered crucial to identify the main trace mineral content in the seaweed samples (WHO, 2022).

The contaminants mercury, lead, cadmium, and arsenic were determined using a method described by the Nordic-Baltic Committee on Food Analysis (NMKL), except for inorganic arsenic, which was determined using the ASU L 25.06-1 (2008-12) method established by Eurofins with some modifications as described in **Papers I-IV**. The NMKL methods are well-known and standardised methods used in food and feed research.

The iodine content was determined by using inductively coupled plasma mass spectrometry (ICP-MS) since ICP-MS has been applied for the identification of trace minerals in food products with good results (Khan et al., 2014). Two different methods, both using ICP-MS, were used to assess the iodine content of the seaweed samples, as described in detail in **Papers I-IV**.

4.2.1.3 Monosaccharide and uronic acid composition

A large part of the dry matter of brown seaweed is carbohydrates (Holdt & Kraan, 2011a). In **Papers I and II**, the monosaccharide and uronic acid composition was analysed to identify appropriate raw materials for processing and changes in the biomass during suggested alternative processing.

Analysing carbohydrates can be difficult due to their structure similarities and chemical characteristics, especially since they lack suitable chromophores for detection in the ultraviolet (UV) light spectrum (Jalaludin & Kim, 2021). High-

pressure liquid chromatography (HPLC) systems can, however, be used to measure carbohydrates and are valuable tools for identifying monosaccharides (Bailey et al., 2014). The analysis of the monosaccharide and uronic acid composition in the current study was performed according to the National Renewable Energy Laboratory (NREL) for determination of total carbohydrates, using the algal biomass laboratory analytical procedure (LAP) (Wychen & Laurens, 2013). The method is described in detail in **Papers I and II**.

4.2.1.4 Total volatile basic nitrogen

The total volatile basic nitrogen (TVB-N) was analysed by using steam distillation on a 7.5% trichloroacetic acid (TCA) extract as described by (Billon & Tao, 1979) and in **Papers III and IV**. TVB-N is a method that is often used to assess the freshness of fish and other seafood products where TVB-N represents the total amount of ammonia (NH_3), dimethylamine (DMA), and trimethylamine (TMA) in a sample (Jinadasa, 2014). The analysis evaluated protein denaturation due to microbial or enzymatic processes of the acid-preserved seaweed samples during storage.

4.2.1.5 Total viable counts (TVC)

Total viable bacteria count (TVC) is commonly used as a quality indicator to assess the efficiency of preservation during storage and to determine the safety and shelf life of food products (Santovito et al., 2023). The NMKL (2013), 86 method, 5th ed., was used to determine the total aerobic viable counts (TVC) as described in **Paper III**.

4.2.1.6 Total phenolic content

The total phenolic content (TPC) of freeze-dried (FD) seaweed extracts was analysed according to the Folin-Ciocalteu procedure as outlined by Singleton and Rossi (1965) with slight modifications as described in **Papers I-IV**. The Folin-Ciocalteu procedure is a simple, inexpensive and the most commonly used method to assess TPC in food extracts (Sánchez-Rangel et al., 2013).

4.2.2 Physical characteristics

Physical properties, such as pH and water activity (A_w), are important factors regarding the quality and safety of food products since manipulation of both parameters is often used to stabilise and prolong the shelf life of food products (Andrés-Bello et al., 2013; Damodaran et al., 2007). To analyse the water activity (A_w) an Aqua lab 4T water activity meter (Decagon Devices, Pullman, WA, USA) was used, and a portable pH meter (Knick, Berlin, Germany) was used to determine the pH of the samples.

Changes in the colour of food products can often serve as indicators for quality changes in the biomass. The colour changes of seaweed can occur both with processing and storage (Nayyar & Skonberg, 2019; Sánchez-García et al., 2021; Zhu et al., 2022). Therefore, a Minolta Chroma Meter CR-300 (Minolta, Osaka, Japan) with the CIE lab system, which records L-, a*- and b*- values, were used to determine the sample colour in the study. The L-values express the lightness on a scale of 0 to 100, where 0 represents black and 100 is white. The a*-values represents colours from green (-) to red (+) and b*-values blue (-) to yellow (+).

4.2.3 Antioxidant properties

Antioxidant properties can differentiate between seaweed species and their compounds. Therefore, three methods were used to assess antioxidant properties: Oxygen radical absorbance capacity (ORAC), 2,2-Diphenyl-1-picrylhydrazylradical (DPPH) radical scavenging activity (DPPH), and metal chelating abilities (MC) to assess both the extracts' abilities to act as primary-, and secondary antioxidants. These methods were chosen to assess seasonal variation in the antioxidant properties of *A. nodosum*, possible uses of seaweed extracts obtained by alternative processing and if and how acid preservation affects the antioxidant properties of *A. esculenta* and *S. latissima*. All analysis was performed on freeze-dried extracts as described in **Papers I-IV**.

ORAC was determined by using a PolarStar optima microplate reader (POLARstar Optima, Ortenberg, BMG Labtech) according to the method described by Dejian Huang et al. (2002) and Ganske (2006) with slight modifications. ORAC is a method commonly used method in the food industry to explore compounds' abilities to inhibit the propagation of lipid oxidation by scavenging peroxyl radicals (Dejian Huang et al., 2002).

The DPPH radical scavenging activity was determined on dried seaweed extracts by using the method described by Sharma and Bhat (2009) as described in **Paper I-IV**. The DPPH radical scavenging method is one of the most applied antioxidant assays to assess a compound's ability to scavenge free radicals due to its simplicity and low cost (Apak et al., 2013).

The MC abilities were determined by using the method described by Boyer et al. (1988) with minor changes as described in **Paper I**. The metal chelation abilities are used to assess the compound's ability to chelate metal ions to retard the rate of oxidation, a secondary antioxidant activity (Apak et al., 2013).

4.2.4 Sensory evaluation

To evaluate the potential effects of acidification on the sensorial properties of the seaweed samples and possible uses of the biomass as a food substance, Generic Descriptive Analysis (GDA) was conducted according to Stone et al. (2020). The sensory panel consisted of nine panellists trained according to the ISO 8586-1 (1993) standard. Acidification is known to affect multiple sensory properties of a treated food component, including taste, odour, and texture (Anyasi et al., 2017). Therefore, a total of 17 sensory attributes were evaluated, which can be divided into three categories: odour (5), flavour (9) and texture (3). A detailed description of how the sensory evaluation was performed is presented in **Papers III and IV**.

4.2.5 Multispectral imaging

Multispectral imaging was applied to assess whether this novel technique could be used as a non-destructive quality monitoring method during seaweed processing.

The multispectral imaging (MSI) device, Videometeter lab 4 (Videometer A/S, Hørsholm, Denmark), was used to take images of the seaweed samples. The device consists of a high-resolution charged-coupled device (CCD) camera and computer system with an advanced digital image analysis tool. The device takes high-resolution spectral images using advanced light-emitting diode (LED) technology to merge 19 wavelengths, from 365 to 970 nm. The size of the obtained images is 2992*2992 pixels, and each pixel within the image corresponds to a reflectance spectrum. When images were taken, the device was operated at 100% reflectance, and calibration was performed according to standard geometry, colour, and self-illumination practices.

The images were captured with the VidoemeterLab 4 system by placing the seaweed samples on a petri dish. A total of 78 images were obtained, 39 of *A. esculenta* and *S. latissima* samples from 2022 and 39 of *A. esculenta* samples from 2023. The VideometerLab software was used to extract spectral information from the images (excluding background). The data was provided as mean values and standard deviations for each wavelength.

4.2.5.1 Image analysis for species classification

Normalised Canonical Discriminant Analysis (nCDA)

Normalised Canonical Discriminant Analysis (nCDA) is a method that uses the transformation of MSI to distinguish effectively between at least two groups by using image analysis. The nCDA determines a relationship between targeted

categorical variables and independent variables by deriving canonical variables that are linear combinations that capture the variation between the predefined classes (Cruz-Castillo et al., 1994). The nCDA method was used for species differentiation through image analysis. The nCDA model was constructed using the VideometerLab software (**Paper IV**).

Support Vector Machine

Support Vector Machine (SVM) is a mathematical method used for modelling regression and classifications using image data. The classification is performed by transforming the original training data into multidimensional space and establishing a hyper-plane at a higher dimensional (Chandra & Bedi, 2021). In the current study, the SVM was used to classify between *A. esculenta* and *S. latissima* (**Paper IV**) using random cross-validation (kernel-type radial basic function) in the Unscrambler software v11 (Aspen Technology, Bedford, MA, USA).

4.3 Statistical analysis

4.3.1 Data collection and statistical analysis of physicochemical and antioxidant properties

Microsoft Excel (Microsoft, Redmond, WA, USA) was used to collect data and calculate averages and standard deviations (SD) of physicochemical and antioxidant properties. Statistical analysis, including one-way ANOVA (analysis of variance) and Tukey's honest significant difference test, was performed on the physicochemical and antioxidant data by using the JMP pro 16 software (SAS, Cary, NC, USA). For the statistical analysis, a significance level of $p \leq 0.05$ was used to determine significant differences between samples or treatments.

4.3.2 Data collection and statistical analysis of sensory data

During the sensory evaluation, data collection was facilitated using the FIZZ software (Version 2.51C, Biosystémes, Couternon, France), while the panellists' performance was monitored by using Panelcheck V1.4.0 (Nofima, Tromsø, Norway). General linear models (GLM) were corrected for the scale used by the panellist, coupled with Duncan's post hoc test using the NCSS software 2000 (NCSS, Utah, USA). A significance level of $p \leq 0.05$ was employed to determine the significant difference between the sensory properties of the samples.

4.3.3 Multivariate analysis

4.3.3.1 Principal Component Analysis (PCA)

Principal component analysis (PCA) is a well-known and widely used method used to transform a dataset of potentially correlated variables into a smaller set of variables, known as principal components (PCs) while retaining the maximum variability in the dataset (James et al., 2023). The PC score plots illustrate how the samples correlate to each other in a reduced-dimensional space, showing similarities, patterns, and outliers in the data (Jolliffe & Cadima, 2016). In **Paper I**, a PCA was conducted to evaluate the relationship between TPC and antioxidant properties, and in **Paper IV**, a PCA was used to explore the differences in the obtained spectral data.

4.3.3.2 Partial Least Squares Regression (PLSR)

Partial least square regression (PLSR) modelling was used to construct prediction models that evaluate the linear relationship between the spectral data, and physicochemical, antioxidant, and sensory parameters of the seaweed samples in **Paper IV.** PLSR is especially beneficial with data that contains many highly correlated predictors, as it effectively manages multicollinearity, and decreases dimensionality with minimal information loss (Helland, 2006). The method is widely used for the modelling of spectral data to identify linear relationships in datasets, where it has been used to build diverse models from spectroscopic and imaging data to use as quality control tools for several applications within the food industry (Cheng & Sun, 2017).

4.3.3.3 Pearson's correlations between reference variables

Pearson's correlation is a statistical method used to analyse potential linear relationships between two continuous variables (Berman, 2018). In the current study, Pearson's correlation was conducted by using the JMP pro 16 (SAS, Cary, NC, USA) software, where it was applied to identify the relationship between the measured variables. Pearson's correlation was applied in **Paper I** between the antioxidant properties and total phenolic content to evaluate whether TPC contributed to the antioxidant properties of the extracts. Furthermore, in **Paper IV**, Pearson's correlation was used to analyse the relationships between the physicochemical, antioxidant, and sensory properties of the seaweed samples.

5 Results and discussions

This chapter presents the study's main findings, while **Papers I-IV** describe more comprehensive results.

5.1 Seasonal variation of *Ascophyllum nodosum* (Papers I and II)

5.1.1 Chemical composition

Seaweed is known to vary significantly in chemical composition due to various factors, including seasonality and environmental factors such as salinity, temperature and maturity (Schiener et al., 2015). Therefore, the seasonal variation of *A. nodosum* was evaluated to explore and identify which raw material would be the most feasible in the production of a liquid side stream and to understand how the seasonal changes in the composition affect the application of the liquid extract.

Variation was observed in the chemical composition of *A. nodosum* between harvesting periods (**Paper I**), with significant differences in the water content, salt-free ash content, and carbohydrate content (Figure 16 A-C). The highest water content was observed in June (79.6±0.2 g/100 g sample) when the algae is in its fertile period, followed by October (71.9±0.3 g/100 g sample) and July (70.3±0.2 g/100 g sample). The higher water content of *A. nodosum* in June might be related to the reproductive stage of the algae due to the presence of fruit bodies/receptacles (Ragan & Jensen, 1978). Conversely, the salt-free ash and carbohydrate contents showed a reverse relationship to the water content, peaking in July and reaching the lowest in June. Additionally, the chemical composition of *A. nodosum* harvested in August 2022 (**Paper II**) was similar to the composition of the seaweed from July 2020 in **Paper I**, indicating consistent proximate composition patterns of the seaweed biomass between years. Overall, the results indicate that there are seasonal changes in the proximate composition of *A. nodosum,* especially during its fertile period.

The trace minerals arsenic, cadmium, lead, mercury and iodine are considered to be major safety hazards in seaweed processing and applications, especially when the product is intended for human consumption (WHO, 2022). The trace mineral analysis showed no significant seasonal variations in the arsenic levels (neither inorganic nor organic arsenic), lead, or mercury within the fresh seaweed samples on a wet-weight basis. Seasonal differences were, however, observed in the cadmium and iodine content. The iodine content was significantly lower in June (156.7±5.8 mg/kg sample) when compared to the other

two harvesting periods (263.3 and 313.3 mg/kg sample in July and October, respectively) and samples from August 2022 (215.5±12.7 mg/kg sample) (Figure 16 D), which aligns with the salt-free ash content of the samples. The cadmium content was, however, lowest in October (0.08 mg/kg sample) and August 2022 (0.11 mg/kg sample) but higher (0.26-0.28 mg/kg sample) in the other two harvesting periods.

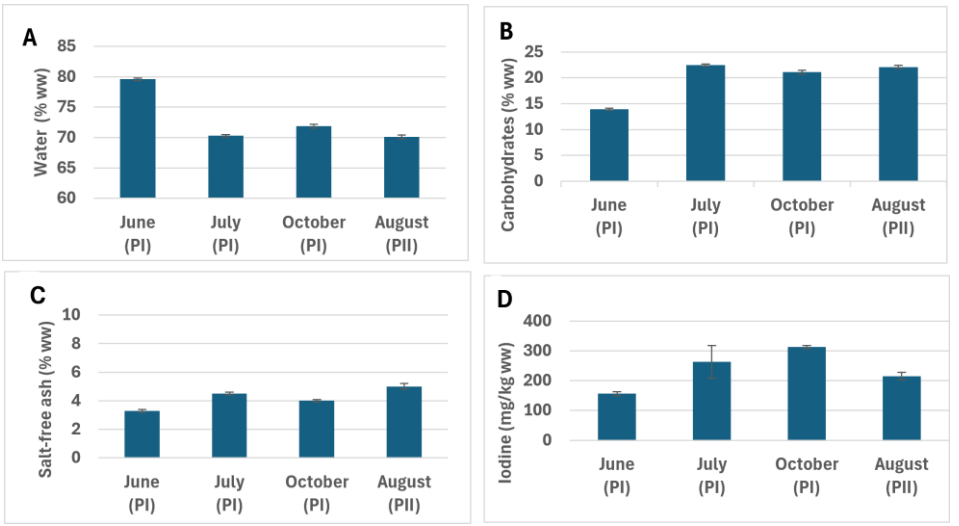

Figure 16 Seasonal changes in water content, carbohydrate content, salt-free ash content and iodine content of fresh Ascophyllum nodosum harvested in June, July and October 2020 (**Paper I**) and August 2022 (**Paper II**).

The monosaccharide composition analysis results revealed that the alginate content and guluronic acid content in the alginate chain were significantly lower in June compared to the other two harvesting periods (Figure 17 A). The alginate levels in the study did not reach the highest reported values (40% of the dw). All alginate values, except for the biomass harvested in June, fell within the range of previously reported values for *A. nodosum,* between approximately 15-40% dw *(Konstantin et al., 2023; Pereira et al., 2020; Schiener et al., 2017)*. Furthermore, the results from **Paper II** indicate that the samples harvested in August 2022 exhibit a higher total alginate content (15.5±0.8 %dw) than the samples collected in June 2022 (11.1±0.7 %dw). However, the mannuronic to guluronic acid rations (M/G ratios) were higher in the samples used in **Paper II**, with an M/G ratio of 2.2±0.8, compared to those used in **Paper I**, which had an average M/G ratio in the range from approximately 0.55 to 0.85. This indicates seasonal differences both in the total alginate content and the alginate composition. Additionally, significantly higher glucose and fucose content was observed in the fresh seaweed samples harvested in October than at other harvesting times, including

the August 2022 samples (Figure 17 B, C). The results show that the *A. nodosum* biomass harvested from July to October is more suitable for alginate production than the biomass harvested in June. Additionally, the results highlight the importance of using appropriate raw materials to obtain higher yields in production and to make the most out of the harvested resources.

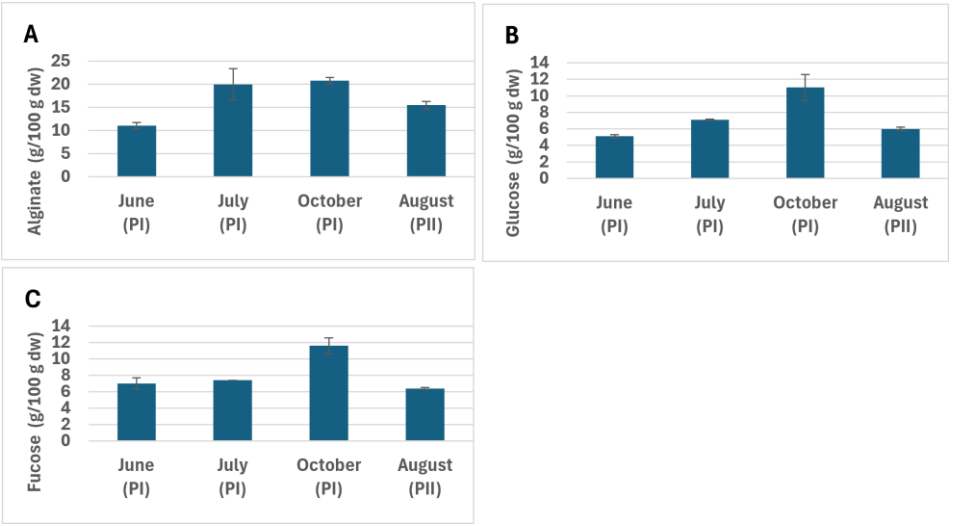

Figure 17 Seasonal changes in (A) alginate-, (B) glucose-, and (C) fucose content of fresh *Ascophyllum nodosum* harvested in June, July and October 2020, and August 2022 (**Paper I and Paper II**).

5.1.2 Total phenolic content and antioxidant properties

The total phenolic content (TPC) and antioxidant properties were analysed to assess the efficiency of the extraction method, seasonal changes in the raw materials used and the possible commercial applications of the liquid. The analysis was performed on different water extracts obtained using either minced fresh, micro-cut, or dried *A. nodosum* in **Paper I** to evaluate if micro-cutting the blend is a necessary step in the alternative processing to extract phenolic compounds, along with other possible valuable compounds that exhibit antioxidant properties. The TPC did not significantly differentiate between the harvesting periods in the extracts obtained by using the fresh minced biomass, while variations were detected in the press-liquid and seaweed meal extracts, highlighting the importance of using appropriate raw materials/methods to extract phenolic compounds from the biomass. The extracts from June 2020 contained significantly lower TPC compared to the obtained press liquid extracts from July and October of the same year, as well as the liquids obtained using materials harvested in August 2022 in the scale-up processing experiment (**Paper II**). The

findings correspond to the results from Konstantin et al. (2023) and Ragan and Jensen (1978), who reported lower TPC of *A. nodosum* in May and June compared to raw materials harvested later the same year.

The antioxidant properties of *A. nodosum* extracts, assessed by oxygen radical scavenging activities (ORAC), varied significantly across harvesting periods. The ORAC values in June were significantly lower compared to the other two harvesting periods, as well as samples from August 2022 (**Paper II**), whereas no significant difference was observed between the values in July and October. Furthermore, no significant difference was observed between harvesting periods in the DPPH radical scavenging activities or metal chelating (MC) abilities. Additionally, the principal component analysis (PCA) (Figure 18, **Paper I**) of antioxidant properties and TPC showed a positive relationship between TPC and ORAC, further confirmed by Pearson's correlation between these variables (r=0.81). This correlation has been reported previously in seaweed extracts of *A. nodosum* and other seaweed species (Agregán et al., 2017; Wang et al., 2009), where TPC correlates with ORAC values, indicating that TPC contributes to the peroxyl scavenging activity in the extract. TPC did, however, not show any correlation with the other antioxidant measurements, even though DPPH radical scavenging activity has been correlated to TPC in some studies (Wang et al., 2009). Therefore, the results indicate that other compounds, such as sterols, fucoxanthin or even some peptides that can act as radical scavengers, contribute to the metal chelating and DPPH radical scavenging activities observed in the extracts (Egusa Saiga & Nishimura, 2013; Wang et al., 2009). Similar observations were also noted in **Paper II** results, where extracts with lower ORAC values typically had lower TPC. The results suggest that the production of the liquid side stream would be relatively stable, with material harvested from July through October. Additionally, the results show that the production would not be suitable in June or when the algae is in its fertile period, as both the antioxidant properties and alginate content are lower than other harvesting periods. However, the biomass harvested in June could be more suitable for other applications, including as fertilizer or a feed enhancer.

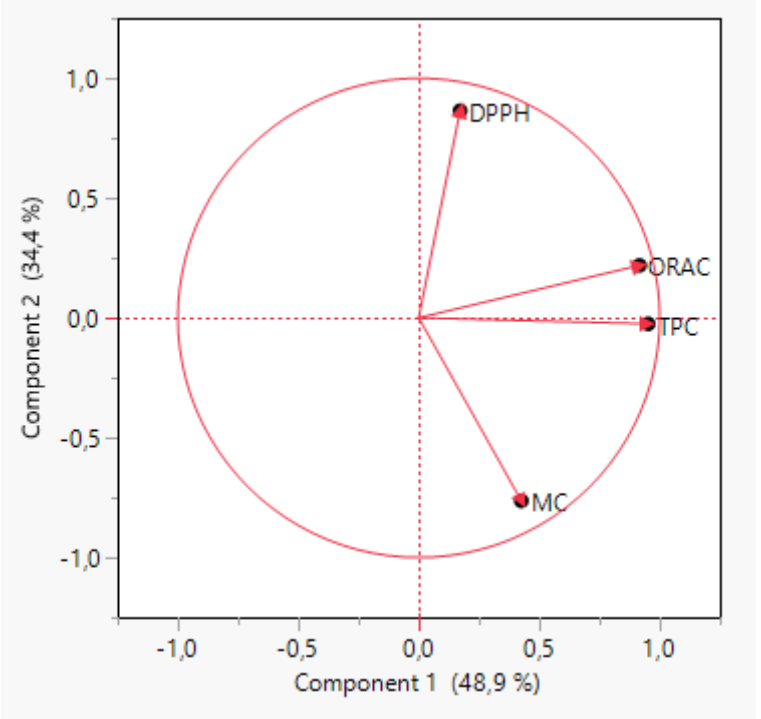

Figure 18 Loading plot of principal component analysis (PCA) of total phenolic content (TPC), DPPH radical scavenging activities, oxygen radical absorbance capacities (ORAC) and metal chelating activities of all extracts used in **Paper I**.

5.2 Evaluation of feasibility and scale-up methods for alternative processing of seaweed meal from Ascophyllum *nodosum* (Paper I and Paper II)

Experiments described in **Paper I** were performed to evaluate the possibilities of producing high-value side products within *Ascophyllum nodosum* seaweed meal processing to increase the utilisation of the biomass at a laboratory scale. To further assess the feasibility of implementing the proposed processing changes within commercial meal processing on an industrial scale, **Paper II** aimed to evaluate several scale-up options for production with an emphasis on the separation between solid and liquid phases, drying of the obtained liquid and the effect of these processing changes on the produced seaweed meal.

5.2.1 Chemical changes during alternative processing of *A. nodosum* during meal processing (Papers I and II)

The chemical analysis results and mass balances from **Paper I** showed that the suggested alternative process affects the distribution of the chemical components

between processing streams. The water content increased significantly, or by approximately 7-10%, in the press cake compared to the raw material, as observed in both lab-scale trials (**Paper I**), and trials performed in an industrial-scale setting (**Paper II**). This increase in water content was primarily caused by the water addition performed prior to the micro-cutting of the raw material. The main components extracted from the biomass into the liquid processing streams (press liquid or decanter liquid) with the alternative process were carbohydrates, ash (including salt), phenolic compounds, arsenic, and small amounts of protein (Table 3).

Both the salt-fee ash content and the salt content reduced significantly in the solid phase in the alternative processing, with a reduction of 28-36% and 54-65%, respectively (**Paper I**) (Table 3). Similar results were obtained in **Paper II,** where a substantial loss of salt content was observed in the solid phase but a lower reduction in salt-free ash content (approximately 18%). The results indicate a loss of mineral content in the solid phase, confirmed by the loss of the iodine content, which was reduced by 25% in the biomass during processing. This would lead to lower mineral content of the seaweed meal if the alternative processing were adopted. By reducing other compounds in the biomass, the alginate content proportionally increases on a dw basis. Therefore, since the pre-extracted seaweed meal is mainly intended for alginate production, the decrease in mineral content is considered positive rather than negative. Furthermore, the results from both **Paper I** and **Paper II** show that the trace minerals arsenic and cadmium were released from the biomass during the process. This especially applies to arsenic, which was reduced by approximately half in the solid phase (press cake) in July and October (**Paper I**). A similar reduction in arsenic content was observed in **Paper II**. The high arsenic and iodine contents in the liquids could affect their applications, highlighting the importance of finding ways to reduce these compounds in the liquid extracts.

The reduction of the carbohydrate content in the press cake and decanter sediments compared to the fresh raw materials can primarily be linked to the increase in the water content of the biomass due to the addition of water before micro-cutting. The main monosaccharide extracted with the press liquid was mannitol (approx. 42-53 g/100 g sample dw) followed by glucose (approx. 12-33 g/100 g sample dw) and fucose (approx.1.2-1.7 g/100 g sample dw) (Table 3) (**Paper I**). Most of the alginate remained in the press cake, as the hexa-mannuronic acid was absent in the press liquid, and only small amounts of the guluronic acid were detected in the press liquid in **Paper I**. These findings were confirmed in **Paper II**, where the same carbohydrates were extracted with the alternative scaled up processing (Table 3), but generally in lower concentrations.

This might be partially explained by the lower content of mannitol, fucose and glucose in the raw material used in **Paper II**.

Since the alginate remains mostly in the sediment after extraction, the results indicate that the proposed alternative processing is feasible and has limited disrupting effects on the current commercial meal intended for alginate production. The proposed alternative processes thus provide opportunities for diverse product development and value-adding from the extracted ingredients in the new liquid stream.

Table 3 Results of the main component affected and of interest during the suggested alternative processing process from **Paper I** and **Paper II**. A lab centrifuge was used for liquid-solid separation in **Paper I**, while both a screw press and decanter were used in **Paper II**.

	Harvesting time	Sample	Water (g/100g)	Salt-free ash (g/100g)	Salt (g/100g)	Carbohydrate (g/100g)	Alginate (g/100g dw)	Glucose (g/100g dw)	Iodine (mg/kg)	Arsenic (mg/kg)
Paper I	July 2020	Fresh seaweed	70.3±0.2^{a}	4.5±0.1^{a}	1.3±0.0	22.5±0.5^{a}	20.0±3.4	7.1±0.1	263±55	7.3±0.5
		Press liquid	94.9±0.1	0.3±0.2	0.9±0.0	3.5±0.1	1.4±0.1	12.3±0.9	169±2	3.8±0.0
		Press cake	80.7±0.1^{b}	2.9±0.2^{b}	0.6±0.0	14.6±0.4^{b}	24.5±3.1	6.8±0.3	183±16	4.4±0.1
		Seaweed meal	6.9±0.2	14.1±0.7	5.8±0.6	67.0±1.4	19.7±1.8	5.8±2.2	893±21	28.5±0.5
	October 2020	Fresh seaweed	71.9±0.3^{a}	4.0±0.1^{a}	1.7±0.0	21.1±0.4^{a}	20.8±0.7	11.0±1.6	313±6	7.5±0.2
		Press liquid	94.8±0.0	0.2±0.1	1.0±0.0	3.8±0.1	1.5±0.1	32.8±1.2	142±4	2.3±0.1
		Press cake	80.4±0.2^{b}	2.9±0.2^{b}	0.6±0.0	14.9±0.3^{b}	23.8±1.3	11.2±1.7	130±0	3.7±0.1
		Seaweed meal	6.2±0.2	15.6±0.2	6.8±0.1	64.9±0.2	12.0±0.3	4.3±0.5	980±20	31.3±1.0
	Processing method									
Paper II	Raw material	Fresh seaweed	70.1±0.3^{g}	5.0±0.2^{c}	1.4±0.0^{c}	22.1±0.3^{c}	15.5±0.8^{c}	6.0±0.2^{d,e}	216±13^{c}	7.3±0.4^{c}
		Micro-cut seaweed	85.1±0.5^{d}	2.3±0.1^{e}	0.8±0.0^{d,e}	11.1±0.6^{e}	NA	NA	NA	NA
	Decanter	Liquid	95.7±0.3^{c}	0.4±0.1^{f}	0.4±0.1^{f}	2.9±0.2^{f}	1.1±0.1^{e}	8.0±1.3^{c,d}	57±29^{e}	2.6±0.2^{e}
		Sediment	77.4±0.6^{d}	4.1±0.1^{d}	4.1±0.1^{d}	16.5±0.5^{d}	17.4±3.1^{c}	5.4±0.9^{e}	168±20^{d}	4.1±0.0^{d}
	Screw press	Press Liquid	94.8±0.2^{a}	0.7±0.2^{f}	0.7±0.2^{f}	3.3±0.2^{f}	NA	NA	NA	NA
		Press cake	80.0±2.9^{e,f}	4.0±0.8^{d}	4.0±0.8^{d}	14.2±2.3^{d}	NA	NA	NA	NA
	Commercial product	Seaweed meal	7.5±0.7	20.7±2.0	5.5±0.2	60.4±1.9	10.0±1.1	6.2±0.8	425±35	29.2±23.2

The different subscript letters a-b indicate significant differences ($p<0.05$) between raw material and press cake results from **Paper I** while the different subscript letters, c-g, are used to indicate significant differences in processing produce results from **Paper II** results.

5.2.2 Total phenolic content (TPC) and antioxidant properties of obtained liquid extracts (Papers I and II)

Total phenolic content and antioxidant properties were analyzed to evaluate the efficiency of extracting phenolic compounds from differently processed raw materials (minced fresh biomass, micro-cut biomass and dried biomass) during three harvesting periods (June, July, and October) and to assess possible uses of the liquid extract in **Paper I**. Additionally, these properties were assessed in liquids obtained from different separation methods, e.g., as affected by different choices of main separation methods (decanter and screw press), and the use of adding a secondary separation/purification method or not (centrifugation), in **Paper II.**

Total phenolic content (TPC)

When comparing extraction methods using differently processed raw materials (minced fresh biomass, micro-cut biomass and dried biomass) in **Paper I**, significant differences were noticed in the TPC. The extracts obtained from July and October showed that by micro-cutting the seaweed blend, higher TPC values were obtained compared to when using the conventional extraction method with only minced fresh seaweed biomass. The results indicate that higher amounts of phenolic compounds are extracted when the biomass is micro-cut. Therefore, the Stephans micro cutter was used as part of the alternative processing process to scale up the production in **Paper II.**

When the main separation methods were compared (**Paper II)**, no differences were observed in TPC between the liquid streams from the decanter and screw-press, resulting in a TPC of 18.0±0.7 and 16.5±1.4 PGE/100 g dw extract, respectively. The centrifuged liquids contained an average TPC in the range of 17.5 to 18.0 PGE/100 g extract. Thus, no significant differences were observed between the centrifuged liquid and the liquids obtained from the main separation. The centrifuged precipitate did, however, contain significantly lower TPC (on average in the range of 9.6 to 12.9 PGE/100 g extract) than the obtained centrifuged liquids. This indicates that the centrifugation does not effectively concentrate the phenolic compounds in the centrifugation liquid. Furthermore, no substantial differences were noticed in the TPC between the tested drying methods, i.e., freeze-drying vs spray drying, of the decanter liquid, with measured TPC of 18.2±0.2 and 18.0±0.7 PGE/100g extract, respectively. This suggests that the short heating process used in the spray drying does not negatively affect TPC, indicating that spray drying could be a feasible option to preserve phenolic compounds in the extract.

Antioxidant properties

The press liquid from June and October exhibited higher ORAC values than the fresh seaweed extracts, whereas the July extracts demonstrated higher ORAC activity in the fresh seaweed extracts. The DPPH radical scavenging activity did not significantly differentiate with the use of differently processed raw materials in the extraction process (**Paper I**). However, the metal chelating abilities (MC) were significantly higher when seaweed meal was used in the extraction process for all harvesting periods, with inhibition in the range of 70.5 to 78.0%, while the other two extraction methods resulted in inhibition in the range of 44.2-50.9%. Thus, the results suggest that if metal chelating properties are of interest, it is more suitable to use dried biomass for extraction than fresh biomass.

The results of **Paper II** suggest that the choice of primary separation method used (decanter vs screw press) does not affect the ORAC values of the obtained liquids, as no significant difference was observed in the ORAC values between the two liquids. In the screw-press processing, the centrifuged liquid showed higher ORAC activities than the precipitate, which corresponds to the TPC content of the liquids. Additionally, these findings align with the PCA from **Paper I**, which demonstrated a relationship between the TPC and ORAC values. In addition, no differences were observed in either TPC or antioxidant properties between drying methods. The results indicate that spray drying could be a feasible option to dry the liquid extracts since spray drying is relatively easy to use, is much faster than freeze drying, is suitable for drying liquids and fits into continuous production processes (Poozesh & Bilgili, 2019). Overall, the results from **Paper II** show that both separation processes are equally efficient in retaining TPC and antioxidant properties, that centrifugation is considered an unnecessary step in the liquid processing, and that spray drying could be used to dry the obtained liquids in the alternative production process.

5.2.3 Evaluation of separation methods for alternative industrial processing of seaweed meal from *Ascophyllum nodosum* (Paper II)

The mass balance calculation provided a comprehensive overview of the separation methods' efficiency in separating the liquid phase from the solid phase during the alternative processes (Figure 19). The results showed that the use of the decanter as the main separation step was more efficient than using the screw press as a higher amount of liquid was retrieved by using the decanter, or 43.3% vs 36.3%. As a result, a slightly higher water content was obtained in the press cake (80±2.9 g/100 g) compared to the decanter sediment (77.4±0.6 g/100 g), indicating better liquid separation of the biomass using the decanter. In addition,

during the processing, through evaluation of the press cake and decanter sediment visually, the same observation was made where the press cake obtained from the decanter appeared noticeably dryer, and the decanter-liquids appeared to be clearer than the screw-press liquid. Additionally, no significant differences were observed in the proximate composition between the decanter sediment and the press cake, nor between the decanter liquid and press liquid. Based on the results and observations, the decanter was considered to be more suitable than the screw press for the main separation method for the suggested alternative processing.

The secondary separation process, centrifugation, was performed to attempt to further refine the liquids obtained from the decanter and screw press processes and to determine if a further separation step would be beneficial. Approximately half of the press liquid ended up as precipitate following centrifugation (18 kg out of 36.6 kg), but 30% of the decanter liquid (12.9 out of 43.3 kg) ended in the precipitate (Figure 19). The results showed that the centrifugation was inefficient in refining the liquid extracts, as little differences were seen in the composition of the liquids pre and post centrifugation, and neither trace minerals were reduced, nor did antioxidant activity improve in the liquid during the centrifugation. Therefore, the centrifugation step was concluded to be an unnecessary step in the process. Further, other methods might be more suitable for refining the liquids. Pressure-driven filtration (nanofiltration, microfiltration, ultrafiltration and reverse osmosis) has, for example, been used to purify carbohydrates and phenolic compounds in seaweed extracts with good results (Díaz-Reinoso, 2020).

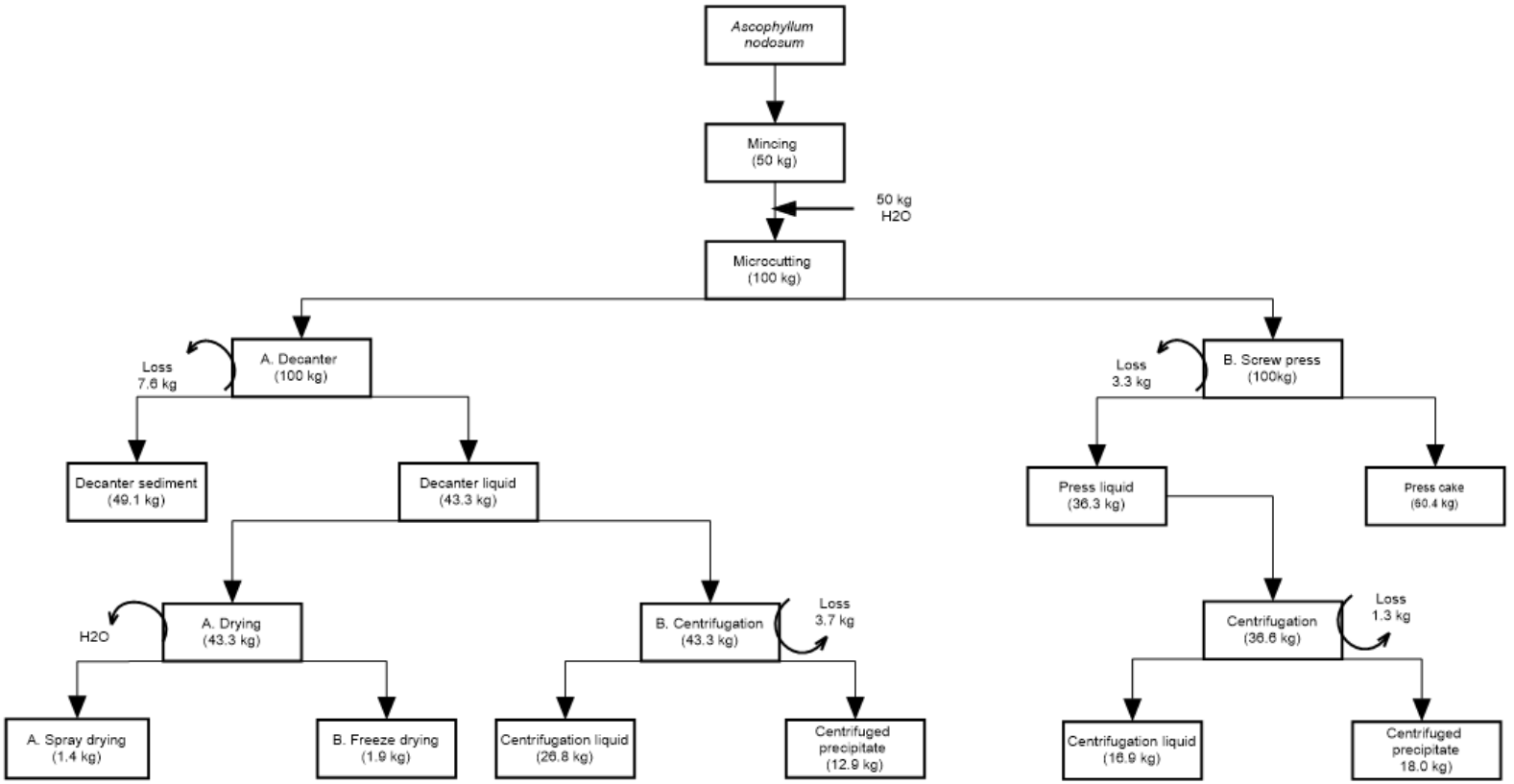

Figure 19 Overview and mass balance of the different processing options, including the primary separation (decanter vs. screw- press), and decanter liquid processed with or without secondary separation of the press liquid by centrifugation, and comparison of processing (**Paper II**).

