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Characterization of three Macroalgae: *Saccharina latissima, Alaria esculenta and Palmaria palmata*

Effect of Different Harvesting Conditions

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Biotechnology
Submission date: May 2016
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Preface

This thesis was written in collaboration between the Norwegian University of Science and Technology (NTNU), at the institute for Biotechnology, and SINTEF Fisheries and Aquaculture AS. The thesis was part of two projects: "Dyrking og foredling av makroalger av høy kvalitet til nye matprodukter", financed by RFF Vest (245326) and owned by Austevoll Seaweed Farm, and "PROMAC: Energy efficient PROcessing of MACroalgae in blue-green value chains" financed by Forskningsrådet (244244/E50).

First of all, I would like to thank my supervisor and co-supervisor at NTNU, Turid Rustad and Olav Andreas Aarstad, for their help and guidance. Turid always answered my questions, even the small ones, and Olav was a tremendous help for me with the carbohydrate analysis. At SINTEF I would like to thank Jorunn Skjermo, Silje Forbord, Rasa Slizyte, Vera Kristinova, Marte Schei, Stine Wiborg Dahle and Merethe Selnes. Especially Vera, Marte and Merethe have been a great help for me in my lab work, so thank you so much for your help and patience. I would also like to thank SINTEF for including me in the SIG Seaweed workshops.

NTNU, Trondheim. 15.05.2016.

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Abstract

Seaweeds have a long tradition of being a part of the diet in many Asian countries. In Europe seaweeds have mainly been utilized industrially, and especially for their content of phycocolloids, this includes Norway. Due to the dramatic increase in the world's population there is a demand for new food sources. However, the nutritional composition in macroalgae varies according to specie and environmental factors. Therefore, more knowledge on the chemical composition in the different macroalgal species is needed. In this thesis the chemical composition of *Saccharina latissima*, *Alaria esculent*a and *Palmaria palmata* was characterised. These are common macroalgal species growing on the Norwegian coast.

In order to characterize the composition of these macroalgae the dry matter, ash, total lipids, total protein and total carbohydrates were determined, in addition to the amino acid and carbohydrate composition. Different seasons and depths were examined in order to find the most optimal harvesting conditions. The *P.palmata* sample was a wild growing sample, while *S.latissima* and *A.esculenta* contained both cultivated and wild samples. The cultivated algae samples were harvested in May and June (*S.latissima* was also harvested in April), and from depths of 2, 5 and 8 meters. In addition, samples containing a mixture of these depths were analysed. The wild samples were harvested in June and contained samples with a mixture of different depths.

The protein content was found by determination of total nitrogen (the C/N method) and determination of the total amount of amino acids (by high-performance liquid chromatography, HPLC). Different hydrolysis methods were tried in order to see which gave the highest amino acid yields. Hydrolysis with 6M HCl containing 0.4% β-mercaptoethanol was found to give the highest yields. Determination of the carbohydrate composition in the macroalgae was performed by high-performance anion-exchange chromatography (HPAE). This method was optimized with regard to different hydrolysis times and different settings on the analysis instrument.

The highest dry matter content could be found in the wild growing samples of *S.latissima* and *A.esculenta*. In cultivated *S.latissima* the dry matter was highest in the samples harvested in May, while the dry matter content in cultivated *A.esculenta* was highest in June.

The ash content was highest in cultivated *S.latissima* and *A.esculenta* in May. The amount of lipids, proteins and carbohydrates were generally highest in cultivated *S.latissima* in May (except for the total carbohydrates), and in cultivated *A.esculenta* in June. Low lipid levels were found in all three macroalgae. When the samples were analysed by HPLC the results showed a higher protein content than when the samples were analysed by the C/N method. The protein content in *S.latissima* and *A.esculenta* was within the values reported in different literature for these species, while the protein content in *P.palmata* was low. The amino acid composition in *S.latissima*, *A.esculenta* and *P.palmata* was dominated by aspartic acid, glutamic acid, alanine and leucine. The carbohydrate content in *S.latissima* and *A.esculenta* was highest in the wild samples. The highest carbohydrate content in the cultivated samples could be found in April for *S.latissima* and in June for *A.esculenta.* The carbohydrate content was highest in *P.palmata*, which had a high amount of mannose/xylose and galactose. In *S.latissima* mannitol dominated the carbohydrate composition, while glucose dominated in *A.esculenta*, especially in the samples from June.

The different depths only provided small variations in the chemical content. However, some of the *S.latissima* and *A.esculenta* samples containing a mixture of depths had a lower dry matter and ash content and a higher content of lipids, proteins and carbohydrates, compared to the other samples. For example, the total protein content was twice as high in some of the samples containing a mixture of depths, than in the samples from individual depths. This was most likely because these samples were washed right after harvesting, and therefore salt and minerals could have been removed.

Sammendrag

I Asia har det lenge vært vanlig å benytte tang og tare i dietten. I Europa har tang og tare hovedsakelig blitt utnyttet industrielt for deres innhold av phycocolloider, inkludert i Norge. Verdens befolkning har økt dramatisk de siste årene og i den forbindelse er det et behov for nye matressurser. Næringsinnholdet i makroalger er miljøpåvirket og varierer mellom artene. På grunnlag av dette er det et behov for mer kunnskap om den kjemiske sammensetningen i de ulike makroalgene. I denne oppgaven har det kjemiske innholdet til *Saccharina latissima*, *Alaria esculent*a og *Palmaria palmata* blitt karakterisert. Disse makroalgene er noen av artene som vokser langs kysten i Norge.

Den kjemiske sammensetningen ble karakterisert ut i fra makroalgenes innhold av tørrstoff, aske, lipider, proteiner, karbohydrater, samt aminosyre og karbohydrat sammensetningen. For å kunne bestemme hvilke høstingsbetingelser som var mest optimal ble makroalgene høstet ved ulike sesonger og fra ulik dybde. I denne oppgaven ble bare en prøve av *Palmaria palmata* karakterisert, og dette var en viltvoksende prøve. *S.latissima* og *A.esculenta* bestod av både dyrkede og viltvoksende prøver. De dyrkede prøvene ble høstet i Mai og Juni (i tillegg ble *S.latissima* også høstet i April), ved 2, 5 og 8 meters dybde. Noen av de dyrkede prøvene inneholdt også en miks av prøver fra disse dybdene.

Proteininnholdet ble bestemt ved å analysere nitrogeninnholdet i prøvene (C/N metoden) og ved å bestemme det totale innholdet av aminosyrer (ved high-performance liquid chromatography, HPLC). I tillegg ble ulike hydrolysemetoder prøvd ut for å se hvilken metode som gav høyest innhold av hver enkelt aminosyre. Det ble funnet at hydrolyse med 6M HCl tilsatt 0.4% β-merkaptoetanol gav best resultater. Makroalgenes sammensetning av karbohydrater ble bestemt ved high-performance anion-exchange chromatography (HPAE). Denne metoden ble optimalisert ut i fra ulike hydrolysetider og ulike innstillinger på analyseinstrumentet.

De viltvoksende prøvene av *S.latissima* og *A.esculenta* inneholdt høyest tørrstoffmengde. I de dyrkede prøvene av *S.latissima* var mengden tørrstoff høyest i mai, mens den var høyest i juni for *A.esculenta*. Askeinnholdet var høyest i mai for både *S.latissima* og *A.esculenta.* Mengden av lipider, proteiner og karbohydrater var generelt høyest i mai for *S.latissima* (bortsett fra mengden av total karbohydrat) og i juni for *A.esculenta*. *S.latissima, A.esculenta* og

P.palmata hadde alle et lavt lipidinnhold. Proteinverdiene var høyere i prøvene analysert ved HPLC enn prøvene analysert ved C/N metoden. Både *S.latissima* og *A.esculenta* hadde proteinverdier innenfor verdiområdet rapportert i litteraturen, mens proteinnivået i *P.palmata* var lavere. Aminosyresammensetningen i *S.latissima, A.esculenta* og *P.palmata* var dominert av asparaginsyre, glutaminsyre, alanin og leucin. Karbohydratinnholdet var høyere i de ville prøvene av *S.latissima* og *A.esculenta.* I de dyrkede prøvene var karbohydratinnholdet høyest i april for *S.latissima* og i juni for *A.esculenta. P.palmata* hadde den høyeste karbohydratmengden og inneholdt hovedsakelig mannose/xylose og galaktose. Mannitol dominerte karbohydratsammensetningen i *S.latissima*, mens glukose dominerte i *A.esculenta*. Glukoseverdien var spesielt høy i juni for *A.esculenta*.

De ulike dybdene utgjorde bare små forskjeller i den kjemiske sammensetningen i makroalgene. Noen av prøvene skilte seg imidlertid ut; algeprøvene som bestod av prøver hentet fra en miks av dybder inneholdt laver tørrstoff og askemengde og en høyere mengde av de andre kjemiske komponentene, sammenlignet med de andre algeprøvene. For eksempel var den totale proteinmengden dobbelt så høy i prøvene som bestod av en miks av dybder, sammenlignet med prøvene som var hentet fra 2, 5 eller 8 meters dybde. Dette skyldtes mest sannsynlig at prøvene som inneholdt en miks av dybder var blitt vasket rett etter de var blitt høstet. Dette kunne ha fjernet salt og andre mineraler.

Contents

1. Introduction

Seaweeds have been utilized for many centuries, and they were used as ”Chinese herbs” dating back as far as to 2700 BC (1). In northern Europe seaweeds were applied as food, feed and fertilizers between the years of 900 to around 1750, before the industrialization (2). Particularly in Asian countries such as China, Japan and Korea, the application of seaweeds in human consumption has been very common (1). According to FAO (3) Asia accounts for 99 % of the total production of cultivated macroalgae in 2012 (4). The macroalgae are mainly used for food and for their content of phycocolloids, including alginate, agar and carrageenan’s (4, 5).

However, seaweed biomass has been exploited all around the world and for several purposes; both in human- and animal food, as food additives, as fertilizers and as soil improvers (4, 6). In Europe the interest in cultivation and exploration of macroalgae have been low compared to the Asian countries (7, 8). Here the use of seaweed in the diet has not been as common as in Asia, and macroalgae have mainly been utilized as animal feed and in the production of food additives, as well as for their phycocolloid content (1, 6). However, in recent years there has also been an increased interest for macroalgae to be used in food production and for production of biofuels (4). In Norway the seaweeds are mainly used for their content of alginate, agar and carrageenan, which are used industrially as thickening, stabilizing and gelling agents (2, 9).

According to FAO (10) the world’s population will reach 9,1 billion people by the year 2050, and due to this there is a demand for new food sources (11). The ocean is rich in raw material, some of which has great potential as resources for food production (1). However, in order to exploit the macroalgae as a food source there is a need for more knowledge on the chemical composition of the different species, and how this varies according to seasons and different growth conditions.

In Norway there are about 480 different macroalgal species, of which 205 are red algal species, 175 are brown algae and 100 are green algae (12). Some of the seaweed species that are common on the coast of Norway are *Gracilaria gracilis*, *Chondrus crispus* and *Palmaria palmata* (red algae), *Ulva lactuca* (green algae), *Saccharina latissima*, *Alaria esculenta*,

Laminaria digitata, Laminaria hyperborea and *Ascophyllum nodosum* (brown algae) (13). Since this thesis centres on the brown algae *Saccharina latissima* and *Alaria esculenta* and the red algae *Palmaria palmata*, the main focus will be on these species.

The main objective of this thesis is the characterization of the three macroalgae: *Saccharina latissima*, *Alaria esculenta* and *Palmaria palmata*, including the effect of different cultivation –and growth conditions. The aim of the thesis is to get a better understanding of the chemical composition of these macroalgae, including the content of dry matter, ash, lipids, proteins and carbohydrates, as well as the amino acid and carbohydrate composition. The macroalgae have been harvested at different times and different depths, and the effect of this will also be reviewed.

1.1 Growth variations in macroalgae

Light and temperature are important for the regulation of seasonal growth. During the autumn and winter months, when the light levels are low, there is little macroalgal growth. In this period the level of nutrients, such as nitrogen, are high. In early spring, the light levels are higher, and growth is initiated. Nutrients therefore decline during this period, since they are being utilized for macroalgal growth. Other factors that also contribute to variations in macroalgal growth include; salinity (low salinity suppress growth), differences in wave exposure, and differences in water depth (14). Hånda et al. (7) examined the growth of *S.latissima* at depths of 2, 5 and 8 meters, and in different seasons. They found that the algae had more optimal growth at depths of 2 and 5 meters, than at 8 meters. They also concluded that the most rapid growth occurred from February to June.

It is ideal that the macroalgae are harvested when their content of carbohydrates, lipids and proteins are highest (4). In the northern hemisphere, the levels of ash, lipids, proteins and certain polysaccharides, such as alginate and fucoidan, reach a peak in late winter/early spring. The storage carbohydrates on the other hand, are at their lowest in this period, since they have been used as an energy source during winter (4, 15, 16).

1.2 Macroalgae

Algae are a heterogeneous group of plants, and can be divided into two main groups; microalgae and macroalgae, the latter is also referred to as seaweeds. Macroalgae can be further divided into brown algae (Phaeophyta), green algae (Chlorophyta) and red algae (Rhodophyta) (17). This thesis includes brown and red algae, and therefore only these are described further.

1.2.1 Brown macroalgae

It is difficult to say exactly how many species the brown seaweeds consist of, as different amounts are described in different literature. For instance, Nilsen et al. (18) claims that the brown seaweeds consist of 1500 species distributed on about 250 genus, while Indergaard (13) states that there are as many as 1750 different species divided on 220 genus. The exact number is therefore uncertain. However, Norway contains around 175 different species of brown algae (19). The brown colour of this seaweed is due to its fucoxanthin content. Fucoxanthin is a xanthophyll pigment. Brown algae also contain other types of xanthophyll pigments, chlorophyll *a* and *c* and β-carotenes (17).

Brown algae can contain high amounts of minerals and antioxidants, as well as high amounts of carbohydrates (4, 19). These seaweeds are mainly exploited industrially for their hydrocolloid content of alginic acid (alginate), laminarin and fucoidan (1). The cell walls in brown algae are made of cellulose and alginic acid, and the main carbohydrate reserve is laminarin and mannitol (17). Brown seaweeds usually have low amounts of proteins, and according to Fleurence (6) the content may vary from 3-15% of the dry matter (dw). The amount of lipids in the laminaria species may reach 1 % (dw) (20).

Saccharina latissima

Saccharina latissima is a type of brown seaweed, and belongs to the Laminariaceae family (15). *S.latissima* is also known as Sugar kelp, due to its content of the sugar alcohol mannitol (18). It can be found growing at clear and turbid coastal waters in the northern hemisphere (7). This macroalgae grow at the sublittoral zones, mainly in sheltered places (20). According to Hånda et al. (7) *S.latissima* has maximum growth in the first half of the year, while it declines during the summer months. Nielsen et al. (14) also observed that the growth of *S.latissima* started in the winter months, reached a maximum during spring and then

decreased during summer and autumn. *S.latissima* cannot grow at temperatures above 22-23°C, but the optimal growth temperature is at 10°C (20).

As mentioned, brown seaweeds are rich in carbohydrates and especially alginate, but also cellulose, laminarin, mannitol and fucoidan are found in brown algae. However, their protein content is more limited compared to that of red and green algae (4).

Alaria esculenta

Alaria esculenta is a member of the Alariaceae, and similarly to *S. latissima* it belongs to the laminariales (21). *A.esculenta*, also known as Winged kelp, can be found in the north Atlantic ocean, and this macroalgae cannot grow at temperatures above 16°C. *A.esculenta* grows in the upper sublittoral zone and can handle more wave-exposed areas unlike *S.latissima* who prefers more sheltered areas (20). *A.esculenta* have been reported to contain between 9-18% (dw) proteins (13), and according to Mæhre et al. (2) *A. esculenta* are among the species that could function as a good source for proteins.

1.2.2 Red macroalgae

The amount of red algal species varies in different literature; according to El Gamal (17) there are about 8000 species of red algae, while Cian et al. (9) and Indergaard (13) claims that red macroalgae consist of about 6000 species. In Norway there is about 250 different red algae (22). The red algae have their colour from the pigment phycoerythrin and phycocyanin. These dominate the other pigments in the algae; chlorophyll *a*, β-carotene and other xanthophyll's (17).

Red algae are used in human food, especially in Eastern Asia. The species mainly used in food is *porphyra spp*. and *Palmaria palmata.* While *porphyra spp*. is utilized mainly in East Asia, *P.palmata* is used in Iceland, Ireland and other European countries (18, 22). Red algae are generally smaller than brown seaweeds, and so the biomass is also less substantial. They are however known to have a high protein content that can be utilized (1, 22). The most significant polysaccharide in the red algae is floridean starch, which functions as the carbohydrate reserve, and the sulphated galactans; agar and carrageenan. The latter two are utilized in the food industry as gelling, thickening and stabilizing agents. They are also applied in pharmaceutical and cosmetics industry (9, 20, 23).

Palmaria palmata

Palmaria palmata, also known as Dulse, grow in the North Atlantic at the intertidal and subtidal zones (17, 20). As mentioned in the section above (1.2.2) red algae can contain a substantial amount of proteins. According to Fleurence (1999) the protein fraction in red algae varies between 10-47% (dw), and for *P.palmata* the protein content can be as high as 35 % (dw). Morgan et al. (24) found the carbohydrate content in *P.palmata* to be around 38-74% (dw) and the lipid level to be about 0.2 -3.8% (dw).

1.3 The chemical composition of macroalgae

1.3.1 Dry matter and ash content

The water content in algae is significantly higher than those found in terrestrial plants, with algae containing between 75-90 % water, compared to 20-40% in terrestrial plants (13). The content of minerals in seaweeds can be determined by measuring the ash content. The ash content in algae varies from 20-45 %, and is a rich source for the minerals sodium (Na), potassium (K), Zink (Zn), iodine (I), and some trace minerals and vitamins (13). In brown seaweeds the ash content is high. Generally the amount of ash in brown seaweeds vary from 15-35% (dw), although it can comprise over 50% of the dry matter (4, 25). According to Dodsen et al. (26) *S.latissima* contains between 22-44% ash (dw), while the ash content in *A.esculenta* is found to be from 14-32% (dw) (25). Red algae usually contain about 8-30% ash (dw), and in *P.palmata* the amount of ash has been found to be around 15-30% (dw) (13, 25).

1.3.2 Lipid content

Coultate defines lipids as "a heterogeneous group of substances associated with living systems, which have the common property of insolubility in water but solubility in non-polar solvents" (27). The level of lipids in seaweed is generally low (1). According to Fleurence et al. (28) the amount can vary from 1.5%-3.3% of the matter (dw). Mourinho et al. (16) found the lipid content in *S.latissima* to vary between 0.62%-3.35% (dw), depending on season. The amount was 0.62-0.88% (dw) in July, while it was 3.33-3.35% (dw) in November. Indergaard (13) reports that the amount of lipids in *A.esculenta* varies from 1-2% (dw), while *P.palmata* contains between 0.3-3.8% (dw) lipids.

Despite low lipid levels, the fat content that has been found in these macroalgae can consist of long-chained polyunsaturated fatty acids (LC-PUFA), such as the omega-3 fatty acids docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), and the omega-6 fatty acid arachidonic acid (ARA) (16). The omega-3 fatty acids EPA and DHA both reduce the risk of developing cardiovascular diseases (CVD) (2). According to Marinho et al. (16) the macroalgae are better sources of these fatty acids than land vegetables.

1.3.3 Protein content and amino acid composition

Proteins are macromolecules and the amino acids are the building units of proteins. Peptide bonds link the amino acids so they form long polypeptide chains (27). The polypeptides can fold into different secondary structures, and these can be further arranged into tertiary and quaternary structure (29). As mentioned in section 1.1, macroalgae are used both as food and feed all over the world, and in Asia they are consumed as marine vegetables. The protein content varies between brown, green and red algae, and between the different species. Brown macroalgae generally have a lower protein content than the green and red macroalgae, and may contain between 3-15% (dw) (6). According to Fleurence (6) especially high amounts of proteins are found in red algae, such as *Porphyra tenera* and *Palmaria palmata,* where the protein value can be as high as 47% and 35% (dw), respectively.

It has also been reported that the protein content in macroalgae can vary according to season. For instance, *P.palmata* has been found to contain a protein content that varies from 9-25 % (dw) depending on season, and the amount is higher during winter then during summer. Harnedy & FitzGerald (30) found the content of glutamic acid, serine and alanine in *P.palmata* to be high in the algae samples harvested in late winter and spring, as well as in September and October, while the level of these were low in samples from July and from November to January. In addition the levels of histidine, leucine, lysine, phenylalanine, tyrosine and threonine decreased in the *P.palmata* samples harvested in the period from April to September. It has also been found that the protein content is lower when the carbohydrate synthesis reaches a peak.

The amino acid composition also varies for the different macroalgae. Some of the most frequent amino acids reported to be found in macroalgae include glycine, alanine, glutamic acid and aspartic acid (1, 6). Aspartic acid and glutamic acid are the amino acids found in the highest amounts. However, these amino acids are reported to make up a smaller fraction in red algae such as *P.palmata* and *P.tenera.* In *P.palmata* the main amino acid fraction consists of leucine, valine and metionine (6). *P.palmata* has also been reported to contain high amounts of glycine (30). Mæhre et al. (2) reviewed the protein content in some Norwegian seaweeds, such as *A.esculenta* and *P.palmata*. In both *A.esculenta and P.palmata* the major amino acids were aspartic acid, glutamic acid, alanine and leucine.

Results from studies done by Mæhre et al. (2) show that brown, red and green macroalgae all cover the human requirements of essential amino acids. The amount and composition of essential amino acids determines the protein quality of a food product. *P.palmata* has been found to contain leucine, valine and methionine (6). It has been found that there is a higher methionine level in some algae than in soy (2). Soybean proteins are used in animal feed. Hence, the use of algal proteins as a supplement in food/feed can increase the nutritional value (2, 28). Glutamate has been used as a flavor additive in food for over a 1000 years, and contains a unique umami taste, which is now known as the fifth basic taste (31). The glutamate content in algae could also be utilized for this purpose.

Protein analysis

Hydrolysis of proteins and other chemical compounds can be performed both chemically and enzymatically. In chemical hydrolysis acid or basic (alkaline) conditions are applied. According to Fountoulakis & Lahm (32) there is no single hydrolysis method available to determine all of the amino acid residues, since the hydrolysis and destruction of the liberated amino acids occur simultaneously. Some of the agents used in protein hydrolysis, such as strong acids, will completely hydrolyse some of the amino acids, while others will be partially or completely destroyed (33). When a sample is acid hydrolysed the peptide linkage between the amino acids is broken, and the amino acids are liberated. In most acid hydrolysis 6M HCl is applied as an agent. Unfortunately some of the amino acids are often destroyed during the hydrolysis with 6M HCl, such as tryptophan. Tyrosine, serine and threonine are all only partially hydrolysed. Cysteine cannot be directly determined (is instead determined as cysteine), and asparagine and glutamine are hydrolysed to aspartic acid and glutamic acid (32).

Alkalic hydrolysis usually applies NaOH or KOH as agents. The advantage of this method is that it can be used for the determination of tryptophan, since tryptophan is stable under alkaline conditions (32, 34). However, the amino acids serine, threonine, arginine and cysteine are destroyed by this method. Hydrolysis can also be performed with sulfonic acids, such as methanesulfonic acid (MSA). Hydrolysis with MSA also allows for determination of tryptophan (32).

In this thesis acid hydrolysis was applied. Since some of the amino acids are destroyed or partially destroyed with this method, it was experimented with different acid and additives in order to see which gave the most optimal hydrolysis (see section 3.1). Methanesulfonic acid was compared with HCl, and β-mercaptoethanol was added to HCl to see if this reduced the losses of amino acids. After the proteins had been hydrolysed, the amino acid content and composition was determined by chromatography.

The principle behind chromatography is that that the sample molecules can be separated based on differences in their physicochemical properties, such as solubility in water and organic solvents, and the charge and size of the molecules (35). In column chromatography the separation occurs in a column that contains a stationary phase, which is a solid or a gel. The mobile phase (eluent) is a liquid, and moves through the stationary phase in a definite direction. The sample molecules are dissolved in the mobile phase and carried through the column where they are distributed between the mobile phase and the stationary phase based on their physicochemical properties (36, 37).
For the separation of amino acids, ion-exchange chromatography (IEC) was used. The sample molecules are separated based on a different net charge. The stationary phase has a positively charged functional group (Na^+). The negatively charged amino acids are attached to it and this ionic interaction is broken by eluent of a specific pH (35, 38, 39). In this thesis the pH value in the mobile phase changed with time. It started out relatively acidic, and then turned more neutral towards the end. Based on this the amino acids were liberated from the column one by one, due to their different net charge.

The samples were injected automatically into the column. The separation occurs when the sample components move through the mobile phase at defined speed and based on the different properties, as mentioned above (38). A detector (usually a UV detector) measures when the molecules emerge form the column, and the time at which this happens is called the retention time. The retention time is based on the interactions between the stationary phase, the mobile phase and the sample molecules (36, 38). In HPLC a high pressure pump is applied, which accelerates the separation of the molecules. A chromatogram shows the separated sample components as peaks. Usually pre-column derivatization is applied to enhance the detection, however in this thesis post-column derivatization was applied (38).

1.3.4 Carbohydrate content and composition

There is no clear definition of carbohydrates, however most of them can be written using the empiric formulae: $(CH_2O)n$. The monosaccharides are the smallest carbohydrate components and can consist of between three to eight carbon-atoms. All of the C-atoms in a monosaccharide contain a hydroxyl group (-OH), except for one that contains a carbonyl group (C=O), also known as the reducing end. When two monosaccharides are linked through glycosidic linkages, a disaccharide is created. Mono-and disaccharides are usually referred to as sugars, such as glucose and sucrose. A chain of a hundred monosaccharides or more is called a polysaccharide, and these are high molecular weight polymers with substantial diversity. An uronic acid is an aldohexose that contains a carboxyl group at carbon atom nr.6 instead of a hydroxyl group. Uronic acids are part of several polysaccharides, such as alginate (27). As mentioned in section 1.1, macroalgae contain several important polysaccharides that can be extracted and used industrially. Both brown and red algae contain important hydrocolloids, such as alginate in brown algae, and agar and carrageenan from red algae. These macroalgae also contain other important sugars and polysaccharides.

Brown algae

Brown macroalgae are rich in carbohydrates, and especially the alginate content is high in these algae. In *Laminaria hyperborea* alginate constitute 40 % of the matter (dw) (40). Schiener et al. (4) found the levels of alginate to be 28.5% (±3.9) in *S.latissima* and 37.4% (±4.0) of the dry matter in *A.esculenta.* Except from alginate, brown seaweeds also contain cellulose, laminarin, mannitol and fuciodan. According to Schiener et al. (4) the average amount of laminarin was reported to be 8.2% (±5.3) in *S.latissima,* and 11.1% (±7.2) of the dry matter in *A.esculenta.* The amount of mannitol was 18.6% (±4.7) and 12.1% (±3.5) of the dry matter in *S.latissima* and *A.esculenta*, respectively. Rather low amounts of cellulose have been found and according to Painter (41) the cellulose content is reported to be around 4.6% (dw), while Black (42) found it to be about 8% (dw). The exact carbohydrate content depends on the different species, and on the different seasons (15).

Alginate is the name for the salt of alginic acids. They occur as the structural component of the cell wall in brown algae (1). Alginate is a family of polysaccharides, and consists of the two monomers: β-D-mannuronic acid (M) and α-L-guluronic acid (G), linked by 1→4 linkages (43). A unique quality of alginate is that it will form gels in the presence of Ca^{2+}-

ions. Four L-guluronate units form a complex with one Ca^{2+}-ion and this gives a characteristic eggbox-structure (27). Alginate is used in several applications. In the food industry it is mainly used as thickening, gelling and stabilizing agents, and is also important as a dietary fiber. Moreover, alginate is applied in biotechnology where they are used as alginate gel beads in cell immobilization. This hydrocolloid is also used in medicine, where it has several beneficial effects, such as stimulating the immune system (1). The highest levels of alginate can be found in early spring and declines during autumn (4, 15).

