Norwegian University
of Life Sciences

Master's Thesis 2018/2019 60 ECTS
Faculty of Chemistry, Biotechnology and Food Science

Characterization of fatty acids in marine macroalgae by GC-MS

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Acknowledgment

The work presented in this thesis was carried out at the Faculty of Chemistry, Biotechnology and Food Science at the Norwegian University of Life Sciences, during the period of September 2018 until May 2019. It represents 60 ECTS of a 120 ECTS master's degree in chemistry.

The practical labor for the thesis was performed at the research group for chemistry of natural products and organic analytical chemistry, and the dissertation is a study in the field of organic analytical chemistry. Working on this thesis has been both exciting and challenging, additionally it has taught me a great deal about analytical chemistry, instrumentation utilized in these processes and method development. I would therefore like to express my gratitude to everyone who contributed and made this project possible.

First and foremost, I would like to thank my main supervisor, Dag Ekeberg, for his help in the development of the thesis and his commitment to the project, as well as being an inexhaustible source of information in the field of organic analytical chemistry. Additionally, I would like to thank to my co-supervisors, Hanne Devle and Lena Foseid, for their endless help in the methodology, the introduction to instrumentation and the contribution of valuable constructive feedback in the writing process. Thank you for your patience, I couldn't have asked for better supervisors.

I would also like to thank my parents for their input on my writing and their continued support throughout my studies, from start to finish.

Ås, May 13th, 2019

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Summary

The main objective of the study was to identify and quantify the fatty acids present in three distinct lipid fractions, namely neutral lipids, free fatty acids and polar lipids, in the macroalgae; *Alaria esculenta, Saccharina latissima* and *Palmaria palmata.* Seaweeds have long since been a part of staple diet in several East-Asian countries but have only just gained popularity in the West, both as food and for the extraction of valuable compounds utilized in industrial production. Biologically important compounds found in macroalgae, such as polysaccharides, proteins, lipids and polyphenols, suggest that incorporating seaweed into human diet could be beneficial for health. With an exponentially increasing population worldwide, securing renewable sources for food are more important than ever. Thus, macroalgae can potentially be utilized in food and feed to a greater extent in the coming decades.

The fatty acid profiles of all lipid fractions in the three species were identified and quantified by the use of gas chromatography coupled to a multisector mass spectrometer with EBE-geometry. The analytical method utilized in the study had previously been established, tested and validated in the laboratories at the group for chemistry of natural products and organic analytical chemistry at our university. The lipids extracted from the macroalgae were fractioned into neutral lipids, free fatty acids and polar lipids using off-line SPE. The fatty acids were then derivatized into fatty acid methyl esters prior to analysis by GC-MS.

The amounts of mono-, and polyunsaturated fatty acids relative to saturated fatty acids and the ratios of n-6/n-3 fatty acids, as well as the presence of important dietary fatty acids such as; linoleic acid, α-linolenic acid, stearidonic acid, arachidonic acid and eicosapentaenoic acid, were subsequently subjected to discussion to evaluate the potential of each species as human food, and effects they may have on human health.

Sammendrag

Hovedmålet med denne studien var å identifisere og kvantifisere fettsyrene tilstede i tre distinkte fraksjoner, henholdsvis, nøytrale lipider, frie fettsyrer og polare lipider, i makroalgene; *Alaria esculenta, Saccharina latissima* og *Palmaria palmata*. Tang har allerede lenge vært et fast innslag i dietten i flere øst-asiatiske land men har bare nettopp blitt populært i vesten, både som mat og for utvinning av verdifulle forbindelser som brukes i industriell produksjon. Biologisk viktige stoffer funnet i makroalger, slik som polysakkarider, proteiner, lipider og polyfenoler, antyder at implementering av tang i humant kosthold kan være gunstig for helsen. Med en eksponentiell populasjonsvekst verden over er sikring av fornybare kilder til mat viktigere enn noen gang. Derfor kan makroalger potensielt utnyttes i en større grad til mat og fòr i de kommende tiårene.

Fettsyreprofilene til alle lipid fraksjonene i de tre artene ble identifisert og kvantifisert ved bruk av en gasskromatograf koplet til et multisektor massespektrometer med EBE-geometri. Den analytiske metoden tatt i bruk har tidligere blitt etablert, testet og validert i laboratoriene for gruppen for naturstoffkjemi og organisk analytisk kjemi ved vårt universitet. De ekstraherte makroalgelipidene ble fraksjonert i nøytrale lipider, frie fettsyrer og polare lipider ved bruk av fast-faseekstraksjon. Fettsyrene ble så derivatisert videre til fettsyremetylestere før analyse med gasskromatografi-massespektroskopi.

Mengden av en-, og flerumettede fettsyrer relativt til mengden mettede fettsyrer og forholdet mellom n-6/n-3 fettsyrer, i tillegg til forekomsten av viktige kostholds fettsyrer som; linolsyre, α-linolensyre, stearidonsyre, arakidonsyre og eikosapentaensyre ble i etterkant benyttet i diskusjonen for å evaluere potensialet til hver art som menneskelig føde, og effekten de kan ha på human helse.

Abbreviations

AA	Arachidonic acid
ALA	α-linolenic acid
CVD	Cardiovascular disease
CHD	Coronary heart disease
DHA	Docosahexaenoic acid
EFA	Essential fatty acid
EPA	Eicosapentaenoic acid
FA	Fatty acid
FAME	Fatty acid methyl ester
FFA	Free fatty acid
FID	Flame ionization detector
GC	Gas chromatography
LLE	Liquid-liquid extraction
LA	Linoleic acid
LOD	Limit of detection
LOQ	Limit of quantification
MS	Mass spectrometer
MUFA	Monounsaturated fatty acid
NL	Neutral lipid
OA	Oleic acid
PL	Polar lipid
PUFA	Polyunsaturated fatty acid
RIC	Reconstructed ion chromatogram
RRF	Relative response factor
SDA	Stearidonic acid
SFA	Saturated fatty acid
SIM	Selected ion monitoring
SPE	Solid-phase extraction
TG	Triglyceride
UFA	Unsaturated fatty acid

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1 General introduction

Since ancient times the human population have harvested and implemented macroalgae in their diet all around the globe, with instances documented as far back as 4th century Japan. The consumption of macroalgae is today largely dominated by the Asian countries China, Korea, and Japan (Yuan *et al.* 2005), but in recent decades macroalgae have gained popularity as a delicacy in some Western countries as well. Macroalgae constitutes of several interesting nutritional components like polysaccharides, minerals, certain vitamins, proteins, lipids and polyphenols, many of which are important bioactive substances (Holdt and Kraan 2011). This gives seaweed a great potential either directly as food or for the extraction of valuable compounds. It is partly due to this that seaweed cultivation has become a major industry worldwide, and in 2016 the total production of aquatic plants was 30.1 million tonnes, of which most were macroalgae (FAO 2018). Today about 150 different seaweed species are utilized as food, with the most consumed species being the brown alga *Laminaria japonica* (kombu), the red alga *Porphyra spp.* (nori) and the brown alga *Undaria pinnatifida* (wakame). With a steadily growing world population, expected to reach 9 billion by 2050, comes an increasing need for sustainable food sources. Thus, a healthy upscaling of cultivation and the commercialization of macroalgae in the food and feed industry can potentially contribute to secure long-term sustenance for the human population. Compared to cereals cultivated on land, macroalgae grows faster due to higher photosynthetic efficiency, they occupy no arable land and consume no fresh water. Additionally, they contain no lignin, eliminating energy intensive removal in pre-treatment (Palatnik and Zilberman 2017). The high carbohydrate content of macroalgae also makes them suitable resources for conversion to biofuels.

Extensive research has been conducted on the subject of fatty acids to determine their biological activity and impact on human health. This is to establish their individual ability to cause, prevent or treat a numerous of amount of diseases. Studies of polyunsaturated fatty acids have revealed that some PUFAs contribute to prevention of diseases like coronary heart disease, cardiovascular disease, diabetes, inflammatory and autoimmune diseases, while other PUFAs may contribute to the same diseases (Adkins and Kelley 2010, Barceló-Coblijn and Murphy 2009, Connor 2000, Russo 2009, Siriwardhana *et al.* 2012). It has been established that dietary intake of n-3 PUFAs is related to overall human health and are potent in the prevention of CVH. The PUFAs arachidonic acid (AA), eicosapentaenoic acid (EPA) and

docosahexaenoic acid (DHA) have been found to be crucial components in normal bodily growth and paramount in the development of the brain and central nervous system, as well as maintaining normal brain function (Harauma *et al.* 2017, Innis 2008). In 2010 FAO established the PUFAs α-linolenic acid (ALA) and linoleic (LA) as essential fatty acids (EFAs), as the human body is unable to synthesize either and therefore must be supplied in the diet. The main nutritional source of the EFAs are plants and plant seeds. LA and ALA act as precursor molecules to the n-6 fatty acid AA and the n-3 fatty acids EPA and DHA, respectively, and the two EFAs compete to partake in metabolic activities. The body can synthesize AA, EPA and DHA through elongation and desaturation. Although able to synthesize EPA and DHA, the conversion rates in the body is relatively low, additional supplementation of EPA and DHA in the diet is therefore recommended by World Health Organization (WHO). These are found abundantly in fatty fish and other seafood.