5.2.4 Effects and implementation of liquid side stream processing

In order to align with the United Nations' sustainability development goals (UN SDGs) regarding responsible consumption and production (SDG 12), as well as life below water (SDG 14), it is imperative to maximise the utilisation of harvested seaweed biomass and to minimise waste in its present applications. Today, the Icelandic company currently harvests approximately 15,000 tons of *A. nodosum* annually. If assumed that the biomass is primarily used for alginate production, 15.000 tons of wild harvested seaweed biomass could be used for the production with the alternative processing, allowing the production of more diverse products from the liquid side stream and the main alginate product. As mentioned, the decanter was evaluated to be the most suitable separation method, whereby during the processing of 100 kg of micro-cut biomass, the decanter produced 43.3 kg of liquid side-stream, with a dry matter content of approximately 4% (Figure 19 and Table 3). That means that a total of 12.990 tons of liquid extract, or approximately 420-570 tons of dried extract on an annual basis, based on the yield of the spray-dried and freeze-dried extracts, respectively, could be produced without impacting the potential of using the meal in the production of alginate.

With increased water content in the initial biomass to be used for seaweed meal processing, the drying time of a product is affected, along with other factors such as convective mass transfer qualities (Singh & Heldman, 2001), calling for adjustments of drying conditions. Based on the analyses from **Paper II**, the decanter sediment contains approximately 7% higher water content than the fresh biomass. This implies that optimisation of the drying protocol may be necessary for drying the decanter sediment compared to current protocols to obtain a water content below 14-15% in the final meal and to ensure the stability and safety of the product (WHO, 2012).

For the processing of the press liquid stream thickening (e.g., sieving and evaporation) may be suitable before freeze drying or spray drying since the press liquids contain approximately 95% water or only 4-5% dry matter. Products with high water content and water activity generally have short shelf life due to their susceptibility to microbial and enzymatic degradation (Damodaran et al., 2007). Furthermore, storage and transportation of such products can be expensive and have a high environmental impact due to their bulk volumes. Therefore, implementing drying and/or concentration processes can be beneficial to obtain a more concentrated and stable product that

requires less storage space. Therefore, concentrating and/or drying of the extracts is recommended for further product development or applications.

The food and feed industry relies heavily on water usage, leading to the overuse of water resources. The high water content (or approximately 15.000 tons annually) in the suggested alternative processing affects its sustainability significantly. Therefore, the focus should be set on examining possibilities of water reduction in the process, as well as taking regional water footprint and water scarcity issues on the production sites (e.g., with the AWARE methodology) into account. Reducing the water content would likely also reduce the drying time of the extract if drying is chosen as a processing step for the extract, and thus reduce both the energy consumption and cost of the operation.

Furthermore, research should be performed to assess the uses of the extract and evaluate how to reduce undesired compounds in the liquid. Our previous trials of using the decanter liquid extract as an antioxidant to retard lipid oxidation in fresh herring in cold storage (by dipping the herring in the liquid extract) show that the extract significantly reduced the production of Thiobarbituric acid reactive substances (TBARS) compared to untreated control samples (unpublished data), indicating that compounds in the extracts act as secondary antioxidants (as oxygen-, and metal chelators) (Shahidi & Zhong, 2015). Additionally, the results show that the extract did not affect the free fatty acid (FFA) content or phospholipid (PL) content (results not published).

5.3 Acid preservation of *Alaria esculenta* and *Saccharina latissima* intended for human consumption (Paper III)

Today, preserving the cultivated brown seaweed biomass is one of the main challenges in the seaweed cultivation sector due to the short harvesting period and high infrastructure cost (Stévant & Rebours, 2021). With increased seaweed production, novel preservation methods that are less energy-consuming and more practical for seaweed cultivators are required. Given these challenges, the work in **Paper III** aimed to evaluate the efficiency of acid preservation, a well-known food preservation method, for brown seaweed biomass.

5.3.1 Chemical properties

The results of **Paper III** show that acid preservation of the *A. esculenta* and *S. latissima* with both lactic and citric acid stabilise the biomass with minimal

changes in the chemical composition. The proximate composition of the biomass remained stable throughout the storage with only slight changes, mainly due to natural variation within the biomass. The results of the proximate composition primarily showed differences between the two seaweed species, as expected (Arlov et al., 2024; Schiener et al., 2015). The main differences were in the water, protein, and carbohydrate content, where *S. latissima* had higher water content but lower carbohydrate and protein content than *A. esculenta*. Since no storage effect was observed, the results suggest that acid preservation with both lactic and citric acid is suitable to preserve the studied brown seaweed biomass for at least 32 weeks.

The discussion of whether seaweed is safe for human consumption and the lack of legislation on the matter has been increasing in the last few years within Europe. The main trace minerals of concern reported in brown seaweed biomass are iodine, arsenic, cadmium, mercury, and lead (WHO, 2022). **Paper III** showed that the trace mineral composition varied significantly between the two species, especially in arsenic, cadmium, and iodine concentrations. *S. latissima* contained a higher content of iodine compared to *A. esculenta* (410±17 vs 100±0 mg/kg ww) but a lower content of cadmium (0.09±0.01 vs 0.36±0.01 mg/kg) and arsenic content (5.5±0.3 vs 9.7±0.5 mg/kg) when compared to *A. esculenta*. The biomass was, however, only rinsed and not blanched. Many methods have been tested to reduce these trace minerals, where blanching has now been established as a good method to reduce the iodine content for seaweed biomass and is currently used in the industry (Hogstad et al., 2023). Previously reported data indicate that blanching the biomass also leads to a significant reduction in dry matter. The main components affected by blanching include ash (such as iodine and salt), arsenic, and carbohydrates (Hogstad et al., 2023; Nielsen et al., 2020). Additionally, the Supplementary Materials of **Paper IV** summarised the efficiency of blanching or soaking on the acid treated biomass and frozen biomass. It has been reported that blanching treatments performed with high heat (or 80°C) can reduce idoine by 90% (Nielsen et al., 2020). The results of the blanching treatments did, however, indicate that blanching the acid treated biomass only effectively reduced the iodine content by 60-70%, while no reduction in iodine was observed when the frozen-thawed biomass was blanched. The lower iodine reduction observed in the acid-preserved biomass might be connected to the smaller amounts of water used in our blanching trials compared to the trials performed by Nielsen et al. (2020). The results, furthermore, suggest that freezing alters the structural characteristics of the biomass, affecting the iodine solubility. However, the mechanisms of these

structural changes need to be studied further. Additionally, the results highlight the importance of blanching before processing the seaweed biomass to effectively reduce the iodine content, especially if it is intended for human consumption.

5.3.2 Physical properties and pH

Physical changes in seaweed biomass can occur after harvesting, as well as during and after processing (Akomea-Frempong et al., 2022; Wirenfeldt et al., 2022). Changes in colour and pH can often indicate chemical degradation of the biomass and the processing efficiency (Andrés-Bello et al., 2013; Pathare et al., 2013). When working with acid-preserved biomass, it is crucial to maintain a stable pH during the storage to prevent the growth of pathogenic bacteria, which can start to grow at a pH above 4.5 (Coban, 2020; Dauthy, 1995).

Both the citric acid and the lactic acid treatments successfully reduced the pH of the seaweed biomass from approximately pH 6.5 to a pH below 3.9 (Table 4). Furthermore, the results show that the pH decreased during storage, with a significant reduction between week 4 and week 32 for both seaweed species and treatments, indicating that the biomass was stable towards microbial spoilage throughout the storage period (Coban, 2020; Theron & Lues, 2010). That agrees with the total viable count (TVC), which remained stable throughout the tested storage period (Figure 20). Overall, the results show that the pH of the acid treated biomass remains stable for at least 32 weeks of storage.

The results of **Paper III** showed colour changes in the biomass during the acidification, resulting in lower a^*-values and higher L-, and b^*-values post-acidification, indicating that the biomass became more yellow and greener after the acid treatment (Table 4). This colour change in the seaweed biomass with acidification is positive since consumers generally preference to consume seaweed products with brighter and greener colours (Blikra et al., 2019; Yamanaka & Akiyama, 1993).

Table 4 Results of physical properties (pH and colour (L-, a*-, and b*-values) of the seaweed samples (control and acid treated samples) from **Paper III**. The subscript letters (a-e) indicate significant differences in the parameters within each species.

Specie	Sample type	Storage duration (weeks)	pH	L-value	a*-value	b*-value
Saccharina latissima	Control	W4	6.47±0.07^{a}	20.4±1.2^{b}	4.6±0.4^{a}	3.5±1.1^{c}
	Lactic acid	W4	3.62±0.02^{c}	26.8±2.8^{a}	2.5±0.9^{b,c}	8.5±1.5^{a}
		W16	3.57±0.01^{c}	26.2±2.2^{a}	2.1±0.6^{c}	7.0±1.4^{a,b}
		W32	3.47±0.01^{d}	26.1±1.6^{a}	1.8±0.4^{c}	7.2±0.8^{a,b}
	Citric acid	W4	3.76±0.01^{b}	26.1±1.5^{a}	3.3±0.6^{b}	8.7±0.7^{a}
		W16	3.73±0.01^{b}	25.2±0.4^{a}	2.7±0.1^{b,c}	7.2±0.4^{a,b}
		W32	3.61±0.01^{c}	26.3±1.5^{a}	2.0±0.3^{c}	6.6±0.8^{b}
Alaria esculenta	Control	W4	6.38±0.02^{a}	23.1±1.3^{c}	3.9±0.5^{a}	4.3±0.8^{b}
	Lactic acid	W4	3.70±0.00^{c,d}	30.5±2.6^{b}	0.4±0.5^{b,c}	8.9±1.4^{a}
		W16	3.67±0.02^{d}	34.6±2.4^{a}	0.0±0.6^{b,c}	10.0±2.3^{a}
		W32	3.53±0.00^{e}	30.7±2.0^{b}	0.5±0.4^{b}	9.7±1.1^{a}
	Citric acid	W4	3.88±0.01^{b}	33.0±2.8^{a,b}	-0.7±0.8^{c}	10.1±1.5^{a}
		W16	3.86±0.02^{b}	31.4±0.8^{a,b}	0.5±0.2^{b}	9.3±0.7^{a}
		W32	3.73±0.00^{c}	31.9±1.0^{a,b}	0.5±0.2^{b}	10.0±0.9^{a}

5.3.3 Total viable count (TVC) and Total volatile basic nitrogen (TVB-N)

Food preservation is used to extend the shelf life of products by preventing the spoilage mechanisms and growth of spoilage microorganisms and pathogenic bacteria. Methods such as freezing, acidification, salting and drying are commonly used (Gram et al., 2002). Acid preservation is performed by lowering the pH down to acidic levels that most pathogenic bacteria cannot grow (usually below 4.6) (CFR, 1979; Dauthy, 1995; Theron & Lues, 2010). Total viable bacteria count (TVC) is commonly used as a quality indicator to assess the efficiency of preservation during storage and to determine the safety and shelf life of food products (Santovito et al., 2023). Results of **Paper III** showed that the frozen control had significantly lower TVC compared to the acid treated samples. However, the TVC of the acid treated biomass remained stable throughout the 32-week storage time (Figure 20). Additionally, the TVC results of the seaweed samples harvested in 2023 were similar to the results obtained in 2022, showing values of 1.1±0.8 log cfu/g sample in frozen controls, 3.9±3.7 log cfu/g sample for lactic acid treated samples and 2.5±1.7

log cfu/g sample for citric acid treated samples. These results indicate that the method efficiently prevents microbial growth and that the production practices are probably stable, as similar results were obtained across the two years. Furthermore, the results indicate that lactic or citric acid are equally effective in inhibiting bacterial growth in both the *A. esculenta* and *S. latissima* samples, indicating that the method effectively stabilises the biomass.

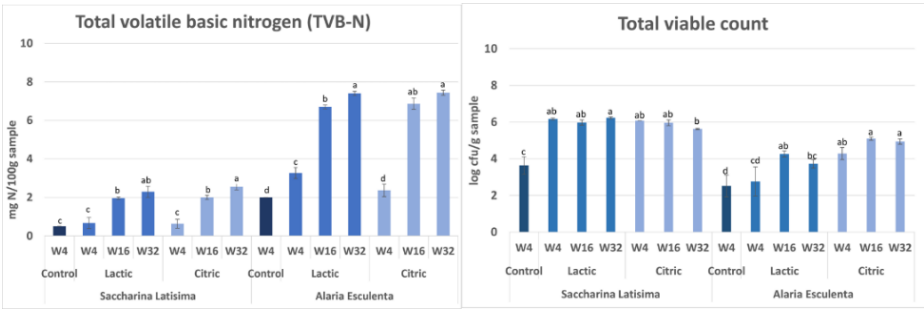

Figure 20 Results of total volatile basic nitrogen (TVB-N) and total viable count (TVC) of seaweed samples from **Paper III**. Results are expressed as means with standard deviation. The TVB-N is presented as mg nitrogen per 100 g sample and TVC as the log of colony-forming units per gram of sample. Significant differences within the same species are indicated by different subscript letters (a-d).

Total volatile basic nitrogen (TVB-N) is a quality control parameter used to assess protein degradation in high-protein food products such as fish and fermented meat products (Bekhit et al., 2021; Jinadasa, 2014). TVB-N has not been used a lot in the assessment of seaweed quality due to the low protein content in the biomass, usually ranging from 3% to 17% dw in brown seaweed (Holdt & Kraan, 2011a). The results in **Paper III** do, however, show that TVB-N gradually increases during storage of the acid-preserved biomass, especially in the *A. esculenta* samples, which have a higher protein content than *S. latissima*. Additionally, results of fermented *A. esculenta* samples (**Paper IV**) showed a significant increase in TVB-N, with TVB-N values from 7.4-16.0 mg N/100 g sample compared to acid treated and control samples (1.7-1.8 mg N/100 g sample) (Figure 20). During fermentation, microorganisms produce nitrogenous compounds to survive (Ekici & Abdullah, 2020), which can explain the higher values of TVB-N in the fermented samples. Additionally, the increase in TVB-N of the acid-preserved samples could be an indication of fermentation of natural microbiota (Stanbury et al., 2013; Sørensen et al., 2021).

5.3.4 Sensory evaluation

The results of the Generic Descriptive Analysis (GDA) in **Paper III** showed distinct textural differences between the two species, and *A. esculenta* samples were tougher and more astringent than the *S. latissima,* which had a more liquid texture, which correlates to the higher water content in the latter species. Additionally, the sensory panel described the treated biomass as not very appealing, and the initial flavour was sour and then changed to very salty. Generally, GDA attributes exceeding a score of 20 out of 100 suggest that all panellists should detect the tested sensory attribute, while values around and above 50 indicate strong characteristics. *A. esculenta* samples, both frozen and acid treated, exhibited strong salty flavour and strong seaweed-, and sour odours and flavours, with a detectable bitterness (**Paper III**). The results show that the acid preservation did not negatively impact the flavour, texture, or odour of *A. esculenta*. Similar results were obtained in the sensory evaluation of samples harvested in 2023, as presented and used in **Paper IV**, where sour, seaweed and salty flavours were detected. The strongly detected flavours of acid and salt in the samples could affect biomass applications, especially for direct consumption. The biomass could be suitable as an ingredient in food products in markets that prefer lower acid and salty flavours. Additionally, pre-processing of the biomass, such as soaking or blanching (Nielsen et al., 2020), should be considered to reduce the strong salt flavours of the biomass to enhance the acceptability of the product.

5.3.5 Total phenolic content (TPC) and antioxidant properties

The results from **Paper III** showed that the total phenolic content (TPC) and antioxidant activity assessed by ORAC and DPPH were low in both the *A. esculenta* and *S. latissima* compared to *A. nodosum* in **Papers I and II.** Specifically, the TPC in the *S. latissima* control samples was 0.5±0.0 PGE/100 g dw extract and 3.0±0.1 PGE/100 g dw extract in the *A. esculenta* control samples (Figure 21A). These species contain much lower TPC compared to *A. nodosum*, which has the lowest TPC content in June (extracts made with dried seaweed meal were 11.0±0.2 g PGE/100 g extract), but a higher content was obtained in October (17.4±0.7 g PGE/100 g extracts) (**Paper I**), which aligns with previously reported data (Stévant, Marfaing, et al., 2017; Wang et al., 2009). The antioxidant properties showed similar trends between seasons and species. Both DPPH radical scavenging activities and ORAC values were lower in the *S. latissima* samples compared to values obtained in *A. esculenta*. However, significantly higher values were observed in the *A. nodosum* samples in **Papers I and II** compared to the two species studied in **Papers III**

and IV. *A nodosum* is thus preferred out of the studied species for product development since the bioactive characteristics, such as antioxidant properties, are important. This is in agreement with earlier studies that state that *Fucus* species generally contain higher total phenolic content and antioxidant activities compared to *A. esculenta* and *S. latissima* (Wang et al., 2009).

The use of seaweed and its extracts has increased over the last decades due to its multiple bioactive properties, including antioxidant properties. Therefore, it is essential to evaluate how processing affects these properties. Sumardianto et al. (2021) showed that short time fermentation of *Gelidium sp*. and *Eucheuma cottonii* increased the total phenolic content and the DPPH radical scavenging activities of their extracts. Additionally, Lee et al. (2015) results showed similar findings as Sumardianto et al. (2021), that short time fermentation increased DPPH radical scavenging activities. In contrast to fermentation, the results of **Paper III** indicate that the acid treatment with both citric and lactic acid decreases the TPC and antioxidant properties (assessed by DPPH radical scavenging activity (IC50)) of *A. esculenta* (Figure 21B). In contrast, no differences were observed in the *S. latissima* samples, but the antioxidant activity was lower than that of *A. esculenta*. Additionally, the IC 50 values, representing the amounts of extracts required to reduce the DPPH radicals by 50%, did not statistically differentiate during the storage of *A. esculenta* samples even though the quantities required to obtain a 50% reduction of DPPH radicals gradually increased during storage. The results, hence, suggest that acid preservation with lactic and citric acid might not be suitable for preserving the antioxidant properties of *A. esculenta*.

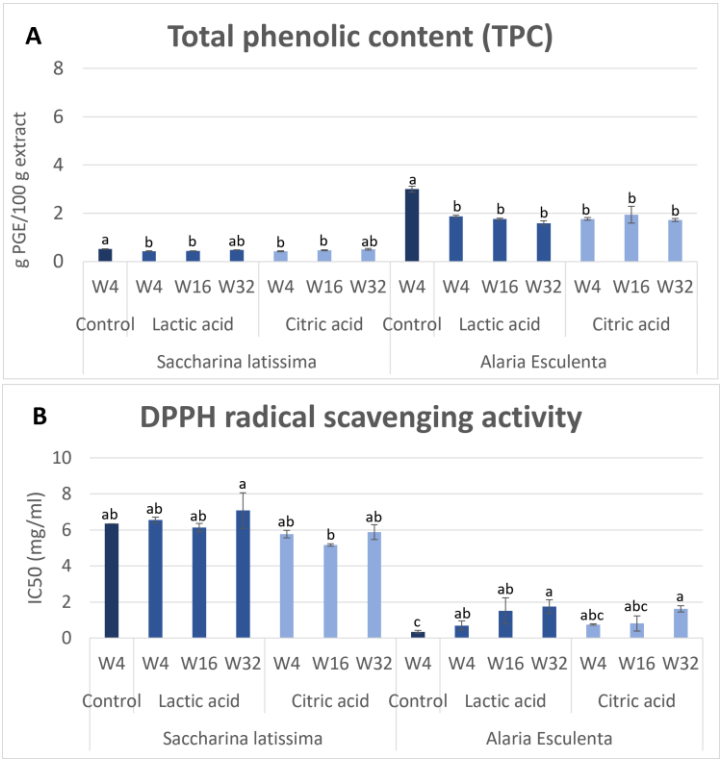

Figure 21 Results of Total phenolic content (TPC) (A) and DPPH radical scavenging activities (B) of water extracts (dry weight) made from control and acid-preserved *A. esculenta* and *S. latissima* for up to 32-week storage (**Paper III**). The results are presented as mean and standard deviation. Different subscript letters indicate significant differences for each species separately.

5.4 Using multispectral imaging (MSI) to assess physicochemical-, and bioactive properties of seaweed (Paper IV)

In seaweed cultivation, the main practices used to ensure the safety of the biomass include microbial and chemical analysis, which are time-consuming, expensive, and destructive methods, alongside the employees' visual inspection. The traditional analyses require expensive instruments, often lots of solvents and other dangerous chemicals, specialised staff, and are very time consuming (Gudjónsdóttir et al., 2011; Lohumi et al., 2015). Less expensive and faster methods could make the processing and harvesting more efficient and make it easier for seaweed cultivators to ensure the quality of the biomass (Lytou et al., 2022). Therefore, the aim of **Paper IV** was to explore the use of

Multispectral imaging (MSI), which is a novel, fast, and easy-to-use technology, to evaluate the quality of the seaweed biomass intended for human consumption.

5.4.1 Spectral data and seaweed recognition

The results of **Paper IV** indicate that multispectral imaging (MSI) technologies could be a valuable tool for assessing multiple parameters during seaweed processing. Slight differences were observed in the visible spectrum, ranging from 515-690 nm, but the main variance in the spectra was obtained in the near-infrared (NIR) region, in the wavelength interval from 690 nm to 970 nm (Figure 22). Absorption at these wavelengths is primarily due to the third overtones and combination bands of chemical bonds, i.e., O-H, C-H and N-H (Osborne, 2006; Ozaki et al., 2006). Different wavelengths thus correspond to the absorption of different chemical components, where the wavelengths 780 and 850 nm are commonly associated with the third overtone stretching of N-H bonds (which can be linked to protein content), while absorption at 880 and 940 nm are due to C-H bond stretching vibrations (often linked to lipids), and at 970 nm are due to stretching vibration of O-H bonds (connected to water) (Ahmed Badr, 2011; Metrohm, 2014; Osborne, 2006).

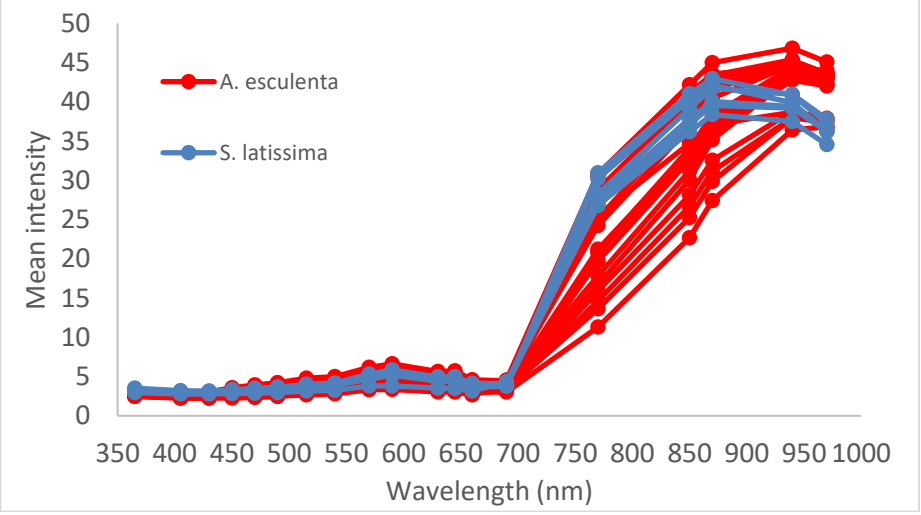

Figure 22 Mean spectra of the seaweed samples, blue spectra represent *S. latissima* samples while red represent *A. esculenta* samples **(Paper IV)**

Differences in both the spectra and Normalized Canonical Discriminant Analysis (nCDA) of the multispectral images obtained from the Videometer Lab showed clear differences between the two tested seaweed species.

Wavelengths from all used spectral ranges (ultraviolet (UV), visible-spectrum (VIS) and near-infrared (NIR)) played a key role in the species differentiation, especially absorption at 365, 490, 570, 590, 690, 780, 850, 940, and 970 nm. A standard red, green and blue (sRGB) image, a NIR absorption image at 970 nm, and a nCDA image are shown in Figure 23. The red pixels in the nCDA image represent *A. esculenta,* while the blue pixels represent *S. latissima* (Figure 23). The green pixels further represent uncertainties in the species classification areas within the samples. Support Vector Machine analysis (SVM) showed excellent (100%) accuracy in discrimination between the two species, confirming differences in the spectral data between the two species.

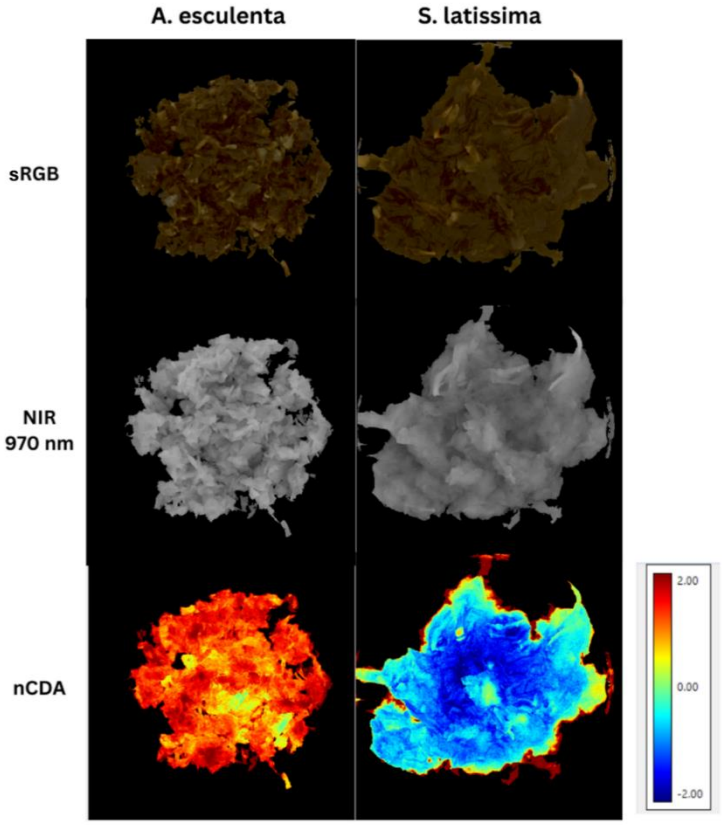

Figure 23 Spectral red, green and blue (sRGB) (top), a corresponding image of the near-infrared (NIR) wavelength at 970 nm (middle) and Normalized canonical discriminant analysis (nCDA) image (bottom) of control samples of *A. esculenta* (left) and *S. latissima (right)* from **Paper IV**.

5.4.2 Principal component analysis (PCA) of spectral data

A PCA was performed on the spectral data obtained from the VideometerLab 4 to assess the distinctions and similarities between samples as described by the MSI spectral data. The first two principal components explained 96% of the variation in the spectra (PC1 = 80% and PC2 =16%) (Figure 24). The PCA showed clear sample groupings, with differences observed between the two seaweed species (*S. latissima* and *A. esculenta)*, treatments (frozen, acid-preserved, fermented, and blanched), between harvesting years (*A. esculenta* samples from 2022 vs 2023), as well as some storage effects of the acid treated samples (Supplementary material, **Paper IV**). The differences between the two species are likely primarily contributed to the variation in the samples' water-, and carbohydrate contents. Additionally, by exploring the spectra obtained in the storage trial presented in **Paper III**, clear groupings were observed between both the treatments (control vs acid treated) and the storage sampling points of the acid preserved seaweed, indicating that storage effects can also be easily detected by the multispectral imaging system. Overall, the results suggest that the spectral data alone can effectively distinguish between the two seaweed species and used processing methods.

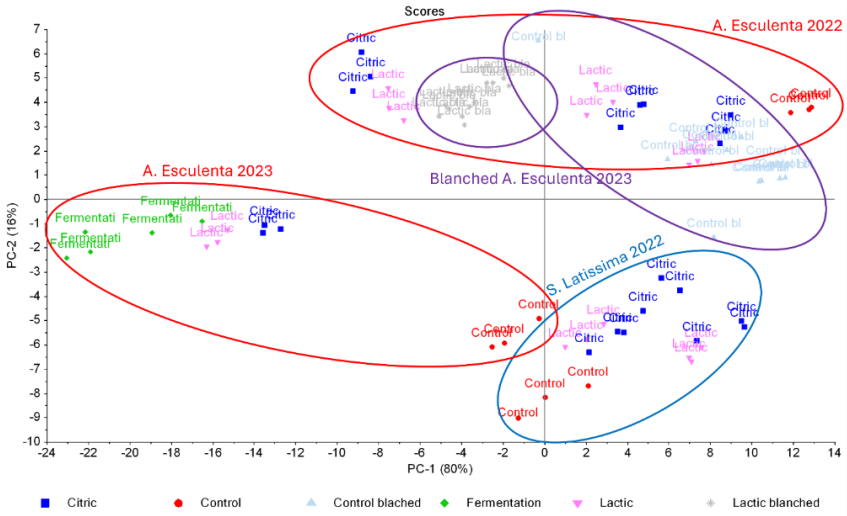

Figure 24 Principal component analysis (PCA) score plot of spectral data (**Paper IV**).

5.4.3 Partial least square regression (PLSR) models

To assess which physicochemical and bioactive characteristics could be reflected in the MSI spectral data, a wide range of parameters were assessed using traditional analytical and sensorial methods and compared to the

spectral data using partial least square regression (PLSR) modelling. PLSR is a common approach for modelling the synergies between physicochemical and spectral data (Cheng & Sun, 2017; Gudjónsdóttir et al., 2024). Physicochemical and bioactive properties of seaweed can vary greatly, not only between species but also between harvesting periods and environmental factors such as temperature, pH, and salinity (Marinho-Soriano et al., 2006; Schiener et al., 2015). Additionally, various factors can influence the final quality of the biomass, such as harvesting protocols, processing methods and efficiency. In addition, storage conditions of the biomass need to be in line with the processing of the biomass as fluctuations in storage temperature and other environmental and chemical factors can affect the product quality (Singh & Heldman, 2001; Sivasankar, 2002). These challenges highlight the importance of effective quality control within seaweed processing and cultivation.

The Partial Least Square Regression (PLSR) model efficiency was assessed based on the models' coefficients of determination and the root mean square errors of both calibration ($\boldsymbol{R_C^2}$ and RMSEC) and cross-validation ($\boldsymbol{R_{CV}^2}$ and RMSECV). A model was assessed as strong when the $\boldsymbol{R_C^2}$ and $\boldsymbol{R_{CV}^2}$ values were higher than 0.75, and the RMSEC and RMSECV were less than 10% of the range of data used to construct the model (Gemperline, 2006; Nepper-Davidsen et al., 2024). It is, however, important to note that the accuracy of the prediction models is limited to the precision of the reference analysis method applied during calibrations (Feinberg, 2007). The range in the number of samples during the model construction ranged from 39-78 samples, with 78 samples in analysis of water, pH, and colour, while the number of samples was in all other cases between 39-54 samples. The parameters used to construct the models are shown in Table 5. Furthermore, the reference analysis results are partially presented in **Paper III** (samples from 2023), while the reference analysis of the biomass harvested in 2024 is described in the appendices in **Paper IV.**

5.4.3.1 Prediction of proximate composition

The PLSR models showed strong correlations between the spectral data and all chemical reference parameters, with $\boldsymbol{R_{CV}^2}$ values ranging from 0.82 to 0.95, with the exception of the lipid content and TVB-N, which were measured in ranges that were too narrow to provide strong modelling conditions (Table 4). The strongest prediction models were constructed for the protein content (R_{CV}^2=0.94, RMSECV =0.12 g/100 g sample), and the total phenolic content (TPC) (R_{CV}^2=0.99, RMSECV=0.24 g PGE/100 g extract), followed by the carbohydrate content (R_{CV}^2=0.89, RMSECV=0.76 g/100 g sample), salt

(R^2_{CV}=0.86, RMSECV=0.41 g/100 g sample), ash (R^2_{CV}=0.80, RMSECV=0.51 g/100 g sample), and lastly, the water content (R^2_{CV}=0.76, RMSECV=1.05 g/100 g sample). The results align with previously reported data, where predictions of protein content (Shalev et al., 2022), TPC, and ash content of seaweed (Nepper-Davidsen et al., 2024), as well as water content in leaves (Tran & Grishko, 2004; Wei et al., 2021), has been sufficiently obtained with VIS-NIR combinations or NIR technologies alone. All these parameters are important for evaluating processing changes and seasonal variation within the biomass (Sánchez-García et al., 2021; Sivasankar, 2002). The samples used to construct the models show the real variation in the biomass and potential changes occurring in it during processing. In addition, the chemical composition was in line with previously reported physicochemical properties of cultivated *A. esculenta* and *S. latissima* (Arlov et al., 2024; Schiener et al., 2015; Stévant, Marfaing, et al., 2017).

The models obtained for the lipid content and the TVB-N were, however, insufficient, as mentioned earlier. The lipid and TVB-N content in the two species was very low and in a very limited range. Furthermore, TVB-N is widely used to assess the quality and freshness of high-protein foods, such as fish and fermented meat products. Since brown seaweeds generally contain a low protein content, the TVB-N is not considered an important factor for assuring the quality of brown seaweed (Holdt & Kraan, 2011a).

The MSI results in **Paper IV** indicate that the technology effectively predicted key chemical parameters in the biomass and that the technology could be considered suitable for evaluating these parameters within seaweed cultivation and processing of the two tested species.

Table 5. Results of model construction by using PLSR with random cross-validation (**Paper IV**) for physicochemical and antioxidant properties of the seaweed samples. The table shows the coefficient of determination of both calibration (R_C^2) and cross-validation (R_{CV}^2), root mean squared error of calibration (RMSEC) and cross-validation (RMSECV), the range of prediction, the sample size, and the number of model factors.

	Calibration		Validation				
Parameter analysed	R_C^2	**RMSEC**	R_{CV}^2	**RMSECV**	**Range**	**Sample size**	**Number of factors**
Water (g/100 g sample)	0.82	0.90	0.76	1.05	83.0-91.5	78	7
Lipids (g/100 g sample)	0.34	0.08	0.18	0.09	0.0-0.5	54	4
Carbohydrates (g/100 g sample)	0.91	0.66	0.89	0.76	3.0-10.9	54	6
Protein (g/100 g sample)	0.95	0.10	0.94	0.12	0.6-2.2	54	4
Salt (g/100 g sample)	0.91	0.33	0.86	0.41	0.1-3.1	54	7
Ash (g/100 g sample)	0.88	0.39	0.80	0.51	1.6-4.4	54	7
Iodine (µg/g sample)	0.89	30.82	0.83	39.38	60-420	39	6
Arsenic (mg/kg sample)	0.87	0.95	0.75	1.33	3.4-10.2	39	6
Cadmium (mg/kg sample)	0.82	0.02	0.73	0.03	0.1-0.3	39	7
Lead (mg/kg sample)	0.86	0.003	0.82	0.004	0.01-0.05	39	5
TVB-N (mg N/100 g sample)	0.55	2.46	0.40	2.92	0.5-15.9	54	4
L-value	0.70	3.00	0.65	3.32	16.8-45	78	5
a*-value	0.93	0.67	0.91	0.79	-6.3-4.6	78	6
b*-value	0.65	3.32	0.53	3.97	2.9-33	78	7
A_w	0.41	0.00	0.25	0.00	0.98-1	54	6
pH	0.96	0.25	0.94	0.28	3.0-6.7	78	6
TPC (g PGE/100 g extract)	0.94	0.20	0.92	0.24	0.4-3.2	39	4
DPPH (IC50)	0.92	0.75	0.91	0.81	0.3-8.2	39	2
ORAC (µmol TE/g extract)	0.93	9.73	0.89	11.70	10-113	39	5
Total viable count (TVC) (log cfu/g sample)	0.72	0.79	0.67	0.93	1.8-6.2	48	4

5.4.3.2 Prediction of trace minerals

MSI captures wavelengths in the UV-VIS, which only measures absorption due to electronic and vibrational excitations, and NIR, which measures absorption by molecular bonds. Trace minerals can generally not be detected with MSI, as they do not absorb in the NIR, visual and UV spectra (Martelo-Vidal & Vázquez, 2016; Osborne, 2006). However, trace element levels may be correlated to the proximate composition, such as water, protein or carbohydrates, making indirect detection of trace minerals possible. It should, though, be kept in mind that their low concentration can fall below the levels of

detection (LOD) or qualification (LOQ), and attempts to predict trace minerals with MSI spectral data should thus be performed and interpreted carefully.

The developed PLSR models for the trace minerals showed some promising results, even though the models were not as efficient as PLSR models obtained for proximate composition. However, the model constructed for the iodine content had a suitable range for processed brown seaweed biomass, from 60-420 mg/kg, although only 39 samples were used (Table 5). The PLSR model showed a strong correlation between the spectral data and the reference measurements of the iodine content, achieving a R^2_{CV} of 0.83, although it had a relatively high RMSECV of 39 mg/kg sample. Additionally, Pearson's correlation results showed that the iodine content showed correlation coefficients around or above 0.8 with the water, protein and carbohydrate content, respectively. Similar results were obtained for other trace minerals, indicating the potential for indirect prediction of these parameters. The results indicate that such modelling should be studied further using a larger sample size and a broader range of heavy metals and iodine content in the tested seaweed samples.

5.4.3.3 Prediction of pH and physical properties

Water activity (A_w), colour, and pH, as well as total microbial counts, are all parameters used to assess the quality and stability of food products (Blikra et al., 2019; Damodaran et al., 2007; Roudaut, 2020). Furthermore, these properties can change significantly with processing and storage (Alves et al., 2021; Damodaran et al., 2007; Dauthy, 1995; Sánchez-García et al., 2021).

The PLSR model for pH was constructed by using reference samples in a range between 3.0 to 6.7, which is considered an appropriate range for the biomass used, where the pH of the fresh seaweed biomass is usually around pH 5-7, and the pH of the acid treated and fermented biomass needs to be lower than 4.6 to prevent the growth of pathogenic bacteria (CFR, 1979; Theron & Lues, 2010). The model effectively predicted the pH with a R^2_{CV} of 0.94 and RMSECV of 0.28. As for the trace minerals, the pH of the biomass is not measured directly with the MSI technology (Jia et al., 2017). Pearson's correlation showed a negative correlation between the pH and the L-value (r=-0.66), indicating that the VIS spectrum contributes to the model's precision. These results align with other published data, showing that wavelengths between 435-993 nm are required to predict the pH of fruits (ElMasry et al., 2007; Rajkumar et al., 2012). The results indicate that the constructed models could effectively predict the pH value of the seaweed samples.

Three prediction models were constructed for the colour parameters based on the CIE-lab system or L-values, a*-values and b*-values, respectively. The model obtained for the a*-values was the most efficient (R^2_{CV}=0.91, RMSECV=0.79), followed by the model predicting the L-value (R^2_{CV}=0.65, RMSECV=3.32), and lastly, the b*-value (R^2_{CV}=0.53, RMSECV=3.97). The results do hence indicate that the constructed models for the L- and a*-values could be used to assess colour changes in the biomass. It should be noted that since the L-, a*- and b*- values can be described as spherical coordinates, a linear modelling approach may not be suitable to assess correlations between the spectral information and the colour parameters. Therefore, non-linear approaches should be evaluated as they could enhance model precision (Dong et al., 2018; Wei et al., 2020; Yu et al., 2019).

The PLSR model for predicting the total viable bacteria count (TVC) was constructed by using a range between 1.8 and 6.2 log cfu/g sample. The model had moderate prediction abilities, with an R^2_{CV} of 0.67 and RMSECV of 0.93. Pearson's correlation showed an intermediate correlation between TVC and water content (r=0.549) and intermediate negative correlations with carbohydrate content (r=-0.547) and protein content (r=-0.510), respectively. Similar results were obtained by Lytou et al. (2022), with prediction efficiencies in the range between 0.8 to 1.18 of TVC, indicating that strengthening of the model is required by evaluating alternative data processing methods.

