Master's thesis

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Biochemical Composition of the Drip Loss From Thawing of the Macroalgae *Saccharina latissima* and *Alaria esculenta*

Effect of freezing and thawing on the macroalgae raw material

Master's thesis in Chemical Engineering and Biotechnology
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Preface

This master's thesis was carried out at the Department of Biotechnology and Food Science, at Faculty of Natural Sciences, at the Norwegian University of Science and Technology (NTNU) in Trondheim, Norway. The laboratory work was performed at the Food Chemistry Laboratory at Gløshaugen. This thesis was executed as an extension of a specialisation project (TBT4500) carried out during the fall of 2019, therefore, some parts of the introduction and some results are based on this work.

I would like to thank my supervisor, Turid Rustad, for her guidance and support throughout the execution of this thesis, and for always answering my questions. Also, thanks to Siri Stavrum for all her help and advice regarding the laboratory work, and for performing the HPLC analyses for the amino acid analyses, and thanks to Olav Andreas Aarstad for all his help with the carbohydrate analysis. Additionally, I would like to thank Marte Schei at SINTEF Fisheries and Aquaculture for performing the CN-analysis, and the people at SINTEF Industry for performing the mineral analysis.

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Randi Sund

Abstract

Macroalgae are used as food for human consumption and raw material for the extraction of additives and functional compounds in various industries such as the food, pharmaceutical and cosmetic industries. Recent years have shown increased scientific and industrial interest in macroalgae because of their potential within the sustainable production of food, energy, and chemicals. There has also been an increased consumer interest due to their beneficial nutritional profile, and their content of potentially health-promoting biologically active compounds. To avoid decay of the biomass after harvesting appropriate preservation methods are essential during transportation and storage. Freezing is a popular preservation method. However, freezing and thawing of macroalgae results in a substantial loss of liquid. In this study, the biochemical composition of drip loss from thawing of frozen samples of the macroalgae species *Saccharina latissima* and *Alaria esculenta* was studied. This was done to assess how the macroalgae raw material was affected by freezing and thawing, and if the drip losses could be used for value-added products in industry, either directly or for extraction of certain compounds. The concentrations of moisture, dry matter, ash, proteins, total amino acids, and some carbohydrates (mannitol, fucose, glucose, galactose, and mannose/xylose) were determined for the macroalgae raw material, drip loss from thawing and leftover raw material after removal of drip loss. Additionally, the concentrations of minerals, phenolic compounds, and free amino acids, as well as the antioxidant activity were determined for the drip losses.

Thawing of *Saccharina latissima* and *Alaria esculenta* raw materials resulted in drip losses of 49% and 55% of the raw material wet weight, respectively. The drip losses consisted of over 90% of moisture and a small amount of dry matter, of which about 70% was minerals. The mineral contents of both drip losses were dominated by sodium and potassium. The iodine concentration was also high, especially for the *Saccharina latissima* sample. Some arsenic, mercury and cadmium were also present in the drip losses. The concentrations of phenolic compounds and proteins were low, and these, as well as the antioxidant activity, were determined to be higher in the *Saccharina latissima* drip loss than in the *Alaria esculenta* drip loss. The protein content seemed to be mostly small peptides or free amino acids. Of the quantified carbohydrates, mannitol was the most abundant in the drip loss samples. Some fucose and glucose also indicated that the samples contained traces of fucoidan and laminaran. Alanine was the most abundant amino acid in the drip losses, followed by lower amounts of glutamic acid and aspartic acid. These results did not indicate any apparent direct uses for the drip losses. The most likely uses seemed to be for extraction of iodine or mannitol for further industrial uses. This indicated that the nutritional value of the *Saccharina latissima* and *Alaria esculenta* raw material was not substantially affected by freezing and thawing. Additionally, freezing and thawing could result in a healthier mineral profile of the leftover raw material, due to removal of iodine and heavy metals. However, loss of mannitol, alanine, glutamic acid and aspartic acid could cause an altered flavour profile of the leftover raw material.

Sammendrag

Makroalger brukes som mat og som råmateriale for ekstraksjon av tilsetningsstoffer og funksjonelle forbindelser i blant annet matindustrien, kosmetikkindustrien og farmasøytisk industri. I løpet av de siste årene har den vitenskapelige og industrielle interessen for makroalger økt på grunn av deres potensiale innen bærekraftig produksjon av mat, energi og kjemikalier. Det har også vært en økt interesse blant forbrukere på grunn av det gunstige næringsinnholdet i makroalger og innholdet av potensielt helsefremmende funksjonelle forbindelser. Passende konserveringsmetoder under frakt og lagring er nødvendige for å unngå forråtnelse av biomassen etter høsting. Frysing er en populær konserveringsmetode, men frysing og tining av makroalger fører til et betydelig drypptap. I denne oppgaven ble den kjemiske sammensetningen av drypptap fra tining, også kalt tinevann, av frosne prøver av makroalgeartene *Saccharina latissima* og *Alaria esculenta* analysert. Dette ble gjort for å se hvordan makroalgene ble påvirket av tining og frysing, og om tinevannprøvene kunne bli brukt som en supplerende inntektskilde i industrien. Konsentrasjonene av fuktighet, tørrstoff, aske, proteiner, totale aminosyrer og noen karbohydrater (mannitol, fukose, glukose, galaktose og mannose/xylose) ble bestemt for råstoffene, tinevannprøvene og restråstoffene etter tining og fjerning av tinevann. I tillegg ble konsentrasjonene av mineraler, fenoler og frie aminosyrer, og antioksidantaktiviteten bestemt for tinevannprøvene.

Tining av frossent *Saccharina latissima* og *Alaria esculenta* råstoff ga tinevann som utgjorde henholdsvis 49% og 55% av råstoffets våtvekt. Tinevannprøvene inneholdt over 90% fuktighet og litt tørrstoff. Over 70% av tørrstoffet var mineraler. Mineralinnholdet var dominert av natrium og kalium i begge tinevannprøvene. Jodinnholdet var også ganske høyt, spesielt for *Saccharina latissima* tinevannet. Det var også noe arsenikk, kvikksølv og kadmium i tinevannprøvene. Fenol- og proteinkonsentrasjonene var lave, begge disse, i tillegg til antioksidantaktiviteten, var høyere i *Saccharina latissima* tinevannet enn i *Alaria esculenta* tinevannet. Proteininnholdet virket som om det besto hovedsakelig av små peptider og frie aminosyrer. Av de målte karbohydratene var mannitol den det var mest av i tinevannprøvene. Noe fukose og glukose antydet også at det var noe fucoidan og laminaran i prøvene. Av aminosyrer var det mest alanin i tinevannprøvene, etterfulgt av mindre mengder glutaminsyre og asparaginsyre. Disse resultatene ga ingen innlysende bruksområder for tinevannprøvene. Ekstraksjon av jod og mannitol for videre industriell bruk virket som de mest realistiske bruksområdene. Dette antydet at næringsverdien av råstoffene ikke ble vesentlig påvirket av frysing og tining. Det er mulig at tining og frysing gjorde at restråstoffene hadde et sunnere mineralinnhold enn råstoffet på grunn av fjerning av jod og giftige tungmetaller. Tap av mannitol, alanin, glutaminsyre og asparaginsyre kan bety at smaken av restråstoffet er annerledes enn av råstoffet.

Contents

1 Introduction

Macroalgae, commonly known as seaweeds, are macroscopic multicellular marine algae. The different species of macroalgae are usually divided into three main groups based on their pigmentation: brown macroalgae (Phaeophyta), red macroalgae (Rhodophyta), and green macroalgae (Chlorophyta) (Banach et al., 2020). Macroalgae have high growth rates and grow by photosynthesis and absorption of nutrients from surrounding waters (Kraan, 2013). Macroalgae are a traditional part of Asian cuisine. However, utilisation of macroalgae also has a long history in Europe where they were used as food for human consumption as well as animal feed and fertilisers, especially in times of food shortage (Mæhre et al., 2014). Today, macroalgae are still used as food for human consumption, in animal feed and as fertilisers (FAO, 2018a). Additionally, they are used as raw material for additives and functional compounds in numerous industries including the food, cosmetic, and pharmaceutical industries (FAO, 2018a; Stévant, 2019).

1.1 Macroalgae Composition and Bioactivities

Macroalgae have a highly variable composition, depending on factors such as species, season and habitat (FAO, 2018a). However, most macroalgae contain many of the same major components. They contain, like other plants, minerals, proteins, lipids, polysaccharides, polyphenols and certain vitamins. Additionally, macroalgae have a very high moisture content, ranging between 61-94% of wet weight (Holdt & Kraan, 2011). From a nutritional perspective, macroalgae contain many important macro- and micronutrients, such as omega-3 and omega-6 fatty acids, vitamin B_{12}, iodine and dietary fibre (Sá Monteiro et al., 2019). Macroalgae are also low in calories due to the overall lipid content being low and because most of the carbohydrate content is dietary fibres not taken up by the human body (Holdt & Kraan, 2011). Additionally, macroalgae contain numerous compounds with demonstrated and suspected biological activities with potential health benefits and applications (Sá Monteiro et al., 2019). This makes macroalgae interesting for extraction of biologically active compounds for cosmetics and pharmaceuticals, amongst others, as well as a potential functional food providing health benefits beyond basic nutrition (Holdt & Kraan, 2011). The ranges of the main components present in brown, red and green macroalgae are given in Table 1.1.

Table 1.1: Ranges of moisture, ash, protein, lipid, carbohydrate and phenolic content of the three main macroalgae groups; brown, red and green macroalgae. Moisture content is given in percent of dry weight, while all other values are given in percent of dry weight. Averages, where found, are given in parentheses.

	Brown macroalgae	**Green macroalgae**	**Red macroalgae**
Moisture	61-94[a]	74-87[b]	70-91[b,c]
Ash	8.7-52[d,e] (30)[f]	11-55[a] (29)[f]	7-39[b] (19)[f]
Protein	1.2-24[a] (12)[f]	4-44[a] (24)[f]	3.8-50[a],[g] (27)[f]
Lipid	0.1-20 (3)[h]	0.3-17 (4.1)[h]	0.05-6.2 (1.4)[h]
Carbohydrate	4-84[a,i]	15-65[a]	24-76[a,b]
Phenolic	0.084-14[a,j]	0.15-0.9[a,k,l]	0.17-1.9[g]

[a]Holdt and Kraan (2011); [b]Olsson et al. (2020); [c]Wen et al. (2006); [d]Nielsen et al. (2016); [d]Forbord et al. (2020); [f]Tamayo Tenorio et al. (2018); [g]Naseri et al. (2019); [h]Wielgosz-Collin et al. (2016); [i]Schiener et al. (2014); [j]Marinho et al. (2019); [k]Uribe et al. (2019); [l]Figueroa et al. (2014)

1.1.1 Minerals

The ash content of macroalgae, reflecting mineral content, is high compared to vegetables. This is attributed to their capacity to retain inorganic marine substances due to the characteristics of their cell-surface polysaccharides (Torres et al., 2019). These minerals and trace elements are absorbed from the surrounding waters, accumulating in the macroalgae (Sá Monteiro et al., 2019). Over 50 different minerals and trace elements are present in macroalgae, many of which are required for the human body's physiological functions. This includes minerals such as potassium, calcium, iron, magnesium, selenium, iodine, manganese, and cobalt (Torres et al., 2019).

Due to their ability to absorb compounds from surrounding waters, macroalgae may also accumulate harmful and persistent contaminants present in their environment. Such contaminants include inorganic arsenic, lead, cadmium and mercury, which pose significant health risks if consumed by humans (Sá Monteiro et al., 2019). Additionally, some macroalgae species, especially brown macroalgae, have been shown to contain very high levels of iodine. This poses another potential health risk associated with consumption of macroalgae, as an excessive iodine intake can affect the thyroid function in humans (Duinker et al., 2016; Holdt & Kraan, 2011; Mæhre et al., 2014). Because of this, monitoring and evaluation of the presence of harmful components and up-to-date guidelines of threshold values for different components in light of macroalgae as food are necessary to assure that consumption of macroalgae and macroalgae-based food products are safe (Barbier et al., 2020).

Despite the concerns around the high iodine content of macroalgae, iodine is

an essential trace mineral for humans and animals (Banach et al., 2020). Iodine deficiency in humans is a known and widespread problem, causing disorders such as goitre and cretinism (Mæhre et al., 2014). Iodine deficiency is also, according to the World Health Organisation (WHO), the most prevalent and easily preventable cause of impaired cognitive development in children (FAO, 2018b). Because of this, the presence of iodine in macroalgae is generally considered beneficial. However, the final concentration of iodine in a macroalgae food product is subject to variations depending on factors such as macroalgae species, season, and environment, as well as the loss of iodine during food processing (Banach et al., 2020). Additionally, the bioavailability of the iodine and the frequency of intake by consumers should be taken into consideration when assessing the risk of excessive iodine intake from consumption of macroalgae (Duinker et al., 2016).

1.1.2 Proteins, Peptides and Amino Acids

Proteins are important nutritional elements in food for human consumption. They have a major role in the growth and maintenance of the human body, and are energy giving nutrients, along with carbohydrates and lipids (Mæhre et al., 2018). Protein, peptide and amino acid composition of macroalgae have been shown to vary with habitat and especially season. The protein concentration has generally been found to be highest amongst red and green macroalgae, while brown macroalgae generally contain much lower amounts of protein (Torres et al., 2019). Several commonly consumed macroalgae species have been shown to have protein contents as high as traditionally consumed pulses, such as soybean and leguminous plants, or eggs (Dumay & Morançais, 2016).

Many macroalgae species have been found to contain significant amounts of high quality proteins for human consumption. This is based on the amino acid composition and the digestibility of the proteins (Bak et al., 2019). Most macroalgae contain all the amino acids that are essential in the human diet (Holdt & Kraan, 2011). However, bioaccessibility of macroalgae proteins, similarly to other plant protein, have been shown to be inferior to that of animal origin. This is due to the complex polysaccharide structure of macroalgae, which could reduce the accessibility of the proteins to the gastrointestinal enzymes, as well as their content of anti-nutritional factors, such as phenolic compounds, phytic acids, and protease inhibitors (Mæhre et al., 2016). Macroalgae proteins have been shown to be tightly bound to polyphenols (Stern et al., 1996), and, especially in brown macroalgae, high phenolic content can limit protein bioaccessibility (Holdt & Kraan, 2011). The formation of protein-phenolic complexes can influence protein structure, solubility, hydrophobicity, thermal stability and the isoelectric point. Additionally, it can result in the blocking of some amino acid residues. This can affect the biological properties of the protein, including the digestibility and utilisation of food proteins (Seczyk et al., 2019). Red and green macroalgae generally contain lower amounts of phenolic compounds and higher amounts of protein, indicating that protein accessibility is most likely not as limited by phenolic content in green and red macroalgae as in brown macroalgae (Mabeau & Fleurence, 1993). The dominant amino acid forms present in macroalgae have been shown to be aspartic and glutamic acids,

giving macroalgae an umami taste (Torres et al., 2019). Additionally, alanine and glycine are contributors to the characteristic taste of Nori (Holdt & Kraan, 2011), a widely consumed macroalga (Fleurence, 2016).

In addition to containing essential amino acids, making seaweed protein very favourable for human consumption, there are also proteins present in seaweeds that have been shown to possess various biological activities. Examples of such proteins are lectins and phycobiliproteins (Holdt & Kraan, 2011; Torres et al., 2019). Demonstrated bioactivities of lectins include antibiotic, anti-HIV, cytotoxic and anti-inflammatory (Holdt & Kraan, 2011). Phycobiliproteins, which are oligomeric coloured proteins found in red seaweeds, have been shown to posses bioactivities such as antimicrobial, antioxidant, anti-inflammatory, and neuroprotective (Torres et al., 2019).

1.1.3 Lipids

Macroalgae have a low calorie content, partly due to their low lipid content, which is usually lower than 5% of dry weight (Uribe et al., 2019). Of the lipids present the levels of polyunsaturated fatty acids are relatively higher than in vegetables. These fatty acids, such as omega-3 and omega-6 fatty acids, are known to have various health benefits in humans (Torres et al., 2019). This increases the nutritional benefit of macroalgae consumption, as these fatty acids are essential in the human diet and macroalgae are one of the only non-fish sources of natural omega-3 long-chain fatty acids (Badmus et al., 2019; FAO, 2018a). Other lipids present in macroalgae include phospholipids, glycolipids, tocopherols, sterols and cartenoids such as β-carotene, lutein, and violaxanthin in red and green algae, and fucoxanthin in brown algae (Holdt & Kraan, 2011).

1.1.4 Polysaccharides

Macroalgae are often rich in polysaccharides. However, which polysaccharides are present is species-specific and varies significantly between the major macroalgae groups. The common cell wall and storage polysaccharides present in brown macroalgae are alginic acid, fucoidan, laminarin and sargassan. While common polysaccharides in green macroalgae are sulphuric acid polysaccharides, sulphated galactans and xylans, and in red macroalgae, agars, carrageenans, xylans, floridean starches, water-soluble sulphated galactans and porphyrans are most common. Other common polysaccharides are cellulose and hemicellulose as cell wall polysaccharides (Holdt & Kraan, 2011). Macroalgae are known for containing agars, carrageenans, and alginates, often called hydrocolloids, that are widely used in the food industry, amongst many others. Many of the polysaccharides present in macroalgae are dietary fibres, meaning that they are not digested by humans. Macroalgae are therefore a good source of dietary fibre compared to many other terrestrial foods. Dietary fibres are important for gut health, providing soothing action, adding bulk to the digestive tract and lowering the cholesterol uptake (Torres et al., 2019). In addition to dietary fibre, macroalgae contain numerous polysaccharides with demonstrated or

suspected bioactivities (Holdt & Kraan, 2011). Especially sulphated polysaccharides from macroalgae have been shown to possess interesting bioactivities, enabling them to act as antithrombotic, antiallergic, anticancer, osteogenic, and cardioprotective agents (Torres et al., 2019).

1.1.5 Phenolic Compounds

In general, green and red macroalgae have low concentrations of phenolic compounds compared to brown macroalgae, which are rich in phlorotannins (Holdt & Kraan, 2011). Macroalgae contain several different types of polyphenols, such as phlorotannins in brown macroalgae (Singh & Sidana, 2013), and flavonoids in brown and red macroalgae (Marinho et al., 2019). The polyphenols present in macroalgae have been shown to possess strong antioxidant activities, and are often more potent than those from terrestrial plants (Tenorio-Rodriguez et al., 2017). Polyphenols from macroalgae have also shown other bioactivities including radiation protective, antibiotic, and antidiabetic effects (Holdt & Kraan, 2011). However, as mentioned in Section 1.1.2, phenolic compounds are often bound to proteins in protein-phenolic complexes, and in the same way that this can impact the biological properties of proteins, it can influence the bioaccessibility and activity of phenolic compounds (Seczyk et al., 2019).

1.1.6 Vitamins

Macroalgae contain both water- and fat-soluble vitamins, and are a good source of vitamins A, B_1, B_{12}, C, D, and E, as well as riboflavin, niacin, pantothenic acid, and folic acid (Baweja et al., 2016). Vitamin E includes α-, β-, and γ- tocopherol (Holdt & Kraan, 2011), all present in brown macroalgae, while green and red macroalgae only contain alpha tocopherol (Baweja et al., 2016). Vitamin K is also present in some species. The presence of vitamin B_{12} in macroalgae is particularly interesting, as this is a vitamin that is generally only present in animal products, and is important to mitigate the effects of aging and anemia (Torres et al., 2019). Macroalgae can therefore be used as a vegan source of vitamin B_{12}. Additionally, some cartenoids present in macroalgae are vitamin precursors, such as β-carotene, which has provitamin A activity (Holdt & Kraan, 2011).

1.2 Macroalgae Industry

In 2016, about 31 million tonnes of aquatic plants were harvested globally for direct consumption or further processing. This included macroalgae and a much smaller volume of microalgae, and comprised just over 27% of all global aquaculture production. The global production of farmed macroalgae in 2016 was just over 30 million tonnes. This represents a large increase in the industry as the global production of aquatic plants in 1995 was 13.5 million tonnes. The main producers in 2016 were China and Indonesia, combined producing over 85% of all farmed macroalgae. Some macroalgae species are produced almost exclusively for human consumption, while others are used in products such as animal feed, fertilisers, pharmaceuticals, and cosmetics. About 221 species of macroalgae are of commercial value (FAO, 2018a).

A major part of the macroalgae industry revolves around the industrial processing of macroalgae for extraction of hydrocolloids such as agars, carrageenans and alginates due to their gelling, emulsifying, thickening and stabilising properties as food additives (Torres et al., 2019). This is a growing industry illustrated by the rapid growth in the farming of tropical macroalgae in Indonesia for the production of carrageenan. The increase in the macroalgae farming output of Indonesia from less than four million tonnes in 2010 to over 11 million tonnes in 2015 and 2016 has been the major contributor to growth in the production of farmed aquatic plants in the recent past (FAO, 2018a).

Due to the increased focus on the sustainable production of food, chemicals, and energy, as well as discoveries of novel bioactivities in compounds isolated from macroalgae, interest in macroalgae as a bioresource has substantially increased in recent years (Barbier et al., 2020; Kraan, 2013; Tenorio-Rodriguez et al., 2017; Torres et al., 2019). This will be discussed further in the following sections.

1.2.1 Food Security for the Future and Macroalgae Sustainability

By 2050 it is estimated that the world's population will reach 9.1 billion (FAO, 2009). Most of this population increase is expected to occur in developing countries, causing an acceleration of urbanisation. In order to feed this growing population food production will need to increase by 70 percent. However, increased biofuel production from food crops and climate change are major concerns regarding long-term food security. Biofuel production increased more than threefold from 2000 to 2008, and in 2007-08 the total usage of coarse grains for the production of ethanol was about 10% of the global production. A continued increase in the use of food resources for the production of biofuels would have serious implications for food security in the future (FAO, 2009). In addition, agriculture is a big contributor to environmental changes due to nitrate and ammonia pollution of ground water, greenhouse gas emissions, and deforestation. A further increase in the agriculture sector could therefore intensify these environmental changes (Mæhre et al., 2016). This implies that while attempting to increase food production, the agriculture sector will also have to adapt and contribute to the mitigation of climate change

and aid in the preservation of natural habitats and biodiversity. While facing these challenges, they will also have to compete for land and water resources with new urban settlements. This implies that to increase food production along with this population growth, farmers will need to produce more food from less land (FAO, 2009). Overcoming these obstacles involves finding new technologies as well as new and sustainable food and protein sources (FAO, 2009; Mæhre et al., 2016).

Macroalgae have been mentioned to have great potential as an alternative food source due to their favourable growth conditions and abundance in marine environments all over the world (Mæhre et al., 2016). They are considered a sustainable resource as they are cultivated in the sea and therefore does not require any arable land or fresh water, as well as minimal human intervention. In addition, they absorb nutrients from the surrounding waters, eliminating the need for fertilisers. They also have high reproduction rates and high biomass yields. Furthermore, due to their fast growth rates they help reduce atmospheric greenhouse carbon through photosynthesis and supply oxygen to the sea. By doing this, they act as short term carbon sinks and help alleviate ocean acidification (Torres et al., 2019). Many macroalgae species have been shown to contain protein of high quality (Bak et al., 2019), making them a possible source of vegan protein (Torres et al., 2019).

Macroalgae have also been proposed as an alternative feedstock for biofuels due to the possibility of sustainable cultivation and their growth rates exceeding those of terrestrial biomass. The only commercially available biofuels today are first generation biofuels, mainly bioethanol and biodiesel. Bioethanol is produced from sugar canes and corn, and biodiesel from rapeseed, this requires arable land and enormous quantities of water. Therefore, increasing production of these first generation biofuels will compete with the increasing need for food. This competition with food production has lead to the development of second generation biofuels from lignocellulosic biomass, such as wood and agricultural waste. These biofuels do not compete directly with food production as the feedstock cannot be used as food, however, they do require land and fresh water resources. The use of macroalgae as feedstock for biofuels could improve the sustainability of the biofuel production due to their higher carbohydrate levels, biomass yields, and widespread availability, as well as their ability to capture carbon dioxide (CO_2) and the fact that their cultivation does not compete with resources needed for food production. Also, their ability to absorb nutrients from surrounding water makes them suitable for integrating in wastewater treatment processes to reduce pollution, which further increases the sustainability of macroalgae-based biofuels (Kraan, 2013). Additionally, waste streams from macroalgae processing, for instance leftover biomass from hydrocolloid production, could be used as feedstock for biofuels in a biorefinery approach to reduce waste and achieve full utilisation of the macroalgae biomass (Torres et al., 2019). Challenges related to macroalgae-based biofuel production includes the development of cost-effective cultivation, harvesting, transport and processing methodologies. Additionally, the high mineral content has been mentioned to be a potential challenge (Kraan, 2013).

Macroalgae biomass has been shown to have potential for use in sustainable production of plastics and other valuable chemicals such as pigments. Cultivation of macroalgae can aid in the recycling of nitrates and phosphates from run-off from agriculture and from fish farming (Kraan, 2013).