It is believed that humans evolved on a diet with n-6 to n-3 ratio of EFAs of about 1/1. In western diets today, we observe ratios of 15-20/1, causing a deficiency of n-3 PUFAs (Simopoulos 2002). The emergence of processed foods, grain fed livestock and fish, hydrogenation and refining of vegetable oils with increased soybean oil in food preparation is likely causes for the reduction of n-3 PUFAs and the increase of n-6 PUFAs in the diet (Saini and Keum 2018). The importance of n-6/n-3 PUFA ratio and the effect it has on human health determined by Simopoulos is reflected in several studies (Liu *et al.* 2013, Russo 2009, Saini and Keum 2018) and the optimal ratio of n-6/n-3 varies from 1/1 to 4/1 depending on the disease in question, as many chronic diseases are multigenetic and multifactorial.

To date, numerous qualitative and quantitative studies have been published in the field of macroalgae, with regard to elucidate potentially beneficial compounds contained in these marine plants. Some have focused on amino acid content and their effects when utilized as animal feed (Gaillard *et al.* 2018). Others have sought to determine seaweeds potential as biofuels, pharmaceuticals and cosmetics (Anyanwu *et al.* 2018). Many research papers have evaluated fatty acid content in a variety of macroalgae species, to assess their effect on human health and potential use in human diet (Bhaskar *et al.* 2004, Biancarosa *et al.* 2018, Colombo *et al.* 2006, Garcia-Vanquero and Hayes 2016, Kumari *et al*. 2010, Schiener *et al.* 2015). However, no study has been carried out to acquire and quantitate fatty acid content in *A. esculenta, S. latissima* and *P. palamata* fractionated into neutral lipids, free fatty acids and polar lipids. Elucidation and quantitation of their complete FA profiles could yield important nutritional information that can potentially mark them as viable for human consumption.

2 Aims of the study

The main aim of this study was to identify and quantify complete FA composition of neutral lipid, free fatty acid and polar lipid fractions in three macroalgae species; *Alaria esculenta*, *Saccharina latissima* and *Palmaria palmata*, utilizing an in-house designed and validated analytical method for derivatized lipids by GC-MS.

The partial objectives are listed below:

- Extraction of lipids from *Alaria esculenta, Saccharina latissima* and *Palmaria palmata* by the use of solvents in a liquid-liquid extraction. Fractioning of the extracted lipids by off-line SPE into three fractions: neutral lipids, free fatty acids and polar lipids, with subsequent analysis by GS-MS to identify and quantify complete FA profiles of the fractions.

- Determine total lipid content and water content of *Alaria esculenta, Saccharina latissima* and *Palmaria palmata.*

- Evaluation of *Alaria esculenta, Saccharina latissima* and *Palmaria palmata* as potential human food, with regard to the FA compositions of the respective fractions and emphasis on PUFA/SFA ratio, amount of MUFAs and PUFAS relative to total FA content, overall n-6/n-3 ratios for each species and the abundance of important dietary FAs like: LA, ALA, SDA, AA, EPA and DHA.

3 Theory

3.1 Macroalgae

Macroalgae, commonly referred to as seaweeds, are a range of multicellular marine plant species. These primitive photosynthetic plants can differ in size from barely visible to several meters long. Generally, macroalgae are divided into three broad groups: green- (*Chlorophyta*), brown- (*Pheaophyta*) and red algae (*Rhodophyta*). Macroalgae can be found in coastal regions in the photic zone, this is where light penetrates the water sufficiently enough for photosynthesis to occur, varying from few meters to upwards of 200 meters deep depending on the clarity of the water. Approximately 11000 different species of macroalgae have been documented, with red algae being the most abundant, with about 7200 species confirmed, followed by brown and green tallying about 2000 and 1800 respectively (Guiry 2019). Seaweeds are strictly benthic plants, meaning they are always attached to seabed or solid surfaces such as rocks, shells, reefs etc. If dislodged, the lifespan of most macroalgae plummets quickly, some may only live for hours (Millar 2011). They derive all their nutrients directly from the surrounding water through diffusion directly into the tissue, thus making them dependent on a constant flow of water for survival. Macroalgae are a crucial part of marine life, in addition to be primary producers they dual as food source and habitat for invertebrates and fish. A large range of species utilize these ocean forests as breeding ground and nurseries (Millar 2011).

3.1.1 *Saccharina latissima*

S. latissima, commonly known as sugar kelp, is a brown alga belonging to the *Laminariaceae* family and the kelp order. Characteristically they have brown-yellowish color with a dimpled center and smooth wavy edges and can reach heights of about 5 meters (tall) with a width of roughly 30 centimeters (**Figure 1)**. Sugar kelp thrives in cold water on rocky surfaces, particularly in bays where it is shielded from high tidal activity. They can be found growing on a wide geographical scale on the northern hemisphere with The North-Eastern Atlantic Ocean and the Barents Sea being the most abundant (Fretwell 2016).

3.1.2 *Alaria esculenta*

A. esculenta, much like *S. latissima*, is a brown alga of the *Alariaceae* family in the kelp order (Laminariales) and are traditionally known as dabberlocks. This macroalga bears a dark brown color and grows to about 2 meters in length. It consists of a strong midrib that runs through the entire frond, which makes it easy to distinguish from other seaweeds (**Fig. 1**). Spawning out of the stipe are small leaves called sporophylls, these contain the spores for reproduction. Dabberlocks preferred habitat are in cold waters on rocky shores, where exposure to tidal activity is high. They are commonly found growing in The North-Eastern Atlantic Ocean, especially around the British Isles (MACOI 2019).

3.1.3 *Palmaria palmata*

The red alga *Palmaria palmata* is a macroalgae belonging to the *Palmariaceae* family and is most often referred to as dulse. *P. palmata* grows directly from a discoid holdfast that gradually widens and subdivides into numerous fronds that range from 20 to 50 centimeters in length with a deep red color (**Fig. 1**). These algae are epilithic and epiphytic, meaning they can grow on both rocky surfaces and anchors on other algae species, especially *Laminaria hyperborea* stipes. The preferred habitat for dulse is in clear sheltered and moderately exposed waters in the subtidal zone, down to a depth of about 20 meters. *Palmata* is most commonly found in The North-Eastern Atlantic Ocean and Canada (Guiry 2019).

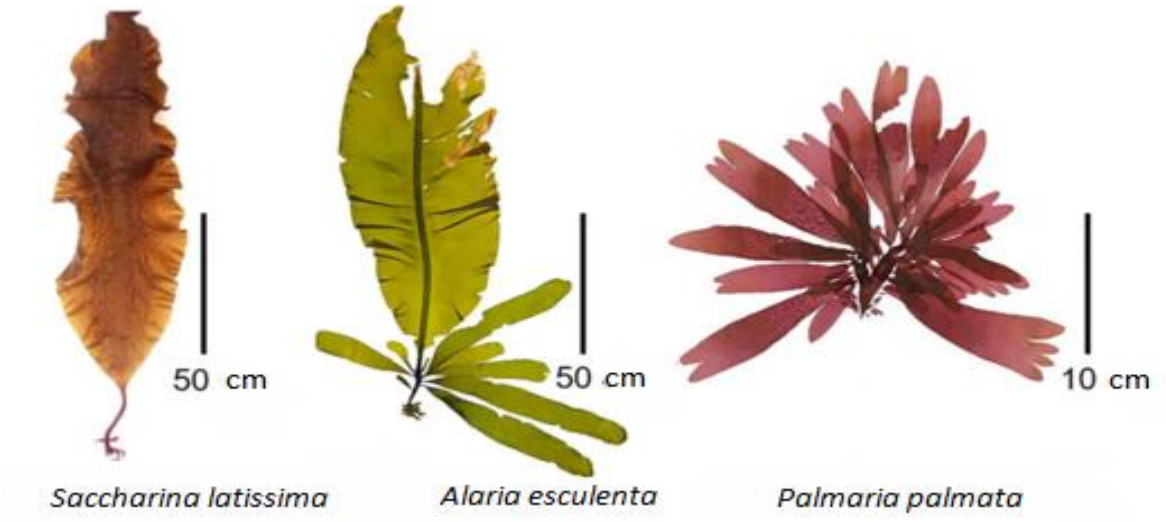


Figure 1: Representative pictures of the three macroalgae *S. latissima*, *A. esculenta* and *P. palmata*, along with indication of size. *Adapted from Roleda *et al.* (accessed 13.05.2019, https://algenett.no/hjem/?Article=69)

3.2 Lipids

The term “lipids” entails a broad group of natural products which includes sterols, waxes, fats, water insoluble vitamins (i.e vitamins A, D, E and K), mono-, di- and triacylglycerols, diglycerides, phospholipids among others. Although no strict definition of a lipid exists, the general consensus seems to define lipids as naturally occurring molecules with a hydrocarbon chain base that is soluble in non-polar, organic solvents (such as diethyl ether, chloroform and hexane). Lipids contribute to several crucial biological processes, some of which are: biological signaling, building blocks in cell membranes and storage of energy in tissue. In biological signaling, lipid molecules are used to convey information between cells. Phospholipids are essential for cell membrane structure; due to the amphiphilic nature of these lipids they can form continuous bilayers (Dewick 2009). As a storage of energy most lipids exist in the form of triglycerides and are often called oils or fats depending on their physical properties in room temperature. These molecules greatly reduce the bulk of diet for many organisms as they are extremely concentrated sources of energy and can be stored in tissue (Holdt and Kraan 2011).