Laminarin and mannitol are storage carbohydrates in Laminariales (4). Laminarin is made up of β-1,3-linked glycopyranoses and some of these are branched with β-1,6-glucose residues. Mannitol is a sugar alcohol derived from mannose (15). Laminarin and mannitol are at their lowest in spring when there is a period of rapid growth (4, 41). However, in autumn and winter the level of these reserve carbohydrates are at their highest. Laminarin has found to be as high as 35% (dw) during autumn and winter (4, 15, 41).

Another polysaccharide present in brown seaweeds is fucoidan. Fucoidan from the Laminariaceae consist of L-fucose and sulphate ester groups (44). In Laminariaceae, fucoidan contains α-1,3-linked fucopyranoses mainly with sulphate substitutions, and also substitutions with 2-*O*-α-L-fucopyranosyl, other glycocyls and/or acetate (15). Fucoidan has several biological activities, such as anticoagulant and antithrombic properties, antiviral and antitumor functions, as well as antioxidant activities (44). Like alginate, fucoidan reaches its maximum level in early spring and is at it lowest in autumn (15).
Cellulose is a linear polymer consisting of β-1,4 linked D-glucose (43), and as mentioned in section 1.2.1, cellulose is present in the cell wall of brown algae.

Red algae

Also red algae contain high amounts of carbohydrates, and the most significant are floridean starch, cellulose, D-mannan, D-xylan, and the sulfated galactans; agar and carrageenan (9, 23). In red algae floridean starch is the storage carbohydrate (45). Floridean starch is composed of branched 1→ 4-linked α-D-glycopyranose units. The cell wall constitutes a large proportion of the dry matter of red algaes, and is composed of a fibrillar wall, amorphous matrix and a glycoprotein domain. The fibrillar polysaccharides include cellulose, and although most red algaes contain cellulose in their cell wall, it is at low amounts. The water-

soluble polysaccharide xylan is composed of β-1,3-linked and β-1,4-linked D-xylose residues (24). Mannan is composed of β-1,4-linked mannose or both mannose and glucose residues. In addition, side chains of α-1,6-inked galactose residues can can occur in this polysaccharide (46). Polymers that contain β-D-mannose or β-D-xylose residues can sometimes substitute the cellulose (9, 23).

The sulfated galactans are situated in the amorphous matrix. Carrageenan is composed of repetitive units of β-D-galactopyranose and α-D-galactopyranose, while agars contain repetitive units of β-D-galactopyranose and α-L-galactopyranose (9, 23). In addition to being used in the food industry, they are applied in the pharmaceutical and cosmetics industry (9). Agar is also used as a growth medium in microbiology due to its gelling ability, in gel electrophoresis, in chromatography and in immunology. Carrageenan's have several applications in food industry; it is for example used as an emulsifier and stabilizer in diary products such as shakes and other milk drinks, in ice crams and in desserts (1).

Carbohydrate analysis

Polysaccharides may have a complex structure and determining their composition can be difficult. Differences in the monosaccharide compositions, complex linkage patterns, different substitutions on the sugar rings, and high molecular weight and polydispersity contribute to the complexity. Separation and detection of monosaccharides can be challenging (47) since many monosaccharides have very similar structure and usually doesn't absorb light in the UV region (48).

Monosaccharides are highly hydrophilic molecules containing several hydroxyl groups. Monosaccharides display low volatility due to its polar nature. Derivatization of the compounds can replace the polar groups in the monosaccharides, so they achieve higher volatility. HPLC and GC are two chromatographic methods used to analyse monosaccharides, providing high sensitivity and accuracy. However, these methods require derivatization of the sugars (47, 49). When the analysis requires derivatization, more steps are necessary so the analysis becomes more time consuming. When applying GC analysis there is also a chance that the derivatization is incomplete, in which case the neutral sugar recovery will be reduced. The drawback with the HPLC analysis is that it doesn't provide an optimal separation of monosaccharides (50).

In this thesis high-performance anion-exchange chromatography (HPAE) combined with pulsed amperometric detection (PAD) was applied. HPAE-PAD separates anionic analytes with high sensitivity and selectivity. The method does not require derivatization and provides direct quantification of the separated carbohydrates. It can be used to analyse carbohydrates such as mono- and oligosaccharides, sugar alcohols and amino sugars. Since carbohydrates are not anionic at neutral pH, they need to be ionized at high pH values in order for separation to occur by anion-exchange chromatography. Therefore the mobile phase needs to have a high pH value (here pH was 13), and the stationary phase must be able to withstand this pH value. In this thesis the CarboPac PA10 column, produced by Dionex Corporation (Thermo Fischer Scientific), was applied. In this column the stationary phase is composed of non-porous/microporous polystyrene-divinylbenzene beads with quaternary amine groups as an anion exchanger, which can tolerate high pH (51, 52).

The mobile phase contains sodium acetate (NaAc) and sodium hydroxide (NaOH). The combination of acetate and hydroxide as a mobile phase makes it possible to separate larger carbohydrates and carbohydrates with a negative charge at neutral pH (51).
The separated carbohydrates are detected by pulsed amperometric detection. PAD is an electrochemical detector based on the ionization of hydroxyl groups at high pH (51, 53). During HPAE-PAD a positive potential is applied and the ionized carbohydrates are electrocatalytically oxidized on the surface of a working (gold) electrode in an amperometry cell. The current can be measured and is proportional to the carbohydrate concentration. In this way the carbohydrates can be detected and quantified. (54, 55).

1.4 The aim of the study

In this thesis the chemical composition of the macroalgae *Saccharina latissima*, *Alaria esculenta* and *Palmaria palmata* was reviewed. These algae are some of the common kelp species that can be found growing on the coast of Norway. There is an interest for more information regarding the chemical content in these macroalgae. Algae samples harvested from different depths were compared and different harvesting periods were examined, in order to find out at which depths and in which months the level of the chemical components were highest.

In the following chapter, the different analytical methods have been described. The "Results-chapter" includes the most important findings in the macroalgae, regarding the chemical composition and at which season and depth the chemical content peaked. In the "Discussion part" the results will be discussed and compared with each other, and with the findings from other studies.

2 Materials and methods

2.1 Sample material

In this thesis three different macroalgae were used; the brown algae *Saccharina latissima* (Sugar kelp) and *Alaria esculenta* (Winged kelp), and the red algae *Palmaria palmata* (Dulse). Most of the *S.latissima* and *A.esculenta* samples were cultivated at Frøya, and these were harvested from depths of 2, 5 and 8 meters. In addition, some of the cultivated algae samples consisted of a mixture of samples harvested from these depths. These "mixed samples" contained equal amounts of samples from each depth. Hence, each of the cultivated brown algae, *S.latissima* and *A.esculenta,* consisted of one sample from 2 meters depth, one sample from 5 meters depth and one sample from 8 meters depth, as well as one sample made up from a mixture of these depths, (all of these samples were harvested in May and June).

Furthermore, two samples of *S.latissima* and *A.esculenta* were harvested from "natural habitats" at Vanvikan, and these are referred to as wild growing algae. These samples were harvested June 01 and June 19. In this thesis only one sample of *P.palmata* was used, and this was a wild growing sample. Three of the algae samples were cultivated at Austevoll and were harvested in April and May. All of these samples were of the specie *Saccharina latissima*. One of the samples harvested in April was a mixture of samples collected from depths of 0-9 meters. The other sample from April was a mixture of samples collected from 1-6 meter depths. The last sample harvested in May was a mixture of samples harvested from depths of 2-8 meters (Table 1).

After being harvested the macroalgae were sent to SINTEF and stored at -80°C. Before this the macroalgae had been stored at outdoor temperatures (12°C) for up to 10 hours. The samples from Austevoll had been stored at -20°C after harvesting, before they were sent to SINTEF. Around 5-6 samples were analysed at the time, and therefore some of the macroalgae were in the freezer for a few months before being freeze-dried and analysed. The samples that consisted of a mixture of depths from 2, 5 and 8 meters were washed with 100, 50 and 0% autoclaved seawater, before being sent to SINTEF. The frozen algae samples were crushed before analysis. In order to get as representative a sample as possible both the stem and leafs from the algae were included in the analysis (inhomogeneous samples). After the samples had been crushed, two parallels of each of the samples were transferred to plastic

containers and freeze-dried. The freeze-dried macroalgae were then milled to powder (by a mill from IKA® Labortechnik, Germany).

Each analysis was performed on all of the samples, except from the alginate determination. This method was only done on the samples from Austevoll. Stine Wiborg Dahle, who works at SINTEF Fisheries and Aquaculture, performed the alginate determination.

Table 1: Overview of the macroalgae analysed and characterized in this thesis. In total 24 algae samples were analysed in this thesis. The table contains information on when and where the algae samples were harvested, from which depths they were harvested and whether the samples were cultivated or wild. The cultivated samples marked with "mix" were a mixture of samples collected from depths of 2, 5 and 8 meter. The wild samples marked with "mix" were a mixture of samples collected from unknown depths.

***Saccharina latissima*, cultivated (Frøya)**	***Alaria esculanta*, wild (Vanvikan)**
Harvested: 20.05.15 (2m, 5m, 8m og mix)	Harvested: 01.06.15 (mix)
Harvested: 18.06.15 (2m, 5m, 8m og mix)	Harvested: 19.06.15 (mix)
***Saccharina latissima*, wild (Vanvikan)**	***Palmaria palmata*, wild (Vanvikan)**
Harvested: 01.06.15 (mix)	Harvested: 19.06.15 (mix)
Harvested: 19.06.15 (mix)	***Saccharina latissima*, cultivated (Austevoll)**
***Alaria esculanta*, cultivated (Frøya)**	Harvested: 27.04.15 (0-9 m)
Harvested: 20.05.15 (2m, 5m, 8m og mix)	Harvested: 27.04.15 (1-6 m)
Harvested: 18.06.15 (2m, 5m, 8m og mix)	Harvested: 19.05.15 (2-8m)

2.2 Determining the dry matter and ash content

In order to determine how much water and dry matter the samples contained per kg, the amount of dry matter in the macroalgae was measured, as well as the ash content. About 0-5 g of the algae samples (wet weight) was weighed in. Three parallels were analysed for each sample. The samples were then heated at 105 °C for 24 hours (in a Termaks B8133 incubator from Labolytic AS, Norway). After incubation the samples were weighed once more, and the dry matter could be calculated. After being heated and weighed the samples were then heated at 600°C for about 12 hours (Hagan elektroovner A/S, Norway). The samples were cooled in a vacuum desiccator and weighed, so the ash content could be calculated.

2.3 Extraction and determination of total lipid

The amount of lipids was determined using the Bligh & Dyer method (56). Since the samples had been freeze-dried and milled the micro method was applied. Between 10 to 50 mg of each sample were weighed in into test tubes (KIMAX®). Each sample consisted of three 3 parallels. 0.8 ml of water, 2 ml of methanol and 1 ml of chloroform was added, and the solution was homogenized for 1 minute by using an Ultra Turrax (IKA®, Germany). Another 1 ml of chloroform was added and the solution was homogenized for 20 seconds. Then 1 ml of water was added and the solution was homogenized for another 20 seconds. The sample solution was centrifuged for 10 minutes at a speed corresponding to 80 %.

After the solution had been centrifuged the lower chloroform layer was extracted by using a pasteur pipette (VWR, Radnor USA). To remove any additional sample residues the chloroform was filtrated through glass wool (VWR, Radnor USA) into a new test tube. In advance small glass valves had been weighed in, and 0.5 ml of the filtrated chloroform for each of the samples were added into these. Since the lipids were dissolved in chloroform, the latter had to be removed before the amount of lipids could be determined. Therefore the glass valves stood over night so the chloroform could evaporate. The amount of lipids could then be calculated. The following formula was used:

$$\% \text{ Lipid} = a \times b \times 100/(c \times v)$$

a = amount (mg) of evaporated fat (weight tube *with fat* - weight tube),
b = total amount of chloroform added (here the amount is 2 ml),
c = amount of evaporated chloroform (here the amount is 0,5 ml)
v = amount (mg) of sample that was weighed in at the beginning

2.4 Determination of protein content by C/N method

The total protein content can be determined by measuring the amount of nitrogen in the sample. The amount of nitrogen is around 16% in most proteins. A conversion factor of 6.25 is usually used to convert the amount of nitrogen in a sample to the amount of proteins (27). However, the amino acid content differs in different food proteins, and this conversion factor is usually too high. Therefore the protein levels will also be too high. An alternative conversion factor of 4.8 was calculated for the algae samples, and this factor may give more accurate protein values (2). For the calculation of this conversion factor see Appendix C.2.

Around 0.7-2.0 mg of the algae samples was accurately weighed into small tin capsules (4x6 mm, fra Santis-analytical, Switzerland). The samples were then analysed in a CN-analyser (ECS 4010 CHNSO analyser, by Costech, Italy) by Marte Schei at SINTEF Fisheries and Aquaculture. The results showed the amount of carbon and nitrogen in each sample. Based on the nitrogen value the amount of proteins in the samples was calculated.

2.5 Extraction of amino acids and determination of the total amount of amino acids

The protein content in the samples can also be measured by quantitative determination of the amino acids. This can be done by determining the amino acid composition, and the method consists of two steps; hydrolysis of the sample to release the amino acids, and quantification of the released amino acids by chromatography (32, 34). The analysis was performed by a high-performance liquid chromatography system (by Agilent Infinity 1260, Agilent Technologies). The detector applied was a multiwave UV-VIS detector, and a Na^+-ion exchange column was used (4.6 x 110 mm, 5 mm). The HPLC-system was coupled to an on-line post-column derivatization module (Pinnacle PCX, Pickering laboratories, Mountain View, CA, USA) using ninhydrin (Trione®) as a derivatizing reagent. All of the reagents, buffers, amino acid standards and the column were obtained from Pickering laboratories (Mountain View, CA, USA). HCl, NaOH, taurine and mercaptoethanol were obtained from Sigma-Aldrich.

As described in section 1.3.3, the mobile phase and temperature had different gradients. The composition of the mobile phase varied throughout the separation, and by using gradients the solvent strength of the eluent entering the column was continuously increased (57). The column also had temperature gradients. The gradients enable better separation and sharper

peaks. About 100 mg (± 20 mg) of the algal samples were weighed into Kimax test tubes, and three parallels were prepared for each sample. The first parallel was used as a "back-up" parallel to see how much sodium hydroxide that should be added in the neutralization, in order for the solution to achieve a pH-value of around 2.2. Some of the back-up parallels were also analysed by HPLC with the other two parallels, while some were discarded because they contained a pH-value that was too high. Therefore the samples analysed contained two or three parallels.

2.5.1 Acid hydrolysis

In this experiment the algae samples were hydrolysed using 6M hydrochloric acid (HCl), containing 0,4% β-mercaptoethanol. 2,0 ml acid was added to the Kimax test tubes, and the sample solutions were incubated at 110°C for 24 hours. The tubes were closed tightly to stop the samples from evaporating. Different acids were tried in this thesis (see section 3.1). During the hydrolysis the amino acids glutamine and asparagine were both converted to glutamic acid and aspartic acid, while cysteine was measured as cystine (cys-cys). The amino acids, taurine and ammonia were quantified from standard curves measured with analytical standards.

After being incubated the samples were allowed to cool down, and 5M NaOH was added in order to achieve a pH-value of 2.2. The samples were filtered using a GFC Whatman micro filter (55 mm ∅, UK), further diluted with a citrate buffer (pH 2.2) and transferred to small bottles/valves before being analyzed by high-performance liquid chromatography (HPLC). Vera Kristinova at SINTEF Fisheries and Aquaculture performed the analysis of the samples by HPLC. In the beginning it was experimented with how much 5M NaOH that was needed in order to get a pH-value of around 2.2. pH-indicator strips (MColorpHast™, Merck Millipore, Germany) were used to check the pH-value. The first eight samples were checked several times with the pH-strips, but it was later concluded that this removed some of the sample, because these samples got lower protein values than what was expected. Therefore it was decided that addition of 2.325 ml 5M NaOH should give the sample a pH-value of around 2.2. Only the pH-value in the "back-up" parallel was measured with pH-strips.

2.6 Extraction of sugar components and determination of the total amount of sugar components

This experiment was conducted in collaboration with Olav Andreas Aarstad, who is a postdoctoral fellow at NTNU. The algal carbohydrates were separated and detected by high-performance anion-exchange chromatography (HPAE) combined with pulsed amperometric detection (PAD). The instrument used was a Dionex ICS 5000+ (by Thermo Fischer Scientific). A Dionex CarboPac PA10, which is developed for separation of monosaccharides, was used as a column.

Monosaccharide analysis of several brown seaweeds has been done previously, for example by Manns et al. (15). They also applied HPAE-PAD and measured the amount of neutral sugars, mannitol and the uronic acids in their seaweed samples. However, Manns et al. tried out several hydrolysis methods, and among them were hydrolysis with trifluoroacetic acid (TFA), which was also applied in this experiment. Standard samples and algae samples were prepared and analysed by HPAE-PAD, in order to see if the monosaccharides could be separated and detected by this method. It was also necessary to try out different parameters on the ICS 5000+ instrument, to see which was most optimal for these samples. The following standard samples were applied in the optimization of the method:

Table 2: Overview of the standard samples analysed by HPAE-PAD in this thesis. Between 0.5 – 2.0 mg of the standard samples were weighed in, and dissolved in deionized water. These stock solutions (1 mg/ml) were further diluted with deionized water to a concentration of 0.01 mg/ml.

Category	**Sugar component**
Monosaccharides:	Galactose, glucose, xylose, fucose, mannose
Di- and trisaccharides:	Maltotriose (trisaccharide), maltose (disaccharide), cellobiose (disaccharide)
Sugar alcohol:	Mannitol, meso inositol (MES)
Uronic acid:	Glucuronic acid, mannuronic acid tetramer (M4), galacturonic acid

The trifluoroacetic acid (TFA) and the standard samples were from Sigma-Aldrich. NaOH and NaAc were acquired from Merck Millipore.

2.6.1 Optimization of the method

In addition to the standard samples, two of the algae samples were also analysed by HPAE-PAD when optimizing the method. As mentioned, all the samples were hydrolysed using 2M trifluoroacetic acid (TFA). Around 30 mg of algae sample was weighed in and 2M TFA was added to a concentration of 10 mg/ml. The samples were incubated for 24 hours at 103°C and then neutralized with 1M NaOH (2 ml of 1M NaOH added to 1 ml of sample) so the sample solutions was not too acidic (a high pH-value is necessary for the mobile phase). The solution was further diluted with deionized water (7 ml), so a 10-fold dilution was achieved for the sample solution. The standard samples and the algae samples were then analysed on the ICS 5000+.

As mentioned in the introduction the HPAE-PAD utilizes a mobile phase that contains NaAc (sodium acetate) and NaOH (sodium hydroxide). Some carbohydrates are charged at neutral pH (such as uronic acids, phosphate/sulphated monosaccharides, sialic acids) and a stronger eluent than NaOH is necessary in order to elute these. NaAc can be used as a stronger eluent, and will accelerate the elution of acidic sugars such as the uronic acids. However sodium hydroxide is also required as an eluent in order for the separation and detection to occur. The detector requires a high pH value in order to function (provided by NaOH). Also the NaOH concentration determines the degree of ionization for the compounds that are to be separated, and therefore determines how strongly they bind to the column components. So even if the detector could function with a lower pH value, NaOH is required as an eluent. (52, 58)

When separating carbohydrates, gradients of the eluents are often used. Usually when separating oligosaccharides the concentration of NaOH is kept constant (isocratic concentration) while a NaAc gradient is applied (58). By applying gradients of one or both eluents it is possible to optimize the separation of the sugar components and make the analysis faster. However in this experiment it was determined that it was unnecessary to use gradients, so isocratic concentrations were applied instead. The best thing would be to analyse all of the sugars in one method, and then it is necessary to apply gradient. However in this experiment this was not feasible. The uronic acids and neutral monosaccharides had to be analysed separately, because this gave better chromatograms (without artefact peaks, labile baseline, long washing procedures and drifting retention times). The method used for the separation of the neutral monosaccharides was with 20 mM NaOH for 30 minutes and for the uronic acids it was with 100 mM NaOH and 100 mM NaAc for 40 minutes.

As mentioned, the first hydrolysis performed on two of the algae samples was done at 103°C for 24 hours after addition of 2M TFA. The hydrolysis had not been optimal since the algal sample had not dissolved completely during incubation. Therefore it was experimented with different hydrolysis conditions, in order to see which gave best results. This is further described in the "Method development"-section (see section 3.2).

2.6.2 Preparation of standard curves

After the method had been optimized and the best hydrolysis conditions found, three mixtures of the standard samples were prepared for analysis. Standard curves were calculated based on these standard mixtures (appendix D.2). When analysing the standard samples in the beginning it was shown that mannose and xylose coeluted, and therefore they had to be analysed separately. The first mixture included the uronic acids, the second contained inositol, mannitol, fucose, glucose and xylose. The third mixture included mannose and galactose. The mixtures were analysed at different concentrations (50, 20, 10, 5, 1 and 0.1 mg/L).

After these had been analysed and the standard curves had been determined, all of the algal samples were analysed. Between 10-20 mg of the samples was weighed in and 2M TFA was added (to a concentration of 10 mg/ml). The samples were incubated, and then diluted 10 times with 1M NaOH (0.18 ml NaOH added to 0.10 ml sample) and deionized water (0.72 ml). The algal samples were further diluted 5-fold (0.8 ml water added to 0.2 ml sample). A 50-fold dilution gave concentrations in the linear area of the standard curve. Also separation will generally be best with as low a concentration as possible. If the sample is not diluted enough it can result in column overload, which means that "the linear capacity of the column is exceeded" (59). This can result in distorted peak shapes and decreased retention time (59).

2.6.3 Challenges

An alginate control sample (SF120 RB alginate, FMC Biopolymer (F_G= 0,48, [η] =1194 ml/g) was also subjected to the same hydrolysis conditions as the alginate samples, and the results showed lower yields of guluronic acid and mannuronic acid than expected. Also the ratio between these two acids (M/G) was highly overestimated. Some precipitation was observed, and this could be due to incomplete degradation of the polymer or because of polymerization between the degraded monosaccharides (from side reactions of released monomers). Guluronic acid is more acid labile than mannuronic acid so a correction factor has to be used in order to calibrate the method. Based on the results obtained, the hydrolysis conditions

should be optimized specifically for the determination of guluronic and mannuronic acid. Alternative methods will probably give a better quantification of the amount of alginate in brown seaweed.

The use of trifluoroacetic acid in the hydrolysis made it challenging to liberate the cellobiose and glucose residues from the cellulose fraction. Furthermore, a positive control, such as a brown macroalgae sample with known composition of monosaccharides and polysaccharides, should have been applied. Since we didn`t have a positive control, the results from this thesis were compared to the results reported in literature on the carbohydrate composition. In addition, decarboxylation in the alginate control samples was observed. Decarboxylation will cause the ratio between the uronic acids to be incorrect.

The interference of carbon dioxide in the mobile phase is a common problem with HPAE-PAD. The mobile phase has a pH of about 13, and when carbon dioxide is dissolved in water at this pH it turns into carbonate (decarboxylation). Carbonate is a divalent ion that will act as an eluent, causing the retention time of the carbohydrates to be reduced, the column selectivity to decrease and give a lower resolution. The carbon dioxide cannot be completely eliminated, but we degased the solution with helium in order to suppress free CO^2 (58, 60). Furthermore, this method did not allow for separation of mannose and xylose, so these are shown together in the results.

2.7 Determination of alginate

Stine Wiborg Dahle, who is a research scientist at SINTEF Fisheries and Aquaculture, performed this method. The extraction of alginate was done according to the method from Østgaard (61). As mentioned in section 1.3.4, alginates consist of mannuronic and guluronic acids, also known as M- and G-blocks. The enzyme M-and G-lyase cleaves the alginate, so that an unsaturated unit at the non-reducing end is formed. This unit has a strong absorbance at 230 nm. The measured absorbance corresponds to the alginate concentration of the macroalgae (according to Beer-Lamberts law) (61). This analysis was performed on three of the macroalgae samples, namely the *S.latissima* samples harvested at Austevoll in April and May.

2.7.1 Extraction of alginate from samples

Before being analysed, the alginate was extracted from the algae samples. About 20 mg of the samples were weighed in, and 10 ml of 0.2M HCl was added to the samples in kimax test tubes. These solutions were then incubated over night, with continuous shaking. The next day the samples were centrifuged for 10 minutes, and the solid material from each sample was extracted. The pellets were washed twice with 0.2M HCl, and centrifuged between each wash. The pellets were re-suspended in 10 ml 0.1M $NaHCO_3$, and incubated for 2 hours. The pH-value of the solution was adjusted to 7.6-7.9 with $NaHCO_3$/HCl, and the samples were incubated over night. The third day the pH was adjusted to a value of 7.0 with $NaHCO_3$/HCl. The sample was centrifuged for 10 minutes, and the supernatant was extracted. This procedure was performed at room temperature.

2.7.2 Enzymatic determination of the alginate content

For the enzymatic determination, 200 µl of the extracted alginate solution was mixed with 400 µl Tris-HCl buffer (50MM, pH 7,5) and 20 µl of each enzyme (1U/ml of alginate M- and G-lyase). A volume of 150 µL of each of these solutions was added to a 96-well UV plate (COSTAR), and the absorbance was measured at 230 nm by using a plate reader (Epoch/Bergman). For validation of the method a positive control containing alginate was also determined, as well as a sample that did not include any of the enzymes.

Alginate control samples (alginate extracted from kelp from SINTEF) were also determined, and from these results a standard curve was calculated. The concentration of the alginate,

extracted from the *S.latissima* samples, could therefore be determined from the standard curve.

2.8 Statistical analysis

For all of the results a Student`s t-test was applied as a statistical analysis, in order to see if there was a significant difference between some of the samples. In this case an unpaired two-sample t-test was used. There was a significant difference between two samples if the p-value was lower than 0.05 ($p<0.05$). In the chapter containing the results, the significant differences found refers to this value, unless stated otherwise.

3 Method development

3.1 Experimentation with acids and additives in the protein hydrolysis

The method development of the amino acid analysis was conducted in collaboration with Vera Kristinova at SINTEF Fisheries and Aquaculture and at their laboratories at SINTEF Sealab. The first experiment included comparison of hydrolysis with two different acids; 6M HCl (containing 0.02% phenol) and 4M methanesulfonic acid. In the second experiment two additives were compared; hydrolysis was performed with 6M HCl including 0.02% phenol, and with 6M HCl containing 0.4% β-mercaptoethanol.

3.1.1 Hydrolysis with hydrochloric acid (HCl) and methanesulfonic acid (MSA)

Extraction of amino acids are usually achieved by conventional acid hydrolysis with 6M HCl at around 110 °C for about 24h. However the essential amino acid tryptophan is often destroyed during this method. According to Muramoto & Kamiya (62) the addition of phenol can prevent destruction of tryptophan. However Fountoulakis & Lahm (32) claim that hydrolysis with methanesulfonic acid allows for determination of tryptophan and methionine sulfoxide.

In accordance with the procedure at SINTEF Fisheries and Aquaculture, 6M HCl containing 0.02 % phenol was used in the hydrolysis. It was also experimented with using 4M methanesulfonic acid (MSA) instead of 6M HCl (containing 0.02% phenol), too see if this gave better extraction of the amino acids. Hydrolysis with MSA was performed at 160°C for 45 minutes. Chiou & Wang describe in their article (63) that hydrolysis with 4M methanesulfonic acid for 45 minutes at 160°C gave results similar to hydrolysis at 110°C for 24 hours.