1.2.2 Consumer Trends and Functional Food

While the scientific and industrial interest in macroalgae has been increasing due to the need for new sustainable solutions for food, chemical, and energy production, there has also been a substantial increase in the consumer interest for macroalgae (Sá Monteiro et al., 2019). Macroalgae have long been common ingredients in the Asian cuisine, while human consumption of macroalgae has been less common in Western cuisine (van den Burg et al., 2012). However, the increased popularity of Asian dishes such as sushi has introduced macroalgae to European palates (Stévant et al., 2017b). In addition to this increased consumer interest in exotic cuisines, there has also been an increasing interest in healthier diets and lifestyles, as well as more sustainable food sources and production (Sá Monteiro et al., 2019; Torres et al., 2019). This has caused an increased interest for macroalgae consumption in Western countries, as macroalgae are low in calories due to low lipid contents and high dietary fibre contents (Holdt & Kraan, 2011), as well as a source of vitamins, minerals and plant-based proteins (FAO, 2018a). They have been labelled as a "superfood", and food products based on macroalgae have seen a substantial increase in recent years (Torres et al., 2019). Additionally, due to their content of biologically active substances such as polysaccharides, proteins, lipids and polyphenols macroalgae have received attention as a functional food that could provide health benefits beyond basic nutrition (Holdt & Kraan, 2011). This implies that food products could be enriched with macroalgae extracts to increase the functionality of the food (Rioux & Turgeon, 2015).

Another growing consumer trend is the preference for food without synthetic additives. This has resulted in the search of natural alternatives for additives such as antioxidants (Kristinová et al., 2009). Macroalgae have been shown to be a rich source of various natural antioxidants, such as polyphenols that can prevent lipid peroxidation in food products (Wang et al., 2009). In addition, the phycocolloids alginate, agar, and carrageenan from macroalgae are already being used as natural additives in foods (Fleurence, 2016).

1.2.3 Macroalgae Industry in Norway

Norway has a long coastline highly suited for aquaculture, and the Northeast Atlantic is home to more than 400 species of brown, red, and green macroalgae (Stévant et al., 2017b). Utilisation of the macroalgae found along the Norwegian coast has a long history. It has been used as food for human consumption, animal feed, fertiliser and for production of chemicals. Already around 1755 burning of kelp for potash used in soda and glass production, was an important income for farmers in the regions between Rogaland and Sør-Trøndelag. The potash was also

later used for iodine production, and in 1870 the first chemical iodide factory in Norway was built in Trondheim, supplied with potash from Hitra in Sør-Trøndelag. Additionally, there are reports of the use of macroalgae to feed livestock in the 19th and early 20th centuries, and the first industries processing *Ascophyllum nodosum* for animal feed and fertiliser were established in 1926 and 1937 (Delaney et al., 2016). Interest in further uses of macroalgae started as early as in the 1890, and in the 1930s research to determine potential uses for potassium, iodine, laminaran, mannitol, carrageenan and alginate from macroalgae was conducted in Norway. Some companies tried producing commercial products based on alginate extracts from macroalgae, although these were often high in impurities, and some even extracted mannitol from the macroalgae. In 1943 the company Algea was sucessful in the extraction of carrageenan for commercial applications. In 1945, the Norwegian company Protan started producing alginate at a commercial level. Their first alginate based product consisted of alginate, sugar, and ascorbic acid which was used as a replacement of the imported product pectin for the making of fruit jams. *Laminaria digitata* was harvested from along the west coast of Norway, and to reduce the transportation distance, Protan moved from Drammen to Vormedal outside Haugesund in 1961. Later, due to problems with the harvesting of *Laminaria digitata* they changed to extraction from *Laminaria hyperborea* (Aasland, 1997). To this day alginate is still produced at the factory in Vormedal by DuPont Nutrition & Biosciences (DuPont Nutrition & Biosciences, n.d.).

Today, Norwegian macroalgae industry includes the production of extracts from macroalgae and seaweed meal for use as soil conditioners, fertilisers and feed supplements, as well as alginate, agar and carrageenan production (Delaney et al., 2016; DuPont Nutrition & Biosciences, n.d.; Mæhre et al., 2014). The majority of macroalgae resources harvested in Norway is from wild resources. In 2015, Norway was only behind Chile and China in the production output of wild macroalgae, with a production of over 147 000 tonnes (FAO, 2018b). According to the Norwegian Directorate of Fisheries, between 130 000-180 000 tonnes of the macroalga *Laminaria hyperborea*, as well as about one tenth of that amount of *Ascophyllum nodosum*, is harvested from wild resources each year. The harvesting is done mechanically, and has been since the 1970s (Directorate of Fisheries, 2015).

Research on macroalgae in Norway has been scattered for several decades, however, since 2008 the number of research projects and participants has increased (Skjermo et al., 2014), and macroalgae cultivation is a new emerging industry in Norway (Sharma et al., 2018). Experimental cultivation of kelps at sea started around 2005 (Stévant et al., 2017b), and the first commercial licences for farming of macroalgae in Norway were distributed in 2014. In 2014, 54 licences were distributed, and in only five years the number of licences had increased to 475 in 2019. There has also been a substantial increase in the amount of harvested farmed macroalgae; from 51 tonnes in 2015 to 178 tonnes in 2018. The main cultivated species in Norway is *Saccharina latissima*, with 174 tonnes harvested in 2018, followed by 2 tonnes of *Alaria esculenta* and 2 tonnes of other species (Directorate of Fisheries, 2019). The main reason behind the increased interest in macroalgae

cultivation in Norway has been the possibility of production of macroalgae based biofuels. However, the cultivation of macroalgae can supply the global food market, as well as be used for animal feed ingredients, pharmaceuticals and fertilisers, and products that can replace petroleum-based materials, such as plastics. Cultivation of macroalgae in Norway is said to have great potential due to the long coast with high-productive areas and strong competence within aquaculture, off-shore constructions and macroalgae biotechnology. It has therefore been proposed that a new Norwegian bioeconomy could be established based on the cultivation and processing of macroalgae (Skjermo et al., 2014).

Norway is a major producer of farmed salmon and trout, which causes excess nutrient discharge that can lead to environmental eutrophication and result in ecological degradation and biodiversity loss. Because of this, the use of macroalgae in Integrated multitrophic aquaculture (IMTA) systems is also an area of great interest in Norway. In IMTA systems macroalgae are cultivated in proximity to fish farms and mitigate the negative effects of the fish farming by absorbing the excess nutrients. Additionally, the excess nutrients could be recovered from the macroalgae, which is especially important for phosphorous, as this is a finite resource, and the abundance of nutrients available to the macroalgae could lead to increased biomass yields (Stévant et al., 2017b).

1.3 Composition of the Macroalgae Species *Saccharina latissima* and *Alaria esculenta*

The macroalgae species *Saccharina latissima* and *Alaria esculenta* are two brown macroalgae species within the order Laminariales, often referred to as kelp (Ometto et al., 2018). Common names for *Saccharina latissima* include sea belt, sweet oarweed, sweet kelp, sugar kombu, and sugar kelp, while *Alaria esculenta* is commonly known as atlantic wakame, bladderlocks, and winged kelp (Bak et al., 2019; Banach et al., 2020). Both species are edible, and have been the focus of large-scale cultivation projects in Europe due to their ability to reach high biomass yields, as well as their valuable nutritional content (Stévant et al., 2017a). Reported ranges of moisture, ash, protein, lipid, carbohydrate, and phenolic content in the two macroalgae are given in Table 1.2.

Table 1.2: Biochemical composition of the brown macroalgae species *Saccharina latissima* and *Alaria esculenta*. Moisture content is given in percent of wet weight. All other values are given in percent of dry weight.

	Saccharina latissima	***Alaria esculenta***
Moisture	63-93[a,b]	72-92[c,d]
Ash	8.7-51.9[a,b]	21-33[e]
Protein	1.1-16[a,f]	7.0-16[d]
Lipids	0.17-5.3[d]	1.5-4.6[d,g]
Carbohydrates	10-77[e,h]	41-72[e,i]
Phenolics	0.07-1.5[j,k]	0.31-6.1[e,k]

[a]Nielsen et al. (2016); [b]Forbord et al. (2020); [c]Ometto et al. (2018); [d]Fische (2016); [e]Schiener et al. (2014); [f]Mols-Mortensen et al. (2017) [g]Mæhre et al. (2014); [h]Manns et al. (2017); [i]Stévant et al. (2017a); [j]Sharma et al. (2018); [k]Roleda et al. (2019)

In a study by Schiener et al. (2014), the seasonal variations of the composition of *S. latissima* and *A. esculenta* was studied. The results reported were based on eight *S. latissima* samples harvested between August 2010 and October 2011, and three *A. esculenta* samples harvested between March 2011 and July 2011. The moisture and ash contents were reported to be at their highest during the winter months followed by a decline into the autumn months, while protein content was said to be highest in the first quarter of the year and lowest in the third quarter of the year. The polyphenol content was reported to peak between May and July and reach a minimum in March, consistent with an accumulation of polyphenols at the onset of the growth phase of the macroalgae. More pronounced seasonal variations were reported for the storage polysaccharides mannitol and laminaran, than for the structural polysaccharides cellulose and alginate. The mannitol content was at its highest during the autumn period, and at its lowest during early spring. The

laminaran content followed a similar trend, peaking in summer and autumn months and dropping to its lowest levels during winter. Alginate content was reported being at its lowest in July, while cellulose had no pronounced seasonal variation (Schiener et al., 2014). Alginate, laminaran, mannitol and fucoidan have several interesting industrial uses discussed in the following sections.

1.3.1 Alginate

The main carbohydrate in brown macroalgae is the structural polysaccharide alginate, reported by Schiener et al. (2014) to account for 28.5% and 37.4% of dry weight in *Saccharina latissima* and *Alaria esculenta*, respectively. Alginate is found in the intercellular matrix of the cell wall, and prevents desiccation, provides flexibility, and is involved in the exchange of ions with seawater. Alginate is a linear polysaccharide composed of β-D-mannuronic acid (M) and α-L-guluronic acid (G). The structure is shown in Figure 1.1. Alginates form gels with divalent ions such as sodium, calcium or magnesium (Rioux & Turgeon, 2015). The gelation of alginates is based on the ability of blocks containing several consecutive G residues (G-blocks) to selectively bind these ions. Because of this, gel strength increases with increasing G content and length of G-blocks (Draget et al., 1997). The composition and molecular weight of an alginate depends on the macroalgae species and extraction method (Rioux & Turgeon, 2015).

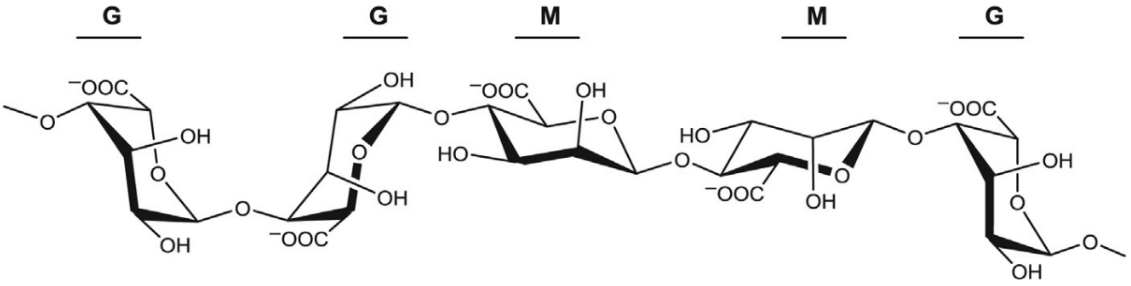

Figure 1.1: Structure of alginate. M is the symbol given to the monomer β-D-mannuronic acid, and G to α-L-guluronic acid. Picture obtained from Rioux and Turgeon (2015).

As the composition of alginates substantially affect their properties, the applications of alginates are many. Alginates are used in the manufacture of pharmaceuticals, cosmetic creams, paper and cardboard, and processed foods (Kraan, 2012). In food products, alginates are used as a stabilising, emulsifying, gelling and thickening agents. Additionally, they can be used to create films used to reduce water loss, control diffusion and manage the shape of a product. Alginate is also used as encapsulation agents for drugs, proteins, and probiotics, amongst others (Rioux & Turgeon, 2015). Alginic acid has been reported to possess bioactivities such as decreasing cholesterol concentration, exerting anti-hypertension effects, preventing absorption of toxic chemical substances, and acting as a dietary fibre. Additionally, sodium alginate has been reported to demonstrate strong antibacterial effect, and alginates with molecular weights over 50kDa have been shown to prevent obesity, and diabetes (Holdt & Kraan, 2011)

1.3.2 Laminaran

Laminaran is a branched or unbranched β1-3 glucan only found in brown seaweed (Jacobsen et al., 2019). The main chain of laminaran consists of (1,3)-β-D-glucose and the branches contain (1,6)-β-D-glucosyl and (1,2)-β-D-glucosyl units. Laminaran exists in both soluble and insoluble forms, where the first is completely soluble in cold water, while the second is only soluble in hot water. Its solubility depends on the number of branches; increasing number of branches corresponds to an increased solubility in cold water. Some laminaran chains also have D-mannitol residues at the end, called M-chains. Chains ending with D-glucose are called G-chains. The chain ratio depends on the macroalgae species (Rioux & Turgeon, 2015). The structures of the two different laminaran chains are shown in Figure 1.2. Average laminaran contents of *S. latissima* and *A. esculenta* were reported by Schiener et al. (2014) to be 8.2% and 11.1% of dry weight, respectively.

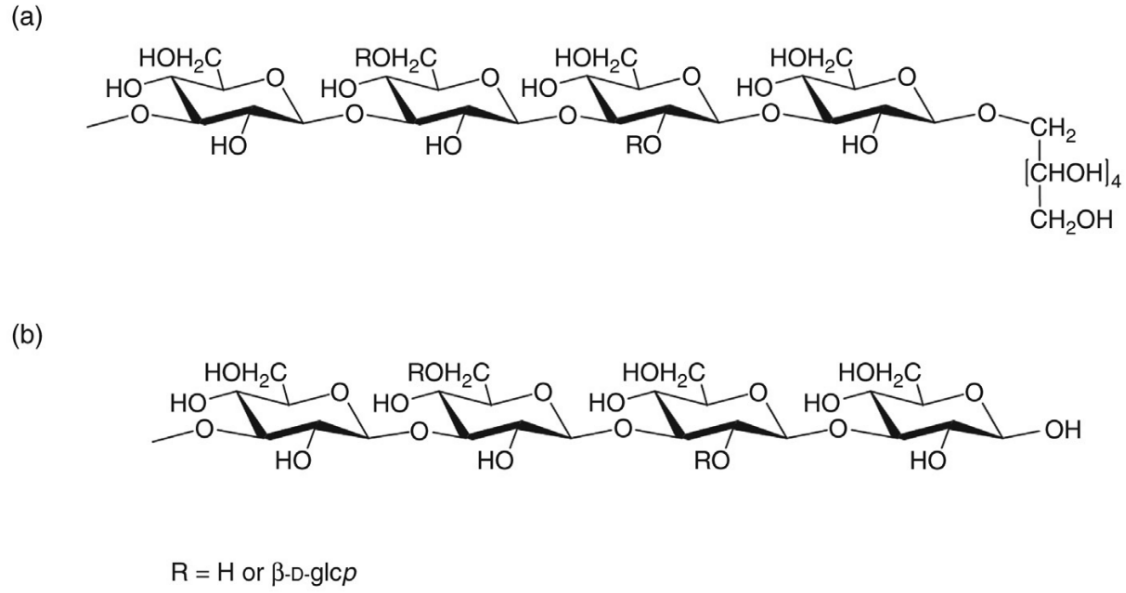

Figure 1.2: Structure of laminarin; (a) shows a M-chain with a D-mannitol residue at the end, and (b) shows a G-chain with a D-glucose residue at the end. R is either hydrogen (H) or branches containing β-D-glycosyl (β-D-glc*p*). The picture is obtained from Rioux and Turgeon (2015).

Laminaran has been shown to have numerous bioactivities including antibacterial, probiotic, and immune system stimulating, as well as being able to reduce cholesterol absorption in the gut and act as a dietary fibre (Holdt & Kraan, 2011). Laminaran is also a possible feedstock for production of bio-ethanol (van den Burg et al., 2012), and has been shown to have potential clinical applications within cancer treatment (Déléris et al., 2016). Commercial applications of the polysaccharide are limited, however, due to its many bioactivities, it has potential for use in the medical and pharmaceutical industries (Kraan, 2012).

1.3.3 Fucoidan

Fucoidans are sulphated fucose-rich polysaccharides found in the cell walls of brown macroalgae (Bruhn et al., 2017). The composition and complexity of fucoidans can vary significantly between different species of macroalgae (Wijesinghe & Jeon, 2012). As the structure varies with species the name fucoidan does not imply a specific structure, but rather polymers from brown macroalgae composed of mainly fucose and sulphate, with lesser amounts of other monosaccharides such as mannose, galactose, glucose, xylose and more (Li et al., 2008). Homopolymers consisting of only sulphated L-fucose are often called homofucans, while the name fucoidan is mostly used for heteropolymers of sulphated L-fucose, also called heterofucans ("Heterofucans from the Brown Seaweed Canistrocarpus cervicornis with Anticoagulant and Antioxidant Activities", 2011). The repeating dimeric unit of fucoidan, consisting of sulphated L-fucose, residues is shown in Figure 1.3. Fucoidans are assumed to be cell wall-reinforcing and seem to associated with protection against effects of desiccation of the macroalgae when exposed at low tide(Holdt & Kraan, 2011).

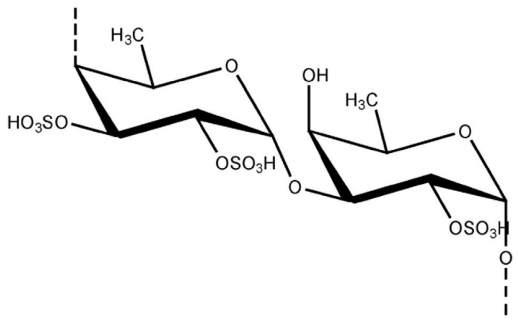

Figure 1.3: Structure of fucoidan; repeating dimeric unit consisting of sulphated-L-fucose residues. Picture obtained from Ahmed et al. (2014).

Fucoidans have been extensively studied in recent years (Li et al., 2008). They have been reported to posses numerous bioactivities including antioxidant, anticancer, anticoagulant, antithrombotic, immunomodulant and antiproliferative effects. Because of this, the scientific and industrial interest in fucoidans has increased in recent years, and they are often mentioned as a key value-added compound for brown macroalgae biorefineries (Bruhn et al., 2017). The variations in structure also causes variations in the bioactivity of the fucoidans. For instance, a fucoidan with large molecular weight can exhibit anticoagulant activity due to a long sugar chain enabling binding to the coagulation protein thrombin present in the bloodstream (Holdt & Kraan, 2011). Today, fucoidans are used in the cosmetic and food industries as an anti-ageing and antiulcer agent, respectively (Ahmed et al., 2014)

1.3.4 Mannitol

Mannitol is present in many species of brown macroalgae and is the sugar alcohol corresponding to mannose (Holdt & Kraan, 2011). It is a monomeric compound used as a storage carbohydrate (Baweja et al., 2016). The average mannitol concentrations in*S. latissima* and *A. esculenta* were reported by Schiener et al. (2014) to be 18.6% and 12.1% of dry weight, respectively. Mannitol have very diverse applications, it is used in pharmaceuticals, chewing gum, the paint and varnish industry, leather and paper manufacturing, plastics industry and in the production of explosives (Holdt & Kraan, 2011). It is also a possible feedstock for bio-ethanol production

(van den Burg et al., 2012). It has a low affinity to water, is chemically inert, and has a relative sweetness of 40-50% compared to sucrose giving it a sweet taste. It is also reported to have a favourable mouthfeel and a pleasantly cool taste (Baweja et al., 2016; Holdt & Kraan, 2011). Due to this it is used as a replacement for sucrose in sugar-free food products and compound coatings, as well as to increase shelf-life and stability by maintaining a proper moisture level. Additionally, it is non-carcinogenic and will not contribute to tooth decay (Holdt & Kraan, 2011).

1.4 Preservation of Macroalgae by Freezing

Macroalgae have a high water content, ranging from 61-94% of wet weight (Holdt & Kraan, 2011), which leads to rapid decay after harvesting (Enríquez et al., 1993). Therefore, to ensure biomass quality and product safety appropriate preservation methods are essential during transportation to processing facilities. Additionally, as the composition and biomass yield of macroalgae varies considerably with season, harvesting is usually seasonal, making preservation and long-term storage of biomass necessary to ensure a year-round supply (Øverland et al., 2019; Schiener et al., 2014). Possible preservation methods include drying, seawater storage, cold storage, silage and freezing (Stévant et al., 2017b). However, these methods can affect the biochemical composition and sensory properties of the macroalgae (Choi et al., 2012; Gupta et al., 2011; Stévant, 2019; Stévant et al., 2017a).

Freezing is one of the most popular means of long-term food storage as it generally preserves taste, texture and nutritional value, by transforming most of the liquid water into ice crystals. This slows down the physical and biochemical changes involved in deterioration and the growth and reproduction of microorganisms. However, freezing can change the quality of the food. This depends on the freezing and thawing protocol followed (Choi et al., 2012). Thawing of frozen macroalgae samples have been reported to result in loss of liquid from the macroalgae blades during thawing (Nielsen et al., 2020; Stévant, 2019; Sund, 2019). This loss of liquid is caused by cell damage due to ice crystal formation and osmotic potential differences during freezing. The ice crystals cause mechanical damage to the cell walls, while freezing of the extracellular matrix causes differences in the osmotic potential between the intracellular space and unfrozen part of the extracellular space causing migration of water out of the cell resulting in cell shrinkage, dehydration, and ultimately membrane damage (Stévant, 2019). The liquid lost during the freezing and thawing process will in this work be called drip loss.

Drip loss from *Saccharina latissima* has been reported by Stévant (2019) and Sund (2019) to be over 40% and 27% of sample wet weight, respectively. Both reported the liquid to be a slightly viscous and brown/orange solution. Drip loss from *Alaria esculenta* was reported by Sund (2019) to be 39% of sample wet weight, as well as being slightly viscous and yellow. These values are quiet high, indicating that a significant amount of the moisture present in fresh macroalgae is lost during freezing and thawing due to the stress the cells undergo in the freezing and thawing processes. Due to this high percentage of drip loss, freezing and thawing of raw macroalgae can make subsequent drying easier. However, as the drip loss samples were reported to be coloured and slightly viscous, it is likely that they contained phytochemical compounds. This could indicate that valuable compounds originally present in the fresh macroalgae could end up in the drip loss. Discarding the drip loss could therefore lead to waste of potentially useful compounds and loss of possible profit, and preserving edible macroalgae by freezing could cause changes in their nutritional profile. Additionally, even though macroalgae are generally viewed as a sustainable resource, full utilisation of the raw material, including what is potentially lost in the drip loss, is important for the sustainable utilisation of the resource.

1.5 Aim of the Study

The aim of this work was to determine the biochemical composition of drip loss from thawing of frozen samples of the macroalgae species *Saccharina latissima* and *Alaria esculenta*. This was done to assess how the macroalgae raw material was affected by freezing and thawing, and if the drip loss could be used as a value-added product in industry, either directly or for extraction of certain compounds.

A similar study of the drip loss from thawing of *S. latissima* and *A. esculenta*, presented in Sund (2019), was executed by the author as a preliminary study prior to this thesis. This study used the same *A. esculenta* raw material to obtain the drip loss sample, while the *S. latissima* raw material was different. Several of the same methods as used in Sund (2019) were used in this study, however some alterations were made due to issues discovered during the preliminary study.

2 Materials and Methods

In this study, the drip loss from thawing of the brown macroalgae species *Saccharina latissima* and *Alaria esculenta* were collected and analysed to determine their biochemical composition. Both the original drip loss samples and freeze dried drip loss samples were analysed. Some analyses were also conducted on the raw material and the leftover raw material after thawing and removal of the drip loss. Analyses conducted on raw material, leftover raw material, and drip loss were used to create mass balances for the distribution of compounds in the raw material between the leftover raw material and drip loss during thawing. A flow chart of the sample preparation is shown in Figure 2.1.