3.2.1 Fatty acids

Fatty acids are carboxylic acids bound to aliphatic hydrocarbon chains of alternate length. This chemical structure gives the molecules both a hydrophilic, polar end and a hydrophobic nonpolar end. Normally the hydrocarbon chains in FAs range from 4 to 28 carbon atoms, with 16, 18, 20 and 22 being the most common lengths as fatty acids tend to exhibit an even number of carbon atoms. Most natural fatty acids appear as straight chains whether they are saturated or unsaturated (Gunstone & Norris 2013).

FAs are classified as either saturated or unsaturated, where saturated fatty acids (SFAs) consists of purely single bonds between the carbon atoms in the chain and unsaturated FAs who have at least one double bond in the carbon chain. UFAs are split into two groups, depending on their degree of unsaturation: monounsaturated fatty acids (MUFAs) containing one double bond and polyunsaturated fatty acids (PUFAs), which contain two or more double bonds in the carbon chain. UFAs exhibit either *trans* or *cis* configuration on the double bonds, with *cis* being the most dominant form, causing the molecules to bend (Gunstone & Norris

2013). The unsaturation of FAs greatly affects their physical properties in room temperature. Triglycerides carrying SFAs exists mostly in solid phase, while triglycerides containing PUFAs tend to appear as liquids of varying viscosity.

In 1979 a systematic method for naming organic chemical compounds was published by the International Union of Pure and Applied Chemistry (IUPAC) along with a recommendation specifically for naming lipids. These reports provide the base for systematic naming (IUPAC names) for organic compounds. The IUPAC nomenclature for FAs includes structural information about the compound, such as number of carbon atoms in the alkyl chain, configuration and position of double bonds present relative to the carboxylic terminus. Trivial names given to FAs before their chemical structure were elucidated are often used interchangeably with the systematic names in literature, often based on their natural source or the plants they were derived from (Gunstone & Norris). Additionally, a shorthand designation that employs numbers and symbols (‘’n’’ or ‘’ ω’’) to describe the chemical structures was developed. This includes information about length of carbon chain, number of unsaturations and position of the double bond closest to the methyl terminus. The different types of nomenclature used to describe six fatty acids commonly found in macroalgae are displayed in **Table 1**.

Table 1. Shorthand designation, IUPAC nomenclature and trivial name of common SFAs, MUFAs and PUFAs typically found in macroalgae

Shorthand designation	IUPAC nomenclature	Trivial name
C14:0	Tetradecanoic acid	Myrstic acid
C16:0	Heksadecanoic acid	Palmitic acid
C16:1n-7	*Cis*-9-heksadeconoic acid	Palmitoleic acid
C18:1n-9	*Cis*-9-octadecanoic acid	Oleic acid
C18:2n-6	*Cis* -9,12-octadecanoic acid	Linoleic acid
C18:3n-3	*Cis*-9,12,15-octadecanoic acid	α-linolenic acid

3.2.2 Phospholipids

Phospholipids are a group of lipids inhabiting crucial structural properties for formation of cell membranes, as previously stated in section 3.2. They consist of a phosphate group coupled together with a glycerol unit and two fatty acid ‘’tails’’ (**Figure 2)**, giving them an amphiphilic nature. Due to their chemical structure they are able to form continuous lipid bilayers that constitute a major part of cell membranes (Dewick 2009).

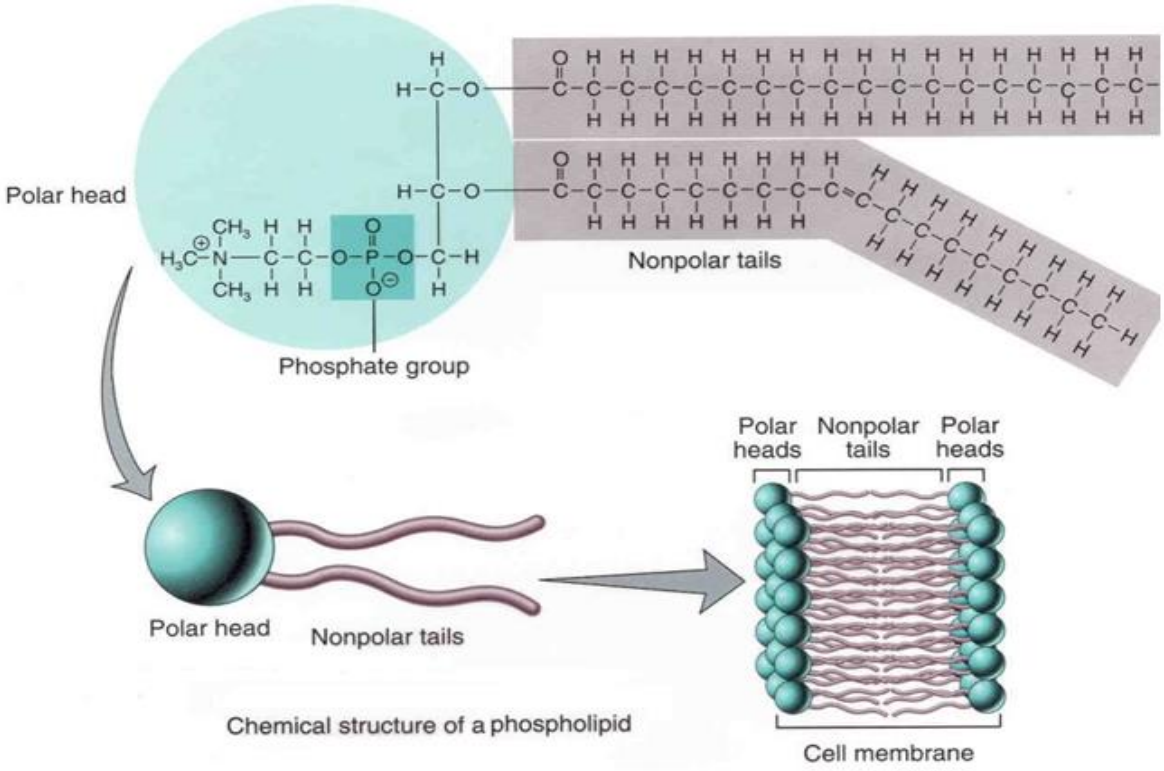


Figure 2: Chemical structure of a phospholipid. The visualization describes the individual components that constitutes a phospholipid, as well as their involvement in the creation of cell membranes. *Adapted from Creative Proteomics (accessed 14.05.2019, https://www.creative-proteomics.com/services/phospholipids-analysis-service.htm).

3.2.3 Acylglycerides

Fatty acids rarely occur in pure form naturally but are found as components of more complex lipid molecules, most commonly forming triglycerides (TGs) with the alcohol glycerol. TGs are formed through esterification of glycerol and three FA units. Although TGs are the most common acylglycerides, they also appear as mono- and diacylglycerides, displayed in **Figure 3**. Natural TGs seldom contain three identical FAs and are referred to as ‘’simple’’ if all three

FAs are identical and ‘’mixed’’ when containing two or more different FAs (Dewick 2009). As previously stated, these lipids are the main form of accumulating energy in plant and animal tissue. TGs are synthesized within the organism, stored in tissue and metabolized when needed.

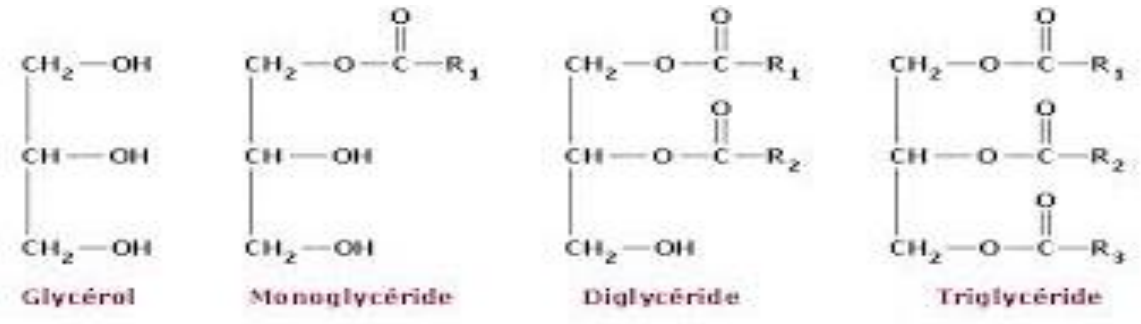


Figure 3: The chemical structure of glycerol, along with the general structure of any given mono-, di-, or triglyceride. *Adapted from Quizlet (accessed 14.05.2019, https://quizlet.com/62551646/fchem-2-extra-part-2-flash-cards/).

3.2.4 Free fatty acids

Free fatty acids are FAs that lack the glycerol backbone that phospholipids and acylglycerides possess. These FAs circulate freely in the plasma and are readily available as energy for the organism. Although mainly used as a source of energy they also play important roles as signaling molecules.