3.1.2 Hydrolysis with HCl containing 0.4% β-mercaptoethanol

Ng. et al (34), describes in their article that the addition of 0.4 % β-ME also prevents tryptophan from being destroyed during hydrolysis. Therefore HCl with the addition of 0.4% β- ME instead of 0.02% phenol was tried.

3.2 Optimization of hydrolysis conditions in the carbohydrate analysis

As mentioned in section 2.6.1 the algal samples had not dissolved properly during incubation at 103°C for 24 hours. According to Garleb et al. (50) it has not been possible to release the glucose residues from the cellulose fraction by the use of TFA, since TFA is incapable of breaking the glycosidic linkages of cellulose. Different hydrolysis conditions were tried to find out if the monosaccharides were decomposed during hydrolysis. The standard samples and one of the algae sample were hydrolysed with 2M TFA and incubated at 103°C for different time periods. The standard samples consisted of a mixture of mannitol, glucose, galactose, fucose, mannose, MES, glucuronic acid, galacturonic acid, mannuronic acid and gulucturonic acid. The standards were incubated for 4, 8, 10.5, 24, 34.5 and 48 hours. The algae sample (4 parallels) was incubated for 9, 24, 33 and 46.5 hours. In the end it was concluded that the best incubation period was 24 hours. After 24 hours more monosaccharides were liberated than after 9 hours, and the degradation of neutral monosaccharides was not significant.

4 Results

4.1 Dry matter and ash content

The dry matter in cultivated *S.latissima* varied from 7.0-11.5% of the wet weight (ww) in May, and from 8.8-10.3% in June. Cultivated *A.esculenta* had dry matter that varied from 8.5-17.6% (ww) in May, and from 14.1-18.2% (ww) in June. The dry matter for *P.palmata* was 15.5% (ww). As mentioned in the "Materials and methods"- chapter, only one sample of *P.palmata* was used in this thesis. This sample was harvested June 19, and consisted of a mixture of different depths. The ash content in *S.latissim*a was between 36.7-47.0% of the dry matter (dw) in May, and between 37.8-44.4% (dw) in June. The ash content in *A.esculenta* varied from 24.5-30.9% (dw) in May, and from 17.3-26.4% (dw) in June. In *P.palmatia* the ash content was 17.4% (dw). All the values can be found in appendix A.

In May, the dry matter in *A.esculenta* was significantly higher than in *S.latissima*, in the samples harvested from 5 and 8 meters depth, and in the samples harvested from 2 meters depth ($p<0.1$). In June, *A.esculenta* also had a significantly higher content of dry matter than *S.latissima* in the sample containing a mixture of depths, and in the sample from 8 meters depth ($p<0.1$). There was only a significant difference in the ash content between the species in June. Here the ash content in *S.latissima* was significantly higher than in *A.esculenta,* in the sample containing a mixture of depths. *S.latissima* was also significantly higher ($p<0.1$) than *A.esculenta* in the samples from of 2, 5 and 8 meters depth (Figure 1 and 2).

Since *P.palmata* was a wild algae sample harvested June 19 (from Vanvikan), this sample was compared to the wild samples of *S.latissima* and *A.esculenta* also harvested June 19 (from Vanvikan). The dry matter was significantly lower in *S.latissima*, compared to the other two species. However, the ash content was at its highest in *S.latissima,* and there was a significant difference ($p<0.1$) between *S.latissima* and *P.palmata* (Fig. 3).

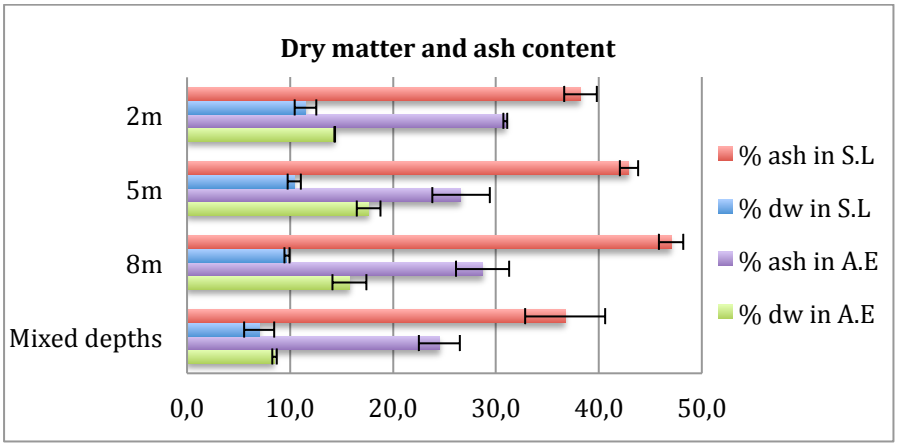


Figure 1: Dry matter (dw) and ash content in *S.latissima* (S.L) and *A.esculenta* (A.E) in May, for each of the different depths the samples were harvested from. The dry matter was calculated from the wet weight (ww), and the ash content was calculated from the dry matter (dw). "Mixed depths" refers to the algae samples containing samples from a mixture of 2, 5 and 8 meters depths. The uncertainty is given as the standard deviation of three parallels, and the results are average value of these.

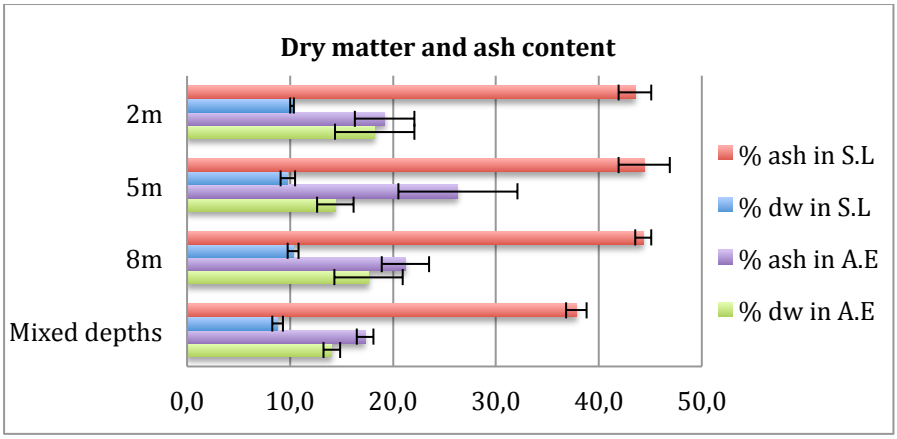


Figure 2: Dry matter (dw) and ash content in *S.latissima* (S.L) and *A.esculenta* (A.E) in June, for each of the different depths the samples were harvested from. The dry matter was calculated from the wet weight (ww), and the ash content was calculated from the dry matter (dw). "Mixed depths" refers to the algae samples containing samples from a mixture of 2, 5 and 8 meters depth. The uncertainty is given as the standard deviation of three parallels, and the results are average value of these.

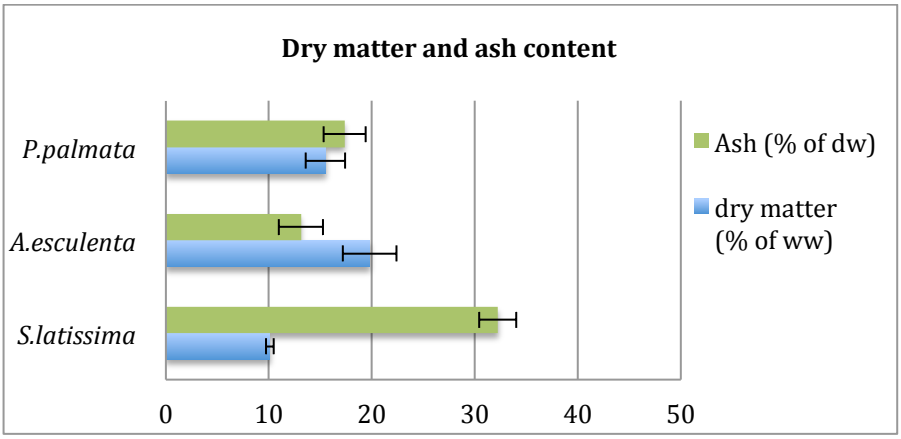


Figure 3: The dry matter (dw) and ash content in *S.latissima*, *A.esculenta* and *P.palmata*. These were wild algae samples harvested June 19, and consisted of samples collected from depths of 2, 5 and 8 meters. The dry matter was calculated from the wet weight (ww), and the ash content was calculated from the dry matter (dw). The uncertainty is given as the standard deviation of three parallels, and the results are average value of these.

The dry matter and ash content also varied significantly between the depths, within *S.latissima* and *A.esculenta*. In May and June the dry matter in *S.latissima* was significantly lower ($p<0.1$) in the samples consisting of a mixture of different depths, compared to the samples from 2 meters depth. For *A.esculenta* there was only a significant difference in the dry matter content within the depths in May. Here the sample containing a mixture of depths had a significantly lower content of dry matter than the samples harvested from depths of 2, 5 and 8 meters. In addition the sample harvested from 2 meters depth was significantly higher than the sample from 5 meters depth (Fig. 4 and 5).

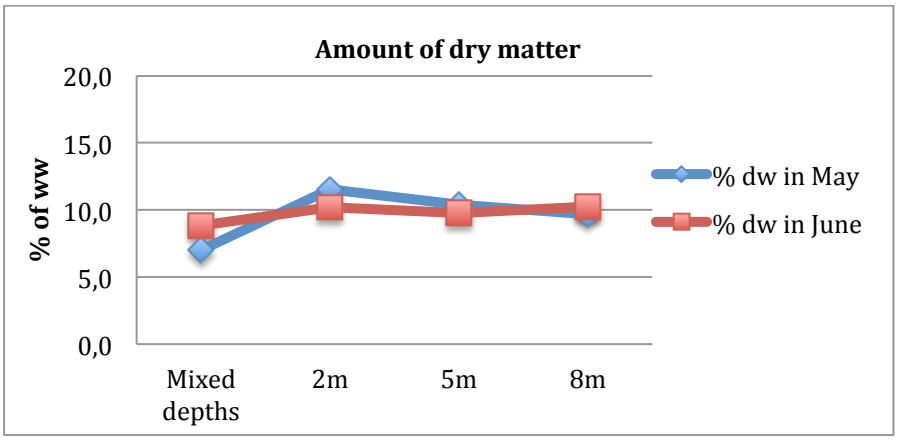


Figure 4: The dry matter (dw) in *S.latissima* in May and June, as a function of different depths. "Mixed depths" refers to the algae samples containing samples from a mixture of 2, 5 and 8 meters depth. The dry matter was calculated from the wet weight (ww). The uncertainty is given as the standard deviation of three parallels, and the results are average value of these.

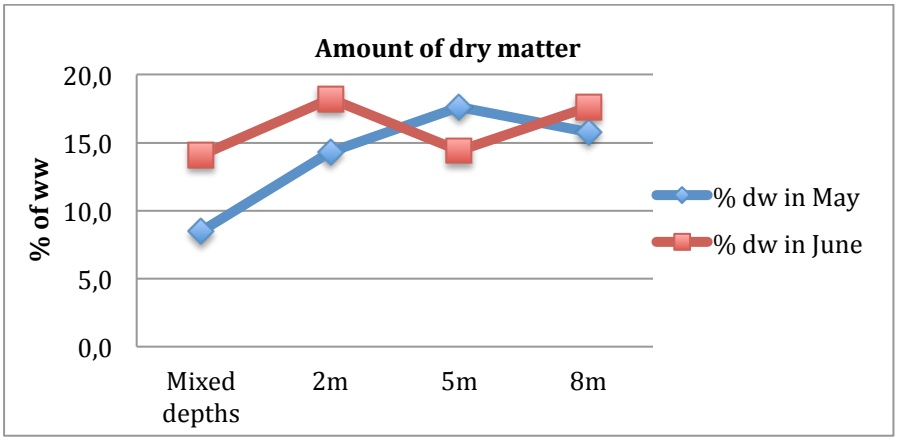


Figure 5: The dry matter (dw) in *A.esculenta* in May and June, as a function of different depths. "Mixed depths" refers to the algae samples containing samples from a mixture of 2, 5 and 8 meters depth. The dry weight was calculated from the wet weight (ww). The uncertainty is given as the standard deviation of three parallels, and the results are average value of these.

The ash content was at its minimum in the samples containing a mixture of depths. In both *S.latissima* and *A.esculenta* there was a significant difference between the samples containing a mixture of depths, and the samples from 2, 5 and 8 meters depth (Fig. 6 and 7).

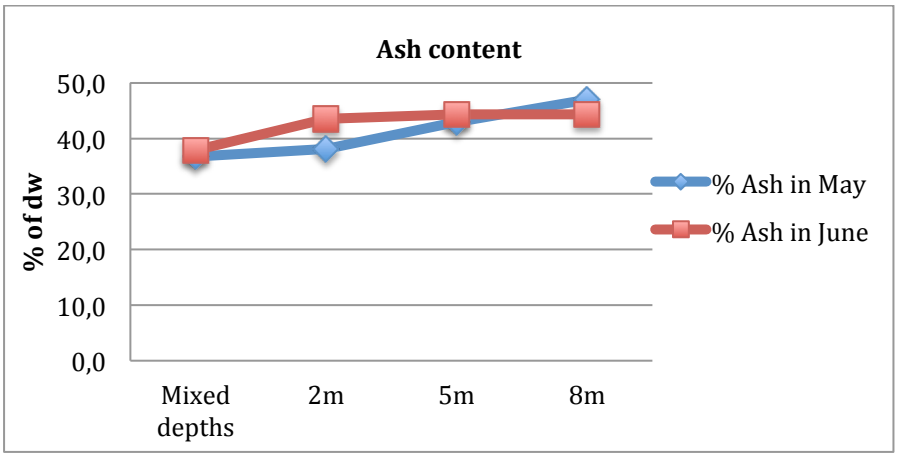


Figure 6: The ash content in *S.latissima* in May and June, as a function of different depths. "Mixed depths" refers to the algae samples containing samples from a mixture of 2, 5 and 8 meters depth. The ash content was calculated from the dry matter (dw). The uncertainty is given as the standard deviation of three parallels, and the results are average value of these.

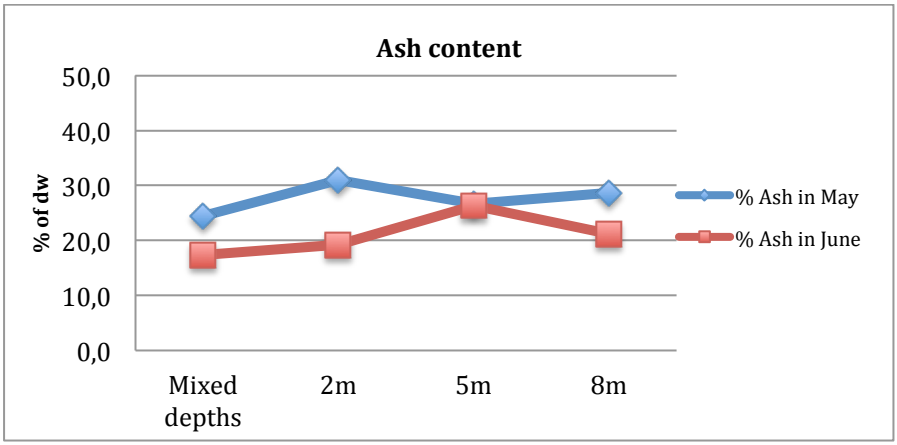


Figure 7: The ash content in *A.esculenta* in May and June, as a function of different depths. "Mixed depths" refers to the algae samples containing samples from a mixture of 2, 5 and 8 meters depth. The ash content was calculated from the dry matter (dw). The uncertainty is given as the standard deviation of three parallels, and the results are average value of these.

The cultivated algae samples of *S.latissima* and *A.esculenta,* harvested June 18 and containing a mixture of depths, was compared to the wild algae samples from Vanvikan, harvested June 01 and June 19 (Fig. 8). In both *S.latissima* and *A.esculenta* there was a significant difference in the dry matter between the cultivated sample and the wild sample harvested June 01. In *S.latissima* there was also a significant difference in the dry matter between the wild samples.

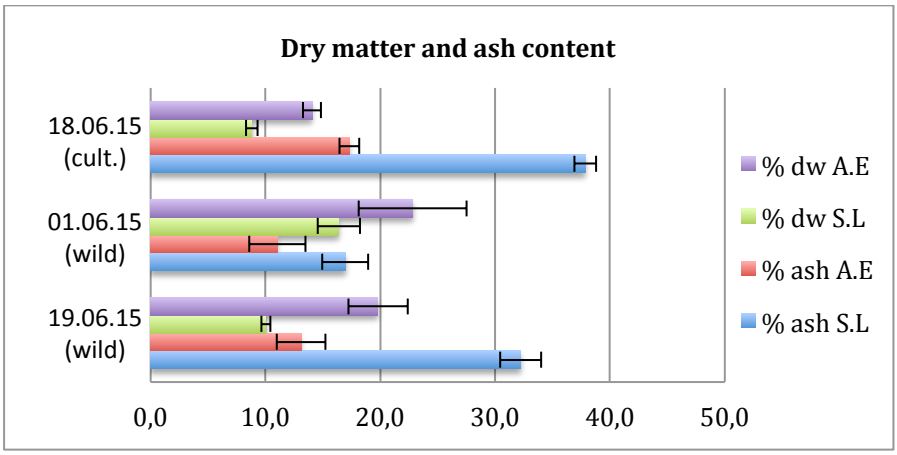


Figure 8: The dry matter (dw) and ash content (ash) in the cultivated (cult.) and wild algae samples of *S.latissima* (S.L) and *A.esculenta* (A.E). The cultivated samples were harvested June 18 (2015), and are here marked as 18.06.15. The wild algae samples were harvested June 01 and June 19 (2015), and are here referred to as 01.06.15 and 19.06.15. The uncertainty is given as the standard deviation of three parallels, and the results are average value of these.

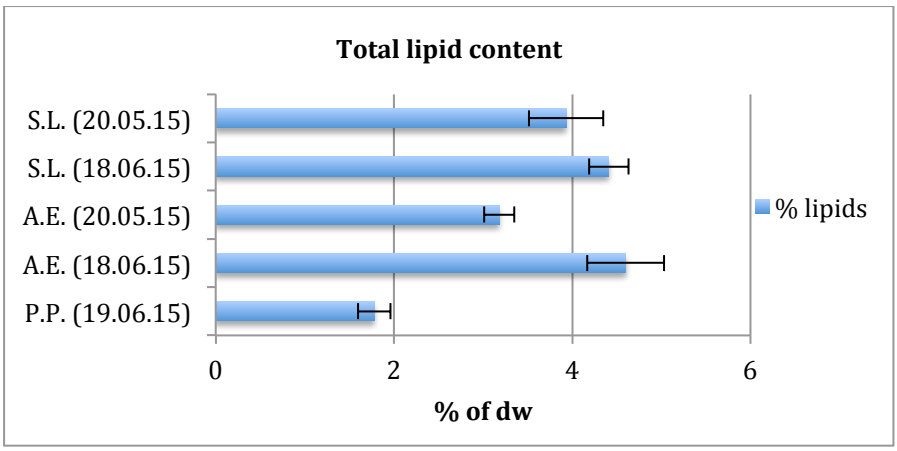
In the *S.latissima* samples cultivated and harvested at Austevoll, the dry matter varied from 8.2-9.1%, with the highest amount in the samples harvested in April. The ash content ranged from 42.2-49.5%, and the highest value could be found in the sample harvested in May (see fig.9-10). The *S.latissima* samples from Frøya had higher dry matter content than the Austevoll samples, except from the samples containing a mixture of depths. There was a significant difference in the dry matter content between the Austevoll-sample harvested May 19 (containing a mixture of depths between 2-8 meter) and the Frøya- sample harvested May 20 (from depths of 2, 5 and 8 meters), and the Frøya- sample harvested June 18 (from 2 and 8 meter depth). The Frøya- sample harvested June 18 had a significantly higher ($p<0.1$) content of dry matter than the Austevoll sample harvested May 19.

The *S.latissima* samples from Austevoll harvested April 27 (containing a mixture of depths from 0-9 meters) had an ash content that was significantly higher ($p<0.1$) than the *S.latissima* sample from Frøya harvested May 20 (at depths of 2 meters). The same Austevoll sample was significantly lower than the *S.latissima* sample from Frøya harvested May 20 (from 8 meters depth). The Austevoll-sample harvested April 27 (containing depths between 1-6 meters) had a significantly higher ash content than the Frøya-sample harvested May 20 (from 2 meters depth). The Austevoll-sample harvested May 19 (consisting of depths from 2-8 meter) had a significantly higher ash content than the Frøya-samples harvested May 20 (from 2 and 5 meters depth and the sample containing a mixture of depths).

The Austevoll- samples harvested in April (containing depths between 0-9 meters and 1-6 meters) had a significantly higher ash content than the Frøya-sample harvested June 18 (containing a mixture of depths). The *S.latissima* sample from Austevoll harvested May 19 (consisting of depths from 2-8 meters) was significantly higher than the Frøya- sample harvested June 18 (from a mixture of depths) and significantly higher ($p<0.1$) than the Frøya samples harvested June 18 (from 2 and 8 meters depth).

4.2 Total lipid content

The total lipid content in *S.latissima* varied from 3.6% - 5.3% (dw) in May, while it was from 1.4%-4.4% (dw) in June. In *A.esculenta* the lipid content ranged from 3.2%-4.1% (dw) in May, and from 3.1%-4.6% (dw) in June (Fig. 9). All the values can be found in appendix B.
In May, *S.latissima* was significantly higher than *A.esculenta* (at 8 meters depth), while *A.esculenta* was significantly higher than *S.latissima* in June (at 2 and 8 meters depth). *P.palmata* had a total lipid content of 1.8% (dw) in June. For the wild algae samples harvested June 19, there was a significant difference between *S.latissima* and *P.palmata*, and a significant difference ($p<0.1$) between *A.esculenta* and *P.palmata* (Fig. 12).



Figur 9: Total lipid content in *S.latissima* (S.L), *A.esculenta* (A.E) and *P.palmata* (P.P). The results for *S.latissima* and *A.esculenta* are from the samples harvested May 20 and June 18, containing a mixture of depths. The *P.palmata* sample also consists of a mixture of depths. The uncertainty is given as the standard deviation of three parallels, and the results are average value of these.

The *S.latissima* samples harvested May 20 had a significantly high lipid content in the sample harvested from 8 meters depth, than in the samples from all the other depths. In June the *S.latissima* sample containing a mixture of depths has a significantly higher lipid content than all the other samples. The sample harvested from 2 meters depth was significantly lower than all the other samples (Fig. 10). In May the *A.esculenta* sample harvested from 2 meters depth had a lipid content that was significantly higher than the content in the samples harvested from 5 meters depth, and the sample containing a mixture of depths. Of the *A.esculenta* samples harvested in June the sample containing a mixture of depths and the sample from 8 meters depth, had a significantly higher lipid content than the sample from 2 meters depth ($p<0.05$) and from 5 meters depth ($p<0.1$) (Fig.11).

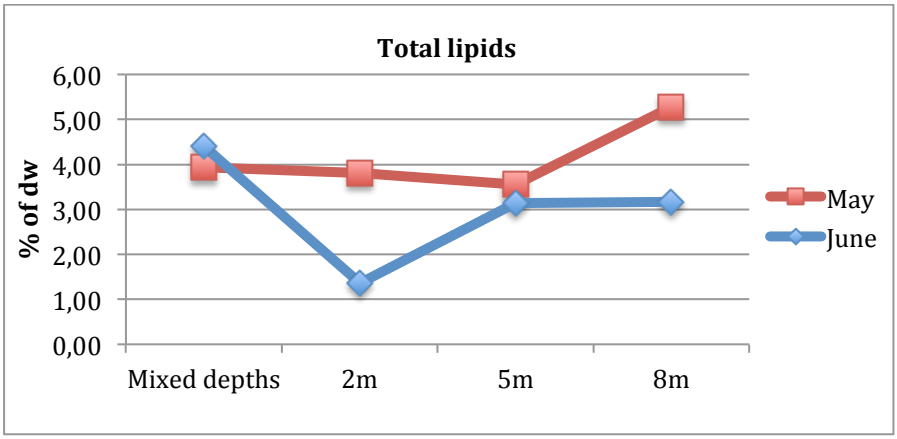


Figure 10: Total lipid content in *S.latissima* in May and June, as a function of different depths. "Mixed depths" refers to the algae samples containing samples from a mixture of 2, 5 and 8 meters depth. The uncertainty is given as the standard deviation of three parallels, and the results are average value of these.

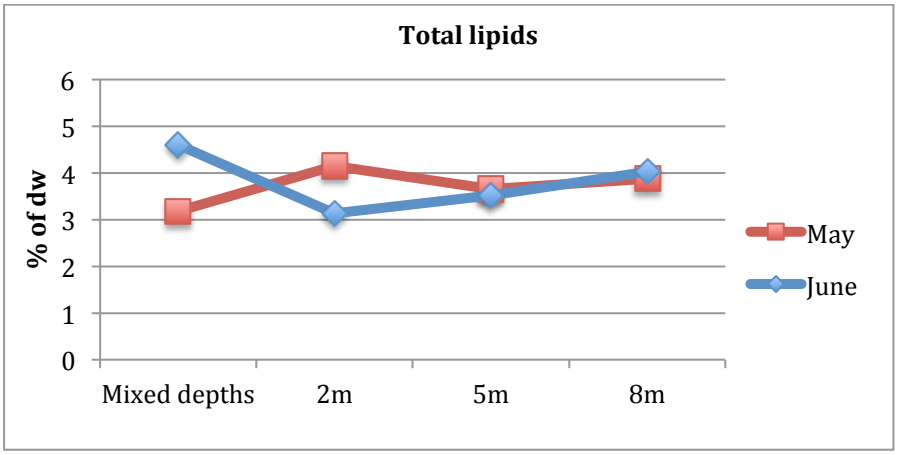
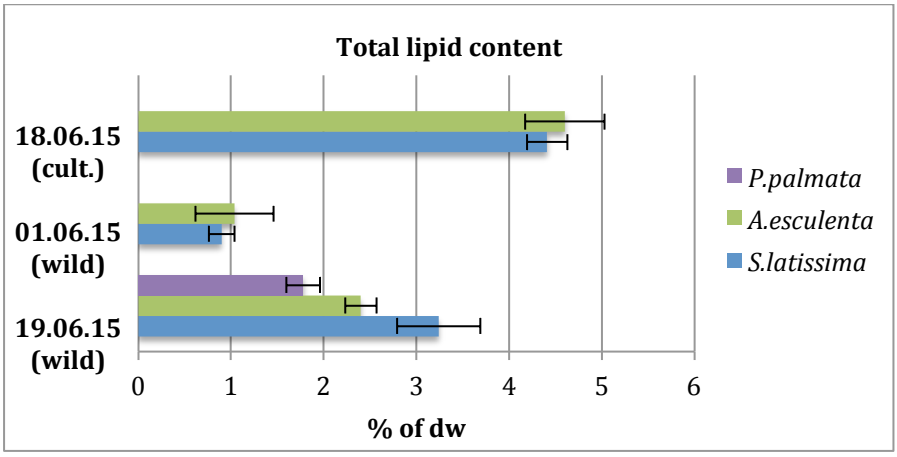


Figure 11: Total lipid content in *A.esculenta* in May and June, as a function of different depths. "Mixed depths" refers to the algae samples containing samples from a mixture of 2, 5 and 8 meters depth. The uncertainty is given as the standard deviation of three parallels, and the results are average value of these.

When comparing the cultivated algae samples harvested June 18, containing a mixture of depths, with the wild algae samples harvested June 01 and June 19, the results showed that for both *S.latissima* and *A.esculenta* the lipid content was significantly higher in the cultivated samples than in the wild samples (Fig. 12). The lipid content in the *S.latissima* sample harvested June 18 was significantly higher ($p<0.1$) than the *S.latissima* sample from June 19.