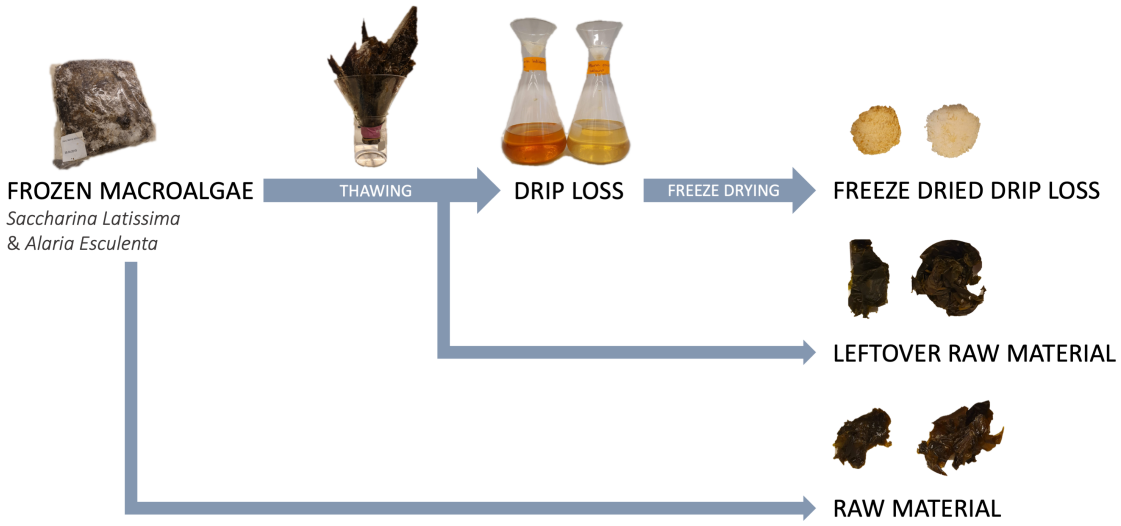

Figure 2.1: Flow chart of sample preparation from frozen blocks of the macroalgae *Saccharina latissima* and *Alaria esculenta* to drip loss samples from thawing, freeze dried drip loss samples, leftover raw material after thawing and raw material.

The weight of the original raw material samples, as well as the two fractions after thawing (leftover raw material and drip loss) were determined. The dry matter and ash content was determined for all samples. The free amino acid composition was determined for the original drip loss samples, while the protein and phenolic content, as well as the antioxidant activity were determined for both the original drip loss samples and the freeze dried drip loss samples. The monosaccharide composition, total amino acid composition, and carbon and nitrogen content was determined for freeze dried drip loss, raw material, and leftover raw material. The mineral content was determined for freeze dried drip loss samples from Sund (2019). To determine these parameters several methods described in the following sections were used. A flow chart showing which analyses were executed on which samples is shown in Figure 2.2.

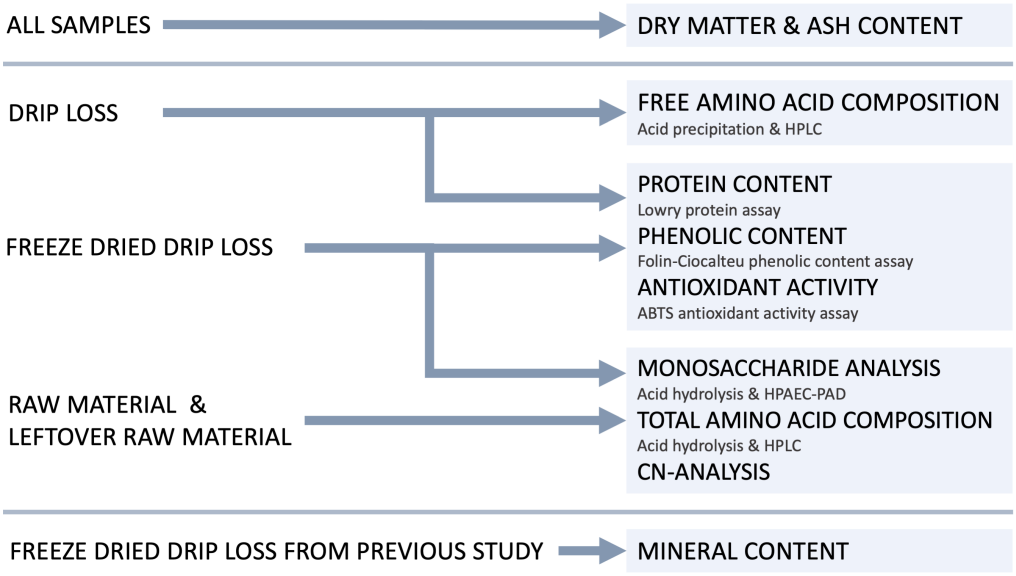

Figure 2.2: Flow chart of analyses conducted on drip loss samples from thawing, freeze dried drip loss samples, leftover raw material after thawing and raw material from samples of the macroalgae species *Saccharina latissima* and *Alaria esculenta*. The freeze dried drip loss used for determination of mineral content was from Sund (2019).

2.1 Sample Preparation

Macroalgae samples of *Saccharina latissima* and *Alaria esculenta* were obtained from Seaweed Energy Solutions (SES). The *S. latissima* was harvested 29.04.2019, and the *A. esculenta* was harvested 06.05.2019. The macroalgae were delivered in large frozen blocks. Samples of 602.93 g and 602.39 g of *S. latissima* and *A. esculenta*, respectively, were cut from the larger blocks and thawed. The samples were thawed in a setup designed to collect the drip loss during thawing. This setup can be seen in Figure 2.3. The setup consisted of a funnel placed in a beaker to collect the drip loss. A mesh was attached to the bottom of the funnel to keep pieces of macroalgae from mixing with the drip loss. As the funnel was a bit too small, plastic sheets were placed between the funnel and the macroalgae samples to act as an extension of the funnel, see Figure 2.3c on the next page. The macroalgae samples were thawed for about 21 hours at 4°C.

(a) Setup used to collect drip loss during thawing of the macroalgae samples.

(b) Mesh attached to bottom of funnel.

(c) Finished setup with the *Saccharina latissima* sample.

Figure 2.3: Pictures of the setup used to collect the drip loss during thawing of the macroalgae samples of *Saccharina latissima* and *Alaria esculenta*.

After thawing, the drip losses and the thawed macroalgae samples were weighed. The drip losses were filtered through glass wool into Erlenmeyer flasks to remove macroalgae debris. The *S. latissima* sample had to be filtered twice to remove all of the debris. The drip loss in percent was calculated from the weight of the drip loss collected (W_{dl}) and the weight of the frozen sample (W_f), as well as from the weight of the frozen sample (W_f) and the weight of the thawed sample (W_t), using Equations 2.1 and 2.2, respectively.

$$\text{Weighed drip loss} = \frac{W_{dl}}{W_f} \cdot 100\% \tag{2.1}$$

$$\text{Calculated drip loss} = \frac{W_f - W_t}{W_f} \cdot 100\% \tag{2.2}$$

Before and after thawing a sample of each macroalgae of about 20 g was put aside for further analysis. The frozen sample was immediately put aside in a freezer, while the thawed sample was kept at 4°C for 11 days before about half of it was freeze dried and the rest frozen.

2.2 Dry Matter and Ash Content

The dry matter and ash content was determined for samples of the frozen and the thawed macroalgae, as well as for the drip loss and freeze dried drip loss samples. This was determined by weighing out a small amount of sample into a crucible and then drying the sample at 105°C for 24 hours, before subsequent combustion in a muffle furnace at 550°C overnight. The crucible with sample is weighed before and after drying as well as after combustion.

2.3 Mineral Content

The concentration of twelve different minerals (Na, Mg, P, K, Ca, Zn, As, Se, Sr, Cd, Hg, Pb and I) present in the drip loss from *Saccharina latissima* and *Alaria esculenta* was determined at SINTEF Industry in Trondheim. The drip loss samples used in this analysis were the samples obtained in Sund (2019) as described in Appendix A.3 on page v. The iodine content was determined in a Tetramethylammonium hydroxide (TMAH) matrix by mixing 0.25 grams of each sample with 4 millilitres of de-ionised water and 1 millilitre of 25% TMAH in water (Acros Organics). The samples were then heated at 90°C for 2-3 hours, before dilution to 10 millilitres with de-ionised water. The samples were then left to settle overnight and diluted 1:5. All standard solutions and dilutions were prepared using ultra-purified water 18.2Ω from a Smart2Pure system from Thermo Scientific. Standards for the calibration curve were prepared in 5% TMAH (v/v) from individual stock solutions containing 1000μg/mL of iodine (Inorganic Ventures, USA). Tellur (Te) was used as an internal standard. The iodine content was measured by an Agilent 8800 Triple Quadrupole ICP-MS (Agilent Technologies, USA) with a SPS4 autosampler (Agilent Technologies, USA) and a standard sample introduction system (Micro Mist glass concentric nebulizer, quartz double pass spray chamber, quartz torch with 2.5 mm id and standard nickel cones). He and O_2 modes were used in this method. Tuning conditions are shown in Table 2.1.

Table 2.1: Agilent 8800 Series Triple Quadrupole ICP-MS System parameters for determination of iodine content.

Parameter	Value
RF Power	1550 W
Plasma Gas Flow	15 L/min
Auxiliary Gas Flow	0.9 L/min
Carrier Gas Flow	1.05 L/min
Option Gas Flow	0.0 L/min
Make Up Gas Flow	0.0 L/min
He Flow Rate	4.3 ml/min
O_2 Flow Rate	30%
Nebulizer Pump	0.1 rps
Sample depth	8.0 mm
Cell tuning modes	He/O_2
S/C Temp	2°C
Scan Type	MS/MS
Replicate/peak pattern/sweeps	4/3/40

The concentrations of the remaining minerals were determined by the following procedure: Freeze dried drip loss samples were decomposed with nitric acid (HNO_3) and hydrogen peroxide (H_2O_2) at 220°C in a Milestone Ethos microwave, before analysis on a Agilent 8800 Triple Quadropole ICP-MS (ICP-QQQ) with a SPS 4 Autosampler. The results were quantified by comparison against standards from Inorganic Ventures and ^{115}In as an internal standard.

2.4 ABTS Assay

The ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) assay was used to determine the radical scavenging activity of the drip loss samples. The assay was executed as described by Re et al. (1999), Nenadis et al. (2004), and Nenadis et al. (2007). The standard curve was created using a dilution series of propyl gallate, and 80% methanol was used as the blank. The radical scavenging activity in propyl gallate equivalents was determined by measurement of absorbance at 734 nm in a Genesys 10S UV-Vis spectrophotometer.

The analysis was conducted on the original drip loss samples and the freeze dried drip loss samples. The freeze dried drip loss samples had a very low solubility in 80% methanol and were therefore dissolved in water before further dilution with 80% methanol. The freeze dried samples were dissolved in water at two different concentrations to see if dissolving the samples in water, before dilution with methanol, could have any effect on the results. The samples were dissolved at concentrations of 0.0622 g/mL and 0.124 g/mL, approximating the "concentration" of the original drip loss samples and double the original concentration. The analysis was also conducted on frozen and thawed drip loss samples and freeze dried drip loss samples kept at 4°C for about 13 weeks.

2.5 Carbohydrate Analysis

A carbohydrate analysis was conducted on samples of the raw material, leftover raw material, and the drip loss resulting from thawing the macroalgae *Saccharina latissima* and *Alaria esculenta*. The samples were freeze dried and crushed into fine powders. Three parallels of approximately 20 mg of each sample were weighed out into hydrolysis tubes and degraded in 5 mL of 1 M trifluoroacetic acid at 100°C for 24 hours. After degradation 0.5 mL of each sample was transferred to an Eppendorf tube. This was done twice to create two parallels. The samples were then dried in a Savant SC250EXP SpeedVac from Thermo Scientific at 40°C and 5 Torr for 4 hours. When dry, the samples were dissolved in 1 mL of Milli-Q water. The samples were then diluted in Milli-Q water to a total volume of 1 mL in 1.5 mL glass vials with precut septum screw cap from Thermo Fisher. The first parallel was run both 50 and five times diluted, while the second parallel was only run five times diluted. The samples were analysed by high performance anion-exchange chromatography with pulsed amperiometric detection (HPAEC-PAD) on an ICS 5000+ system equipped with a pulsed amperometric detector (carboquad waveform) using a 4 x 50 mm CarboPac PA1 guard column and a 4x 250 mm main column. The samples were eluted at a flow rate of 1 ml/min using the conditions shown in Table 2.2. The method was based on the method described by Zhang et al. (2012). The data obtained were processed using a Chromeleon 7 Chromatography Data System software, version 7.2.1 and the peaks were identified using monosaccharide standards.

Table 2.2: Conditions used for the high performance anion-exchange chromatography with pulsed amperiometric detection (HPAEC-PAD) analysis. NaOH is sodium hydroxide, and NaAc is sodium acetate.

Time [min]	NaOH [mM]	NaAc [mM]	Gradient
0-25	10	0	Isocratic
25-30	10-100	0	Linear
30-40	100	0-200	Linear
40-55	100	200	Isocratic
55-60	100	0	Isocratic
60-75	10	0	Isocratic

The monosaccharide standards used for determination of the carbohydrate composition of the samples were two different mixes containing inositol, mannitol, fucose, glucose and xylose, and rhamnose, galactose and mannose, named "Mix 1" and "Mix 4", respectively. The standards were run at concentrations of 0.1, 0.5, 1.0, 2.5, 5.0, 7.5 and 10.0 mg/L to create standard curves for the concentration of the different monosaccharides. In the method used the retention times of mannose and xylose are very similar and these are therefore not possible to adequately separate in the results for the samples. An average standard curve for mannose and xylose was therefore created to attempt to quantify the combined concentration of mannose and xylose in the samples. This average standard curve was created using the concentrations of the standards and corresponding average peak areas of the mannose and xylose standards.

2.6 Protein Precipitation

To uncover possible interference of phenols in the Lowry protein assay and proteins in the Folin-Ciocalteu assay two different protein precipitation methods were tested on the original drip loss samples and the freeze dried drip loss samples dissolved in water. The methods used were ethanol precipitation and trichloroacetic acid (TCA) precipitation and these were conducted as described below.

Ethanol precipitation was done by mixing the sample with 96% ethanol to a volume percentage of 75%. The solution was then centrifuged in a Heraeus Multifuge X1R centrifuge from Thermo Scientific at 4 997×g for 30 minutes. The precipitate and supernatant were then separated by decantation. The precipitate was solubilised in distilled water. The supernatant was freeze dried and then dissolved in distilled water.

Trichloroacetic acid (TCA) precipitation was done as described by Hoyle and Merrit (1994). The sample was mixed with 20% TCA in a 1:1 ratio, resulting in a final concentration of TCA of 10%. The solution was incubated at room temperature for 30 minutes. The precipitate was removed by filtration through a Whatman Qualitative Grade 1 filter paper.

2.7 Folin-Ciocalteu Phenolic Content Assay

The total phenolic content of the drip loss samples was determined by the Folin-Ciocalteu assay. The assay was executed as described by Nenadis et al. (2007) and Singleton et al. (1999). The standard curve was created using a dilution series of propyl gallate, and 80% methanol was used as the blank. The total phenolic content in propyl gallate equivalents was determined by measurement of absorbance at 725 nm in a Genesys 10S UV-Vis spectrophotometer. All analyses were done in triplicates.

The analysis was conducted on the original drip loss samples and the freeze dried drip loss samples. The analysis was also conducted on frozen and thawed drip loss samples and freeze dried drip loss samples kept at 4°C for about 10 weeks. Additionally, the analysis was conducted on the supernatants from the protein precipitations describes in Section 2.6 on the previous page.

2.8 Lowry Protein Assay

The Lowry protein assay was used to determine the protein content of the drip loss from *Saccharina latissima* and *Alaria esculenta*. The method was executed as described by Lowry et al. (1951). Bovine serum albumin (BSA) was used as a standard. The protein content in BSA equivalents was determined by measurement of absorbance at 750 nm in a Genesys 10S UV-Vis spectrophotometer. All analyses were conducted in triplicates.

The analysis was conducted on original drip loss samples and the freeze dried drip loss samples, as well as on frozen and thawed drip loss samples and freeze dried drip loss samples kept at 4°C for about 12 weeks. Additionally, the analysis was conducted on the supernatants and precipitates from the ethanol precipitation, and the supernatants from the TCA precipitation. Both the original drip loss samples and dissolved freeze dried drip loss samples were subjected to these precipitation methods, as previously described in Section 2.6 on the preceding page.

2.9 CN-analysis

This CN-analysis was executed on freeze dried samples of original macroalgae raw material, leftover macroalgae after thawing, and drip loss. Two parallels of 500-800 μg of each sample were packed into tin capsules and delivered to Marte Schei at SINTEF Fisheries and Aquaculture. The CN-analysis was conducted by Marthe Schei on a CN-analyser (ECS 4010 CHNSO analyser, Costech, Italy).

2.10 Free Amino Acid Content

The amount and composition of free amino acids were determined for the original drip loss samples from *Saccharina latissima* and *Alaria esculenta* as described by (Osnes & Mohr, 1985). Three parallels of one millilitre of each drip loss sample were thoroughly mixed with 0.25 millilitres 10% sulphosalisylic acid and incubated at 4°C for 30 minutes, before the precipitated protein was removed by centrifugation for 10 minutes at 9 168 × g in a eppendorf 5415 R centrifuge. The samples were diluted with de-ionised water, filtered through 0.22 μm syringe filters, and analysed by reverse phase HPLC using a Nova-Pak C18 column on an UltiMate® 3000 HPLC (Thermo Scientific) with a Dionex RF 2000 fluorescence detector with OPA derivatisation. The internal standard used was Aba. The HPLC analysis was performed by Siri Stavrum at NTNU.

2.11 Total Amino Acid Content

The amount and composition of total amino acids was determined for freeze dried samples of original macroalgae raw material, leftover raw material, and drip loss was determined as described by (Blackburn, 1978). Three parallels of each sample were hydrolysed in 6M hydrochloric acid (HCl) for 22 hours at 105°C, neutralised with sodium hydroxide (NaOH), and filtered using Whatman glass microfiber filter GF/C and a vacuum filtering pump. The samples were diluted with de-ionised water, filtered through 0.22 μm syringe filters and analysed by reverse phase HPLC using a Nova-Pak C18 column on an UltiMate® 3000 HPLC (Thermo Scientific) with a Dionex RF 2000 fluorescence detector with OPA derivatisation. The internal standard used was Aba. The HPLC analysis was performed by Siri Stavrum at NTNU.

2.12 Statistical Analysis

The analyses, except the CN analysis and the mineral analysis, were conducted in triplicates. The CN-analysis was conducted for two parallels. For the analyses conducted in triplicates, as well as the CN-analysis, the standard deviation was found using the Excel function "STDEV.S". Statistically significant differences were determined using p-values from a unpaired two-sample Student's t-test found by using the Excel function "T.TEST". The limit for statistical significance was set at a 5% level. The statistical analysis for the mineral analysis was done by SINTEF Industry.

3 Results and Discussion

The drip loss obtained from thawing frozen samples of the macroalgae *Saccharina latissima* and *Alaria esculenta* was calculated in two ways. The weighed drip loss, based on the weights of the obtained drip losses, was determined to be 49.1% and 55.0% of the original sample wet weight for *S. latissima* and *A. esculenta*, respectively. While the calculated drip loss, based on the difference in weight between the original macroalgae samples and the leftover macroalgae samples after removal of the drip loss, was determined to be 52.5% and 57.3% of the original sample wet weight for *S. latissima* and *A. esculenta*, respectively. The weights of the initial raw material and leftover raw material, as well as the weight of the collected drip loss and the drip loss in percent of the wet weight of the raw materials are given in Table 3.1. The observed differences in weighed and calculated drip losses could be due to evaporation of water from the macroalgae samples during thawing or incomplete transfer from the beaker the drip loss was collected in to the beaker it was weighed in. Because the amounts of different biochemical compounds measured in this work are based on the concentration present in the drip loss that was actually collected, all further calculations in this work will be based on the weighed drip loss. The drip loss from *S. latissima* was bright orange in colour and the *A. esculenta* drip loss was a lighter yellow colour. The *S. latissima* sample seemed slightly more viscous than the *A. esculenta* sample. The drip loss samples are shown in Figure 3.1. The raw data from the sample preparation is given in Appendix A.1 on page i.

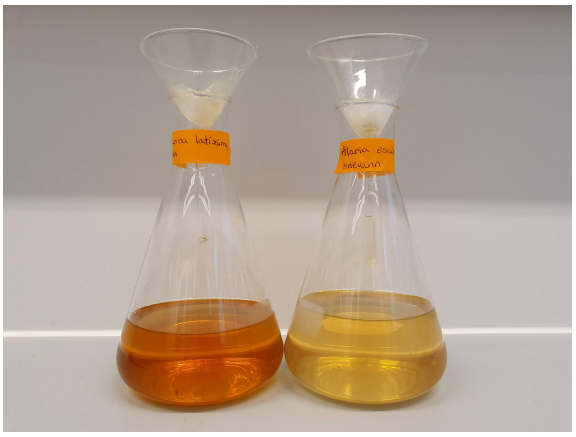

Figure 3.1: The drip loss samples from the thawed macroalgae *Saccharina latissima* (left) and *Alaria esculenta* (right).

Table 3.1: Weights of the frozen macroalgae raw material samples, the leftover raw material samples, and the drip loss samples of *Saccharina latissima* and *Alaria esculenta*, as well as the drip loss in percent of initial sample weight. The weighed drip loss is the drip loss in percent based on the weighed amount of drip loss collected, while the calculated drip loss is the drip loss in percent based on the difference in weight between the frozen and thawed macroalgae samples.

	Saccharina latissima	***Alaria esculenta***
Weight frozen raw material [g]	602.93	602.39
Weight leftover raw material [g]	286.49	257.01
Weight drip loss	295.98	331.4
Weighed drip loss [%]	**49.09**	**55.01**
Calculated drip loss [%]	**52.48**	**57.33**

The drip loss from the *A. esculenta* sample was larger than the drip loss from the *S. latissima* sample. This is consistent with earlier findings in Sund (2019). Additionally, the leftover *A. esculenta* raw material seemed slightly more dry than the leftover *S. latissima* sample. This indicates that the *A. esculenta* sample might have lost more liquid during the thawing process. This could be due to the two macroalgae samples not being cut in equal pieces when thawing or simply that the *A. esculenta* raw material loses more water during thawing. In Sund (2019), both drip losses were considerably lower than found in this work, being 27% and 39% of original sample wet weight for *S. latissima* and *A. esculenta*, respectively. This might be due to the different thawing techniques, as the macroalgae samples in Sund (2019) were thawed in beakers with no separation between the drip loss and the macroalgae samples. This thawing technique makes it more challenging to separate the drip loss from the leftover raw material, resulting in a lower perceived drip loss. It could also be possible that having the macroalgae samples partly submerged in drip loss while thawing could affect the amount of drip loss from the thawing of the samples. The drip loss from the *S. latissima* sample is in this study markedly higher than the drip loss observed in Stévant (2019), at around 40%.

3.1 Dry Matter and Ash Content

The dry matter and ash content was determined for raw material and leftover raw material samples of *Saccharina latissima* and *Alaria esculenta*, as well as for the drip loss and freeze dried drip loss from the thawing of the macroalgae. The compositions of moisture, dry matter and ash of the different samples are shown in Figure 3.2. Figure 3.3 shows the moisture and ash content of the samples in percent of wet weight and dry weight of the original raw materials, respectively. The contents of the leftover raw material and the drip loss are stacked to illustrate the mass balance of the moisture and ash content before and after thawing. The raw data for the dry matter and ash content determination and the determined dry matter and ash contents of the samples are given in Tables A.4 and A.5 in Appendix A.2 on page iii.

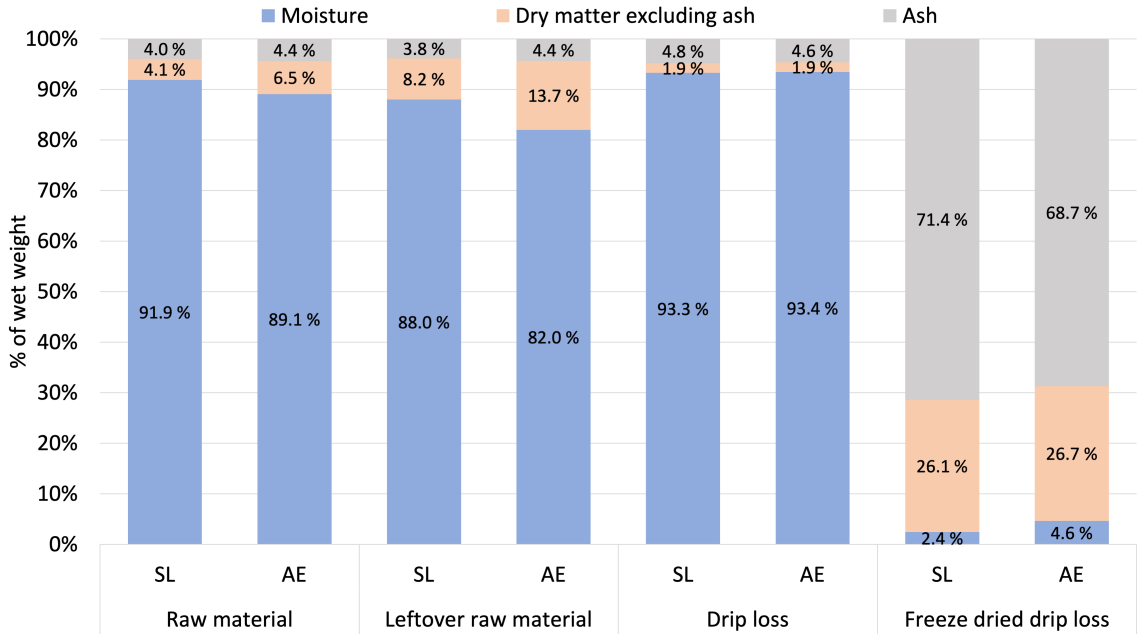

Figure 3.2: Distribution of moisture, dry matter excluding ash content, and ash content of raw material, leftover raw material, drip loss, and freeze dried drip loss from the macroalgae species *Saccharina latissima* (SL) and *Alaria esculenta* (AE). All values are given in percent of sample wet weight. Dry matter excluding ash content is calculated by subtracting the determined ash content from the determined dry matter content.