3.2.5 Fatty acids effect on human health

In recent decades a numerous amount of studies and investigations have been launched to discover the metabolism of PUFAs and the biological effect these have on human health, with especially high focus on n-3 fatty acids. We currently know that several n-3 FAs play crucial roles in growth and development of the human body and may play a role in the prevention of diseases like diabetes, coronary artery disease, cancer, inflammatory and autoimmune disorders etc (Simopoulos 1999). It has been suggested that a balanced ratio of consumed n-6 fatty acids to n-3 fatty acids are important for good health (Simopoulos 2002).

The PUFAs linoleic acid (LA) and alpha-linolenic acid (ALA) were deemed the only two essential fatty acids (EFAs) by FAO (2010) with convincing evidence that these must be

contained in the diet, as the human body is unable to synthesize neither. LA and ALA are n-6 and n-3 PUFAs (**Fig. 4)**, respectively, and the amount consumed relative to each other are crucial for normal metabolism, where a ratio of 1-4:1 of LA to ALA was found to be optimal. Through elongation and desaturation LA and ALA act as precursors to long chained PUFAs like arachidonic acid (AA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), as seen in **Figure 4**. As the human body is unable to convert n-6 fatty acids to n-3 fatty acids, a shortage of ALA in the diet has been directly linked to an overall deficiency of n-3 PUFAs (Barceló-Coblijn and Murphy 2009). The eicosanoids (signaling molecules made by oxidation of PUFAs) produced in metabolism of n-3 PUFAs have been found to possess anti-inflammatory, anti-allergic and anti-thrombotic effects, while eicosanoids of n-6 PUFAs exhibit the opposite attributes (Simopoulus 1999). In a study of chronic headaches, patients who increased the consumption of n-3 nutrients and lowered the n-6 intake shifted their PUFA balance significantly, and the need for medication was reduced to nearly half of baseline amount (Ramsden *et al.* 2013).

The n-3 PUFAs EPA and DHA are known to have several benefits against cardiovascular diseases (CVDs) with well established anti-inflammatory and hypotriglyceridemic effects (Siriwardhana *et al.* 2012). The anti-inflammatory effects of EPA and DHA have several mechanisms, one of the more important ones is competitive inhibition of AA and subsequently the formation of the eicosanoid PGE_2, a proinflammatory prostaglandin mediator of AA. EPA and DHA possess antiobesity effects, this is due to their beneficial alteration of the metabolic process by reducing adiposity and increasing lipid oxidation (Siriwardhana *et al.* 2012). A study done by Harauma *et al.* (2017) concluded that both AA and DHA was necessary for the development of brain and central nervous system and for normal bodily growth. DHA is also required for optimal neuronal and retinal function, it plays a key role in maintaining the membrane fluidity which is essential to neurological and cognitive tasks (Saini and Keum 2018).

In human diet, the most prevalent MUFA is oleic acid (OA, C18:1n-9), a fatty acid often found in plant oils and animal-derived fats. OA has been found to possess a slightly LDL-cholesterol lowering effects, as well as modest anti-inflammatory, small blood pressure-lowering effect and may improve glucose control and insulin sensitivity (Calder 2015). Most of the positive health effects of MUFAs were observed when OA replaced SFAs in the diet.

Studies have shown that diets containing high amounts of SFAs can be detrimental to human health. SFAs have several negative health impacts such as raise in total and LDL cholesterol concentrations and increased coagulation, inflammation and insulin resistance. Saturated fatty acids, specially lauric (C12:0), myristic (C14:0) and palmitic (C:16:0), is linked to higher risk of coronary heart disease (CHD), cardiovascular disease (CVD) and type 2 diabetes (Calder 2015). Convincing evidence have been presented to claim that by substituting SFAs with MUFAs and PUFAs in the diet, a decrease in several severe health effects caused by SFAs is observed, such as reduced LDL cholesterol concentration and total/HDL cholesterol ratio, as well as reduced risk of CHD (FAO 2010).

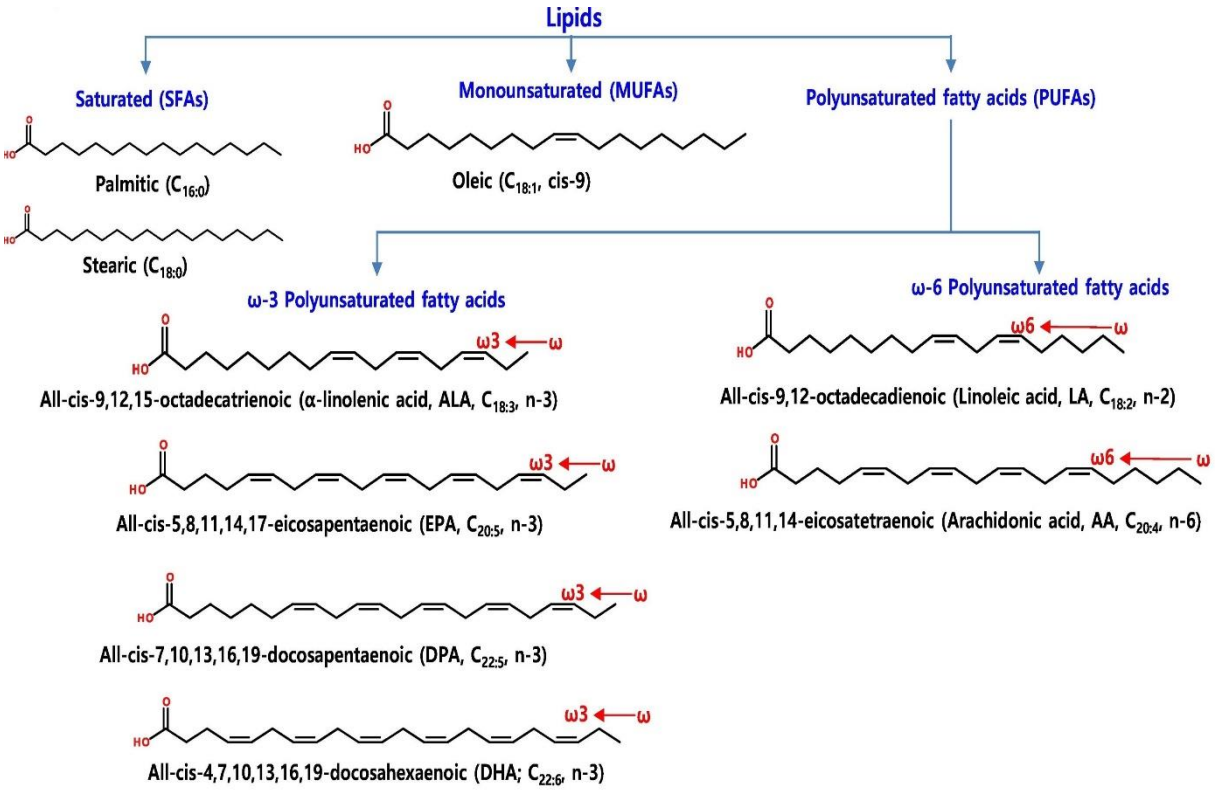


Figure 4: The chemical structures of a SFA, a MUFA and PUFAs with well established effects on human health, many of whom are found abundantly in macroalgae. *Adapted from Saini and Keum (2018).

3.3 Macroalgae as a source of food

Macroalgae have been utilized in the diet for coastal human populations for centuries, and although the vast majority of seaweed is consumed in Asia, we are witnessing an increasing interest in western countries. Typically, macroalgae is used as a part of staple diet in China,

Japan and Korea but is more often seen as a delicacy in the West, with a higher focus on the use of seaweeds for phycocolloids (Kumar *et al.* 2010). Phycocolloids are thickening and gelling agents used in a various assortment of foods and other industrial applications. Today about 250 species of macroalgae are used directly as food or for phycocolloid production, with brown algae represent roughly 66.5 % of the consumed species while red and green algae account for 33 % and 0,5 %, respectively (Kumar *et al.* 2010).

Macroalgae have been found to contain a wide range of substances, and many species are believed to positively effect human health. Constituents found in seaweed include polysaccharides, proteins, lipids, trace elements, polyphenols among others (Anyanwu *et al.* 2018, Holdt and Kraan 2011), many of which are biologically active compounds implicated in the prevention of chronic diseases (Allsopp *et al.* 2016, Holdt and Kraan 2011). The chemical composition of macroalgae can vary greatly both inter- and intraspecies throughout the year with main factors being; habitat, temperature, salinity, light intensity, nutrient availability and other environmental conditions (Anyanwu *et al.* 2018, Kumar *et al.* 2010). Displayed in **Table 2** are the nutritional composition of nine macroalgae species, this includes the content of protein, lipid and carbohydrates relative to dry matter.

Table 2: Proximate distribution of protein, lipid and carbohydrate in selected macroalgae. Displayed as percentage of total dry weight.