5.4.3.4 Prediction of antioxidant properties

Even though the species used in the current study are not known for high antioxidant properties (Wang et al., 2009), an assessment was performed on the efficiency of the MSI technology to predict the oxygen radical absorbance capacity (ORAC) and DPPH radical scavenging activities. The PLSR model for ORAC had a relatively high RMSECV despite having a strong linear relationship (R^2_{CV}=0.89). Like for some of the other parameters, the antioxidant activity cannot be directly measured with the spectral data, but Pearson's correlation analysis showed strong correlations between ORAC and water, protein, and carbohydrates (r=-0.900, r=0.884, and r=0.902 respectively), indicating that these factors contribute to the model accuracy. The DPPH model showed similar results as the ORAC models, obtaining a strong linear relationship (R^2_{CV}=0.81) but with moderate accuracy (RMSECV=0.81), accompanied with Pearson's correlations to the same parameters as the ORAC. The results show that MSI could be an effective screening tool to evaluate the antioxidant properties of the two brown seaweed species. However, models should be strengthened by adding other brown seaweed

species to increase both the sample size and the range in these parameters for effective industrial applications.

5.4.3.5 Prediction of sensory parameters

The models constructed showed that MSI could be used to effectively predict sour and seaweed odour, flavour, and textural properties, as these parameters had a wider range for the model construction than other sensory parameters. Other parameters, such as spoilage odour and flavour, rancid odour and flavour, and metallic and sweet flavours, had a narrower range (**Paper III**), affecting the significance of the results and strength of the prediction models in **Paper IV**. The general descriptive sensory analysis is performed by a group of trained sensory panellists, who all have personal sense differences, even though the panellists are well trained. However, using MSI or other mechanical techniques could limit the variability and personal biases by the human judges. Overall, the results indicate that the MSI is a promising technology to effectively predict multiple sensory parameters simultaneously, but implementations of the technique in practical applications require further research.

6 Conclusion

Growing interest in seaweed cultivation for food purposes and increased pressure on sustainable production practices highlight the importance of research for further growth of the industry. Therefore, the main goal of this thesis was to evaluate and improve the current utilisation, preservation, and quality control practices within the seaweed industry, with emphasis on the two most cultivated seaweed species in Europe, *A. esculenta* and *S. latissima*, as well as a wild-harvested seaweed species, *A. nodosum*.

A. nodosum is the most harvested seaweed species in Iceland, where most of the biomass is exported as dried seaweed meal, mainly used for alginate production. The *A. nodosum* harvested from June to October contained up to approximately 21% alginate per its dry weight (**Paper I**). That leaves roughly 80% of the biomass unused, presenting opportunities for side-stream utilisation in seaweed meal production when the biomass is intended for alginate production. The results of **Paper I** showed that the proposed alternative processing can extract valuable compounds from the biomass without affecting the alginate content in the final commercial seaweed meal. This was further confirmed in **Paper II,** making the production of liquid extract feasible within the meal production. If phenolic compounds are of interest in the liquid extract, the production would not be feasible in all harvesting periods due to the seasonal variation in chemical composition and antioxidant properties of *A. nodosum*. This variation can be directly linked to the reproduction stage of the algae, but the *A. nodosum* contained lower phenolic content in June compared to other evaluated harvesting periods. Based on the results, the algal meal and press liquid production should be relatively stable from July through October. In the case of scaling up the production, mass balance calculations and chemical analysis in **Paper II,** showed that by using a decanter, higher yields of liquid extracts were obtained compared to using a screw-press in a primary separation step where the liquid and solid phases are separated. Additionally, the results indicated that spray drying could be a suitable drying method to preserve the liquid extract. Using these alternative processing methods, approximately 420 thousand tons of spray dried liquid extract could be produced annually from the harvested 15.000 tons. Future research should, however, evaluate if further processing of the liquid extract is required to reduce the heavy metals, iodine, and salt content of the extracts. Additionally, future studies should emphasise on evaluating applications of the press liquid, such as its use as bio-stimulants or extraction of active components for cosmetics.

Seaweed cultivation is expected to grow significantly within Europe in the next few decades. With increased volumes, reasonable preservation and production methods are required to ensure the quality and safety of the seaweed biomass for human consumption. Therefore, **Paper III** aimed to evaluate the efficiency and stability of acids to preserve brown seaweed biomass, and **Paper IV** aimed to assess the quality of seaweed biomass using novel technologies or multispectral imaging (MSI).

The results of **Paper III** indicated that acid preservation with both lactic and citric acid stabilises the seaweed biomass for up to seven months if stored at room temperature. However, minor changes in TVB-N were observed, indicating protein deterioration or increased formation of biogenic amines during storage. The high iodine, salt, arsenic and cadmium content and strong salt and acid flavours could, however, affect its applications for food applications, indicating that pre-processing is required, such as blanching of the biomass. The acid treatment did affect the total phenolic content and radical scavenging abilities of *A. esculenta*, suggesting that the method might not be suitable if the biomass is intended for bioactive purposes such as antioxidant activities. However, the biomass might be ideal as an ingredient in a wide range of value-added products. Future research should focus on evaluating the efficiency of acid preservation on blanched seaweed biomass if intended for human consumption and on evaluating if and how acid-preserved biomass affects physicochemical changes when incorporated into food products.

The results of **Paper IV** showed that multispectral imaging (MSI) is a promising technology that can be used for species recognition and can effectively predict multiple parameters in the studied brown seaweed biomass, including pH, proximate composition, total phenolic content, and some trace minerals. Also, clear sample groupings in the spectral data were observed between processing methods. Even though the results are promising, there are some limitations to the applications of MSI to seaweed quality monitoring, including the precision of the reference analysis methods used during the calibrations, which affects the accuracy of the models. Future research should focus on exploring the use of MSI during different processing steps within seaweed cultivation, from harvesting to fully processed products, to increase the variability in the tested chemical composition. Additionally, using a larger number of samples to strengthen the models further and evaluate different pre-processing methods for the spectral data and non-linear modelling techniques is recommended.

7 Future prospectives

The study's results showed how seaweed processing and quality control could evolve and highlighted the importance of fully exploring all aspects of each process. As seaweed production increases, mechanisation and good production practices are crucial. Even though many questions were addressed in the study, numerous questions emerge as well, such as what food and feed applications can the liquid extract be used for, whether the liquid needs purification, how acid preservation works on blanched seaweed biomass, how can the biomass be used in food products, is it possible to improve the models obtained by MSI by using higher sample size and more extensive range, and can the technology be applied for other seaweed species as well? Ongoing research within the “QualiSea” BlueBio Cofund project aims to address some of these questions, including how blanched acid-preserved seaweed biomass affects food products' physicochemical and sensory properties. Additionally, further research will be performed on assessing multispectral imaging as a quality control tool within the seaweed industry by evaluating its efficiency to predict multiple properties of seaweed. For example, MSI will be applied to red and green seaweed species in indoor cultivation studies in the new project “Indoor cultivation and product development from seaweed in borehole seawater” funded by the Icelandic Food Research Fund (Matvælasjóður). Additionally, the plan is to explore the uses of MSI for other seaweed species throughout various processing stages in other future projects. The unanswered and answered questions are all interconnected as they all address bottlenecks in the seaweed industry from different perspectives, all aiming to preserve and increase the value of the harvested seaweed biomass, which is crucial for the growth of the seaweed industry.

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Paper I

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Article

Added Value of *Ascophyllum nodosum* Side Stream Utilization during Seaweed Meal Processing

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Abstract: *Ascophyllum nodosum* contains many valuable compounds, including polyphenols, peptides, and carotenoids that have been shown to exhibit biological activities. These compounds are not a priority ingredient in seaweed meal products for the current users. Hence, the aim of the study was to investigate the chemical and bioactive characteristics of *A. nodosum* as affected by seasonal variation and evaluate the potential benefits of alternative processing and the utilization of side streams for product development. The analysis of raw materials, press liquid, and press cake from alternative processing and the commercial seaweed meal at different harvesting periods indicated that the chemical composition is linked to the reproductive state of the algae. Phenolic content and ORAC activity increased following the seaweed's fertile period, making alternative processing more promising in July and October compared to June. Several valuable ingredients were obtained in the press liquid, including polyphenols, which can be used in the development of new high-value bioactive products. The suggested alternative processing does not have a negative effect on the composition and quality of the current seaweed meal products. Hence, the extraction of valuable ingredients from the fresh biomass during the processing of seaweed meal could be a feasible option to increase the value and sustainability of seaweed processing.

Keywords: *Ascophyllum nodosum*; brown algae; macroalgae; seasonal variation; proximal composition; trace minerals; monosaccharide composition; bioactive compounds; polyphenols; antioxidant activity

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1. Introduction

Macroalgae, or seaweed, are plant-like organisms that can be divided into three main categories based on their pigmentation characteristic, i.e., brown seaweed (*Phaeophyceae*), red seaweed (*Rhodophyceae*), and green seaweed (*Chlorophyceae*) [1,2]. At least 221 seaweed species (32 species of green seaweed, 125 species of red seaweed, and 64 species of brown seaweed) are used worldwide in seaweed processing. Generally, seaweed is used for human consumption in three different ways, consumed directly as food (fresh, dried, liquid extracts, canned, salted, or prepared directly as food), as a food supplement, or as a thickening agent (e.g., alginate (from brown algae), agar (from red algae), and carrageenan (from red algae) in a wide range of food products [1–3]. As well as direct consumption, seaweed is used as an ingredient in bio-stimulants for agriculture, to enhance nutraceutical properties of plants and fruits for human consumption [4], and to increase plant resilience to abiotic stresses [5]. Seaweed can be both cultivated or wild harvested. The worlds' production of seaweed in 2005 was 14.7 million tons, where only 1.2 million tons came from wild seaweed. In the year of 2015, the total production doubled to 30.4 million tons, where 29.4 million tons originated from cultivated seaweed, and only 1.1 million tons were obtained from wild resources [1]. Recently, the production has increased even further, and in 2018 the world's seaweed production reached 32.4 million tons, where 97.1% was obtained from cultivated seaweeds [6].

Seaweed is recognized as a good source of food with high nutritional value. It is known for its high concentrations of carbohydrates, low content of fat, and its richness in polyunsaturated fatty acids, bioactive compounds (including polyphenols, peptides, and carotenoids), vitamins, and minerals [7]. Brown seaweed contains various bioactive compounds that have been proven to have multiple biological activities, such as antioxidant, antiviral, antifungal, and antibacterial properties [8,9]. Seaweed is exposed to a broad range of stressors in its environment, such as fluctuations in temperature, changes in light, desiccation, and osmotic stress. These environmental stress factors can lead to the formation of strong oxidizing agents, such as free radicals. Despite the formation of oxidizing agents, seaweed rarely undergoes major photodynamic damage, which suggests that seaweed cells possess protective mechanisms and compounds [10]. Compounds found in seaweed, such as peptides, low molecular weight sulphated polysaccharides (fucoidan), amino acids, carotenoids, tocopherols, and polyphenols have been reported to have antioxidant activities [9].

Wild brown seaweed, including *Ascophyllum nodosum*, are currently processed into seaweed meal in Iceland and other countries such as Canada and Ireland. In the current production process of seaweed meal, the biomass is mechanically harvested, transported to the factory, chopped, dried, ground, sieved, and packed. The meal is mostly sent out of the country and used in the extraction of the hydrocolloid alginate, used as a fertilizer, or as animal feed. However, to produce 23,000 tons of alginate, approximately 85,000 tons of dried seaweed is needed [11]. The leftover pulp from hydrocolloid production is usually sent to landfills, pumped back to the sea, or occasionally used as soil conditioner [2,12,13], meaning that around 70% of the dried seaweed biomass is not utilized to its full potential. Furthermore, seaweed meal is mainly used as a feed enhancer for animals due to its high content of carbohydrates, vitamins, and minerals (especially iodine) [2] but not due to their bioactive compounds.

Some studies have shown that some bioactive compounds, such as polyphenols, are extractable with water, and extracts retrieved possess antioxidant abilities [14,15]. However, after the extraction of seaweed, many compounds still remain in the leftover biomass, including compounds in the seaweed that are less soluble in water, such as lipids and cellulose [2,16]. Therefore, there might be some opportunities in the valorization of the seaweed meal processing process where an extraction step could possibly be added to part of the process to retrieve valuable compounds from the fresh seaweed biomass to both reduce waste in the seaweed value chain and increase the value of the production. Hence, the aim of the present study was to evaluate the chemical composition and antioxidant activity of *Ascophyllum nodosum* and its produce, as affected by seasonal variation, and to assess the feasibility of developing alternative processes and high-quality side products in a sustainable manner without affecting the buyers' desired chemical composition of the commercial seaweed meal.

2. Results and Discussion

2.1. Chemical Composition

The basic chemical composition (moisture, fat, protein, salt, ash, and carbohydrate content) was determined for each harvesting period (June (where the seaweed contained fruit bodies/receptacles), July, October 2020) at four different production stages of *A. nodosum*, i.e., the fresh seaweed, press liquid (PL), press cake (seaweed after extraction), and commercial dried seaweed meal from the production of Thorverk (Table 1). The press liquid and press cake were only measured in July and October due to their antioxidant potentials and their possibilities for product development. The fresh seaweed and seaweed meal were, however, analyzed at all three harvesting periods to evaluate the seasonal variation in *A. nodosum*.

Table 1. Chemical composition of the four processing stages of seaweed during seaweed meal production. The results are expressed as mean ± standard deviation.

Harvesting Time	Sample ID	Moisture (g/100 g Sample) (n = 3)	Lipids (g/100 g Sample) (n = 4)	Protein (g/100 g Sample) (n = 4)	Salt (g/100 g Sample) (n = 4)	Salt free ash (g/100 g Sample) (n = 4)	Carbohydrates (g/100 g Sample) (n = 3)
June	Fresh seaweed	79.6 ± 0.2^{a}	0.3 ± 0.2	1.6 ± 0.1	1.4 ± 0.0^{b}	3.3 ± 0.1^{c}	13.9 ± 0.2^{c}
	Press liquid	NA	NA	NA	NA	NA	NA
	Press cake	NA	NA	NA	NA	NA	NA
	Seaweed meal	9.1 ± 0.2^{a}	0.5 ± 0.1^{c}	6.0 ± 0.3^{a}	5.8 ± 0.3^{b}	13.7 ± 0.3^{b}	64.0 ± 2.1^{b}
July	Fresh seaweed	$70.3 \pm 0.2^{a,y}$	0.2 ± 0.1	1.2 ± 0.2	1.3 ± 0.0^{b}	$4.5 \pm 0.1^{a,x}$	$22.5 \pm 0.2^{a,x}$
	Press liquid	94.9 ± 0.1	<0.01	0.2 ± 0.0	0.9 ± 0.0	0.3 ± 0.2^{a}	3.5 ± 0.1^{b}
	Press cake	80.7 ± 0.1^{x}	0.2 ± 0.1^{b}	1.0 ± 0.1	0.6 ± 0.0	2.9 ± 0.2^{y}	14.6 ± 0.4^{y}
	Seaweed meal	6.9 ± 0.2^{b}	1.6 ± 0.0^{a}	4.7 ± 0.2^{c}	5.8 ± 0.6^{b}	14.1 ± 0.7^{b}	67.0 ± 1.4^{a}
October	Fresh seaweed	$71.9 \pm 0.3^{b,y}$	0.2 ± 0.2	1.1 ± 0.3	1.7 ± 0.0^{a}	$4.0 \pm 0.1^{b,x}$	$21.1 \pm 0.4^{b,x}$
	Press liquid	94.8 ± 0.0	<0.01	0.2 ± 0.0	1.0 ± 0.0	0.2 ± 0.1^{b}	3.8 ± 0.1^{a}
	Press cake	80.4 ± 0.2^{x}	0.4 ± 0.1^{a}	0.9 ± 0.2	0.6 ± 0.0	2.9 ± 0.2^{y}	14.9 ± 0.3^{y}
	Seaweed meal	6.2 ± 0.2^{c}	1.1 ± 0.1^{b}	5.5 ± 0.2^{b}	6.8 ± 0.1^{a}	15.6 ± 0.2^{a}	64.9 ± 0.2^{b}

Different subscript letters (a–c) indicate significant differences ($p < 0.05$) at each processing step between harvesting periods (June, July, October). Different subscript letters (x and y) indicate significant difference between fresh seaweed and fresh press cake within the same harvesting period ($p < 0.05$). NA: Composition analyses of the press liquid and press cake from seaweed harvested in June were not performed due to the low antioxidant potential of the press liquid during this season.

Fresh seaweed generally contains a high moisture content, where the content can reach up to 94% in brown seaweed [17]. In the present study a significant difference was observed in the moisture content of the fresh seaweed between all harvesting periods as well as in the seaweed meal samples. The samples from June contained the highest water content in both fresh seaweed and seaweed meal. The water content might, hence, be related to the reproduction stage of the algae since fruit bodies/receptacles were observed on the seaweed in June, indicating that the seaweed was in its fertile period [18]. As expected, the press liquids contained high moisture contents, or 94.9 ± 0.1 g/100 g sample and 94.8 ± 0.0 g/100 g sample in July and October, respectively. Interestingly, the moisture content observed in the press cake compared well to the fresh seaweed, indicating that the biomass absorbed a significant amount of the added water. The moisture content of the dried seaweed meal was 6.2–9.1 g/100 g sample at all seasons, and thus below the 14–15% threshold as recommended during production for dried feed [19]. When the moisture content of the dried feed/food is lower than 15%, the water activity is lower than 0.6, meaning that there is limited available water for microorganism growth, and the probability of lipid oxidation is low, and hence a stable dried product was obtained [16,20].

Many factors can influence the lipid content and fatty acid composition of seaweed, including temperature, light intensity, and the salinity of the sea. Some studies have also reported seasonal fluctuations where in some species the content is highest in winter, but for others such as fucus species the content has been recorded higher in the summertime [17,21–23]. This correlates well with the results from the seaweed meal in the present study, where the content was highest in July and lower in October. The lipid content of seaweed is typically low, where the content for *A. nodosum* has been reported to be in the range between 1.8–4 g/100 g DW [24–26]. In the present study, as expected, the total lipid content of the seaweed samples was low in all harvesting periods. The fresh seaweed samples contained 0.2–0.3 g/100 g sample, and the press liquid contained less than 0.01 g lipids/100 g sample at all seasons, indicating that the lipid content in the raw material remained almost completely in the press cake during pressing. Hence any utilization of the press liquid would not affect the lipid amount or composition of the seaweed meal. The low lipid content in the press liquid agrees with the fact that lipids are not water soluble and only dissolve in organic solvents [16]. The low presence of lipids in the press liquid can furthermore be beneficial during the processing and storage of any potential press liquid-derived products, since no or little lipid degradation will happen during storage of the liquid or the dried press liquids.

A protein conversion factor of 5 was used instead of the commonly used 6.25 to calculate the crude protein content. This protein conversion factor was applied, as recommended for seaweed by Angell, et al. [27] due to the high amounts of non-protein nitrogen (NPN) present in seaweed. A significant difference was only observed in the protein content between harvesting times of the seaweed meal, with none in the fresh seaweed, press liquid, or press cake. The protein content was recorded highest in the samples from June, where the seaweed meal contained 6.0 ± 0.3 g protein/100 g sample and the fresh seaweed samples 1.6 ± 0.1 g protein/100 g sample. The results show that some of the proteins goes out with the press liquid during pressing (confirmed by the press liquid protein content of 0.2 ± 0.0 g/100 g sample in both July and October), resulting in a slight lowering in protein content of the press cake. However, the amount of protein extracted with the pressing liquid does not have a significant impact on the protein composition of the press cake when compared to the fresh seaweed samples, indicating that the loss of protein during pressing should not affect the protein yield of the final product substantially. The press liquid, however, contains some protein, which might have some impact on the antioxidant activities of the press liquid extracts since some amino acids and peptides in seaweed have been suggested to have bioactive activities, including antioxidant activities [28–30]. Therefore, the protein and peptides might have some effect on antioxidant activities of the press liquid extracts since they have both been reported as potential radical scavengers and ion chelators [28–30].

A large part of the seaweed ash content was salt (NaCl), or approximately 17% to 74% of the total ash content. A significant difference was found in salt content between harvesting times in both the fresh seaweed samples and the seaweed meal samples. The salt content of the fresh seaweed and seaweed meal accounted for 29–30% of the total ash content and was recorded to be significantly higher in October (1.7 ± 0.0 g/100 g sample) when compared with June and July (1.3–1.4 g/100 g sample). The salt content of the seaweed meal showed a similar trend to the fresh samples, being lowest in July. Since a large part of the salt content went out with the press liquid, lower salt contents were detected in the press cake compared to the fresh seaweed. The high amounts of salt in the press liquid might affect the use of it in food products, e.g., by intensifying flavors from the extract, especially if used in large quantities. However, if the aim is to use the press liquid as a natural antioxidant in food products, only a small amount would be used, which should not affect the flavor greatly.

The ash content was determined, and the salt free ash content calculated. The salt free ash content of the fresh seaweed samples was significantly differentiated between all three harvesting periods, where the highest content was recorded in July (4.5 ± 0.1 g/100 g sample), followed by October (4.0 ± 0.1 g/100 g sample), and lastly June (3.3 ± 0.1 g/100 g sample). The results, hence, indicate that the ash of the seaweed from July contains proportionally higher quantities of minerals than during the other seasons. Minerals commonly found in brown seaweed include magnesium, sodium, calcium, chlorine, potassium, phosphorus, sulfur, iodine, copper, iron zinc, fluoride, selenium, manganese, molybdenum, nickel, cobalt, and boron [1]. However, in the production of the press liquid, some of the minerals seems to be washed out with the process (0.2–0.3 g/g sample). When the fresh seaweed and press cake were compared, the press cake contained significantly lower amounts of salt free ash, or 28–36% lower values, that would result in lower salt free ash/mineral content of the final seaweed meal as well if the press cake was used in the meal production.

The carbohydrate content of the fresh seaweed samples was significantly differentiated between all harvesting periods, where the highest content was observed in July (22.5 ± 0.2 g/100 g seaweed), followed by October (21.1 ± 0.4 g/100 g seaweed), and lastly June (13.9 ± 0.2 g/100 g seaweed). The same trend was seen in the carbohydrate content of the seaweed meal, where the content was highest in July when compared to the other harvesting periods. The carbohydrate values obtained in June correlated well to values presented earlier [31], that showed a carbohydrate content of *A. nodosum* of 13.1 g/100 g sample. Some carbohydrates seem to go out with the pressing of seaweed, where the content

was at an average of 3.5–3.8 g/100 g of press liquid, leaving less content of carbohydrates in the press cake. A significant difference was observed between the carbohydrate content of the fresh seaweed samples from July and October when compared to the press cake samples, which contained 30–35% less carbohydrates, indicating that the extraction process could affect the final carbohydrate content and composition of the final dried product. However, this also shows the potential utilization of the press liquid for the development of products with bioactive characteristics, based on the carbohydrate composition.

2.2. Monosaccharide and Uronic Acid Composition

The monosaccharide and uronic acid composition of the four tested production streams were evaluated to predict the final composition in the seaweed meal after extraction (Table 2).

Table 2. Monosaccharide and uronic acid composition in percentage (%) of sample dry weight (dw) of the four phases of production.

		Monosaccharides				Uronic Acids	
Harvesting Time	**Sample ID**	**Mannitol**	**Fucose**	**Glucose**	**Xylose/ Mannose**	**Hexa-Mannuronic Acid**	**Glucuronic Acid**
June	Fresh seaweed	7.1 ± 0.3^{b}	7.0 ± 0.7^{b}	5.1 ± 0.2^{b}	2.8 ± 0.3^{b}	5.1 ± 0.3	6.0 ± 0.4^{b}
	Press liquid	NA	NA	NA	NA	NA	NA
	Press cake	NA	NA	NA	NA	NA	NA
	Seaweed meal	13.6 ± 0.9^{b}	11.8 ± 0.9^{a}	5.4 ± 0.5	5.1 ± 0.5	7.6 ± 0.8	8.0 ± 0.9
July	Fresh seaweed	10.4 ± 1.2^{a}	7.4 ± 0.0^{b}	7.1 ± 0.1^{b}	3.5 ± 0.1^{b}	7.1 ± 3.1	12.9 ± 0.3^{a}
	Press liquid	41.9 ± 3.4^{b}	1.7 ± 0.1^{a}	12.3 ± 0.9^{b}	1.1 ± 0.1^{a}	ND	1.4 ± 0.1
	Press cake	8.5 ± 0.6	14.1 ± 1.0^{b}	6.8 ± 0.3^{b}	5.8 ± 0.4	10.4 ± 1.1	14.1 ± 2.0
	Seaweed meal	20.0 ± 1.6^{a}	7.1 ± 3.0^{b}	5.8 ± 2.2	3.7 ± 1.6	8.7 ± 0.5	11.0 ± 1.3
October	Fresh seaweed	11.1 ± 0.8^{a}	11.6 ± 1.0^{a}	11.0 ± 1.6^{a}	4.6 ± 0.45^{a}	8.4 ± 0.4	12.4 ± 0.3^{a}
	Press liquid	52.4 ± 2.5^{a}	1.2 ± 0.1^{b}	32.8 ± 1.2^{a}	0.6 ± 0.1^{b}	ND	1.5 ± 0.1
	Press cake	8.2 ± 1.5	17.2 ± 1.6^{a}	11.2 ± 1.7^{a}	7.0 ± 1.0	9.9 ± 0.7	13.9 ± 0.5
	Seaweed meal	18.4 ± 2.3^{a}	5.6 ± 0.4^{b}	4.3 ± 0.5	2.7 ± 0.3	5.1 ± 0.2	6.9 ± 0.1

Different subscript letters (a,b) of same produce between different harvesting periods (e.g., mannitol of fresh seaweed from June, July, and October, and so on) indicate significant differences ($p < 0.05$). NA: Composition analyses of the press liquid and press cake from seaweed harvested in June were not performed due to the low antioxidant potential of the press liquid during this season. ND: not detected

Brown seaweed has been reported to accumulate carbohydrates, and then mannitol, over the summer and autumn months, and the levels decrease in winter [32,33]. Mannitol is a non-hygroscopic sugar alcohol derived from mannose and is naturally present in most species of brown seaweed [9]. It is a straight chain of six carbons with six hydroxyl groups with the chemical formula C6H14O6 [34,35]. The mannitol content in the fresh seaweed samples was significantly lower in June (7.1 ± 0.3% dry weight (dw)) when compared to the other two harvesting periods where similar results were obtained. However, the mannitol content of the seaweed samples was recorded highest in the press liquids (PL), 41.9 ± 3.4% dw and 52.4 ± 2.5% dw in July and October, respectively, leaving the press cake with a low mannitol content.

Glucose units are the main building blocks of the polysaccharide laminarin found in brown seaweed. Laminarin is composed of 20–25 glucose units of (1,3)-β-d-glucan with β-(1,6) branching. They have two main forms, i.e., M (end with mannitol residue) and G (end with glucose residue) chain structures. They are low molecular weight and water-soluble compounds and is one of the main storage polysaccharides in brown seaweed [7,36]. The growth and structure of laminarin depends on both environmental conditions and the species. Specifically, laminarian synthesis of seaweed is directly linked to nitrate and nitrite content in the ocean, which is also connected to the rapid growth phase of the seaweed in the spring. When nitrite/nitrate content is high, the algae grow fast, but when nitrate decreases over the winter a synthesis of laminarin starts and the growth of the

seaweed decreases or stops [36–38], meaning that laminarin content, and hence the glucose content of brown seaweed, is usually higher in winter as the results from the present study indicate. The glucose seemed to be washed out in some amounts with the press liquid, where the measured content was significantly higher in the press liquid from October (or 32.8 ± 1.2% dw) when compared to the press liquid made in July. However, the press cake seemed to contain similar amounts of glucose after the extraction.

Sulphated fucose (L-fucose 4-sulpfate) is the main building block of fucoidan, which is a sulphated ester polysaccharide. Fucoidan is mainly found in the cell wall of brown seaweed and as an intracellular material. It is both a water-soluble and acid-soluble compound [7,39]. In the present study, the fucose content in the fresh seaweed samples was recorded as significantly higher in October when compared to the other two harvesting periods. Even though the fucose content was higher in October, the press liquid from July contained significantly higher amounts of fucose than the press liquid from October. Some of the fucose, 1.7 ± 0.1% dw and 1.2 ± 0.1% dw, went out with the press liquid in July and October, respectively. However, some of the fucose remained in the press cake. As discussed earlier, fucose is both water soluble and acid soluble. Studies have been performed on different extraction methods for fucoidan that indicate that hot water extraction (with some pre-purification treatment) gives a better yield of fucoidan when compared to acid extraction. The extraction treatments also affect the chemical composition or ratios of the fucoidan [40–42]. However, even though water extraction of fucoidan has been more successful than using other solvents, the majority of the fucose content remained in the press cake in the present study. This indicates that factors other than the solvent used in extraction might matter in the case of fucoidan extraction. For example, results from a study conducted by Ferreira, et al. [43] show that the extraction yield increases with a longer extraction time and by using heat in the process. In the present study, no heating was involved, only water at room temperature was used. However, if a higher content of fucoidan was desired in the press liquid, an evaluation of the production process would be required including potential heating steps or an increase in the extraction time.

Xylose/mannose content in the fresh seaweed samples and press liquid was significantly differentiated between harvesting periods, where the content was higher in October in the fresh seaweed samples when compared to the other harvesting periods but higher in July in the press liquid. The content was recorded to be the highest in the press liquids, from 5.8 ± 0.4% dw up to 7.0 ± 1.0% dw. In the other seaweed samples, small amounts of xylose/mannose were recorded of 5% dw or less.

Alginic acid (alginate, algin) is a high molecular and alkali soluble compound. Alginates are linear binary arranged chains made of the uronic acids 1,4-linked β-D-mannuronic (M) and α-L-guluronic acid (G). Alginates are structural components in brown seaweed and are, hence, mainly found in the cell walls but are also an intracellular material. The sequence of M- and G-blocks can vary not only between species but can also be dependent on the harvesting time [44,45]. Alginates are hydrocolloids, and the gel-formation ability of alginates is directly linked to the content of guluronic acids and the length of the G-blocks, where alginates with a higher content and longer G-blocks make gels with a higher strength [16]. In the present study the glucuronic acid content in the fresh seaweed samples was significantly lower in June when compared to the other two harvesting periods. The hexa-mannuronic acid was, however, similar in all harvesting periods. The results, hence, indicate that both the total alginate content and the gel strength in June is lower than the seaweed samples from July and October. The calculated total alginate content of the fresh seaweed samples from June contained the lowest amounts, approximately 11% dw, and the samples from July and October contained approximately 20% dw and 21% dw, respectively. The uronic acid hexa-mannuronic acid was not detected in the press liquids along with only small amounts of Guluronic acid, leaving the majority of the uronic acids in the press cake. Since the press liquid contains such small amounts of uronic acids, the results indicate that the production of the press liquid should not affect the alginate content greatly in the final product.

2.3. Trace Elements

One of the main issues in the production of wild seaweed is the possibility of the contamination of heavy metals, such as arsenic and mercury. Iodine is also found in large quantities in seaweed [1,46,47]. Hence, the arsenic (As), cadmium (Cd), mercury (Hg), lead (Pb), and iodine (I) contents were determined in the present study for each harvesting period for the four different production stages. However, as for other measurements of the chemical composition, the press liquid and press cake were only measured in July and October (Table 3).

Table 3. Arsenic, cadmium, mercury, lead, and iodine content of A. nodosum samples and their produce. The reported values can have margin of error of +/− 20% due to the method used. Results are expressed as mean ± standard deviation of wet weight ($n = 3$).

Harvesting Time	Sample ID	Mercury (mg/kg Sample)	Cadmium (mg/kg Sample)	Arsenic (mg/kg Sample)	Inorganic Arsenic (mg/kg Sample)	Lead (mg/kg Sample)	Iodine (ug/g Sample)
June	Fresh seaweed	<0.01	0.263 ± 0.006^{a}	7.1 ± 0.2	<0.01	<0.01	156.7 ± 5.8^{b}
	Press liquid	NA	NA	NA	NA	NA	NA
	Press cake	NA	NA	NA	NA	NA	NA
	Seaweed meal	0.023 ± 0.001	0.950 ± 0.034^{b}	30.8 ± 0.8^{a}	<0.01	0.039 ± 0.005^{b}	670.0 ± 112.7^{b}
July	Fresh seaweed	<0.01	0.28 ± 0.02^{a}	7.3 ± 0.5	<0.01	<0.01	263.3 ± 55.1^{a}
	Press liquid	<0.01	0.04 ± 0.0^{a}	3.8 ± 0.01^{a}	<0.01	<0.01	169.3 ± 1.7^{a}
	Press cake	0.015 ± 0.001	0.18 ± 0.01^{a}	4.4 ± 0.1^{a}	<0.01	0.010 ± 0.005^{b}	183.3 ± 15.8^{a}
	Seaweed meal	0.022 ± 0.002	0.933 ± 0.026^{b}	28.5 ± 0.5^{b}	0.19 ± 0.01	0.056 ± 0.004^{a}	893.3 ± 21.2^{a}
October	Fresh seaweed	<0.01	0.083 ± 0.002^{b}	7.5 ± 0.2	<0.01	<0.01	313.3 ± 5.8^{a}
	Press liquid	<0.01	$<0.01^{b}$	2.3 ± 0.1^{b}	<0.01	<0.01	142.2 ± 4.1^{b}
	Press cake	0.014 ± 0.002	0.063 ± 0.004^{b}	3.7 ± 0.1^{b}	<0.01	0.023 ± 0.002^{a}	130.0 ± 0.0^{b}
	Seaweed meal	0.020 ± 0.002	$1.43 \pm 0.03^{a*}$	31.3 ± 1.0^{a}	0.12 ± 0.00	0.035 ± 0.002^{b}	980.0 ± 20.0^{a}

Different subscript letters of same produce between different harvesting periods (e.g., mercury of fresh seaweed from June, July, and October, and so on) indicate significant differences ($p < 0.05$). An asterisk (*) indicates values above the set limits according to European Commission for dried animal feed. NA: Composition analyses of the press liquid and press cake from seaweed harvested in June were not performed due to the low antioxidant potential of the press liquid during this season.

Arsenic (As) is a metalloid that is found in the environment in both inorganic and organic forms. The organic form is considered to be less toxic than the inorganic one since inorganic arsenic is acknowledged as carcinogenic for humans [48]. In the present study, no significant difference was observed in arsenic content between the harvesting times of the fresh seaweed samples, and the inorganic arsenic content was less than 0.01 in all cases. The total arsenic content of the press liquid was significantly higher in July when compared to the content in October, but was still within the set limits according to the European Commission for dried animal feed. The arsenic content in the press cake was significantly differentiated between the two harvesting periods and contained around 40–50% less arsenic when compared to the fresh seaweed samples. It is known that rinsing food and cooking with high amounts of water, e.g., rice, can reduce the arsenic content of the food component [49], which correlates with the results from the present study, where during the production of the press liquid, the arsenic seemed to be extracted with the water, hence reducing the arsenic content in the press cake. The seaweed meal from June contained significantly lower amounts of arsenic than the other two harvesting periods, 28.5 ± 0.5 mg/kg. Seaweed meal intended for feed should not contain higher amounts of arsenic than 40 mg/kg, where the inorganic arsenic content should not exceed 2 mg/kg [50], but no regulations have been set on the maximum arsenic content of seaweed used as food or supplements for human consumption. Seaweed meal is often used as feed for animals, and, according to the results in the present study, both the inorganic and organic arsenic were below the set limits of 40 mg/kg of organic and 2 mg/kg of inorganic arsenic.

The heavy metal cadmium (Cd) is found in the environment, both naturally occurring and from anthropogenic sources (industrial and agricultural sources). The main source of cadmium exposure for humans, except from smoking, is foodstuffs [51]. In the present

study the fresh seaweed samples from October contained significantly lower amounts of cadmium compared to the other two harvesting periods (June and July). The press liquid produced from July-harvested seaweed contained a significantly higher cadmium content, 0.04 ± 0.00 mg/kg, when compared to that from October, which contained less than 0.01 mg/kg, indicating that when a higher Cd content is in the raw material, a higher content will be in the press liquid, leaving less content in the press cake in both cases. However, the seaweed meal from October contained a significantly higher cadmium content than the other two harvesting periods and contained 1.43 ± 0.03 mg/kg of cadmium. The limits of cadmium in seaweed used for animal feed is set to 1 mg/kg [50], meaning that the seaweed meal from both June and July were below that limit but the meal from October exceeded the limits.

Mercury (Hg) is present in the environment, both from anthropogenic and natural sources, in three different chemical forms (metallic mercury, inorganic mercury, and organic mercury) [52]. The mercury content in the present study was recorded to be slightly higher in the seaweed meals independent of season, and in the press cake from July (0.02 mg/kg), than in other samples. However, no significant differences were recorded in the mercury content between the tested processing streams. The set limits of mercury in animal feed with water content below 12% are 10 mg/kg [50] and 0.1 mg/kg for supplements that contain seaweed. Thus, the Hg levels of all seaweed meal samples and press liquid samples were under the limit for mercury to use as both animal feed and as a supplement for human consumption [53].

The environmental contaminant lead (Pb) occurs mainly by anthropogenic sources (including battery manufacturing, mining, and smiting) but also naturally. Humans can be exposed to lead through water, food, soil, dust, and air, but the main source of exposure is through food [54]. A significant difference was observed in the seaweed samples regarding lead content between the harvesting periods, where the seaweed meal harvested in July contained significantly higher amounts, 0.056 ± 0.004 mg/kg, in the meal compared to the other harvesting periods. Slightly higher Pb content was observed in the press cake from October when compared to the press cake obtained in July. However, according to regulations, the lead content of animal feed cannot exceed 0.1 mg/kg (European Commission, 2002). The seaweed meal used in the present study was in no case higher than 0.06 mg/kg, and thus lower than the set limits for lead content at each harvesting periods. The set value for supplements for human consumption is, however, set higher than for animal feed, at 3 mg/kg [53], so all samples were below the limit for supplement production as well.

Iodine (I) is an essential nutrient for the function of the thyroid gland. In the diet, iodine mostly comes from the consumption of seafood [47], and seaweed has been reported to be a very good source of iodine [24,31]. The iodine content in the fresh seaweed samples contained high amounts of iodine, as expected, but the content was differentiated significantly between harvesting periods. The fresh seaweed samples from June contained significantly lower amounts when compared to the other two harvesting periods. The iodine content of the press liquid and press cake from July was then significantly higher when compared to October, even though the content in the fresh seaweed samples was higher in October than in July, indicating that the extracted iodine content may not solely be related to the initial iodine content of the raw material and might be influenced by other factors too, such as availability and extractability. The press cake contained 30.4–58.5% lower amounts of iodine than the fresh seaweed samples, indicating that a significant amount of the iodine would be lost with the production of the press liquid. The iodine content of the seaweed meal was significantly lower in June (670 ± 113 mg/kg sample) when compared to the other two harvesting periods The high iodine content in the press liquid might also affect the possibility of usage of the chemical in food production, especially if it is dried or condensed. The recommended dietary allowance (RDA) of iodine, set by the Nordic council of ministers, for adults is 150 µg/day, and the upper-level intake is set at 600 µg/day [55]. Hence, very small amounts of fresh *A. nodosum* are needed to be consumed to obtain the RDA of iodine.