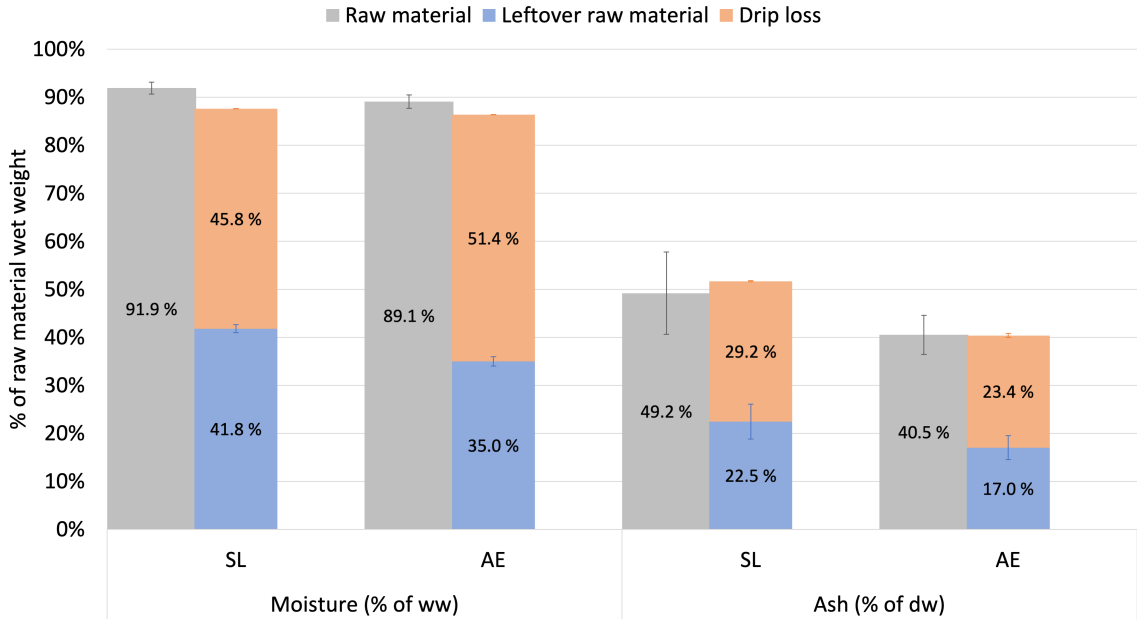

Figure 3.3: Moisture and ash content of macroalgae raw material, leftover raw material, and drip loss from *Saccharina latissima* (SL) and *Alaria esculenta* (AE) in percent of wet weight of original raw material sample.

The drip losses were very similar in overall composition with just under 93.5% moisture and just over 6.5% dry matter of wet weight. The ash content was about 70% of dry matter. This resulted in the distributions shown in Figure 3.2, with dry matter excluding ash constituting 1.92±0.08% and 1.91±0.06% of wet weight and the mineral content constituting 4.82±0.02% and 4.64±0.08% of wet weight. This implies that 23% and 16% of the respective dry matter excluding ash contents of the *S. latissima* and *A. esculenta* raw material samples were lost to the drip losses during thawing. The *S. latissima* and *A. esculenta* raw materials also lost just under 60% of their ash content to the drip losses. Consequently, after freezing and thawing the leftover *S. latssima* and *A. esculenta* raw materials contained 77% and 84% of the dry matter excluding ash originally present in the raw materials, and less than half of the original mineral content of the raw material. The high loss of moisture could make subsequent drying of the leftover raw materials easier, and the mineral loss could reduce the content of unwanted minerals such as iodine and heavy metals.

From Figures 3.2 and 3.3 it can be seen that the leftover *A. esculenta* raw material contained less moisture in percent of wet weight than the leftover *S. latissima* raw material, 82% versus 88%. Additionally, it can be seen that a larger proportion of the moisture content from the *A. esculenta* raw material was lost to the drip loss than for the *S. latissima* raw material, 58% compared to 50%. This supports the previously mentioned observations of a more dry leftover *A. esculenta* raw material, and contributes to the higher amount of obtained *A. esculenta* drip loss per gram of raw material.

The moisture contents of the raw material samples of both macroalgae were quite high, at 91.9±1.3% and 89.1±1.4% of wet weight of *S. latissima* and *A. esculenta*, respectively. These values are just barely below the highest values found in literature of respectively 93% (Forbord et al., 2020) and 92% (Nielsen, 2018). In Fische (2016) the moisture content of the two species were reported to be higher in May compared to June, and in Schiener et al. (2014) moisture content was reported to be highest during the winter months followed by a decline into the autumn months. The samples analysed in this study were harvested in the end of April (*S. latissima*) and beginning of May (*A. esculenta*), and a high moisture content was therefore expected based on the previously reported seasonal variations.

The ash content of the *S. latissima* raw material of 49±9% of dry weight was also among the highest levels of previously reported *S. latissima* ash content. The ash content of the *A. esculenta* raw material, of 41±4% of dry weight was slightly higher than expected, as the highest value found in literature was 33% of dry weight (Schiener et al., 2014). According to (Schiener et al., 2014) the ash content has a similar seasonal variation as the moisture content, with high values during the winter months and a decrease into the autumn months. Additionally, Fische (2016) reported higher ash contents of both macroalgae in May than in June. High ash contents of the samples studied in this work were therefore expected. The elevated ash content of *A. esculenta*, when compared to previously reported values, could be due to geographical variations.

3.2 Mineral Content

The mineral content was determined for freeze dried drip loss samples prepared as a part of the preliminary study described in Sund (2019). The drip loss samples were obtained from a different *Saccharina latissima* raw material, but the same *Alaria esculenta* raw material. The method for obtaining the drip loss samples was somewhat different compared to this study, details of the preparation of the drip loss from Sund (2019) are given in Appendix A.3 on page v. The concentrations of the minerals potassium (K), sodium (Na), iodine (I), phosphorus (P), magnesium (Mg) and calcium (Ca) are given in milligrams per gram freeze dried drip loss in Figure 3.4, while the concentrations of the minerals strontium (Sr), arsenic (As), mercury (Hg) and cadmium (Cd) are given in micrograms per gram freeze dried drip loss in Figure 3.5. The samples were also analysed for lead (Pb), selenium (Se), and zinc (Zn), but these were not present in concentrations above the limit of detection for the individual minerals; less than 0.2, 10, and 2 μg/g, respectively.

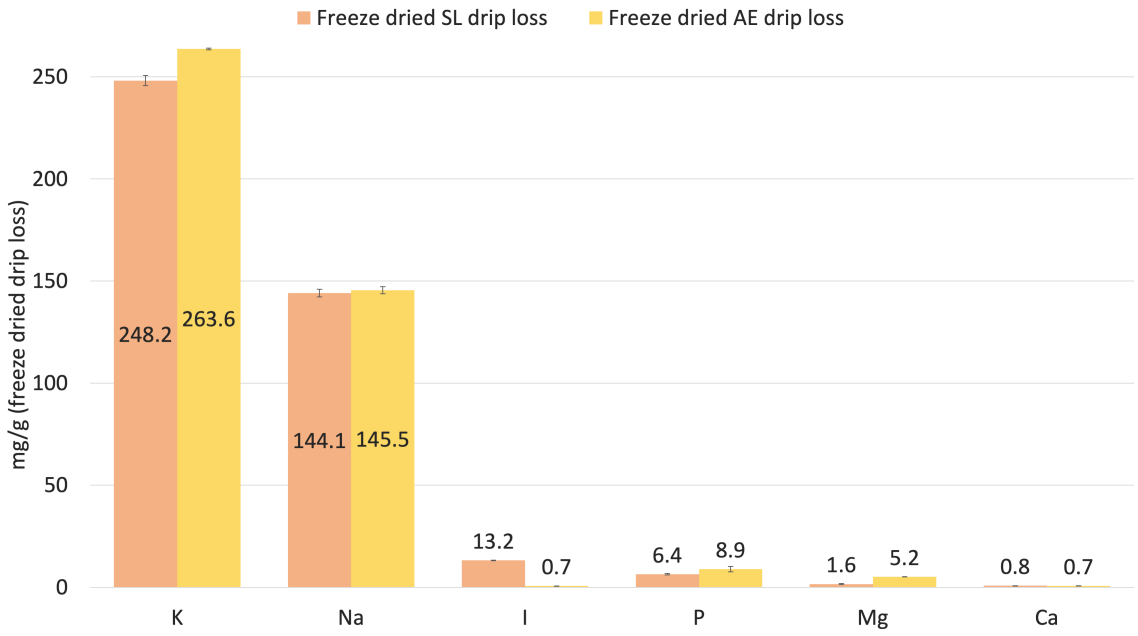

Figure 3.4: Amount of the minerals potassium (K), sodium (Na), iodine (I), phosphorus (P), magnesium (Mg) and calcium (Ca) present in the freeze dried drip loss samples from *Saccharina latissima* (SL) and *Alaria esculenta* (AE) given in milligrams per gram freeze dried drip loss sample.

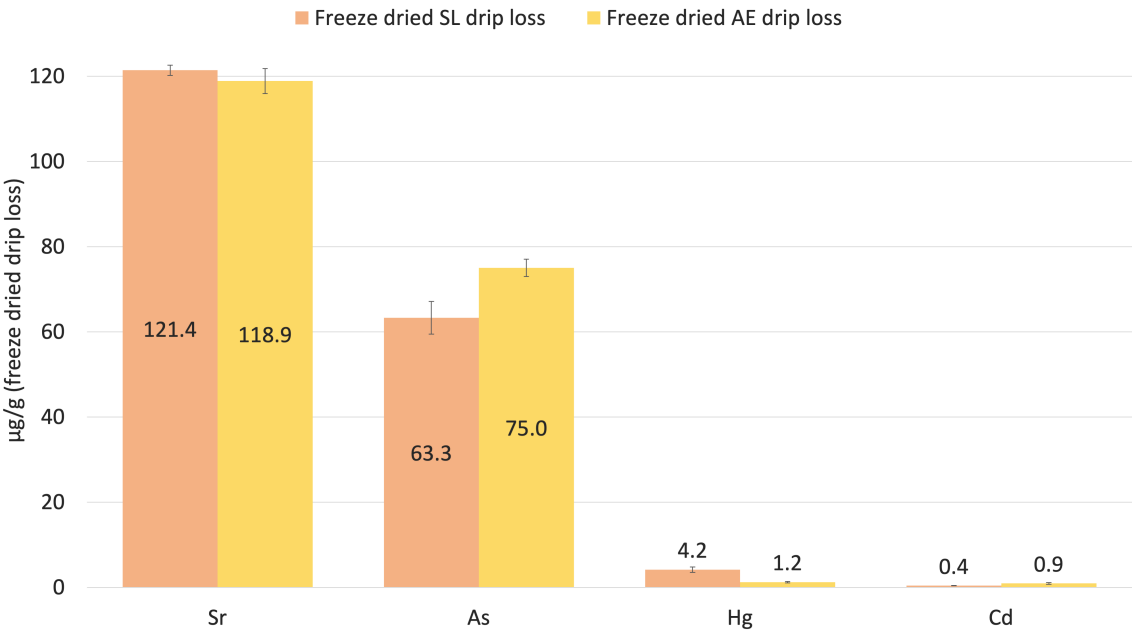

Figure 3.5: Amount of the minerals strontium (Sr), arsenic (As), mercury (Hg) and cadmium (Cd) present in the freeze dried drip loss samples from *Saccharina latissima* (SL) and *Alaria esculenta* (AE) given in micrograms per gram freeze dried drip loss sample.

From Figures 3.4 and 3.5 it can be seen that the main minerals present in the drip loss samples was potassium, followed by sodium. The *A. esculenta* drip loss contained slightly more potassium, phosphorus, magnesium, arsenic and cadmium than the *S. latissima* sample, while the *S. latissima* drip loss contained more iodine, strontium, and mercury than the *A. esculenta* sample. The remaining minerals were present in similar amounts in the two drip loss samples. The mineral content of macroalgae have been shown to vary substantially with factors such as cultivation depth, site location and season (Schiener et al., 2014; Sharma et al., 2018), which makes it difficult to assess if the overall mineral composition in drip loss reflects that of the raw material. However, it seems that potassium, sodium, iodine, phosphorus, magnesium, and calcium are usually the top six minerals present in *S. latissima* and *A. esculenta*, although some variations are seen within these elements (Mæhre et al., 2014; Olsson et al., 2020; Schiener et al., 2014; Sharma et al., 2018; Stévant et al., 2017a). Seeing as these also are the six top minerals present in the drip loss it is plausible that the mineral content of the drip loss was similar to that of the raw material. The minerals potassium, iodine, phosphorus, magnesium, and calcium are all important in a healthy diet (Nordic Council of Ministers, 2014), meaning that their presence in the drip loss is beneficial if it were to be used as a food additive. However, the concentration of these, except iodine, in the drip loss samples are very low compared to the daily intake recommended by Nordic Council of Ministers (2014), and consumption of one gram of drip loss would cover less than 2% of the recommended daily intake of these minerals.

Macroalgae have been under investigation for use as salt-replacing ingredient in manufactured food products by lowering the sodium to potassium (Na/K) ratio (Stévant et al., 2017a). Diets with high Na/K ratios are associated with health risks, such as high blood pressure and cardiovascular diseases (Perez & Chang, 2014). The Na/K ratio recommended by the World Health Organisation (WHO) is close to 1.0 (Stévant, 2019). The Na/K ratios of the drip loss samples are 0.58 and 0.55 for *S. latissima* and *A. esculenta*, respectively. This shows a potential use of the drip losses as salt-replacing ingredients in the food industry. However, Stévant (2019) also reported a Na/K ratio of 0.25 for the red macroalgae *Palmaria palmata*, indicating that other macroalgae might be better for use as salt-replacing ingredients than the *S. latissima* and *A. esculenta* drip losses. Stévant et al. (2017a) reported a Na/K ratio of 0.56 for *S. latissima* and 0.94 for *A. esculenta*, however, Sharma et al. (2018), reported very varying sodium and potassium concentrations in *S. latissima* at different depths and harvest times, giving a Na/K ratio ranging from 0.55 to 1.55. This implies that without knowing the mineral content of the raw material the drip loss samples were obtained from it is not possible to conclude if the Na/K ratio in the drip loss reflects that of the raw material. This could be included in further research to investigate if the Na/K ratio of the leftover raw material is increased or decreased after thawing and removal of drip loss.

Iodine is an essential trace mineral for humans (Banach et al., 2020). However, excessive intake of iodine can also be dangerous (Duinker et al., 2016). Brown macroalgae have been reported to contain higher amounts of iodine than red and green macroalgae (Kilinç et al., 2013), and it has been shown that *S. latissima* contains very high amounts of iodine (Lüning & Mortensen, 2015; Nielsen et al., 2020). Due to this, consumption of *S. latissima* could easily become a source of excessive iodine intake. High iodine content of macroalgae has therefore been identified as a major risk of macroalgae consumption in several studies (Banach et al., 2020; Duinker et al., 2016; Sá Monteiro et al., 2019). Because of this, many studies have looked at methods for reducing the iodine content of *S. latissima*, *A. esculenta*, and other macroalgae (Lüning & Mortensen, 2015; Nielsen et al., 2020; Nitschke & Stengel, 2016). The European Food Safety Authority (EFSA) has recommended a tolerable upper intake level for iodine at 600μg/day (European Food Safety Authority, 2006). The iodine content of the freeze dried drip loss samples was determined to be 13 200$\pm$400mg/kg and 710$\pm$60mg/kg for *S. latissima* and *A. esculenta*, respectively. This shows that consumption of one gram of freeze dried drip loss from *S. latissima* or *A. esculenta* would result in intakes of respectively 22 and 1.2 times the EFSA recommended upper tolerable limit. Due to this, some considerations should be taken to the iodine content before using the drip loss samples, especially from *S. latissima*, directly in food products. Reported iodine contents of *S. latissima* range from 39-4800mg/kg dry weight (Lüning & Mortensen, 2015; Nielsen et al., 2020; Schiener et al., 2014), and of *A. esculenta* from 220-1238 mg/kg dry weight (Mæhre et al., 2014; Nitschke & Stengel, 2016; Schiener et al., 2014). The high iodine content of the *S. latissima* drip loss compared to *A. esculenta* was therefore expected due to its famously high iodine content. Due to the high iodine content of the drip loss, freezing and thawing could be investigated as a method for reducing

iodine contents of macroalgae. Additionally, the drip loss could be used for iodine extraction for the use in iodine food supplements.

Dietary exposure to inorganic arsenic can cause numerous adverse health effects, including cancer of the lung, urinary bladder, and skin, at very low amounts. Research into the doses likely to cause such health effects have resulted in the withdrawal of a previous provisional tolerable weekly intake (PTWI) established by WHO and FAO of 15 micrograms per kilo of body weight, due to results showing that even very low exposure levels can be unsafe. No new guidelines have been established (European Food Safety Authority (EFSA), 2009). The presence of respectively 63±4 and 75±2 micrograms of arsenic per gram of freeze dried *S. latissima* and *A. esculenta* drip loss, is therefore a potential hazard associated with use of the drip loss samples in food products. However, it is the inorganic form of arsenic that has been shown to be the most toxic (Mæhre et al., 2014), and the arsenic content presented here is the total arsenic content, including both organic and inorganic arsenic. Additionally, a study by Díaz et al. (2012) showed that the majority of the arsenic in macroalgae is in the organic form. This implies that the arsenic content is likely to be in organic form and therefore not pose as big of a threat as if all of it was in the inorganic form. To determine the actual hazard posed by this arsenic content it would be necessary to determine the proportion of inorganic arsenic.

Mercury is another a heavy metal that can cause adverse health effects if consumed by humans (Mæhre et al., 2014). Mercury is most toxic in the form of methylmercury, which is classified as a possible carcinogenic and can damage the neurodevelopment of children. The tolerable weekly intake (TWI) of mercury is 4 micrograms per kilogram body weight, while for methylmercury it is 1.3 micrograms per kilogram body weight. The mercury present in fish and seafood is mostly in the form of methylmercury, but not much is known on the speciation of mercury in macroalgae (Sá Monteiro et al., 2019). The mercury contents of the drip loss samples were determined to be 4.3±0.6 and 1.22±0.14 micrograms per gram of freeze dried *S. latissima* and *A. esculenta* drip loss, respectively. As the form of this content has not been determined, it is safest to assume that it is in the form of methylmercury. Based on this assumption, an intake above 22 and 76 grams a week of the respective drip loss samples would cause excessive intake for a person weighing 70 kilograms. If the drip loss was only used as a food additive in small amounts, and some of the mercury was not in the form of methylmercury, this would most likely not pose a health threat for the consumers. However, it should be taken into consideration when evaluating the safety of consumption of the drip loss samples. Speciation studies determining the portion of methylmercury could help evaluate the hazard posed by the mercury content.

Cadmium is also a heavy metal unsuitable for human consumption (Mæhre et al., 2014). It has been shown to be toxic to the kidneys and bones, and a TWI of 2.5 micrograms per kilogram bodyweight has been determined (Sá Monteiro et al., 2019). The cadmium levels of the drip loss samples were determined to be 0.40±0.03 and 0.9±0.2 micrograms per gram freeze dried *S. latissima* and *A.*

esculenta drip loss, respectively. These values imply that to overstep the TWI for cadmium, a person weighing 70kg would have to consume over 400 grams or around 200 grams a week of the respective drip losses. These are quite high quantities indicating that the cadmium content of the drip losses would not be the main concern if they were to be used in food products.

The mineral content of the drip loss can give it potential for use as fertiliser or to enrich the soil with nutrients. However, the phosphorus content determined here is much too low for use of the drip loss directly as fertiliser or as the only fertiliser, as 20kg of phosphorous per hectare is a normal dosing (Olsson et al., 2020). This would mean that to obtain the required phosphorous content for one hectare of land would require 3 125 kg and 2247 kg of the respective *S. latissima* and *A. esculenta* drip losses. This is not a realistic scenario, and indicates that if the phosphorus content from the drip loss samples were to be used in fertilisers, it would have to be extracted from the drip loss. However, for small scale organic cultivation, drip loss from macroalgae might be useful for enriching the soil.

3.3 Phenolic Content

The phenolic content of the original drip loss samples and the freeze dried drip loss from thawing of the macroalgae *Saccharina latissima* and *Alaria esculenta* was determined using the Folin-Ciocalteu phenolic content assay. In addition to fresh untreated samples of the drip loss and freeze dried drip loss, the supernatants from TCA and ethanol protein precipitations were analysed to attempt to remove proteins interacting with the analysis (Singleton et al., 1999). Additionally, frozen and thawed drip loss samples, and freeze dried drip loss samples stored at 4°C for about 10 weeks were analysed. The ethanol precipitation was conducted on the fresh drip loss samples and the TCA precipitation was conducted on the frozen and thawed and stored drip loss samples. The results of these precipitations should therefore be compared accordingly; ethanol precipitation results should be compared to results for the fresh drip loss samples, and TCA precipitation results should be compared to results for frozen and thawed and stored drip loss samples. The results for the original drip loss samples and the freeze dried drip loss samples are given in Tables 3.2 and 3.3, respectively.

Table 3.2: Phenolic content of the original drip loss samples from thawing of the macroalgae *Saccharina latissima* and *Alaria esculenta*. The phenolic content is given in micromoles of propyl gallate (PG) equivalents per litre of drip loss as well as percent of dry weight (DW) original macroalgae sample. The trichloroacetic acid (TCA) precipitation was conducted on the frozen and thawed drip loss samples and the corresponding values should therefore be compared to the values for the frozen and thawed drip loss samples, while the values from the ethanol precipitation should be compared to the values for the fresh drip loss samples.

Phenolic content	[μ**mol** $_{\textbf{PG eq.}}$/$\mathbf{L_{drip\ loss}}$]	[**% of DW**]
Saccharina latissima		
Fresh drip loss	843 ± 5	0.1045 ± 0.0007
After ethanol precipitation	760 ± 20	0.094 ± 0.003
Frozen and thawed drip loss	845 ± 6	0.1046 ± 0.0007
After TCA precipitation	800 ± 30	0.100 ± 0.003
Alaria esculenta		
Fresh drip loss	896 ± 17	0.0928 ± 0.0018
After ethanol precipitation	639 ± 18	0.0663 ± 0.0019
Frozen and thawed drip loss	780 ± 4	0.0808 ± 0.0004
After TCA precipitation	750 ± 20	0.078 ± 0.002

Table 3.3: Phenolic content of the freeze dried drip loss samples from thawing of the macroalgae *Saccharina latissima* and *Alaria esculenta*. The phenolic content is given in micromoles of propyl gallate (PG) equivalents per gram of freeze dried drip loss as well as percent of dry weight (DW) original macroalgae sample. The trichloroacetic acid (TCA) precipitation was conducted on the frozen and thawed drip loss samples and the corresponding values should therefore be compared to the values for the frozen and thawed drip loss samples, while the values from the ethanol precipitation should be compared to the values for the fresh drip loss samples.

Phenolic content	[μmol $_{\text{PG eq.}}$/g$_{\text{drip loss}}$]	[% of DW]
Saccharina latissima		
Freeze dried drip loss	11.2 ± 0.6	0.086 ± 0.005
After ethanol precipitation	9.14 ± 0.17	0.0704 ± 0.0013
Stored freeze dried drip loss	12.7 ± 0.3	0.098 ± 0.002
After TCA precipitation	13.4 ± 0.3	0.104 ± 0.003
Alaria esculenta		
Freeze dried drip loss	10.2 ± 0.3	0.0622 ± 0.0018
After ethanol precipitation	9.5 ± 0.3	0.0581 ± 0.0014
Stored freeze dried drip loss	10.85 ± 0.09	0.0663 ± 0.0006
After TCA precipitation	11.5 ± 0.5	0.070 ± 0.003

As phenolic compounds are generally more soluble in polar organic solvents than in water, and with the drip loss is essentially being a water extract, the phenolic compound yield of the drip loss was expected to be low. The highest phenolic contents determined for both species were in the fresh original drip loss samples, of 0.1045±0.0007% and 0.0928±0.0018% of the dry weight of the raw material samples of *S. latissima* and *A. esculenta*, respectively. Considering that the lowest reported phenolic contents of the two species found in the literature are 0.07% and 0.31% of dry weight for *S. latissima* and *A. esculenta*, respectively, the amount of phenolic content transferred to the drip loss during thawing seems to be quite low, especially for *A. esculenta*. As the phenolic content of the original raw material is unknown it is not possible to know how much of the phenolic content of the raw material ended up in the drip loss. Seasonal variations in polyphenol content reported by Schiener et al. (2014) and Roleda et al. (2019) seem to indicate that the polyphenol content of the species should be at its lowest from March to the beginning of May, followed by increasing content during summer and autumn. The raw material samples used in this study were harvested in the end of April and the start of May, making it likely that they have low phenolic content. However, as the samples were harvested quite late in the reported minimum period, the polyphenol content could have started to increase. Either way, it seems that only a small amount of the phenolic content of the *A. esculenta* raw material was transferred to the drip loss during thawing, while for the *S. latissima* raw material the fraction of phenolic content transferred to the drip loss during thawing seems to be somewhat higher.