Seaweed species	Protein [%]	Lipid [%]	Carbohydrate [%]
Green			
Ulva species	24.4	1.8	61.8
Ulva rigida	6.4	0.3	18.1
Geldidium pristodies	11.8	0.9	43.1
Red			
Gracilaria cervicornis	19.7	0.4	63.1
Porphyra tenara	34.2	0.7	40.7
Alaria	17.1	3.6	39.8
Brown			
Padina	18.8	1.7	31.6
Sargassum vulgare	13.6	0.5	61.6
Laminaria	16.1	2.4	39.3

*Adapted from Chandini *et al.* (2008)

Seaweed contains high amounts of polysaccharides, both in cell wall structural and as storage polysaccharides (Chiandini *et al.* 2008). Macroalgae are reported to contain quantities of up to 74 % of dry weight (Holdt and Kraan 2011) where most of the polysaccharides are dietary fibers. The other major polysaccharides found in macroalgae are phococolloids. These water-soluble carbohydrates are subdivided into three groups; alginates, carrageenan and agar. Phococolloids are applied in food as thickening and gelling compounds and are used to produce ice cream, mayonnaise etc. (FAO 2003).

The protein content of macroalgae vary greatly with species but are generally small in brown algae with a maximal of 24 % of dry weight. Green and red algae express higher levels of protein and have been found to contain up to 44 % of dry weight (Holdt and Kraan 2011), which is comparable to vegetables like soybeans (Chiandini *et al.* 2008). It has been established that most seaweed species contain all essential amino acids. They are rich in acidic amino acids (glutamatic and aspartic) with sulphur amino acids (cysteine and methionine), lysine, tryptophan and histidine found to be the limiting amino acids (Gaillard *et al.* 2018, Holdt and Kraan 2011). To determine the nutritional value of proteins, referred to as ‘’amino acid score’’, the composition of essential amino acids is evaluated. Macroalgae typically express amino acid score values ranging from 60 to 100, which is higher than for proteins found in vegetables and cereals (Murata and Nakazoe 2001).

The lipid content of seaweed is generally low across all species, with a maximum of 4,5 % of dry weight (Chiandini *et al.* 2008, Holdt and Kraan 2011) The majority of lipids in macroalgae is stated by Murata and Nakazoe (2001) to be phospholipids followed by neutral and glycolipids, contrary, Bhasker *et al.* (2004) found glycolipids to be the main class of lipids, followed by neutral and phospholipids . Just like the other biochemical components in macroalgae, fatty acids vary depending on season and environmental factors. It is established that macroalgae contain high contents of PUFAs and MUFAs relative to SFAs as well as high n-3 fatty acid contents. The FA composition of macroalgae typically consist of the fatty acids C14:0, C16:0, C16:1, C18:0, OA, LA, ALA, stearidonic acid (SA), AA and EPA (Biancarosa *et al.* 2018).

3.4 Lipid analysis

Throughout scientific history a number of different procedures have been employed to analyze biological matrices. In the field of qualitative and quantitative lipid analysis, several thin layer chromatography (TLC) techniques were early in use. This is mainly used to separate and determine the concentration of different types of lipid groups, the effect is achieved through ‘’spotting’’ of a matrix on a TLC plate coated in a suitable absorbing material placed in an appropriate solvent (Malis and Mangold 1960). As science progresses so does the equipment and techniques, and in recent decades more novel methods for lipid analysis have been developed, such as: electrospray ionization mass spectrometry (ESI-MS), matrix-assisted laser desorption and ionization time-of-flight mass spectrometry (MALDI-TOF-MS) and high-performance liquid chromatography (HPLC). However, gas chromatography mass spectrometry (GC-MS) remain the most commonly utilized method, as it is regarded as ‘’gold standard’’ for identification of substances. This method requires the analytes to be volatile, as such, lipids are derivatized into methyl ester prior to analysis.

3.4.1 Lipid extraction

For the extraction of lipids from biological matrices two vastly utilized methods are deployed, namely those of Folch *et al.* (1957) and Bligh and Dyer (1959). Folch *et al.* developed a simple method for isolating total lipid content from tissue by exposing it to a mixture of 2:1 choloroform methanol solution (v/v) and water containing a mineral salt for a liquid-liquid extraction. The combined use of non-polar and polar solvents is necessary in order to extract both neutral and polar lipids from the tissue. Additionally, the method is applicable to tissues containing relatively low contents of lipids (Folch *et al.* 1957). The method developed by Bligh and Dyer (1959) is a rapid and simple technique to extract and purify lipids from biological materials. Contrary to Folch *et al.* this method employs a 1:2 chloroform methanol mixture (v/v) to separate lipids from the tissue. The method was designed to extract lipids from fish tissue with high water content (~80 %), but many alterations are permissible to adapt the method for use with other tissues (Bligh and Dyer 1959).

Although many methods have been employed to study and elucidate the FA profiles of macroalgae (Kumari *et al.* 2010, Matanjun *et al.* 2008, Sanchez-Machado *et al.* 2004), the Bligh and Dyer and Folch methods based on chloroform/methanol solvent systems are

invariably used as standard techniques (Kumari *et al.* 2011). Other solvent systems like dichloromethane/methanol (Graeve *et al.* 2002) and diethyl ether (El-Shoubaky *et al.* 2008) have been employed, as there are no established criteria for the selection of appropriate solvents. Thus, it is left to the individual researcher to decide. However, comparison and verification of extraction and derivatization methods are still required to evaluate which best suits different matrices as to obtain accurate qualitative and quantitative results.

In order to obtain representative sample results it is vitally important that the biological matrices exposed to solvent extraction is representative for the species in its entirety. This problem is mainly solved through homogenization of the sample materials. Homogenous samples are achieved by disruption of the biological tissue, and several chemical and mechanical disruption methods exist. Cryopulverization is a commonly utilized method, where the samples are flash frozen with liquid nitrogen and subsequently homogenized through use of mortar and pestle (Smucker and Pfister 1975). The cryopulverization serves a dual purpose; submerging the samples in liquid nitrogen causes it to become brittle due to the water contained in biological tissue, thus making the pulverization process easier. Additionally, the cells in the tissue are disrupted prompting the release of lipids contained inside the cell and in the cell membranes (Burden 2012). The drawbacks of this method are potential loss of small sample amounts and the finite capacity to process a large sample quantity (Burden 2012).

3.4.2 Solid-phase extraction

Solid-phase extraction (SPE) is an extensively used sample preparation technique in analytical chemistry (Andrade-Eiroa *et al.* 20016, Hennion 1999, Poole 2003) and can be performed either off-line or on-line. When on-line SPE is employed, the sample preparation method is directly coupled to the chromatographic system used for analysis, this eliminates the need for further treatment of the samples (Hennion 1999). With the use of off-line SPE, the samples require further handling prior to analysis. Initially SPE was developed as a complement or replacement method to liquid-liquid extraction (LLE), as LLE is labor intensive, difficult to automate and consumes relatively large amount of solvents (Poole 2003).

SPE uses two major principles; retention and elution, based on the affinity the sample compounds have towards the stationary phase or the mobile phase (Simpson 2000). The

stationary phase is comprised of a solid material that acts as a sorbent for the analytes. Typically, the sorbent consists of n-alkylsilica, that comes in disposable cartridges (Poole 2003). However, the main factor in choice of sorbent material is the analyte of interest, as different analytes require sorbent materials with different attributes.

In the SPE process the analytes are deposited on the column with the stationary phase, often subsequent to conditioning of the sorbent with an appropriate solvent (hexane or heptane). The analytes are then distributed between the liquid sample and the solid phase, by either adsorption to the surface or through penetration of the other layer of molecules, which creates an equilibrium (Simpson 2000). Elution occurs when a mobile phase the analytes have a greater affinity to is introduced, thus, the compound of interest is desorbed from the stationary phase and collected. An additional washing step may be utilized, where a solvent with no affinity to the analyte of interest is used to eliminate unwanted compounds from the sample. This principle can be applied to separate different classes of lipids into fractions, by use of distinct solvents as mobile phases. Presented in **figure 1** is a graphical representation of the SPE process.

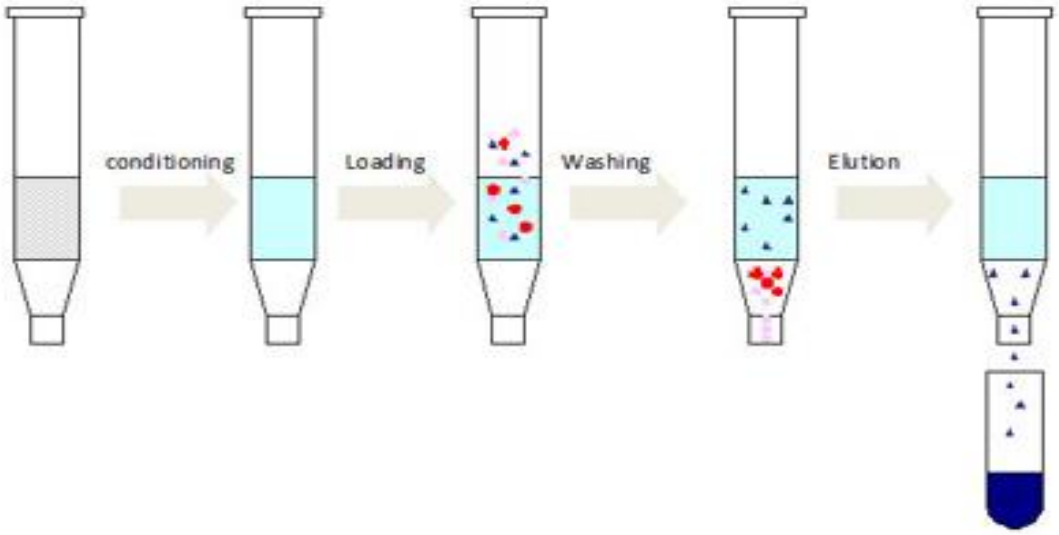


Figure 5: The basic principles of SPE, highlighting the retention of analyte molecules in the sorbent, and elution by the use of a solvent as a mobile phase. With an additional washing phase displayed. *Adapted from Affinisep (accessed 05.04.2019, https://www.affinisep.com/technology/solid-phase-extraction/).