2.4. Total Phenolic Content (TPC) and Antioxidant Activities of Press Liquid and Seaweed Extracts

2.4.1. Total Phenolic Content (TPC)

The total phenolic content (TPC) was evaluated of the freeze-dried (FD) extracts (press liquids, fresh seaweed extracts, and seaweed meal extracts) from different harvesting periods. The meal extracts from October presented the highest TPC (17.4 ± 0.7 g PGE/100 g extract), followed by the meal extracts from July and the press liquid extracts from July and October (Table 4). A significant difference was observed in the TPC between harvesting periods in both the press liquid and meal extracts, where the extracts from June contained significantly lower amounts of polyphenols. The press liquid extracts did, however, not differentiate significantly between July and October. However, the meal extracts from October contained significantly higher amounts when compared to the extracts from July. The seasonal variation in polyphenol content of *A. nodosum* has been evaluated by Apostolidis, et al. [56] and Parys, et al. [57]. In both studies, two peaks in polyphenol content were observed, one in summer (July in both cases) and another in autumn (September and October). The results from the present study also show a similar trend, or higher content detected in the extracts from July and October when compared to June, where the algae is still in its fertile period. Results have shown that there are many factors that can affect the levels of phlorotannins available in the algae, including factors such as the reproductive state of the algae, the age of the thallus, temperature, salinity of the sea, ambient nutrients, and light intensity [58–60]. Some studies have also indicated that herbivore grazing can increase the levels of phlorotannins in *A. nodosum* [61,62], and the grazing of seaweed in Scandinavia and Scotland seems to be at its highest in the summer [57], which can be one of the contributors to the high content of polyphenols in July. Therefore, the difference in the extracted TPC from *A. nodosum* in the present study can be linked partially to the reproductive state and seasonal variation, but other factors can also be contributors to the levels accumulated in the algae. When extraction methods were compared, a significant difference was observed in the TPC between the extracts in all harvesting periods. However, the extracts from June did not show the same results as the other two harvesting periods or indicate higher TPC when the fresh seaweed extraction was used. The other two harvesting periods showed similar results when the extraction methods were compared, where the results indicate that a higher polyphenol content was extractable by using pressure in the extraction process of the fresh seaweed. Furthermore, results from October indicated higher polyphenols were extracted from the seaweed meal and press liquid, which indicates that the potential of the alternative processing methods and the utilization of the press liquid increases as the seaweed matures during the season.

2.4.2. Oxygen Radical Scavenging Activity (ORAC)

Oxygen radical absorbance capacity (ORAC) is used to determine the antioxidant capacity of an extraction by evaluating their abilities to inhibit lipid oxidation by scavenging peroxyl radicals. Thus, a higher ORAC value (Ø) indicates a stronger peroxyl radical scavenging ability. The method is based on the antioxidant abilities to inhibit oxidation induced by peroxyl radicals, which are initiated by the thermal decomposition of 2,2′-azobis (2-amidino propane) dihydrochloride (AAPH). With time, fewer antioxidants can donate hydrogen atoms to the peroxyl radicals as the reaction progresses, leading to the combining of radicals and fluorescent molecules, and hence the loss of fluorescence [63–65]. The results are shown in Table 5.

Table 4. Total phenolic content (TPC) of the freeze-dried extracts (fresh seaweed extracts, press liquid extracts and seaweed meal extracts as affected by harvesting season. Results are expressed as mean ± standard deviation.

Harvesting Time	Extracts	TPC * (g PGE/100 g Extract) (n = 3)
June	Fresh seaweed extract	12.0 ± 0.2 ^{a}
	Press liquid extract	10.8 ± 0.5 ^{b,y}
	Seaweed meal extract	11.0 ± 0.2 ^{b,y}
July	Fresh seaweed extract	13.1 ± 0.3 ^{b}
	Press liquid extract	15.2 ± 0.7 ^{a,x}
	Seaweed meal extract	15.5 ± 0.8 ^{a,y}
October	Fresh seaweed extract	12.5 ± 0.8 ^{c}
	Press liquid extract	15.1 ± 0.1 ^{b,x}
	Seaweed meal extract	17.4 ± 0.7 ^{a,x}

Different subscript letters (a–c) within columns indicate significant differences in extraction method ($p < 0.05$). Different subscript letters (x,y) of same produce between different harvesting periods (e.g., PSE/PL/SME from June, July, and October) indicate significant differences ($p < 0.05$). * A typical equation for the standard curve was 3.7741x + 0.0316, with the correlation coefficient R^2 = 0.9983, a level of detection LOD = 0.010026, and level of quantitation LOQ = 0.432747.

Table 5. Oxygen radical scavenging activity (ORAC), DPPH radical scavenging activity (DPPH), and metal chelating ability (MC) of extracts from different harvesting periods. Results are expressed as mean ± standard deviation (n = 3). Measurements of DPPH and MC were made with concentration of 5 mg/mL.

Harvesting Time	Extracts	ORAC Value (µmol of TE/g Extract)	DPPH (% Inhibition)	MC (% Inhibition)
June	Fresh seaweed extract	507 ± 11	94.1 ± 0.3	50.9 ± 1.3
	Press liquid extract	625 ± 9	94.8 ± 0.3	50.2 ± 3.8
	Seaweed meal extract	680 ± 71	94.8 ± 0.4	70.5 ± 2.9
July	Fresh seaweed	1648 ± 54	95.1 ± 0.5	49.2 ± 2.8
	Press liquid extract	1452 ± 62	94.9 ± 0.4	44.2 ± 4.9
	Seaweed meal extract	1640 ± 72	93.4 ± 0.3	76.6 ± 1.9
October	Fresh seaweed extract	1314 ± 143	94.6 ± 0.1	44.7 ± 3.8
	Press liquid extract	1476 ± 109	95.3 ± 0.5	45.7 ± 3.7
	Seaweed meal extract	1637 ± 179	94.9 ± 0.2	78.0 ± 1.8

The highest ORAC value was observed in the fresh seaweed extract from July (1648 ± 54 µmol of TE/g extract), followed by the meal extract from July and October (1640 ± 72 and 1637 ± 179 µmol of TE/g extract). The ORAC values were significantly differentiated between harvesting periods, where June was significantly lower in ORAC values when compared to the other periods, but no difference was observed between July and October. As previously discussed, the algae were still in their fertile period in June, which led to less content of polyphenols in the extracts. ORAC values and polyphenol content have been reported to be connected with each other [14], which might explain the low ORAC activities in extracts from June. Furthermore, the same study investigated the antioxidant activities of Icelandic seaweed and showed that the Fucus species showed good antioxidant abilities, including high ORAC values. The ORAC values of water extracts of dried seaweed from *A. nodosum* harvested in May was approximately 1350 µmol of TE/g extract. The ORAC values reported by the discussed study were much higher when compared to the meal extract from June, and lower when compared to the meal extracts from July and October in the present study. The difference in values could mainly be explained by the reproductive state of the algae (which varies between April and June) [66] when compared to the values from July and October, or the method used for measurements and extraction. In the extraction of fresh seaweed extracts, slightly higher amounts of seaweed,

1.1/1 (*w*/*w*) of seaweed/water, were used compared to the amounts used in the extraction of press liquids. However, when fresh seaweed extracts are compared to press liquid, the press liquids had higher ORAC values in June and October but lower in July, indicating that the higher amounts used in the fresh seaweed extracts were not a dominating factor for the results of the ORAC measurements. In general, the results indicate that *A. nodosum* abilities to scavenge peroxyl radicals were lower in June when the seaweed is in its fertile period, compared to the other assessed seasons.

2.4.3. DPPH (2,2-Diphenyl-2-picrylhydrazyl hydrate) Radical Scavenging Activity

Due to its low cost and simplicity, DPPH radical scavenging activity is one of the most popular antioxidant assays to evaluate the capacity of antioxidants to scavenge free radicals [67]. DPPH activity was evaluated for extracts from each assessed processing step obtained at different seasons at the concentrations of 5 mg/mL. The results from the DPPH assay of the seaweed samples are expressed as %inhibition (Table 5).

The DPPH assay performed on the extracts in the present study showed very high radical scavenging activity of all extracts. No significant difference was observed in the DPPH radical scavenging activities when harvesting time (June, July, and October) and the extracts were compared. The DPPH activities were similar in all extracts, ranging from 94.1% to 95.1% inhibition. Some studies have examined the DPPH radical scavenging activity of seaweed extract and brown seaweed species. Fucus species typically have rather high DPPH radical scavenging abilities compared to red and green algae [14,68]. It is difficult to compare the results of a DPPH assay to results from other studies due to the lack of a standard protocol for the assay. The results in similar studies often have different extraction methods, use other solvents during the extraction, and/or results are expressed in different units, e.g., as IC_{50} number, antiradical power ($1/EC_{50}$), or as mg GAE/g DW. DPPH is often expressed with an IC_{50} number, which describes the concentration of an antioxidant that is required to reduce the DPPH absorbance by 50% [67]. The results in this study did not allow the calculations of an IC_{50} number since the inhibition concentration was above 75% for all concentrations measured and samples. Therefore, the DHHP radical scavenging activity results were expressed as %inhibition. The %inhibition indicates how well the extract scavenges free radicals, hence, the higher the inhibition, the better radical scavenging abilities the extract has. Free radical scavengers used in foods e.g., butylated hydroxytoluene (BHT), tocopherols, and plant phenolics have high radical scavenging activities [16,69]. Since the extracts in the present study had very high %inhabitation of DPPH, it might indicate that it could be of good use to inhibit the lipid oxidation of some food products.

2.4.4. Metal Chelating Abilities (MC)

The metal chelating (MC) properties of the seaweed extracts were determined by measuring the formation of a ferrozine and Fe^{2+} complex. The metal chelating was measured at a concentration of 5 mg/mL and results are expressed as %inhibition in Table 5. The highest MC inhibition of seaweed samples was found in the seaweed meal extracts from October (78 ± 2 %inhibition), followed by the seaweed meal extracts from July (76.6 ± 2 %inhibition) and June (70.5 ± 3 %inhibition). The fresh seaweed extracts typically exhibited similar inhibition as press liquids, indicating that even though the fresh seaweed extract was made with a slightly higher seaweed content, it did not have significantly higher MC activities when compared to press liquid extracts and did not affect the results greatly. A significant difference was observed in metal chelating inhibition between extract types where the meal extracts exhibited higher MC activities when compared to the other two extraction methods. No significant difference was observed in the metal chelating abilities between harvesting periods.

The metal chelating activities of Icelandic seaweed reported by Wang, Jónsdóttir and Ólafsdóttir [14] showed that the water extracts of *A. nodosum* (in concentration of 5 mg/mL) exhibited metal chelating inhibition of approximately 95%. The study also indicated that

other compounds than phenolic compounds exhibit metal chelating abilities. Other studies, such as Saiga et al. [29] and Wang, et al. [70], have reported some peptides and proteins to have metal chelating abilities. The press liquids from July and October contained some protein content, 0.3 and 0.2 g/100 g sample, respectively (Table 1), which might contribute to the metal chelating activities.

2.4.5. Correlation between Total Phenolic Content and Antioxidant Activities

Principal component analysis (PCA) was performed to assess the relationship between TPC and antioxidant measurements. A PCA loading plot is shown in Figure 1 The first two components explained 48.9% and 34.4% of the total data variance, respectively. Component 1 showed a correlation between TPC and ORAC values. A Pearson correlation was performed, and the test showed a correlation between TPC and ORAC with a Pearson correlation coefficient (r) = 0.81.However, TPC did not correlate to any of the other antioxidant measurements. A study conducted by Wang, Jónsdóttir and Ólafsdóttir [14] indicated that the higher the phenolic content in a seaweed extract, the higher antioxidant capacity of the extract, including both DPPH and ORAC values. Other studies on both seaweed and plant extracts have also indicated that TPC correlates with antioxidant activities [71–74]. However, in the present study, ORAC only correlated with TPC and not to DPPH. However, Wang, Jónsdóttir and Ólafsdóttir [14] also demonstrated that other compounds, such as sterols or fucoxanthin found in brown seaweed, could also work as radical scavengers during measurements of the DPPH assay. The high DPPH antioxidant activities of the extracts in the present study may therefore be due to compounds other than polyphenols.

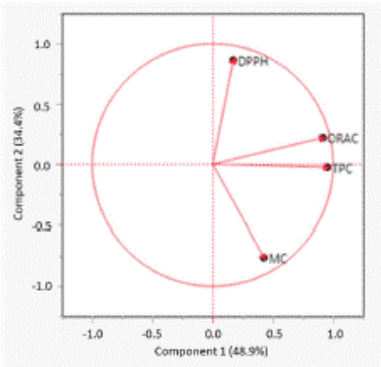

Figure 1. PCA loading plot of total phenolic content (TPC), oxygen radical scavenging capacity (ORAC), DPPH radical scavenging activity (DPPH), and metal chelating (MC) measurements.

3. Materials and Methods

3.1. Seaweed Sampling and Preparation

Ascophyllum nodosum was mechanically harvested by Thorverk hf and collected in June, July, and October 2020. Approximately 20 kg of fresh seaweed from each harvest were stone cleaned by hand and minced with a Mainca CR-40 mincer (Mainca, Barcelona, Spain). Approximately 5 kg of the minced seaweed was collected in a bin, and the rest used for production of press liquid and press cake. All samples were stored at −25 °C till further use and analysis. Seaweed meal from Thorverk hf was used to examine typical chemical composition of the algal meal. The meal was processed (the biomass is harvested mechanically, transported to the factory, chopped, dried, grinded, sieved, and packed) by Thorverk from the same harvesting times as the collected fresh biomass used for the press liquid and press cake assessment. The meal was collected right after drying and stored at room temperature till further use.

3.2. Preperation of Press Liquid and Water Extracts

To produce the press liquid, an equal amount of tap water and fresh minced seaweed (1/1 *w*/*w*) were mixed and put through a Stephan micro cutter (Stephan Machinery GmbH, Germany). The blend was centrifuged at 5100 revolutions per minute (RPM) at 4 °C for 10 min, and both the supernatant (press liquid) and press cake were collected. The extraction of fresh minced seaweed and seaweed meal was performed with distilled water as solvent. For each seaweed meal extraction, 30 g of seaweed meal was put in a 500 mL Erlenmeyer flask, which was filled up with water to the 300 mL mark. For each fresh seaweed extraction, 150 mL of fresh seaweed and 150 mL of water (approximately 1.1/1 *w*/*w*) were put in 500 mL Erlenmeyer flask. All extracts were shaken for 1 h and centrifuged at 5100 RPM and 4 °C for 10 min. The extracts and press liquid samples were finally freeze-dried in a Genesis 25 SQ EL freeze dryer (SP industry, Philadelphia United States of America) and stored at −25 °C till further use to minimize degradation of the extract.

3.3. Nutritional and Mineral Composition of Seaweed and Its Produce

The water content of the seaweed samples was determined as the difference in weight of the sample before and after drying for at least 4 h at 103 ± 2 °C [75]. The total lipid content was determined according to AOCS official method Ba-3-38 (2009) [76]. The protein content of the seaweed samples was determined by using the Kjeldahl method according to ISO 5983-1:2005 [77] and ISO 5983-2:2009 [78], where the nitrogen content was multiplied by factor 5 as recommended for seaweed [27] to obtain the crude protein content in the samples. The ash content was determined by burning 2 g samples at 550 °C for 3 h, and the residue weighed and compared to the sample weight before burning [79]. The salt content was determined with the Volhard titration method [80]. Carbohydrate content of the seaweed samples was determined by calculation, subtracting water, protein, fat, and ash from 100 g of the sample.

The trace elements, arsenic, cadmium, mercury, and lead were determined according to the NMKL-186 method [81]. The inorganic arsenic content was determined by hydride generation atomic absorption spectroscopy according to the ASU method (2008-12) with modification, CON-PV 01288 (2020-05). The iodine content was determined by inductively coupled plasma mass spectrometry according to the DIN EN 15111 (2007-06) method with modification., CON-PV 01187 (2017-08).

3.4. Monosaccharide and Uronic Acid Composition

The monosaccharide and uronic acid contents were determined in duplicates of 25 ± 2.5 mg of the freeze-dried samples. The samples were hydrolyzed according to Wychen and Laurens [82] with sulfuric acid where 250 uL of 72% (*w*/*w*) sulfuric acid was added, the tubes were placed into 30 °C water-bath for an hour, and finally vortexed every 5 to 10 min. Afterwards, 7 mL of deionized water was added to the tubes to bring the sulfuric acid concentration to 4% (*w*/*w*). The samples were vortexed, autoclaved for one hour at 121 °C and cooled down. The hydrolyzed samples were neutralized by transferring 1 mL of hydrolysate to a white Sarstedt tube. The aliquot was neutralized to a pH 6–8 by using 1 M calcium carbonate. The aliquot was centrifuged and filtered through a 0.2 µm nylon filter (Phenomenex-Phenex, 15 mm, Torrance, CA, USA).

The monosaccharide composition of the hydrolyzed samples was analyzed according to method by Wychen and Laurens [82] and the uronic acid according to method by Basumallick and Rohrer [83]. Both measurements were performed with Dionex HPAED-PAD 5000+ on Dionex Carbo Pack PA 20 (3 × 150 mm), 6 µm column by using either two or three eluents, depending on the method used. For the monosaccharide measurements, two eluents were used, or 200 mM NaOH and deionized water, but for the uronic acid measurements three eluents were used, the same as used for the monosaccharides as well as 1 M sodium acetate in 200 mM NaOH. The flow rate of the eluents for the monosaccharide composition was set at 0.5 mL/min, and at 0.4 mL/min for the uronic acid assessment. The %monomeric sugar was calculated by using linear regression coefficients and oven dry weight of sample.

3.5. Total Phenolic Content (TPC)

The total polyphenol content (TPC) of the seaweed extracts were determined according to the Folin–Ciocalteu procedure as described by Singleton and Rossi [84] with slight modifications. For the measurements, 20 µL of each sample/standard (gallic acid and phloroglucinol) was put in a microplate with 100 µL of 0.2 N Folin–Ciocalteu and let sit at a room temperature for 5 min. Approximately 80 µL of 7.5% (*w*/*w*) Na_2CO_3 solution was then added, and the microplate put in a microwave for 10 s at 800 W. The microplate was shaken for 30 min, and then the absorbance was read at 720 nm with a Cytaion5 (Agilent Technologies, Santa Clara, CA, USA) microplate reader. The TPC was determined from the standard curves of phloroglucinol made with solutions ranging from 0 µg/mL to 100 µg/mL. Each extract was measured in triplicate ($n = 3$).

3.6. Antioxidant Activity

3.6.1. DPPH (2,2-Diphenyl-1-picrylhydrazy) Radical Scavenging

2,2-Diphenyl-1-picrylhydrazy (DPPH) radical scavenging activity was performed according to Sharma and Bhat [85]. Quantities of 150 µL sample/ 70% (*w*/*w*) ethanol were mixed with 50 µL of 2,2 diphenyl-1-picrylhydrazy (DPPH) or 70% (*w*/*w*) ethanol in a 96-well plate. A sample was prepared by mixing the sample and DPPH solution, The microplate was covered and shaken for 30 min at room temperature at 320 rpm. The sample absorbances were read at 520 nm in a Cytaion5 (Agilent Technologies, Santa Clara, CA, USA) microplate reader. Each sample was measured in triplicate. The inhibition percentage was calculated as follows

$$\%\ inhibition = \frac{A_{blank} - \left(A_{sample} - A_{control}\right)}{A_{blank}} \times 100$$

where A_{blank} is the absorbance of the blank, A_{sample} is the absorbance of the sample, and $A_{control}$ is the absorbance of the control samples at 520 nm.

3.6.2. Metal Chelating Ability (MC)

The metal chelating ability was determined according to the method described by Boyer, et al. [86] with slight modifications. A quantity of 100 µL of sample/water, 100 µL of 0.5 mM ferrozine/water, and 50 µL of 0.2 mM $FeCl_2$ was added to a 96-well microplate. The samples were prepared with the sample, ferrozine, and $FeCl_2$, the control sample with sample, water, and $FeCl_2$, the control blank with 200 µL of water and $FeCl_2$, and the blank with water, ferrozine, and $FeCl_2$. The microplate was covered and shaken for 30 min at room temperature. The absorbance was read at 560 nm with a Cytaion5 (Agilent Technologies, Santa Clara, CA, USA) microplate reader. Each sample was measured in triplicate. %Chelating activity was calculated as follows

$$\text{Chelating activity } (\%) = \frac{(A_{Net\ blank}) - \left(A_{Net\ sample}\right)}{(A_{Net\ blank})} \times 100$$

where $A_{Net\ blank}$ is the absorbance of the blank minus the blank control, and $A_{Net\ sample}$ is the absorbance difference between the sample and the sample control.

3.6.3. Oxygen Radical Absorbance Capacity (ORAC)

The oxygen radical absorbance capacity (ORAC assay) was analyzed according to Huang, Ou, M., Flanagan and Prior [64] and Ganske [65] with slight modifications. Approximately 60 µL of 10 mM fluorescein was added into a black 96-well microplate (Costar, cat no. 3694) along with 10 µL of sample (seaweed extract), standard (Trolox) or water (blank). Approximately 40 µL of phosphate buffer solution (pH 7.4) was added in one well for gain adjustment. The mixture was incubated at 37 °C for 10 min. After incubation, 30 µL of 120 mM 2,2 azobis (2-methylpropionamidine) dihydrochloride solution (AAPH solution) was added and the fluorescence emission (excitation at 485 nm, emission at 520 nm) was

read every minute for 100 min in a POLARstar optima fluorescence analyzer (BMG Labtech, Ortenberg, Germany). The area under the fluorescence curve (AUC) was calculated by the normalized curves. Each sample was measured in triplicate.

3.7. Statistical Analysis

Statistical analysis of the chemical composition and antioxidant measurements was performed by using Microsoft Excel 2013 (Microsoft Inc., Redmond, WA, USA) and JMP pro15 (SAS Institute Inc., Cary, NC, USA). Averages and standard deviation (SD) were calculated in Microsoft Excel. One way analysis of variance (ANOVA) and Tukey's honest significant difference test were performed in the JMP pro15 software (SAS, Cary, NC, USA) for each sample of each group. For all samples, statistical significance was set to $p < 0.05$. Principal components analysis (PCA) was performed in JMP pro15 to evaluate the relationship between TPC and antioxidant activity of the extracts where a correlation probability test (Pearson product-moment correlation) was used to determine the correlation between variables.

4. Conclusions

The results from the present study indicate that the chemical composition of *A. nodosum* changes with the reproductive state of the algae and thus with harvesting seasons. Both the chemical composition and antioxidant activities of the press liquids from July and October were very similar, meaning that if the press liquid would be utilized for product development on an industrial scale, it would probably be a relatively stable production from July to October. Furthermore, due to the removal of compounds from the seaweed biomass with the production of the press liquid, the production increases the total alginate content and purity of the press cake, meaning that alginate producers could obtain an even higher yield of alginate from the pressed seaweed.

Alternative processing is more promising for high value product development from seaweed harvested in July and October compared to June due to higher total phenolic content and antioxidant activities (assessed by ORAC) in the seaweed biomass. Furthermore, product development from the ingredients present in the press liquid is in line with the current demands of full utilization and no-waste policies. The proposed alternative processing can both increase the value and sustainability of seaweed processing. Further research for applications of the press liquid is, however, needed to assess its potential for food or cosmetics applications.

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Paper II

Alternative industrial processing of seaweed meal from *Ascophyllum nodosum*: Value adding and product development potential of liquid side-streams obtained with different separation methods

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Abstract

Ascophyllum nodosum is harvested from wild sources and used in seaweed meal production. It contains multiple valuable compounds, including polyphenols, polysaccharides and carotenoids that have bioactive properties. In current uses of the biomass e.g. as a substance for alginate production, these compounds are not of any special interest, hence there is an opportunity for better utilization of the biomass. Therefore, the aim of the study was to investigate the potential of producing and upscaling production of a byproduct (targeted seaweed extract produced with mixture of fresh seaweed and water) within traditional seaweed meal processing. That was performed by evaluating liquid/solid separation (screw press vs decanter), centrifuging as secondary separation step as well as drying methods (spray drying vs freeze drying) on the obtained liquid extracts. Chemical and antioxidant properties were evaluated throughout the alternative processing and mass balance calculated. The results suggests that the decanter was more efficient for separation compared to the screw press due to higher yield of liquid retrieved. The chemical composition (water, protein, ash, and monosaccharide content) and antioxidant properties (assessed by DPPH and ORAC) did not differentiate significantly neither between the separation methods nor during further processing of the liquids (centrifugation or drying methods). Furthermore, the alginate remained in the solid phase during separation and the main components extracted with the liquid phase included salt, minerals, polyphenols, and carbohydrates while the main monosaccharides in the liquid phase included mannitol and glucose. The results hence indicate that freeze-drying and spray-drying are equally beneficial to maintain the antioxidant properties of the liquid seaweed extracts, and that the alternative processing with a decanter as the primary separation step could be a feasible solution to meet the demands of full utilization and no-waste policies within seaweed production.

Keywords (4-6 words): *Ascophyllum nodosum*, Seaweed processing, Separation methods, Side stream utilization, Drying methods, Bioactive properties

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1 Introduction

Seaweed production worldwide more than tripled between the years 2000 and 2020. During these years Asia contributed over 97% of the total harvested biomass, while Europe contributed less than 0.8%. However, unlike in Asia, the majority of harvested seaweed biomass in Europe is from wild sources, not cultivation (FAO, 2020, 2022). The interest in seaweed has been growing in Europe over the years, and the focus has been shifting towards cultivation of brown seaweed species for food and other high value products. The total seaweed production in Europe has been estimated to grow significantly in the next few years, and according to the recently published report “Hidden champion of the ocean” by Seaweed for Europe, the overall production could grow from the 300 thousand tons that were harvested in 2020 to 8 million tons in 2030 (A Vincent et al., 2020).

Responsible consumption and production, and life below water are two of the 17 Sustainable development goals (UN-SDGs) that were set and adopted by the United Nation member states in 2015. These development goals are directed towards sustainable production and consumption, as well as to preserve and protect ocean resources (United Nations, 2015). To follow the above-mentioned development goals, there is a need to ensure proper utilization of the harvested seaweed biomass before increasing production to minimize the waste in its current applications.

Ascophyllum nodosum is a brown seaweed species that grows all around Iceland and is likely the most abundant seaweed specie found on the Icelandic coast. Generally, *A. nodosum* contains high moisture (68-80% wet weight), carbohydrate (42-64% dry weight (dw)) and ash content, (15-31% dw) but lower protein (4-12% dw), and lipid content (1-8% dw). Furthermore, it is known for its richness in vitamins, minerals, and bioactive compounds (Hrólfsdóttir et al., 2022; Lorenzo et al., 2017; Morrissey et al., 2001; Peinado et al., 2014). Furthermore, like other seaweed species, *A.nodosum* accumulates trace minerals from its surroundings, such as arsenic, cadmium, and lead. which can affect their applications, especially for human consumption (Holdt & Kraan, 2011b; Lozano Muñoz & Díaz, 2020) *A. nodosum* has been wild harvested in Europe for a long time, and the main harvester in Iceland harvests around 15.000 tons (wet basis) annually to produce seaweed meal. Seaweed meal produced with *A. nodosum* is used for multiple applications today, including for extraction of the hydrocolloid alginate, as a feed enhancer, as an ingredient in cosmetics products, and in production of bio-stimulants for plants (Lindsey White & Wilson, 2015; Pereira et al., 2020). In many of those applications, including alginate production, multiple side-streams and components are currently not considered of any specific interest, and the biomass is therefore not used to its full potential. However, *A. nodosum* contains multiple bioactive ingredients, including polyphenols, carotenoids, tocopherols, sulphated polysaccharides and fucoxanthin. These bioactive components could be used for other valuable purposes, including as ingredients for cosmetic products (Gager et al., 2020; Resende et al., 2021), as antioxidants or functional ingredients in food products (Agregán et al., 2017; O'Sullivan et al., 2016; Wang et al., 2010), or as an ingredient in bio stimulant formulations (Frioni et al.,

2018; Goñi et al., 2018; Shukla et al., 2019). In recent years, discussions on biorefineries and full utilization of resources, including seaweed biomass have gained interest (Baghel, 2023; Torres et al., 2019). Many studies have focused on exploring the possibilities of different biorefinery approaches on lab scale with seaweed biomass to extract compounds before and after hydrocolloid extraction (Baghel et al., 2020; Baghel et al., 2016; Hrólfsdóttir et al., 2022; Meinita et al., 2019; Zhang et al., 2020). However, few studies have been performed on what processing equipment could be used on an industrial scale for full utilization of this biomass. Zhang and Thomsen (2021) evaluated the possibilities of scale up of sequential extraction with three brow seaweed species aiming to obtain different products, e.g. laminarin, fucoidan and feed supplement, where a decanter centrifuge was used as a solid-liquid separation method.

In Thorverk, the main seaweed harvester in Iceland, the seaweed meal processes from wild harvested *A. nodosum* currently involve the following steps. First, the biomass is mechanically harvested, collected in nets that are allowed to float in the ocean, collected, landed on the dock, and transported to the factory. The biomass is then processed to seaweed meal by chopping, drying with geothermal heat, and milling of the biomass. Within seaweed meal processing there are some opportunities in adding pre-processing steps before the drying of the biomass to promote separation of valuable ingredients from the biomass. Furthermore, several studies have shown that water extractions can result in high yields of phenolic compounds as well as other compounds such as carbohydrates and protein that can exhibit bioactive properties (Hermund et al., 2022; Hrólfsdóttir et al., 2022; Wang et al., 2009). Thus, soaking, or targeted ingredient extraction might be suitable additional processing steps to effectively extract bioactive compounds from the seaweed biomass before drying, without affecting the alginate concentration of the final meal product (main product). To implement such changes within large scale processing facilities multiple things needs to be considered, including what kind of pre-processing and equipment fit within the processing, their physical and economic feasibility, the environmental effects and sustainability of the process, and how these changes would affect the final products, both from the main production (seaweed meal), and from the side-streams (new products) (Damodaran et al., 2008; Singh & Heldman, 2001). Therefore, the objective of the study was to assess the potential of producing a targeted seaweed extract from the liquid side-stream obtained when water is added to the fresh minced seaweed biomass, followed by micro cutting. The efficiency of using either a decanter or a screw press for the primary liquid separation step during the seaweed meal processing was assessed, by analyzing the chemical composition (proximate composition, monosaccharide composition, and trace minerals) throughout the process, and the antioxidant properties of the obtained liquid extracts. Furthermore, the potential need for further purification or treatment of the collected liquid via centrifugation was assessed, and whether this additional purification is needed to ensure high quality and pure extracts. Finally, the effects of using different drying methods to dry the obtained liquids from the side-stream were assessed to evaluate if, and then how, spray- and freeze-drying affects the antioxidant properties of the press liquid. The research questions were thus addressed by:

a) Evaluating the efficiency of separation of the liquid stream from the solids of the micro cut seaweed-blend, by comparing two separation methods (decanter vs. screw press) and analyzing the resulting

chemical properties (proximate composition, monosaccharide composition and trace minerals) and mass balances throughout the processes.

b) Assessing possible effects of using a disc centrifuge as a secondary separation step during seaweed liquid processing, focusing on the chemical composition and antioxidant properties of the liquid extracts.

c) Estimating the effect of different drying methods, freeze-drying and spray drying, on the chemical and bioactive properties of the obtained liquid extracts.

2 Materials and methods

2.1 Sample collection and pre-processing

Ascophyllum nodosum was harvested in late August 2022 by Thorverk hf. Approximately 300 kg of fresh seaweed biomass was collected in a 460 L plastic tub and transported to the processing facility, where the seaweed was minced with a Wolfking C.250 Universal grinder (Koncept Tech ApS, Slagelse, Denmark). Approximately 10 kg of the minced fresh seaweed biomass were collected, frozen in a -24°C freezer, where they were stored until analyzed as a reference sample. The remaining 290 kg of the biomass were used for further processing. Seaweed was mixed with tap water in a 1:1 ratio by weight, mixed, and put through a Stephan micro cutter (Stephan Machinery GmbH, Germany) obtaining a particle size of 1 mm or less. The micro cut seaweed blend was then frozen and stored at -24°C until it was processed approximately one month later. Commercial dry seaweed meal samples made from *A. nodosum,* processed by Thorverk hf. as described in the introduction, were also retrieved from a similar processing time to compare the obtained sediment from proposed alternative processing to conventional processing of the seaweed. This comparison was made to evaluate whether the new liquid processing would affect the quality of the commercial seaweed meal.

2.2 Assessment of processing alternatives

Primary separation step – decanter vs. screw press

To evaluate different processing methods' ability to effectively separate the liquids from the micro cut seaweed blend, two different types of separation methods were tested, or separation with i) a GEA Westfalia CA 22 decanter (GEA Westfalia separator AG, Germany) and ii) a screw press at a speed of approximately 30 rpm (Bibun, Japan) (Figure 1). Each process was performed in triplicate (20-30 kg batches), and samples were collected from each run. The composition results of the ***liquid*** and ***sediment*** from the decanter were compared to the ***press liquid*** and ***press cake*** deriving from the screw-press, respectively.

Secondary separation – centrifugation

The liquids retrieved from both separation processes were then put through an ASE 20-03-077 disk centrifuge (GEA, Düsseldorf, Germany). The centrifuge was operated at room temperature (20°C) at maximum velocity (12.000 rpm), and the material was pumped in at a rate of 200 L/hour (3.33 L/min) with counter current pressure of 1.5 bars during separation. The resulting ***centrifuged liquid*** and the ***precipitates*** were retrieved for analysis.

Drying method alternatives

Due to the high-water content of the liquid side-streams, drying was evaluated as a necessary processing step to reduce the high cost and environmental impacts of transport. To estimate the effect of different drying methods on the chemical and bioactive properties of the liquid extracts, the decanter liquid samples were either freeze dried with a Genesis 25 SQ EL freeze dryer (SP industry, Philadelphia, USA) or spray dried with a Mobile minor spray dryer (GEA Niro, Copenhagen, Denmark) with inlet temperature of approximately 170°C and outlet temperature around 90-100°C. Only samples from the decanter liquid were chosen to be used for evaluation of drying method efficiency since better separation of the liquid and solid phases were obtained with the decanter. Samples were collected after each processing step throughout processing and stored at -24°C until analyzed.

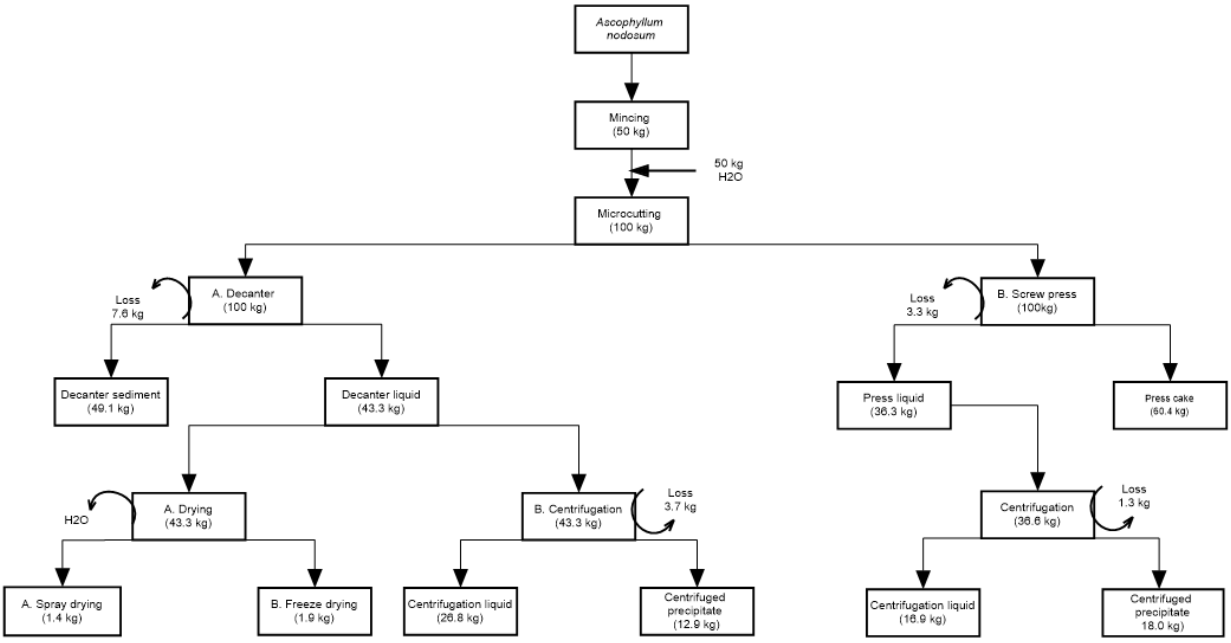

Figure 25 Overview of the different processing options, including the primary separation (decanter vs. screw-press), and decanter liquid processed with or without secondary separation of the press liquid by centrifugation, and comparison of processing streams using two drying methods (spray vs. freeze drying).

2.3 Mass and material balance calculations

To assess the separation efficiency of the decanter and screw-press mass and material balances were calculated for the proximate and trace element compositions of the streams throughout the processes (Figure 1 and Supplementary material 1-4). Materials were thus weighed before and after each step throughout the process. The focus was set on balancing the chemical composition of the streams, including water, carbohydrate, lipids, proteins, salt, ash, and trace element composition, for the different separation process alternatives (decanter vs. screw press). In the calculation an input weight of 50 kg fresh seaweed biomass (assuming no loss in the micro cutting process) were used for each scenario, to give a 100 kg basis after the 1:1 water addition, for assessment of the efficiency of the separation and processing steps.

2.4 Nutritional and trace element composition

Proximate composition

The water content of the samples throughout the process was determined according to ISO 6496:1999, from the difference in samples' weight before and after drying at 103 ± 2°C for at least 4 hours (ISO, 1999). The Dumas method, as described by ISO 16634-1:2008 (E) method (ISO, 2008), was used to determine the crude protein content, using a nitrogen conversion factor of 5 due to the high content of non-protein nitrogen products present in seaweeds (Alex R. Angell et al., 2016). The lipid content was determined by using a Soxhlet extractor, according to the AOCS Ba 3-38 (2009) method (AOCS, 2009a). The ash content of the samples was determined according to ISO 5984 (2002), where approximately 2 g of sample were burned for 3 hours at 550°C, and the weight of the burned residues then compared to the original weight of the sample (ISO, 2002). Salt content was determined according to the AOAC 17th ed no.976.18 method (AOAC, 2000). The carbohydrate content (TC) of the seaweed samples was then calculated as follows:

$$TC = 100\ g\ sample - (crude\ protein + fat\ content + ash\ content + water\ content)$$

Results of water, lipids, protein, ash, and carbohydrates were all expressed as mean± standard deviation as g/100 g wet weight (ww) from three sample replicates (n=3).

Trace element analysis

To determine trace elements (lead, cadmium, arsenic and mercury), the NMKL-186 (2007) method was used (NMKL, 2007b). Analysis of iodine were performed by the GBA group, according to the DIN 51727: 2011-11/ DIN EN ISO 10304-1: 2009-07 method. Since the mass balances indicated higher efficiency of the decanter separation than the press separation, only the decanter separation streams were analyzed for trace elements and iodine. For all trace element analyses, three samples analyzed for each sample type (n=3), and results expressed as mg/kg on sample basis.

2.5 Monosaccharide composition and alginate content

The monosaccharide composition and alginate content were only analyzed in the processing streams where the decanter was used during the primary separation step. For the monosaccharide, total phenolic content, alginate content, and antioxidant potential assessments, all samples from the assessed processing streams were freeze-dried with a Genesis 25 SQ EL freeze dryer (SP industry, Philadelphia United States of America) prior to analysis, except for the commercial algal meal samples and the spray-dried decanter liquid samples, which were already well dehydrated. All samples were prepared in duplicate according to the National Renewable Energy Laboratory (NREL) determination of total carbohydrates using the algal biomass laboratory analytical procedure (LAP) (Wychen & Laurens, 2013). Aliquots of hydrolyzed samples were neutralized with 1 M calcium carbonate, centrifuged at 5530×g for 10 min in a Beckman Coulter TJ-25 centrifuge (Beckman Coulter, Germany), and the supernatant was filtered through a 0.45 µm syringe filter. Samples were diluted and analyzed with a Dionex ICS 5000+ (Thermo Scientific, USA) High-Performance Anion-Exchange chromatography with a Pulsed Amperometric Detection (HPAE-PAD) and a Dionex Carbo Pack PA 20 (3 × 150 mm) column (Thermo Scientific, USA). A gold electrode and a silver/silver chloride reference electrode were used. For monosaccharide composition, the eluent composition was A: 200 mM NaOH, B: Distilled water, and the flow rate was 0.5 mL/min. Used reference standards for the monosaccharides determination were mannitol, fucose, galactose, glucose, and xylose.