Figur 12: Total lipid content in the cultivated *S.latissima* and *A.esculenta* samples (harvested 18.06.15) with the wild samples of *S.latissima*, *A.esculenta* and *P.palmata* (harvested 01.06 15 and 19.06.15). The uncertainty is given as the standard deviation of three parallels, and the results are average value of these.

Among the *S.latissima* samples cultivated and harvested at Austevoll the sample harvested May 19, containing a mixture of samples from depths between 2-8 meters, had the highest content of total lipids. The lipid content in this sample was at 2.82% (±0.3). The sample harvested 27 April, containing samples from depths between 1-6 meters, had a lipid content of 2.79% (±0.1). For the last sample harvested in April, containing depths between 0-9 meters, the lipid amount was found to be 2.01% (±0.4).

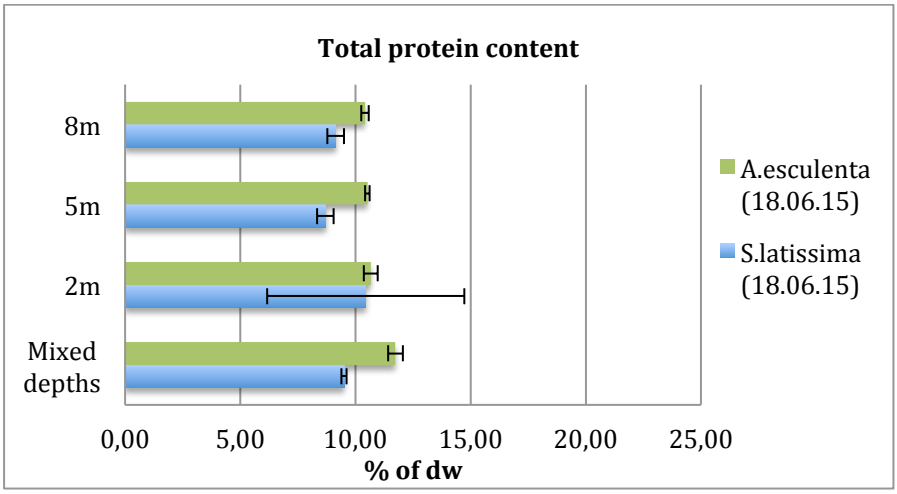
Most of the *S.latissima* samples from Austevoll had a significantly lower lipid content than the *S.latissima* samples from Frøya. The Austevoll- samples harvested April 27, containing depths from 0-9 meters and from 1-6 meters, were significantly lower than the Frøya- samples harvested May 20 and June 18, in most of the depths. The *S.latissima* sample from Austevoll harvested May 19, containing depths from 2-8 meter, had a significantly lower lipid content than the Frøya sample harvested May 20 from 8 meters depth. This Austevoll-sample also had a significantly lower lipid content than the Frøya sample harvested June 18, containing a mixture of depth. However the same Austevoll-sample had a significantly higher lipid level than the Frøya sample harvested June 18 from 2 meters depth.

4.3 Protein content determined by C/N analysis

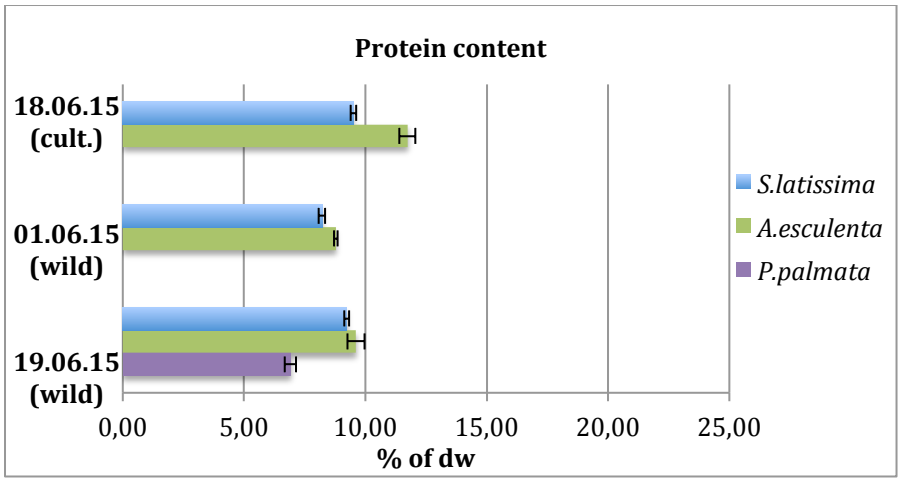
Due to problems with the C/N analyser (ECS 4010 CHNSO analyser, by Costech, Italy) not all of the algae samples were analysed, and the *S.latissima* and *A.esculenta* samples harvested from 2, 5 and 8 meters depth in May, are therefore not included. As mentioned in section 2.4, a conversion factor of 4.8 was applied when calculating the protein content in the algal samples (all the values can be found in appendix C.1).

In May the protein value for *S.latissima* was 11.8% of the dry matter (dw), while it was at 13.6% for *A.esculenta.* In June the protein content in *S.latissima* ranged from 8.7-10.5% (dw). In *A.esculenta* the protein content varied from 10.4-11.7% (dw) (Figure 13.). *P.palmata* had a total protein content of 6.9% (dw). The protein value for *A.esculenta* was found to be significantly higher than for *S.latissima* in May and in June, (except from at 2 meters depth). When comparing the *P.palmata* sample with the wild algae samples of *S.latissima* and *A.esculenta*, all harvested June 19, the red algae was significantly lower than both of the brown algae.
The *S.latissima* sample containing a mixture of 2, 5 and 8 meters depth in June was significantly higher ($p<0.1$) than the *S.latissima* sample harvested from 5 meters depth. The *A.esculenta* sample harvested in June, and containing a mixture of depths, was significantly higher than the *A.esculenta* samples from 2, 5 and 8 meters depth.

When comparing the wild growing samples of *S.latissima*, harvested June 01 and June 19 with the cultivated *S.latissima* sample from June 18, it was found that the wild sample from June 01 was significantly lower than the two other samples.
The cultivated sample of *A.esculenta,* harvested June 18, was significantly higher than both the wild growing samples. The *S.latissima* samples cultivated and harvested at Austevoll, had protein values from 6.47-8.48% (dw), and the highest value were from the sample harvested May 19, collected at depths from 2-8 meter. This value was significantly higher than for the Austevoll- sample harvested April 27, at depths from 0-9 meter. The two Austevoll- samples from April, containing samples from 0-9 meter depth and from 1-6 meter, was significantly lower than almost all of the Frøya- samples. The Austevoll- sample harvested May 19, was only significantly lower than the Frøya- sample harvested June 18, containing a mixture of depths.



Figur 13: Total protein content in cultivated *S.latissima* and *A.esculenta*, harvested in June (18.06.15), from different depths. The uncertainty is given as the standard deviation of four parallels, and the results are average value of these.



Figur 14: Total protein content for cultivated *S.latissima* and *A.esculenta* (cult.) harvested June 18, and for wild growing (wild) *S.latissima, A.esculenta* (harvested June 01 and June 19) and *P.palmata* (harvested June 19). The uncertainty is given as the standard deviation of four parallels, and the results are average value of these.

4.4 Protein content and amino acid composition determined by HPLC

As mentioned in section 3.1, different acids and additives were applied in the hydrolysis of the macroalgae samples, when extracting the amino acids. The aim was to find the most optimal hydrolysis conditions when determining the amino acid composition in the macroalgae. The total protein content in the algae samples are all calculated based on the total amount of amino acids, as % of the dry weight (% total AA).

4.4.1 Hydrolysis with HCl (w/0.02% phenol) and MSA

In order for the experiment to be less time consuming, the hydrolysis with HCl and methanesulphonic acid (MSA) was performed on only five of the samples, namely *S.latissima* (S.L) and *A.esculenta* (A.L) harvested June 18 (cultivated) and June 19 (wild), as well as *P.palmata* (P.P) harvested June 19 (wild). All of the cultivated samples consisted of samples harvested from a mixture of depths from 2, 5 and 8 meter (Fig. 15). From the results it was clear that hydrolysis with 6M HCl containing 0.02% phenol gave higher yields of amino acids than hydrolysis with 4M MSA. There was a significant difference between the two methods for the *S.latissima* (S.L) and *A.esculenta* (A.E) samples harvested June 18, and the *P.palmata* (P.P) sample harvested June 19.

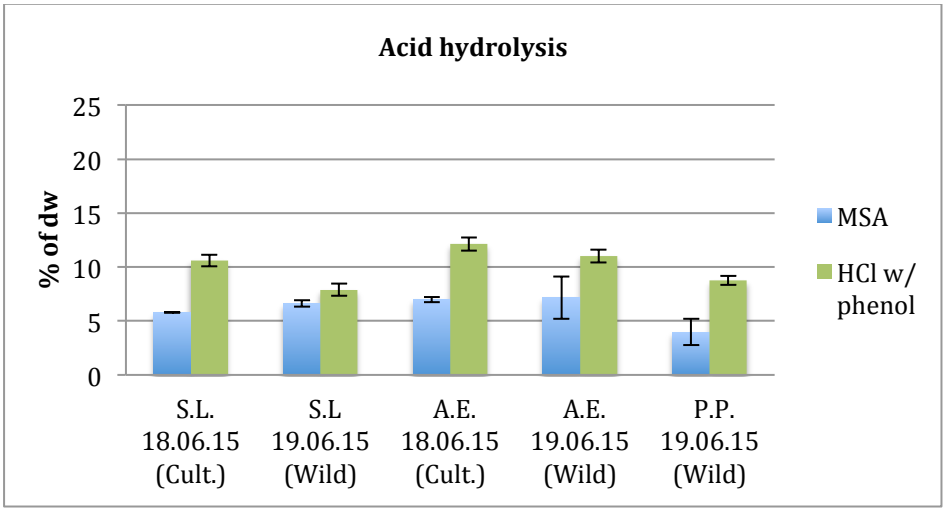


Figure 15: Total amount of amino acids after hydrolysis with HCl and MSA. The Cultivated (Cult.) *S.latissima* (S.L) and *A.esculenta* (A.E) samples harvested June 18 were compared with the wild growing sampled (wild) of *S.latissima*, *A.esculenta* and *P.palmata* (P.P) harvested June 19. The uncertainty is given as the standard deviation of two or three parallels, and the results are average value of these.

4.4.2 Hydrolysis with HCl containing 0.4% β-mercaptoethanol

When comparing hydrolysis with 6M HCl containing 0.02% phenol, and with 6M HCl containing 0.4% β-mercaptoethanol (β-ME) as additives, the results showed that the addition of β-ME generally gave higher yields of amino acids (Fig. 16). This hydrolysis method was done on the same five samples analysed in section 1.4.1. From the results it could be observed that the addition of mercaptoethanol to HCl, instead of phenol, gave better results. All but one sample (*A.esculenta* harvested June 19) showed a significant difference between these two methods. Therefore all the samples were hydrolysed with HCl containing 0.4% β-ME.

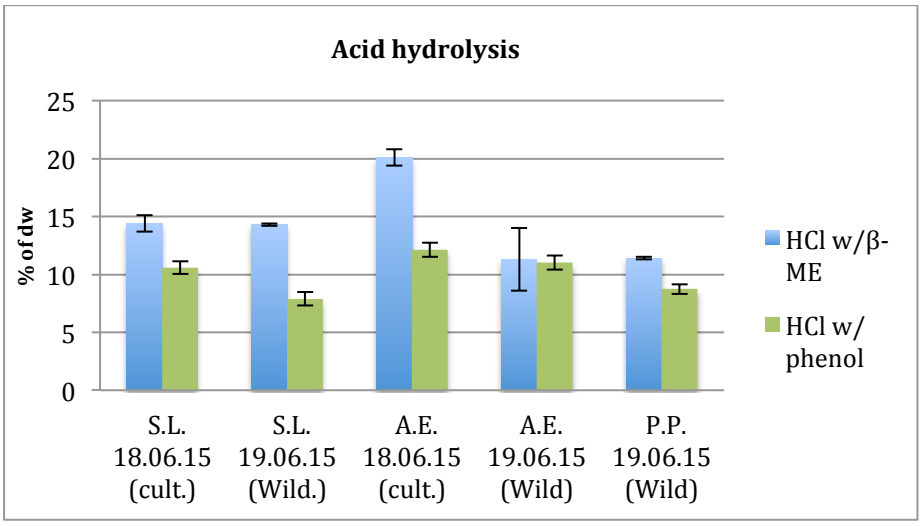


Figure 16: Total amount of amino acids were compared after hydrolysis with HCl containing 0.02% phenol and containing 0.4% β-mercaptoethanol, as additives. The five samples hydrolysed were the cultivated (Cult.) *S.latissima* (S.L) and *A.esculenta* (A.E) samples harvested June 18, and the wild growing sampled (wild) of *S.latissima*, *A.esculenta* and *P.palmata* (P.P) harvested June 19. The uncertainty is given as the standard deviation of two or three parallels, and the results are average value of these.

4.4.3 Total amino acid content (% AA)

The results show that *A.esculenta* generally had the highest content of total protein, and the amount varied from 7.0-15.7% of dry matter (dw). The protein content for *A.esculenta* reached a peak in June in the sample containing a mixture of depths, while the lowest value also could be found in June in the sample from 2 meters depth. For *S.latissima* the protein content varied from 6.4-12.7% (dw). The protein content was at its highest in May at a mixture of different depths, while it was at its lowest in June at 2 meters depth. *P.palmata* had the lowest protein content of all the macroalgae (9.1% of the dry matter) (Fig. 17). All the values can be found in appendix C.3.

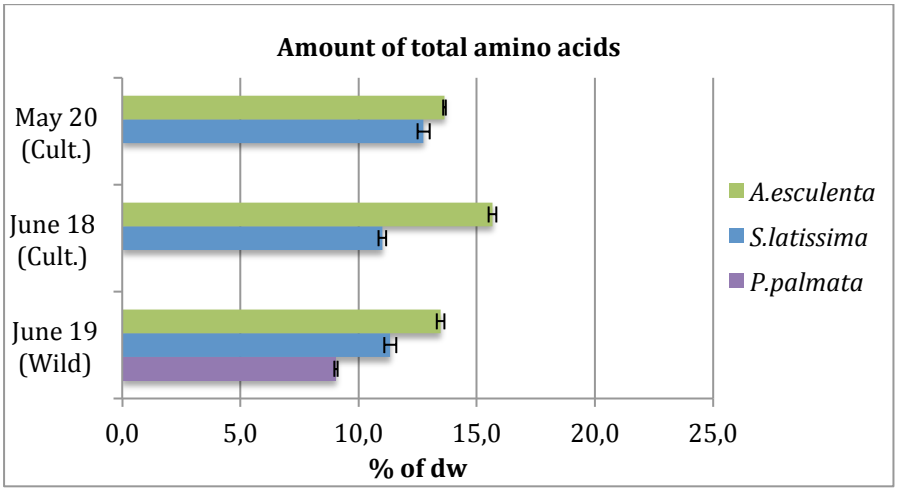


Figure 17: Total amount of amino acids in *S.latissima*, *A.esculenta* and *P.palmata*. The cultivated (cult.) samples of *S.latissima* and *A.esculenta* were harvested May 20 and June 18. The wild samples (wild) of *S.latissima, A.esculenta* and *P.palmata* were harvested June 19. All the samples contain a mixture of samples from 2, 5 and 8 meters depth. The uncertainty is given as the standard deviation of two or three parallels, and the results are average value of these.

Protein content between species

In May, the protein content in *A.esulenta* was significantly higher than *S.latissima* at all depths. In June there was also a significant difference between the species at almost all depths, except from at 2 meters depth (Fig.18-19). When comparing the protein content in the wild growing samples of all three algae, harvested June 19, *A.esculenta* had a significantly higher content than the wild sample of *S.latissima,* while the content in *P.palmata* was significantly lower than in both the brown algae (Fig.17).

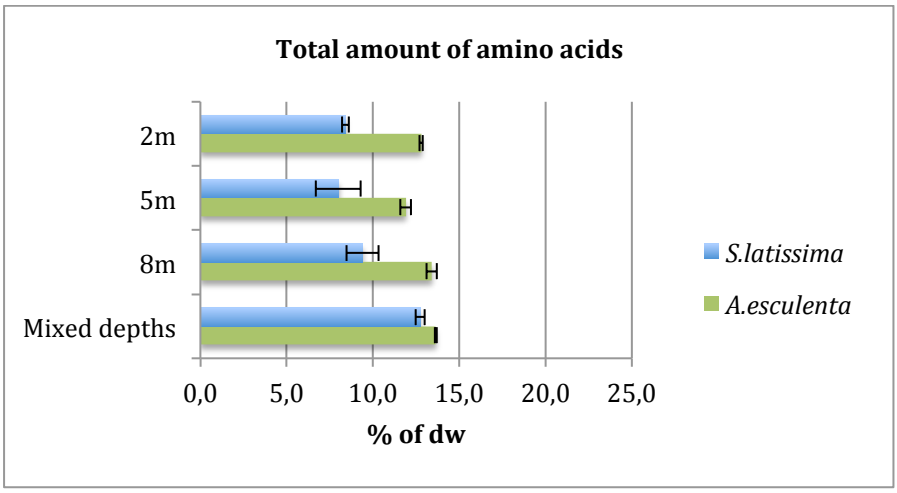


Figure 18: Total amount of amino acids in *S.latissima* and *A.esculenta* in May. "Mixed depths" refers to the algae samples containing samples from a mixture of 2, 5 and 8 meters depths. The uncertainty is given as the standard deviation of two or three parallels, and the results are average value of these

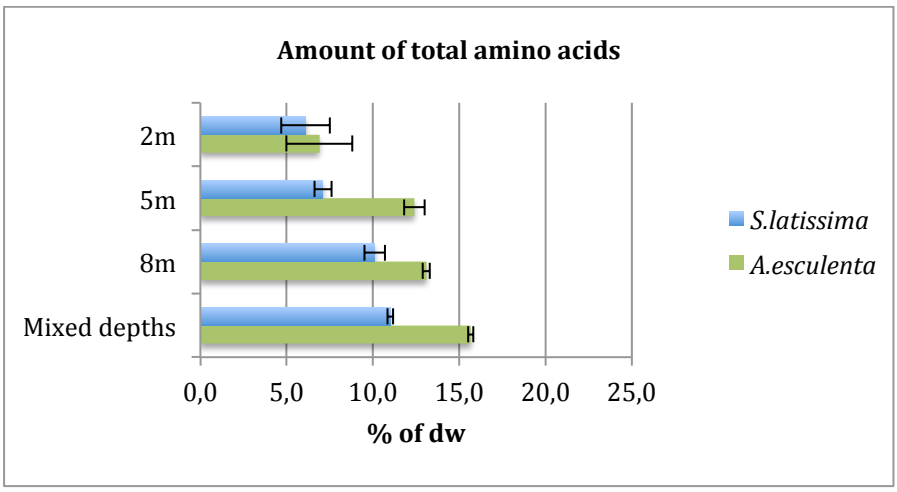
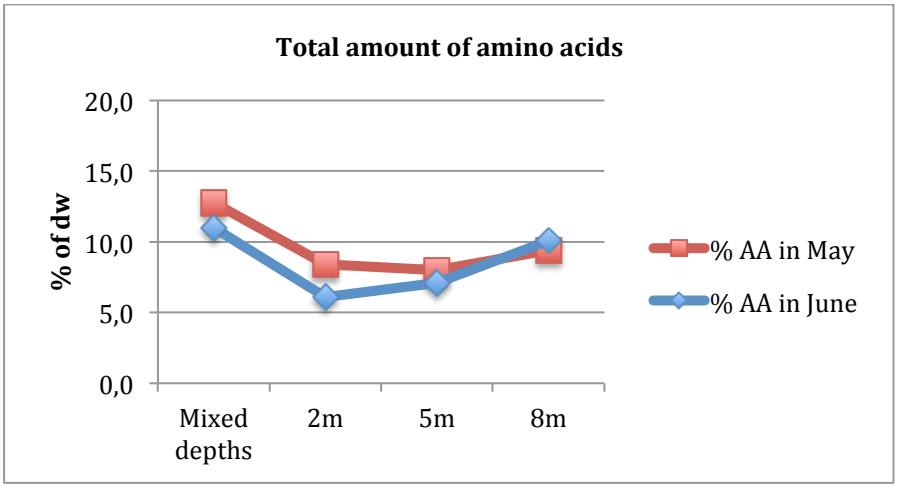


Figure 19: Total amount of amino acids in *S.latissima* and *A.esculenta* in June. "Mixed depths" refers to the algae samples containing samples from a mixture of 2, 5 and 8 meters depths. The uncertainty is given as the standard deviation of two or three parallels, and the results are average value of these.

Protein content at different depths

The total protein content in the *S.latissima* sample harvested May 20, containing a mixture of depths, was significantly higher compared to the *S.latissima* samples from 2, 5 and 8 meters depth. In June the *S.latissima* sample containing a mixture of depths and the sample from 8 meters depth, had a significantly higher protein content than the *S.latissima* samples from 2 and 5 meters depth (the *S.latissima* sample harvested from 8 meter was significantly higher ($p<0.1$) than the sample from 5 meter depth) (Fig. 17). For *A.esculenta* the sample containing a mixture of depths had a protein content that was significantly higher than the protein content in the *A.esculenta* samples from 2 and 5 meters depth, in May. The sample from 5 meters depth was significantly lower than all the other *S.latissima* samples from May. In June the *A.esculenta* sample containing a mixture of depths had a significantly higher protein content than the *A.esculenta* samples from all the other depths. The *A.esculenta* sample from 2 meters depth has a protein content that was significantly lower than the content in all the other *A.esculenta* samples from June (the *A.esculenta* sample harvested from 2 meters was significantly lower ($p<0.1$) than the sample from 5 meter depth) (Fig. 20-21).



Figur 20: Total amount of amino acids in *S.latissima* in May and June, as a function of different depths. "Mixed depths" refers to the algae samples containing samples from a mixture of 2, 5 and 8 meters depths. The uncertainty is given as the standard deviation of two or three parallels, and the results are average value of these.

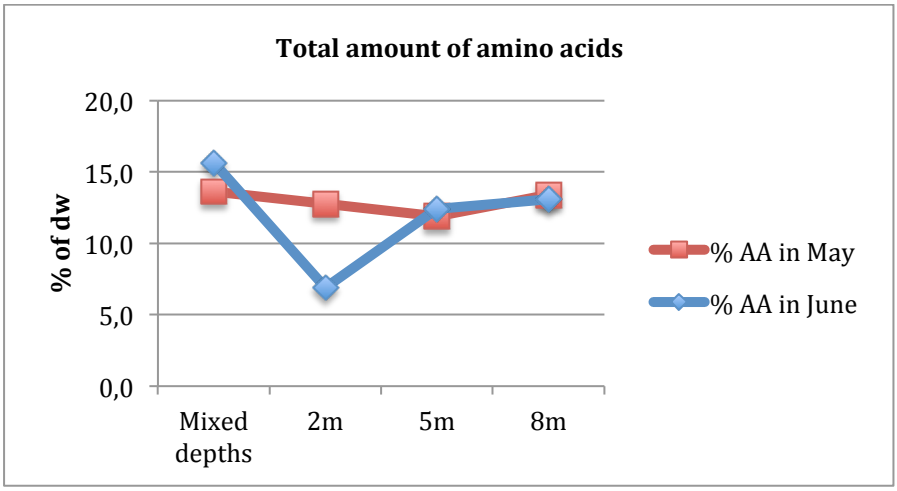


Figure 21: Total amount of amino acids in *A.esculenta* in May and June, as a function of different depths. "Mixed depths" refers to the algae samples containing samples from a mixture of 2, 5 and 8 meters depths. The uncertainty is given as the standard deviation of two or three parallels, and the results are average value of these.

The protein content in the cultivated samples of *S.latissima* and *A.esculenta*, harvested June 18, was compared with the protein content in the wild growing *S.latissima* and *A.esculenta* samples harvested June 01 and June 19, as well as the *P.palmata* sample harvested June 19 (Fig. 22). The cultivated sample of *S.latissima* as well as the wild growing *S.latissima* sample from June 19 had a significantly higher protein content than the wild *S.latissima* sample harvested June 01. For *A.esculenta* the cultivated sample had a significantly higher protein content than both of the wild growing *A.esculenta* samples of. The protein content in *P.palmata* was significantly lower than the protein content in most of the cultivated and wild growing samples of *S.latissima* and *A.esculenta.*

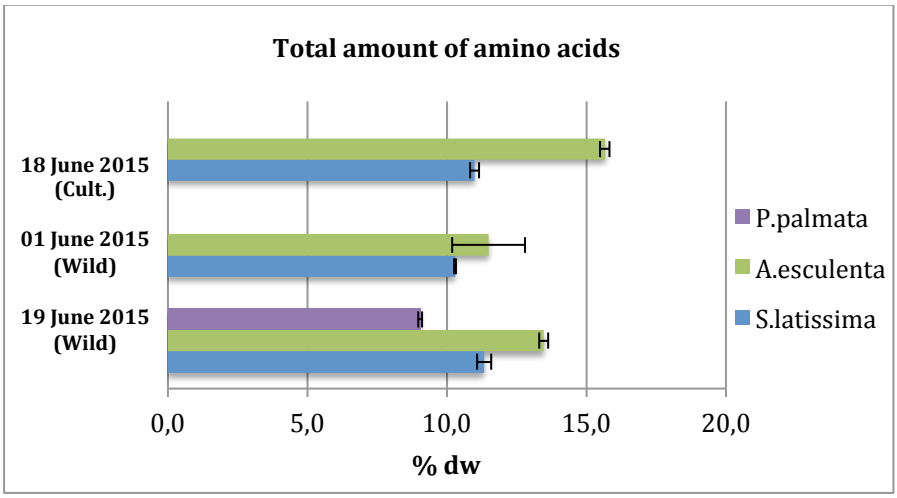


Figure 22: Total amount of amino acids in the cultivated (Cult.) and wild algae samples of *S.latissima*, *A.esculenta and P.palmata*. The uncertainty is given as the standard deviation of two or three parallels, and the results are average value of these.

The *S.latissima* samples cultivated at Austevoll, were harvested April 27 and May 19. The results showed that the protein content was considerably higher in the *S.latissima* sample harvested May 19, containing depths from 2-8 meters, than in the *S.latissima* samples harvested in April. The sample harvested in May had a protein value of 7.3% of the dry matter (dw). The two *S.latissima* samples harvested in April, containing depths from 1-6 meter and 0-9 meter, had a protein content of 6.6% and 6.7% (dw), respectively (Fig. 23).

The sample harvested April 27, containing depths from 1-6 meters, was significantly lower than the sample harvested May 19.

The *S.latissima* samples from Austevoll had a significantly lower protein content than the *S.latissima* samples from Frøya in both May and June. In May the protein content was significantly different in the samples from 2 and 8 meters depth, and in the sample containing a mixture of depths. In June the protein content was significantly different in the samples from 8 meters depth and the sample containing a mixture of depths.

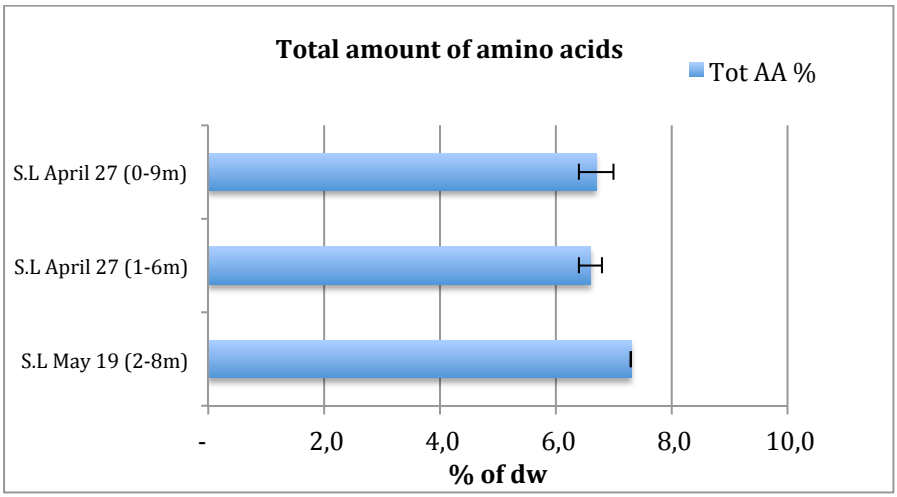


Figure 23: Total amount of amino acids in the *S.latissima* samples cultivated and harvested from Austevoll. The samples were harvested May 19 (sample containing a mixture of depths from 2-8 meters), and April 27 (samples containing a mixture of depths from 0-9 meters and 1-6 meters). The uncertainty is given as the standard deviation of two or three parallels, and the results are average value of these.