Brown macroalgae have been shown to be higher in phenolic compounds than green and red macroalgae (Wang et al., 2009), also seen in Table 1.1 on page 2. In Table 1.2 on page 11, it can be seen that the phenolic content of *A. esculenta* has been shown to reach 6.1 percent of dry weight, while the phenolic content of *S. latissima* only seems to reach 1.5 percent of dry weight (Roleda et al., 2019). This implies that of the two species only *A. esculenta* is defined as a high-phenolic species with a phenolic content higher than 2% of dry weight (Van Alstyne & Paul, 1990). With this quite large difference in phenolic content it could be expected that the drip loss from *A. esculenta* would have a higher phenolic content than the *S. latissima* drip loss. However, for both the original and freeze dried drip loss samples, the phenolic content was determined to be significantly higher in the *S. latissima* samples, at 843±5 micromoles per litre and 11.2±0.6 micromoles per gram for *S. latissima* original and freeze dried drip losses, and 896±17 micromoles per litre and 10.2±0.3 micromoles per gram for *A. esculenta* original and freeze dried drip loss. This difference was also found in Sund (2019). Without analysing the phenolic content of the raw material it is not possible to know if this is due to *S. latissima* containing more water-soluble phenols, or if, at the time of harvest for the different species, they contained similar amounts of phenolic compounds. Considering the fact that when comparing the phenolic content of the drip loss samples in percent of dry weight of the raw material it also seems as if the *S. latissima* raw material lost a larger proportion of its phenolic content to the drip loss than the *A. esculenta* raw material, this might be the case. This is something that should be investigated in future studies to determine if *S. latissima* loses a higher fraction of its phenolic content to drip loss during thawing than *A. esculenta*.

For both species the phenolic content of the original drip loss samples were significantly higher than for the freeze dried drip loss samples; 0.1045±0.0007% and 0.086±0.005% of the dry weight of *S. latissima* raw material, for original and freeze dried drip loss samples, respectively, and 0.0928±0.0018% and 0.0622±0.0018% of dry weight of *A. esculenta* raw material, for original and freeze dried drip loss samples, respectively. This is most likely due to aggregation of either phenolic compounds or interfering proteins during the freeze drying process.

All fresh drip loss samples, both original and freeze dried, showed significantly higher phenolic content than after ethanol precipitation. This indicates that some non-ethanol-soluble proteins present in the drip loss samples interfere with the phenolic content assay, or that some of these larger non-ethanol-soluble proteins are bound to phenols, causing removal of phenolic content along with the protein precipitation. As phenolic compounds are generally more soluble in polar organic solvents such as ethanol (Wang et al., 2009), it seems unlikely that phenolic content has been precipitated in the ethanol precipitation. This implies that the decrease in phenolic content might actually be due to a decrease in interfering proteins, and that the actual phenolic content is closer to the content of the ethanol precipitation supernatant. Additionally, the ethanol precipitation supernatant was concentrated by freeze drying, which may have affected the phenolic or protein content as it seems to have done in the freeze dried drip loss samples.

The drip loss from *A. esculenta* showed a significant decrease in phenolic content after freezing and thawing. This difference was not observed for the *S. latissima* drip loss. This implies that some of the phenolic compounds present in the *A. esculenta* drip loss, and not in the *S. latissima* drip loss, are sensitive to freezing and thawing.

The phenolic content of stored, freeze dried *S. latissima* drip loss was determined to be significantly higher than the fresh freeze dried drip loss. This was not expected as it is unlikely that the phenolic content increases during storage. However, this could be due to changes during storage of interfering compounds, making them quantifiable in the Folin-Ciocalteu assay after storage.

Additionally, the stored freeze dried *S. latissima* drip loss phenolic content was determined to be significantly lower than the phenolic content after the TCA-precipitation. This is not expected either, as the TCA-precipitation should remove proteins that possibly interact with the assay and decrease the apparent phenolic content, or, if none of the non-acid-soluble proteins interfere with the assay, not change the phenolic content. The difference might be caused by a slight difference in the absorbance of the solution due to the presence of TCA in the assay, or TCA might interfere with the actual chemical reactions in the assay. Another explanation could be that some protein-phenolic complexes are broken as the protein is precipitated by the addition of TCA, which might cause the phenolic compound that is left in the supernatant to react more strongly with the Folin-Ciocalteu assay and therefore give a higher phenolic content. None of the other samples showed a significant difference after the TCA-precipitation. This implies that none of the non-acid-soluble proteins present in the drip loss samples from either species interfere with the assessment of phenolic content by the Folin-Ciocalteu phenolic content assay.

Regardless of possible protein interactions in the assay, the phenolic content of the drip loss samples from thawing of the macroalgae *S. latissima* and *A. esculenta* is quite low. Phenolic compounds from macroalgae, and in particular phlorotannins from brown macroalgae, have been shown to possess strong antioxidant activities, making them interesting for use as natural antioxidant additives for food products (Wang et al., 2009). As the phenolic content of the drip loss is quite low, drip loss is not likely to be a good source for extracting macroalgae phenolics. However, if the drip loss is to be used as a food additive without much further treatment, some phenolic content can be favourable. Nonetheless, most of the phenolic compounds present in the original raw material is most likely left in the leftover raw material, especially for *A. esculenta*.

3.4 Antioxidant Activity

The antioxidant activity of the drip loss samples from the thawing of frozen samples of the macroalgae *Saccharina latissima* and *Alaria esculenta* was measured using the ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) assay. The antioxidant activity was analysed for the fresh original and freeze dried drip loss samples, as well as the frozen and thawed original drip loss samples and the freeze dried drip loss samples after storage at 4°C for about 13 weeks. The ABTS assay was favoured over the DPPH assay due to challenges with the solubility of the freeze dried drip loss samples in methanol and associated issues uncovered in a preliminary study, described in Sund (2019). The results are shown in Tables 3.4 and 3.5 for original drip loss samples and freeze dried drip loss samples, respectively.

Because the freeze dried drip loss samples were insoluble in methanol they were first dissolved in water and then diluted with methanol as the samples in the ABTS assay should be diluted with methanol. To determine if this had any impact on the result the freeze dried drip loss samples were dissolved in water at two different concentrations, one approximating the original drip loss sample concentration of dry matter, and one at double this concentration. The samples were then diluted to the same concentration with 80% methanol before they were analysed in the ABTS assay. The different approaches to dissolution and dilution showed no significant differences, implying that dissolving the freeze dried samples in water before diluting with 80% methanol does not affect the results from the assay.

Table 3.4: Antioxidant activity in micromoles propyl gallate (PG) equivalents per litre of drip loss and per gram of raw material dry weight for the original, fresh and frozen and thawed drip loss samples from thawing of the macroalgae *Saccharina latissima* and *Alaria esculenta*.

Antioxidant activity	$[\mu\mathbf{mol}_{\mathbf{PG\ eq.}}/\mathbf{L}_{\mathbf{drip\ loss}}]$	$[\mu\mathbf{mol}_{\mathbf{PG\ eq.}}/\mathbf{g}_{\mathbf{DW}}]$
Saccharina latissima		
Drip loss	830 ± 80	4.9 ± 0.4
Frozen and thawed drip loss	640 ± 90	3.8 ± 0.5
Alaria esculenta		
Drip loss	340 ± 30	1.68 ± 0.17
Frozen and thawed drip loss	380 ± 70	1.9 ± 0.4

Table 3.5: Antioxidant activity in micromoles propyl gallate (PG) equivalents per gram of freeze dried drip loss and per gram of raw material dry weight for the freeze dried drip loss samples, both fresh and stored at 4°C for about 13 weeks, from thawing of the macroalgae *Saccharina latissima* and *Alaria esculenta*.

Antioxidant activity	[μmol $_{\text{PG eq.}}$/g$_{\text{drip loss}}$]	[μmol $_{\text{PG eq.}}$/g$_{\text{DW}}$]
Saccharina latissima		
Freeze dried drip loss	10.1 ± 1.0	3.7 ± 0.4
Stored freeze dried drip loss	10.0 ± 1.5	3.6 ± 0.6
Alaria esculenta		
Freeze dried drip loss	5.2 ± 0.6	1.51 ± 0.16
Stored freeze dried drip loss	5.6 ± 1.5	1.63 ± 0.42

Both the original and freeze dried drip loss samples were significantly different between the species, with the *S. latissima* samples showing about double the antioxidant activity. The original drip loss samples showed antioxidant activities of 830±80 and 340±30 μmoles PG equivalents per litre drip loss, for *S. latissima* and *A. esculenta*, respectively. While the freeze dried drip loss samples showed antioxidant activities of 10.1±1.0 and 5.2±0.6 μmoles PG equivalents per gram of freeze dried drip loss, for *S. latissima* and *A. esculenta*, respectively. As phenolic compounds are known to have antioxidant properties (Wang et al., 2009), it could be expected that the phenolic content of the samples coincided with the antioxidant activity. However, even though the *S. latissima* drip loss sample contained slightly more phenolic compounds than the *A. esculenta* sample, it is not comparable to the large difference in the antioxidant activity. This implies that some of the phenolics present in the drip loss from *A. esculenta* do not possess antioxidant activity measurable with the ABTS assay, or that the *S. latissima* drip loss sample contained other compounds with antioxidant activity. These compounds could either be present in higher concentrations in the *S. latissima* drip loss or not present at all in the *A. esculenta* drip loss sample.

There are other compounds present in brown macroalgae that have been shown to possess antioxidant activity, including sulphated polysaccharides such as fucoidan, and cartenoid pigments such as fucoxanthin (Holdt & Kraan, 2011; Wang et al., 2009). The results from the carbohydrate analysis, which will be discussed in Section 3.5 on page 43, showed that the drip loss samples contained small amounts of fucose, indicating that there was some fucoidan present in the drip loss samples. This is in agreement with findings from a heteronuclear single quantum coherence (HSQC) fingerprint analysis done as a part of the previously mentioned preliminary study described in Sund (2019), where the analysis showed traces of homofucans in the *S. latissima* sample, and heterofucans in the *A. esculenta* sample. As can be seen from Figure 3.8 on page 46 in Section 3.5, the fucose content is higher in the *S. latissima* drip loss sample, than in the *A. esculenta* drip loss sample. This implies that the *S. latissima* drip loss contains more fucose residues as a part of the fucoidan content. Fucose residues can be sulphated, and in a study by Wang

et al. (2008), it was suggested that the ratio of sulphate content to fucose was an effective indicator of antioxidant activity. This difference in fucose content, and consequently fucoidan content, in the drip loss samples might be the reason for the higher antioxidant activity exhibited by the *S. latissima* drip loss sample. Additionally, the *S. latissima* drip loss was darker in colour than the *A. esculenta* drip loss. Seen in relation with the antioxidant activity there could be a possibility that the darker colour and the increased antioxidant activity was caused by an increased fucoxanthin content. In a study by Stévant et al. (2017a), the effects of seawater storage on the chemical composition of *S. latissima* and *A. esculenta* was studied. This showed that *A. esculenta* had a higher fucoxanthin content than *S. latissima*, but that only the *S. latissima* sample lost fucoxanthin during seawater storage. This could be an indication that the *S. latissima* raw material loses fucoxanthin content more easily, and that a similar response might be seen during thawing.

There was no significant differences between fresh original and freeze dried drip loss samples, and frozen and thawed original and stored freeze dried drip loss samples. However, the standard deviations increased quite a lot. This could be due to instability of some of the compounds with antioxidant properties, causing larger variations in the antioxidant activity between parallels taken from the samples.

The original *S. latissima* drip loss sample showed a significantly higher antioxidant activity than the freeze dried drip loss. There was no significant difference observed between the original and freeze dried *A. esculenta* drip loss samples. This might indicate that some of the compounds with antioxidant activity present in the *S. latissima* drip loss, but not in the *A. esculenta* drip loss, are sensitive to the freeze drying process.

3.5 Carbohydrate Analysis

The concentrations of the carbohydrates mannitol, fucose, glucose, galactose, rhamnose, mannose and xylose in the *Saccharina latissima* and *Alaria esculenta* raw material, leftover raw material and freeze dried drip loss from thawing were determined through a carbohydrate analysis. The results are shown as percent of dry weight of raw material in Figures 3.6 and 3.7 on the next page, for *S. latissima* and *A. esculenta*, respectively. All values are given as weight of the sugar monomers after hydrolysis of the polysaccharides. During hydrolysis the glycosidic bonds in the polysaccharides are broken by the addition of water molecules, the concentrations presented here are therefore slightly overestimated compared to the actual polysaccharide content. The concentrations of there carbohydrates in percent of each sample weight are given in Appendix A.4.

The rhamnose content was very low in all samples and is therefore not presented. Mannose and xylose content is presented together as these were not possible to separate with the method used. The sum of the carbohydrates quantified in this analysis does not reflect the total carbohydrate content as the hydrolysis protocol used in does not hydrolyse cellulose, and mannuronic and guluronic acids were not quantified. Three parallels of each sample were weighed out and hydrolysed and two parallells were prepared from each hydrolysis tube, resulting in a total of 6 parallels for each sample. However, parallel two for the leftover raw material of *S. latissima* deviated substantially from the remaining parallels and was therefore removed from the calculations. Additionally, due to a much higher mannitol concentration, mannitol had to be quantified from 50 times diluted samples, while the other carbohydrates were quantified from five times diluted samples. Due to laboratory closures because of the Covid-19 pandemic, only one of the two parallels from after the hydrolysis were analysed at a 50 times dilution. The mannitol content is therefore based on three parallels, and only two parallels for the leftover raw material.

There were some issues in the execution of the carbohydrate analysis, such as lower signals and bigger variations between samples than normally expected, as well as decreasing values for consecutive parallels. This was suspected to be due to the gold electrode used in the HPLC being slightly old and worn. This is suspected to be the reason why some of the samples have quite high standard deviations, as well as why the mass balances seem to be a bit skewed, with the combined carbohydrate contents of the leftover raw material and the drip loss samples appearing to be higher than the contents of the raw material. Due to thess issues the carbohydrate analysis should have been repeated to verify the results presented here. However, due to laboratory closures because of the Covid-19 pandemic, this was not possible. Therefore, the following results might not be completely reliable. Nonetheless, they give an indication of the carbohydrate content of raw material and drip loss from thawing, and the effect freezing and thawing has on the carbohydrate composition of *S. latissima* and *A. esculenta* raw matereial. This is something that could be repeated in future studies to ensure more reliable results.

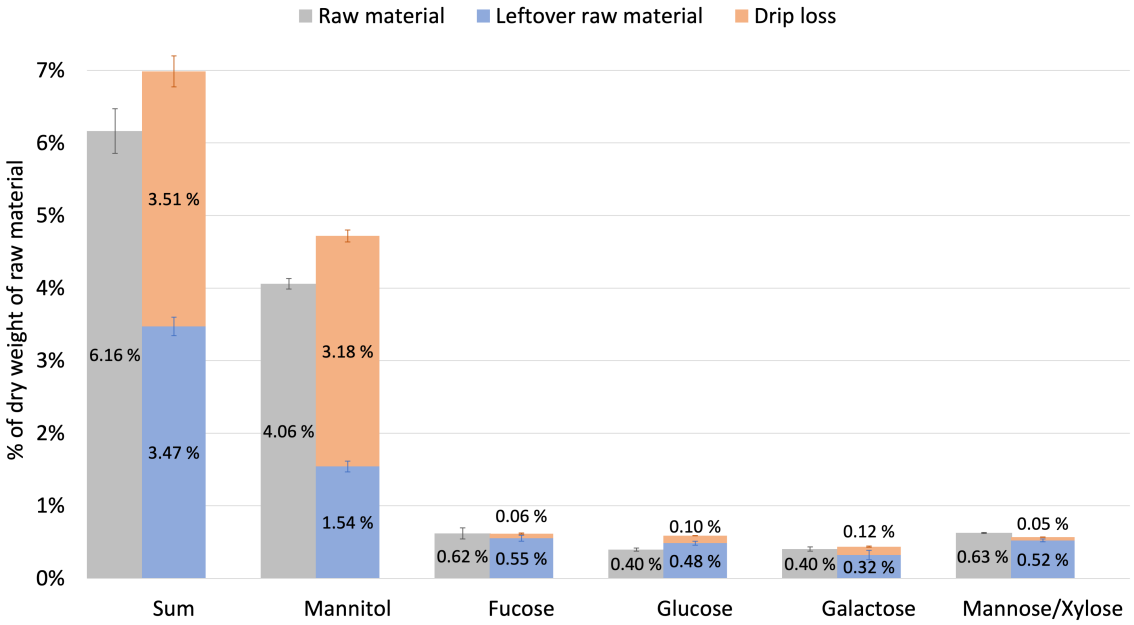

Figure 3.6: Sum of measured carbohydrate content, as well as mannitol, fucose, glucose, galactose, and combined mannose and xylose content of raw material, leftover raw material and drip loss of *Saccharina latissima*.

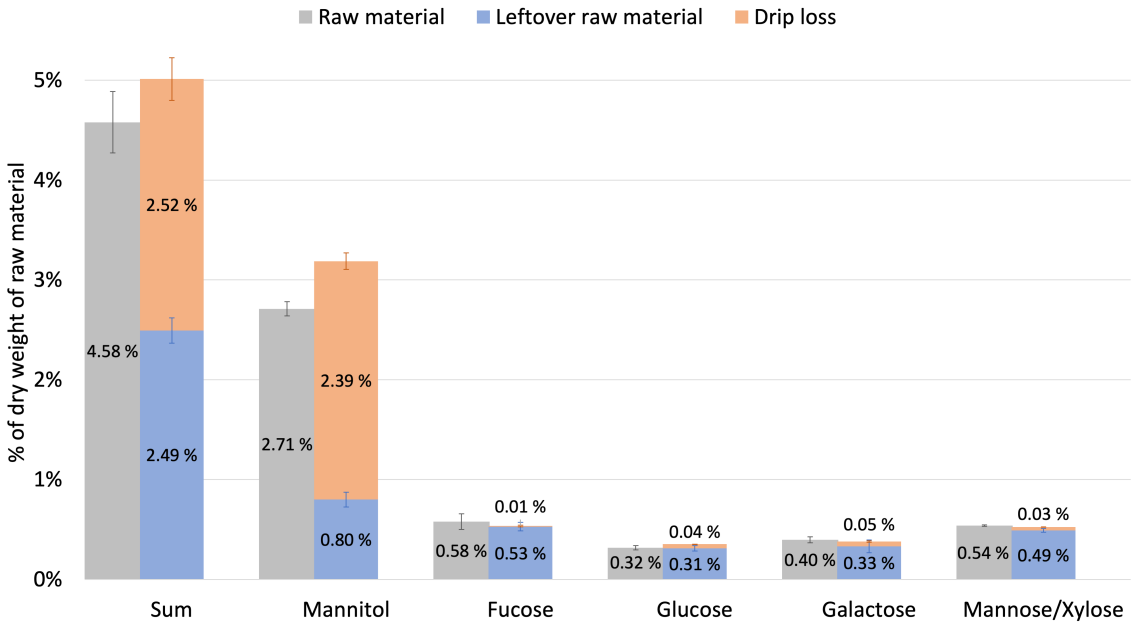

Figure 3.7: Sum of measured carbohydrate content, as well as mannitol, fucose, glucose, galactose, and combined mannose and xylose content of raw material, leftover raw material and drip loss of *Alaria esculenta*.

As can be seen from Figures 3.6 and 3.7, mannitol was the main carbohydrate present in all the samples. Additionally, as seen in the mass balances presented in Figures 3.6 and 3.7, for both *S. latissima* and *A. esculenta* the majority of the

mannitol content present in the raw material was transferred to the drip loss during thawing. Of the other carbohydrates analysed the majority was retained in the leftover raw material.

3.5.1 Carbohydrate Composition of Raw Material

From Figures 3.6 and 3.7 it can be seen that the *S. latissima* raw material contained more carbohydrates than the *A. esculenta* raw material, determined to be 6.2±0.3% compared to 4.58±0.08% of dry weight. The sum of the carbohydrate content of both raw material samples was much lower than the reported carbohydrate contents of the species, up to 77% and 72% of dry weight for *S. latissima* and *A. esculenta*, respectively (Schiener et al., 2014). The main reason for this is due to cellulose not being broken down during hydrolysis and no quantification of mannuronic and guluronic acids, the components of alginate. Alginate and cellulose were reported by Schiener et al. (2014) to constitute 28.5% and 11.0% of dry weight in *S. latissima*, and 37.4% and 11.3% of dry weight in *A. esculenta*. It was therefore expected that the sum of the carbohydrates quantified in this work would be much lower than the expected total sum of carbohydrates.

Mannitol is usually present as a monomeric compound (Baweja et al., 2016), however, some of the mannitol content could also be from laminaran polymers, as they sometimes contain some mannitol residues (Jacobsen et al., 2019). The mannitol contents of the raw materials were determined to be 4.1±0.3% and 2.71±0.07% for *S. latissima* and *A. esculenta*, respectively. The average mannitol content reported by Schiener et al. (2014) was 18.6% and 12.1% for *S. latissima* and *A. esculenta*, respectively. The mannitol content determined in this study is much lower than the average reported by Schiener et al. (2014). However, they also reported a minimum in the mannitol content during early spring, which is around when the macroalgae samples used in this study were harvested. Additionaly, Handå et al. (2013) reported variations in the mannitol content of *S. latissima* between 2-18% of dry weight. However, they reported a peak in the mannitol content in April with a decrease into May. Due to conflicting reports of the seasonal variation it is difficult to know if the mannitol content should be at a maximum or at a minimum. Nonetheless, the mannitol contents are within the reported ranges, but in the lower part of these.

The fucose content of the samples is indicative of the fucoidan content, as L-fucose is the main monosaccharide constituent of fucoidans (Usov et al., 2001). Fucose content in *S. latissima* and *A. esculenta* was reported by (Stévant et al., 2017a) to be 0.76% and 1.25% of dry weight, respectively. These values are somewhat higher than the ones determined in this study, however, the difference could be due to seasonal or geographic variations.

Glucose in macroalgae in the Laminariales order is mainly found within the cell walls in the form of cellulose and laminaran (Stévant et al., 2017a). As cellulose was not broken down in the hydrolysis protocol used in the carbohydrate

analysis in this work, the glucose content presented here represents the laminaran content of the samples. Laminaran can also contain 6-9% uronic acids and some mannitol (Jacobsen et al., 2019), implying that some of the mannitol content present in the samples could also come from laminaran polymers. The average laminaran content reported by Schiener et al. (2014) was 8.2% and 11.1% of dry weight for *S. latissima* and *A. esculenta*. This is much higher than the glucose concentrations of 0.40±0.03% and 0.316±0.008% of dry weight determined for respectively *S. latissima* and *A. esculenta* in this work. However, looking into the seasonal variations for the laminaran content reported by Schiener et al. (2014), the laminaran content seems to be highly variable with low content in the first part of the year up until April. Because of this, low laminaran content is reasonable for the samples analysed in this work.

3.5.2 Carbohydrate Composition of Freeze Dried Drip Loss

Figure 3.8 shows the carbohydrate composition of the freeze dried drip loss samples from *S. latissima* and *A. esculenta* in percent of the freeze dried drip loss sample dry weight. From this it can be seen that the *S. latissima* drip loss contained more carbohydrates than the *A. esculenta* drip loss, determined to be 9.7±0.6% compared to 8.8±0.3% of dry weight.

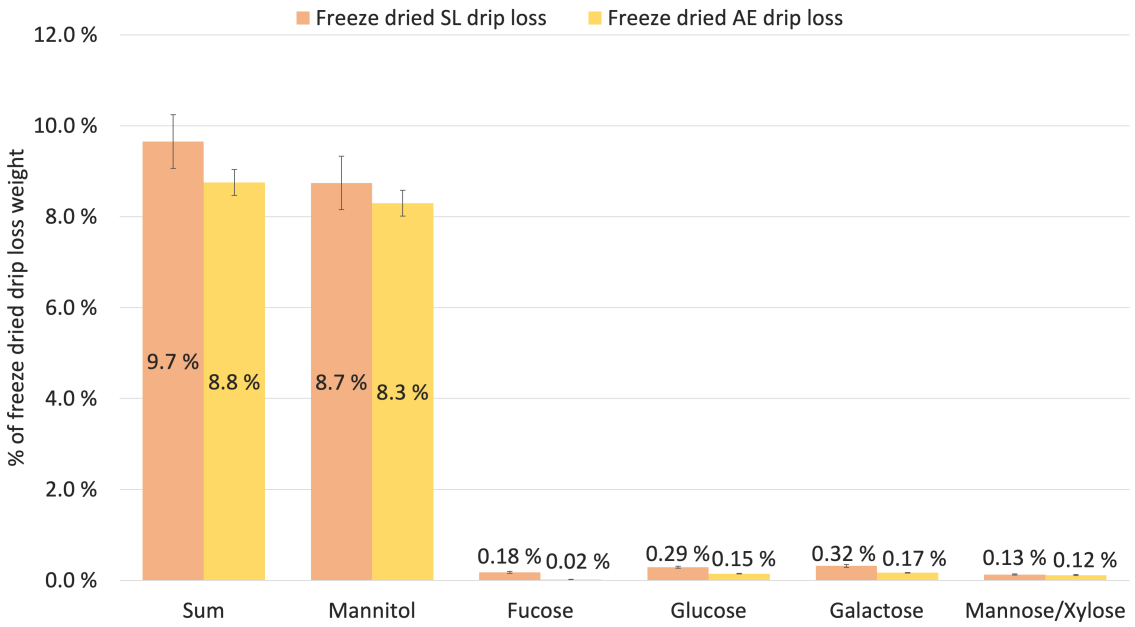

Figure 3.8: The carbohydrate composition of the freeze dried drip loss samples from *Saccharina latissima* (SL) and *Alaria esculenta* (AE). The sum represents the sum of all the shown carbohydrates. The concentrations are given in percent of freeze dried drip loss dry weight.