For analysis of uronic acids, the eluent composition was A: 200 mM NaOH, B: 1 M Sodium acetate in 200 mM NaOH, and C: Milli-Q water, and the flow rate was 0.5 mL/min. Reference standards used for the uronic acids determination were guluronic acid and mannuronic acid. The results are expressed as average ± standard deviation as g/100 g dry weight (dw) from three sample replicates (n=3).

2.6 Total phenolic content

The Folin–Ciocalteu method, as described by Singleton and Rossi (Singleton & Rossi, 1965), was used to determine the total phenolic content (TPC) of the dried liquid extracts. Approximately 100 mg of each dried extract were dissolved in 10 mL of distilled water, shaken for 1 hour, and centrifuged for five minutes at 3500 rotations per minute (RPM) at 4°C. For the measurements, the samples, and standards (gallic acid and phloroglucinol, in concentrations ranging from 0 µg/mL to 100 µg/mL), were diluted in distilled water, and 20 µL of each put in a 96-well microplate. Quantities of 100 µL of 0.2 N Folin–Ciocalteu solution were added, and the samples kept at room temperature for 5 minutes. Approximately 80wasof 7.5% (*w*/*w*) Na_2CO_3 solution were added, and the microplate put in a microwave for 10 s at 600 W, and then placed on a shaker for 30 minutes at room temperature at 320 RPM. A Cytaion5 (Agilent Technologies, Santa Clara, CA, USA) microplate reader was used to read the absorbance at 720 nm. Standard curves from phloroglucinol were used to determine the total phenolic content, and results were expressed as gram phloroglucinol equivalent/100 g of dried (freeze dried or spray dried) liquid extract. Each extract (n=3 from

each processing stream) was measured in duplicate, and the mean calculated to represent each extract. Results were then expressed as mean and standard deviation calculated from the three extract replicates.

2.7 Antioxidant properties

2.7.1 DPPH (2,2-Diphenyl-1-picrylhydrazy) radical scavenging activities

The method described by Sharma and Bhat (2009) was used to determine the DPPH radical scavenging activity of the dried (freeze dried and spray dried) seaweed liquids. To prepare samples for measurements, samples were dissolved in 70% ethanol, shaken for 1 hour, and centrifuged at 3500 rpm at 4°C for 5 minutes. For measurements, a 96-well plate was used, where either 150 µL of diluted sample or 70% ethanol (w/w) were mixed with either DPPH solution (2,2 diphenyl-1-picrylhydrazy in methanol and 70% ethanol) or 70% ethanol (w/w) in quantities of 50 µL in the wells. The 96-well plate was shaken at 320 rpm on a plate shaker for 30 minutes, while protected from light, and the absorbance read in a Cytaion5 (Agilent Technologies, Santa Clara, CA, USA) microplate reader at 520 nm. To calculate %inhibition of DPPH radicals, following equation was used:

$$\% \text{ inhibition} = \frac{(A_{blank} - A_{control\ blank}) - (A_{sample} - A_{control\ sample})}{(A_{blank} - A_{control\ blank})} \times 100$$

where A_{blank} is the absorbance of the blank solution (70% ethanol in DPPH solution), $A_{control\ blank}$ is the absorbance of ethanol, A_{sample} is the absorbance of the sample in the DPPH solution, and $A_{control}$ is the absorbance of the control samples in ethanol at 520 nm.

Each extract (n=3) was measured in duplicate, and the mean calculated to represent the %inhibition of each extract. Results were then expressed as mean and standard deviation calculated from the three extract replicates.

2.7.2 Oxygen radical absorbance capacity (ORAC)

The oxygen radical absorbance activity (ORAC) was analyzed with the method described by D. Huang et al. (2002) and Ganske (2006) with minor modifications. The dried extracts were prepared as mentioned above for TPC measurements and diluted to fit the standard curve. Briefly, 10 µL standard/sample were added in a 96-well black microplate, with the addition of 60 µL of 10 nM fluorescein solution. Approximately 40 µL of phosphate buffer were used as gain adjustment. The microplate was incubated at 37°C for 10 minutes, and then 30 µL of 120 mM AAPH (2,2 azobis (2-methylpropionamidine) dihydrochloride) solution were added. The fluorescence (excitation/emission 485nm/520 nm) spectra were recorded every minute for 100 minutes in a PolarStar optima microplate reader (POLARstar Optima, Ortenberg, BMG Labtech). Each extract (n=3) was measured in duplicate, and the mean calculated to represent the ORAC value (µmol Trolox equivalent (TE)/g extract) of each extract. Results were then expressed as mean and standard deviation calculated from the three extract replicates.

2.8 Statistical analysis

Microsoft excel (Microsoft, Redmond, WA, USA) was used for data collection, calculation of averages and standard deviation (SD), as well as for table formatting. JMP pro 16 (SAS, Cary, NC, USA) was used for statistical analysis, including one way ANOVA (analysis of variance), pooled t-tests, and Tukey's honest significant difference test. For all statistical analysis, a significance level of $p \leq 0.05$ was used to determine significant difference between samples during alternative processing.

3 Results and discussions

3.1 Nutritional composition

The nutritional composition (water, fat, protein, salt-free ash, carbohydrate, and salt content) of the seaweed and liquid samples throughout processing is presented in Table 1. Both the water content and carbohydrate content of the fresh minced *A. nodosum* were high, or 70.1 ± 0.3 g/100 and 22.1 ± 0.3 g/100 g sample, respectively. Furthermore, the lipid content of the fresh *A. nodosum* was low, the ash content relatively high, which fits well to previous studies on *A. nodosum* harvested in Iceland (Hrólfsdóttir et al., 2022) and internationally (Lorenzo et al., 2017; Samarasinghe et al., 2021). The water content of the biomass increased from 70.1±0.3 g/100 g sample to 85.1±0.5 g/100 g sample due to the water added to the minced seaweed prior to the micro cutting process. The aim of the primary separation step (decanter or screw press) is to retrieve as much liquid as possible from the seaweed biomass. Therefore, the water content remaining in the press cake or decanter sediment is a good indicator for the efficiency of the separation step. A significant decrease in water content was observed in the decanter sediment (77.4 ± 0.6 g/100 g sample) and the screw press cake (80.0 ± 2.9 g/ 100 g sample) when compared to the micro cut biomass, indicating that both separation steps were effective in removing water from the biomass. Although the water content of the decanter sediment and press cake did not differentiate significantly, mass balances showed that the amount of liquid retrieved from the two processes were 43.3 kg from the decanter versus 36.3 kg from the screw-press (Figure 1 and Supplementary materials 1 and 2). This indicates that the decanter is a more effective liquid separation technique than the screw-press for this process. However, that leaves unanswered which method is more effective for retrieving valuable ingredients with potential functional or bioactive properties, such as carbohydrates. Furthermore, when material losses during processing were estimated by mass balance calculations, higher material loss was observed when the decanter (7.6%) was used as the primary separation step compared to the screw press (3.3%). This material loss is mainly due to the raw material stuck inside the processing equipment after processing. If these separation methods were used in an industrial setting with continuous flow the same ratios of raw material losses would be

expected between the two methods during daily processing or 3.3 versus 7.6 kg approximately. These differences are hence not expected to have an impact on the final sediment production and applications.

Regarding other macro nutrients, similar trends were observed during the processing, where salt, protein, salt-free ash content, and carbohydrate content were significantly lower in the micro cut seaweed compared to the fresh biomass due to added water to the micro cut biomass. The carbohydrate content was significantly lower in the decanter sediment and press cake compared to the minced biomass. Furthermore, after the primary separation the carbohydrate content increased in the press cake (14.2 ± 2.3 g/100 g sample) and decanter sediment (16.5 ± 0.5 g/100 g sample) compared to the micro cut seaweed, in agreement with the liquid removal during these processing steps.

The decanter liquids and press liquids were then centrifuged to assess whether valuable compounds could be better purified from these liquid streams through centrifugation. Approximately half of the press liquid stream (18 kg out of 36.6 kg), and only 30% (12.9 kg of the 43.3 kg) of the decanter liquid stream was lost (ended up as precipitate) after centrifugation. However, little difference was seen in the composition of the liquid and precipitate after the centrifugations, indicating that the step was unnecessary for the isolation of potentially valuable components.

Table 6 Nutritional composition of seaweed samples. Values mean ± standard deviation of three sample replicates (n=3). Results are expressed as g/100g sample wet weight (ww).

	Sample	Water	Lipid	Protein	Salt	Salt free ash	Carbohydrates
Raw materials							
	Minced seaweed	70.1 ± 0.3^{e}	<0.1	1.5 ± 0.4^{a}	1.4 ± 0.0^{a}	5.0 ± 0.2^{a}	22.1 ± 0.3^{a}
	Micro cut seaweed	85.1 ± 0.5^{b}	<0.1	0.6 ± 0.0^{c,d}	0.8 ± 0.0^{b,c}	2.3 ± 0.1^{c}	11.1 ± 0.6^{c}
After primary separation							
Decanter	**Liquid**	95.7 ± 0.3^{a}	<0.1	0.2 ±0.0^{d,e}	0.8 ± 0.1^{b}	0.4 ± 0.1^{d}	2.9 ± 0.2^{d}
	Sediment	77.4 ± 0.6^{d}	0.3 ± 0.1	1.1 ± 0.2^{c,d}	0.6 ± 0.0^{d,e}	4.1 ± 0.1^{b}	16.5 ± 0.5^{b}
	Centrifuged liquid	96.7 ± 0.2^{a}	<0.1	0.1 ± 0.0^{e}	0.7 ± 0.1^{b}	0.3 ± 0.0^{d}	1.6 ± 0.1^{d}
	Centrifuged precipitate	97.6 ± 0.2^{a}	<0.1	0.2 ± 0.1^{d,e}	0.5 ± 0.1^{c,d}	0.2 ± 0.1^{d}	1.1 ± 0.2^{d}
Screw press	**Press liquid**	94.8 ± 0.2^{a}	0.1 ± 0.0	0.2 ± 0.1^{d,e}	0.9 ± 0.0^{b}	0.7 ± 0.2^{d}	3.3 ± 0.2^{d}
	Press cake	80.0 ± 2.9^{c,d}	0.2 ± 0.0	0.9 ± 0.2^{b,c}	0.6 ± 0.0^{d}	4.0 ± 0.8^{b}	14.2 ± 2.3^{b}
	Centrifuged press liquid	96.9 ± 0.1^{a}	<0.1	0.1 ± 0.0^{e}	Na	Na	2.2 ± 0.1^{d}
	Centrifuged precipitate	95.5 ± 2.2^{a}	<0.1	0.5 ± 0.2^{c,d,e}	Na	Na	2.3 ± 2.0^{d}
After drying of decanter liquids							
Freeze-dried	**Liquid**	3.8 ± 0.2	0.9 ± 0.4	4.0 ± 0.3	18.2 ± 1.1	10.8 ± 0.4^{x}	62.1 ± 1.1
Spray-dried	**Liquid**	4.2 ± 2.0	0.4 ± 0.1	4.1±0.4	17.2±1.0	9.4 ± 0.4^{y}	64.6 ± 1.9
Main product composition							
	Commercial seaweed meal	7.5 ± 0.7	1.1 ± 0.2	4.9 ± 0.5	5.5 ± 0.2	20.7 ± 2.0	60.4 ± 1.9

Different subscript letters (a-e) indicate significant difference ($p<0.05$) between sample type in alternative processing line. Different subscript letters (x-y) indicate significant difference ($p<0.05$) between drying methods (freeze drying vs spray drying) of press liquids.

No significant difference was seen in the proximate composition of the liquid (press vs. decanter liquid) and solid (press cake vs. decanter sediment) streams, respectively, after the primary separation. Furthermore, no difference was seen between the composition of the centrifuged streams between the two primary separation methods. Hence, the results indicate that the separation method did not affect the chemical composition of neither the solid nor liquid phases obtained from each method. However, the results from the mass balance (Supplementary material 1 and 2) show that larger quantities of liquid were

retrieved with the decanter compared to the screw press, indicating that the choice of method affects how well the method separates the liquid and solid phase apart.

3.2 Trace minerals

As mentioned above, based on the mass balance (Supplementary material 1-4) calculation and basic chemical composition (Table 1), the decanter separation process was chosen for further chemical analysis, including for evaluation of trace minerals (Table 2). Iodine (I), arsenic (As), cadmium (Cd), lead (Pb), and mercury (Hg) are minerals that have been of concern in seaweed, but even though they are considered hazardous, legislations for processing, utilization, and uses of the biomass as an food source for human consumption is lacking from the authorities for European seaweed producers and/or consumers (Hurtado et al., 2022). There are, however, some legislations regarding the above-mentioned trace minerals when used for feed applications (Table 2) and as a food supplement.

Table 7 Mineral composition of seaweed processing streams. Values mean ± standard deviation of three sample replicates (n=3). Results are expressed as mg/kg of samples weight within the production.

		Iodine	Mercury	Lead	Arsenic	Cadmium
Allowed limits						
	Feed*		<10.0	<0.1	<40.0	<1.0
	Food supplement**	<600	<0.1	<3.0		
Raw materials						
	Minced seaweed	215.5 ± 12.7[a]	<0.03	<0.03	7.26 ± 0.41a	0.11 ± 0.09
After primary separation with decanter						
Decanter	**Liquid**	56.7 ± 28.9[c]	<0.03	<0.03	2.62± 0.24[c]	0.08 ± 0.11
	Sediment	168.2 ± 20.0[b]	0.04±0.01	<0.03	4.11 ± 0.04[b]	0.16 ± 0.01
	Centrifuged liquid	33.3±2.9[c]	<0.03	<0.03	2.25±0.61[c,d]	0.07±0.10
	Centrifuged precipitate	25.0±0.0[c]	<0.03	<0.03	1.57±0.27[d]	0.01±0.01
After drying of decanter liquids						
Freeze dried	**Liquid**	1233±230	0.25±0.27[x]	0.08±0.04	62.76±0.22	0.36±0.09
Spray dried	**Liquid**	1167±58	<0.03[y]	0.07±0.00	65.4±2.2	0.39±0.04
Main product composition						
	Commercial seaweed meal	425±35.4	<0.03	0.17±0.18	29.15±23.22	1.14±0.20

*Limits for feed applications for seaweed meal with water content 12% or less (European Commission, 2002)

** European Commission (2006), Directive (2002)

Different subscript letters (a-e) indicate significant difference ($p<0.05$) between sample type in alternative processing line.

Generally, brown seaweed contains high mineral content where the iodine content can contribute up to 1.2% of the total dry weight of some species. The content varies between species, and *A. nodosum* is known to have a relatively high iodine content (Holdt & Kraan, 2011b). The iodine content of the minced seaweed biomass was 215.5 ± 12.7 µg/g on a wet basis. During processing of the biomass with the decanter separation, approximately 25% of the iodine content was washed out of the solid biomass with

the liquid phase or was lost during processing, while around 70-75% of the iodine remained in the sediment after processing (supplementary material 4). After drying of the liquids the iodine content of the freeze-dried and spray-dried liquids reached over 1000 µg/g, due to the low dry matter content of the liquid, which might affect the application possibilities of the dried products for human consumption.

The mercury and lead content of the minced seaweed biomass was low, and in all cases under the set limits for usage as feed, or less than 0.03 mg/kg (ww), and therefore remained low throughout the decanter process. However, the arsenic and cadmium contents were higher, but the arsenic content of the initial biomass was 7.26 ± 0.41 and cadmium 0.11 ± 0.09 mg/kg ww. The arsenic content significantly differentiated throughout the decanter process, and the decanter sediment contained significantly lower arsenic content, or 4.11 ± 0.04 mg/kg ww, when compared to the initial biomass (7.26 ± 0.41 mg/kg ww). This indicates that some of the arsenic was released from the biomass during primary separation within the decanter where the liquid contained 2.62± 0.24 mg arsenic/kg ww. During secondary separation step, the majority of the arsenic content remained in the centrifuged decanter liquid (2.25±0.61 mg/kg ww) but some content was also analyzed in the centrifuged decanter precipitate (1.57±0.27 mg/kg ww). The results hence indicate that the centrifugation method was not efficient in reducing the arsenic content in the liquid phase. In the dried seaweed liquids, the arsenic content was 62.76±0.22 mg/kg for the freeze dried and 65.4±2.2 mg/kg for the spray dried samples and were hence higher than the limits set for animal feeds (40 mg/kg for meal with water content 12% or less) in both cases. This confirms prior results that showed that the arsenic is only partially extracted from the biomass during the liquid processing (Hrólfsdóttir et al., 2022). However, it is expected that the liquid could be used either as is, in concentrated or dried form, or as an ingredient in value added products. If the liquid is concentrated or dried, it is important that the arsenic and cadmium content remains under those set threshold values for feed and food supplement applications (Table 2). Therefore, it would be necessary to keep in mind the allowed limits of trace minerals for the intended product application each time during process optimization.

3.3 Monosaccharide and uronic acid composition

The main polysaccharides reported to be present in brown algae and in *A. nodosum* are alginate, laminarian, fucoidan, and cellulose, as well as the sugar alcohol mannitol (Øverland et al., 2019). Therefore, the monosaccharides quantified in the research were mannitol, fucose, glucose, xylose, and galactose as well as uronic acid. The sugar composition was determined for the processing streams within the decanter processing to assess the effect of applying the suggested extraction pre-processing step on the intended final product (commercial seaweed meal) as a material for alginate production, and to determine possible applications for the produced liquid extracts. Results from the monosaccharide and uronic acid composition analysis are represented in Table 3.

Table 8 Monosaccharide and uronic acid composition of seaweed and its processing streams during alternative processing with a decanter as the primary liquid separation step. Values mean ± standard deviation of three sample replicates (n=3). The results are expressed as g/100 g dry wight (dw).

		Mannitol	Fucose	Glucose	Xylose	GulA	ManA	Galactose	Alginate	Measured	Alginate	M/G
		g/100 g dw	g/100 g dw	g/100 g dw	g/100 g dw	g/100 g dw	g/100 g dw	g/100 g dw	G+M g/100 g dw	Total carbs g/100 g dw	% of total	ratio
Raw materials												
	Minced seaweed	5.5±0,3^{c}	6.4±0.1^{a,b}	6.0±0.2^{b,c}	1.9±0.02^{a}	4.9±0.2^{a}	10.6±0.6^{a}	0.6±0.0^{a}	15.5±0.8^{a}	36.0±1.3^{a}	43.0±0.9^{b}	2.2±0.0^{a}
After primary separation with decanter												
Decanter	**Liquid**	15.0±3.2^{a,b}	0.6±0.1^{c}	8.0±1.3^{a,b}	0.2±0.03^{b}	0.43±0.0^{c}	0.7±0.1^{c}	0.2±0.0^{b}	1.1±0.1^{c}	25.3±4.2^{b}	4.6±1.2^{d}	1.5±0.3^{b}
	Sediment	2.8±0.6^{c}	7.4±1.2^{a}	5.4±0.9^{c}	2.4±0.5^{a}	5.4±0.9^{a}	12.0±2.1^{a}	0.6±0.1^{a}	17.4±3.1^{a}	36.1±6.1^{a}	48.3±1.9^{a}	2.2±0.1^{a}
	Centrifugation liquid	17.3±0.1^{a}	0.6±0.1^{c}	9.2±0.9^{a}	0.2±0.0^{b}	0.4±0.1^{c}	0.6±0.1^{c}	0.2±0.0^{b}	1.0±0.2^{c}	28.7±1.7^{a,b}	3.6±0.5^{d}	1.4±0.1^{b}
	Centrifuged precipitate	12.9±1.4^{b}	0.6±0.1^{c}	7.2±1.0^{a,b,c}	0.3±0.0^{b}	0.5±0.0^{c}	0.4±0.1^{c}	0.3±0.0^{b}	0.9±0.1^{c}	22.2±2.5^{b}	4.1±0.3^{d}	0.9±0.1^{c}
After drying of decanter liquids												
Freeze drying	**Liquid**	15.0±3.2^{a,b}	0.6±0.1^{c}	8.0±1.3^{a,b}	0.2±0.03^{b}	0.43±0.0^{c}	0.7±0.1^{c}	0.2±0.0^{b}	1.1±0.1^{c}	25.3±4.2^{b}	4.6±1.2^{d}	1.5±0.3^{b}
Spray drying	**Liquid**	18.8±1.3^{a}	0.5±0.0^{c}	9.3±0.4^{a}	0.3±0.1^{b}	0.4±0.2^{c}	0.7±0.1^{c}	0.2±0.0^{b}	1.1±0.3^{c}	30.1±2.0^{a,b}	4.0±0.0^{d}	1.9±0.6^{a,b}
Main product composition												
	Commercial seaweed meal	6.3±0.7^{c}	5.6±0.3^{b}	6.2±0.8^{b,c}	1.9±0.1^{a}	3.1±0.2^{b}	6.9±0.9^{b}	0.7±0.1^{a}	10.0±1.1^{b}	30.6±2.0^{a,b}	32.6±2.0^{c}	2.2±0.1

Different subscript letters (a-d) indicate significant difference ($p<0.05$) between sample type.

Abbreviations: GulA + G Guluronic acid: ManA + M: Mannuronic acid

Based on the chemical composition of the decanter liquid, the main monosaccharides that are extracted from the seaweed biomass with the process are mannitol and glucose. The mannitol content of the decanter liquid was 15.0±3.2 g/100 g dw and glucose 8.0±1.3 g/100 g dw. Furthermore, no difference was observed in either the monosaccharide or uronic acid composition of the dried liquids, indicating that the short heating during spray drying did not have a significant effect on the monosaccharide composition of the decanter liquid.

Brown seaweeds have been used as a substrate for production of the hydrocolloid alginate since 1927, where it was first used to seal cans, but applications for it have extended since to multiple purposes, including as a food thickener, textile printing, and pharmaceutical industries (McHugh, 1987). For alginate producers, the alginate yield is understandably of uttermost importance. Therefore, the alginate content and the M/G (mannuronic acid/guluronic acid) ratio within the decanter sediment need to remain the same or increase for this alternative processing to be feasible.

The analysis of samples from the decanter processing showed neither any significant difference between the minced seaweed and the decanter sediment in total alginate content (15.5±0.8 and 17.4±3.1 g/100 g dw, respectively), nor in mannuronic acid/guluronic acid (M/G) ratio (2.2±0.0, and 2.2±0.1 g/100 g dw, respectively). However, during the decanter processing, the removal of other monosaccharides, such as mannitol and glucose, from the biomass affected the total amount of alginate per total carbohydrate content. A significant difference was observed in the alginate content after the decanter processing compared to the minced seaweed biomass, where the decanter sediment samples contained higher amounts or 48.3±1.9 g/100 g dw compared to minced seaweed samples with 43.0±0.9 g/100 g dw. These results confirm, like prior research on lab scale suggests (Hrólfsdóttir et al., 2022), that the alginate content in the sediment does not decrease with the liquid extraction process, and that the sediment can be used for production of seaweed meal. Furthermore, the alginate content in the decanter sediment was higher than in the commercial seaweed product. The decanter sediment

from this alternative process is therefore considered suitable for alginate production.

3.4 Total phenolic content and antioxidant properties of liquids

Phenolic compounds have been studied a lot in recent years, especially due to their bioactive potentials, including antioxidant properties (Obluchinskaya et al., 2022; Singh & Sidana, 2013; Wang et al., 2009). The main phenolic compounds present in brown seaweed are phlorotannin's, which are polymers of phloroglucinol. They are generally found in high quantities in Fucus species, including *A. nodosum* (Sabeena Farvin & Jacobsen, 2013; Wang et al., 2009). Therefore, to evaluate the potential usage of the liquid streams, the total phenolic content (TPC) and the antioxidant properties (oxygen radical absorbance capacity (ORAC) and DPPH radical scavenging activities (DPPH)) of the decanter and screw-press liquid streams, both unprocessed and centrifuged, were analyzed (Table 4).

Table 9 Total phenolic content (TPC), Oxygen radical absorbance capacity (ORAC) and DPPH radical scavenging activity (DPPH) of the liquid streams retrieved with different processing methods. TPC is expressed as g phloroglucinol equivalent (PGE)/100 g extract, ORAC is expressed as µmol Trolox equivalent (TE)/g extract and DPPH results are expressed as %inhibition by using a concentration of 0.5 mg/mL in the analysis. Results are expressed as mean and standard deviation of three samples (n=3) were analyzed from each liquid stream and each sample was measured in duplicate.

	Sample	**TPC**	**ORAC**	**DPPH**
Liquid extracts from decanter processing				
Freeze drying	**Decanter liquid**	18.0±0.7^{a}	1873±322^{a}	90.1±1.0 ^{a,b}
	Decanter centrifuged liquid	17.5±1.4^{a}	1776±288^{a,b}	91.6±0.5^{a,b}
	Decanter centrifuged precipitate	12.9±1.1^{b}	1572±123^{a,b}	87.5±1.0^{a,b}
Spray drying	**Decanter liquid**	18.2±0.2^{a}	2259±397^{a}	86.3±3,4^{a,b}
Liquid extracts from the screw press processing				
Freeze drying	**Press liquid**	16.5±1.4^{a}	1636±111^{a,b}	92.8±1.4^{a}
	Centrifuged press liquid	18.0±0.5^{a}	2033±28^{a}	91.5±0.7^{a,b}
	Centrifuged precipitate	9.6±2.3^{b}	1096±374^{b}	85.2±5.3^{b}

Different subscript letters (a-c) indicate significant difference ($p<0.05$) between sample type.

The TPC of the liquids retrieved from the two different separation methods did not significantly differ, where the obtained TPC was 18.0±0.7 and 16.5±1.4 g PGE/100 g extract in the decanter and screw-press liquids, respectively. The results hence indicate that both separation methods would be suitable to extract phenolic compounds from the biomass. However, a significant difference was observed when the fluids from both separation methods were centrifuged but the centrifuge precipitates contained significantly lower phenolic content compared to the centrifugation liquids. Furthermore, no significant difference was observed in TPC between the spray

dried and freeze-dried decanter liquids, resulting in TPC values of 18.2±0.2 and 18.0±0.7 g PGE/100 g extract, respectively.

The oxygen radical absorbance capacities (ORAC) assay was performed to assess the extracts potential as an antioxidant in peroxyl radical induced oxidation, where higher ORAC values indicate stronger antioxidant properties exhibited by the assessed compound (Borlinghaus et al., 2020; Ganske, 2006). ORAC values ranged from 1096±374 up to 2259±397 µmol of TE/g, where the highest activity was recorded in the spray dried decanter liquid and lowest in the screw press centrifugation precipitate. No significant difference was observed in ORAC values when the decanter liquid (1873±322 µmol TE/g) and screw press (1636±111 µmol TE/g extract) liquid were compared, indicating that the primary separation method does not affect the oxygen radical absorbance capacities of the liquids. Furthermore, in the screw-press processing, higher activities were obtained in the centrifugation liquid compared to the centrifugation precipitate. ORAC values of the screw press liquids hence align with the TPC content of the screw press liquid obtained from the primary and secondary separations, both lower TPC and ORAC activity were observed in the centrifugation precipitate compared to the centrifuged liquid. However, no significant difference was observed between neither the liquids of the decanter processing nor drying methods.

DPPH (2,2-Diphenyl-1-picrylhydrazy) radical scavenging activity is a widely used method to assess radical scavenging properties of compounds that exhibit antioxidant properties. The DPPH activity was relatively high, showing more than 80% inhibition of radicals in all samples at 0.5 mg/ml concentrations, when compared to extracts from other brown seaweed species growing in Icelandic waters, such as *Alaria esculenta, Laminaria digitata* and *Saccharina latissima* (Sabeena Farvin & Jacobsen, 2013; Wang et al., 2009). However, Wang, 2009 (Wang, 2009) showed that to achieve inhibition of 80% or higher using commercial antioxidants, such as Butylated hydroxytoluene (BHT) and ascorbic acid, concentrations of only 5-10 µl/ml were needed. The results hence show that the extracts are not potent as radical scavengers or as antioxidants used in the food industry and could hence be more suitable for

other applications e.g. as bio-stimulants or ingredients in cosmetic products. A similar pattern as obtained in the ORAC values was observed in the DPPH radical scavenging activities, where the lowest activity was observed in the screw press centrifugation precipitate (85.2±5.3%) compared to other samples. The lower activity of the screw press centrifugation precipitate, assessed by both ORAC and DPPH could be connected to a lower TPC, since the quantities of TPC of seaweed extracts has often been connected directly to antioxidant activities (Sabeena Farvin & Jacobsen, 2013; Wang et al., 2009). Overall, the liquids obtained from both separation processes exhibited similar total phenolic contents and antioxidant properties (assessed by DPPH and ORAC), indicating that both separation methods are equally viable for when retaining the antioxidant properties of the obtained products.

4 Conclusion

The results from chemical analysis and mass balance calculations indicate that the alternative processing where a decanter was used gives better separation of the liquid from the material stream compared to using a screw-press. The water content, as well as other proximate compounds were, however, not significantly different in the obtained press cake and decanter sediment. Furthermore, both proximate composition and antioxidant properties of liquids obtained from the two separation processes (decanter and screw press) were similar, indicating that the separation method itself did not affect the composition of the liquids. However, to obtain higher yields of the liquids, the decanter was considered more efficient, and is hence the method recommended. Furthermore, since the results show that the alginate content remains stable in the sediment post-decanter separation, an alternative process using a decanter can be suggested. Additionally, the results from the current study indicate that using disc centrifugation to purify valuable compounds within the extracts or to decrease quantities of heavy metals or other unwanted compounds was inefficient. Today, Thorverk harvests up to

15.000 tons of *A. nodosum* annually. As mentioned, the decanter was evaluated to be the most suitable separation method, whereby during processing 100 kg of micro cut biomass with the decanter produced 43.3 kg of liquid side-stream. That means that a total of 12.990 tons could be produced of liquid, or approximately 420-570 tons of dried liquid extract, dependent on yield of the spray dried and freeze-dried extract, respectively. However, drying conditions for the seaweed alginate meal would have to be optimized prior to commercialization due to the increase in water use and biomass water content during processing compared to processing of fresh *A. nodosum*. Additionally, the results indicated that drying of the decanter liquid could be performed with a spray dryer without effecting the chemical and antioxidant properties of the liquid, indicating further processing of targeted extracts from the seaweed are possible.

For future applications, further processing might be necessary to decrease quantities of heavy metals, salts, iodine, or other unwanted compounds and increase yield of antioxidant compounds in the liquid, which might limit its potential applications. Pressure-driven membrane filtration methods, such as reverse osmosis, nanofiltration, ultrafiltration and microfiltration methods have been used to target specific bioactive compounds in seaweed extracts. These purification methods might thus be more appropriate treatments than the centrifugation tested here, although dependent on the intended application. Testing of the effectiveness of further purification is, however, left for further studies.

5 Declarations

Author contribution:

Anna Þóra Hrólfsdóttir.; Conceptualization, methodology, software, validation, formal analysis, investigation, data curation, writing original draft preparation, writing - review and editing and visualization,

Hildur Inga Sveinsdóttir.; Conceptualization, methodology, validation, writing - review and editing, visualization, supervision, project administration, and funding acquisition.

Sigurjón Arason.; Conceptualization, methodology, validation, writing - review and editing, visualization, supervision, project administration, and funding acquisition.

María Gudjónsdóttir; Conceptualization, methodology, validation, writing—review and editing, visualization, supervision, project administration, and funding acquisition.

All authors have read and agreed to the published version of the manuscript.

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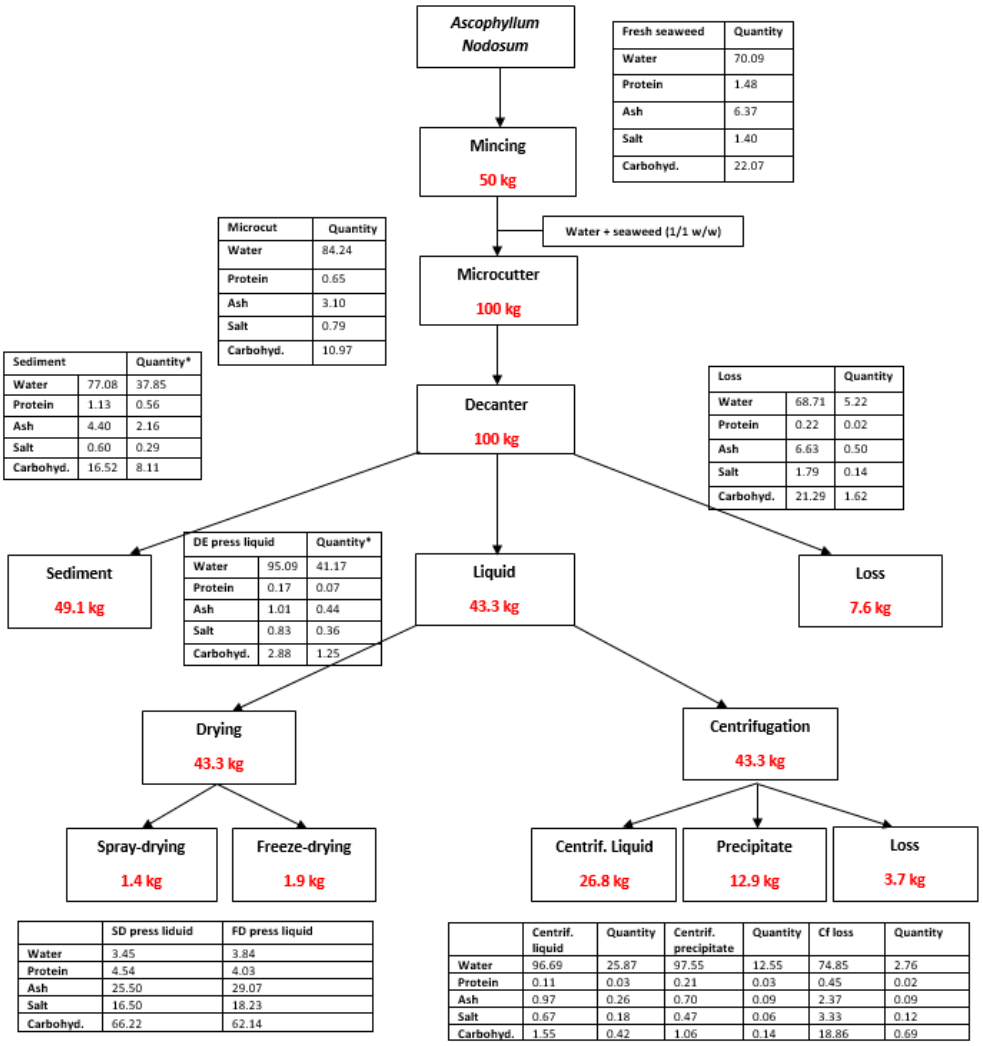
7 Supplementary materials



Fresh seaweed	Quantity
Water	70.09
Protein	1.48
Ash	6.37
Salt	1.40
Carbohyd.	22.07

Microcut	Quantity
Water	84.24
Protein	0.65
Ash	3.10
Salt	0.79
Carbohyd.	10.97

Sediment		Quantity*
Water	77.08	37.85
Protein	1.13	0.56
Ash	4.40	2.16
Salt	0.60	0.29
Carbohyd.	16.52	8.11

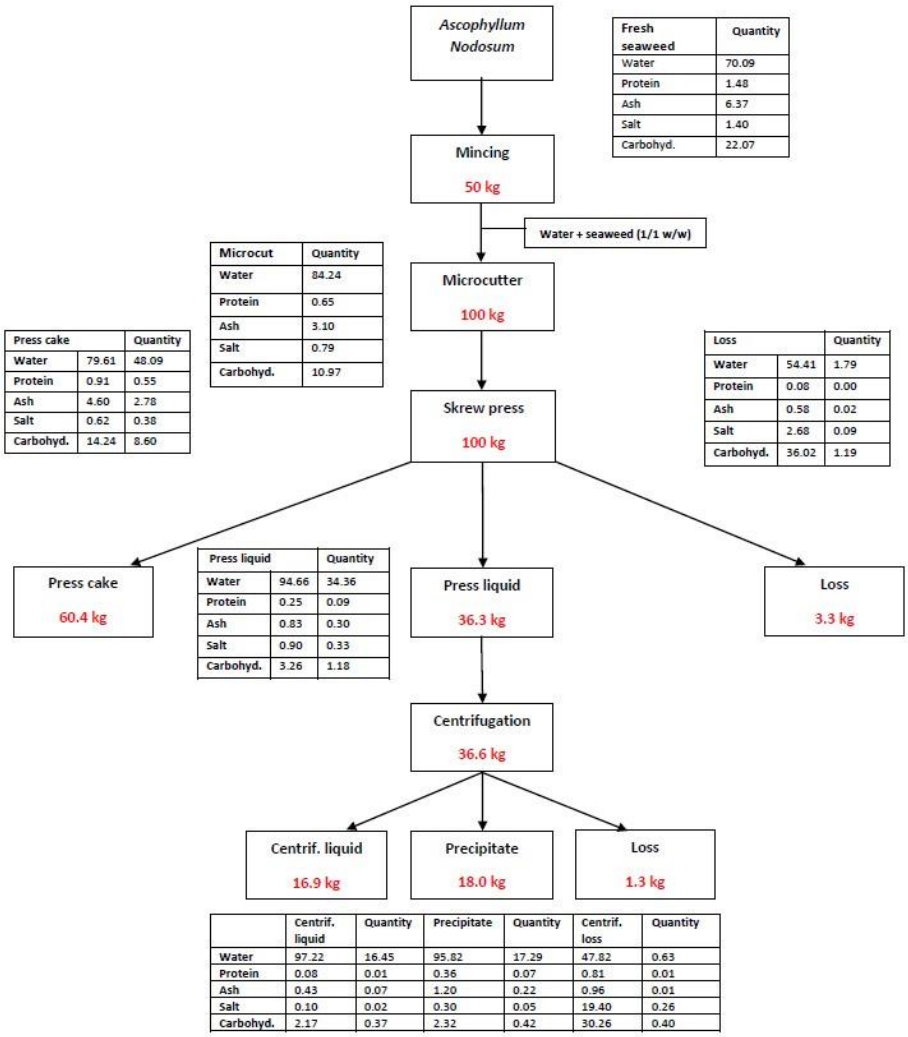
Loss		Quantity
Water	68.71	5.22
Protein	0.22	0.02
Ash	6.63	0.50
Salt	1.79	0.14
Carbohyd.	21.29	1.62

DE press liquid		Quantity*
Water	95.09	41.17
Protein	0.17	0.07
Ash	1.01	0.44
Salt	0.83	0.36
Carbohyd.	2.88	1.25

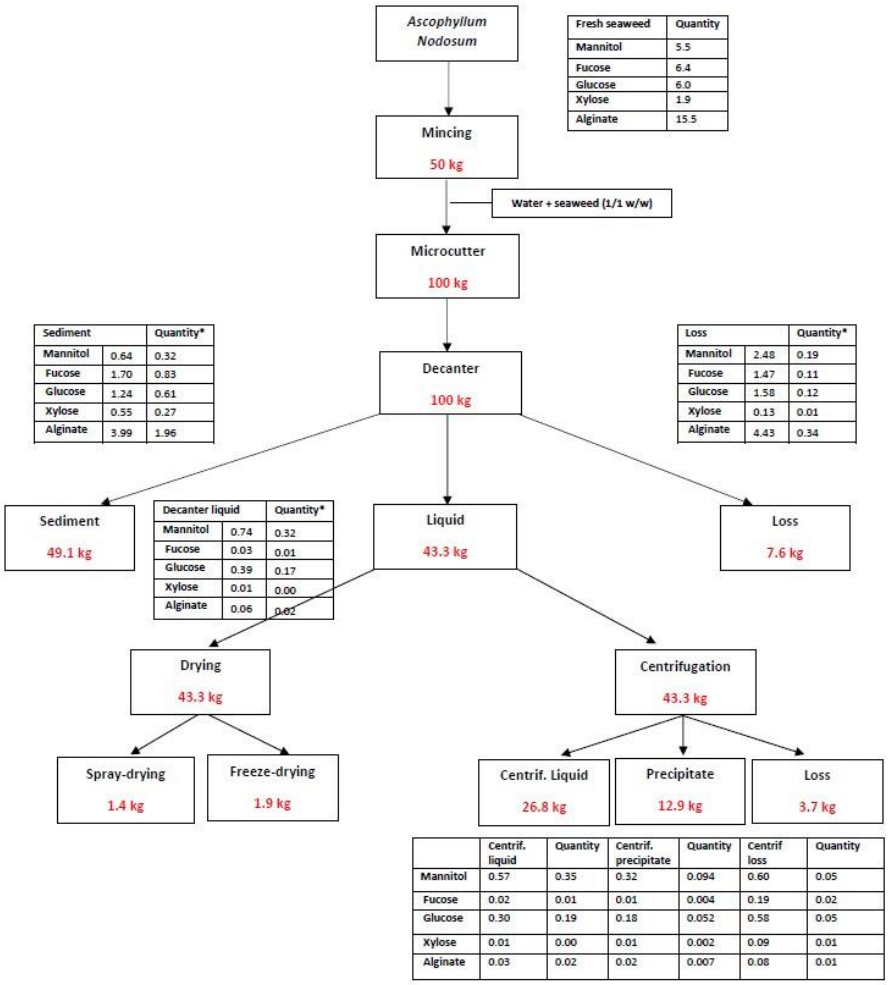
	SD press liduid	FD press liquid
Water	3.45	3.84
Protein	4.54	4.03
Ash	25.50	29.07
Salt	16.50	18.23
Carbohyd.	66.22	62.14

	Centrif. liquid	Quantity	Centrif. precipitate	Quantity	Cf loss	Quantity
Water	96.69	25.87	97.55	12.55	74.85	2.76
Protein	0.11	0.03	0.21	0.03	0.45	0.02
Ash	0.97	0.26	0.70	0.09	2.37	0.09
Salt	0.67	0.18	0.47	0.06	3.33	0.12
Carbohyd.	1.55	0.42	1.06	0.14	18.86	0.69

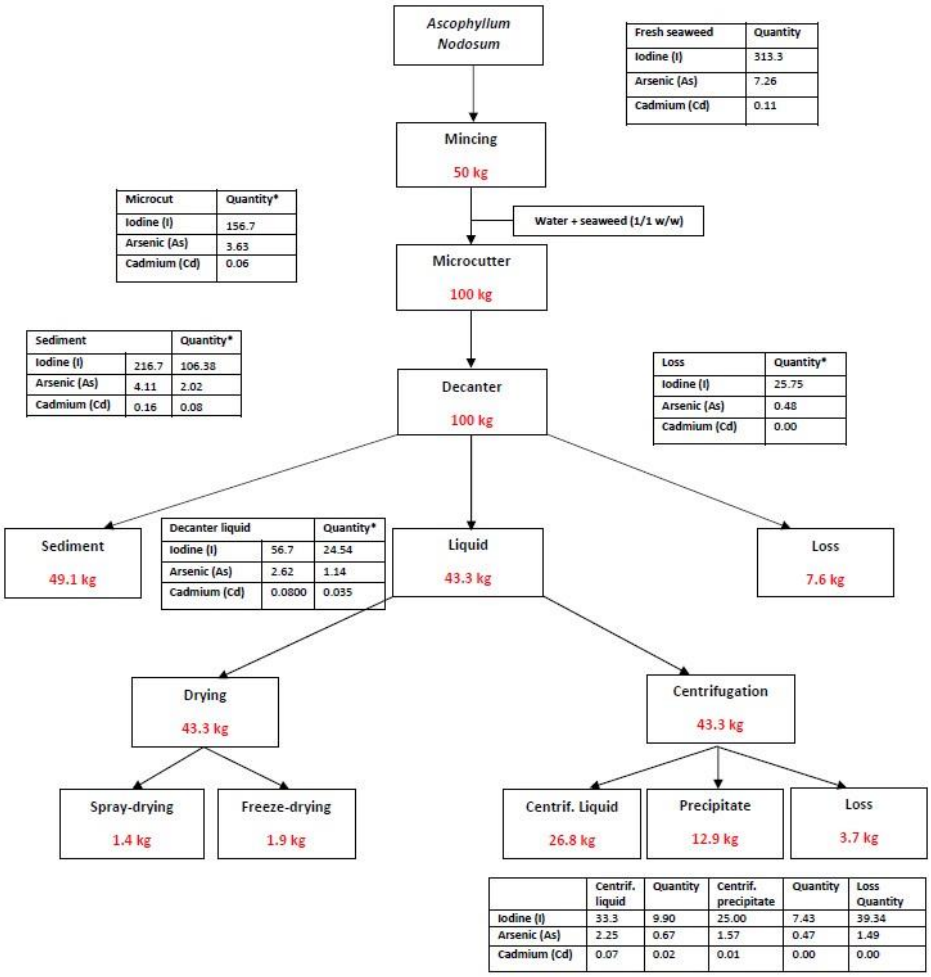
Supplementary material 1. Results of mass balance calculations of the proximate composition of streams during processing with the decanter as primary separation step. Results are calculated with 50 kg of fresh seaweed biomass (assuming no loss in the micro cutting process) for each scenario to give a 100 kg basis for assessment of the primary separation steps. The calculations are performed based on the chemical analysis and the weight of the biomass entering and leaving each processing step. The results of the proximate composition are expressed as g/100 g sample.