4.4.4 Amino acid composition

The amino acid composition in *S.latissima, A.esculenta* and *P.palmata* was dominated by aspartic acid, glutamic acid, alanine and leucine. Some tryptophan and taurine were determined, although in low amounts. The essential amino acids (EAA) were present in all the samples of *S.latissima, A.esculenta* and *P.palmata* in thesis, however leucine, lysine, phenylalanine and valine dominated the composition. In addition, *A.esculenta* contained a high amount of threonine in the sample harvested in May, from 2 meters depth (the values for each amino acid can be found in appendix C.4).

Since glutamic acid, aspartic acid, alanine and leucine dominate the amino acid composition in *S.latissima*, *A.esculenta* and *P.palmata*, figure 24-30 only show the content of these amino acids in both species in May and June, as a function of different depths. The highest value of aspartic acid in *S.latissima* was 1.3%, while it was 1.7% in *A.esculenta.* Glutamic acid peaked at 1.9% in *S.latissima* and at 4.1% in *A.esculenta*. Alanine and leucine reached values of 1.5% and 1.1% in *S.latissima*, and 2.2% and 1.1% in *A.esculenta*, respectively. In *P.palmata* the levels of aspartic acid, glutamic acid, alanine and leucine were 0.9%, 1.4%, 0.8% and 0.7%, respectively. The highest levels of aspartic acid and leucine were found in the *S.latissima* sample from May, containing a mixture of depths. In *A.esculenta* aspartic acid and leucine were highest in the sample from June, containing a mixture of depths (Fig. 24-27). For both *S.latissima* and *A.esculenta* the highest amount of glutamic acid and alanine were found in the wild samples (harvested June 01 and June 19) (Fig. 28 and 29).
The amount of aspartic acid, glutamic acid, alanine and leucine were significantly lower ($p<0.05$ and $p<0.1$) in *S.latissima* compared to *A.esculenta,* in most of the samples from May and June.

Furthermore, the values for each amino acid in May were compared to the values found in June; the cultivated *S.latissima* sample containing a mixture of depths had a significantly higher amount of each amino acid in May, compared to the sample from June. The *A.esculenta* samples harvested from 2 and 8 meters depth had a significantly higher amount of each amino acid in May compared to June, while the *A.esculenta* sample containing a mixture of depths had a significantly higher amount of each amino acid in June (Fig. 22-25).
The wild growing *P.palmata* sample was compared to the wild samples of *S.latissima* and *A.esculenta*. All of these samples were harvested June 19. *A.esculenta* had a significantly

higher amount of glutamic acid and alanine, compared to *P.palmata*. While the content of leucine and aspartic acid was significantly higher in *S.latissima* (Fig. 30).

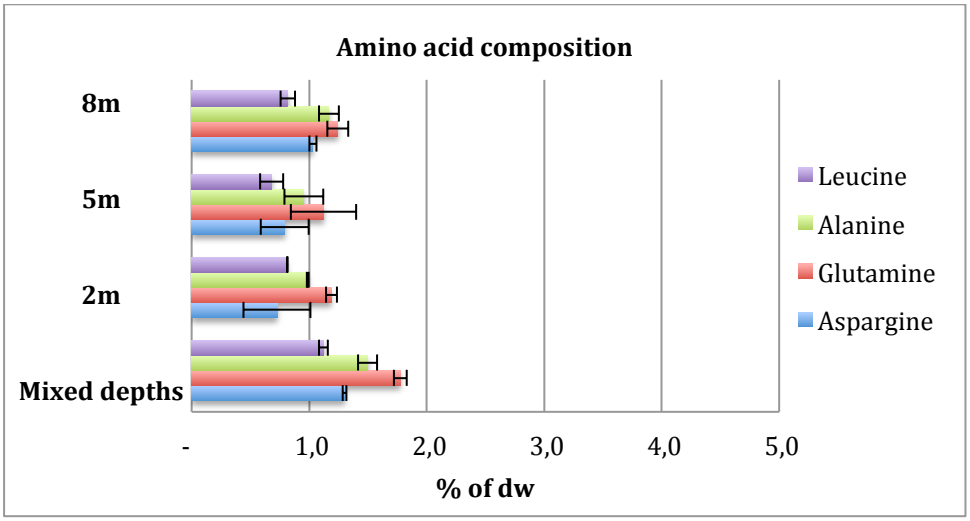


Figure 24: The amount of the different amino acids in *S.latissima* in May, as a function of different depths. Only the four most abundant amino acids are shown here, and these were aspartic acid, glutamic acid, alanin and leucin. The uncertainty is given as the standard deviation of two or three parallels, and the results are average value of these.

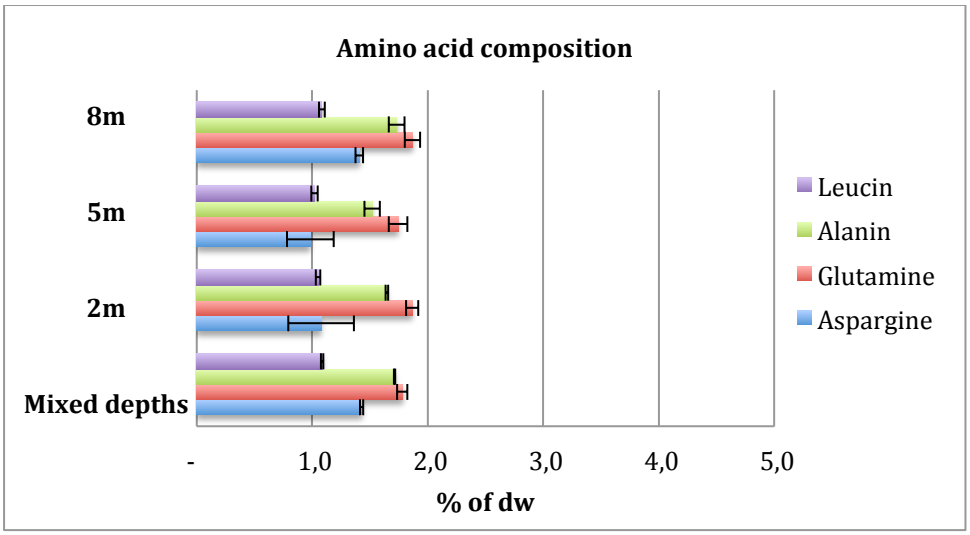


Figure 25: The amount of the different amino acids in *A.esculenta* in May, as a function of different depths. Only the four most abundant amino acids are shown here, and these were aspartic acid, glutamic acid, alanin and leucin. The uncertainty is given as the standard deviation of two or three parallels, and the results are average value of these.

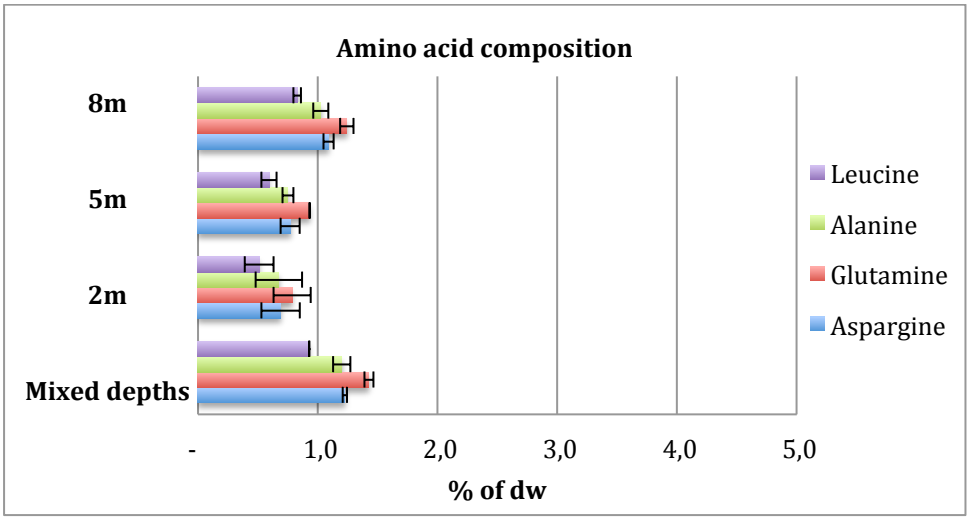
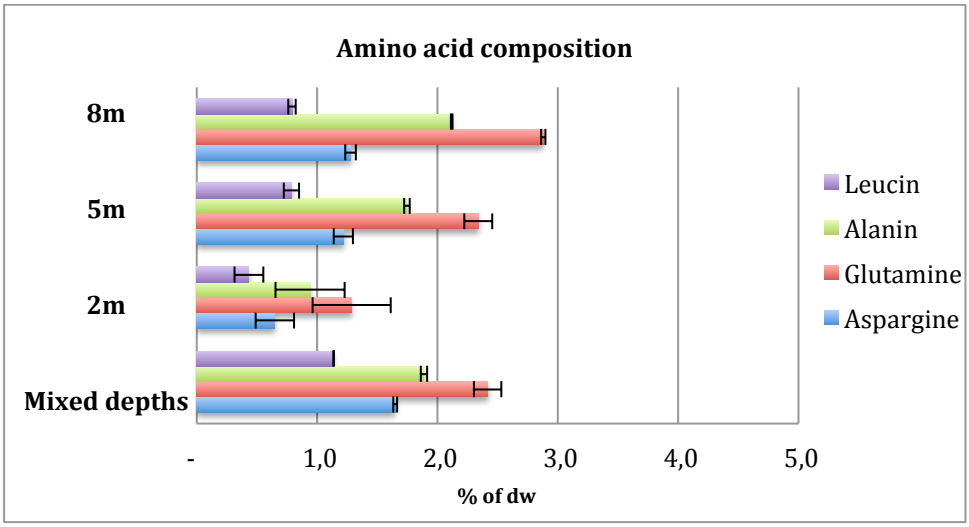
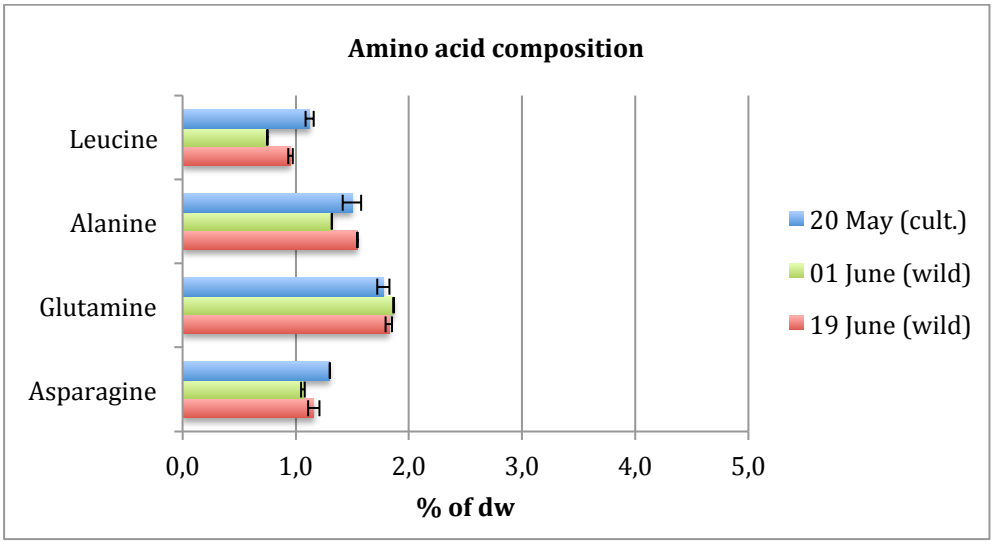


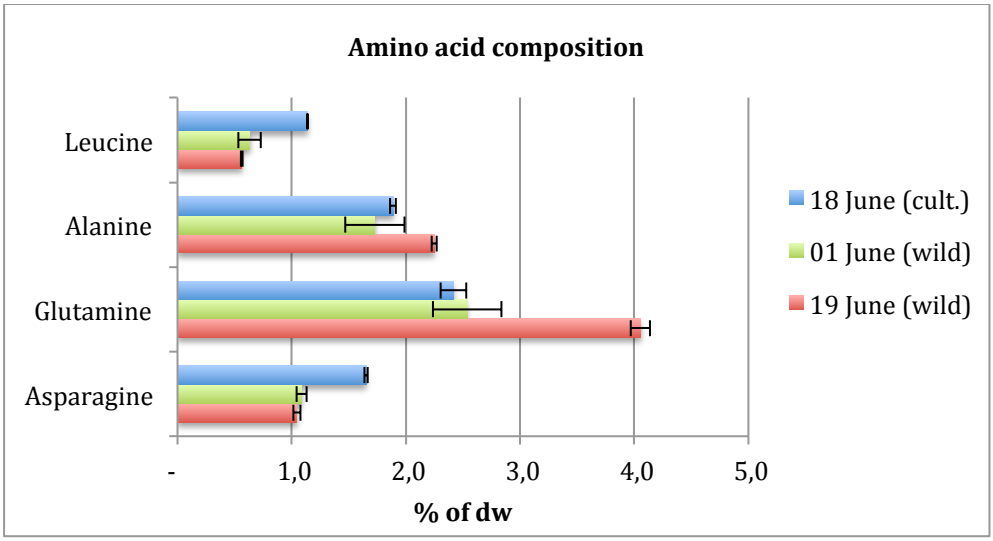
Figure 26: The amount of the different amino acids in *S.latissima* in June, as a function of different depths. Only the four most abundant amino acids are shown here, and these were aspartic acid, glutamic acid, alanin and leucin. The uncertainty is given as the standard deviation of two or three parallels, and the results are average value of these.



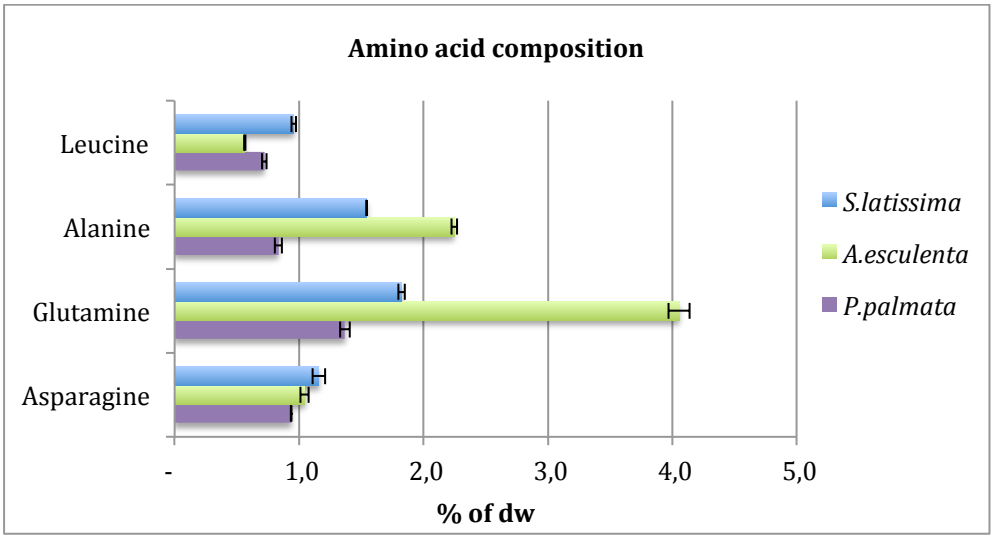
Figur 27: The amount of the different amino acids in *A.esculenta* in June, as a function of different depths. Only the four most abundant amino acids are shown here, and these were aspartic acid, glutamic acid, alanin and leucin. The uncertainty is given as the standard deviation of two or three parallels, and the results are average value of these.



Figur 28: The amino acid composition in cultivated (cult.) and wild growing (wild) *S.latissima*. Of the cultivated samples, the levels of these amino acids were highest in the sample harvested in May, containing a mixture of depths. Therefore this sample was compared to the wild samples. The uncertainty is given as the standard deviation of two or three parallels, and the results are average value of these.



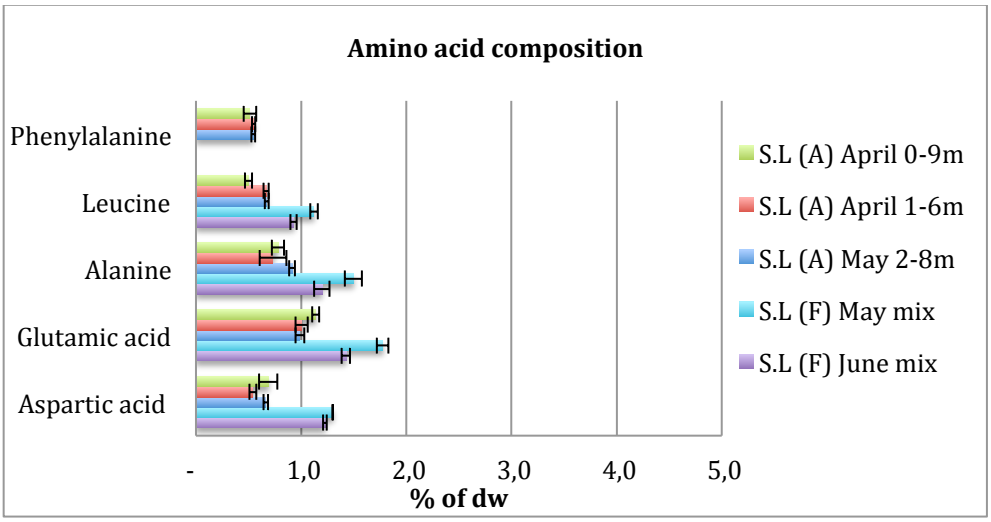
Figur 29: The amino acid composition in cultivated (cult.) and wild growing (wild.) *A.esculenta*. Of the cultivated samples, the levels of these amino acids were highest in the sample harvested in June, containing a mixture of depths. Therefore this sample was compared to the wild samples. The uncertainty is given as the standard deviation of two or three parallels, and the results are average value of these.



Figur 30: The amount of the most abundant amino acids (aspartic acid, glutamic acid, alanin and leucin) in *P.palmata*, *S.latissima* and *A.esculenta.* All the samples were harvested June 19, containing a mixture of depths. The uncertainty is given as the standard deviation of two or three parallels, and the results are average value of these.

The *S.latissima* samples from Austevoll

In accordance with the other algae samples, the amino acids composition in the *S.latissima* samples from Austevoll was dominated by asparagine, glutamine, alanine and leucine. In addition high levels of phenylalanine were found in these samples. The highest level of alanine was found in the sample harvested in May (containing samples from depths between 2-8 meters), while aspartic acid and glutamic acid was highest in sample from April (containing samples from depths between 0-9 meters). The sample harvested in April, containing depths from 0-9 meters, had a significantly lower amount of leucine than the other two Austevoll- samples. The *S.latissima* samples from Frøya generally contained a higher content of aspartic acid, glutamic acid, alanine and leucine, compared to the *S.latissima* samples from Austevoll. Especially the Frøya samples harvested in May and June, containing a mixture of depths, were significantly higher than all of the Austevoll samples (Fig. 31).



Figur 31: The amino acid composition the *S.latissima* samples harvested at Austevoll (S.L (A)), compared to two of the samples harvested at Frøya (S.L (F)). Two of the Austevoll- samples were harvested April 27, and contained depths from 0-9 meter and 1-6 meter. The last Austevoll- sample was harvested May 19, and contained depths from 2-8 meter. The Frøya-samples were harvested in May and June, and contained a mixture of depths from 2, 5 and 8 meters. The uncertainty is given as the standard deviation of two or three parallels, and the results are average value of these.

4.5 Carbohydrate composition

As mentioned in section 2.6, the following carbohydrate compounds were determined by this analysis; mannitol, fucose, galactose, glucose, mannose/xylose (the latter two could not be separated with the method applied), as well as mannuronic acid and guluronic acid. Therefore the total carbohydrate amount was calculated based on the amount of these, except for the uronic acids. Because of the problems with determining mannuronic and guluronic acid, these are not included in the total carbohydrate. However, since the amount of alginate was determined enzymatically for the Austevoll-samples, the amount of uronic acids is included in the total carbohydrate amount for these samples (the total carbohydrate value and the value of each carbohydrate component for all the algae samples, can be found in appendix D.1).

The results are all calculated from the 10 mg concentration area of the standard curves. Only two of the samples were analysed with three parallels; the wild *A.esculenta* sample harvested June 19, and the Austevoll- sample harvested April 27, from depths of 0-9 meter. Therefore the significant difference has not been calculated for these samples.

Comparing the total carbohydrate content in all three species

The highest amount of total carbohydrate was found in *P.palmata*, with a value of 49.9% of the dry matter (dw). For *S.latissima* the value of total carbohydrate varied from 8.1-16.3% (dw), and the content reached a peak in May. The amount varied from 12.3-23.9% (dw) in *A.esculenta,* and the highest value was found in June (Fig.32). *S.latissima* generally had a higher carbohydrate content than *A.esculenta* in May (except from at 8 meters depth), while *A.esculenta* had a higher amount of carbohydrates in June (Fig. 33-34).

When comparing the cultivated sample harvested June 18, containing a mixture of depths from 2, 5 and 8 meters, with the wild growing samples harvested June 01 and June 19, it was observed that the carbohydrate content was higher in the wild samples (Fig. 35).

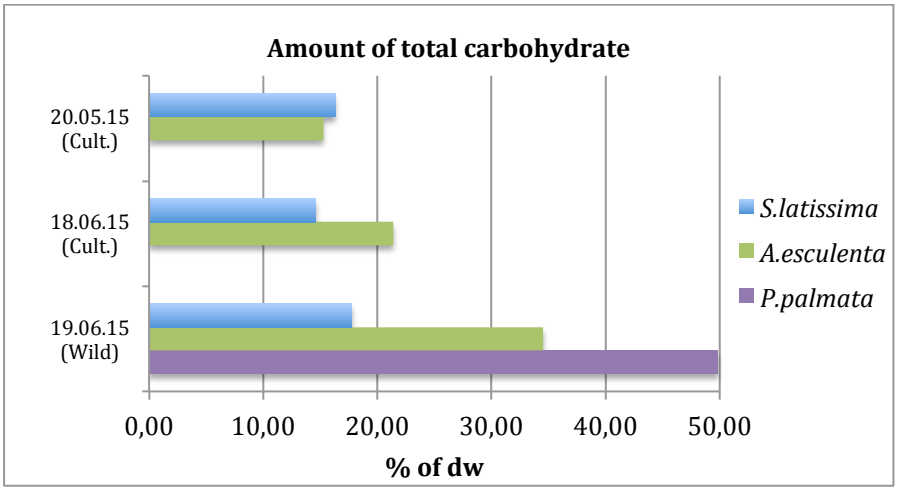


Figure 32: Amount of total carbohydrate in cultivated (Cult.) *S.latissima* and *A.esculenta*, harvested May 20 and June 18, and wild growing (Wild) *S.latissima, A.esculenta* and *P.palmata,* harvested June 19. All the samples contain a mixture of depths. The carbohydrate content is given in % of dry matter (dw).

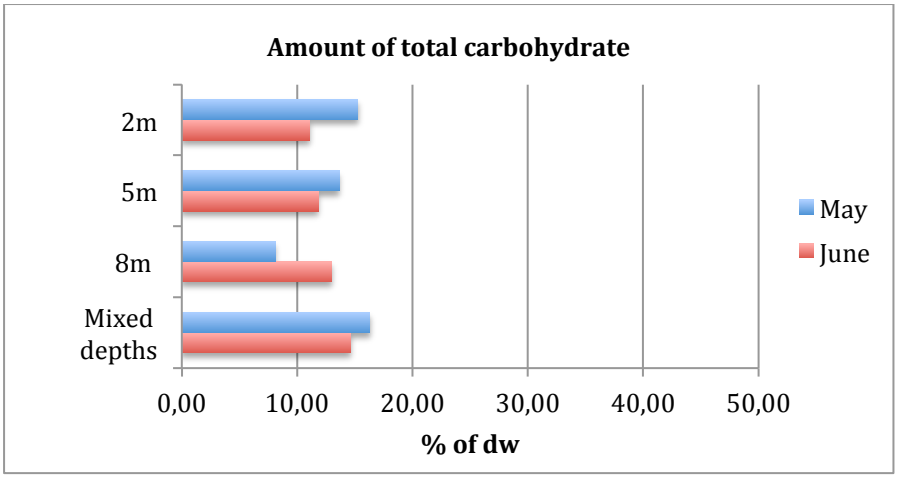


Figure 33: The total carbohydrate content in *S.latissima* as a function of different depths, in May and June. "Mixed depths" refers to the algae samples containing samples from a mixture of 2, 5 and 8 meter depths. The carbohydrate content is given in % of dry matter (dw).

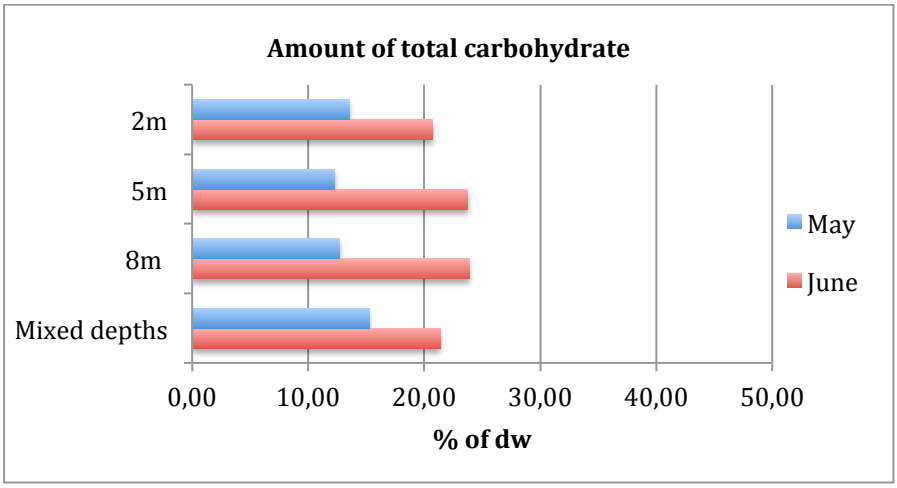


Figure 34: The total carbohydrate content in *A.esculenta* as a function of different depths, in May and June. "Mixed depths" refers to the algae samples containing samples from a mixture of 2, 5 and 8 meter depths. The carbohydrate content is given in % of dry matter (dw).