The *S. latissima* drip loss had a slightly higher mannitol concentration than the *A. esculenta* sample, at 8.5±0.6% of dry weight, compared to 7.9±0.3% of

dry weight. Mannitol has a relative sweetness of 40-50% compared to sucrose (Baweja et al., 2016), making it a common sweetener in sugar-free products (Holdt & Kraan, 2011). As substantial amounts of the mannitol present in *S. latissima* and *A. esculenta* raw material is transferred to the drip loss during thawing (seen in Figures 3.6 and 3.7), drip loss from thawing of these macroalgae could be used for further extraction of mannitol as a value-added product in a processing facility. Additionally, the drip losses could be used to enhance the flavour of food products by adding a sweet taste. If this was the case, it would probably be necessary to freeze dry or at least concentrate the drip loss samples as the water content is high.

As mentioned in the previous section, the fucose content of the samples are indicative of the fucoidan content. The fucose contents of the drip loss samples from *S. latissima* and *A. esculenta* were determined to be 0.17±0.02% and 0.0214±0.0009% of freeze dried drip loss dry weight, respectively. This implies that the drip loss samples contain some fucoidan, however, these are small amounts, especially in the *A. esculenta* drip loss. In a preliminary study, described in Sund (2019), a heteronuclear single quantum coherence (HSQC) fingerprint analysis of drip loss samples showed traces of homofucans in the *S. latissima* drip loss and heterofucans in the *A. esculenta* drip loss. This, along with the determined fucose content in this work, suggests that drip loss from thawing of these macroalgae species contain small amounts of fucoidan polymers. Additionally, fucoidans have been shown to be viscous in very low concentrations (Kraan, 2012), which indicates that the observed viscosity of the drip loss solutions could be due to the fucoidan content. This is consistent with the observed higher viscosity of the *S. latissima* drip loss compared to the *A. esculenta* drip loss, as the *S. latissima* drip loss seems to have a higher fucoidan concentration.

3.5.3 Impact of Freezing and Thawing

In Figures 3.6 and 3.7 mass balances show the carbohydrate content in the raw material before thawing and the distribution of the same carbohydrates between the leftover raw material and the drip loss after thawing for samples of *S. latissima* and *A. esculenta*, respectively. From these figures it is possible to assess the impact freezing and subsequent thawing had on the macroalgae raw material.

The sum of the carbohydrates quantified in this work (mannitol, fucose, glucose, galactose, and mannose/xylose) changes substantially after thawing. Only about half of the quantified carbohydrate content of the raw material is left in the leftover raw material after removing the drip loss. However, this is mainly due to the majority of the mannitol content of the raw material ending up in the drip loss after thawing. Of the remaining carbohydrates only very small amounts are transferred to the drip loss, and most is left in the leftover raw material. Therefore, the impact of freezing and thawing of *S. latissima* and *A. esculenta* raw material seems to be low for most carbohydrates, except for mannitol. This could cause a change in flavour of the frozen and thawed macroalgae as mannitol gives them a sweet taste. If the mannitol content is not of import, then freezing and thawing seems to be a suitable preservation method regarding the carbohydrate content.

3.6 Protein Content

There are many different analytical methods for determining protein content. These are based on different analytical principles, determining protein content directly or indirectly. Direct protein determination is based on analysis of amino acid residues, while indirect methods can be based on calculations from nitrogen content or after chemical reactions with functional groups within the protein. This complicates comparison of protein content determined in different studies, as they might be based on different analytical methods causing considerable variations in the reported content. In addition, protein extraction method further affects the result due to different extraction yields (Mæhre et al., 2018). In a study by Mæhre et al. (2018), different analytical methods for protein determination were tested on several food products. The methods they compared were a total amino acid analysis, Kjeldahl's method, Bradford's method and a modified version of the Lowry method. For the mehtods requiring extraction of protein two different methods were used and extraction yield was determined. Their study showed that using the Kjeldahl method to convert nitrogen content into protein content overestimated the protein content compared to the amino acid analysis, and that the spectrophotometric methods also overestimated the protein content due to interfering substances. They concluded that protein determination using amino acid analysis was the only method where interfering substances did not affect the results, and recommended this method as a preferred method for food protein determination. This is consistent with the recommendation from the Food and Agriculture Organisation of the United Nations (FAO) in FAO (2002) that, when possible, protein content in food products should be determined by the sum of the amino acid content.

However, there are issues concerning protein determination by amino acid composition analysis as well, well described in Fountoulakis and Lahm (1998). A proper hydrolysis step is a prerequisite for a successful analysis, but as the hydrolysis and destruction of liberated residues proceed simultaneously, no hydrolysis method can liberate all amino acids while still enabling quantitative recovery of them in the hydrolysate. The completeness of the hydrolysis method is dependent on temperature, time, hydrolysis agent and additives. Treatment with hydrochloric acid (HCl) under high temperature is the most common hydrolysis method. While effective and convenient, there are limitations to this method: The amino acids asparagine and glutamine are completely hydrolysed to aspartic and glutamic acid, respectively. Tyrosine is partially destroyed, while tryptophan is completely destroyed, and cysteine cannot be directly determined from the acid-hydrolysed samples. Serine and threonine are partially hydrolysed as well (Fountoulakis & Lahm, 1998). Methionine has also been shown to suffer transformation during acid hydrolysis (Mustățea et al., 2019). Additionally, some bonds can be difficult to cleave due to steric hindrance and the degree of unfolding, causing an incomplete release of all amino acid residues (Fountoulakis & Lahm, 1998). The method also quantifies free amino acids, which causes an overestimation of the total protein content, however, it is generally accepted that this is evened out by the reduction caused by partial or complete destruction of certain amino acids during the acid hydrolysis (Bak et al., 2019).

Four different methods were used to analyse protein content in this study; the Lowry protein assay, a CN-analysis, a total amino acid analysis and a free amino acid analysis. The results from each analysis are given and discussed in the following sections.

3.6.1 Lowry Protein Assay

The protein contents determined in the original drip loss samples, frozen and thawed drip loss samples, supernatant and pellet from ethanol precipitation, and supernatant from TCA-precipitation are given in Table 3.6. The corresponding results for the freeze dried drip loss samples are given in Table 3.7.

Table 3.6: Protein content of the original drip loss samples from thawing of the macroalgae *Saccharina latissima* and *Alaria esculenta* measured using the Lowry protein assay. The protein content is given in grams per litre of drip loss as well as milligrams per gram dry weight (DW) original macroalgae sample. The ethanol-soluble peptides were measured in the supernatant from an ethanol protein precipitation, and the non-ethanol-soluble proteins were measured in the pellet from the same precipitation. The acid soluble peptides were measured from the supernatant from a trichloroacetic acid (TCA) precipitation. The TCA precipitation was conducted on the frozen and thawed drip loss samples and the corresponding values should therefore be compared to the values for the frozen and thawed drip loss samples, while the values from the ethanol precipitation should be compared to the values for the fresh drip loss samples.

Protein content	$[\mathbf{g/L_{drip\ loss}}]$	$[\mathbf{mg/g_{DW}}]$
Saccharina latissima		
Fresh drip loss	1.41 ± 0.02	8.22 ± 0.11
Ethanol-soluble peptides	1.175 ± 0.007	6.86 ± 0.04
Non-ethanol-soluble proteins	0.276 ± 0.008	1.61 ± 0.05
Frozen and thawed drip loss	1.52 ± 0.03	8.85 ± 0.16
Acid soluble peptides	1.539 ± 0.012	8.99 ± 0.07
Alaria esculenta		
Fresh drip loss	1.11 ± 0.02	5.44 ± 0.11
Ethanol-soluble peptides	0.977 ± 0.009	4.77 ± 0.04
Non-ethanol-soluble proteins	0.200 ± 0.15	0.97 ± 0.07
Frozen and thawed drip loss	1.19 ± 0.04	5.8 ± 0.2
Acid soluble peptides	0.963 ± 0.015	4.70 ± 0.07

Table 3.7: Protein content of the freeze dried drip loss samples from thawing of the macroalgae *Saccharina latissima* and *Alaria esculenta* measured using the Lowry protein assay. The protein content is given in grams per litre of drip loss as well as milligrams per gram dry weight (DW) original macroalgae sample. The stored freeze dried drip loss samples were stored for 12 weeks at 4°C. The ethanol-soluble peptides were measured in the supernatant from an ethanol protein precipitation, and the non-ethanol-soluble proteins were measured in the pellet from the same precipitation. The acid soluble peptides were measured from the supernatant from a trichloroacetic acid (TCA) precipitation. The TCA precipitation was conducted on the 12 weeks old drip loss samples and the corresponding values should therefore be compared to the values for the stored drip loss samples, while the values from the ethanol precipitation should be compared to the values for the fresh freeze dried drip loss samples.

Protein content	$[\mathbf{mg/g_{drip\ loss}}]$	$[\mathbf{mg/g_{DW}}]$
Saccharina latissima		
Freeze dried drip loss	21.2 ± 0.5	7.70 ± 0.17
Ethanol-soluble peptides	15.1 ± 0.2	5.47 ± 0.06
Non-ethanol-soluble proteins	3.935 ± 0.013	$(1.385 \pm 0.005)\cdot 10^{-3}$
Stored freeze dried drip loss	22.5 ± 1.1	8.2 ± 0.4
Acid soluble peptides	22.9 ± 0.3	8.33 ± 0.10
Alaria esculenta		
Freeze dried drip loss	15.8 ± 0.4	4.55 ± 0.10
Ethanol-soluble peptides	14.36 ± 0.07	4.13 ± 0.02
Non-ethanol-soluble proteins	1.66 ± 0.07	$(0.46 \pm 0.02)\cdot 10^{-3}$
Stored freeze dried drip loss	16.4 ± 0.6	4.73 ± 0.17
Acid soluble peptides	14.4 ± 0.2	4.14 ± 0.05

The *S. latissima* drip loss samples contained more protein than the *A. esculenta* samples, both in the original drip loss and the freeze dried drip loss. This is consistent with the findings in Sund (2019), however, in the aforementioned study, the protein content of the *S. latissima* drip loss was determined to be somewhat higher than found in this study. This might have been because the *S. latissima* raw material used to obtain the drip loss analysed in this previous study had not been frozen immediately after harvest, and was therefore suspected to be slightly degraded, giving a higher protein concentration in the drip loss. In general, the protein concentration in *A. esculenta* raw material is reported to be higher than in *S. latissima* raw material (Fische, 2016; Schiener et al., 2014), from this it could be expected that the *A. esculenta* drip loss would contain more proteins, as more proteins are available to be transferred into the drip loss during thawing. However, it is also reported that *A. esculenta* contains more phenolic compounds than *S. latissima* (Roleda et al., 2019; Schiener et al., 2014). This could imply that more proteins in *A. esculenta* are bound to non-water-soluble phenolic compounds, keeping them from being transferred to the drip loss during thawing.

For both *S. latissima* and *A. esculenta* the original drip loss samples have a statistically significant higher protein content than the freeze dried drip loss. This could be due to aggregation or other changes in the protein content during the freeze drying. Additionally, this could be caused by the aggregation or changes of phenolic compounds, as the Folin-Ciocalteu phenolic content assay showed that the phenolic content was lower after freeze drying (see Section 3.3 on page 36), or due to changes in other compounds that interfere with the Lowry protein assay. However, the non-ethanol-soluble protein concentration, which should only include larger proteins, is much higher in the original drip loss samples than in the freeze dried drip loss samples, indicating that something happens to the larger proteins present in the drip loss during freeze drying. This could be due to aggregation or complex formation with soluble phenols, or cross-linking with minerals.

There is also a statistically significant difference between the fresh original drip loss and the frozen and thawed drip loss from *S. latissima*. However, the measured protein concentration is higher in the frozen and thawed drip loss. This might be due to a change in the proteins present during the freezing or thawing that makes them interact with the assay in a different way, or, as the difference is not large, it might just be due to the analyses being conducted at different times. Interfering compounds could also have changed during freezing and thawing, making them interfere more strongly with the assay. There are no significant differences in the protein content before and after freezing and thawing of the original drip loss from *A. esculenta*, or before and after 12 weeks storage at 4°C in either of the freeze dried drip loss samples.

All original drip loss and freeze dried drip loss samples contain significantly higher amounts of protein than both the pellet and supernatant from the ethanol precipitation. The pellet and the supernatant from this precipitation contains non-ethanol-soluble proteins and ethanol-soluble peptides, respectively. There is more ethanol-soluble peptides than non-ethanol-soluble proteins in the drip loss samples. This indicates that a major part of the protein present in the drip loss are small peptides or free amino acids.

Both the original drip loss and freeze dried drip loss from *A. esculenta* contain significantly higher concentrations than the corresponding trichloroacetic acid (TCA) precipitation supernatants. This indicates that the *A. esculenta* drip loss might have a higher concentration of larger proteins than *S. latissima*, as there seems to be no larger proteins in *S. latissima* that precipitated in the TCA-precipitation.

The Lowry protein assay has been widely used for analysis of protein content for many decades due to its simplicity and availability (Mæhre et al., 2018). However, there are some issues with the method that can cause inaccurate results. It uses the Folin phenol reagent and the colour indicating the amount of protein present in the sample is developed through two reactions: First a reaction with the copper in the alkaline copper reagent, and then a reduction of the

phosphomolybdic-phosphotungstic reagent by the copper-treated protein.(Lowry et al., 1951) The main chromogenic amino acids are tyrosine and tryptophan, and to a lesser extent cysteine, cystine and histidine. The peptide linkages are also chromogenic.(Peterson, 1979) Free amino acids give a lower colour yield (Lowry et al., 1951). The sequence, chain length, and exposure of functional groups in peptides can all affect the colour yield positively or negatively, causing the results in absorbance to not be completely proportional to the protein content. Additionally, many substances have been found to interfere with the Lowry method, most of these interfering substances reduce the Folin phenol reagent (Peterson, 1979). Interfering substances relevant for this study are; phenols, glycine, potassium ions, mannose, glucose and xylose (Bensadoun & Weinstein, 1976). The method also uses a reference protein for creation of a standard curve, which can cause the determined concentration to be inaccurate for the sample being analysed.

3.6.2 CN-analysis

Determination of protein content from the total nitrogen content of macroalgae is a commonly used method (Biancarosa et al., 2017). In fact, Angell et al. (2016) reported that 52% of all studies regarding determination of protein content of macroalgae used nitrogen content determination to estimate the protein content, applying a nitrogen-to-protein conversion factor of 6.25. This traditional nitrogen-to-protein conversion factor is based on the assumption that the sample contains protein with 16% nitrogen and an insignificant amount of non-protein nitrogen (Lourenço et al., 2002). However, macroalgae contain considerable amounts of non-protein nitrogen, such as pigments, free amino acids and inorganic nitrogen, which can lead to overestimation of protein content when using the nitrogen-to-protein conversion factor of 6.25 (Angell et al., 2016). Additionally, a variable amino acid composition will cause variations in the nitrogen to protein ratio (Mæhre et al., 2014). Therefore, the accuracy of protein determination from nitrogen content is dependent on establishment of species-specific nitrogen-to-protein conversion factors (Lourenço et al., 2002).

Many studies have calculated new nitrogen-to-protein conversion factors for macroalgae, all reporting conversion factors below 6.25 (Aitken et al., 1991; Bak et al., 2019; Biancarosa et al., 2017; Diniz et al., 2011; Lourenço et al., 2002; Schiener et al., 2014; Shuuluka et al., 2013). In Angell et al. (2016), after a study of available literature on nitrogen-to-protein conversion factors for macroalgae, a default conversion factor for macroalgae of 5 was proposed.

Table 3.8: Nitrogen to protein conversion factors for *Saccharina latissima* and *Alaria esculenta* found in litterature.

	Nitrogen-to-protein conversion factor	
Study	*Saccharina latissima*	*Alaria esculenta*
Schiener et al. (2014)	5.3	6.0
Biancarosa et al. (2017)	4.37	4.45
Manns et al. (2017)	2.1-5.9*	-
Bak et al. (2019)	2.0	-
Forbord et al. (2020)	3.8	-

*:High during summer, low during winter.

As seen in Table 3.8, there are large variations in calculated nitrogen-to-protein conversion factors for *Saccharina latissima* and *Alaria esculenta*. In Manns et al. (2017), a seasonal variation in the conversion factors for *S. latissima* was found, with higher factors during the summer (peak in July), and lower factors during the winter (lowest in February). The calculations in this study are based on the nitrogen-to-protein conversion factors found in Biancarosa et al. (2017), as these are based on samples harvested in Norway. These factors are 4.37 and 4.45 for *S. latissima* and *A. esculenta*, respectively.

The protein contents calculated from the nitrogen content of the raw material, leftover raw material, and the freeze dried drip loss, as well as the nitrogen and carbon contents of the samples are shown in Figure 3.9. The values are given in percent of raw material dry weight, and the leftover raw material and drip loss concentrations are stacked to illustrate the mass balance before and after freezing and thawing. The carbon, nitrogen and protein concentrations for each sample are given in Table A.9 in Appendix A.5 on page vii.

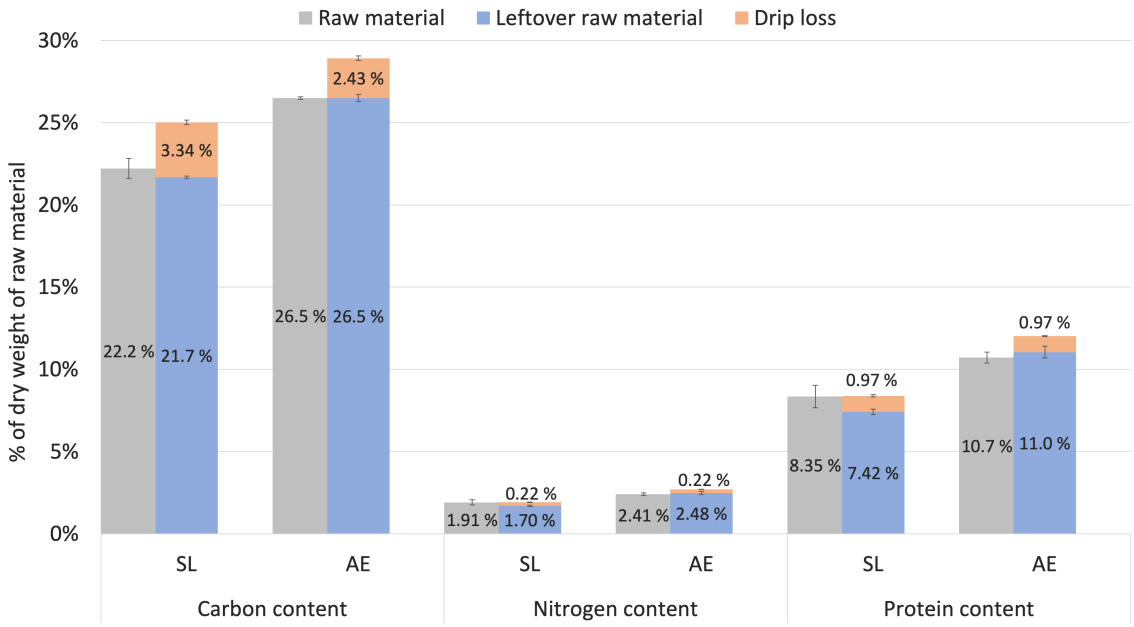

Figure 3.9: Mass balances for carbon, nitrogen and protein content from CN-analysis for *Saccharina latissima* (SL) and *Alaria esculenta* (AE) raw material, leftover raw material and freeze dried drip loss from thawing. The values are given in percent of dry weight of the raw material.

The mass balances shown in Figure 3.9 seems to be a bit skewed, especially for *A. esculenta*, with the combined concentrations of the leftover raw material and the drip loss being higher than the concentration in the original raw material. This might be due to inhomogeneities in the raw material and leftover raw material samples, as no considerations were made to separate blades and stipes, which may have different compositions (Holdt & Kraan, 2011). The samples were crushed to fine powders in an attempt to get homogeneous samples, however, if the samples contained a different blade to stipe ratio, this could affect the results. Additionally, only two parallels of each sample were run, so it is a possibility that the results could even out somewhat if more parallels were tested.

As can be seen from Figure 3.9, most of the carbon, nitrogen and protein content present in the raw material was left in the leftover raw material. Only small amounts were transferred to the drip loss during thawing. This implies that the nutritional value of the leftover raw material when it comes to protein content was not substantially altered in the freezing and thawing processes. Additionally, most of the carbon containing compounds present in the raw material, were retained in the leftover raw material. This was also seen in the carbohydrate analysis (see Section 3.5 on page 43), where mannitol was the only carbohydrate where the majority was transferred to the drip loss. Of the other carbohydrates quantified the majority were retained in the leftover raw material.

The carbon contents of the *S. latissima* and *A. esculenta* raw materials were determined to be 22.2±0.6% and 26.50±0.07% of dry weight, respectively. Schiener

et al. (2014) reported carbon content ranging from 21.1-30.5% of dry weight for *S. latissima* and 28.5-31.2% of dry weight for *A. esculenta*, and seasonal variations giving a maximum content for *S. latissima* in autumn, and between May an July for *A. esculenta*. Minimum carbon contents for both species were reported in March. The value determined for *S. latissima* was within the lowest part of the range reported by Schiener et al. (2014), and just slightly lower than the lowest value reported in Handå et al. (2013). The carbon content determined for the *A. esculenta* raw material was slightly lower than the range reported by Schiener et al. (2014). Low carbon contents were expected from the reported seasonal variations.

The nitrogen contents of the *S. latissima* and *A. esculenta* raw materials were determined to be 1.91±0.16% and 2.41±0.08% of dry weight, respectively. Schiener et al. (2014) reported nitrogen contents ranging from 0.8-2.2% of dry weight in *S. latissima*, and 1.5-2.1% of dry weight in *A. esculenta*, with maximum values between February and May. The values determined in this study were therefore in line with the reported seasonal variations, being in the high end of the range reported for *S. latissima* and higher than the maximum value reported for *A. esculenta*.

The protein contents of the *S. latissima* and *A. esculenta* raw materials were determined to be 8.3±0.7% and 10.7±0.3% of dry weight, respectively. Protein content for the two macroalgae are reported to range between 1.1-13% and 7.0-16% of dry weight for *S. latissima* and *A. esculenta*, respectively (Fische, 2016; Nielsen et al., 2016). Schiener et al. (2014) reported maximum protein levels during the first quarter of the year and minimum during the third quarter of the year. The determined protein values were therefore within the reported ranges and in accordance with the reported seasonal variations, as they were somewhat below the maximum values and would according to the findings of Schiener et al. (2014) be in a transition period with decreasing protein content.

The carbon and nitrogen content, as well as the the protein content calculated from the nitrogen content, for the freeze dried drip loss samples from *S. latissima* and *A. esculenta* are shown in Figure 3.10.

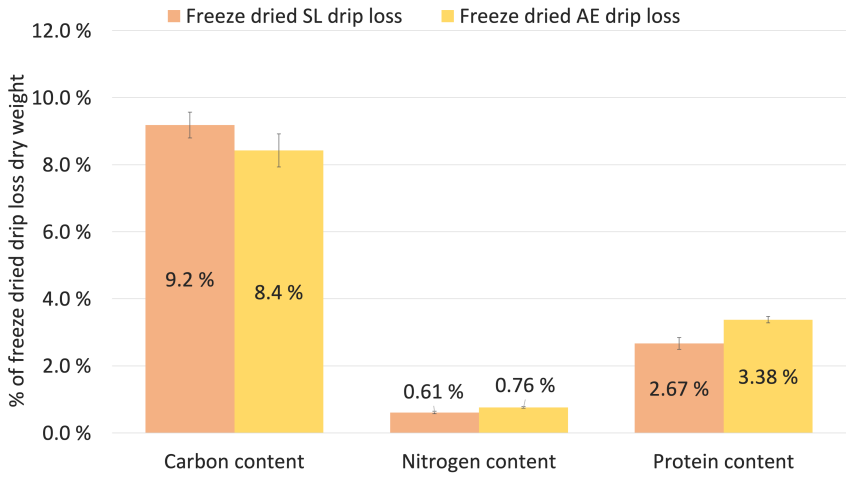

Figure 3.10: Carbon, nitrogen and protein content of freeze dried drip loss samples from thawing of the macroalgae *Saccharina latissima* (SL) and *Alaria esculenta* (AE). The values are given in percent of drip loss weight.