3.4.3 Transestrification

Preliminary to analysis of samples by GC-MS, FAs are required to be derivatized into fatty acid methyl esters (FAMEs), due to their limited volatility (Devle 2013). Normally FAs are esterified through heating in the presence of excess of anhydrous methanol with an acidic catalyst or rapid esterification in anhydrous methanol with a basic catalyst (Christie 2012), this results in the elimination of the glycerol unit from the FAs (**Figure 6**). The use of the basic catalyst, sodium methoxide, is the most common method used for glycerolipids, as this causes a complete transesterification at room temperatures in few minutes. The mild conditions this technique employs prevent undesirable additional reactions, like isomerization of double bonds in MUFAs and PUFAs (Christie 2012). Free fatty acids are not esterified under basic conditions but are easily transesterified with an acidic catalyst. Boron trifluoride in methanol (10-14%, w/v) is commonly used for rapid transesterification of FFAs, although additional heating is required for complete reaction. The method also results in few undesirable reactions and may be utilized for transesterification of both FFAs and PLs (Christie 2012).

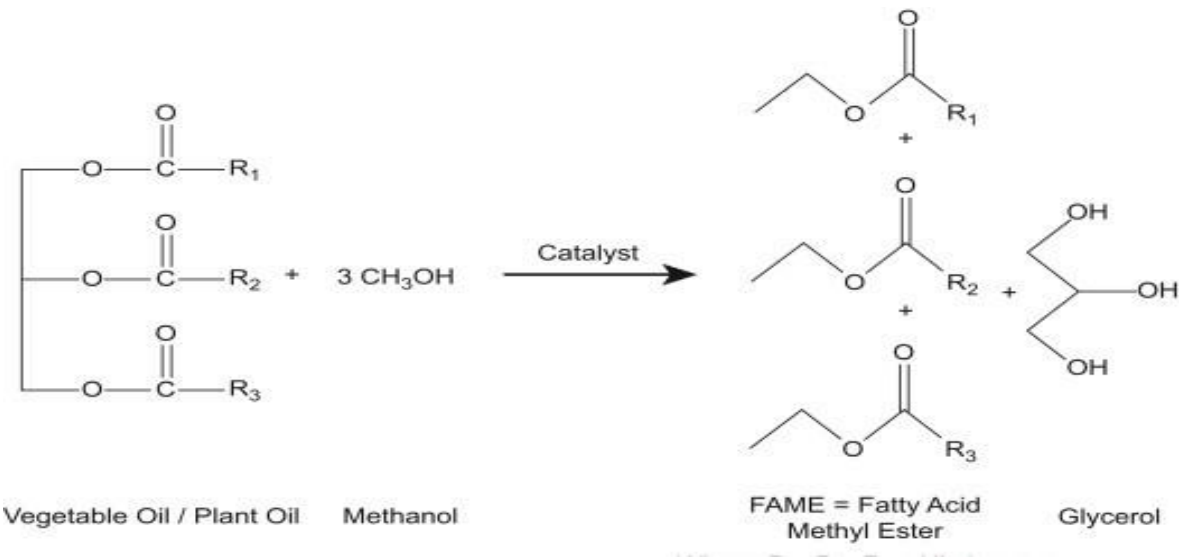


Figure 6: Basic reaction scheme for the transesterification of a triglyceride into fatty acid methyl esters, in the presence of anhydrous methanol and a catalyst. *Adapted from Patel and Shah (2015).

3.4.4 Gas chromatography mass spectrometry

In analytical chemistry, upon the completion of any sample preparation, an analysis is needed in order to identify and quantify the compounds contained in the matrix. The instrumentation utilized in the analysis must be able to separate complex sample mixtures to the extent that all analytes contained within may be identified. Currently gas chromatography (GC) is among the most commonly used technologies for the analysis of fatty acids. Present-day GC methods allows for sensitive and reproducible fatty acid analyses, as well as the characterization of complex mixtures in combination with other chromatographic separations and spectroscopic identification (Seppänen-Laakso *et al.* 2002). The molecules separated by the GC are handled by a directly coupled detector instrument, most commonly used is flame-ionization detector (FID) or mass spectrometer (MS).

The GC-MS process of analyzing FAMEs consist of several steps, including injection, separation, identification and quantification. High precision and accuracy are attained through optimization of these. In the injection phase, the FAMEs are deposited on the instrument and vaporized. The vaporized FAMEs are then carried through the GC column stationary phase by an inert gas, most commonly helium or in some cases hydrogen. The separation occurs as the analytes passes through the column, interactions between the compounds and the stationary phase affects the time each individual analyte require for elution. The most commonly used stationary phase are silica-fused capillary columns, these offer an exponentially higher resolution capacity than the previously used packed columns (Eder 1995). The drawback of utilizing silica-fused capillary columns is the need for detectors with higher response and sensitivity (Eder 1995).

For identification and quantification of the FAMEs, FID or MS detectors are commonly used. MS detectors have the ability to provide spectrometric data such as molecular weight, empirical formula, structural information and even complete structures of unknown compounds (Christie 2012), while FID depends solely on comparing retention times to reference standards to identify FAMEs (Devle 2013). As such, GC coupled to a MS detector is the most utilized method for identification and quantification of FAMEs.

4 Materials and methods

4.1 Chemicals and standards

For quantification of FAMEs a total of three different internal standards were chosen, all provided by Larodan AB (Malmö, Sweden). Nonadecanoic acid (C19:0), trinonadecanoin (C19:0), 1,2- Dinonadecanoyl-sn-Glycero-3-phosphatidylcholine (C19:0) used to quantify FFA-, NL- and PL fractions, respectively. The IS solutions were made by dissolving standard in chloroform to a final concentration of 10 mg/mL. To identify the FAMEs a 37-component Food Industry Fame Mix (Restek, Bellefonte, PA, USA) was utilized.
The methanol used in the extraction process, the SPE procedure and to make the sodium methoxide solution, was provided by Sigma-Aldrich (Steinheim, Germany) and of Chromasolv quality. Chloroform used for the Folch mix and for elution in SPE was supplied by Sigma-Aldrich and of Chromasov quality. A mix of diethyl ether puriss. p.a. ≥99,8% and acetic acid 96% puriss. p.a was used in the eluation of FFAs in SPE, these were supplied by Sigma-Aldrich and Riedel-de Haën (Germany), respectively. In the transestrifiction of lipids to FAMEs 10% (~1,3 M) boron-trifluoride-methanol was utilized, procured from Sigma-Aldrich. Prior to GC-MS the FAMEs were dissolved in heptane ≥99% n-heptane provided by Sigma-Aldrich.

4.2 Pretreatment of the samples

The macroalgae was provided by Seaweed Energy Solutions and harvested on the west coast of Norway, outside Sør-Trøndelag County Municipality in May and June 2018. It was vacuum packed and frozen prior to the shipment to the university. On arrival it was placed in a freezer holding -20°C until usage. The algae were thawed, crudely rinsed (the algae were cultivated on rope, therefore extensive cleaning was unnecessary) and cut into pieces (approximately 1x1 cm). The algae were frozen with liquid N_2 (99,9999%, AGA, the Linde Group, Munich, Germany) and freeze dried (Alpha 2–4 LD plus, Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany) for 72 hours to ensure that all water was extracted. Further the freeze-dried material were milled into a particle size of 1x1 mm or less using a Retsch SM 2000 (F.kurt RETSCH GmbH & Co. KG, Haan, Germany), no differentiation was made between stipe and blade.

4.3 Water content

Samples of the three algae unused in the previously described process were utilized to determine total water content of each species. The macroalgae were dried of external water and placed in crucibles. Four sample replicates of each species containing ~10 g was prepared. The crucibles were incubated at 105°C (VD-23, Binder GmbH, Germany), until constant mass was obtained. After incubation, the crucibles were cooled at room temperature in a desiccator, and subsequently weighed. Water content of the samples was expressed as $\%H_2O$ per gram algae.