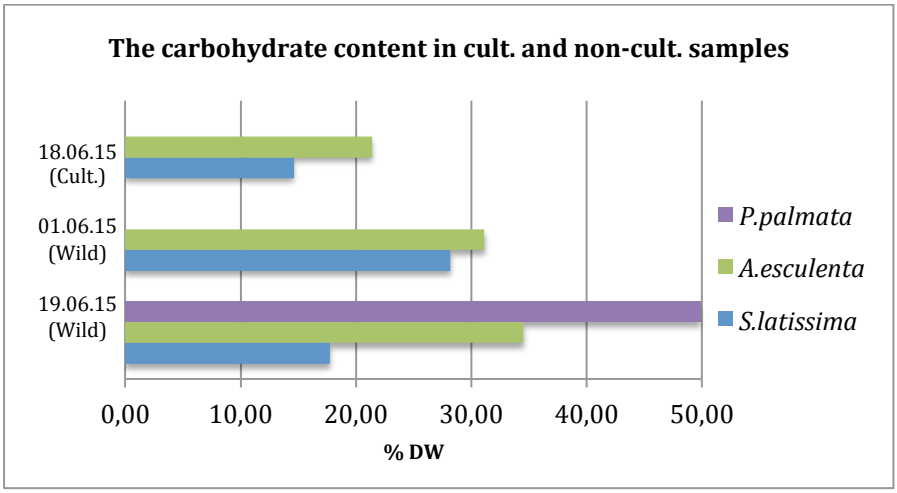


Figure 35: The total carbohydrate content in cultivated (Cult.) *S.latissima* and *A.esculenta*, as well as wild growing (Wild) *S.latissima, A.esculenta* and *P.palmata*. All the samples contain a mixture of samples from 2, 5 and 8 meters depth. The carbohydrate content is given in % of dry matter (dw).

Total carbohydrate content at different depths

For *S.latissima* the highest content of total carbohydrate was found in the algae samples containing a mixture of depths, both in May and June. The *A.esculenta* samples from May also had the highest amount of total carbohydrate in the sample containing a mixture of depths, while in June the highest carbohydrate amount was found in the samples from 5 and 8 meters depth (Fig. 36-37).

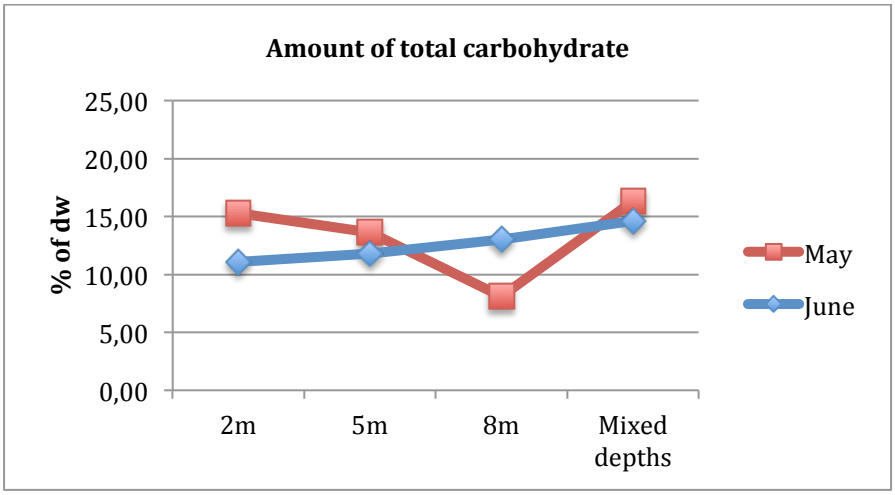


Figure 36: Amount of total carbohydrate in *S.latissima* in May and June, as a function of different depths. All the samples contain a mixture of samples from 2, 5 and 8 meters depth. The carbohydrate content is given in % of dry matter (dw).

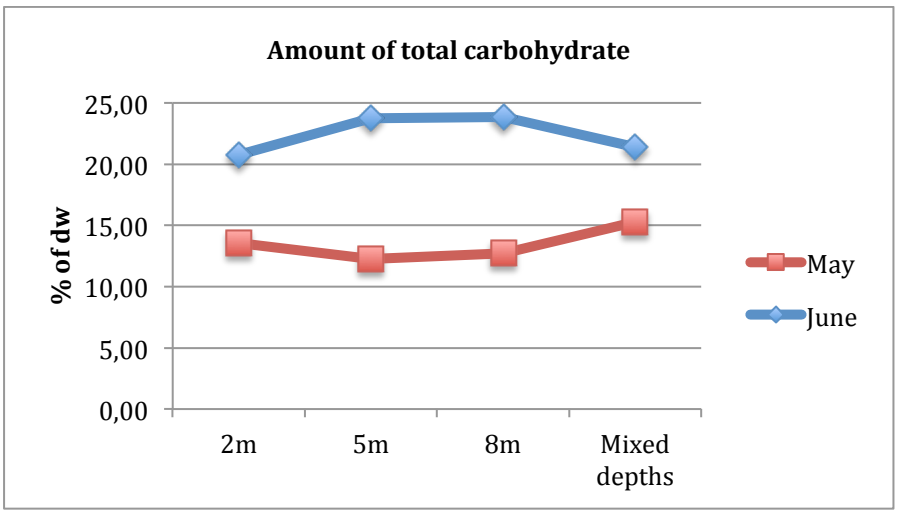


Figure 37: Amount of total carbohydrate in *A.esculenta* in May and June, as a function of different depths. All the samples contain a mixture of samples from 2, 5 and 8 meters depth. The carbohydrate content is given in % of dry matter (dw).

Total carbohydrate content in the samples from Austevoll

Among the *S.latissima* samples harvested at Austevoll in April and May, the *S.latissima* sample harvested in April, containing depths from 0-9 meter, had the highest amount of total carbohydrate with a value of 24.0% (dw). In the other sample harvested in April, containing depths from 1-6 meter, the total carbohydrate was 20.8% (dw). The *S.latissima* sample harvested in May, containing depths from 2-8 m, had a value of 18.7% (dw) (Fig. 38). (These samples did not include the alginate value that was determined enzymatically).

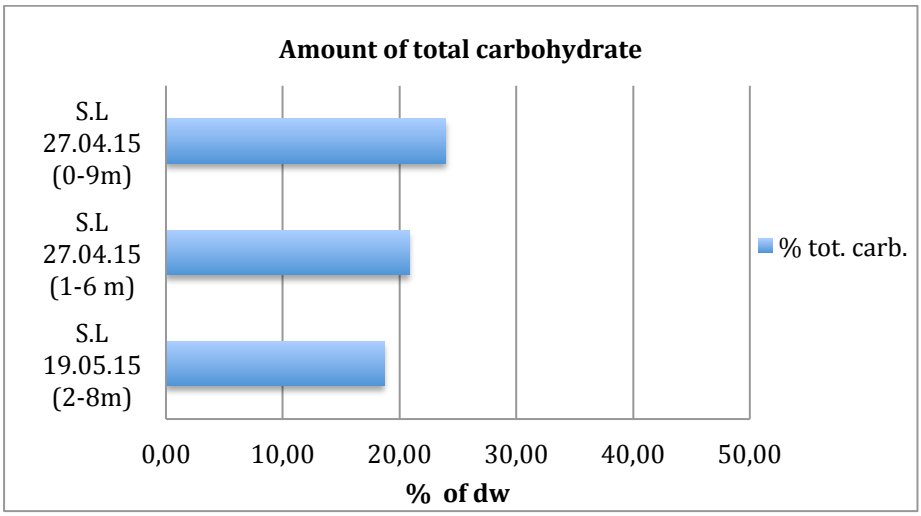


Figure 38: The total carbohydrate content (% tot. carb.) in the *S.latissima* (S.L) samples harvested from Austevoll in April and May. The samples from April contain depths from 0-9 meters and 1-6 meters. The sample from May contain depths from 2-8 meters. The carbohydrate content is given in % of dry matter (dw).

4.5.1 The carbohydrate composition

In both May and June, *S.latissima* had a high amount of the sugar alcohol mannitol and mannuronic acid (ManA). In May there was also a high level of glucose (Fig. 39-40). High amounts of mannitol, glucose and mannuronic acid could also be found in *A.esculenta*, in May and June (Fig. 41-42). In *S.latissima* the amounts of mannitol, glucose and mannuronic acid were slightly higher in May than in June. The mannitol content in *S.latissima* varied from 5.21-10.54% (dw) in May, and from 8.13-10.32% (dw) in June. The amount of glucose in *S.latissima* reached a peak in May, with 2.88% (dw). In *A.esculenta* the content of these carbohydrates were slightly higher in June. The amount of mannitol in *A.esculenta* varied from 7.8-9.6% (dw) in May, and from 8.06-10.00% (dw) in June. In June, the amount of glucose in *A.esculenta* reached a peak with 13.41% (dw). In both *S.latissima* and *A.esculenta* the highest levels of the carbohydrate components were generally found in the samples containing a mixture of depths.

P.palmata had especially high amounts of mannose/xylose (26.81%) and galactose (19.24%), as well as relatively high amounts of glucose (3.47%) (Fig. 43).

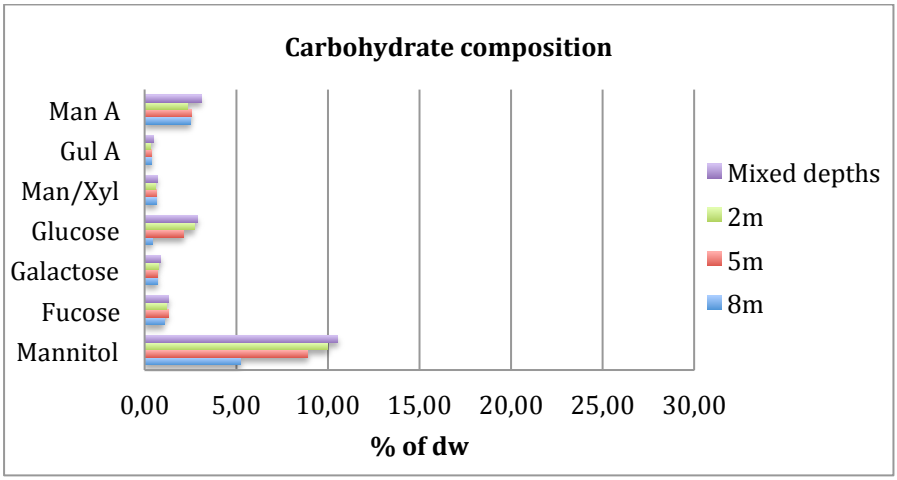


Figure 39: The carbohydrate composition in *S.latissima* in May, as a function of different depths. Mannose and xylose couldn not be separated, and these are given toghether. Despite the problems with determining mannuronic acid (ManA) and guluronic acid (GulA), these are both included. Mannose and Xylose (Man/Xyl) could not be separated, and are therefore given together.

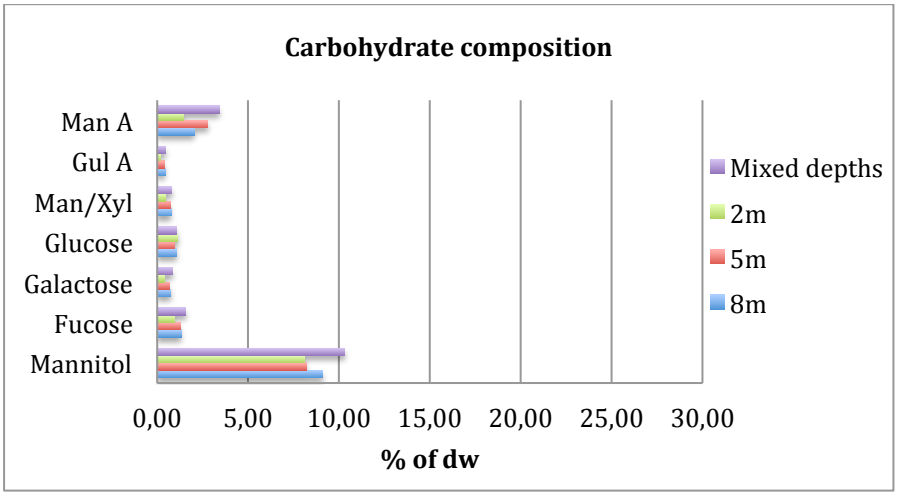


Figure 40: The carbohydrate composition in *S.latissima* in June, as a function of different depths. Mannose and xylose couldn not be separated, and these are given toghether. Despite the problems with determining mannuronic acid (ManA) and guluronic acid (GulA), these are both included. Mannose and Xylose (Man/Xyl) could not be separated, and are therefore given together.

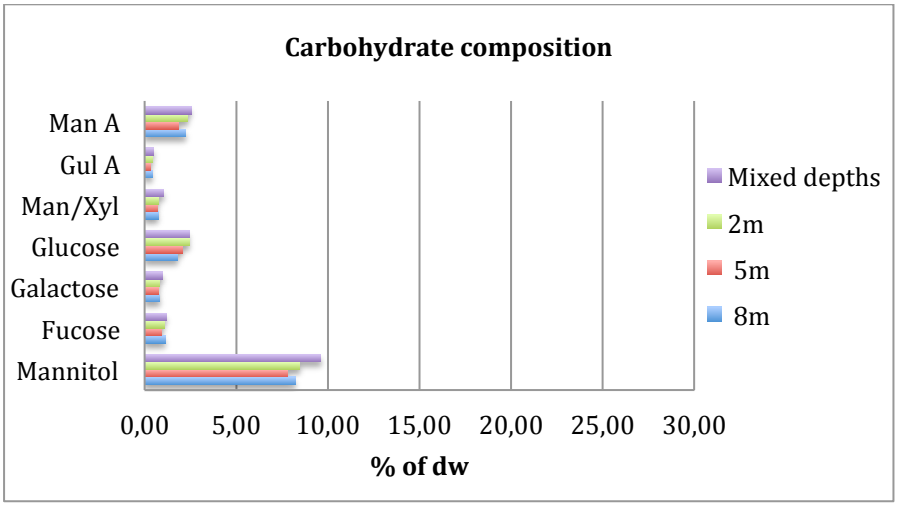


Figure 41: The carbohydrate composition in *A.esculenta* in May, as a function of different depths. Mannose and xylose couldn not be separated, and these are given toghether. Despite the problems with determining mannuronic acid (ManA) and guluronic acid (GulA), these are both included. Mannose and Xylose (Man/Xyl) could not be separated, and are therefore given together.

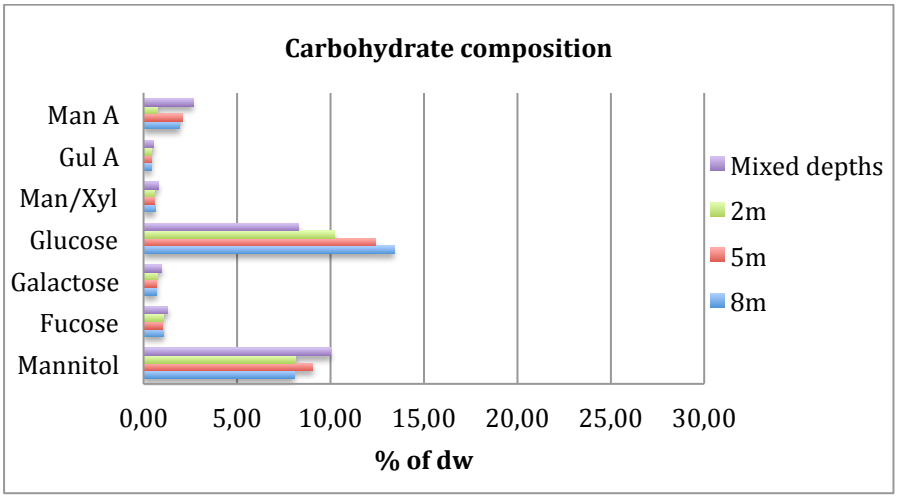


Figure 42: The carbohydrate composition in *A.esculenta* in June, as a function of different depths. Mannose and xylose couldn not be separated, and these are given toghether. Despite the problems with determining mannuronic acid (ManA) and guluronic acid (GulA), these are both included. Mannose and Xylose (Man/Xyl) could not be separated, and are therefore given together.

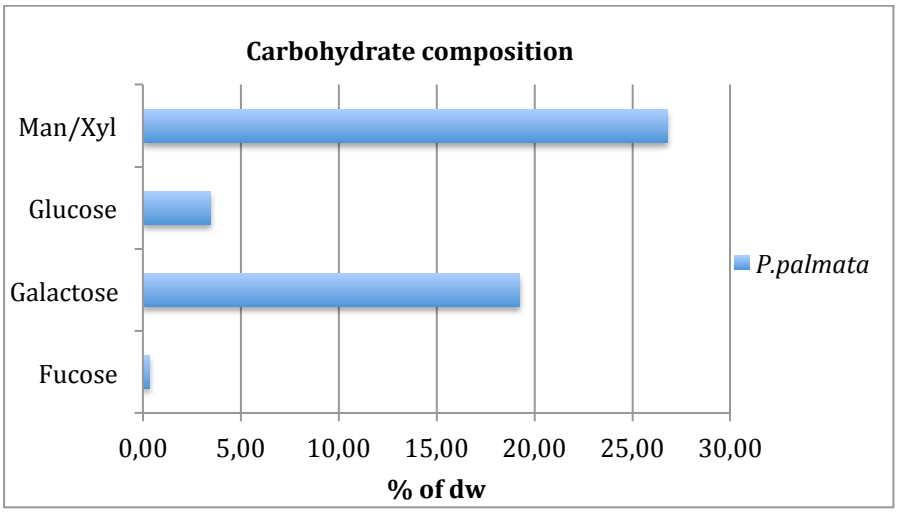


Figure 43: The carbohydrate composition in *P.palmata*. *P.palmata* does not contain alginate, and therefore the uronic acids have not been included. In addition, no content of mannitol was found in the red algae. Mannose and Xylose (Man/Xyl) could not be separated, and are therefore given together.

As mentioned in section 1.5.1, the wild growing samples of both *S.latissima* and *A.esculenta* contained higher amounts of total carbohydrate than the cultivated samples. When comparing the wild samples harvested June 01 and 19, with the cultivated sample harvested June 18, it was observed that the amount of glucose and mannitol were particularly high in the wild samples (Fig. 44-45).

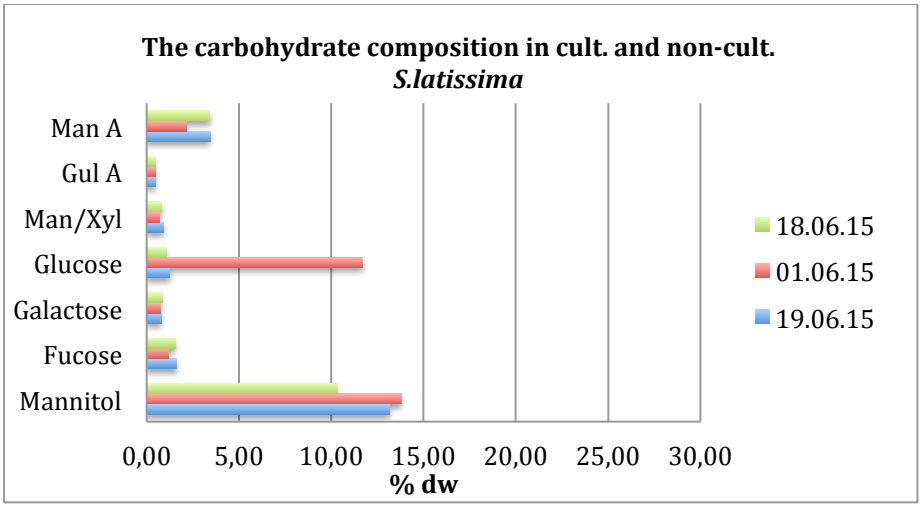


Figure 44: The carbohydrate composition in cultivated (cult.) and wild growing (Wild) *S.latissima*. Mannose and xylose couldn not be separated, and these are given toghether. Despite the problems with determining mannuronic acid (ManA) and guluronic acid (GulA), these are both included. Mannose and Xylose (Man/Xyl) could not be separated, and are therefore given together.

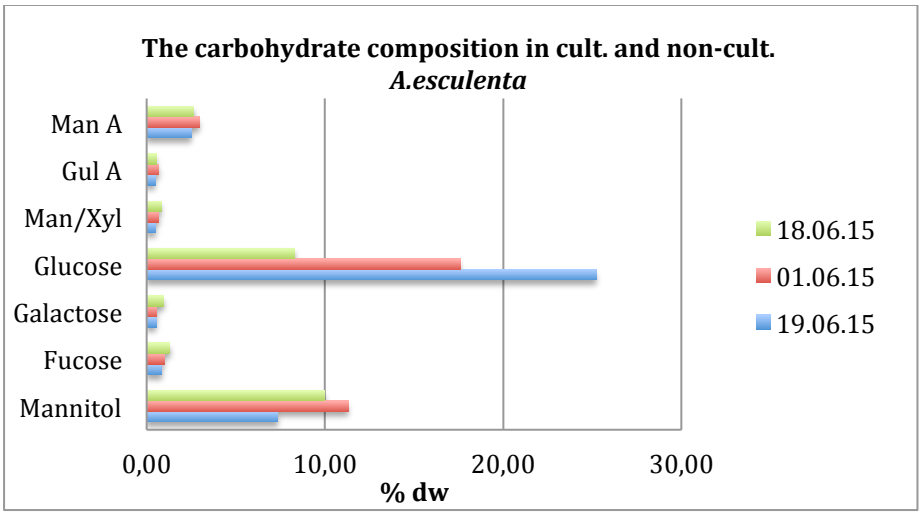


Figure 45: The carbohydrate composition in cultivated (cult.) and non-cultivated (non-cult.) *A.esculenta*. Mannose and xylose couldn not be separated, and these are given toghether. Despite the problems with determining mannuronic acid (ManA) and guluronic acid (GulA), these are both included. Mannose and Xylose (Man/Xyl) could not be separated, and are therefore given together.

The carbohydrate composition in the *S.latissima* samples harvested at Austevoll, were relatively similar to the composition in the *S.latissima* samples harvested at Frøya. The amount of mannitol was higher in the Austevoll-samples, while the amount of mannuronic acid was lower than in the Frøya-samples. The amount of mannitol was higher in the *S.latissima* samples harvested in April, than in the sample harvested in May, and reached a peak in the sample containing depths from 0-9 meter (Fig. 46).

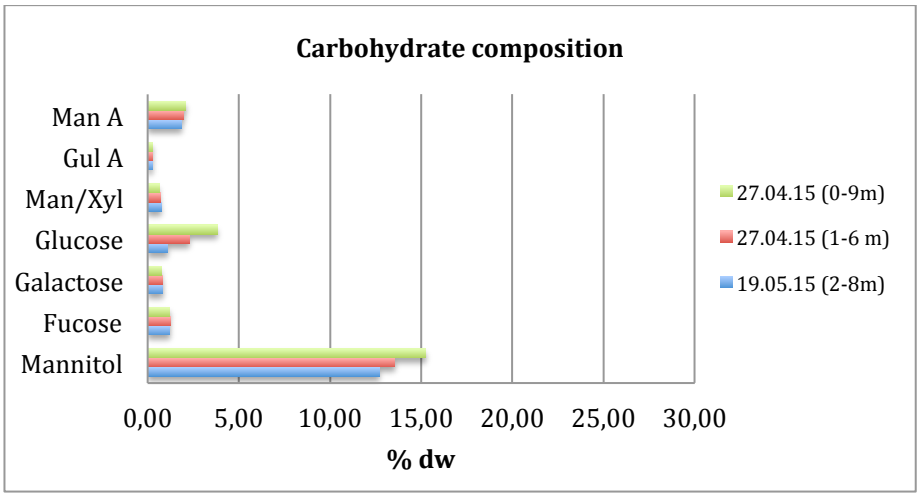


Figure 46: The carbohydrate composition in the samples harvested at Austevoll April 27, containing depths from 0-9 meter and 1-6 meter, and May 19, containing depths from 2-8 meter. Mannose and xylose couldn not be separated, and these are given toghether. Despite the problems with determining mannuronic acid (ManA) and guluronic acid (GulA), these are both included. Mannose and Xylose (Man/Xyl) could not be separated, and are therefore given together.

4.6 Alginate determination

As mentioned in section 2.7, the alginate determination was only performed on the *Saccharina latissima* samples cultivated and harvested at Austevoll. The alginate content was significantly higher than the content found for the samples analysed by HPAE-PAD. For the samples harvested in April at depths from 0-9 meters and 1-6 meters depth, the alginate content was 22.1% and 27.0% (dw), respectively. The sample harvested in May at depths from 2-8 meters had an alginate content of 24.3% (dw).

The Austevoll-samples analysed by HPAE-PAD, had an alginate content of 2.4% for the sample harvested at depths from 0-9 m, 2.1% for the sample harvested at depths from 1-6 m, and 2.3% for the sample harvested at depths from 2-8 m in May.

Table 3: The amount of alginate in each of the *S.latissima* samples from Austevoll, determined by enzymatic analysis and HPAE-PAD analysis.

Sample	Enzymatic	HPAE-PAD
27.04.15 (0-9m)	22.1 (±1.50)	2.4
27.04.15 (1-6m)	27.0 (±0.65)	2.1
19.05.15 (2-8m)	24.3 (±2.10)	2.3

4.7 Total mass balance for each sample

The tables show the total value for each of the chemical components analysed in this thesis. The uronic acids are not included in the total carbohydrate content for each sample, with the exception of the Austevoll-samples. The Austevoll-samples contain the alginate values from the alginate determination performed by Stine Wiborg Dahle. All the values are % of the dry matter (dw). Since the *S.latissima* samples from Austevoll had alginate values included in the total mass content, these samples reached a mass content of around 100. Since the alginate content was not included in the other algae samples, the total mass value for these samples were lower.

Table 4: The total mass content for the cultivated *Saccharina latissima* samples harvested in May and June at Frøya, and the wild *S.latissima* samples harvested in June at Vanvikan.

Sample	Ash (%)	Lipids (%)	Proteins (% AA)	Carbohydrates (%)	Alginate (%)	Totale mass (% dw)
20.05.15	36,09	1,96	8,90	16,31	-	63,26
2m	38,04	5,89	8,60	15,25	-	67,79
5m	42,93	5,55	8,20	13,64	-	70,32
8m	47,00	5,28	9,60	8,10	-	69,98
18.06.15	37,90	4,41	6,60	14,60	-	63,51
2m	43,49	1,36	6,40	11,05	-	62,30
5m	44,21	3,14	7,30	11,81	-	66,46
8m	44,25	3,17	10,20	13,00	-	70,62
01.06.15	16,77	0,9	6,90	28,16	-	52,73
19.06.15	32,21	3,24	7,90	17,75	-	61,10

Table 5: The total mass content for the cultivated *Alaria esculenta* samples harvested in May and June at Frøya, and the wild *A.esculenta* samples harvested in June at Vanvikan.

Sample	Ash (%)	Lipids (%)	Proteins (% AA)	Carbohydrates (%)	Alginate (%)	Totale mass (% dw)
20.05.15	24,38	3,18	13,10	15,26	-	55,92
2m	30,91	8,92	13,10	13,57	-	66,50
5m	26,49	7,03	12,20	12,26	-	57,98
8m	28,50	5,98	13,70	12,70	-	60,88
18.06.15	17,27	4,6	10,30	21,39	-	53,56
2m	18,70	3,13	7,00	20,75	-	49,57
5m	27,38	3,52	12,60	23,76	-	67,25
8m	20,87	4,02	13,40	23,87	-	62,17

01.06.15	10,69	1,04	11,50	31,13	-	54,36
19.06.15	12,92	2,4	7,90	34,49	-	57,72

Table 6: The total mass content in *Palmaria palmata,* harvested in June at Vanvikan.

Sample	Ash (%)	Lipids (%)	Proteins (% AA)	Carbohydrates (%)	Alginate (%)	Totale mass (% dw)
19.06.15	17,2	1,78	7,40	49,86	-	76,24

Table 7: The total mass content in the *Saccharina latissima* samples harvested at Austevoll in April and May.

Sample	Ash (%)	Lipids (%)	Proteins (% AA)	Carbohydrates (%)	Alginate (%)	Totale mass (% dw)
27.04.15 0-9m	42,23	2,07	6,80	21,62	22,13	94,85
27.04.15 1-6m	44,68	2,79	6,70	18,56	27,03	99,76
19.05.15 2-8m	49,51	2,82	7,40	16,54	24,25	100,52

5 Discussion

5.1 Factors that might influence the chemical composition

The chemical composition in macroalgae varies with season, and it is therefore important that the seaweeds are harvested at a time when the chemical components are at their highest.
The samples from Frøya were harvested from depths of 2, 5 and 8 meter, and in addition some of the Frøya-samples consisted of samples harvested from a mixture of these depths. These samples containing a mixture of depths should represent the average value of all three depths. However, most of the results from the chemical analysis of the macroalgae showed that the samples containing a mixture of depths were higher or lower than the samples from depths of 2, 5 and 8 meters depth. This has made it difficult to compare the samples containing a mixture of depths with the samples from depths of 2, 5 or 8 meters.