The differences in carbon and nitrogen contents of the two drip loss samples were statistically significant. The carbon contents in both samples were much higher than the nitrogen content. The protein contents of the *S. latissima* and *A. esculenta* drip loss samples were determined to be 2.67±0.18% and 3.38±0.09% of freeze dried drip loss weight, respectively. This was not a significant difference (p=0.063). However, this is not in agreement with the results from the Lowry protein assay (see Section 3.6.1 on page 49), where the results showed that the *S. latissima* drip loss had a higher protein concentration than the *A. esculenta* drip loss. This could be due to the inaccurate species-specific nitrogen-to-protein conversion factors, as the factor used for *A. esculenta* (4.45) was slightly higher than the one for *S. latissima* (4.37).

3.6.3 Total Amino Acids Content

The total amino acid content was determined for the raw material, leftover raw material, and freeze dried drip loss samples. The determined total amino acid compositions of raw material, leftover raw material and drip loss for *S. latissima* and *A. esculenta* are shown in Figures 3.11 and 3.12, respectively. The amino acid content is given in percent of raw material dry weight, and the results for the leftover raw material and drip loss are stacked and displayed next to the raw material to illustrate the distribution of the amino acids from the raw material between the leftover raw material and drip loss. The total amino acid content for each sample is given in Appendix A.6.

The total amino acid content does not include cysteine, proline or tryptophan, as these were not measured. Additionally, the acid hydrolysis causes glutamine and asparagine to be hydrolysed into glutamic acid and aspartic acid, respectively. Threonine and serine are also partially hydrolysed, tyrosine is partially destroyed, and methionine has been shown to suffer transformation during the acid hydrolysis

(Fountoulakis & Lahm, 1998; Mustățea et al., 2019). The amino acid concentrations were calculated using molar weights as if all the amino acids were bound in protein; without the weight of water (18 g/mol).

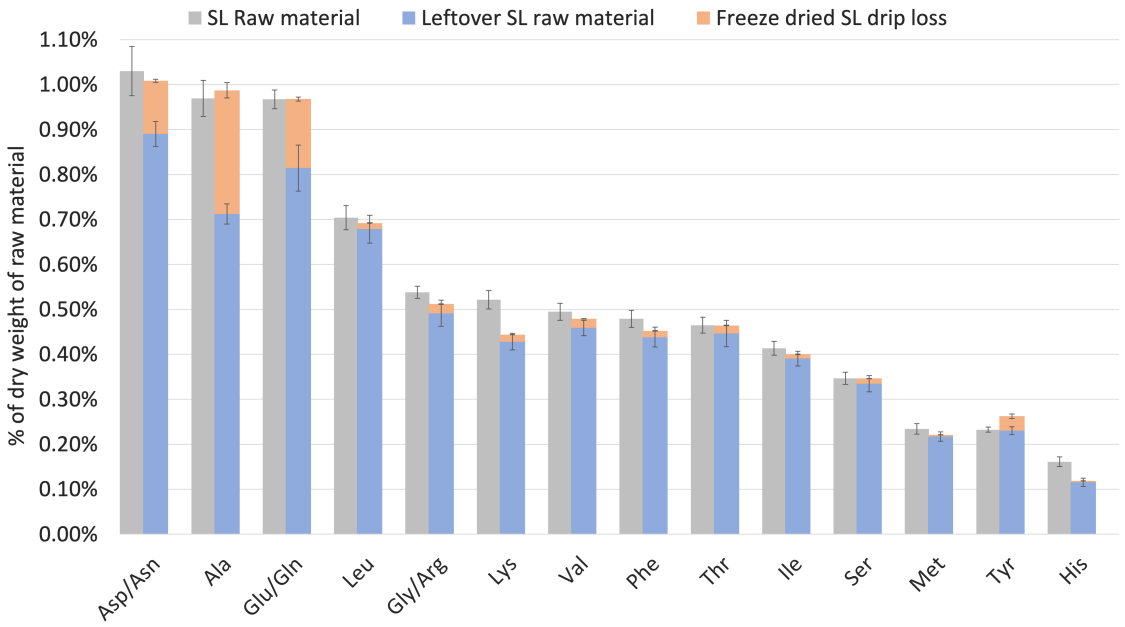

Figure 3.11: Mass balances for total amino acid composititon of *Saccharina latissima* (SL) raw material, leftover raw material and freeze dried drip loss from thawing. The values are given in percent of dry weight of the macroalgae raw material.

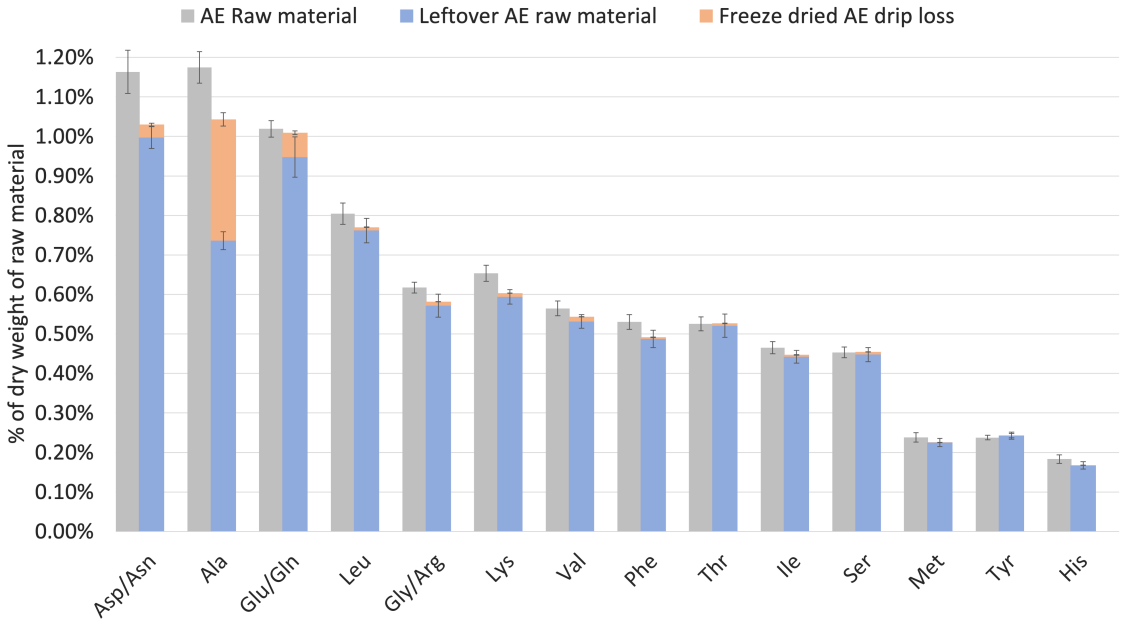

Figure 3.12: Mass balances for total amino acid composititon of *Alaria esculenta* (AE) raw material, leftover raw material and freeze dried drip loss from thawing. The values are given in percent of dry weight of the macroalgae raw material.

From Figures 3.11 and 3.12, it can be seen that alanine was the most abundant amino acid in both drip loss samples, followed by glutamic acid/glutamine, and aspartic acid/asparagine. These most abundant amino acids present in the raw material samples were aspartic acid/asparagine, followed by equal amounts of alanine and glutamic acid/asparagine for *S. latissima*, and aspartic acid/asparagine and alanine in almost equal amounts, followed by glutamic acid/glutamine for *A. esculenta*. In the leftover raw material samples aspartic acid/asparagine, followed by glutamic acid/glutamine were the most abundant for both species, in *S. latissima* these were followed by alanine, while in *A. esculenta* they were followed by leucine and then alanine. This shows that the main amino acid lost to the drip loss samples during thawing, either as free amino acids or as part of proteins, was alanine. Both raw materials lost just under 30% of their alanine content to the drip losses. Some aspartic acid and glutamic acid were also lost to the drip loss, however, in smaller amounts. Of the remaining amino acids only very small amounts were transferred to the drip loss. Glutamic and aspartic acid gives an umami flavour (Torres et al., 2019), and alanine gives a sweet flavour (Wen et al., 2006), indicating that the loss of these amino acids to the drip loss might affect the taste of the leftover raw material.

The sum of total amino acids in the *S. latissima* raw material, estimating the total protein content, was determined to be 7.60±0.09% of dry weight. This was much lower than the April protein content reported by Mols-Mortensen et al. (2017) (14.8%-16.1% of dry weight), while it was higher than the April protein content reported by Bak et al. (2019) (4.4-5.9% of dry weight), and the May protein content reported by Marinho et al. (2015) (1.5-5.2% of dry weight). The protein content is compared to the April and May protein contents as the *S. latissima* sample was harvested on the 29th of April. These reported protein contents of *S. latissima* indicates substantial geographical variations, making it difficult to compare the protein content determined in this study to literature. However, the protein content is within expectations, as it is within the reported ranges of protein content for *S. latissima*. Although some variations, the relative amounts of each amino acid of the *S. latissima* raw material seemed to be consistent with previous findings, with a very similar amino acid profile as reported by Biancarosa et al. (2017).

The sum of total amino acids in *A. esculenta* raw material, estimating the total protein content, was determined to be 8.66±0.12% of dry weight. This was lower than the average around 11% based on literature (Biancarosa et al., 2017; Fische, 2016; Mæhre et al., 2016; Mæhre et al., 2014). However, it was still within the range reported by Fische (2016). The relative amounts of each amino acid of the *A. esculenta* raw material seemed to deviate slightly from literature values, with somewhat higher relative alanine content and lower relative glutamic acid/glutamine content (Biancarosa et al., 2017; Mæhre et al., 2016; Mæhre et al., 2014). Nonetheless, the overall amino acid profile was comparable to the reported values. The deviations from expected protein content and amino acid composition could be due to seasonal or geographical variations.

The determined protein content of the *A. esculenta* raw material in this study was about 14% higher than that of the *S. latissima* raw material. This was expected as literature values seem to indicate that the average protein content of *A. esculenta* is higher than of *S. latissima* (Biancarosa et al., 2017; Fische, 2016; Schiener et al., 2014). The relative amounts of each amino acid were very similar in both samples.

The sum of total amino acids for the freeze dried drip loss samples were 0.724±0.019% and 0.482±0.005% of raw material dry weight, for *S. latissima* and *A. esculenta*, respectively. This shows that the *S. latissima* raw material lost almost 50% more protein per gram of dry weight to the drip loss than the *A. esculenta* raw material. This resulted in protein concentrations of 1.95±0.08% and 1.67±0.03% of the freeze dried drip loss samples of *S. latissima* and *A. esculenta*, respectively. Considering that the *A. esculenta* raw material had a higher protein concentration than *S. latissima* it could be expected that the *A. esculenta* drip loss sample also had a higher protein concentration than the *S. latissima* drip loss. However, *A. esculenta* has also been reported to have a higher phenolic content than *S. latissima* (Roleda et al., 2019), which could mean that more proteins are bound to non-water-soluble phenolic compounds, preventing them from being transferred to the drip loss.

3.6.4 Free Amino Acid Content of Drip Loss

The free amino acid content was determined for the original drip loss samples (frozen and thawed). The concentrations of these free amino acids were calculated using the same molar weights as for the total amino acid concentrations, that is the molar weight as if the amino acid is bound in a protein, to enable comparison with the total amino acid content. The concentrations of alanine, glutamic acid, glutamine, aspartic acid and asparagine present in the drip loss are compared to the total content of the corresponding amino acids in Figure 3.13 for both *S. latissima* and *A. esculenta*. As glutamine and asparagine are hydrolysed to glutamic and aspartic acid during the acid hydrolysis, the total amino acid concentrations for glutamic and aspartic acid also include glutamine and asparagine. Because of this, the free glutamine and asparagine concentrations are stacked on top of the free glutamic and aspartic acid concentrations for accurate comparison between the concentrations of the total and free content of these amino acids. The free amino acid concentrations of the remaining amino acids are compared to the corresponding total amino acid concentrations in Figures 3.14 and 3.15. The concentrations are given in percent of raw material dry weight. The free amino acid content in grams per litre of drip loss is given in Appendix A.7.

Figure 3.13: Comparison of total amino acids and free amino acids for alanine, glutamic acid and aspartic acid in *Saccharina latissima* (SL) and *Alaria esculenta* (AE) drip loss from thawing. The values are given in percent of dry weight of the macroalgae raw material. The slightly lighter green sections in the columns for glutamic acid and aspartic acid as free amino acids represent the free glutamine and asparagine, respectively. These are added here as they are hydrolysed to glutamic acid and aspartic acid in the total amino acid analysis.

The determined alanine content in the drip loss samples from both species, as well as the aspartic acid content of the *A. esculenta* drip loss, were significantly higher than the determined total content of these amino acids. This is not reasonable as the total content of an amino acid should be the sum of the free and bound concentrations of the amino acid. There is no obvious reason for free alanine or aspartic acid to be destroyed during the acid hydrolysis, making it difficult to explain why this happens. The determination of free and total amino acid content of drip loss samples from *S. latissima* and *A. esculenta* should therefore be repeated in further research to ascertain if this is an experimental error or if the free alanine and aspartic acid content is affected by the freeze drying process, is destroyed during acid hydrolysis or is incompatible with the OPA-derivatisation used in the quantification. Because of this, it not possible to determine how much of the alanine in the drip loss samples is free alanine compared to total alanine, nor is it possible for the aspartic acid content of the *A. esculenta* drip loss.

Despite the results being questionable, both samples seem to contain significant amounts of free alanine, which is known to be flavourful providing a sweet taste (Holdt & Kraan, 2011). The presence of free alanine could be beneficial for the use of the drip loss samples in food products. The free alanine concentrations of the original *S. latissima* and *A. esculenta* samples were determined to be 0.55±0.03 and 0.90±0.01 g/L of drip loss. The free glutamic and aspartic acid content of the drip loss samples could also be relevant for the use of the drip loss samples in

food products as thew provide umami flavour (Biancarosa et al., 2017). However, from Figure 3.13 it can be seen that the glutamic acid seems to be mostly bound in protein, and the concentrations of free glutamic acid in the drip loss samples are as low as 15.7±1.0 and 5.40±0.02 mg/L drip loss for *S. latissima* and *A. esculenta*, respectively. This indicates that glutamic acid, which is the main component in the taste sensation of umami (Pangestuti & Kim, 2015), is not the main flavour component of the drip loss samples. The amount of free aspartic acid content is higher. As discussed previously the concentration of free aspartic acid in the *A. esculenta* drip loss was determined to be higher than the total aspartic acid content which makes the results questionable and makes it difficult to discuss the free aspartic acid content. However, in the *S. latissima* drip loss sample, the free aspartic acid concentration seems to constitute the majority of the total aspartic acid content, with free asparagine making up the rest. The free aspartic acid content of the *S. latissima* drip loss was determined to be 0.180±0.009g/L drip loss.

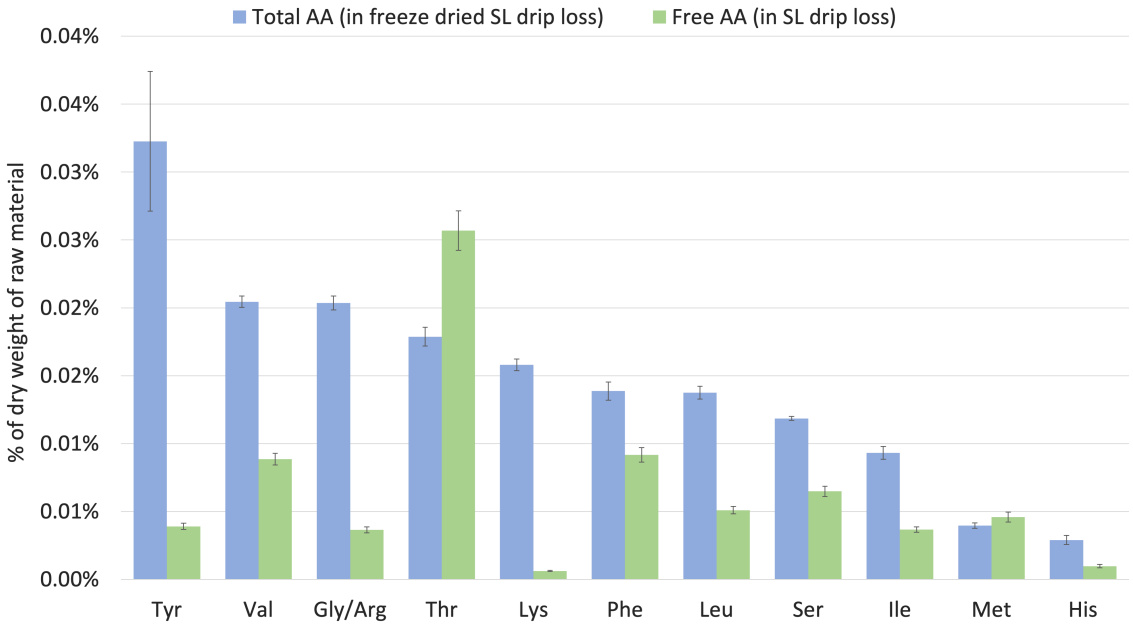

Figure 3.14: Comparison of total amino acids and free amino acids for *Saccharina latissima* (SL) drip loss from thawing. The values are given in percent of dry weight of the macroalgae raw material.

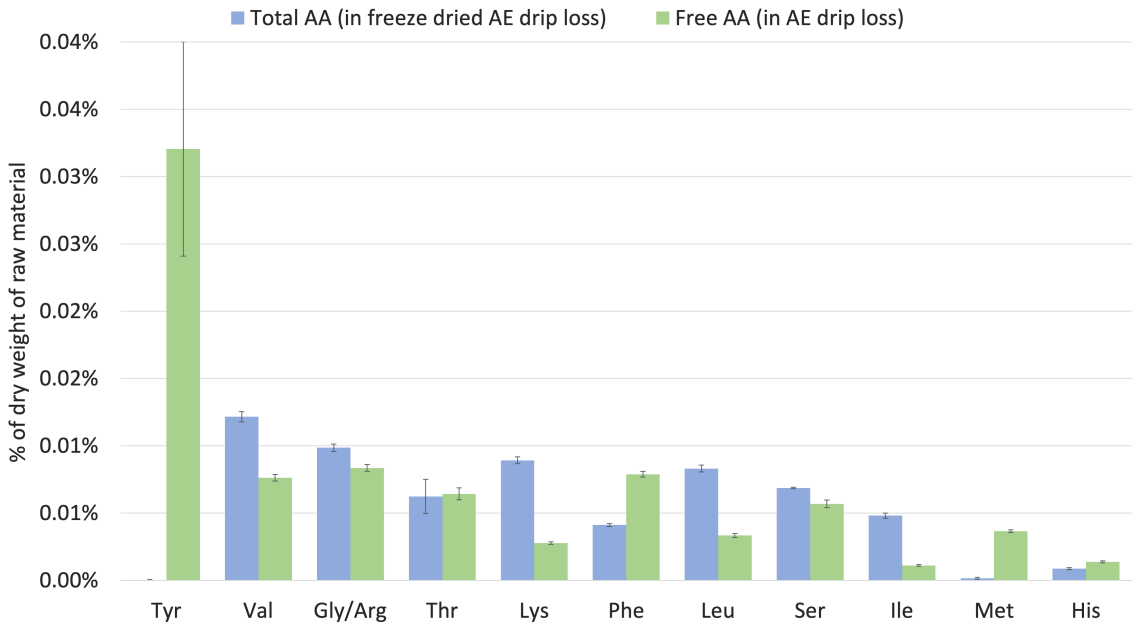

Figure 3.15: Comparison of total amino acids and free amino acids for *Alaria esculenta* (AE) drip loss from thawing. The values are given in percent of dry weight of the macroalgae raw material.

Of the less abundant amino acids present in the drip loss samples the proportion of the total concentration that was present as free amino acids varied. From Figures 3.14 and 3.15, it can be seen that the concentration of free threonine is higher than the total threonine concentration, this is most likely due to the partial hydrolysation of threonine that happens during the acid hydrolysis. The acid hydrolysis is also known to cause partial destruction of tyrosine, this can be seen clearly for the *A. esculenta* sample (see Figure 3.15), where a complete destruction of a substantial amount of tyrosine seems to have occurred. Destruction of tyrosine is not evident in the *S. latissima* sample. The total methionine levels also seem to be affected by the acid hydrolysis, especially in the *A. esculenta* sample. These apparent reductions in the concentrations of certain amino acids caused by the acid hydrolysis contribute to a reduction in the protein content calculated from the sum of total amino acids. However, the protein content from the sum of total amino acids also include the free amino acids, and as most of the amino acids are also present in free forms to some degree this will most likely even out this decrease in protein content caused by the acid hydrolysis. This is a generally accepted assumption in protein determination from amino acid content (Bak et al., 2019).

The protein content of *A. esculenta* seems to be more affected by the acid hydrolysis than the *S. latissima* protein content. This is especially noticable for tyrosine and methionine. Additionally, the proportion of free amino acids to total amino acids seems to be higher in the *A. esculenta* drip loss than in the *S. latissima* drip loss. This could simply be due to an overall higher proportion of free amino acids in the *A. esculenta* drip loss, or it could be a further indication of the protein content of the *A. esculenta* drip loss being more sensitive to the acid hydrolysis.

Addition of phenol has been shown to act as a protective substance to increase the recovery yield of certain residues, such as tyrosine, phenylalanine and histidine, in the hydrolysate (Fountoulakis & Lahm, 1998; Mustățea et al., 2019). In light of this, the observed difference in the destruction of tyrosine in the two drip loss samples could indicate that the phenolic content of the *S. latissima* drip loss, which was determined to be higher than that of the *A. esculenta* drip loss (see Section 3.3), might protect the tyrosine from destruction. Additionally, phenylalanine and histidine contents of the *A. esculenta* drip loss samples also show higher concentrations of free than total amino acids, a trend which is not seen in the *S. latissima* drip loss sample. This indicates that these amino acids could be affected by the acid hydrolysis as well, and might also be protected by phenolic compounds in the *S. latissima* drip loss sample.

3.6.5 Comparisons of Methods

For the freeze dried drip loss samples the Lowry protein assay and the total amino acid content analysis indicated a significantly higher protein concentration in the *S. latissima* sample. However, the protein content from the CN-analysis showed no significant difference between the two samples. For the *S. latissima* sample both Lowry assay and the CN-analysis gave higher protein concentrations than the total amino acids analysis, however, only the difference between the Lowry assay and the total amino acid content analysis was significant. For the *A. esculenta* freeze dried drip loss the CN analysis gave a significantly higher protein concentration than the Lowry assay and total amino acids analysis. The fact that two out of three analyses gave significantly higher protein concentrations for the *S. latissima* drip loss implies that this is the case. Additionally, as there are significant differences between the protein concentration determined using the different methods, and because the total amino acid analysis is generally regarded as the most accurate method for protein determination (Mæhre et al., 2014), the results from the total amino acid analysis are regarded as the most accurate. The results from the Lowry protein assay are also quite close to the protein determined from the total amino acid content, making it more plausible that the protein determined from the total amino acid content is the most accurate.

For the *S. latissima* raw material and leftover raw material the difference between the protein content determined from total amino acid content and the CN-analysis was only statistically different in for the leftover raw material. For *A. esculenta* the difference between the protein content determined from total amino acid content and the CN-analysis was significant for both the raw material and leftover raw material. In all cases, the protein concentrations for the CN-analysis were higher than those determined from the total amino acid content indicating that this analysis overestimates the protein content. This is most likely due to inaccurate nitrogen-to-protein conversion factors. However, this could also be due to the hydrolysis and destruction of certain amino acids during the acid hydrolysis reducing the measured amino acid content. As the nitrogen-to-protein conversion factors can be affected by seasonal and geographical variations, and the CN-analysis was only based on two

parallels rather than three, it is assumed that the protein contents determined from the total amino acid analysis are most accurate.

3.7 Further Work

The dry matter excluding ash quantified for the freeze dried drip loss in this work leaves more than half of the dry matter unidentified. This could be other carbohydrates from the polysaccharide content. The cellulose and alginate content of the drip losses should also be determined. However, a heteronuclear single quantum coherence (HSQC) fingerprint analysis conducted in the preliminary work described in Sund (2019), showed no traces of alginate in the drip loss samples, and cellulose is generally considered to be insoluble in water, so the samples are not expected to contain these polymers. Nonetheless, it should be checked. Additionally, the nitrogen-to-protein conversion factors of 4.37 and 4.45 for *S. latissima* and *A. esculenta*, respectively (Biancarosa et al., 2017), seemed to overestimate the protein content when compared to the protein content determined from the Lowry protein assay and the total amino acid analysis. These factors were below the traditional factor of 6.25, which assumes that all nitrogen present is bound in protein containing 16% nitrogen (Lourenço et al., 2002). This implies that the content of non-protein nitrogen, such as nitrate, in the drip loss is quite high and could contribute to the unidentified portion of the dry matter. As the samples are both coloured, the pigment content should also be determined.