Supplementary material 2. Results of the mass balance calculations of the proximate composition during processing with the screw press as the primary separation step. Results are calculated with 50 kg of fresh seaweed biomass (assuming no loss in the micro cutting process) entering the process for each scenario to give a 100 kg basis for assessment of the primary separation steps. The calculations are performed based on the chemical analysis and the weight of the biomass entering and leaving each processing step. The results of the proximate composition are expressed as g/100 g sample.



Supplementary material 3. Results of the mass balance calculations of the monosaccharide composition and alginate content during processing with the decanter as primary separation step. Results are calculated with 50 kg of fresh seaweed biomass (assuming no loss in the micro cutting process) entering the process for each scenario to give a 100 kg basis for assessment of the primary separation steps. The calculations are performed based on the chemical analysis and the weight of the biomass entering and leaving each processing step. The results are expressed as g/100 g dw sample.



Supplementary material 4. Results of the mass balance calculations of the trace mineral content during processing with the decanter as the primary separation step. Results are calculated with 50 kg of fresh seaweed biomass (assuming no loss in the micro cutting process) entering the process for each scenario to give a 100 kg basis for assessment of the primary separation steps. The calculations are performed based on the chemical analysis and the weight of the biomass entering and leaving each processing step. The results of the trace mineral content are expressed as g/100 g sample.

Paper III

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Physicochemical- and bioactive properties of acid preserved *Alaria esculenta* and *Saccharina latissima* during storage

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Sensorial properties

ABSTRACT

The short harvesting period of cultivated brown seaweed in Europe can make it difficult for cultivators to produce high quality seaweed biomass all year around. Hence there is a need for novel processing and preservation methods. Acid preservation is a well-known method to preserve food, where the aim is to reduce the pH below 4.5 to inhibit microbial growth. To evaluate the effectiveness of acid preservation, a shelf-life experiment was conducted with *Saccharina latissima* and *Alaria esculenta*. The biomass was either treated with lactic- or citric acid and stored for approximately seven months. Physicochemical (including proximate composition, trace minerals, total phenolic content (TPC), texture and pH), microbial-, sensory attributes, and antioxidant (ORAC, DPPH) analyses were performed on the preserved biomass during storage. The proximate composition, color, pH, and texture of the acid-preserved seaweed were relatively stable throughout the storage. However, a decrease was observed in TPC and antioxidant properties (assessed by DPPH) with the acid treatments. Acid preservation is, thus, a good method to stabilize the studied biomass for food and feed applications but less applicable if intended for antioxidant purposes. However, the acid treated biomass might be suitable as an ingredient for a wide range of value-added products.

1. Introduction

During the last decades, aquaculture has become the fastest-growing food processing technology sector (Garlock et al., 2020). The total aquaculture production in 2018 was 114.5 million tons, whereof seaweed contributed 32.4 million tons, or 28.3% of the total produced biomass. Approximately 97% of the seaweed production originates from cultivation, which is mainly practiced in Asia (FAO, 2020). However, seaweed cultivation has recently gained more interest in Europe where according to the report "Hidden champion of the ocean" the seaweed sector in Europe could grow from the 300.000 tons annual wet weight produced today up to 8 million tons in the future (Vincent et al., 2020). However, before starting scaling up production it is crucial to solve some of the main bottlenecks within the sector to ensure high quality of the harvested biomass.

One of the main bottlenecks in the seaweed cultivation sector in Europe lies in the preservation of seaweed biomass. When brown seaweed species, such as *Alaria esculenta* and *Saccharina latissima*, are cultivated, sporelings are deployed on ropes into the ocean in October to November (depending on locations) and allowed to grow over approximately 5–6 months. The seaweed is then harvested in late April throughout May, before biofouling of the biomass which starts in June–July. The short harvesting period makes it difficult for seaweed cultivators to provide their customers with high quality seaweed biomass all year around and makes them reliant on using various preservation methods to stabilize their products. The main methods used to preserve the biomass are drying, freezing, salting, and fermentation (Choi et al., 2012; Singh & Heldman, 2001; Stévant, et al., 2018a). Freezing of the biomass can increase the shelf-life of the seaweed for long periods, but the method is, however, both energy and infrastructure demanding and can lead to substantial nutritional loss and quality deterioration due to drip loss (Blikra et al., 2021). Dehydration or drying

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of seaweed biomass in northern countries is expensive due to its high energy consumption and infrastructure demands. Furthermore, the drying can effect nutritional content of the biomass (Singh & Heldman, 2001; Stévant et al., 2017b). With rising energy prices in Europe, seaweed cultivators in Europe cannot rely on drying and freezing processes for biomass preservation alone. Therefore, it is important to explore other alternatives to preserve seaweed biomass.

Acidification is a less energy-demanding preservation method than freezing or drying, and does not require expensive equipment (Blikra et al., 2021; Sandbakken et al., 2018). The acidification can be obtained by two different processes, by addition of acids, or fermentation. The addition of acids to foods, also called artificial acidification, is a widely employed method aimed at improving the safety, stability, and shelf-life of food products. This technique involves the use of acidifying agents, commonly organic acids like lactic, citric, malic, and acetic acids, or their salts (Dauthy, 1995; Theron & Lues, 2010). These acids serve various purposes within the food industry, and may function as antioxidants, flavor enhancers, acidulants, and pH adjusters. One of their key roles is to lower the pH of the food product below pH 4.5 to inhibit the growth of pathogenic bacteria, such as *Clostridium botulinum*, *Escherichia coli*, *Salmonella*, and *Listeria monocytogenes*, that are known to cause foodborne illnesses (Adams, Moss, & Moss, 2000; CFR, 1979; Dauthy, 1995; Theron and Lues, 2007). Acid preservation does not only inhibit growth of pathogenic bacteria but is also known to affect the sensory attributes of foods, including the texture, odor, and taste, depending on both type of acid used and their concentrations (Dauthy, 1995).

Currently, the knowledge and literature on the effects of acid preservation of seaweed intended for human consumption and its effect on storage shelf-life is limited. Some studies have described acid preservation of seaweed biomass intended for biofuel purposes (Sandbakken et al., 2018), feed applications (Novoa-Garrido et al., 2020), quality of alginate and cellulose post acid treatment (Nøkling-Eide et al., 2023), and protein quality during short storage periods (Standal et al., 2023). Due to the gap in knowledge and the need to explore alternative methods for preservation of cultivated seaweed biomass, the objective of the current study was to evaluate the efficiency and stability of acid preserved seaweeds intended for human consumption, with focus on two of the most cultivated brown seaweeds in Europe, *Alaria esculenta* and *Saccharina latissima*.

2. Material and methods

2.1. Seaweed sampling, preparation, and acidification

Saccharina latissima and *Alaria esculenta* were cultivated and harvested by Seaweed Solutions AS (SES) in Norway. The seaweed spores were produced in Trondheim, Norway, where seedlings of *S. latissima* were deployed in the ocean in November 2021, and seedlings of *A. esculenta* were deployed in January 2022. The ropes were approximately 1–4 m below the surface at the SES farming sites in Frøya, Måsskjæra (N63'44, E8'53 N) in Norway, where the seaweed was allowed to grow over the winter and spring. Both species were harvested on the May 30, 2022. Following harvesting, the seaweed was landed at Hitra, Norway, and transported to the laboratory facilities of SINTEF in Trondheim, Norway, where the biomass was stored in a cold room (0–4 °C) until the following day. Each species was minced separately, and then either acid preserved or frozen (control). Due to the fast deterioration and long shipment of samples across countries for analysis, frozen seaweed biomass was chosen instead of fresh as a control to evaluate the physicochemical and sensory changes obtained in the acid treated biomass during acidification. Freezing is a widely employed and efficient preservation method to prevent deterioration while having minimal impact on the chemical composition of food products (Singh & Heldman, 2001). This especially applies if the freezing is fast, and the samples are kept under stable frozen conditions. The frozen samples were thus stored at −25 to −30 °C till they were analyzed (after approximately two months). The acid treatment was performed with either lactic or citric acid. Lactic acid was added as a 3 M solution, while citric acid was added dry. The acids were added until the pH reached approximately 3.7. The final lactic acid concentration in the samples were 106 mmol/kg for *A. esculenta* and 60 mmol/kg for *S. latissima*. The corresponding concentrations of citric acid were 28 and 17 mmol/kg. After adding the acids, the seaweed samples were divided into several 1 kg plastic bags, which were vacuum packed and stored at room temperature until used. The seaweed samples were transported to Matis, Iceland where physicochemical, bioactive, and sensorial properties of the samples were analyzed to assess the quality and storage stability of the biomass.

Sampling took place on three occasions during the 32-week storage time where the first sampling occurred 4 weeks (W4) post acid treatment, and the other two samplings were on week 16 (W16), and week 32 (W32) post acid treatment. One bag (1 kg) from each treatment was divided into three samples replicates (n = 3) per sampling point. At least 70 gr of each sample replicate was freeze-dried in a Genesis 25 SQ EL freeze dryer (SP industry, Philadelphia United States of America), and the rest of the biomass was used for chemical, microbial, and physiological analysis.

2.2. Proximate composition of seaweed samples

The water content of the seaweed samples was analyzed according to the ISO 6496:1999 method, where around 5 g of each sample were dried in an oven at 102°C–104 °C for at least 4 h. The weight of the evaporated water from the sample was used to assess the water content of sample. The protein content was determined with the Kjeldahl method as described by ISO 5983-1:2005 (2005) and ISO 5983-2:2009 (2009), where a conversion factor of 5 was used to calculate crude protein content as recommended for seaweed samples due to their high non-protein-nitrogen content (Angell et al., 2016). To determine the total lipid content of the seaweed samples, the Soxhlet extraction method was used according to the OCS Official Method Ba-3-38 (AOCS, 2009). Ash content was determined by weight loss of the sample after burning approximately 2 g of seaweed sample at 550 °C for 3 h (ISO 5984:2002). The Volhard titration method was used to determine the salt content (AOAC, 1990). The total carbohydrate (TC) content of seaweed samples was then calculated for each sample based on results from the chemical analysis of the abovementioned water, protein, lipid, and ash, measurements, using the following equation:

$$TC = 100 - (water + protein + ash + lipids)$$

2.3. Trace minerals

The iodine content was analyzed with inductively coupled plasma mass spectrometry (ICP-MS) with the method described by DIN EN 15111 (2007–06), with modifications according to CON-PV 01187 (2022–06). The contaminants lead, mercury, arsenic (inorganic and organic), and cadmium were analyzed in the control samples to assess the safety of the seaweed biomass for human consumption. The total arsenic content was analyzed with graphite furnace atomic absorption spectrometry (GF-AAS), performed according to ASU L 00.00–19/3 (2004–07), and modified for expansion of analytes according to the CON-PV 00508 (2020–01) method. Inorganic arsenic was analyzed according to the ASU L 25.06–1 (2008–12) method with hydride generation (HG-ASS) after acidic extraction with some modifications (CON-PV 01288 (2020–05). Cadmium, lead, and mercury were determined according to NMKL (2007), method 186 (NMKL, 2007b).

2.4. Physical parameters

The water activity of the samples was determined with an Aqua lab 4

T water activity meter (Decagon Devices, Pullman, WA, USA). About 2 gr of sample were put in a plastic cup, and placed in the water activity meter, and the water activity was read at a temperature between 24 °C and 25 °C. The pH value was analyzed by using a portable pH meter (Knick, Berlin, Germany). Color was analyzed by using Minolta Chroma Meter CR-300 (Minolta, Oska, Japan) with use of CIE Lab system. L-, a*- and b*-values are recorded by the instrument. L-values indicate lightness on the scale from 0 to 100 from black to white, respectively, a-values represent green color (−) to red (+), and the b-value blue (−) to yellow (+). Each sample (n = 3 per treatment) was measured in duplicate, or at two different points within each sample at each sampling point.

2.5. Total volatile basic nitrogen (TVB-N) and microbial analysis

TVB-N was determined during storage by steam distillation of a 7.5% trichloroacetic acid (TCA) extract (Billon & Tao, 1979). NaOH was added to the extract, and the blend was steam distilled with a Struers TVN steam distillatory and collected in a beaker glass containing 4% boric acid, along with methyl red and bromocresol green indicators. The solution was then titrated with 0.025 N H_2SO_4 to the equivalence point for steam distillation along with 10% NaOH.

Total aerobic microbial counts (TVC) were determined according to the NMKL (2013), 86 method, 5th ed., where the preparation of sample dilution series were performed according to general microbiological principles. Dilutions were pour plated on Petri dishes with unspecific agar medium, followed by incubation under aerobic conditions for 72 ± 6 h at 30.0 ± 1.0 °C. Lactic acid bacteria were determined according to NMKL 140, 2nd ed. 2007 method (NMKL, 2007a), where preparation of the dilution series was performed according to general microbiological principles, and dilutions spread with spiral plating on a surface of a De Man, Rogosa, and Sharpe (M.R.S.) agar. The plates were incubated in anaerobic jars for 72 h at 30 °C. For both methods, colonies were counted, and the number of viable microorganisms were calculated by multiplying the number of counted colonies by the appropriate dilution factor. The results are expressed as log of colony forming units/gram sample (log CFU/g) (n = 3 per each treatment) on each sampling occasion.

2.6. Sensory evaluation

Generic descriptive analysis (GDA) (Stone et al., 2020) was performed on the seaweed samples after 16 weeks storage by nine trained panelists, who were trained according to ISO. (1993) standard. The training sessions were two, where both acid-preserved and frozen-thawed samples of *A. esculenta* and *S. latissima* were used. The panel training involved the determination of appropriate descriptors for the seaweed products, including 17 sensory attributes in three categories, i.e., odor (5), flavor (9), and texture (3), which were chosen during the sensory training sessions. The tested sensory attributes for odor were sour, seaweed, extra odor, rancid, and spoilage, while the tested flavor attributed included sour, bitter, salt, sweet, seaweed, metal, extra flavor, rancid, and spoilage. The texture attributes examined included liquid, tender and astringent (Table S1, Supplementary material 1). The intensity of each sensory attribute was described by using an unstructured scale from 0 to 100. The two species were evaluated on separate sensory evaluation occasions.

Approximately 24 h prior to the sensory evaluation the frozen control *A. esculenta* samples were put in a refrigerator (4 °C) and allowed to thaw. In preparation prior to the sensory evaluation, approximately two tablespoons of each sample were put in each aluminum tray, which were each marked with random three-digit numbers. The seaweed samples were served to the sensory panelists at room temperature, and red lights were used to hide the appearance of the samples. Prior to sensory evaluation, the frozen-thawed *S. latissima* samples were evaluated as spoiled and were hence not included in the sensory evaluation. Each panelist (n = 9) evaluated all available samples, two for *S. latissima* and three samples of *A. esculenta*, which were each provided in duplicate.

2.7. Extract preparations, total phenolic content (TPC) and antioxidant properties

The total phenolic content (TPC) and antioxidant properties of water extracts of the seaweed samples were performed to evaluate potential bioactive properties of the seaweed samples. Approximately 4 g of freeze-dried seaweed sample were added to 40 ml of water, the samples were shaken on an IKA KS 130 basic plate shaker (IKA, Staufen, Germany) for 1 h, and then centrifuged in a TJ-25 centrifuge (Beckman coulter, California, United States) for 20 min at 5100 rotations per minute (RPM). The supernatant was collected and filtered through a Whatman filtration paper (no 4, CAT No. 1004–150). The extracts were freeze-dried in a Genesis 25 SQ EL freeze dryer (SP industry, Philadelphia United States of America) freeze dryer, and stored at −25 °C till analysis of antioxidant properties. One extract was made from each sample replicate, or three extracts per treatment for each species (n = 3) on each sampling occasion.

To evaluate the total phenolic content (TPC) of the seaweed samples, analysis was performed on the freeze-dried water extracts made from each seaweed sample. The Folin-Ciocalteu procedure (17) was used to determine TPC, with slight modifications as described by Hrólfsdóttir et al, 2022. Each extract (n = 3 per treatment) was measured in duplicate, and results expressed as grams phloroglucinol equivalent (PGE)/100 g freeze dried extract.

To determine the antioxidant properties of seaweed samples, 2,2-Diphenyl-1-picrylhydrazy radical scavenging activity (DPPH) and Oxygen radical absorbance capacities (ORAC) were analyzed. DPPH was determined with method according to Sharma and Bhat (2009), as described by Hrólfsdóttir et al. (2022). Results are expressed as %inhibition of DPPH radicals, and measurements were performed with multiple concentrations (from 0.1 to 10 mg/ml) of the seaweed extracts to calculate the half inhibitory effect (IC50 number). The ORAC measurements were performed according to the method described by and Huang et al. (2002) and Ganske and Dell (2006) with slight modifications as described by Hrólfsdóttir et al. (2022). The results are expressed as ORAC value (Ø), or µmol of trolox equivalent (TE)/gram freeze-dried extract. All measurements were performed in duplicate with three samples for each treatment (n = 3) on each sampling point.

2.8. Statistical analysis

Collection of data and calculation of averages and standard deviations (SD) were performed in Microsoft excel (Microsoft, Redmond, WA, USA). JMP pro 16 (SAS, Cary, NC, USA) was used to perform statistical analysis, e.g. one way ANOVA (analysis of variance) and Tukey's honest significant difference test for the chemical composition, trace minerals.

The FIZZ software (Version 2.51C, Biosystémes, Couternon, France) was used to collect data during sensory analysis and Panelcheck V1.4.0 (Nofima, Tromsø, Norway) was used to monitor the panelists performance. For statistical analysis of sensory data, a General linear model (GLM), corrected for use of scale by the panelist, was applied by using the NCSS software 2000 (NCSS, Utah, USA) coupled with Duncan's post hoc test. A significance level of $p \leq 0.05$ was used for all statistical analysis to determine significant difference between samples during storage and in sensory analysis.

3. Results and discussion

3.1. Proximate composition

Proximate composition of acid-preserved *A. esculenta* and *S. latissima* was assessed during storage (Table 1). As expected, the seaweed samples

Table 1
Nutritional composition of seaweed samples, untreated (control) and acid preserved (with lactic or citric acid) during storage of 28 weeks. Results are expressed as g/100 g sample written as average ± standard deviation (n = 3).

Specie	Sample type	Storage time	Water	Lipid	Protein	Ash	Carbohydrates	Salt
Saccharina latissima	Control	W4	90.8 ± 0.2	0.3 ± 0.1^{a,b}	0.7 ± 0.0^{a,b}	4.3 ± 0.0	3.9 ± 0.2^{a,b}	3.0 ± 0.1^{a,b}
	Lactic acid	W4	90.6 ± 0.6	0.2 ± 0.1^{a,b}	0.7 ± 0.1^{a,b}	4.2 ± 0.0	4.3 ± 0.6^{a,b}	2.9 ± 0.0^{b}
		W16	90.2 ± 0.4	0.1 ± 0.0^{b}	0.8 ± 0.0^{a}	4.2 ± 0.1	4.7 ± 0.5^{a,b}	3.0 ± 0.1^{a,b}
		W32	89.8 ± 0.9	0.1 ± 0.0^{b}	0.6 ± 0.1^{b}	4.2 ± 0.0	5.2 ± 0.8^{a}	3.0 ± 0.0^{a,b}
	Citric acid	W4	91.0 ± 0.5	0.4 ± 0.2^{a}	0.7 ± 0.0^{a,b}	4.3 ± 0.0	3.6 ± 0.6^{b}	3.0 ± 0.0^{a,b}
		W16	90.6 ± 0.1	0.3 ± 0.1^{a,b}	0.8 ± 0.0^{a}	4.3 ± 0.1	4.2 ± 0.3^{a,b}	3.0 ± 0.1^{a,b}
		W32	90.0 ± 0.2	0.1 ± 0.0^{b}	0.7 ± 0.0^{a,b}	4.4 ± 0.0	4.9 ± 0.2^{a,b}	3.1 ± 0.0^{a}
Alaria esculenta	Control	W4	84.7 ± 0.4	0.1 ± 0.0	1.9 ± 0.1^{a,b}	4.1 ± 0.1^{a,b}	9.3 ± 0.5	2.4 ± 0.1^{c}
	Lactic acid	W4	85.7 ± 0.4	0.2 ± 0.1	1.5 ± 0.2^{c}	4.0 ± 0.0^{b}	8.6 ± 0.3	2.5 ± 0.0^{b,c}
		W16	83.8 ± 0.5	<0.1	1.7 ± 0.0^{a,b,c}	4.1 ± 0.1^{a,b}	10.4 ± 0.6	2.5 ± 0.1^{b,c}
		W32	86.0 ± 1.5	<0.1	1.5 ± 0.0^{c}	4.0 ± 0.0^{b}	8.5 ± 1.5	2.6 ± 0.0^{a,b}
	Citric acid	W4	84.7 ± 1.2	0.1 ± 0.0	1.9 ± 0.1^{a}	4.2 ± 0.0^{a}	9.0 ± 1.0	2.5 ± 0.0^{b,c}
		W16	86.1 ± 0.9	0.1 ± 0.0	1.6 ± 0.1^{b,c}	4.1 ± 0.1^{a,b}	8.2 ± 0.8	2.6 ± 0.1^{a}
		W32	84.6 ± 0.1	<0.1	1.7 ± 0.0^{a,b,c}	4.2 ± 0.0^{a}	9.6 ± 0.2	2.6 ± 0.0^{a}

Subscript letters (a-c) show significant differences ($p < 0.05$) in nutritional composition between samples for each species separately.
Storage time is written in weeks, where W4 is first sampling point, W16 sampling at 16 weeks storage and W32 sampling at week 32 storage.

had a high water content, and the main proportion of dry matter content originated from ash and carbohydrates for both species, while having lower content of protein and lipids (Schiener et al., 2015; Stévant et al., 2017a). Only minor changes were observed in the chemical composition of the acid preserved seaweed species throughout the 32-week storage duration, which can mainly be explained by natural variation in chemical composition of the biomass itself and partially due to methodological uncertainty during the analysis, rather than due to spoilage mechanisms. However, some changes were observed during storage of *S. latissima* samples in carbohydrate content, but none can be directly linked to each treatment. Overall, the result indicates that both lactic acid and citric acid treatments seem to be suitable to preserve the biomass for at least 32 weeks duration.

3.1.1. Trace elements

To assess the safety of seaweed as a food ingredient, some of the main trace elements that have been reported to be of concern in seaweed for food applications were analyzed in the frozen control samples (Table 2). These trace minerals are total arsenic (As), inorganic arsenic (IAs), mercury (Hg), lead (Pb), cadmium (Cd), and iodine (I). A significant difference was observed between species in arsenic, lead, cadmium, and iodine content, where the *A. esculenta* contained significantly lower amounts of iodine, while simultaneously containing significantly higher content of the other trace minerals.

While comprehensive regulations and guidance have been established for various fishery resources, food safety of seaweed has been overlooked. European Union (EU) legislations do only exist for maximum levels of lead, cadmium, and mercury in feed and supplement applications. With increased seaweed interest within Europe, the urgent need of legislation for seaweed as a food material must be highlighted to address this significant gap in regulatory aspects (WHO, 2022). Even though the European union has not yet set direct legislations for heavy metals and iodine for seaweed intended for food applications, some guidelines have been published both in Norway and France (ANSES, 2018, 2020; Høgstad et al., 2023). Seaweed producers in Norway have also established guidelines for cultivation and handling of *A. esculenta* and *S. latissima*, addressing food safety concerns, focusing on cadmium, inorganic arsenic, and iodine (Høgstad et al., 2023). The French authorities, ANSES (French Agency for Food; Environmental and Occupational Health & Safety), have set guidelines regarding above the mentioned trace minerals, where recommended maximum levels of cadmium are set to 0.5 mg/kg dry weight (dw), for lead maximum levels of 5 mg/kg dw, for mercury maximum levels of 0.1 mg/kg dw, for inorganic arsenic 3 mg/kg dw (ANSES, 2020), and for iodine maximum levels of 2000 mg/kg dw apply (ANSES, 2018). If results from analysis on dry matter basis are compared to values recommended from ANSES for seaweed intended for human consumption, the cadmium levels in both species were too high (1.0 ± 0.1 mg/kg dw in *S. latissima* and 2.4 ± 0.1 mg/kg dw in *A. esculenta*), and the same applies to iodine in the *S. latissima* samples (4462 ± 123 mg/kg dw). Furthermore, even though there are not any specific set limits of the total arsenic content in the ANSES recommendation, the total arsenic content of both species exceeded the allowed limits for seaweed used as animal feed, or <40.0 mg/kg dw (European Commission, 2002). It is important to highlight that Inorganic arsenic, which is the toxic form of arsenic, is below set limits by ANSES for both species. Several studies have assessed the efficiency of applying different processing methods for trace element reduction, including blanching, soaking in water, or ultrasound-assisted approaches (Correia et al., 2021; Nielsen et al., 2020; Noriega-Fernández et al., 2021; Stévant et al., 2018b). Nielsen et al. (2020) showed that the iodine content can be reduced up to 90% by blanching *S. latissima* or from approximately 4600 mg/kg dw to roughly 300 mg/kg dw. Furthermore, Noriega-Fernández et al. (2021) showed that by using mild heating and ultrasound assisted approaches, iodine,

Table 2
Trace elements of control samples (frozen, nontreated samples). Results are expressed as mg/kg of sample of wet weight and dry weight. Results are expressed as average ± standard deviation (n = 3).

	Sample type	Sample type	Total Arsenic	Inorganic Arsenic	Mercury	Lead	Cadmium	Iodine
Wet weight (ww)	*Saccharina latissima*	Control	5.47 ± 0.25^{a}	<0.10	<0.005	0.01 ± 0.00^{a}	0.09 ± 0.01^{a}	410 ± 17.0^{a}
	Alaria esculenta	Control	9.67 ± 0.49^{b}	<0.10	<0.005	0.03 ± 0.00^{b}	0.36 ± 0.01^{b}	100 ± 0.0^{b}
Dry weight (dw)	*Saccharina latissima*	Control	59.55 ± 3.4	<0.10	<0.005	0.07 ± 0.00^{b}	1.0 ± 0.1^{b}	4462 ± 123^{a}
	Alaria esculenta	Control	63.24 ± 4.10	<0.10	<0.005	0.2 ± 0.02^{a}	2.4 ± 0.1^{a}	654 ± 17^{b}
	Max content[a] *(mg/kg dw)*			3	0.1	5	0.5	2000
	Maximum daily intake[b]							600 µg

Subscript letters (a-b) show significant differences ($p < 0.05$) in trace element composition between species, separately for wet weight and dry weight.
[a] As defined by French authorities ANSES (ANSES, 2018, 2020).
[b] Maximum levels of daily intake as defined by the Icelandic Directory of Health (Icelandic Director of Health, 2017).

cadmium, and arsenic content could be effectively reduced in *Laminaria hyperborea*. The result from the present study thus highlights the importance of such trace element reducing pre-treatments of the seaweed biomass prior to development of food applications thereof.

3.2. Physical properties

Both the pH and color were recorded of seaweed samples throughout the storage period (Table 3) as well as water activity (aw). The aw of the samples was stable throughout the storage time, where it ranged from 0.985 to 0.992. The untreated *S. latissima* and *A. esculenta* had pH of approximately 6.4–6.5, and the acid treated samples pH values between 3.4 and 3.9, showing that the acid preservation was performed efficiently since the achieved pH was lower than 4.6 (CFR, 1979; Dauthy, 1995). Furthermore, the pH was relatively stable, but statistically significant decrease was observed throughout the storage duration, for both the lactic acid and citric acid treated samples. Nøkling-Eide et al. (2023) observed similar trends in pH within their study on formic acid preserved *A. esculenta*, which showed a slight decrease in pH from week 4 post acid treatment to week 16 of storage. Furthermore, Sørensen et al, 2021 showed that both *A. esculenta* and *S. latissima* contain natural microbiota that can contribute to natural fermentation of the biomass. The natural microbiota within the biomass might contribute to the pH decrease, since acids are produced during this natural fermentation process (Stanbury et al., 2013; Sørensen et al., 2021).

Table 3
Physical properties of the seaweed samples untreated (control) and acid preserved (with lactic or citric acid) during storage of 32-weeks.

Specie	Sample type	Storage duration (weeks)	pH	L*-value	a*-value	b*-value
Saccharina latissima	Control	W4	6.47 ± 0.07^{a}	20.4 ± 1.2^{b}	4.6 ± 0.4^{a}	3.5 ± 1.1^{c}
	Lactic acid	W4	3.62 ± 0.02^{c}	26.8 ± 2.8^{a}	2.5 ± 0.9^{b,c}	8.5 ± 1.5^{a}
		W16	3.57 ± 0.01^{c}	26.2 ± 2.2^{a}	2.1 ± 0.6^{c}	7.0 ± 1.4^{a,b}
		W32	3.47 ± 0.01^{d}	26.1 ± 1.6^{a}	1.8 ± 0.4^{c}	7.2 ± 0.8^{a,b}
	Citric acid	W4	3.76 ± 0.01^{b}	26.1 ± 1.5^{a}	3.3 ± 0.6^{b}	8.7 ± 0.7^{a}
		W16	3.73 ± 0.01^{b}	25.2 ± 0.4^{a}	2.7 ± 0.1^{b,c}	7.2 ± 0.4^{a,b}
		W32	3.61 ± 0.01^{c}	26.3 ± 1.5^{a}	2.0 ± 0.3^{c}	6.6 ± 0.8^{b}
Alaria esculenta	Control	W4	6.38 ± 0.02^{a}	23.1 ± 1.3^{c}	3.9 ± 0.5^{a}	4.3 ± 0.8^{b}
	Lactic acid	W4	3.70 ± 0.00^{c-d}	30.5 ± 2.6^{b}	0.4 ± 0.5^{b,c}	8.9 ± 1.4^{a}
		W16	3.67 ± 0.02^{d}	34.6 ± 2.4^{a}	0.0 ± 0.6^{b,c}	10.0 ± 2.3^{a}
		W32	3.53 ± 0.00^{e}	30.7 ± 2.0^{b}	0.5 ± 0.4^{b}	9.7 ± 1.1^{a}
	Citric acid	W4	3.88 ± 0.01^{b}	33.0 ± 2.8^{a,b}	−0.7 ± 0.8^{c}	10.1 ± 1.5^{a}
		W16	3.86 ± 0.02^{b}	31.4 ± 0.8^{a,b}	0.5 ± 0.2^{b}	9.3 ± 0.7^{a}
		W32	3.73 ± 0.00^{c}	31.9 ± 1.0^{a,b}	0.5 ± 0.2^{b}	10.0 ± 0.9^{a}

Subscript letters (a-e) show significant differences ($p < 0.05$) in pH and color parameters (L*-,a*-, b*-values) between samples for each species separately.

The CIE system was used to assess potential color changes of the seaweed samples during the storage period. Sensory aspects such as appearance and color are possible barriers for increased consumption of seaweed biomass within the European populations (Blikra et al., 2021). The color of the brown seaweed biomass can play an important role in increasing interest in seaweed as a food source and processing of the biomass can contribute to more appealing color of the seaweed. Blanching, where biomass is emerged in hot water for a short time is e.g., used in wakame processing to retrieve the bright green color that consumers prefer and are used to from vegetables as well as to reduce undesired compounds (Blikra et al., 2019; Yamanaka and Akiyama, 1993). In the current study higher L*- and b*-values and lower a*-values were obtained in both species after acid treatment compared to the frozen control samples. The results hence imply that when seaweed is treated with acid, the seaweed gets lighter, greener, and more yellow in color, which is considered a positive change rather than negative, especially if the final product is intended for human consumption. During storage a significant decrease was observed in the a*- and b*-values in the citric acid treated *S. latissima* between the first (week 4) and last sampling point (week 32). Furthermore, a significant increase was observed in the a*-value of the citric acid treated *A. esculenta* samples during storage. These changes were not observed in the lactic acid treated samples during storage, independent of species, which indicates that the citric acid has more effect on the color compared to lactic acid during storage. However, it is important to evaluate further how the color changes could change if the biomass is blanched prior to the acid treatment. Furthermore, it is important to note that the color of the biomass itself is not homogenous which could affect the results. Blikra et al. (2019) showed for example that the distal parts of *A. esculenta* were less red and yellow than the proximal parts of the plant, while the proximal parts of both *A. esculenta* and *S. latissima* were lighter in color compared to the distal parts.

3.3. Total viable count (TVC) and total volatile basic nitrogen (TVB-N)

To assess the effectiveness and safety of the treatment to stabilize the biomass and prevent microbial growth of the acid preserved seaweed samples, the total viable count (TVC) was analyzed. The method is often used as a quality indicator to assess efficiency of preservation method during storage and to determine the safety and shelf-life of food products. Significantly lower TVC were observed in the frozen control samples compared to the lactic acid and citric acid treated samples for all sampling points in the *S. latissima*, and for all except for week 4 of lactic acid treated *A. esculenta* samples (Fig. 1). Freezing is known to prevent or reduce the growth rate of microorganisms as well as reducing reaction rates, e.g. in enzyme and oxidative reactions, and additionally the freezing and thawing processes cause injuries and even death of some microorganisms (Archer, 2004; Singh & Heldman, 2001). The results hence show that the freezing of the seaweed biomass was efficient in inhibiting microbial growth. Furthermore, the TVC did not significantly differentiate over the storage time in the lactic acid and citric acid treated *S. latissima* samples, where TVC was ranging from 5.6 to 6.2 log cfu/g. Notably, in week 32, the TVC of the lactic acid treated *S. latissima* (6.2 ± 0.1 log cfu/g) was significantly higher compared to the citric acid treated samples (5.6 ± 0.0 log cfu/g). However, higher TVC was observed in the citric acid samples (4.9 ± 0.1 log cfu/g) compared to the lactic acid treated samples (3.7 ± 0.2 log cfu/g) in the *A. esculenta*. The results hence indicate that different acids might have different effects on different seaweed species. When the two species are compared, higher TVC was observed in the *S. latissima* samples compared to the *A. esculenta*.

Sánchez-Garcia et al, (2021) suggested that TVB-N and

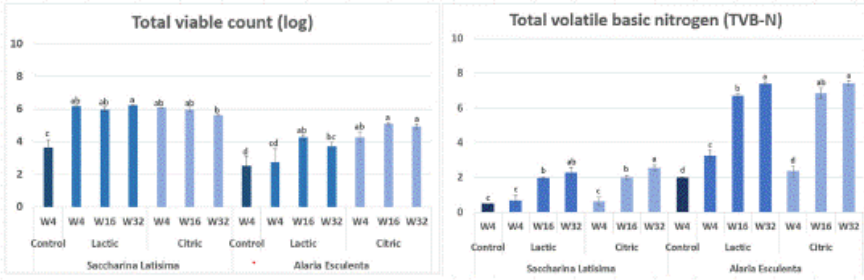

Fig. 1. Results of analysis of Total volatile basic nitrogen (TVB-N) and total viable count (TVC) during storage for 32 weeks. Results are expressed as mean ± standard deviation of three sample replicates. The results from TVB-N are expressed as mg nitrogen (N)/100 g sample, and the TVC as log colony-forming units per gram sample (log cfu/g). Different subscript letters indicate significant differences of samples of same species.