There could be several explanations for this; first of all, only the samples from a mixture of depths were washed after harvesting. The washing would have removed the salt, and possibly other minerals as well. Secondly, the macroalgae may not have been sufficiently mixed after being freeze-dried and milled (the samples were not homogeneous, since they included both the stem and leafs of the macroalgae samples). Thirdly, some of the samples containing a mixture of depths were freeze dried separately from the other samples (these were used for the determination of lipids, proteins and carbohydrates), as well as being incubated and ash dried separately from the other samples (for determination of dry matter and ash). Lastly, the dried and milled samples could have been subjected to some moisture from the environment, before being analysed. However, the reason why the samples containing a mixture of depths didn't represent the average value of all three depths was most likely due to the washing.
.

5.2 Dry matter and ash content

The dry matter in macroalgae should according to literature be 10-30% of the wet weight (ww) (13). In this thesis the dry matter was within the reported range for all of the algae. The ash content is high in brown algae and has been found to vary from 22-44% in *S.latissima*, from 14-32% (DW) in *A.esculenta*, and from 15-30% in *P.palmata* (25, 26). The ash content found for *S.latissima* and *A.esculenta* in this thesis were higher than the reported values. The algae samples analysed in this thesis contained both the stem and leaf from the algae plant, and whether this has been measure in the macroalgae from the literature as well, were not reported. Therefore this could be the reason as to why the values in this thesis were higher than the ones reported in the literature. The highest ash content in macroalgae has been reported to be in early spring (15), and in this thesis the ash content for both *S.latissima* and *A.esculenta* were found to be higher in May than in June.

Both *S.latissima* and *A.esculenta* had highest dry matter in the samples from 2 meters depth, and so this might be the most ideal harvesting depth. Furthermore, the dry matter content was higher in the wild growing samples (from Vanvikan), than in the cultivated samples (from Frøya), and especially the wild samples harvested June 01 had a high content of dry matter.
The ash content in the *S.latissima* sample from Frøya seemed to increase with increasing depth, and was highest in the sample harvested from 8 meters depth. In the *S.latissima* sample from Austevoll, the highest amount of ash was found in the sample harvested in May, containing a mixture of depths from 2-8 meters. This is in accordance with the results for the *S.latissima* samples from Frøya. In *A.esculenta* the ash content was highest in the samples from 2 and 8 meters depth. Consequently, samples harvested from 8 meters depth seems to contain a high ash content for both *S.latissima* and *A.esculenta*.

The lowest amount of dry matter and ash was found in the *S.latissima* and *A.esculenta* samples containing a mixture of depths. The reason for this is most likely the fact that these samples were washed after harvesting. This would remove some of the chemical components, such as salt and other minerals. In addition, the dry matter and ash content was determined by incubation and by being dried in an ash oven, while the rest of the samples were freeze-dried and then analysed for lipids, proteins and carbohydrates. The dry matter and ash content should therefore probably have been measured in the freeze-dried samples as well, in order to get more correct values.

Some of the algae samples had dry matter values with a particularly high standard deviation between the parallels. These samples were analysed again, but the new results also showed a high standard deviation between the parallels. The explanation for this could be that the samples were inhomogeneous, since both stem and leaf was included in the analysed samples (as mentioned in section 2.1). Therefore the parallels might differ from one another and give an unexpectedly high standard deviation.

5.3 Total lipid content

According to the studies done by Marinho et al. (16) with *S.latissima* in Denmark, the lipid content was found to be higher in the winter months while it declined in the summer. Marinho et al. also followed the Bligh & Dyer method for lipid extraction. They found the lipid content in *S.latissima* to vary from 0.6-3.4% (dw), with the highest value in November and the lowest value in July. Indergaard (13) found the amount of lipids to range from 1-2% in *A.esculenta* and from 0.3-3.8% in *P.palmata* (the method applied for lipid determination was not included in the literature by Indergaard). Both *S.latissima* and *A.esculenta* contained a higher amount of lipids than reported by Marinho et al. while the lipid content in *P.palmata* was within the reported range. However, the lipid content found in *S.latissima A.esculenta* and *P.palmata* in this thesis was low. Mæhre et al. (2) found significantly higher lipid values in *A.esculenta* and *P.palmata*. In their article, the lipid content in *A.esculenta* was around 53.9 and 37.8 % of the dry weigth (g/kg), respectively. However, Mæhre et al. used petroleum to extract the lipids, and determined the lipid content gravimetrically. Therefore it is difficult to compare their results with the results found in this thesis.

The lipid content in the algae samples did not vary that much between the different depths. For the *S.latissima* sample from Frøya the lipid content was highest in May, at 8 meters depth. In the *S.latissima* samples from Austevoll the highest lipid content was also found in the sample harvested in May, containing depths from 2-8 meters. This coincides with the results for the *S.latissima* samples from Frøya. In *A.esculenta* the highest amount of lipids was not found in May, but in June

5.4 Protein content

According to Fleurence (6) brown algae are found to have a protein content varying from 3-15% (dw). The protein content in red algae is significantly higher than the content found in brown algae, and in *P.palmata* the content can vary between 9-25%, and be as high as 35%. However, the *P.palmata* studied by Fleurence were harvested from the French Atlantic coast, and so the chemical composition in those algae may vary from the composition in the macroalgae analysed in this thesis. Furthermore, the article by Fleurence does not state which method was applied for the determination of the protein content. Schiener et al. (4) found the protein content in *S.latissima* to be 7.6% in May, and 6.0% in July. In *A.esculenta* the protein content was 11.6% in May and 9.4% in July. Schiener et al. used a different method when determining the protein content in *S.latissima* and *A.esculenta,* and this may influence the results. Indergaard & Jensen (25) reported that the protein content was 9-18% (dw) in *A.esculenta*, and 8-25% (dw) in *P.palmata*. They measured the protein content by converting nitrogen to protein using a conversion factor of 6.25. This method was also applied in this thesis (C/N method). In addition the protein content was also determined by ion exchange chromatography (HPLC), in this thesis.

Protein results from the C/N method

The protein results obtained by C/N method were generally lower than the results achieved by high-performance liquid chromatography (HPLC). One reason for this could be that the conversion factor (4.8) used was too low. Usually a factor of 6.25 is used to convert nitrogen to protein. However, as mentioned in section 2.4, a new conversion factor was calculated in this thesis. This new conversion factor was calculated based on the ratio between the protein values found by the C/N- method (by using a factor of 6.25) and by the HPLC- method. The results from the HPLC-analysis showed low values of tryptophan (tryptophan was partially destroyed during the hydrolysis), and asparagine and glutamine were determined as aspartic and glutamic acid (and these contain less nitrogen than asparagine and glutamine, and will therefore give a lower protein value). Based on this, the protein results found by the HPLC-method was not completely accurate, and the conversion factor of 4.8 may have been too low. The protein results for *S.latissima* and *A.esculenta* were higher than the values found by Schiener et al. (4), and *A.esculenta* was within the range of the protein values reported by Indergaard & Jensen (25). The protein value found for *P.palmata* (6.9%) was lower than the protein values reported by Indergaard & Jensen for *P.palmata* (8-25%). This could also be

due to the fact that they used a higher conversion factor (6.25) than the one applied in this thesis (4.8).

According to literature (4, 15), the protein content has been found to be highest in winter/spring. The highest protein content in *S.latissima* and *A.esculenta* (from Frøya) was found in the samples harvested in May. For the *S.latissima* samples from Austevoll the protein content was also highest in the sample harvested in May, and containing depths from 2-8 meter. Due to problems with the C/N analyser, the samples harvested from different depths in May were not analysed by this method, and are not included in this thesis. Therefore the protein values for the different depths in June were compared. For both *S.latissima* and *A.esculenta* the highest amount of proteins was found in the samples containing a mixture of depths, and in the samples harvested from 2 meters depth. Consequently, it would seem that the highest amount of proteins found in *S.latissima* and *A.esculenta* are in the samples harvested in May.

Furthermore, the *S.latissima* sample harvested in June from 2 meters depth had a high standard deviation. This sample was going to be re-analysed, but due to the C/N analyser not working, this could not be done before this thesis was delivered.

Protein results from the HPLC -method

The protein values obtained by acid hydrolysis and HPLC were also higher than the results reported in the literature for *S.latissima* and *A.esculenta.* Similarly to the protein results found for *P.palmata* by the C/N method, the protein value for *P.palmata* in the sample analysed by HPLC was lower than the values found for *S.latissima* and *A.esculenta*. However, the protein value was in the lower range of the protein values reported by Fleurence (6) and by Indergaard & Jensen (25). The *S.latissima* samples from Austevoll and Frøya both had highest protein content in May, while the protein content in *A.esculenta* was highest in June. In *S.latissima* and *A.esculenta* the highest amount of proteins were in the samples harvested from 8 meters depth, and in the samples containing a mixture of depths. The protein content in *S.latissima* and *A.esculenta* seemed to increase with increasing depth (Figure 18-19). However, there was nothing in the literature that could substantiate if the protein content increases with increasing depth.

The protein results from the C/N-method and the HPLC-method both showed a high level of proteins in the samples containing a mixture of depths. Once again it was unexpected that these samples had a protein value that was higher than the values for all the other depths.
As mentioned, the protein results determined by high-performance liquid chromatography (HPLC) were generally higher than the protein values determined by the C/N method. It would therefore seem that the most optimal method for determining the total protein content is by acid hydrolysis combined with HPLC. Still, the calculated conversion factor (4.8) used in the C/N method might have been too low, and higher protein values could be achieved by using a more accurate conversion factor. Moreover, determining the total amino acid content by acid hydrolysis and HPLC was demanding seeing as it required several steps. In this thesis two or three parallels were applied for each sample. However, because the analysis method included so many steps, more than three parallels should be applied, although this would be more time consuming and more costly.

Method development

The conventional hydrolysis method with 6M HCl destroys tryptophan, and partially destroys tyrosine, serine and threonine (32). Addition of phenol has been found to prevent tryptophan from being destroyed during the hydrolysis (62), and therefore hydrolysis with 6M HCl containing 0.02% phenol has been used in order to get higher tryptophan yields (32). 4 M methanesulfonic acid (MSA) and addition of β-mercaptoethanol to HCl has also been reported to prevent destruction of tryptophan and other residues (32, 62). Therefore the samples were hydrolysed with 4 M methanesulfonic acid for 45 min at 160°C, as described by Fountoulakis (32). The results showed a lower yield of tryptophan and of the other amino acids compared to the hydrolysis with 6M HCl (w/0.02% phenol). Consequently the method with MSA as an agent was discarded. Muramoto & Kamiya (62) found that the addition of 0.4 % β-mercaptoethanol gave higher yield of tryptophan. In this thesis it was also found that adding 0.4% mercaptoethanol to 6M HCl gave a higher amount of total amino acids. However, the literature did not provide an explanation as to why β-mercaptoethanol gave higher yields of tryptophan.

5.5 Amino acid composition

Most studies done on the amino acid composition in macroalgae report that aspartic acid and glutamic acid are the amino acids usually found in the highest amount, the exception is in red algae. *P.palmata* has been found to contain a higher content of glycine, leucine, valine and methionine (1, 2, 6). In this thesis the results from the amino acid analysis showed that aspartic acid, glutamic acid, alanine and the essential amino acid leucine dominated the amino acid content in *S.latissima*, *A.esculenta* and *P.palmata*. In addition to leucine, the essential amino acids (EAA) leucine, lysine, phenylalanine and valine were also found in high amounts. The amount of each amino acid was generally higher in *A.esculenta*, compared to *S.latissima* and *P.palmata*.

Mæhre et al. (2) also found the major amino acids in *A.esculenta* and *P.palmata* to be aspartic acid, glutamic acid, alanine and leucine, while the major EAA were leucine, valine and lysine. The level of each amino acid found by Mæhre et al. were significantly higher than the values found for each amino acid this thesis. The explanation for this could be that different methods were applied for the determination of the amino acid composition. Contrary to the findings reported by Fleurence (6), the results found in *P.palmata* in this thesis and by Mæhre et al. showed a high content of aspartic acid and glutamic acid. The macroalgae studied in this thesis and by Mæhre et al. were harvested from the Norwegian coast, while the algae studied by Fleurence was harvested from the French Atlantic coast. Since the protein content and the amino acid composition can be affected by seasonal and environmental conditions (1), these differences found in the levels of aspartic acid and glutamic acid in *P.palmata* is most likely due to the macroalgae being harvested from different locations.

The level of nutrients and nitrogen also influence the amino acid composition in macroalgae, as high nitrogen availability has been found to give higher protein levels (30). According to Harnedy & FitzGerald (30) the nitrogen levels are high in the winter months and in early spring. In *S.latissima* the level of each amino acid was generally higher in May, while for *A.esculenta* there were no great variations in the level of amino acids between May and June. Moreover, *S.latissima* and *A.esculenta* contained high amounts of each amino acid in the samples containing a mixture of depths. The wild samples of *S.latissima* and *A.esculenta* also contained high amino acid levels, and in *A.esculenta* the amount of glutamic acid and aspartic acid was especially high in the wild sample from June 19.

5.6 Carbohydrate composition

The highest total carbohydrate content was found in *Palmaria palmata,* due to its high content of xylose/mannose and galactose. Both agar and carrageenan contain galactose units, so this *P.palmata* sample most likely contain a high content of these hydrocolloids. *P.palmata* also contained a relatively high amount of glucose. As described earlier, floridean starch function as the carbohydrate storage in red algae, and is composed of glucose-units (23). Mannose and xylose units are known to sometimes substitute the cellulose content in red algae.

The carbohydrate content was higher in *A.esculenta* compared to *S.latissima*. The total carbohydrate content in *S.latissima* were higher in May than in June, while in *A.esculenta* the carbohydrate content was higher in the samples from June. For *S.latissima* and *A.esculenta* the highest amount of the carbohydrate components could generally be found in the samples containing a mixture of depths from 2, 5 and 8 meters. However, the glucose content in *A.esculenta* in June was higher in the samples from depths of 5 and 8 meters. For both *S.latissima* and *A.esculenta*, the carbohydrate content was higher in the wild growing samples than in the cultivated samples. The *S.latissima* samples from Austevoll had a higher carbohydrate level than the *S.latissima* samples from Frøya. The Austevoll samples harvested in April contained the highest amount of total carbohydrate.

As reported by Schiener et al. (4), *S.latissima* and *A.esculenta* can contain around 18.6% and 12.1% mannitol (dw), and around 8.2% and 11.1% (dw) laminarin, respectively. In addition *S.latissima* and *A.esculenta* can contain 28.5% and 37.4% alginate, respectively.
S.latissima and *A.esculenta* both contained high amounts of mannitol, glucose and mannuronic acid. Mannitol dominated the carbohydrate content in *S.latissima*, however the mannitol levels for *S.latissima* were lower than the levels reported by Schiener et al. In *A.esculenta* the major carbohydrate component was glucose, and particularly the wild growing *A.esculenta* sample harvested June 19 contained a high amount of glucose. Both laminarin and cellulose consist of glucose units. In this thesis the hydrolysis of the cellulose fraction proved challenging, and the amount of cellulose has not been found to be particularly high in brown algae (41). Therefore, it might seems that the high content of glucose is due to *A.esculenta* containing a high amount of the storage carbohydrate laminarin.

The results showed that both *S.latissima* and *A.esculenta* contained a relatively high amount of mannuronic acid and a lower amount of guluronic acid. Concequently it would seem that mannuronic acid dominate the alginate content. The samples analysed by anion-exchange chromatography had alginate- values far below the ones reported by Schiener et al. (28.5% and 37.4), due to challenges with the analysis (as mentioned in section 2.6.3). However, the alginate content in the *S.latissima* samples from Austevoll was determined enzymatically, and gave more accurate values. These samples contained between 22.1-27.0% alginate.

According to Manns et al. (15) the amount of storage carbohydrates should decline during spring and summer, when the content of alginate and fucoidan peaks. However, the carbohydrate results from this thesis showed that the carbohydrate composition in the *S.latissima* and *A.esculenta* samples harvested in May and June, were dominated by the storage carbohydrates mannitol and laminarin. In *S.latissima* the content of mannitol and glucose were higher in May than in June, while the amount of glucose in *A.esculenta* reached a peak in June. In addition the mannitol and glucose (laminarin) content was higher in the *S.latissima* samples harvested in April at Austevoll. This seems likely seeing as the storage carbohydrates should decline in spring when macroalgal growth is initiated. Contrary to what was reported by Manns et al., the storage carbohydrates still dominated the carbohydrate composition in *S.latissima* and *A.esculenta* in May and June. The reason for this could be environmental, since the macroalgae analysed in this thesis was harvested from the Norwegian coast.

6 Further work

There were several challenges with the carbohydrate analysis performed by HPAE-PAD; the levels of the uronic acids were too low, and decarboxylation interfered with the results for the uronic acids. Xylose and mannose could not be separated, and hydrolysis of the cellulose fraction was challenging when trifluoroacetic acid was applied as an agent. Furthermore, the column applied in this analysis was not ideal.

Even though the determination of mannuronic and guluronic acid can be optimized, HPAE-PAD does not seem to be the most ideal method for determination of the uronic acids. Instead enzymatic determination of alginate and determination by NMR (nuclear magnetic resonance) may be more optimal (43, 61). In this thesis enzymatic determination of alginate was performed by Stine Wiborg Dahle (according to the procedure by Østgaard), and this method gave significantly higher alginate levels than the results found by HPAE-PAD. The results of the carbohydrate content determined by HPAE-PAD should also be compared with results found by GC-MS, in order to see if this is a more optimal method. Better liberation of the cellulose fraction can be obtained by hydrolysis with sulfuric acid as a hydrolysis agent, instead of trifluoroacetic acid (64). Positive controls, where the polysaccharide composition is known, should be applied together with a red and brown algae sample where the content of monosaccharides, as well as the amount and type of polysaccharides, have been identified.

Washing of the macroalgae after harvesting should be avoided, seeing salt and other minerals are removed, which gives the algae samples a lower dry matter and ash content.

7 Conclusion

In this thesis the chemical composition in *Saccharina latissima*, *Alaria esculenta* and *Palmaria palmata* was determined. Both wild and cultivated samples of *S.latissima* and *A.esculenta* were analysed. In addition the cultivated *S.latissima* and *A.esculenta* samples harvested from different depths (2, 5 and 8 meters depth, as well as samples containing a mixture of these depths), and from different months (April, May and June), were compared. Furthermore, methods for determination of amino acids and carbohydrate components were developed and optimized.

The dry matter content was within the range that was reported in literature (10-30%). The highest dry matter content could be found in the wild growing samples of *S.latissima* and *A.esculenta* harvested June 01 and June 19. In cultivated *S.latissima* the dry matter was at its highest in the sample harvested in May, while the dry matter content in cultivated *A.esculenta* was at its highest in June. For both *S.latissima* and *A.esculenta* the dry matter reached a peak in the samples harvested from 2 meters depth. According to literature the ash content is highest in spring, and for both *S.latissima* and *A.esculenta* the ash content reached a peak in May, in the samples from 2 and 8 meters depth.
The level of lipids was low in all three macroalgae. The lipid content in *S.latissima* was highest in May at depths of 8 meters. In *A.esculenta* the maximum lipid content was found in the sample harvested in June, and containing a mixture of depths.

The protein content was found by determining the total amino acid content (by HPLC) and by the C/N method. HPLC gave higher levels of total protein. Hydrolysis with 6M HCl containing 0.4% β-mercaptoethanol gave higher amino acid yields than the other hydrolysis methods. The protein level in *S.latissima* and *A.esculenta* was in accordance with the protein levels reported in the literature. However, *P.palmata* contained a low protein content.
For *S.latissima* the protein content was highest in May, while the protein content in *A.esculenta* reached a peak in June. The protein content was higher in the samples from 8 meters depth and in the samples containing a mixture of depths, for both *S.latissima* and *A.esculenta*. The amino acid composition was dominated by aspartic acid, glutamic acid, alanine and leucine in all of the samples. Lysine, phenylalanine, leucine and valine constituted the major fraction of the essential amino acids.

The highest total carbohydrate was found in *P.palmata,* and the major carbohydrate components were xylose/mannose and galactose (residue in agar and carrageenan). The total carbohydrate content was higher in the wild samples than in the cultivated samples of *S.latissima* and *A.esculenta*. And mannitol and glucose dominated the carbohydrate composition in *S.latissima* and *A.esculenta*. Consequently, it would seem that the levels of the storage carbohydrates were higher than the other carbohydrates. The highest amount of carbohydrates was found in April for *S.latissima*, and in June for *A.esculenta*. However, there was no significant variation between the depths.

Generally it would seem that the highest content of the chemical components was in May for *S.latissima* and in June for *A.esculenta*. None of the different depths gave a higher content of the chemical components, and the macroalgae can be harvested at depths from 2-8 meter.

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Appendix

Contents

A. Dry matter and ash content

Table 8: The dry matter and ash content in the *Saccharina latissima* (S.L) samples cultivated and harvested at Austevoll. The samples were harvested April 27 (containing a mixture of samples harvested from 0-9 meters depth, and from 1-6 meters depth), and May 19 (containing a mixture of samples harvested from 2-8 meters depth). The dry matter was calculated from the wet weight (% of ww). The ash content was calculated from the dry matter (% of dw). The uncertainty is given as the standard deviation (stdev.) of three parallels, and the results are average value of these.

	Sample	**% DW**	**stdev.**	**% Ash**	**stdev**
S.latissima (April: 27.04.15)	S.L 0-9 meters	9,07	1,11	42,23	0,89
S.latissima (April: 27.04.15)	S.L 1-6 meters	9,02	1,27	44,68	1,56
S.latissima (May: 19.05.15)	S.L 2-8 meters	8,16	0,15	49,51	2,14

Table 9: The dry matter and ash content in the *S.latissima* (S.L) and *A.esculenta* (A.E) samples cultivated and harvested at Frøya. The samples were harvested in May and June, from depths of 2, 5 and 8 meters. In addition, some of the samples contained a minture of these depths ("mixed depths"). The dry matter was calculated from the wet weight (% of ww). The ash content was calculated from the dry matter (% of dw). The uncertainty is given as the standard deviation (stdev.) of three parallels, and the results are average value of these.

	Sample	**% DW**	**stdev.**	**% Ash**	**stdev**
	May (20.05.15)				
S.latissima	S.L mixed depths	7,01	1,46	36,73	3,9
	S.L 2 meter	11,54	1,05	38,16	1,6
	S.L 5 meter	10,39	0,65	42,94	0,9
	S.L 8 meter	9,68	0,25	46,99	1,2
A.esculenta	A.E mixed depths	8,49	0,24	24,46	2,0
	A.E 2 meter	14,33	0,05	30,94	0,2
	A.E 5 meter	17,63	1,17	26,64	2,8
	A.E 8 meter	15,79	1,65	28,71	2,6
	June (18.06.15)				
S.latissima	S.L mixed depths	8,84	0,51	37,84	1,0
	S.L 2 meter	10,21	0,17	43,50	1,6
	S.L 5 meter	9,75	0,69	44,35	2,5
	S.L 8 meter	10,26	0,55	44,27	0,8
A.esculenta	A.E mixed depths	14,07	0,8	17,33	0,8
	A.E 2 meter	18,24	3,86	19,18	2,9
	A.E 5 meter	14,41	1,76	26,35	5,8
	A.E 8 meter	17,63	3,31	21,22	2,3

Table 10: The dry matter and ash content in the wild growing samples of *S.latissima* (S.L), *A.esculenta* (A.E) and *P.palmata* (P.P), all harvested at Vanvikan. The *S.latissima* and *A.esculenta* samples were harvested June 01 and June 19 (2015), while *P.palmata* was harvested June 19 (2015). The dry matter was calculated from the wet weight (% of ww). The ash content was calculated from the dry matter (% of dw). The uncertainty is given as the standard deviation (stdev.) of three parallels, and the results are average value of these.

	Sample	**% DW**	**stdev.**	**% Ash**	**stdev**
S.latissima	S.L 01.06.15	16,40	1,85	16,96	2,0
S.latissima	S.L 19.06.15	10,06	0,39	32,23	1,8
A.esculenta	A.E 01.06.15	22,83	4,69	11,04	2,5
A.esculenta	A.E 19.06.15	19,81	2,59	13,11	2,1
P.palmata	P.P 19.06.15	15,49	1,9	17,36	2,0

B. Total lipids

Table 11: Total lipid content (% of dry matter) in the *Saccharina latissima* (S.L) samples cultivated and harvested at Austevoll. The samples were harvested April 27 (containing a mixture of samples harvested from 0-9 meters depth, and from 1-6 meters depth), and May 19 (containing a mixture of samples harvested from 2-8 meters depth). The uncertainty is given as the standard deviation (stdev.) of three parallels, and the results are average value of these.

	Prøve	**% Tot. Lipids**	**stdev.**
S.latissima (April: 27.04.15)	S.L 0-9 meters	2,01	0,38
S.latissima (April: 27.04.15)	S.L 1-6 meters	2,79	0,10
S.latissima (May: 19.05.15)	S.L 2-8 meters	2,82	0,32

Table 12: Total lipid content (% of dw) in the *S.latissima* (S.L) and *A.esculenta* (A.E) samples cultivated and harvested at Frøya. The samples were harvested in May and June, from depths of 2, 5 and 8 meters. In addition, some of the samples contained a minture of these depths ("mixed depths"). The uncertainty is given as the standard deviation (stdev.) of three parallels, and the results are average value of these.

	Prøve	**% Tot. Lipids**	**stdev.**
	May (20.05.15)		
S.latissima	S.L mixed depths	3,93	0,42
	S.L 2 meter	3,80	0,43
	S.L 5 meter	3,55	0,14
	S.L 8 meter	5,28	0,39
A.esculenta	A.E mixed depths	3,18	0,17
	A.E 2 meter	4,14	0,03
	A.E 5 meter	3,66	0,08
	A.E 8 meter	3,87	0,26
	June (18.06.15)		
S.latissima	S.L mixed depths	4,41	0,22
	S.L 2 meter	1,36	0,42
	S.L 5 meter	3,14	0,14
	S.L 8 meter	3,17	0,07
A.esculenta	A.E mixed depths	4,60	0,43
	A.E 2 meter	3,13	0,21
	A.E 5 meter	3,52	0,17
	A.E 8 meter	4,02	0,07

Table 13: Total lipid content (% of dw) in wild growing samples of *S.latissima* (S.L), *A.esculenta* (A.E) and *P.palmata* (P.P) all harvested at Vanvikan. The uncertainty is given as the standard deviation (stdev.) of three parallels, and the results are average value of these.

	Prøve	**% Tot. Lipids**	**stdev.**
S.latissima	S.L 01.06.15	0,90	0,14
S.latissima	S.L 19.06.15	3,24	0,45
A.esculenta	A.E 01.06.15	1,04	0,42
A.esculenta	A.E 19.06.15	2,40	0,17
P.palmata	P.P 19.06.15	1,78	0,18

C. Total protein

C.1 Protein content determined by C/N- method

Table 14: Total protein content (% of dry matter) in *Saccharina latissima* (S.L) samples cultivated and harvested at Austevoll. The samples were harvested April 27 (containing a mixture of samples harvested from 0-9 meters depth, and from 1-6 meters depth), and May 19 (containing a mixture of samples harvested from 2-8 meters depth). The uncertainty is given as the standard deviation (stdev.) of four parallels, and the results are average value of these.