Future studies should also analyse the mineral content mass balance to see ascertain if freezing and thawing of the raw materials decreases or increases the sodium to potassium ratio, as well as if the amounts of iodine and heavy metals present in the drip loss samples are large enough to substantially decrease the content of these minerals in the raw material. Speciation studies of the arsenic and mercury content of the drip loss samples should also be conducted to determine the hazard the presence of these minerals pose for use of the drip loss in food products.

A material balance for the phenolic content of the raw material, drip loss, and leftover raw material could also be attempted to determine if the *S. latissima* raw material loses more of its polyphenol content to the drip loss than *A. esculenta*. Why the *S. latissima* drip loss had a higher antioxidant activity than the *A. esculenta* drip loss could also be looked at in future studies.

4 Conclusion

Freezing and thawing of *Saccharina latissima* and *Alaria esculenta* raw materials resulted in a substantial loss in moisture; the drip loss samples weighed 49% and 55% of the original frozen raw material, respectively. The drip losses were very similar in overall composition with just under 93.5% moisture and just over 6.5% dry matter of wet weight, of which just over 70% was ash content. This implied that the *S. latissima* and *A. esculenta* raw materials respectively lost 50% and 58% of the original moisture content, 23% and 16% of the original dry matter excluding ash content, and 59% and 57% of the original mineral content during thawing.

From the mineral analysis, it was determined that the main minerals present were potassium and sodium. The sodium to potassium ratio of the samples were 0.58 and 0.55 for *S. latissima* and *A. esculenta*, respectively. The *S. latissima* drip loss contained almost 19 times as much iodine per gram freeze dried drip loss than the *A. esculenta* drip loss. However, at concentrations of 13.2±4 and 0.71±0.06 milligrams per gram of freeze dried drip loss, consumption of one gram of drip loss would result in iodine intakes 22 and 1.2 times the recommended upper tolerable limit. The drip loss samples also contained some arsenic, mercury, and cadmium.

The phenolic content of the *S. latissima* drip loss was determined to be higher than for the *A. esculenta* drip loss. However, the amount of phenolic compounds lost to the drip loss per gram dry weight of the raw materials was low compared to literature values for the total phenolic content of the two macroalgae, especially for *A. esculenta*. Therefore, it seems as if only a small amount of the phenolic content of the raw material was lost to the drip loss during thawing. Ethanol precipitation of proteins caused an apparent reduction of phenolic content. This implied that some larger proteins might have interfered with the Folin-Ciocalteu phenolic content assay, and that the actual phenolic content was lower than what was determined from the untreated drip loss samples.

The antioxidant activity of the *S. latissima* drip loss was almost double that of the *A. esculenta* drip loss. As phenolic compounds from macroalgae have been shown to have antioxidant activities, this was possibly related to the higher phenolic content of the *S. latissima* sample. However, the difference in antioxidant activity between the two drip loss samples was much larger than the difference in phenolic content. This was suggested to be due to the *S. latissima* phenolic compounds having higher antioxidant activities, an apparent higher content of fucoidan, or possibly a higher fucoxanthin concentration.

The carbohydrate analysis showed that, in both species, over half of the mannitol content present in the raw material was lost to the drip loss during thawing. Of the other quantified carbohydrates (fucose, glucose, galactose and mannose/xylose) only small amounts were lost to the drip loss. The mannitol content of the drip losses were determined to be 8.7±0.6% and 8.3±0.3% of freeze dried drip loss weight, for *S. latissima* and *A. esculenta*. Mannitol has many industrial uses and

the extraction of mannitol from drip losses could, therefore, have potential as a value-added product in macroalgae industry. Small amounts of fucose and glucose content indicated that the drip losses contained traces of fucoidan and laminaran.

The Lowry protein assay combined with ethanol protein precipitation indicated that the major part of the proteins present in the drip losses were ethanol-soluble small peptides or free amino acids. The CN-analysis showed that the majority of the carbon and nitrogen content, and consequently the protein content, was retained in the leftover raw material. Both species lost just under 30% of the alanine present in the raw material to the drip loss. The alanine and aspartic acid content seemed to be mostly in free form, while most of the glutamic acid content seemed to be bound in protein. All protein concentrations seemed to be overestimated when calculated from the nitrogen content, the Lowry assay and the total amino acid analysis gave more similar answers. The total amino acid analysis was decided to be the most reliable. The protein concentrations, calculated from the sum of total amino acids, of the drip loss samples showed that the *S. latissima* raw material lost over 50% more protein per gram of dry weight to the drip loss. This caused a significantly higher protein concentration of the *S. latissima* drip loss of 1.95±0.08% of freeze dried drip loss weight, compared to 1.67±0.03% of freeze dried *A. esculenta* drip loss weight.

Several uses of the drip losses from thawing of the macroalgae *S. latissima* and *A. esculenta* were discussed, including salt-replacer, flavour enhancer, food additive and fertiliser. However, the drip loss in itself does not seem to have any apparent direct uses. The sodium to potassium ratio was quite low, but there seem to be other options that are better suited as salt-replacing ingredients. As a fertiliser, it was seen that it is not a viable option to use the drip loss as the only phosphorous source in large scale production due to quite low phosphorous content. The use of the drip losses as food additives or flavour enhancers seem to be the most feasible utilisations. The concentrations of free alanine and aspartic acid could bring a complex sweet flavour and umami flavour to food products. However, the content of free glutamic acid was low, which is the main component in the umami taste. The mannitol content could also be relevant in this aspect as it has a sweet taste and can replace sugar content. The protein content and phenolic content, along with some antioxidant activity, could increase the functionality of a food, but the concentrations are low, and large amounts of drip loss would be needed for it to make a substantial difference. The high iodine contents, especially of the *S. latissima* drip loss, as well as the presence of the toxic heavy metals arsenic, mercury and cadmium in the drip losses complicate and limit their use as food additives or flavour enhancers. There seems to be an imbalance in the ratio of flavourful or functional compounds to mineral content, that causes the addition of enough of the drip losses to enhance the flavour or increase the functionality of a food product to result in a mineral content that could potentially be dangerous for the consumer. Therefore, the most likely use of the drip loss samples seems to be for the extraction of functional compounds, especially iodine or mannitol, for further industrial uses. Over half of the dry matter excluding ash was not identified

in this study, therefore, attempts to identify this fraction should be done in future studies.

The results showed that the raw material was affected by freezing and thawing, losing substantial amounts of moisture and mineral content, as well as some organic dry matter. The loss of moisture could make subsequent drying of the leftover raw material easier. The mineral content of the drip loss indicated that freezing and thawing could be used for removal of iodine and heavy metals from the raw material, making the leftover raw material safer to consume. Additionally, the loss of sodium and potassium could either improve (lower) or worsen (elevate) the sodium to potassium ratio of the raw material. A complete mass balance of the mineral content before and after thawing would be needed to determine which was the case. The loss of phenolic compounds and proteins seemed to be very low, indicating that the nutritional and functional value that proteins and phenolic compounds represent in the macroalgae raw material was not considerably altered after freezing and thawing. It was, however, suspected that the flavour of the leftover raw material could be different than that of the raw material due to substantial losses of free alanine and the carbohydrate mannitol, as well as the loss of some free glutamic and aspartic acid.

In conclusion, the drip loss from thawing of the macroalgae *Saccharina latissima* and *Alaria esculenta* does not seem to have any apparent direct uses. The main components seem to be water, the minerals sodium and potassium, the carbohydrate mannitol, and a small amount of proteins, mainly in the form of small peptides and free amino acids. The most likely uses for the drip losses seems to be for extraction of iodine or mannitol for further industrial uses. Therefore, it seems as if the nutritional value of *Saccharina latissima* and *Alaria esculenta* raw material is not substantially affected by freezing and thawing, and that freezing and thawing could result in a healthier mineral profile of the leftover raw material. However, the flavour could be slightly altered due to the loss of flavourful compounds. Further research is needed to determine the exact effect of freezing and thawing on the mineral content of the raw material, as well as to determine what is present in the unidentified part of the drip loss dry matter.

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A Raw Data

The raw data for the preparation of drip loss samples from thawing of the two macroalgae *Saccharina latissima* and *Alaria esculenta* and some analyses conducted during this study are given in the following sections.

A.1 Sample Preparation

The weights recorded during thawing of frozen samples of the macroalgae *Saccharina latissima* and *Alaria esculenta* and collection of the resulting drip loss from the macroalgae samples are shown in Table A.1.

Table A.1: Weight of initial *Saccharina latissima* and *Alaria esculenta* samples and weight of the drip loss from thawing of the samples.

	Saccharina latissima	***Alaria esculenta***
Weight initial sample [g]	602.93	602.39
Weight thawed sample [g]	286.49	257.02
Weight drip loss [g]	316.46	345.35

Two different samples of each drip loss sample were removed for further analysis before freeze drying. These samples of 2 mL and 30 mL were weighed. The weight of these samples were used to calculate an average density of the drip loss samples. These values and the calculated density are given in Table A.2.

Table A.2: Weight of the 30 mL and 2 mL drip loss samples from *Saccharina latissima* and *Alaria esculenta* removed for further analysis, and the density of the drip loss samples calculated from these values.

	Saccharina latissima	***Alaria esculenta***
Weight 30 mL sample of drip loss [g]	31.194	30.9781
Weight 2 mL sample of drip loss [g]	2.0749	2.0584
Density of drip loss [g/mL]	1.039	1.031

The drip loss that was freeze dried was weighed before ($W_{\text{before freeze drying}}$) and after freeze drying ($W_{\text{freeze dried}}$). These values, as well as the calculated density of the drip loss samples ($\rho_{\text{drip loss}}$) were used to calculate the ratio of grams of freeze dried sample per millilitre of original drip loss sample as shown in Equation A.1. The weights of the drip loss samples before and after freeze drying and the calculated values for grams of freeze dried sample per millilitre of original drip loss sample are given in Table A.3.

$$\begin{matrix}\text{Grams of freeze dried sample per} \\ \text{millilitre original drip loss sample}\end{matrix} = \frac{W_{\text{freeze dried}}}{W_{\text{before freeze drying}}} \cdot \rho_{\text{drip loss}} \tag{A.1}$$

Table A.3: Weight of the drip loss samples from *Saccharina latissima* and *Alaria esculenta* before and after freeze drying, and a calculated value for grams of freeze dried sample per millilitre of original drip loss sample.

	Saccharina latissima	***Alaria esculenta***
Weight drip loss before freeze drying [g]	260.55	298.50
Weight freeze dried drip loss [g]	15.6028	17.0618
Grams of freeze dried sample per millilitre of original drip loss sample [g/mL]	0.062	0.059

A.2 Dry Matter and Ash Content

The raw data for one of the parallels used for determination of the dry matter and ash content in frozen and thawed samples of *Saccharina latissima* and *Alaria esculenta*, and drip loss and freeze dried drip loss from thawing of these macroalgae is given in Table A.4. The values presented in the results section are based on two more parallels, however, as the numbers were quite similar only the first parallel is shown here.

Table A.4: Raw data from the first parallel used for determination of the dry matter and ash content of frozen and thawed *Saccharina latissima* and *Alaria esculenta* samples, as well as drip loss and freeze dried drip loss samples from thawing of the two macroalgae samples. The table lists the weight of the samples analysed before drying at 105°C for 24 hours, after drying and after combustion at 550°C overnight.

Weight sample:	**At start [g]**	**After drying [g]**	**After combustion [g]**
Saccharina latissima			
Frozen sample	2.5824	0.2049	0.1097
Thawed sample	2.4346	0.2472	0.0768
Drip loss	2.0749	0.1412	0.0995
Freeze dried drip loss	0.5216	0.5100	0.3753
Alaria esculenta			
Frozen sample	2.5689	0.2402	0.1030
Thawed sample	2.4354	0.4999	0.1239
Drip loss	2.0584	0.1358	0.0959
Freeze dried drip loss	0.5168	0.5033	0.3611

The determined dry matter and ash contents for the raw material, leftover raw material, original drip loss and freeze dried drip loss samples from *S. latissima* and *A. esculenta* are given in Table A.5. These values are only based on the first parallel.

Table A.5: Dry matter and ash content raw material and leftover raw material samples of *Saccharina latissima* and *Alaria esculenta*, and of the drip loss and freeze dried drip loss from the thawing of the macroalgae. Dry matter is given in percent of wet weight of the sample. Ash is given in percent of wet weight of the sample and in percent of dry weight of the sample.

	Dry matter [%]	**Ash [%]**	**Ash [% of dw]**
Saccharina latissima			
Raw material	7.93	4.25	53.54
Leftover raw material	10.15	3.15	31.07
Drip loss	6.81	4.80	70.47
Freeze dried drip loss	97.78	71.95	73.59
Alaria esculenta			
Frozen sample	9.35	4.01	42.88
Thawed sample	20.53	5.09	24.78
Drip loss	6.60	4.66	70.62
Freeze dried drip loss	97.39	69.87	71.75

A.3 Preparation of Drip Loss Samples Used for Mineral Analysis

The drip loss samples from *Saccharina latissima* and *Alaria esculenta* were prepared in Sund (2019). The *A. esculenta* raw material was from the same batch as the *A. esculenta* used in this thesis. The *S. latissima* sample was obtained from Sintef Ocean. The sample was harvested 27.06.2019, and had the ID-tag RA-SR-270619. This sample was frozen approximately 12 to 18 hours after harvesting.

Table A.6: Dry matter and ash content of the drip loss samples from Sund (2019).

	Saccharina latissima		***Alaria esculenta***	
Sample	**Original**	**Freeze dried**	**Original**	**Freeze dried**
Dry matter content [%]	5.9	98.2	5.4	96.6
Ash content [%]	3.7	62.7	3.8	67.6

A.4 Carbohydrate Content

The quantified carbohydrate content of the raw material, leftover raw material, and drip loss from *Saccharina latissima* and *Alaria esculenta* are given in Tables A.7 and A.8, respectively.

Table A.7: The quantified carbohydrate content of the raw material, leftover raw material, and drip loss from *Saccharina latissima*. The values are given in percent of dry matter of raw material and leftover raw material for the respective samples, and in percent of the freeze dried drip loss weight.

	Raw material [% of DW]	SD	Leftover raw material [% of DW]	SD	Drip loss [% of freeze dried weight]	SD
Mannitol	4.058	0.308	2.188	0.181	8.745	0.589
Fucose	0.619	0.077	0.784	0.061	0.175	0.023
Glucose	0.398	0.029	0.688	0.091	0.288	0.022
Galactose	0.403	0.050	0.456	0.069	0.319	0.030
Mannose/Xylose	0.626	0.072	0.742	0.082	0.128	0.014

Table A.8: The quantified carbohydrate content of the raw material, leftover raw material, and drip loss from *Alaria esculenta*. The values are given in percent of dry matter of raw material and leftover raw material for the respective samples, and in percent of the freeze dried drip loss weight.

	Raw material [% of DW]	SD	Leftover raw material [% of DW]	SD	Drip loss [% of freeze dried weight]	SD
Mannitol	2.710	0.071	1.136	0.106	8.299	0.284
Fucose	0.578	0.021	0.750	0.038	0.022	0.001
Glucose	0.316	0.008	0.440	0.025	0.148	0.004
Galactose	0.397	0.012	0.470	0.026	0.168	0.006
Mannose/Xylose	0.538	0.021	0.696	0.036	0.117	0.013

A.5 CN-analysis

The carbon, nitrogen and protein content calculated from the nitrogen content of the raw material, leftover raw material and drip loss samples from thawing of the macroalgae *Saccharina latissima* and *Alaria esculenta* are shown in Table A.9.

Table A.9: Carbon, nitrogen and protein content of raw material, leftover raw material and freeze dried drip loss from thawing of frozen raw material of the macroalgae *Saccharina latissima* and *Alaria esculenta*. The results for the raw material and leftover raw material are given in percent of dry weight of the respective samples, while the results for the freeze dried drip loss are given in percent of the sample weight. The protein content is calculated from the nitrogen content using the N-to-protein conversion factors from Biancarosa et al. (2017) of 4.37 and 4.45 for *S. latissima* and *A. esculenta*, respectively.

	Saccharina latissima		
	Raw material	Leftover raw material	Drip loss
Carbon content	22.2 ± 0.6	30.75 ± 0.11	9.2 ± 0.4
Nitrogen content	1.91 ± 0.16	2.41 ± 0.05	0.61 ± 0.04
Protein content	8.3 ± 0.7	10.5 ± 0.2	2.67 ± 0.18
	Alaria esculenta		
	Raw material	Leftover raw material	Drip loss
Carbon content	26.50 ± 0.07	37.6 ± 0.3	8.4 ± 0.5
Nitrogen content	2.41 ± 0.08	3.53 ± 0.12	0.76 ± 0.02
Protein content	10.7 ± 0.3	15.7 ± 0.5	3.38 ± 0.09

A.6 Total Amino Acid Content

The total amino acid contents of the raw material, leftover raw material, and drip loss from *Saccharina latissima* and *Alaria esculenta* are given in Tables A.10 and A.11, respectively.

Table A.10: The total amino acid content of the raw material, leftover raw material, and drip loss from *Saccharina latissima*. The values are given in percent of dry matter of raw material and leftover raw material for the respective samples, and in percent of the freeze dried drip loss weight.

Amino acid	Raw material (% of DW)	SD	Leftover raw material (% of DW)	SD	Freeze dried drip loss (% of weight)	SD
Asp (+Asn)	1.03	0.05	1.26	0.04	0.325	0.010
Glu (+Gln)	0.97	0.02	1.16	0.07	0.423	0.012
Asn	0.01	0.00	0.01	0.00	0.000	0.000
His	0.16	0.01	0.16	0.01	0.008	0.001
Ser	0.35	0.01	0.47	0.03	0.033	0.000
Gln	0.02	0.00	0.02	0.01	0.002	0.000
Gly/Arg	0.54	0.01	0.70	0.04	0.056	0.001
Thr	0.46	0.02	0.63	0.04	0.049	0.002
Ala	0.97	0.04	1.01	0.03	0.76	0.05
Tyr	0.23	0.01	0.33	0.01	0.089	0.014
Met	0.23	0.01	0.31	0.01	0.011	0.001
Val	0.49	0.02	0.65	0.02	0.056	0.001
Phe	0.48	0.02	0.62	0.03	0.038	0.002
Ile	0.41	0.02	0.55	0.02	0.026	0.001
Leu	0.70	0.03	0.96	0.04	0.038	0.001
Lys	0.52	0.02	0.61	0.03	0.044	0.001
Sum	7.578	0.277	9.458	0.427	1.954	0.077

Table A.11: The total amino acid content of the raw material, leftover raw material, and drip loss from *Alaria esculenta*. The values are given in percent of dry matter of raw material and leftover raw material for the respective samples, and in percent of the freeze dried drip loss weight.

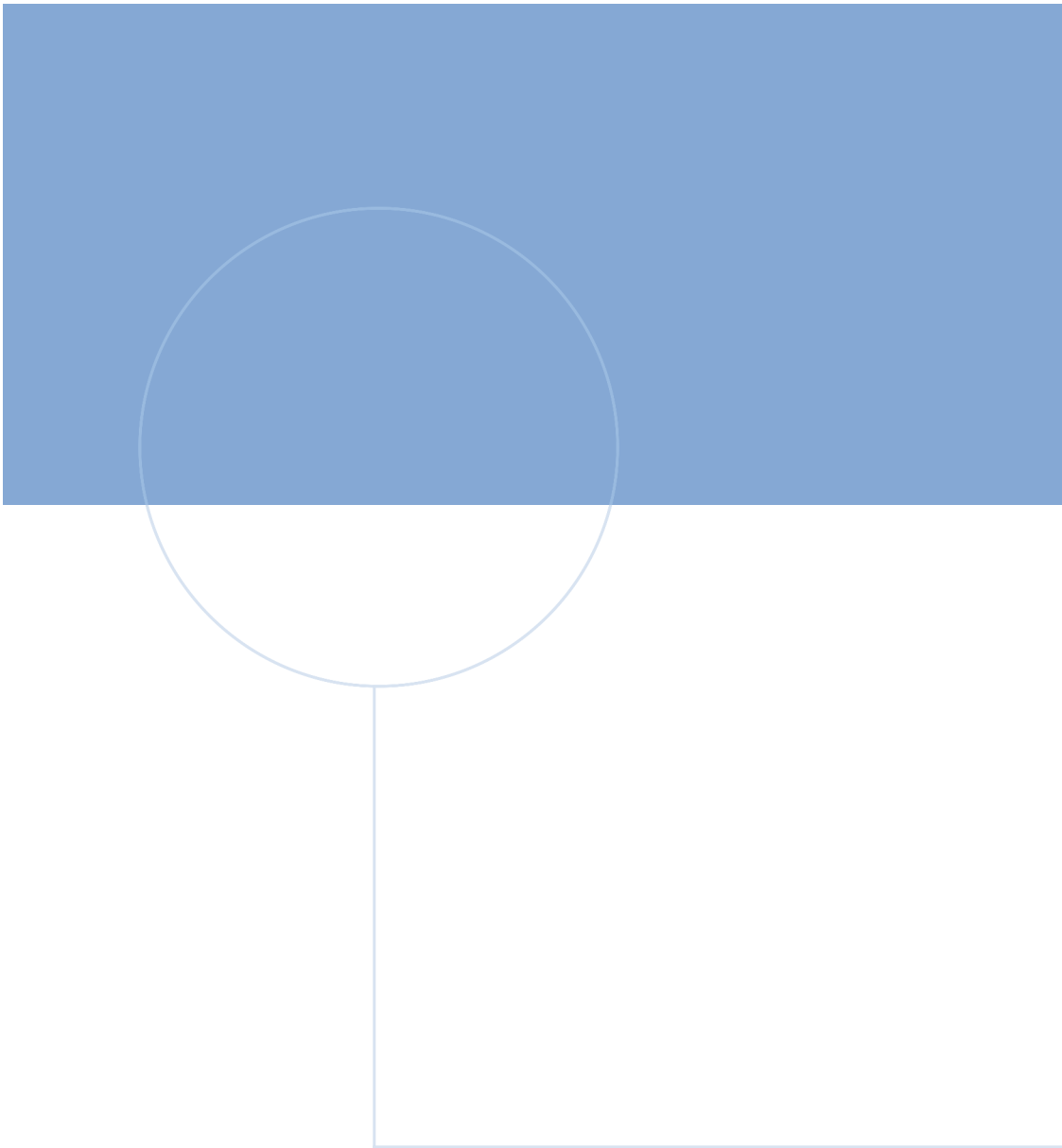
Amino acid	Raw material (% of DW)	SD	Leftover raw material (% of DW)	SD	Freeze dried drip loss (% of weight)	SD
Asp (+Asn)	1.16	0.04	1.42	0.05	0.114	0.003
Glu (+Gln)	1.02	0.04	1.35	0.10	0.214	0.006
Asn	0.00	0.00	0.00	0.00	0.000	0.000
His	0.18	0.02	0.24	0.03	0.003	0.000
Ser	0.45	0.02	0.64	0.05	0.024	0.000
Gln	0.02	0.00	0.02	0.01	0.002	0.000
Gly/Arg	0.62	0.03	0.81	0.08	0.034	0.001
Thr	0.53	0.02	0.74	0.07	0.022	0.004
Ala	1.17	0.04	1.05	0.08	1.067	0.016
Tyr	0.24	0.02	0.34	0.04	0.000	0.000
Met	0.24	0.01	0.32	0.03	0.001	0.000
Val	0.56	0.04	0.76	0.06	0.042	0.001
Phe	0.53	0.04	0.69	0.05	0.014	0.000
Ile	0.47	0.03	0.63	0.05	0.017	0.001
Leu	0.80	0.04	1.08	0.09	0.029	0.001
Lys	0.65	0.03	0.84	0.07	0.031	0.001
Sum	8.681	0.411	10.937	0.832	1.673	0.031

A.7 Free Amino Acid Content

The free amino acid content of the drip loss from *Sachharina latissima* and *Alaria esculenta* is given in Table A.12.

Table A.12: Free amino acid contents of drip loss samples from *Sachharina latissima* (SL) and *Alaria esculenta* (AE). Values are given in grams per litre of drip loss.

Amino acid	SL [g/L]	SD	AE [g/L]	SD
Asp	0.1800	0.0091	0.1735	0.0032
Glu	0.0157	0.0010	0.0054	0.0000
Asn	0.0203	0.0015	0.0013	0.0000
His	0.0017	0.0002	0.0028	0.0001
Ser	0.0111	0.0007	0.0116	0.0006
Gln	0.0977	0.0063	0.0018	0.0001
Gly/Arg	0.0062	0.0004	0.0171	0.0005
Thr	0.0440	0.0025	0.0132	0.0009
Ala	0.5548	0.0257	0.8977	0.0114
Tyr	0.0067	0.0004	0.0656	0.0163
Met	0.0079	0.0006	0.0075	0.0002
Val	0.0152	0.0007	0.0156	0.0005
Phe	0.0157	0.0009	0.0161	0.0004
Ile	0.0063	0.0003	0.0023	0.0001
Leu	0.0087	0.0005	0.0068	0.0003
Lys	0.0011	0.0001	0.0057	0.0002
SUM	1.0562	0.0349	1.3012	0.0305



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