Trimethylamine nitrogen TMA-N might be useful quality parameters to determine degradation due to either microbial or enzyme activity in seaweed. Since TVB-N might be an indicator of these changes, total volatile basic nitrogen (TVB-N) was analyzed. The analysis in the present study showed that the TVB-N gradually increased throughout the storage period in both species, but the values were low, e.g. for fish products set limit for TVB-N is 25–35 mg N/100 g sample (European Commission, 2008). Higher TVB-N values were observed in *A. esculenta* samples compared to *S. latissima* samples, which might be due to *A. esculenta's* higher crude protein content, which is available for degradation through microbial or enzymatic processes. Furthermore, nitrogenous compounds such as biogenic amines can be produced by microorganisms during fermentation to survive (Ekici & Abdullah, 2020) Thus, the increased TVB-N during storage might relate to the fermentation of natural microbiota, but these changes require further research to evaluate possible causes.

3.4. Sensory analysis

To evaluate the acid preserved seaweed as an ingredient for products for human consumption, a sensory evaluation was performed (Table 4). Differences between the two species were mainly observed in the textural properties. Overall, the texture of the *A. esculenta* was more astringent and tough compared to the texture of *S. latissima*. Furthermore, citric acid treated *S. latissima* samples contained significantly more liquid assessed by texture compared to all *A. esculenta* samples. These results align with the proximate chemical composition (Table 1), which showed that the *S. latissima* had a higher water content than *A. esculenta*. Generally, sensory values above 20 indicate that the tested component should be detectable by all panelists, and values around and above 50 indicate strong properties. The results hence show that both the acid treated and frozen *A. esculenta* possess strong seaweed and sour odor and strong salt, seaweed, and sour flavor. Furthermore, results show values above 20 in bitter flavor that should hence be easily detectable by most. Due to the strong flavor and odor properties, the products tested might not be favored by consumers to consume directly but could be used as an ingredient. The results hence imply that the acid preserved biomass needs to be improved if intended for direct human consumption. Therefore, methods such as blanching prior acidification is a feasible option to explore, since the method is often performed on seaweed before it is added into food products, since e.g. the salt and iodine content of the seaweed biomass has been shown to decreases significantly during blanching (Nielsen et al., 2020), changing both the safety and flavor of the biomass. Overall, the sensory evaluation indicated that acid preservation of *A. esculenta* did not affect flavor, odor, and texture negatively.

3.5. Total phenolic content and antioxidant properties

To evaluate the antioxidant properties of the two seaweed species,

Table 4
Results from Generic descriptive analysis (GDA) of seaweed samples. Results are presented as mean values of the responses from nine trained sensory panelists.

	Sensory attribute	Alaria control	Alaria citric	Alaria lactic	Saccharina citric	Saccharina lactic	p-value
Odor							
	Sour	41	49	44	45	44	0.540
	Seaweed	58^{a}	49	52	46	40^{b}	**0.018**
	Extra smell	8	9	12	17	20	0.140
	Rancid	2	1	1	2	2	0.548
	Spoilage	2	1	1	1	2	0.927
Flavor							
	Sour	43	54	54	49	51	0.293
	Bitter	28	21	21	23	24	0.279
	Salt	47	40	36	45	48	0.046
	Sweet	9	11	11	8	8	0.362
	Seaweed	54^{a}	55	54^{a}	49	38^{b}	**0.040**
	Metal	10	7	8	8	7	0.500
	Extra taste	10	6^{b}	6	18	20^{a}	**0.017**
	Rancid	2	1	1	3	2	0.191
	Spoilage	2	1	1	6	2	0.271
Texture							
	Liquid	43^{c}	$43^{b,c}$	$42^{b,c}$	64^{a}	$58^{a,b}$	**0,002**
	Tough	51^{a}	46^{a}	43^{a}	26^{b}	24^{b}	**0,000**
	Astringent	49^{a}	41^{a}	39^{a}	21^{b}	24^{b}	**0,000**

Subscript letters (a-c) show significant differences ($p < 0.05$) in sensory attributes between samples as determined by general linear model (ANOVA) and Duncans test.

and how they are affected by acid preservation, analysis of DPPH radical scavenging activities and oxygen radical absorbance capacities (ORAC) were performed. The phenolic compounds in seaweed have often been associated to the antioxidant properties of seaweed and extracts retrieved from seaweed (Jiménez-Escrig et al., 2012; Sabeena Farvin and Jacobsen, 2013; Wang et al., 2009). Hence the total phenolic content (TPC) was evaluated to assess the relationship between ORAC, DPPH and TPC for the two seaweed species (Table 5).

The TPC was low in all cases in the *S. latissima* samples (around 0.5 g/100 g dw extract) where the small differences can also be linked to variation within the biomass itself rather than changes in the biomass due to storage (Table 5). Higher concentrations were, however, observed in the *A. esculenta* samples, where the content ranged from 1.59 ± 0.09 to 3.00 ± 0.12 g PGE/100 g dw extract. For the *A. esculenta* samples, the TPC in the control samples were significantly higher compared to all acid treated samples, for all sampling points, indicating that the acid treatment caused deterioration of the phenolic compounds (Table 5). The phenolic content of both species is, however, relatively low compared to the water extracts of other brown seaweed species, such as *Ascophyllum nodosum* and *Fucus vesiculosus*, which have 4–6 times higher phenolic content than observed in the present study. The TPC in Fucus species, however, may depend both on the harvesting time of the biomass and the extraction method used (Hrólfsdóttir et al., 2022; Wang et al., 2009). For future research, more accurate quantitative methods such as High-performance liquid chromatography (HPLC), might be useful to further estimate the deterioration of TPC of the acid treated seaweed biomass.

Table 5
Total phenolic content (TPC), Oxygen radical absorbance capacity (ORAC), and DPPH radical scavenging activities of dry weight (dw) extracts of acid preserved seaweed samples for 32 weeks storage. Results are expressed as mean ± standard deviation (n = 3), where TPC results are expressed as g phloroglucinol equivalent (PGE)/100 g dw extract, ORAC results expressed as ORAC value (Ø) (μmol of tocopherol equivalent (TE)/g dw extract), and DPPH results expressed as IC50 number (amount of active component required to reduce the absorbance of DPPH radicals by 50%).

Species	Sample type	Storage	TPC	ORAC	DPPH
Unit		Weeks	PGE/100 g dw extract	TE/g dw extract	IC50
Saccharina latissima	Control	W4	0.53 ± 0.01^{a}	17.3 ± 1.9^{a,b}	6.35 ± 0.75^{a,b}
	Lactic acid	W4	0.43 ± 0.02^{b}	10.7 ± 1.4^{b}	6.55 ± 0.17^{a,b}
		W16	0.44 ± 0.01^{b}	19.7 ± 0.6^{a}	6.13 ± 0.23^{a,b}
		W32	0.48 ± 0.01^{a,b}	21.6 ± 0.9^{a}	7.08 ± 0.99^{a}
	Citric acid	W4	0.42 ± 0.03^{b}	16.3 ± 5.6^{a,b}	5.77 ± 0.22^{a,b}
		W16	0.47 ± 0.04^{b}	20.7 ± 1.6^{a}	5.17 ± 0.06^{b}
		W32	0.50 ± 0.04^{a,b}	19.6 ± 1.8^{a}	5.88 ± 0.41^{a,b}
Alaria esculenta	Control	W4	3.00 ± 0.12^{a}	94.0 ± 6.3^{a,b}	0.35 ± 0.07^{c}
	Lactic acid	W4	1.87 ± 0.05^{b}	68.7 ± 7.9^{b,c}	0.70 ± 0.25^{a,b}
		W16	1.76 ± 0.04^{b}	93.4 ± 15.5^{a,b}	1.51 ± 0.72^{a,b}
		W32	1.59 ± 0.09^{b}	92.2 ± 7.6^{a,b}	1.75 ± 0.38^{a}
	Citric acid	W4	1.77 ± 0.05^{b}	65.3 ± 3.6^{c}	0.76 ± 0.03^{a,b,c}
		W16	1.94 ± 0.35^{b}	105.6 ± 14.3^{a}	0.82 ± 0.42^{a,b,c}
		W32	1.72 ± 0.07^{b}	104.1 ± 2.3^{a}	1.62 ± 0.17^{a}

Subscript letters (a-c) show significant differences ($p < 0.05$) in TPC and antioxidant properties (ORAC, DPPH) between samples for each species separately. Storage time is written in weeks, where W4 is the first sampling point, W16 corresponds to sampling at 16 weeks storage and W32 to sampling at 32 weeks of storage.

Similar trends were observed in the DPPH radical scavenging activities as in the TPC, where the acid treatments appeared to decrease the activity of the *A. esculenta* when treated with acid (both lactic and citric acid samples). The IC50 of the *A. esculenta* control samples were significantly lower when compared to the *A. esculenta* acid preserved seaweed samples, but the IC50 value of the control samples was 0.35 ± 0.07 mg/ml, 0.70 ± 0.25 to 1.75 ± 0.38 mg/ml for the lactic acid treated samples and 0.76 ± 0.03 to 1.62 ± 0.17 mg/ml for the citric acid treated samples. The results hence indicate that antioxidant properties assessed by DPPH decrease with acid treatment. That is the opposite of what studies have shown when seaweed is fermented for short time periods (Lee et al., 2015; Sumardianto, PH, AD, & Rianingsih, 2021). Sumardianto et al. (2021) showed an increase in both polyphenols and antioxidant properties (assessed by DPPH) of extracts obtained from the red seaweed species *Gelidium* sp. and *Eucheuma cottonii* that had been fermented for 24 h with *Lactobacillus plantarum* and *Lactobacillus acidophilus* cultures when compared to the starting point of fermentation. Furthermore, Lee et al. (2015) showed an increase in DPPH scavenging activity with fermentation of *Sargassum siliquanstrum* biomass. Even though no significant difference was observed in the IC50 numbers within each species during the storage period of the acid treated samples, the extract quantities (mg/ml) needed to reduce the DPPH radicals decreased with storage time. For the *S. latissima* samples, no specific tendencies were observed in either the TPC or DPPH activities which might also be explained by the low overall antioxidant activity and low amounts of phenolic compounds present in the biomass.

ORAC values were low for both species, ranging from 10.7 ± 1.4 to 21.6 ± 0.9 μmol of TE/g dw extract in the *S. latissima* samples, and from 65.3 ± 3.6 to 105.6 ± 14.3 μmol of TE/g dw extract in the *A. esculenta*. No specific trends were observed in the ORAC values during storage or with acid treatment. The results correspond to earlier studies, where both *A. esculenta* and *S. latissima* have shown low antioxidant properties when assessed by ORAC (Mildenberger, Stangeland, & Rebours, 2022; Wang et al., 2009) compared to extracts from other brown seaweed species (e.g. Fucus species), which can have been reported to have ORAC values of 1500–3000 μmol of TE/g dw extract (Hrólfsdóttir et al., 2022; Wang et al., 2009). Therefore, *S. latissima* and *A. esculenta* might be more suitable for applications that do not rely on antioxidant properties, such as ingredients in food products post blanching treatments.

4. Conclusion

The results show that the proximate composition, color, pH, and microbial counts of the acid-preserved seaweed remained relatively stable for at least seven-month (32-week) storage at room temperature, with minimal differences observed between lactic acid and citric acid treated seaweed. However, acid preservation of seaweeds might cause protein deterioration or increase in biogenic amines as evidenced by increase in TVB-N over the storage period. Additionally, the study revealed that the seaweed biomass has relatively high cadmium, arsenic, and iodine content on a dry weight basis, along with high salt content that could limit the application of the biomass as it is for food and feed applications Furthermore, the strong salt and acid flavor detected in the samples might also influence the possible uses of the biomass as a food ingredient. Notably, a decrease was observed in the total phenolic content (TPC) and antioxidant properties (assessed by DPPH) during the storage of acid preserved biomass of *A. esculenta*. In conclusion, acid preservation using lactic acid or citric acid was effective in stabilizing the seaweed biomass and could possibly be used as a stabilization method for *A. esculenta* and *S. latissima* as well as other seaweed species. Low antioxidant characteristics of the tested *S. latissima* and *A. esculenta*, however, imply that these species might not be ideal if the seaweed is intended for applications for its antioxidant properties. Nevertheless, the

acid treated biomass might be suitable for seaweed as an ingredient in a wide range of value-added products.

CRediT authorship contribution statement

Anna Þóra Hrólfsdóttir: Writing – review & editing, Writing – original draft, Visualization, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Sigurjón Arason:** Writing – review & editing, Visualization, Validation, Supervision, Methodology, Conceptualization. **Hildur Inga Sveinsdóttir:** Writing – review & editing, Visualization, Validation, Supervision, Methodology, Conceptualization. **Maren Sæther:** Writing – review & editing, Methodology, Conceptualization. **Inga Marie Aasen:** Writing – review & editing, Project administration, Methodology, Funding acquisition, Conceptualization. **María Guðjónsdóttir:** Writing – review & editing, Visualization, Validation, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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Paper IV

Multispectral imaging techniques for evaluating physicochemical-, antioxidant, and sensory properties of *Alaria esculenta* and *Saccharina latissima*

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Abstract

With the increased seaweed production worldwide, there is a need to focus on improved production practices to produce high-quality seaweed biomass, especially if the biomass is intended for high-quality products and human consumption. Multispectral imaging (MSI) is a novel technique used as a quality control tool within the food industry due to its rapid, real-time measurements. Therefore, the study aimed to assess the possibilities of using MSI as a quality control tool within seaweed cultivation to predict the physicochemical (including proximate composition, trace minerals, pH, and color), microbiological (total viable counts (TVC)), sensory, and antioxidant properties of *A. esculenta* and *S. latissima* throughout diverse processing and handling. The results showed differences in spectra between species, and species classification got 100% accuracy when using a Support Vector Machine (SVM) with the spectral data. Furthermore, results indicated that the partial least square regression (PLSR) models developed with cross-validation of the MSI data effectively predicted multiple quality parameters, including pH (R^2_{CV}=0.94, RMSECV=0.278), carbohydrate content (R^2_{CV}=0.89, RMSECV=0.76), protein content (R^2_{CV}=0.94, RMSECV =0.12), ash content (R^2_{CV}=0.80, RMSECV=0.51), and phenolic content (R^2_{CV}=0.992,

RMSECV=0.24). In addition, the results showed possibilities of using the technology to assess several sensory properties. In conclusion, the results show the potential of using the MSI technology as an effective quality control tool within the seaweed industry to simultaneously evaluate multiple physicochemical properties of brown seaweed biomass.

Keywords: Seaweed quality, Multispectral imaging, Chemometrics, Quality assessment, Macroalgae, Proximate composition, Trace minerals, Sensory evaluation, Antioxidant properties.

1 Introduction

The seaweed industry in Europe is in its infancy compared to Asia, where only around 0.8% of the total seaweed biomass produced in 2019 (35,756.5 thousand tons) was contributed by Europe, while 97% was contributed by Asia. Most of the biomass from Asia was obtained by cultivation, but the main sources of the harvested biomass in Europe were from wild sources (FAO, 2021; Farghali et al., 2023). In Europe, seaweed cultivation has recently gained more interest, and the industry is expected to grow extensively in the future, with the aim of using the cultivated biomass for, e.g., food and other value-added products (A Vincent et al., 2020). Currently, many European consumers consider seaweed a novel food, even though seaweed has been consumed since ancient times by humans and animals in many European coastal communities, including Norway, Iceland, Ireland, France, and the United Kingdom (Delaney et al., 2016; McHugh, 2003; van den Burg et al., 2019). However, the Western world population has increased the utilization of brown seaweed as an ingredient in food products and for direct consumption (WHO, 2022), mainly due to indications of its possible health benefits and sustainable production practices (Blikra et al., 2021; Buschmann et al., 2017). With the potential for increased seaweed production and consumption in Europe, it is essential to establish methods for responsible production and quality assessment of seaweed biomass.

Non-destructive methods to evaluate the quality and physicochemical parameters of food products are increasingly valued for their ability to preserve sample integrity while providing rapid and real-time analysis (Nicolaï et al., 2007). These techniques are not only cost-effective and more environmentally friendly, but they also allow for repeated measurements without destroying the sample (Liu et al., 2015). Their application in food quality assessment is particularly noteworthy, as they offer comprehensive data crucial for ensuring product safety and consumer acceptance, making them an indispensable tool in modern quality monitoring and assessment processes (El-Mesery et al., 2019; Liu et al., 2015).

Among the various non-destructive methods, near-infrared (NIR) spectroscopy has emerged as a standard analytical technique for analyzing the chemical composition, quality, and sensorial properties of food products over the past decade (Osborne, 2006). The technology focuses on capturing spectral data within the near-infrared range (780-2500 nm), offering precise insights into the chemical composition of various food items (Niemöller & Behmer, 2007). Building upon the spectroscopy principles, multispectral

imaging (MSI) emerges as a novel, non-destructive approach that extends beyond NIR spectroscopy by integrating aspects of both spectroscopy and image analysis (Da-Wen, 2010). MSI is designed to simultaneously acquire spatial and spectral information from an object, thereby providing a comprehensive analysis of its characteristics (Boelt et al., 2018; Liu et al., 2014). This fusion of imaging with spectral analysis allows MSI to detect various quality-related factors of food products effectively and quickly, especially when these attributes exhibit distinct spectral responses across different spatial regions. MSI is recognized for its role in quality analysis and assessments in various food industries, with growing interest in its potential as a rapid, real-time method for evaluating food quality and safety through spectroscopic data (Tsakanikas et al., 2020; van Ginneken & de Vries, 2017). While the use of MSI in seaweed research and industry is still emerging, some studies have begun to examine how the MSI technology can be applied to seaweed. For instance, van Ginneken and de Vries (2017) employed imaging spectroscopy to analyze the reflectance characteristics of six seaweed species: two green (*Codium* and *Ulva* spp), two red (*Chondrus crispus* and *Osmundea pinnatifida*), and two brown (*Fucus* spp and *Laminaria ochroleuca*) species. Additionally, MSI has been used as an instrument to map intertidal reefs and estimate the distribution and density of seaweed biomass (Borges et al., 2023; Rossiter et al., 2020).MSI has also been evaluated as a rapid assessment of the microbial quality of edible seaweeds (Lytou et al., 2022). However, there remains a gap in the literature when it comes to the evaluation of using MSI for chemical, bioactive, and physical properties, as well as its potential as a quality control tool for seaweed biomass.

Seaweed cultivation is gaining increasing interest in Europe, with *Saccharina latissima* and *Alaria esculenta* being the two most cultivated species. As the industry grows, there is a need for efficient and reliable methods to evaluate the chemical composition of seaweed biomass for process monitoring, quality control, and optimization of production. MSI has emerged as a promising non-destructive technique that could potentially address this need. This study aimed to investigate if MSI can be effectively used to predict the physicochemical and antioxidant properties of *Saccharina latissima* and *Alaria esculenta*, and whether this technology is suitable as a quality control tool within seaweed processing. By examining the potential of MSI in this context, the study seeks to provide new opportunities for the seaweed industry to enhance their operations and ensure the quality of their products.

2 Material and methods

2.1 Seaweed sampling and preparation

A. esculenta and *S. latissima* harvested in 2022, and *A. esculenta* samples harvested in 2023, were cultivated on ropes at Frøya, Måsskjæra (N63'44, E8'53N) in Norway by Seaweed Solutions AS (SES). The seaweed was harvested on the 30th of May 2022 and 7th of May 2023, transported to the processing facilities, and stored in a cooler at 4°C overnight till further processing. The following samples were used in the current study:

1. Frozen controls of *A. esculenta* and *S. latissima* from 2022
2. Lactic acid and Citric acid treated *A. esculenta* and *S. latissima* harvested in 2022
3. Frozen controls of *A. esculenta* harvested in 2023
4. Lactic acid and Citric acid treated *A. esculenta* harvested in 2023
5. Fermented *A. esculenta* harvested in 2023
6. Blanched/soaked lactic acid treated and frozen-thawed *A. esculenta* harvested in 2023

A detailed description of the processing of the seaweed samples is given in appendix A and an overview of the samples and treatments is represented in Table A.1 in appendix A.

2.2 Multispectral imaging equipment

The seaweed samples were imaged using the VideometerLab 4 system, a multispectral imaging (MSI) device from Videometer A/S (Herlev, Denmark). The system consists of a high-resolution charged-coupled device (CCD) camera and computer technology with advanced digital image analysis. The VideometerLab 4 uses a strobed light-emitting diode (LED) technology to combine 19 different wavelengths, ranging from 365 nm to 970 nm, into a single high-resolution spectral image with 19 bands. Every pixel in the spectral image is represented as a reflectance and scattering spectrum. The system was calibrated according to standard practice with respect to color, geometry, and self-illumination. The VideometerLab 4 was operated at 100% reflectance mode, and the size of the obtained MSI images is 2992 x 2992 pixels.

2.3 Data acquisition and extraction

During the data acquisition, seaweed samples were placed onto a petri dish and imaged using the VideometerLab 4. In total were 78 images captured, and each sample was measured in triplicate. During the storage trial in 2022, 39 images were taken, and another 39 images were taken of differently processed seaweed in 2023. The VideometerLab software was used to extract the spectral information from these images (without the background), providing both mean values and standard deviations of the spectra for each captured wavelength.

2.4 Multivariate analysis of spectral data

2.4.1 Principal Component Analysis (PCA)

The chemometric software Unscrambler© ver. 11 (Aspen Technology, Bedford, MA, USA) was used to perform a PCA on the spectral data in the current study. Singular Value Decomposition (SVD) was used for the PCA, using cross-validation with 20 random segments for validation. The total number of components used was 7, but the optimal number of components was 3. Outliers were neither specifically labeled nor removed.

2.4.2 Partial Least Squares Regression (PLSR)

PLSR models were built for the quantitative analysis of various physicochemical parameters, using the spectral data as input variables and the results of the physicochemical and antioxidant analysis as the output variables using the chemometric software Unscrambler© ver. 11 (Aspen Technology, Bedford, MA, USA). The models were evaluated using a cross-validation approach with 15-20 random segments, depending on the size of the dataset. he performance of these PLSR models was evaluated by several key metrics: the calculation of the root mean square error of calibration (RMSEC), the root mean square error of cross-validation (RMSECV), the correlation coefficient in the calibration sample set (R_C^2), and the correlation coefficient in the cross-validation test set (R_{CV}^2) (Martens & Naes, 1992; Otto, 2023).

2.4.3 Pearson's correlations between reference variables

Pearson's correlations between variables were calculated using the JMP pro 16 (SAS, Cary, NC, USA) software. This method was used to identify potential relationships between the different physicochemical and antioxidant parameters applied in the study.

2.5 Image analysis for species classification

2.5.1 Normalized Canonical Discriminant Analysis

Normalized Canonical Discriminant analysis (nCDA) is a supervised model based on MSI transformation of the images that determines how to best separate or discriminate two or more groups of individuals, given quantitative measurements of several variables on these individuals. nCDA is designed to optimize the variance across two different groups while minimizing the variance within each group, enhancing group separability (Cruz-Castillo et al., 1994). In the study, nDCA was used to model species differentiation. The VideometerLab software was used to develop the nCDA model.

2.5.2 Support Vector Machine

In this study, the Support Vector Machine (SVM) approach was used for the classification of seaweed species based on the spectral data. SVM performs classification by transforming the original training data into a multidimensional space and constructs a hyper-plane in a higher dimensional. SVM can classify both linear and non-linear data (Chandra & Bedi, 2021). The SVM classification model for the two seaweed species was done with a 10-fold cross-validation using the kernel-type radial basis function.

2.6 Reference analysis

Several physical, chemical, microbiological, antioxidant, and sensory quality parameters were assessed in the seaweed samples after the different processing and treatments to allow comparison and benchmarking of the MSI data to known and accredited reference measurements.

2.6.1 Chemical analysis

Water content was determined according to the ISO 6496:1999 (1999) method. The Kjeldahl method described by ISO 5983-2:2009 (2009) and ISO 5983-1:2005 (2005), was used to determine the protein content of the seaweed samples from 2022, while the Dumas method ISO 16634-1:2008 (E) method (ISO, 2008) was applied for samples from 2023. A conversion factor of 5 was used to calculate the final protein content as recommended for seaweed (Alex R Angell et al., 2016). The ash content was analyzed according to the ISO 5984:2002 (2002) method. The total lipid content was determined using the Soxhlet extraction method according to the AOCS Official Method Ba-3-38 (AOCS, 2009b). To determine the samples' salt content, the Volhard titration method, as described by AOAC (1990) was used. Results from water, lipid,

protein, and ash content analysis were used to calculate the carbohydrate content (C) by using the following equation:

$$C = 100 - (water + protein + ash + lipids) \qquad \text{(Eq. 1)}$$

To determine the iodine content, DIN EN 15111 (2007-06) was used with modifications according to CON-PV 01187 (2022-06) applying Inductively Coupled Plasma Mass Spectrometry (ICP-MS). The arsenic content was determined according to the ASU L 00.00-19/3 (2004-07) method with modifications according to the CON-PV 00508 (2020-01) by using graphite furnace atomic absorption spectrometry (GF-AAS). The cadmium and lead content of the biomass was analyzed using Method 186 (NMKL, 2007a). Steam distillation of a 7.5% trichloroacetic acid (TCA) extract, as described by Billon and Tao (1979) was used to analyze the Total volatile basic nitrogen (TVB-N) of the seaweed samples.

2.6.2 Physical properties

Color analysis was conducted using a Minolta Chroma Meter CR-300 (Minolta, Osaka, Japan), applying the CIE Lab system. The pH of the biomass was recorded using a Knick portable pH meter (Knick, Berlin, Germany).

2.6.3 Total phenolic content (TPC) and antioxidant properties

Antioxidant properties (DPPH radical scavenging activities (DPPH), oxygen radical absorbance capacity (ORAC), and total phenolic content (TPC)) were determined on water extracts. The extracts were produced with 4 g of seaweed sample in 40 mL of distilled water as described by (Hrólfsdóttir et al., 2024). TPC was analyzed on the freeze-dried extracts by using the Folin-Ciocalteu procedure as described by Hrólfsdóttir et al. (2022). The ORAC was analyzed as described by Dejian Huang et al. (2002) and Ganske and Dell (2006) with some modifications (Hrólfsdóttir et al., 2022). The DPPH was determined as outlined by Sharma and Bhat (2009), as described by Hrólfsdóttir et al. (2022).

2.6.4 Sensory evaluation

Generic descriptive analysis (GDA) was performed on the seaweed samples according to Stone et al. (2020). A total of 17 sensory attributes (in three categories, odor (5), flavor (9), and texture (3)) were assessed on *A. esculenta* and *S. latissima* from 2022, including acid treated samples stored for 16 weeks and frozen-thawed *A. esculenta* (Table B.2 in Appendix B), while 9 sensory attributes (in two categories, odor (3) and flavor (6)), were assessed on differently processed *A. esculenta* samples (fermented, lactic acid and citric acid treated) harvested in 2023. The sensory analysis was performed by nine

panelists trained according to (ISO., 1993) as described by Hrólfsdóttir et al. (2024).

3 Results and discussions

3.1 Spectra of the seaweed samples

Averages of mean reflectance spectra were plotted for each sample group/sample occasion, with a total of three sample replicates for each group across the 19 wavelengths obtained in the range of 365-970 nm, hence covering wavelengths in the ultraviolet spectrum (UV) (365 nm), visible spectrum (VIS) (400-690), and the near-infrared spectrum (NIR) (780-970) (Martelo-Vidal & Vázquez, 2016; Osborne, 2006). Slight variations were observed between the spectra at the visible spectrum at wavelengths between 515 and 690 nm (Figure 1). The main differences were, however, observed within the NIR spectrum range, indicating that the wavelengths between 780 and 970 play a key role in detecting the differences among the seaweed samples.

The peak in the NIR range (780-970 nm) is connected to the third overtones of various chemical bonds, including C-H, O-H, and N-H, providing insights into molecular interactions and changes due to processes like lipid oxidation, protein degradation, and variations in water content (Osborne, 2006; Ozaki et al., 2006). Specifically, the wavelength 970 nm corresponds to water content, capturing the stretching vibrations of O-H bonds. Similarly, the wavelengths at 880 nm and 940 nm are indicative of features related to lipids, primarily through stretching vibrations of C-H bonds. These wavelengths thus help identify changes in lipid structures that occur during lipid oxidation. Additionally, the wavelengths 780 and 850 nm correspond to the third overtone stretching of N-H bonds, which are key structural components of amides, which are prevalent in peptides and proteins (Ahmed Badr, 2011; Metrohm, 2014).

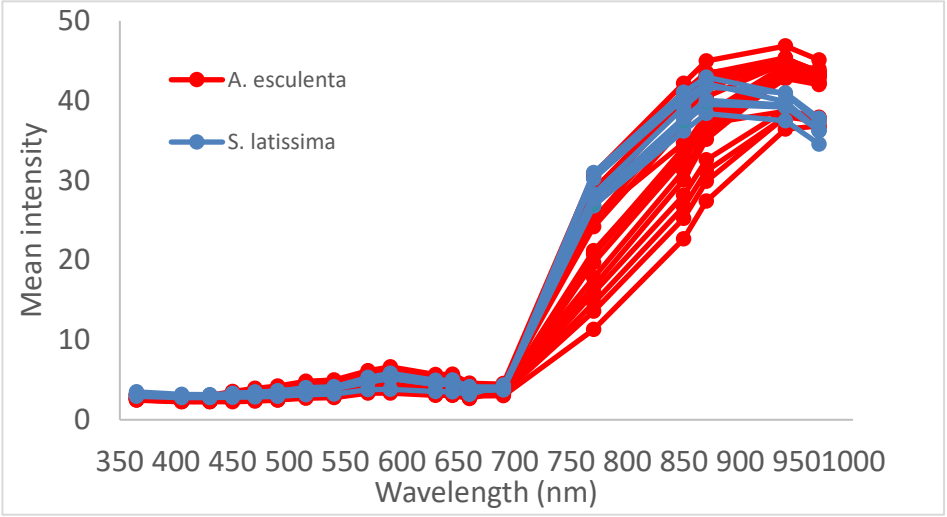

Figure 26 Mean reflectance spectra of brown seaweed samples used in the study. The red spectra are of A. esculenta samples, while the blue spectra are of S. latissima samples.

3.2 Principal component analysis (PCA) of spectra

Principal component analysis (PCA) was performed on all the spectral data obtained (Figure 2) to provide an overview of the dataset. The PCA explained 96% of the variation within the spectral data, where PC1 explained 80% and PC2 16%. The PCA effectively distinguished differences between the two species (*A. esculenta* and *S. latissima*) and between treatments (control, acid preservation, and fermentation) as effects of both treatment and the storage duration of the acid-treated samples could be distinguished from the PCA. Figure C.1 (in appendix C) shows the PCA plot where samples are labeled by sampling point. Additionally, the spectral data from the two different harvesting periods (2022 and 2023) show two clear groupings within the PCA score plot if the blanched seaweed is excluded. The chemical properties, particularly water content and carbohydrates, which vary the most between species, are likely the main contributors to the observed groupings in the final PCA plot. The results hence indicate that the spectral data could be used effectively to differentiate between both the seaweed species and processing methods.

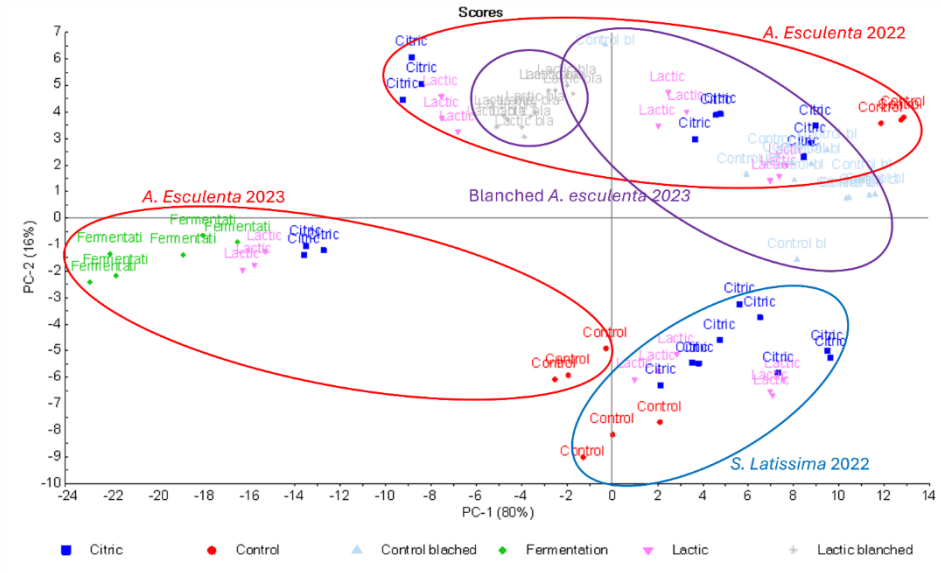

Figure 27 PCA score plot of spectra obtained of frozen control, acid preserved, fermented, and blanched seaweed samples used in the study. The blue oval shape shows S.latissima samples, the red oval shapes represent A. esculenta samples from either 2023 or 2022 (excluding blanched samples), and the purple shows blanched A. esculenta samples obtained from 2023.

3.3 Species recognition by multispectral image analysis

The Normalized Canonical Discriminant Analysis (nCDA) model was developed to evaluate the potential of using multispectral image analysis to differentiate between the two seaweed species. The model was developed using control samples, which had not been subjected to any treatments. Figure 3 presents an sRGB image of the species, a near-infrared (NIR) image at 970 nm, followed by the outcomes of the nCDA model. In this visualization, red pixels signify *A. esculenta*, while blue pixels indicate *S. latissim*a. Pixels that appear closer to green represent areas where the model is uncertain about the species classification. The nCDA model used wavelengths from the UV, visible, and NIR spectra to distinguish between the species, specifically wavelengths at 365, 490, 570, 590, 690, 780, 850, 940, and 970 nm. These wavelengths correlated well with the variations observed in the mean reflectance spectra of the two seaweed species in Figure 1. Notably, the NIR bands showed the greatest variation, which emphasizes their role in distinguishing between the seaweed species. Additionally, a small peak in the visual (VIS) spectral range, particularly from 570 nm to 690 nm, aligned with spectral features that also aid in distinguishing between the seaweed species.

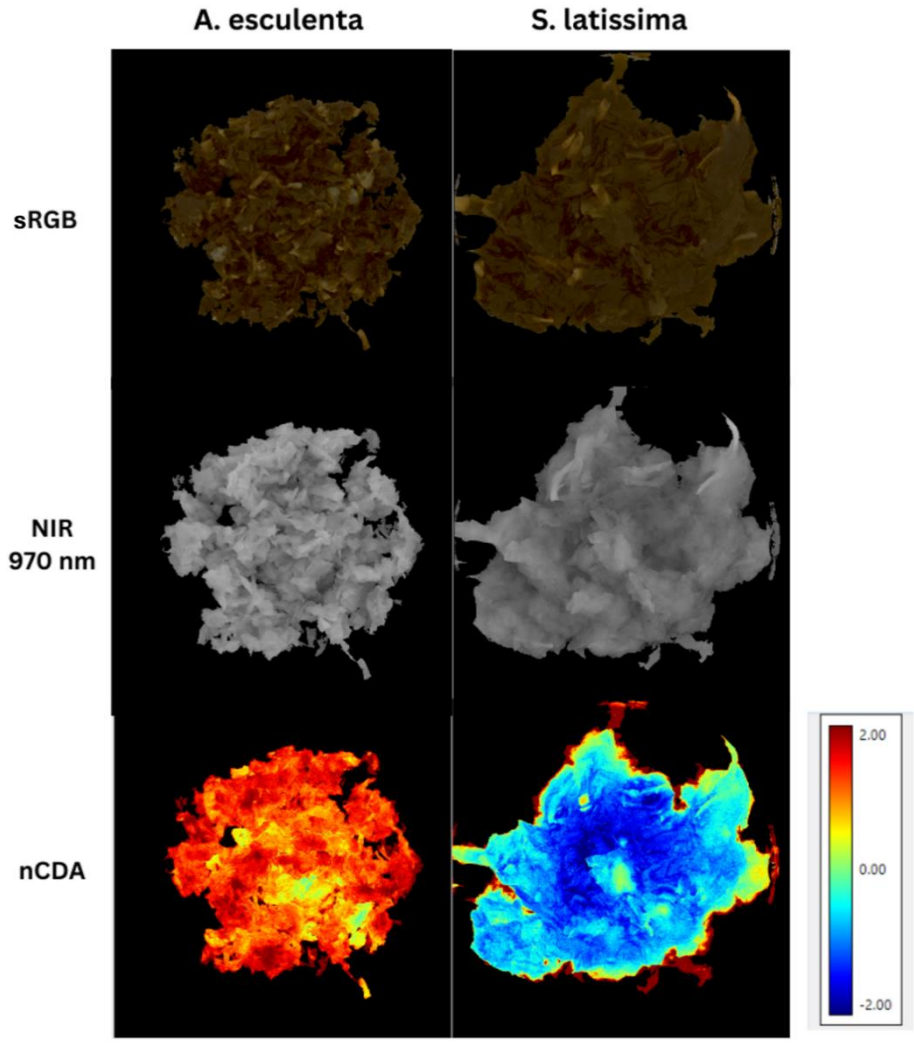

Figure 3 sRGB images of the two seaweed species, with a corresponding image of NIR wavelength 970 nm, and the nCDA transformation image.

Additionally, a Support Vector Machine (SVM) classification model was constructed using the spectral data from all 78 samples. Both the training and validation phases of the SVM model achieved a 100% accuracy rate. It is noteworthy that 77% of the analyzed samples were *A. esculenta*. Despite this imbalance, the results indicated that image analysis holds potential as a method for differentiating between the seaweed species.

3.4 PLSR model performance

A comparison of the MSI spectral data to multiple physicochemical-, and bioactive parameters of the seaweed biomass was performed (Table 1) to identify what parameters the equipment could predict, allowing the MSI to be used as a nondestructive analytical quality control equipment for the brown seaweed biomass. The results of the chemical analysis performed on samples from 2022 are presented in a published article by Hrólfsdóttir et al. (2024), and all results are described thoroughly in Appendix D. A PLSR model was developed for each analyzed parameter with a sample size ranging between 39 and 78 samples. Color, pH, and water were assessed in all 78 samples, while sample sizes of other parameters were in the range of 39 to 54 (Table 1). An effective model is defined by high values of the coefficients of determination R_C^2 and R_{CV}^2 (typically above 0.75), which indicate strong correlations and high predictive accuracy during both the calibration and cross-validation phases, respectively. Conversely, low values of root mean square errors of calibration (RMSEC) and cross-validation (RMSECV) are indicative of a model's precision. Ideally, these should be below 10% of the range of the observed data, denoting minimal deviation between the predicted and actual values in the calibration and cross-validation datasets, respectively (Gemperline, 2006; Nepper-Davidsen et al., 2024). These performance metrics are necessary for verifying the reliability and applicability of the PLSR models in accurately predicting the physicochemical and antioxidant analyses based on spectral data. Additionally, since these models are based on reference measurements, they inherently cannot be more accurate than the reference method itself. Recognizing this limitation is important for a comprehensive understanding of the capabilities and constraints of the models. While PLSR provided valuable insights, using different preprocessing methods for the spectral data could further enhance model performance. Techniques such as normalization, baseline correction, and smoothing could optimize the spectral input for analysis, potentially improving the accuracy and robustness of the predictions (Gholizadeh et al., 2015). Additionally, using alternative machine learning and deep learning models could offer enhanced predictive capabilities and a deeper understanding of complex, non-linear relationships within the spectral data, as PLSR cannot effectively extract nonlinear informative features from the MSI data (Wei et al., 2020; Yu et al., 2019).