4.4 Lipid extraction

Four sample replicates of each macroalgae were prepared and treated separately during sample preparation, all samples were weighed out within the parameters of 5 g ± 0.03. Additionally, a blank replicate was prepared for each alga. A modified version of Folch's method (Devle 2009) was used to extract lipids from the macroalgae. 100 mL $CHCl_3$:MeOH (2:1) was added to a Pyrex 250 mL bottle with screw cap, containing the sample. 50 µL of each IS solution was then added using a Hamilton syringe. The sample tube was shaken horizontally for 20 min (Biosan Ltd., PSU 10i, Riga, Latvia) at 350 rpm. The sample solution was then transferred to a separatory funnel, where 20 ml 0.9% NaCl(aq) was added to induce phase separation. After approximately 20 min the organic phase was transferred to a beaker. The polar phase was then reextracted two additional times with 66 mL $CHCl_3$. The organic phases were combined and evaporated with a vacuum evaporator (Q-101, Buchi Labortechnik AG, Flawil, Switzerland) at 35 °C. After evaporation the sample was re-dissolved with 5 mL $CHCl_3$, transferred to centrifugal vials (1,5 mL) and centrifuged (Sigma 1-14, Sigma Laborzentrifugen GmbH, Germany) at 14800 rpm for 5 min to remove particle matter. The sample was then evaporated with nitrogen gas (N_2) at 30 °C, for then to be re-dissolved with 1 mL $CHCl_3$ and transferred to vials prior to solid phase extraction (SPE).

4.5 Solid phase extraction

The off-line SPE procedure was carried out by a GX-274 ASPEC (Gilson, Middleton, WI, USA) and use of the software program TRILUTION® LH Software version 3.0 (Gilson, Middleton, WI, USA). As stationary phases, the SPE Bond Elut NH2 500 mg, 3 mL columns (Agilent Technologies, USA) were used and were conditioned with 7.5 mL hexane. 500 µL of

the sample was then applied to the columns. The NLs were eluted with 5 mL chloroform, the FFAs were eluted using 5 mL diethyl ether:acetic acid (98:2 v/v%) and the PLs were eluted with 5 mL methanol. The lipid fractions were transferred to 6 mL Duran® GL 14 culture tubes (Mainz, Germany). The fractions were then evaporated using nitrogen gas at 40 ℃.

4.6 Derivatization to FAMEs

For the formation of FAMEs, the dried NL and PL fractions were re-dissolved in 2 mL heptane, before addition of 1.5 mL (3.3 mg/mL) sodium methoxide. The sodium methoxide was made by dissolving metallic sodium (purum, Merck, Darmstadt, Germany) in methanol. The tubes were then placed on an orbital shaker (Biosan Ltd., PSU 10i) and shaken for 30 min at 350 rpm, before left to settle vertically for 10 min. The heptane phase was so transferred to vials and stored at -20 ℃ prior to analysis by GC-MS. The FFA fractions were added 1 mL 10% boron-trifluoride-methanol (Sigma-Aldrich) solution and placed in water bath for 5 min at 70 ℃. 1 mL of heptane was added to each tube and the contents stirred with a vortex mixer. The heptane phases were transferred to GC vials and stored at -20 ℃ before analysis.

4.7 GC-MS analysis of FAMEs

Identifying the FAMEs was done by utilizing an Autospec Ultima GC-MS (Micromass Ltd. Manchester, England). The MS was a three-sector instrument with an EBE geometry. The ionization method used was electron ionization (EI) in positive mode, where electrons were accelerated to 70 eV before collision with analyte molecules at 250 ℃. The range scanned was set to 40-600 *m/z* and the resolution was tuned to 1000. The transfer line temperature was kept at 270 ℃. Coupled with the mass spectrometer was an Agilent 6890 Series gas chromatograph (Agilent Technology, Wilmington, DE, USA). The GC was equipped with a CTCPAL Autosampler (CYC Analytics, AG, Zwingen, Switzerland), with a split ratio of 1/10 and injections of 1 µL sample. The separation was carried out on a 60 m Restek column (Rtx®-2330) with 0.25 mm I.D. and a 0.2 µm film thickness of fused silica biscyanopropyl cyanopropylphenyl polysiloxane stationary phase (Restek Corporation, 256 Bellefonte, PA, USA). As carrier gas, helium (99,9999%, Yara, Rjukan, Norway) was used at a constant flow of 1 mL/min. Run time set for the analysis were 92 minutes total, with the initial oven temperature set to 65 °C for 3 minutes. Then, at a rate of 40 °C/min temperature was increased to 150 °C and held for 13 minutes. Next, it was held at 151 °C for 20 minutes. The

temperature was then increased at a rate of 2 °C/min to 230 °C and held for 10 minutes. Lastly, the temperature was held at 240 °C for 3.7 minutes after an increase of 50 °C/min. Four parallels and an additional blank of undiluted replicates where subjected to GC-MS analysis, with a single injection per replicate. Prior to analysis of the algae replicates, heptane replicate injections were employed to clean the instrument of any possible contaminants. Masslynx 4.0 (Waters, Milford, MA, USA) was the software used in the GC-MS analysis. Identification of the FAMEs was made possible by comparing retention times with standards along with searches in the MS library NIST 2014 Mass Spectral Library (Gaithersburg, MD, USA).

4.8 Method validation

The method validation of GC-MS as an analytical method for identification and quantification of FAMEs was carried out by Devle *et al.* (2009). The analytical parameters subjected to testing in their study were; linearity, sensitivity, selectivity, accuracy, repeatability, LOD and LOQ, using three acquisition modes: full scan, SIM and RIC. For the method validation, FAs derivatized from ewe milk samples and a 38 FAME mix was utilized. Satisfying results were reported for all analytical parameters and values for LOD and LOQ were in the ng/mL range for all acquisition modes.

4.9 Quantification of FAMEs

The quantification of the FAMEs required the use of relative response factors, displayed in **appendix III**, previously determined by Devle *et al.* (2009). The RRF-values were obtained through analysis of Restek Food Industry FAME Mix in four concentrations: 150, 300, 600 and 1200 µg/mL. Duplicates of each concentrations, as well as two injection replicates of each concentration were subjected to GC-MS analysis (Devle *et al.* 2009). FAMEs not represented in the Restek Food Industry FAME Mix were assigned reasonable RRF-values (e.g. C18:1n-7c and C20:4n-3c were assigned the same RRF-value as C18:1n-9c and C20:4n-6c, respectively). A total of three internal standards were used to quantify the FAMEs, one for each of the FAME fractions (NL, FFA and PL). The concentrations and volumes of the internal standards are displayed in **appendix II**. The quantitation of each respective FA was calculated according to equation 1, displayed below.

Eq. 1

$$Mass_{FAME} = \frac{Area_{SAMPLE} * Cons_{IS} * Volume_{sample} * 1000}{Area_{IS} * RRF * g_{ALGAE}}$$

5 Results and discussion

To identify and quantify FAs present in miniscule concentrations, the highly selective and sensitive method of GC-MS coupled with full scan acquisition was selected for this study. Although SIM and RIC offer better selectivity and specificity by scanning for pre-determined ions (Devle *et al.* 2013), full scan acquisition was chosen due to its ability to identify FAMEs based on spectral information and library searches. This provides the ability to identify FAMEs with no representative reference standards. The analysis of the three macroalgae by GC-MS resulted in the identification and quantification of 32, 27 and 28 total FAs for *A. esculenta, S. latissima* and *P. palmata*, respectively, containing between 10 and 24 carbon atoms. This is a higher number than previously reported for *A. esculenta* and *P. palmata* (Mæhre *et al.* 2014, Sánchez-Machado 2004), but coincides well with reported finds for *S. latissima* (Marinho *et al.* 2015).

Due to large variations in peak response from the internal standards within the fractions, the calculations to determine amount (μg/g DW) of every respective FA present in the lipid fractions yielded false values. Thus, the results are presented as percent of total response for each fraction. This provides viable results as the response of each peak is directly proportional to the total amount of all registered peaks in the fraction. The likely cause of the large variations in IS response is non-homogenized internal standards during addition to the samples. The internal standards were dissolved in chloroform and kept at -20 ℃ prior to the lipid extraction process, as such the internal standards may have precipitated out of the solution. Calculated average fatty acid concentration along with standard deviation for all NL, FFA and PL fractions can be found in **appendix IV** through **XII**. Presented in these appendices are also retention times and average peak areas, along with values for matchfactor, reverse matchfactor and probability, acquired through library searches in NIST 2014 based on spectral information. Lack of sample material made it impossible to repeat the lipid extraction.

5.1 Total lipid and water content

The total lipid content in macroalgae is generally low across all species, >1-4.5 % (Holdt and Kraan 2011). Total lipid content relative to dry weight in *A. esculenta, S. latissima* and *P. palmata* were found to be 1.80, 3.25 and 3.20 %, respectively, which is consistent with values reported in previous studies of the same species (Barbosa *et al.* 2017, Biancarosa *et al.* 2018). Water content, displayed in **Table 3** along with total lipid content, of the three macroalgae ranged from 76-87 %, similar amounts was described by Schiener *et al.* (2015) regarding *A. esculenta* and *S. latissima*. No previous publication describing water content in *P. palmata* was found. The lipid content of the three macroalgae were fractionated into neutral lipids, free fatty acids and polar lipids, and the fatty acid profiles for each fraction were determined in all species.

Table 3: Total lipid content (mg/g DW) of *Alaria esculenta, Saccharina latissima* and *Palmaria plamata*, presented in the table is also water content for the same species given as %water per g alga (n=4, for both total lipid and water content).