	Prøve	**% Tot. Protein**	**stdev.**
S.latissima (April: 27.04.15)	S.L 0-9 meters	8,4	0,23
S.latissima (April: 27.04.15)	S.L 1-6 meters	9,8	0,26
S.latissima (May: 19.05.15)	S.L 2-8 meters	11,0	0,18

Table 15: Total protein content (% of dw) in the *S.latissima* (S.L) and *A.esculenta* (A.E) samples cultivated and harvested at Frøya. The samples were harvested in May and June. The samples in June contained samples harvested from depths of 2, 5 and 8 meters. In both May and June some of the samples contained a mixture of these depths ("mixed depths"). The uncertainty is given as the standard deviation (stdev.) of four parallels, and the results are average value of these.

	Prøve	**% Tot. Protein**	**stdev.**
	May (20.05.15)		
S.latissima	S.L mixed depths	15,4	0,24
A.esculenta	A.E mixed depths	17,7	0,32
	June (18.06.15)		
S.latissima	S.L mixed depths	12,4	0,14
	S.L 2 meter	13,6	5,58
	S.L 5 meter	11,3	0,46
	S.L 8 meter	11,9	0,48
A.esculenta	A.E mixed depths	15,3	0,43
	A.E 2 meter	13,9	0,39
	A.E 5 meter	13,7	0,13
	A.E 8 meter	13,6	0,21

Table 16: Total protein content (% of dw) in wild growing samples of *S.latissima* (S.L), *A.esculenta* (A.E) and *P.palmata* (P.P) all harvested at Vanvikan. The *S.latissima* and *A.esculenta* samples were harvested June 01 and June 19 (2015), while *P.palmata* was harvested June 19 (2015). The uncertainty is given as the standard deviation (stdev.) of four parallels, and the results are average value of these.

	Prøve	**% Tot. Protein**	**stdev.**
S.latissima	S.L 01.06.15	10,7	0,18
S.latissima	S.L 19.06.15	12,0	0,12
A.esculenta	A.E 01.06.15	11,4	0,10
A.esculenta	A.E 19.06.15	12,5	0,46
P.palmata	P.P 19.06.15	9,0	0,30

C.2. Calculation of conversion factor

Table 17: The results found by the C/N-method was first calculated by using a conversion factor of 6.25. The ratio between these results, and the protein results found by HPLC, was calculated. The average ratio value was found to be 1.3. The average ratio- value was used to calculate a new conversion factor.

Samples:	CN- results	HPLC- results	Ratio (CN/HPLC)
S.L May 20 (mix)	15,4	8,7	1,8
S.L June 18 (mix)	12,4	6,5	1,9
S.L June 18 (2m)	10,8	6,2	1,7
S.L June 18 (5m)	11,3	7,1	1,6
S.L June 18 (8m)	11,9	10,1	1,2
S.L June 01 (mix)	10,7	6,8	1,6
S.L June 19 (mix)	12,0	7,7	1,6
A.E May 20 (mix)	17,7	12,8	1,4
A.E June 18 (mix)	15,3	10,1	1,5
A.E June 18 (2m)	13,9	6,9	2,0
A.E June 18 (5m)	13,7	12,4	1,1
A.E June 18 (8m)	13,6	13,1	1,0
A.E June 01 (mix)	11,4	11,2	1,0
A.E June 19 (mix)	12,5	7,6	1,6
P.P June 19 (mix)	9,0	7,3	1,2
S.L April 27 (0-9m)	8,4	6,6	1,3
S.L April 27 (1-6m)	9,8	6,6	1,5
S.L May 19 (2-8m)	11,0	7,3	1,5
Average			**1,3**

$$\text{New value:} \quad \frac{6.25}{1.3} = \mathbf{4.8}$$

Formel 1. The new conversion factor was calculated by dividing the conversion facrot of 6.25 with the average ratio.

C.3 Protein content determined by HPLC-method

Table 18: Total protein content (% of dry matter) in the *Saccharina latissima* (S.L) samples cultivated and harvested at Austevoll. The samples were harvested April 27 (containing a mixture of samples harvested from 0-9 meters depth, and from 1-6 meters depth), and May 19 (containing a mixture of samples harvested from 2-8 meters depth). The uncertainty is given as the standard deviation (St.dev.) of two or three parallels, and the results are average value of these.

	Prøve	**Tot. Protein (% AA)**	**St.dev.**
S.latissima (April: 27.04.15)	S.L 0-9 meters	6,7	0,3
S.latissima (April: 27.04.15)	S.L 1-6 meters	6,6	0,2
S.latissima (May: 19.05.15)	S.L 2-8 meters	7,3	0,0

Table 19: Total protein content (% of dw) in the *S.latissima* (S.L) and *A.esculenta* (A.E) samples cultivated and harvested at Frøya. The samples were harvested in May and June. The samples in June contained samples harvested from depths of 2, 5 and 8 meters. In both May and June some of the samples contained a mixture of these depths ("mixed depths"). The uncertainty is given as the standard deviation (St.dev.) of four parallels, and the results are average value of these. The protein values for some of the samples were found by Vera kristinova, at SINTEF Fisheries and Aquaculture.

	Prøve	**Tot. Protein (% AA)**	**St.dev.**
	May (20.05.15)		
S.latissima	S.L mixed depths	12,7	0,3
	S.L 2 meter	8,4	0,2
	S.L 5 meter	8,0	1,3
	S.L 8 meter	9,4	0,9
A.esculenta	A.E mixed depths	13,6	0,1
	A.E 2 meter	12,8	0,1
	A.E 5 meter	11,9	0,3
	A.E 8 meter	13,4	0,3
	June (18.06.15)		
S.latissima	S.L mixed depths	11,0	0,2
	S.L 2 meter	6,1	1,4
	S.L 5 meter	7,1	0,5
	S.L 8 meter	10,1	0,6
A.esculenta	A.E mixed depths	15,7	0,2
	A.E 2 meter	6,9	1,9
	A.E 5 meter	12,4	0,6
	A.E 8 meter	13,1	0,2

Table 20: Total protein content (% of dw) in wild growing samples of *S.latissima* (S.L), *A.esculenta* (A.E) and *P.palmata* (P.P) all harvested at Vanvikan. The *S.latissima* and *A.esculenta* samples were harvested June 01 and June 19 (2015), while *P.palmata* was harvested June 19 (2015). The uncertainty is given as the standard deviation (St.dev.) of two or three parallels, and the results are average value of these. The protein values for some of the samples were found by Vera kristinova, at SINTEF Fisheries and Aquaculture.

	Prøve	**Tot. Protein (% AA)**	**St.dev.**
S.latissima	S.L 01.06.15	10,3	0,03
S.latissima	S.L 19.06.15	11,3	0,3
A.esculenta	A.E 01.06.15	11,1	1,4
A.esculenta	A.E 19.06.15	13,5	0,2
P.palmata	P.P 19.06.15	9,1	0,1

C.4 Amino acid composition:

Table 21: Amino acid composition in the *S.latissima* samples cultivated and harvested at Austevoll. The samples were harvested April 27 (containing a mixture of samples harvested from 0-9 meters depth, and from 1-6 meters depth), and May 19 (containing a mixture of samples harvested from 2-8 meters depth). The uncertainty is given as the standard deviation (St.dev.) of two or three parallels, and the results are average value of these

	27.04.15 (0-9m)		**27.04.15 (1-6m)**		**19.05.15 (2-8m)**	
Amino acid	**Average**	**St.dev.**	**Average**	**St.dev.**	**Average**	**St.dev.**
Taurin	0,00	0,00	0,02	0,02	0,02	0,02
Aspartic acid + Asparagine	**0,69**	0,09	**0,54**	0,03	**0,67**	0,02
Threonine	0,23	0,01	0,18	0,01	0,25	0,01
Serine	0,32	0,04	0,26	0,00	0,32	0,00
Glutamic acid + Glutamine	**1,14**	0,03	**1,01**	0,06	**0,99**	0,04
Proline	0,20	0,01	0,29	0,01	0,20	0,04
Glycine	0,33	0,03	0,31	0,05	0,35	0,01
Alanine	**0,78**	0,06	**0,73**	0,13	**0,91**	0,03
Cystine (Cys-Cys)	0,15	0,06	0,19	0,01	0,34	0,02
Valine	0,39	0,01	0,34	0,04	0,36	0,01
Methionine	0,12	0,01	0,13	0,00	0,15	0,00
Isoleucine	0,28	0,02	0,29	0,01	0,33	0,00
Leucine	**0,50**	0,03	**0,67**	0,02	**0,67**	0,02
Tyrosine	0,18	0,02	0,19	0,00	0,20	0,01
Phenylalanine	**0,51**	0,06	**0,55**	0,02	**0,54**	0,02
Histidine	0,14	0,01	0,14	0,01	0,14	0,01
Lysine	0,35	0,02	0,42	0,00	0,44	0,01
Tryptophan	0,14	0,03	0,00	0,00	0,00	0,00
Arginine	0,26	0,01	0,30	0,01	0,36	0,00
Sum (AA)	6,7	0,6	6,6	0,4	7,3	0,3

Table 22: Amino acid composition in the *S.latissima* samples cultivated and harvested at Frøya in May. The samples samples were harvested from depths of 2, 5 and 8 meters. In addition, one sample consists of a mixture of these depths (”mixed depths”). The amino acid values for the sample containing a mixture of depths, were found by Vera kristinova, at SINTEF Fisheries and Aquaculture. The uncertainty is given as the standard deviation (St.dev.) of two or three parallels, and the results are average value of these

	Mixed depths		**2m**		**5m**		**8m**	
Amino acid	**Average**	**St.dev.**	**Average**	**St.dev.**	**Average**	**St.dev.**	**Average**	**St.dev.**
Taurin	0,0	0,0	0,02	0,02	0,02	0,02	0,06	0,00
Aspartic acid + Asparagine	1,3	0,0	0,73	0,06	0,79	0,18	1,03	0,18
Threonine	0,5	0,0	0,24	0,04	0,27	0,05	0,40	0,09
Serine	0,7	0,0	0,42	0,03	0,44	0,15	0,53	0,12
Glutamic acid + Glutamine	1,8	0,1	1,19	0,05	1,12	0,28	1,24	0,09
Proline	0,4	0,0	0,26	0,01	0,24	0,03	0,43	0,02
Glycine	0,7	0,0	0,44	0,03	0,40	0,09	0,53	0,04
Alanine	1,5	0,1	0,99	0,01	0,96	0,16	1,17	0,09
Cystine (Cys-Cys)	0,3	0,1	0,39	0,01	0,31	0,01	0,24	0,01
Valine	0,8	0,0	0,39	0,02	0,44	0,12	0,47	0,02
Methionine	0,3	0,0	0,14	0,01	0,15	0,02	0,17	0,03
Isoleucine	0,6	0,0	0,36	0,01	0,36	0,07	0,46	0,03
Leucine	1,1	0,0	0,81	0,00	0,68	0,10	0,82	0,06
Tyrosine	0,3	0,0	0,24	0,01	0,21	0,02	0,27	0,02
Phenylalanine	0,8	0,1	0,57	0,01	0,53	0,03	0,55	0,04
Histidine	0,3	0,0	0,18	0,01	0,15	0,04	0,18	0,02
Lysine	0,7	0,0	0,53	0,00	0,45	0,06	0,44	0,03
Tryptophan	0,2	0,0	0,00	0,00	0,09	0,06	0,01	0,00
Arginine	0,5	0,0	0,43	0,03	0,34	0,01	0,41	0,05
Sum (AA)	12,7	0,5	8,4	0,4	8,0	1,5	9,4	0,9

Table 23: Amino acid composition in the *A.esculenta* samples cultivated and harvested at Frøya in May. The samples samples were harvested from depths of 2, 5 and 8 meters. In addition, one sample consists of a mixture of these depths (”mixed depths”). The amino acid values for the sample containing a mixture of depths, were found by Vera kristinova, at SINTEF Fisheries and Aquaculture. The uncertainty is given as the standard deviation (St.dev.) of two or three parallels, and the results are average value of these

	Mix depths		**2m**		**5m**		**8m**	
Amino acid	**Average**	**St.dev.**	**Average**	**St.dev.**	**Average**	**St.dev.**	**Average**	**St.dev.**
Taurin	0,1	0,0	0,05	0,01	0,07	0,03	0,11	0,02
Aspartic acid +Asparagine	1,4	0,0	1,08	0,28	0,98	0,20	1,41	0,03
Threonine	0,5	0,0	**0,91**	0,32	0,36	0,03	0,62	0,01
Serine	0,8	0,0	0,59	0,14	0,74	0,11	0,75	0,02
Glutamic acid +Glutamine	1,8	0,0	1,87	0,05	1,74	0,08	1,87	0,07
Proline	0,5	0,0	0,44	0,04	0,48	0,00	0,57	0,00
Glycine	0,7	0,0	0,70	0,02	0,68	0,03	0,70	0,02
Alanine	1,7	0,0	1,65	0,01	1,52	0,07	1,73	0,07
Cystine (Cys-Cys)	0,3	0,0	0,33	0,02	0,32	0,02	0,36	0,03
Valine	0,8	0,0	0,64	0,03	0,61	0,02	0,66	0,00
Methionine	0,2	0,0	0,22	0,01	0,23	0,01	0,22	0,00
Isoleucine	0,6	0,0	0,61	0,01	0,59	0,03	0,62	0,02
Leucine	1,1	0,0	1,05	0,02	1,02	0,03	1,08	0,03
Tyrosine	0,4	0,0	0,38	0,04	0,38	0,00	0,39	0,00
Phenylalanine	0,8	0,0	0,69	0,04	0,69	0,04	0,70	0,01
Histidine	0,3	0,0	0,29	0,02	0,26	0,01	0,27	0,01
Lysine	0,8	0,0	0,66	0,01	0,64	0,02	0,67	0,01
Tryptophan	0,2	0,0	0,03	0,01	0,02	0,01	0,04	0,00
Arginine	0,1	0,0	0,05	0,01	0,07	0,03	0,11	0,02
Sum (AA)	13,6	0,2	12,8	1,1	11,9	0,8	13,4	0,4

Table 24: Amino acid composition in the *S.latissima* samples cultivated and harvested at Frøya in June. The samples samples were harvested from depths of 2, 5 and 8 meters. In addition, one sample consists of a mixture of these depths (”mixed depths”). The amino acid values for the sample containing a mixture of depths, were found by Vera kristinova, at SINTEF Fisheries and Aquaculture. The uncertainty is given as the standard deviation (St.dev.) of two or three parallels, and the results are average value of these

	Mix depths		**2m**		**5m**		**8m**	
Amino acid	**Average**	**St.dev.**	**Average**	**St.dev.**	**Average**	**St.dev.**	**Average**	**St.dev.**
Taurin	0,1	0,0	0,01	0,01	0,05	0,01	0,09	0,00
Aspartic acid + Asparagine	1,2	0,0	0,69	0,10	0,77	0,04	1,09	0,15
Threonine	0,4	0,0	0,19	0,01	0,29	0,01	0,45	0,02
Serine	0,6	0,0	0,33	0,07	0,39	0,02	0,60	0,03
Glutamic acid + Glutamine	1,4	0,0	0,79	0,15	0,93	0,00	1,24	0,05
Proline	0,4	0,1	0,26	0,06	0,24	0,01	0,33	0,01
Glycine	0,7	0,0	0,37	0,09	0,41	0,04	0,64	0,03
Alanine	1,2	0,1	0,68	0,19	0,75	0,05	1,03	0,06
Cystine (Cys-Cys)	0,2	0,1	0,06	0,03	0,16	0,06	0,18	0,00
Valine	0,7	0,0	0,39	0,10	0,43	0,03	0,63	0,03
Methionine	0,2	0,0	0,12	0,04	0,14	0,01	0,20	0,01
Isoleucine	0,5	0,0	0,29	0,08	0,33	0,03	0,46	0,03
Leucine	0,9	0,0	0,51	0,14	0,59	0,06	0,83	0,05
Tyrosine	0,3	0,0	0,18	0,05	0,20	0,00	0,29	0,01
Phenylalanine	0,6	0,0	0,43	0,09	0,45	0,02	0,64	0,03
Histidine	0,2	0,0	0,15	0,03	0,15	0,01	0,20	0,01
Lysine	0,6	0,0	0,36	0,09	0,40	0,04	0,58	0,03
Tryptophan	0,2	0,0	0,00	0,00	0,07	0,07	0,13	0,04
Arginine	0,4	0,0	0,27	0,08	0,32	0,04	0,45	0,03
Sum (AA)	11,0	0,5	6,1	1,4	7,1	0,5	10,1	0,6

Tabell 25: Amino acid composition in the *A.esculenta* samples cultivated and harvested at Frøya in June. The samples samples were harvested from depths of 2, 5 and 8 meters. In addition, one sample consists of a mixture of these depths (”mixed depths”). The amino acid values for the sample containing a mixture of depths, were found by Vera kristinova, at SINTEF Fisheries and Aquaculture. The uncertainty is given as the standard deviation (St.dev.) of two or three parallels, and the results are average value of these

	Mix depths		**2m**		**5m**		**8m**	
Amino acid	**Average**	**St.dev.**	**Average**	**St.dev.**	**Average**	**St.dev.**	**Average**	**St.dev.**
Taurin	0,1	0,1	0,02	0,02	0,07	0,00	0,04	0,00
Aspartic acid + Asparagine	1,7	0,0	0,65	0,16	1,22	0,08	1,28	0,04
Threonine	0,6	0,0	0,27	0,04	0,45	0,04	0,42	0,01
Serine	0,9	0,0	0,35	0,09	0,66	0,04	0,70	0,02
Glutamic acid + Glutamine	2,4	0,1	1,29	0,33	2,34	0,12	2,88	0,02
Proline	0,5	0,0	0,20	0,10	0,33	0,05	0,40	0,02
Glycine	0,8	0,0	0,31	0,08	0,59	0,04	0,59	0,02
Alanine	1,9	0,0	0,94	0,29	1,75	0,02	2,12	0,01
Cystine (Cys-Cys)	0,5	0,1	0,21	0,12	0,28	0,07	0,22	0,03
Valine	0,9	0,0	0,36	0,10	0,69	0,08	0,62	0,03
Methionine	0,3	0,0	0,09	0,02	0,18	0,02	0,16	0,00
Isoleucine	0,6	0,0	0,26	0,07	0,47	0,03	0,46	0,02
Leucine	1,1	0,0	0,43	0,12	0,79	0,06	0,79	0,03
Tyrosine	0,5	0,1	0,19	0,05	0,35	0,05	0,33	0,01
Phenylalanine	0,8	0,1	0,43	0,07	0,64	0,07	0,59	0,04
Histidine	0,3	0,0	0,15	0,04	0,25	0,01	0,24	0,01
Lysine	0,9	0,0	0,39	0,10	0,71	0,04	0,67	0,03
Tryptophan	0,2	0,0	0,12	0,04	0,19	0,01	0,17	0,01
Arginine	0,6	0,0	0,24	0,06	0,46	0,04	0,47	0,02
Sum (AA)	15,7	0,5	6,9	1,9	12,4	0,9	13,1	0,4

Table 26: Amino acid composition in the wild growing samples of *S.latissima*. The samples were harvested June 01 and June 19 (2015), at Vanvikan. The uncertainty is given as the standard deviation (St.dev.) of two or three parallels, and the results are average value of these. The amino acid values for these samples were found by Vera kristinova, at SINTEF Fisheries and Aquaculture.

	01.06.15 (mix)		**19.06.15 (mix)**	
Amino acid	**Average**	**St.dev.**	**Average**	**St.dev.**
Taurin	-	-	0	0
Aspartic acid + Asparagine	1,1	0,0	1,2	0,1
Threonine	0,4	0,0	0,4	0,0
Serine	0,5	0,0	0,6	0,0
Glutamic acid + Glutamine	1,9	0,0	1,8	0,0
Proline	0,4	0,0	0,4	0,0
Glycine	0,6	0,0	0,6	0,0
Alanine	1,3	0,0	1,5	0,0
Cystine (Cys-Cys)	0,2	0,0	0,2	0,0
Valine	0,6	0,0	0,6	0,0
Methionine	0,2	0,0	0,2	0,0
Isoleucine	0,4	0,0	0,5	0,0
Leucine	0,7	0,0	1,0	0,0
Tyrosine	0,2	0,0	0,2	0,0
Phenylalanine	0,6	0,0	0,6	0,0
Histidine	0,2	0,0	0,2	0,0
Lysine	0,5	0,0	0,6	0,0
Tryptophan	0,2	0,0	0,2	0,0
Arginine	0,3	0,0	0,5	0,0
Sum (AA)	10,3	0,2	11,3	0,3

Table 27: Amino acid composition in the wild growing samples of *A.esculenta*. The samples were harvested June 01 and June 19 (2015), at Vanvikan. The uncertainty is given as the standard deviation (St.dev.) of two or three parallels, and the results are average value of these. The amino acid values for the sample harvested June 19 were found by Vera kristinova, at SINTEF Fisheries and Aquaculture.

	01.06.15 (mix)		**19.06.15 (mix)**	
Amino acid	**Average**	**St.dev.**	**Average**	**St.dev.**
Taurin	0,08	0,00	0,1	0,0
Aspartic acid + Asparagine	1,09	0,04	1,0	0,0
Threonine	0,38	0,05	0,4	0,0
Serine	0,50	0,01	0,6	0,0
Glutamic acid + Glutamine	2,54	0,30	4,1	0,1
Proline	0,31	0,05	0,3	0,0
Glycine	0,47	0,06	0,5	0,0
Alanine	1,73	0,26	2,2	0,0
Cystine (Cys-Cys)	0,16	0,02	0,4	0,1
Valine	0,56	0,09	0,5	0,0
Methionine	0,12	0,02	0,1	0,0
Isoleucine	0,37	0,06	0,3	0,0
Leucine	0,63	0,10	0,6	0,0
Tyrosine	0,32	0,04	0,3	0,0
Phenylalanine	0,54	0,00	0,4	0,0
Histidine	0,22	0,02	0,2	0,0
Lysine	0,55	0,08	0,6	0,0
Tryptophan	0,16	0,16	0,4	0,0
Arginine	0,34	0,04	0,4	0,0
Sum (AA)	11,1	1,4	13,5	0,4

Table 28: Amino acid composition in the *P.palmata* sample, harvested June 19. The uncertainty is given as the standard deviation (St.dev.) of two or three parallels, and the results are average value of these. The amino acid values for this sample was found by Vera kristinova, at SINTEF Fisheries and Aquaculture.

	19.06.15 (mix)	
Amino acid	**Average**	**St.dev.**
Taurin	-	-
Aspartic acid + Asparagine	0,9	0,0
Threonine	0,4	0,0
Serine	0,5	0,0
Glutamic acid + Glutamine	1,4	0,0
Proline	0,2	0,0
Glycine	0,5	0,0
Alanine	0,8	0,0
Cystine (Cys-Cys)	0,1	0,0
Valine	0,6	0,0
Methionine	0,1	0,0
Isoleucine	0,4	0,0
Leucine	0,7	0,0
Tyrosine	0,3	0,0
Phenylalanine	0,6	0,0
Histidine	0,2	0,0
Lysine	0,6	0,0
Tryptophan	0,2	0,0
Arginine	0,4	0,0
Sum (AA)	9,1	0,3

D. Total carbohydrate

D.1 Carbohydrate composition

Table 29: The carbohydrate composition in the *S.latissima* samples from Austevoll. The samples were harvested April 27 (containing a mixture of samples harvested from 0-9 meters depth, and from 1-6 meters depth), and May 19 (containing a mixture of samples harvested from 2-8 meters depth). (Gal. = galactose. Glc. = glucose, Man/Xyl = mannose and xylose. GalA=guluronic acid. ManA= mannuronic acid.)

		Mannitol	**Fucose**	**Gal.**	**Glc.**	**Man/Xyl**	**GulA**	**ManA**	**Tot. carb**
April 27	0-9 m	15,23	1,21	0,74	3,81	0,63	0,28	2,08	23,98
April 27	1-6 m	13,55	1,23	0,81	2,29	0,68	0,28	1,98	20,82
May 19	2-8 m	12,69	1,20	0,83	1,08	0,75	0,27	1,86	18,67

Table 30: The carbohydrate composition in the *S.latissima* (S.L) and *A.esculenta* (A.E) samples cultivated and harvested at Frøya. The samples were harvested in May and June, from depths of 2, 5 and 8 meters. In addition, some of the samples contained a minture of these depths ("mixed depths"). (Gal. = galactose. Glc. = glucose, Man/Xyl = mannose and xylose. GalA=guluronic acid. ManA= mannuronic acid.)

		Mannitol	**Fucose**	**Gal**	**Glc**	**Man/Xyl**	**Gul A**	**Man A**	**Tot. carb**
May (20.05.15)									
S.latissima	Mixed depths	10,54	1,31	0,88	2,88	0,70	0,49	3,08	16,3
	2m	9,98	1,20	0,78	2,70	0,60	0,34	2,36	15,3
	5m	8,90	1,28	0,70	2,13	0,63	0,37	2,56	13,6
	8m	5,21	1,08	0,72	0,41	0,67	0,40	2,53	**8,1**
A.esculetna	Mixed depths	9,60	1,19	1,00	2,43	1,03	0,49	2,53	15,3
	2m	8,46	1,07	0,82	2,44	0,78	0,43	2,37	13,6
	5m	7,80	0,93	0,76	2,06	0,70	0,33	1,88	12,3
	8m	8,21	1,12	0,83	1,79	0,73	0,43	2,25	12,7
June (18.06.15)									
S.latissima	Mixed depths	10,32	1,56	0,84	1,08	0,81	0,46	3,43	14,6
	2m	8,13	0,93	0,41	1,14	0,45	0,19	1,47	11,1
	5m	8,23	1,26	0,65	0,96	0,71	0,41	2,79	11,8
	8m	9,11	1,32	0,75	1,05	0,77	0,43	2,05	13,0
A.esculenta	Mixed depths	10,00	1,29	0,96	8,32	0,82	0,55	2,65	21,4
	2m	8,14	1,05	0,75	10,21	0,59	0,44	0,77	20,7
	5m	9,06	1,04	0,70	12,39	0,57	0,44	2,06	23,8
	8m	8,06	1,08	0,70	13,41	0,62	0,40	1,92	23,9

Table 31: The carbohydrate composition in the wild growing samples of *S.latissima* (S.L)*, A.esculenta* (A.E) and *P.palmata* (P.P), all harvested at Vanvikan. The *S.latissima* and *A.esculenta* samples were harvested June 01 and June 19 (2015), while *P.palmata* was harvested June 19 (2015). (Gal.= galactose. Glc.= glucose, Man/Xyl = mannose and xylose. GalA= guluronic acid. ManA= mannuronic acid).

		Mannitol	**Fucose**	**Gal.**	**Glc.**	**Man/Xyl**	**Gul A**	**Man A**	**Tot. carb**
S.latissima	S.L 01.06.15	13,80	1,20	0,75	11,71	0,70	0,48	2,14	**28,2**
S.latissima	S.L 19.06.15	13,15	1,64	0,79	1,24	0,93	0,48	3,44	17,8
A.esculenta	A.E 01.06.15	11,29	1,00	0,57	17,58	0,68	0,67	2,94	31,1
A.esculenta	A.E 19.06.15	7,37	0,85	0,53	25,23	0,51	0,49	2,51	34,5
P.palmata	P.P 19.06.15			19,24	3,47	26,81			49,5

D.2 Calculation of the concentration for each carbohydrate component

Table 32: Standard curves were made for each standard mix (1, 2 and 3). The regression formulas shown here, were used to calculate the concentration of each carbohydrate component.

Carbohydrates	Equation	r^2
Guluronic acid	y = 1,7028x - 0,1297	0,99998
Mannuronic acid	y = 1,1230x + 0,0445	0,99985
Mannitol	y = 2,8498x + 2,9749	0,98096
Fucose	y = 2,4721x + 0,8518	0,99798
Glucose	y = 4,0888x - 0,0481	0,99989
Galactose	y = 3,2093x + 2,2170	0,99845
Mannose/xylose	y = 2,7270x + 2,1679	0,99846