Table 1. Coefficient of determination in calibration (R_C^2) and in cross-validation (R_{CV}^2), root mean squared error of calibration (RMSEC) and cross-validation (RMSECV), valid prediction range, sample size (n), and number of model factors (latent variables) of physicochemical and antioxidant parameters of seaweed samples used for PLSR cross-validation.

	Calibration		Validation				
Parameter analyzed	R_C^2	**RMSEC**	R_{CV}^2	**RMSECV**	**Range**	**Sample size**	**Number of factors**
Water (g/100 g sample)	0.82	0.90	0.76	1.05	83.0-91.5	78	7
Lipids (g/100 g sample)	0.34	0.08	0.18	0.09	0.0-0.5	54	4
Carbohydrates (g/100 g sample)	0.91	0.66	0.89	0.76	3.0-10.9	54	6
Protein (g/100 g sample)	0.95	0.10	0.94	0.12	0.6-2.2	54	4
Salt (g/100 g sample)	0.91	0.33	0.86	0.41	0.1-3.1	54	7
Ash (g/100 g sample)	0.88	0.39	0.80	0.51	1.6-4.4	54	7
Iodine (mg/kg sample)	0.89	30.82	0.83	39.38	60-420	39	6
Arsenic (mg/kg sample)	0.87	0.95	0.75	1.33	3.4-10.2	39	6
Cadmium (mg/kg sample)	0.82	0.02	0.73	0.03	0.1-0.3	39	7
Lead (mg/kg sample)	0.86	0.003	0.82	0.004	0.01-0.05	39	5
TVB-N (mg N/100 g sample)	0.55	2.46	0.40	2.92	0.5-15.9	54	4
L-value	0.70	3.00	0.65	3.32	16.8-45	78	5
a^*-value	0.93	0.67	0.91	0.79	-6.3-4.6	78	6
b^*-value	0.65	3.32	0.53	3.97	2.9-33	78	7
A_w	0.41	0.00	0.25	0.00	0.98-1	54	6
pH	0.96	0.25	0.94	0.28	3.0-6.7	78	6
TPC (g PGE/100 g extract)	0.94	0.20	0.92	0.24	0.4-3.2	39	4
DPPH (IC50)	0.92	0.75	0.91	0.81	0.3-8.2	39	2
ORAC (µmol TE/g extract)	0.93	9.73	0.89	11.70	10-113	39	5
Total viable count (TVC) (log cfu/g sample)	0.72	0.79	0.67	0.93	1.8-6.2	48	4

3.4.1 Chemical properties prediction

The chemical composition of seaweed varies significantly between species and is dependent on the harvesting period and environmental factors such as temperature, salinity, and pH (Marinho-Soriano et al., 2006; Schiener et al., 2015). This variability highlights the importance of finding new non-destructive methods for seaweed producers to use within their processing to evaluate the

chemical and physical parameters of their products. The brown seaweed species, *A. esculenta* and *S. latissima,* generally contain water content of between 80-90%, are relatively high in ash and carbohydrates, and contain lower amounts of protein and lipids (Schiener et al., 2015; Tibbetts et al., 2016). The chemical composition of samples used in the study is similar to previously reported values for both species (Arlov et al., 2024; Schiener et al., 2015; Stévant, Marfaing, et al., 2017), indicating that the constructed PLSR models would be suitable for assessing these parameters within seaweed cultivation and processing of *A. esculenta* and *S. latissima*. Table 1 presents the specific ranges for each chemical variable used in this study.

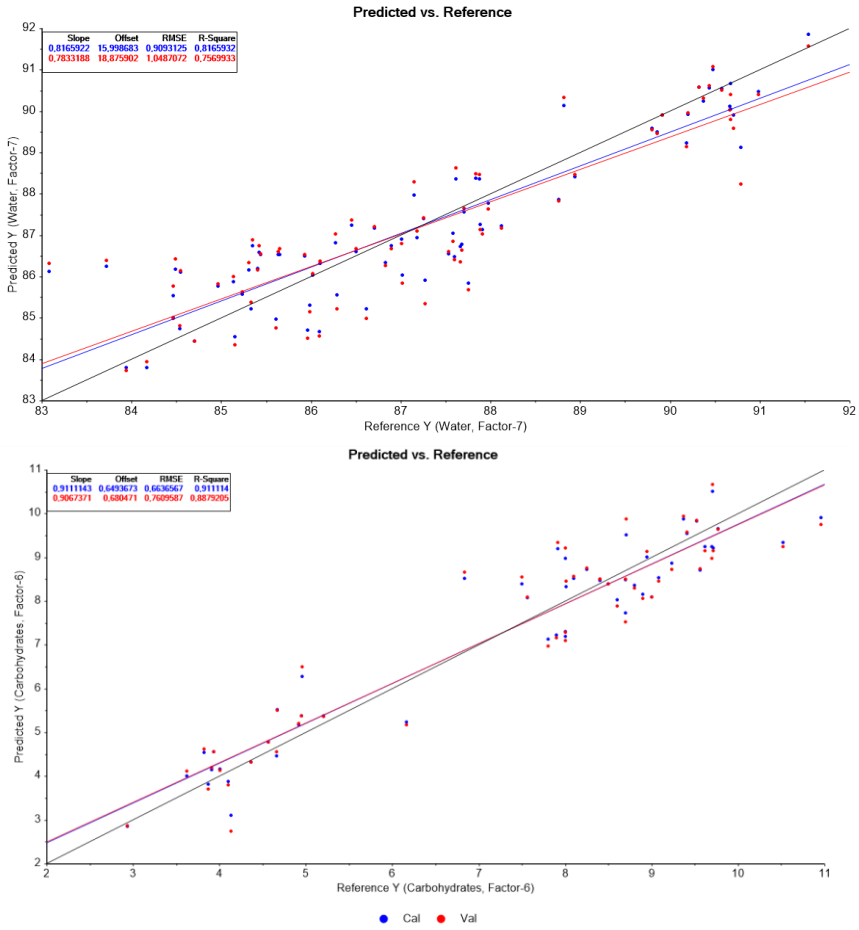

Figure 4. PLS-R models of (a) water content and (b) carbohydrate content. Calibration data points used for model training are shown in blue, and validation data points are shown in red.

The results of the PLSR modeling indicated that all parameters, except the lipid content, had R^2_{CV} between 0.82 and 0.95, indicating strong correlations between the spectral data and the reference parameters. The best results were observed in the protein prediction model, followed by the modeling of the total phenolic content (TPC), carbohydrates, salt, ash, and water contents. Shalev et al. (2022) also reported the successful use of NIR spectroscopy within the VIS-NIR region combined with machine learning to determine protein content in seaweed. This further suggests that MSI could potentially be a reliable technique for assessing protein content in seaweed. Even though two different analytical methods were used in the determination of protein content between

the two harvesting periods, the PLSR model showed excellent prediction, indicating that the difference in analysis did not affect the model performance. The PLSR model for water content covered a range of 83-92%, including the highest number of samples (n=78), resulting in a R^2_{CV} of 0.76 (n=54) and RMSECV of 1.05 (Figure 4a). Several studies have demonstrated the feasibility of MSI in predicting water content, for example, within the context of leaves (Tran & Grishko, 2004; Wei et al., 2021). The carbohydrate content ranged between 3-10.9%, with the PLSR model achieving a R^2_{CV} of 0.89 and RMSECV of 0.76 (Figure 4b). The range in TPC, protein, salt, and ash content of the studied biomass was relatively low, with parameters varying by 2-3%, while a wider range was observed in the water and carbohydrate content. While the models effectively analyze these specific parameters within the used ranges, the potential to further validate and refine the models lies in incorporating a broader variability within the parameters in future studies. The PLSR models for ash and TPC achieved R^2_{CV} value of 0.8 and RMSECV of 0.51 for ash, and R^2_{CV} of 0.92 and RMSECV of 0.24 for TPC. These results are similar to those achieved by Nepper-Davidsen et al. (2024)) who used NIR spectroscopy (range 1000–2500 nm) and PLSR for biomass profiling of the kelp *Ecklonia radiata* after freeze-drying. Their study also produced comparable models for ash and phlorotannins. Although the studies differ in the specific wavelengths used, the similar performance observed in this study for ash and TPC suggests that MSI, even when applied within varying ranges of the VIS/NIR spectrum, could be a suitable technique to predict ash and TPC in seaweed.

The lipid content range within this study was minimal, or from 0-0.5, which proved to be an insufficient range to construct a reliable calibration model. This result is consistent with the inherently low lipid content typically found in brown seaweed (Holdt & Kraan, 2011a). Due to the low lipid content, it is not commonly thought of as an important parameter for evaluating the quality of seaweed and its produce and would hence not be considered to affect the efficiency of using the MSI technology as a quality control within seaweed processing.

The construction of calibration models for total volatile basic nitrogen (TVB-N) for seaweed was insufficient, even though a broad range of TVB-N content (0.5 to 15.9 mg N/100 g sample) was detected in the samples. Hyperspectral imaging (HSI), coupled with deep learning, has been used to evaluate TVB-N content in fish fillets with promising results (Moosavi-Nasab et al., 2021). TVB-N is considered one of the most important freshness indicators for monitoring the quality and safety of high-protein foods, including seafood

products and fermented meat products (Jinadasa, 2014; Wang et al., 2016). However, since brown seaweed typically contains low protein contents (Holdt & Kraan, 2011a), TVB-N is not considered an important factor in evaluating the quality of seaweed in the same way as fish products. Model inaccuracies for TVB-N do hence not affect the use of MSI as a quality control tool in the seaweed industry.

3.4.2 Trace mineral content prediction

One of the key limiting factors of seaweed used as a food source is chemical hazards, including heavy metals (e.g., arsenic, cadmium, lead, and mercury) that are well-known food safety hazards. Furthermore, seaweed can accumulate iodine in high concentrations, especially some brown seaweed species. Iodine is an essential mineral used to synthesize thyroid hormones in the human body. Excessive intake of iodine can, however, lead to the inhabitation of both the synthesis and release of thyroid hormones (FAO & WHO, 2022). Therefore, if seaweed is intended for human consumption, monitoring trace mineral levels within the biomass is necessary to ensure safety before consumption.

The current monitoring methods for trace minerals include analysis with expensive instruments, such as Inductively coupled plasma mass spectrometry (ICP-MS), which are expensive, time-consuming, and destructive methods. Direct measurements or detection of trace minerals by using MSI are challenging because they do not absorb in the NIR, visual, and UV spectra. As MSI primarily measures absorption by molecular bonds (by NIR) (Osborne, 2006), and absorption due to electronic and vibrational excitations (by UV-VIS) (Martelo-Vidal & Vázquez, 2016), the detection of trace minerals by MSI is predominantly indirect. However, some transitional elements (such as Ni, Cu, Co, and Cr) can exhibit absorption features in the VIS/NIR regions under certain conditions, particularly when present at very high concentrations (>4000 mg/kg) (Shi et al., 2014). The quantitative presence of trace minerals can correlate with other substances that can be detected in the obtained spectra, such as water, protein, and carbohydrates in the same sample. Accurate detection will, therefore, depend on the trace minerals' interactions with other chemical components within the seaweed sample. Additionally, low concentration levels of some of the trace minerals could fall below the levels of detection (LOD) or quantification (LOQ) of typical MSI systems. However, research has shown that HSI and fluorescence HSI with mathematical modeling methods can effectively be used for secondary determination of the

content of heavy metals, such as cadmium and lead, in lettuce (Zhou et al., 2020; Zhou et al., 2023).

The PLSR model constructed to predict iodine (n=39) content in the seaweed samples achieved R^2_{CV}=0.83 and RMSECV=39.38, with iodine content ranging from approximately 60-420 mg/kg. This range is considered a suitable range for developing prediction models for the iodine content of *A. esculenta* and *S. latissima*, both before and after processing. The variability in iodine content across these species can be attributed to seasonal changes and can decrease by up to 90% when the biomass is blanched (Nielsen et al., 2020). However, the relatively high RMSECV indicates that while the technology performs adequately as a screening tool to determine if iodine is within a safe or dangerous range, it is less effective for precise measurements. Therefore, to strengthen the models further for applications in the seaweed industry, both biomass with lower and higher iodine content (since the content can be even lower or higher than values used in the current study (Krook et al., 2023; Nielsen et al., 2020)) and larger sample sizes should be considered. Using Pearson's correlation, iodine levels showed a correlation to water (r=-0.805), protein (r=0.899), and carbohydrates (r=0.914), indicating the indirect prediction of iodine could be correlated with these substances under the set conditions. Similar conclusions can be drawn from the PLSR models for predicting cadmium, arsenic, and lead levels, although the range for these trace minerals was much smaller. Pearson's correlation analysis showed a correlation between arsenic and carbohydrates (r=0.915) and protein (r=0.838), while cadmium showed a correlation between carbohydrates (r=-0.958), protein (r=-0.928) and water content (r=0.535), respectively. Lead showed a correlation with the carbohydrate (r=0.861) and protein (r=0.887) content.

3.4.3 Physical properties prediction

Physical properties, including pH, water activity (A_w), and color, are parameters that are considered to be important factors in evaluating the quality and stability of food products (Blikra et al., 2019; Damodaran et al., 2007; Roudaut, 2020). The pH is often analyzed in marine products to assess microbial and enzymatic activities during processing (Sánchez-García et al., 2021). Processing methods, such as acidification and fermentation, used in the food industry affect the pH of the biomass (Dauthy, 1995), highlighting the importance of evaluating pH in the quality assessment of processed seaweed if intended for human consumption. The obtained model for pH showed strong prediction potential, with R^2_{CV} of 0.94 and an RMSECV=0.28. The range of the pH

measurements was from approximately 3.0 to 6.7, indicating that the model is reliable in predicting pH levels during diverse seaweed processing, e.g. from harvesting where pH is often higher or around 6 to fully processed, acid-treated or fermented product which typically has a lower pH (Healy et al., 2023; Hrólfsdóttir et al., 2024; Sørensen et al., 2021). The spectral data does, however, not estimate the pH through direct measurements as done with H^+ sensitive electrodes but through evaluating the influence of pH levels on other chemical spectral properties of the samples (Jia et al., 2017). Hence, the pH levels can correlate to other factors seen within the spectral data, as mentioned earlier. Analysis of the data, as well as the negative Pearson's correlation observed between the pH and L-values (r=-0.660), show that the visible light spectrum contributes to the precise model prediction. Furthermore, the results of studies performed by Khodabakhshian et al. (2017), Rajkumar et al. (2012), and ElMasry et al. (2007) on multispectral and hyperspectral imaging to predict the pH of fruits showed that few wavelengths in the 435-993 nm range are optimal for predicting the pH value.

Color is often used in the food industry, including marine food products, to assess quality and homogeneity during production since the loss of quality is often associated with color change (Sánchez-García et al., 2021). Furthermore, color is an extremely important factor regarding consumers' food choices and acceptability (Rico et al., 2007). The PLSR models obtained for color (L-, a*-, and b*-values) varied between the parameters assessed. The model construction for predicting the b*-values (R^2_{CV}=0.53, RMSECV=3.97) was the least efficient, but most efficient for the a*-values (R^2_{CV}=0.91, RMSECV=0.79). This might indicate that the b*-value is less relevant for these seaweed species than the L- and a*-values. The color of *A. esculenta* and *S. latissima* varies significantly and can be affected by treatments such as blanching and acidification (Blikra et al., 2019; Hrólfsdóttir et al., 2024; Zhu et al., 2022). The range in L-, a*-, and b*-values used in the construction of the models are within a similar range as has been previously reported for both species (Stévant, Marfaing, et al., 2017; Zhu et al., 2022). However, if used as a quality control tool within the industry, further increase in the variability within the range would be required, where both fresh seaweed samples and samples in worse condition might be relevant to further distinguish between high-quality and low-quality biomass as the color changes when the seaweed biomass is stored fresh. Additionally, since the color parameters as measured on the CIE-lab scale are not linear variables and the spectral information has been reduced, using another modeling approach could enhance the accuracy of the predictions. Results, however, indicate that the PLSR models obtained for L-,

and a*-values could effectively be used to assess changes in the lightness and green color of the seaweed biomass.

3.4.4 Microbial properties prediction

Another quality control parameter to assure the safety of seaweed biomass is microbial contamination, where seaweed can contain various pathogenic microorganisms, most often the same microorganisms that are associated with fishery products, e.g. *Bacillus, Salmonella, pathogenic Escherichia coli, Staphylococcus aureus, Listeria,* and *Vibrio* species (FAO & WHO, 2022). The obtained PLSR model for total viable bacteria count (TVC) was made from only 48 samples in the range of 1.8-6.2 log cfu/g sample. The model had a moderate ability to predict TVC with R^2_{CV} of 0.67 and 0.93 RMSECV. Furthermore, when data is explored further, the TVC correlated somewhat with water content (r=0.549) and negatively with the protein and carbohydrate contents (r=-0.510 and -0.547, respectively). Lytou et al. (2022), obtained similar prediction efficiency in *A. esculenta* samples (RMSE ranging from 0.81-1.18, depending on the sample set used). The results indicate that either a larger dataset or other data processing and analysis methods were required.

3.4.5 Antioxidant properties prediction

The bioactive properties of seaweed have been explored a lot in the last few years, and some compounds in brown seaweed species have been proven to exhibit bioactive properties, including antioxidant properties (Holdt & Kraan, 2011a; Qin, 2018). These substances are then often extracted from the biomass and used for multiple applications, including cosmetic-, and pharmaceutical industries (Jacobsen et al., 2019). Currently, the methods used to identify active compounds (such as phenolic compounds) include expensive equipment, such as high-performance liquid chromatography (HPLC). Furthermore, to identify the compound's antioxidant performance, methods including oxygen radical absorbance capacities (ORAC), DPPH radical scavenging activities, and metal chelating (MC) can be used, which can be expensive and time-consuming to perform. However, the species used in the current study are not known for high antioxidant properties when measured by ORAC and DPPH.

The models for ORAC were obtained by using ORAC values between 10-113, which are considered low when compared to other brown seaweed species such as *Ascophyllum nodosum* or Fucus species (Wang et al., 2009). ORAC showed a strong linear relationship (with an R^2_{CV} of 0.89), but the RMSECV was relatively high, or 11.7. The ORAC values do, however, show a

negative correlation with water (r=-0.900), and a positive correlation with protein (r=0.884) and carbohydrates (r=0.902), which can hence be the main contributing factors to the model accuracy. The models for DPPH obtained a strong linear relationship, with moderate RMSEPCV (0.81), but these models were constructed from a narrower data range or IC50 numbers in the range of 0.3-8.2, compared to the model constructed for ORAC. Furthermore, models for DPPH radical scavenging activities correlated positively with water (r=0.907) and negatively with carbohydrates (r=-0.893) and protein (r=-0.945). The results indicate that these methods could be used to evaluate the antioxidant properties of the two brown seaweed species used in the current study, but models should, however, be strengthened by increasing the sample size and range.

3.4.6 Sensory quality prediction

Generic descriptive analysis (GDA) was performed on part of the seaweed samples. The constructed PLSR models for the sensory properties thus used smaller sample sizes - either 15 or 24 samples - compared to those developed for physicochemical and antioxidant properties. All parameters were assessed using samples harvested in 2022, while fewer parameters were assessed on samples harvested in 2023 (Table 2). The scale for sensory evaluation spans from 0 to 100, providing a broad spectrum for analysis. Certain parameters, such as sour, seaweed, and additional odors and flavors, along with texture parameters, offer a wide and applicable range for constructing reliable models. However, others, including rancid odor and flavor, spoilage odor and flavor, as well as metallic and sweet flavors, have a more limited range, which may affect the significance of the results. Nonetheless, using MSI or other mechanical techniques as a measurement tool for assessing sensory attributes offers an advantage that, unlike human judges, mechanical methods are not subject to the variability and personal taste differences that can affect the consistency of sensory evaluations, even among well-trained panelists. While there may be indications of potential trends, these findings should be approached with caution and require further validation. The results do, however, show indications that some of the sensorial properties can be predicted by using spectral data obtained from MSI, including textural attributes, as well as sour and seaweed odor and flavor.

Table 2. The coefficient of determination during calibration (R_C^2) and cross-validation (R_{CV}^2), the root mean squared error of calibration (RMSEC) and cross-validation (RMSECV), the range and sample size of sensory data obtained by Generic descriptive analysis (GDA), and the number of factors (latent variables) used for PLSR cross-validation.

	Calibration		Validation				
Sensory attribute	R_C^2	**RMSEC**	R_{CV}^2	**RMSECV**	**Range**	**Sample size**	**Number of factors**
Odor							
Sour	0.96	0.86	0.87	1.48	41-55	24	7
Seaweed	0.89	1.56	0.84	1.94	40-58	24	4
Extra	0.95	1.08	0.92	1.37	8-20	15	3
Rancid	0.99	0.06	0.98	0.07	1-2	15	2
Spoilage	0.98	0.06	0.93	0.14	1-2	15	7
Flavor							
Sour	0.95	1.56	0.79	3.35	43-69	24	7
Bitter	0.99	0.60	0.99	0.75	11-28	24	4
Salt	0.98	0.53	0.96	0.90	36-48	24	7
Sweet	0.94	0.32	0.88	0.47	8-11	24	7
Seaweed	0.95	1.25	0.88	1.98	38-55	24	7
Metal	0.98	0.71	0.98	0.90	7-20	24	4
Extra	0.98	0.90	0.97	1.09	6-20	15	2
Rancid	0.99	0.08	0.96	0.17	1-3	15	7
Spoilage	0.98	0.28	0.91	0.61	1-6	15	7
Texture							
Liquid	0.99	0.71	0.98	1.33	42-64	15	6
Tender	0.98	1.48	0.98	1.79	24-51	15	2
Astringent	0.97	1.95	0.96	2.30	21-49	15	1

The results are presented as responses from seven to nine trained panelists by using the GDA scale from 0-100. Values above 20 suggest that the tested attribute should be detectable by all, while values above 50 indicate strong properties.

4 Conclusion

By integrating the MSI technology into quality control processes within the seaweed industry, companies could ensure the quality and safety of their seaweed products. The ability to assess the quality of seaweed accurately and efficiently can lead to improved product standards, provide consumers with information about the biomass, and ensure transparency regarding its origin and composition. The results indicate that MSI is a promising tool for species recognition, as well as to evaluate the proximate composition, TPC, pH, and potentially some trace minerals, showing its potential as a non-invasive, rapid, and precise method for seaweed quality control. The strongest PLSR models obtained in the study included predictions of water content, carbohydrates, salt, ash, and pH with R^2_{CV} above 0.82 and relatively low RMSECV values. While the study demonstrated the use of MSI in seaweed for physicochemical and antioxidant properties, it is important to acknowledge its limitations, which is the need for both more data and a more extensive range within some of the parameters. Additionally, the accuracy of the technique is limited by the precision of the reference measurements used in the models' calibration. However, the non-invasive approach and rapidity of the MSI analysis represent considerable benefits. Exploring models focused on individual species, with a more extensive dataset and a greater variety of property ranges, could be advantageous. Additionally, standardizing these methods may further enhance their applicability and accuracy.

While the study focused on the application of MSI in assessing various physicochemical and antioxidant properties in seaweed biomass, there is a potential for exploring alternative predictive modeling methods. Given the exploratory nature of the study and the broad array of properties examined, using PLSR was considered the most appropriate due to its effectiveness in handling multiple correlated response variables and its suitability for initial exploratory analysis. Although PLSR provides valuable information, using different preprocessing methods for spectral data could further enhance the models' performance. Exploring alternative machine learning and deep learning methods may also provide improved predictive capabilities and better handle non-linear relationships in MSI data, addressing the limitations of PLSR in extracting non-linear features.

In conclusion, the results highlight the potential for broader applications of MSI within the seaweed industry. Considering the potential of MSI to assess the seaweed proximate composition and physicochemical and antioxidant properties, future research should explore its use in various stages

of processing, from the harvesting site to the evaluation of fully processed, ready-to-consume products. Expanding MSI applications and standardization could greatly improve quality control and safety measures across different seaweed species and throughout the entire production chain.

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6 CRediT authorship contribution statement

Anna Þóra Hrólfsdóttir: Writing – original draft, Writing – review & editing, Visualization, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

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Sigurjón Arason: Writing – review & editing, Supervision, Conceptualization.

María Gudjónsdóttir: Writing – review & editing, Validation, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization.

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7 Declaration of competing interest

The authors declare no competing interest.

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9 Appendices

9.1 Appendix A

9.1.1.1 Sample preparation and sampling 2022

The day following harvest, the *S. latissima* and *A. esculenta* samples harvested in 2022 were minced individually and either frozen in 1 kg plastic bags or treated with acid (lactic acid or citric acid) with a ratio of 60 mmol/kg for *S. latissima* and 106 mmol/kg for *A. esculenta*. Following the acid treatments, the samples were split into 1 kg plastic bags, vacuum packed, and thereafter stored at room temperature. The samples were then transported to the laboratory in Iceland. Sampling on acid-treated samples occurred three times over a 32-week storage duration, at week 4, week 16, and week 32 post-acid treatment. At each sampling point, one bag per treatment was split into three sample replicates (n=3). A part of the untreated seaweed biomass was frozen to allow analysis of the untreated biomass. This was chosen as a control since the analytical activities were performed in a laboratory in Iceland, requiring lengthy transport and fast deterioration of fresh seaweed biomass. Analyzing the fresh biomass was, therefore, not an option. The frozen biomass was stored at approximately -24°C till analyzed, but analysis of the frozen control samples took place at the same time as the first sampling of the acid-preserved biomass (at week 4).

9.1.2 Sample preparation and sampling 2023

Most of the *A. esculenta* biomass harvested in 2023 was transported to the processing facilities of SES and stored overnight in a cooler at approximately 4°C. The following morning, the seaweed was processed as follows. The biomass was minced to a particle size of approximately 5 mm in a meat grinder. Acids (either lactic or citric acid) in ratios 106 mmol acid/kg seaweed were then manually added to the minced biomass. The acid-treated biomass was then divided into 1 kg bags, which were vacuum-packed and sealed. The biomass was stored in a cold room (4°C) overnight, transported to Iceland via air-plane transportation (approximately 2 days), and then stored at room temperature (20-25°C) till analyzed approximately a month later. Raw material samples were prepared, as the year before, by adding minced biomass in 1 kg plastic bags, which were partially vacuum packed and frozen in a freezer at -18°C. The frozen control biomass was transported with the acid treated samples to Iceland, where it was stored at -24 °C at the laboratory until analyzed one month later.

Part of the harvested biomass was shipped fresh directly to Belgium in cooled transport. Upon arrival, the seaweed biomass was minced and fermented with two different inoculum, Flora Italia (a blend of *Pediococcus acidilactici, Lactobacillus curvatus, Staphylococcus xylosus*, and *Lactobacillus Sakei* cultures) and Vege inoculum (a blend of *Lactobacillus rhamnosus* and *Propionibacterium freudenreichii subsp. shermanii*). The fermentation process of the samples was performed by adding $1.0x10^{-9}$ CFU/g dry matter of seaweed. The blend was divided into 400 g plastic bags and then vacuum packed and stored at approximately 20°C till used one month later. One bag per treatment (frozen, acid-treated, and fermented) was split into three sample replicates.

9.1.2.1 Blanching and soaking treatments

Blanching treatments were performed on the seaweed biomass from 2023 to reduce its iodine content. Four blanching methods were performed: frozen minced and lactic acid-treated seaweed samples were soaked at 25°C for either 10 or 30 minutes or blanched at 80°C for either 20 seconds or 2 minutes. All four methods were performed on both the lactic acid treated and the minced frozen biomass. For the frozen minced biomass, only blanching at 80°C (for 20 seconds and 2 minutes) was performed.

Soaking treatments at room temperature (25°C) were performed by adding 150 g of seaweed biomass to an Erlenmeyer flask containing approximately 600 mL of tap water at room temperature for either 10 or 30 minutes. The flask was then emptied into a strainer with a 100 µm filter cloth. The samples were put in plastic containers and stored until used a few hours later.

Blanching treatments were performed by adding 150 g of seaweed biomass to approximately 600 mL of preheated 80°C hot tap water. The flasks were then placed into the heating bath at 80°C for either 20 seconds or 2 minutes, respectively. The samples were collected by pouring the blend into a strainer covered with 100 µm filter cloth. The samples were allowed to cool and stored in plastic containers until used a few hours later. Each treatment, e.g., blanching and soaking, was performed in triplicate.

An overview of the samples and treatments is given in Table SM 1.

Table A.1. Overview of seaweed samples used in the study. Storage time is in weeks post acid treatment of samples from storage trial in 2022.

Year	Sample name	Treatment	Specie	Storage time
2022	AE Control	Frozen	AE	
	P0 AE lactic acid	Treated with lactic acid	AE	4 weeks
	P1 AE lactic acid	Treated with lactic acid	AE	16 weeks
	P2 AE lactic acid	Treated with lactic acid	AE	32 weeks
	P0 AE citric acid	Treated with citric acid	AE	4 weeks
	P1 AE citric acid	Treated with citric acid	AE	16 weeks
	P2 AE citric acid	Treated with citric acid	AE	32 weeks
	SL control	Frozen	SL	
	P0 SL lactic acid	Treated with lactic acid	SL	4 weeks
	P1 SL lactic acid	Treated with lactic acid	SL	16 weeks
	P2 SL lactic acid	Treated with lactic acid	SL	32 weeks
	P0 SL citric acid	Treated with citric acid	SL	4 weeks
	P1 SL citric acid	Treated with citric acid	SL	16 weeks
	P2 SL citric acid	Treated with citric acid	SL	32 weeks
2023	AE - Control	Frozen	AE	NA
	AE - Lactic acid	Treated with lactic acid	AE	NA
	AE - Citric acid	Treated with citric acid	AE	NA
	AE - Fermented FI	Fermented with Flora Italia inoculum*	AE	NA
	AE - Fermented Vege	Fermented with Vege inoculum**	AE	NA
	AE – Control 80C 20 sec	Frozen, defrosted + blanched for 20 sec at 80°C	AE	NA
	AE – Control 80C 2 min	Frozen, defrosted + blanched for 20 sec at 80°C	AE	NA
	AE – Control 25C 10 min	Frozen, defrosted + blanched for 20 sec at 80°C	AE	NA
	AE – Control 25C 30 min	Frozen, defrosted + blanched for 20 sec at 80°C	AE	NA
	AE – Lactic 80C 20 sec	Treated with lactic acid + blanched for 20 sec at 80°C	AE	NA
	AE – Lactic 80C 2 min	Treated with lactic acid + blanched for 2 min at 80°C	AE	NA
	AE – Lactic 25C 10 min	Treated with lactic acid + blanched for 10 min at 25°C	AE	NA
	AE – Lactic 25C 30 min	Treated with lactic acid + blanched for 30 min at 25°C	AE	NA

9.2 Appendix B

Table B.1. Sensory attributes assessed in samples from 2022 and 2023. All attributes were evaluated in samples from 2022, but sensory attributes in Italian font were assessed in 2023.

Sensory attribute		Scale	Description
Odor			
	Sour	none || much	Acid, not sour due to spoilage
	Seaweed	none || much	Seaweed smell
	Extra odor	none || much	Extra smell, chemical smell, iodine, plastic paint
	Rancid	none || much	Rancid smell
	Spoilage odor	none || much	Overall spoilage odor due to microbial- and oxidation spoilage
Flavor			
	Sour	none || much	Sour, basic flavor
	Bitter	none || much	Bitter basic flavor
	Salt	none || much	Salt basic flavor
	Sweet	none || much	Sweet basic flavor
	Seaweed	none || much	Seaweed flavor
	Metal	none || much	Metallic-, iron flavor
	Extra flavor	none || much	Extra flavor, chemical, iodine
	Rancid	none || much	Rancid flavor
	Spoilage	none || much	Overall spoilage flavor due to microbial- and oxidation spoilage
Texture			
	Liquid	none || much	Amount of liquid in sample
	Tender	tough|| tender	Evaluated by chewing
	Astringent	none || much	Dried mouth

9.3 Appendix C

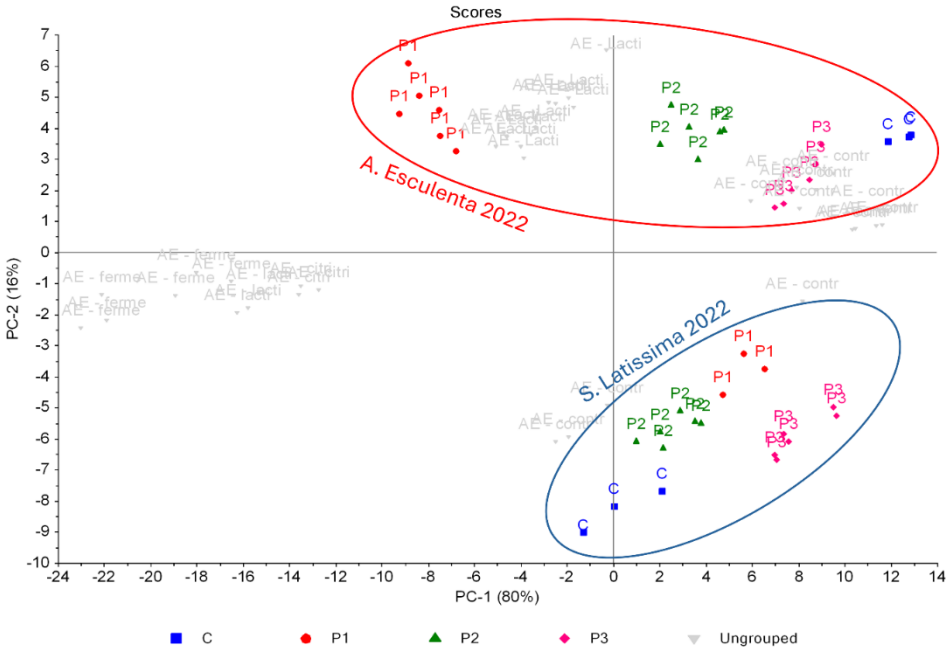

Figure C.1. PCA plot of spectra obtained of frozen control, acid preserved, fermented, and blanched seaweed samples used in the study. The blue oval shape shows S.latissima samples from 2022, and the red oval shape represent A. esculenta samples from 2022 (excluding blanched samples). Samples are labeled by their sampling point (P1, P2, P3) and C for control samples. Fermented and blanched samples are labeled as ungrouped as they were not stored.

9.4 Appendix D

9.4.1 Physicochemical and bioactive properties of the seaweed samples

9.4.1.1 Chemical properties

The chemical composition of the seaweed samples varied between species, while minor differences were observed within species (Table 1). A detailed and comprehensive description of the physicochemical and bioactive properties of samples from 2022 can be seen in a recent publication by Hrólfsdóttir et al. (2024). The water content of the *S. latissima* samples used in the study was in the range of 88.9-91.5 and A. esculenta 83.0-88.8 g/100 g sample, respectively. Both species had low lipid content but higher ash, carbohydrate, and salt content, as most brown seaweed species are known for (Holdt & Kraan, 2011a). *A. esculenta* had notably higher protein (1.3-1.8 g/100 g sample) and carbohydrate (6.9-10.9 g/100 g sample) content compared to *S. latissima* samples (0.6-0.8 g/100 g of protein and 2.9-6.2 g/100 g of carbohydrates). The protein content of the seaweed samples was determined by using two different methods, the Kjeldahl method and the Dumas method. Both methods estimate the total nitrogen within the biomass, and the crude protein content is calculated with a conversion factor of 5. Even though studies have indicated that the Dumas method often reports slightly higher crude protein levels (Jung et al., 2003; Thompson et al., 2002), the protein content of *A. esculenta* samples that were analyzed with different methods was within the same range, indicating that method used did not affect the results significantly. Trace mineral analysis indicated higher levels of arsenic, lead, and cadmium in *A. esculenta*, while the iodine concentrations in *S. latissima* contained higher levels of iodine. Across the different treatments and years (2022 and 2023), the chemical and trace mineral profiles of A. esculenta remained consistent, except for an increased iodine content in the 2023 samples. Additionally, blanching performed in 2023 reduced the iodine, salt, and ash content in the samples when compared to the control samples.

The total volatile basic nitrogen (TVB-N) of the seaweed samples ranged from 0.5 to 16 mg N/100 g sample. Overall, the A. *esculenta* samples exhibited higher TVB-N levels, ranging from 2-15.9 mg N/100 g, compared to *S. latissima* (0.5-2.7 mg N/100 g sample), which is likely due to *A. esculenta's* higher protein content. Additionally, treatments and storage times significantly influenced the TVB-N levels, where fermented seaweed samples showed the highest values (7.1-15.9 mg N/100 g sample), and the TVB-N levels gradually

increased of the acid-preserved biomass throughout the storage time of both species.

9.4.1.2 Physical properties

The pH of the seaweed samples varied depending on the treatment, harvesting period, and storage time, but a slight decrease was noted in the acid-treated samples during storage. The frozen control samples exhibited higher pH values, ranging from 5.2 to 6.0, compared to the acid-treated (3.5-4.1) and fermented samples (3.7-3.9). The control samples harvested in 2022 had higher pH values than those from 2023, and acid-treated samples from 2022 were slightly more acidic than samples harvested in 2023. The water activity (a_w) of the samples remained consistent, ranging from 0.98-0.99.

The color was assessed by recording L-, a^*-, and b^*-values. The acid-treated samples of both species had significantly higher L-values compared to the control samples from 2022. The a^*-values were higher in *S. latissima* samples (1.6-4.9) compared to *A. esculenta* samples (-1.3 to 4.1), which significantly increased with acid treatment for both species. Additionally, the a^*-values in *A. esculenta* samples harvested in 2022 were higher (-1.3 to 4.1) than the values obtained in 2023 (-4.1 to -0.3). The b^*-values increased in samples from 2022 with acid treatment with slight variation between species, e.g. ranging from 2.9 to 8.8 in *S. latissima* and 3.5 to 11.1 in *A. esculenta* samples. Furthermore, higher b^*-values were obtained in the control, the acid preserved, and fermented *A. esculenta* samples harvested in 2023 than in 2022.

9.4.1.3 Microbial properties

The total viable count (TVC) of the seaweed samples was higher in the *S. latissima* samples than in the *A. esculenta* samples. Additionally, the TVC was significantly lower in the frozen control samples than in the acid-treated samples for both *A. esculenta* and *S. latissima*. The *A. esculenta* samples harvested in 2023 showed similar results as samples from 2022, with a higher TVC in the acid-treated samples than in the frozen controls.

9.4.1.4 Total phenolic content and antioxidant properties of the seaweed samples

The total phenolic content (TPC) of *S. latissima* samples harvested in 2022 contained significantly lower TPC, ranging from 0.4-0.6 g PGE/100 g extract compared to *A. esculenta* samples, which ranged from 1.5-3.2 g PGE/100 g extract. The highest TPC content was observed in the control samples of *A.*

esculenta, which then decreased with acid treatment. The assessment of antioxidant properties showed similar tendencies, with *S. latissima* showing both lower ORAC values (11-23 µmol TE/g extract) and DPPH radical scavenging activities (IC50 value of 5.1-8.2 mg/ml) than *A. esculenta* (ORAC value of 114 µmol TE/g extract and DPPH IC50 value of 0.3-2.0 mg/ml). Additionally, the DPPH radical scavenging activity of the *A. esculenta* samples followed the same tendency as the TPC, indicating a decrease in antioxidant activities with acid treatment.

9.4.1.5 Sensory evaluation

The main differences in the sensory data from 2022 were observed in the textural properties of the seaweed samples. The texture of *A. esculenta* was more astringent and tougher compared to the texture of *S. latissima*. Additionally, the preserved seaweed samples exhibited strong flavors and odors of seaweed and sourness, with values generally around or above 50, except for seaweed flavor and odor of lactic acid treated *S. latissima.* Fermented samples notably reached a score of 69 on the GDA scale for sour flavor. Salt flavor was also dominant in all the samples, reaching up to a score of 48. Other attributes tested in the seaweed samples scored lower, as shown in the range provided in Table 1.