Species	Total lipid [mg/g DW]	Water content [%]
Alaria esculenta	17.96 ± 2.85	78.2 ± 0.8
Saccharina latissima	32.45 ± 2.98	87.2 ± 0.2
Palmaria palmata	31.96 ± 6.00	76.6 ± 0.5

5.2 Neutral lipid fraction in *A. esculenta, S. latissima* and *P. palmata*

In the NL fraction 27, 26 and 18 FAs were identified and quantified in *A. esculenta, S. latissima* and *P. palmata,* respectively. These constitutes 56.9 % of the total FA content in *A. esculenta*, while only 21.6 and 25.4 % for *S. latissima* and *P. palmata*. Although several FAs were identified in the fraction, the same 10 fatty acids predominated in *A. esculenta* and *S. latissima.* While most of the same FAs predominated in *P. palmata,* some of the PUFAs were found in significantly smaller proportions. Among the predominating FAs, three were SFAs (C14:0, C16:0 and C18:0), two were MUFAs (C16:1n-7c and C18:1n-9c) and five were PUFAs (C18:2n-6c, C18:3n-3c, C18:4n-3c, C20:4n-6c and C20:5n-3c). These FAs constitute more than 95 % of total fatty acids in the NL fraction for all three species. A fatty acid was deemed predominant if it was present in more than 2 % in one of the species. Previous studies

on the same species performed by Biancarosa *et al.* (2018), Marinho *et al.* (2015) and Robertson *et al.* (2015) reported correlating values for the predominating FAs, with the exception of C18:4n-3c in *A. esculenta* (Biancarosa *et al.* 2018). The variations are most likely due to seasonal, geographical or environmental factors. Displayed in **Figure 7** is a graphical representation of the predominating FAs in the three seaweeds. Total fatty acid profile for *A. esculenta, S. latissima* and *P. palmata*, is presented in **Table 4**.

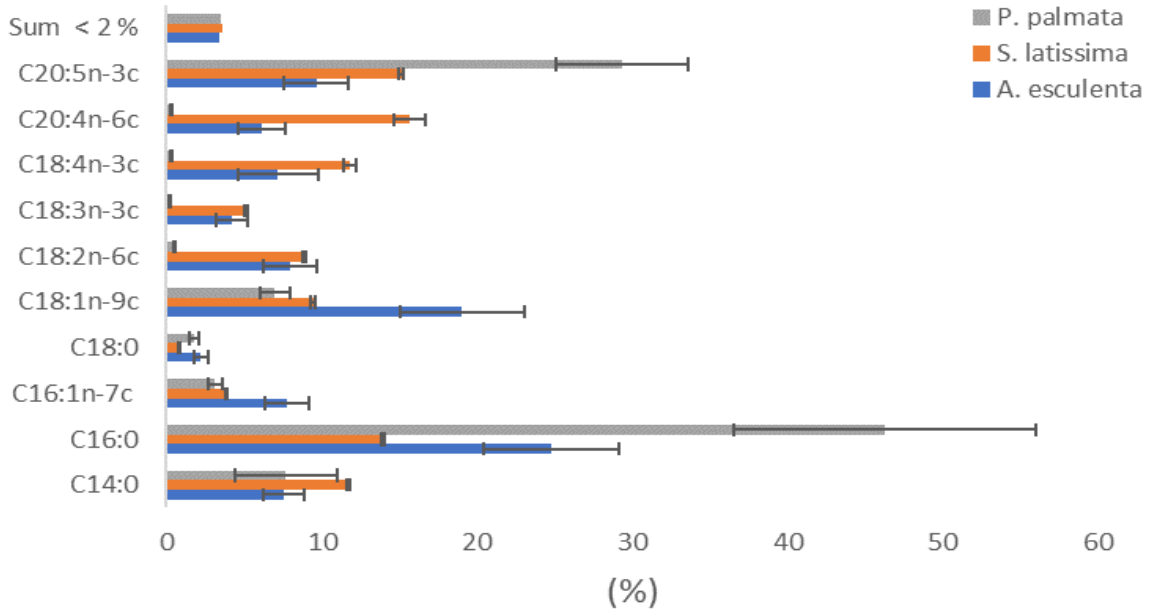


Figure 7: Fatty acid profile for fatty acids in the NL fraction that constitutes of more than 2 % of total fatty acid content, in at least one of the fractions. SUM < 2% is the summarized contribution of the remaining fatty acids (n=4 for *A. esculenta* and *P. palmata*, n=3 for *S. latissima*, error bars = ±SD).

Of the predominant fatty acids several are important dietary PUFAs. Included in these are the two essential fatty acids linoleic acid (LA, C18:2n-6c) and α-linolenic acid (ALA, C18:3n-3c), as well as stearidonic acid (SDA, C18:4n-3c), arachidonic acid (AA, C20:4n-6c) and eicosapentaenoic acid (EPA, C20:5n-3c). Although *P. palmata* contained significantly less LA, ALA, SDA and AA, the content of EPA was exponentially higher than in *A. esculenta* and *S. latissima* (29.4 compared to 9.7 and 15.0 % of the NL fraction). The NL fractions also contain relatively high amounts of the MUFA oleic acid (OA, C18.1n-9c) across all species. Several factors contribute to the evaluation of macroalgae as a potentially health enhancing food. Although protein and polysaccharides are more likely to affect human health than lipids through consumption of macroalgae, as seaweed contain substantially larger amounts off these compounds compared to lipids. When evaluating macroalgae as potentially health

beneficial from a purely FA point of view, factors such as the ratio of PUFAs/SFAs, amount of EFAs (LA and ALA) and n-3/n-6 ratio are considered. The amount of SFAs, MUFAs and PUFAs vary greatly intraspecies in the NL fraction. The highest amount of SFAs was observed in *P. palmata* with 56.1 % compared to 35.6 and 27.6 % for *A. esculenta* and *S. latissima*, respectively. While MUFAs and PUFAs constituted of 27.2 and 37.2 %, 13.8 and 58.6 %, 13.3 and 30.6 % of total fatty acids in *A. esculenta* and *S. latissima* and *P. palmata*, respectively.

S. latissima contain the largest quantities of both LA (8.9 %) and ALA (5.2 %), which is only slightly higher than for *A. esculenta* containing 8.0 and 4.2 %, respectively, but exponentially higher than for *P. palmata* who only exhibit amounts of 0.5 and 0.1 % of LA and ALA. As highlighted in **Figure 7**, *S. latissima* contain the absolute highest amounts of SDA and AA, while *P. palmata* exhibit exceptionally large amounts of EPA compared to the other two. The same trends are reported by Biancarosa *et al.* (2018) for all three species. In the neutral lipid fraction *S. latissima* also expressed the highest values of both n-3 and n-6 PUFAs, only somewhat more abundant than *A. esculenta.* Although *P. palmata* had similar values of n-3, almost no n-6 PUFAs were found. Shown in **Figure 8** is a comparison of the SFA, MUFA, PUFA, n-3 and n-6 content in the NL fraction for the three macroalgae.

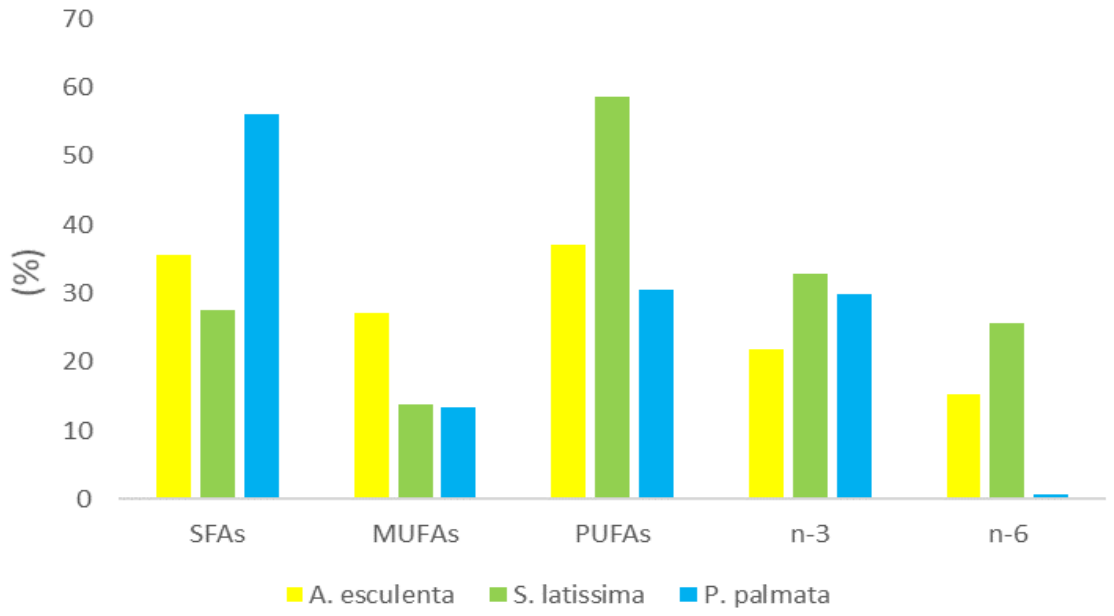


Figure 8: A graphical representation of SFA, MUFA, PUFA, n-3 and n-6 distribution relative to total FA content in the NL fraction for each respective species (n=4 for *A. esculenta* and *P. palmata*, n=3 for *S. latissima*).

Table 4: Fatty acid content (%) for each respective FA in the NL fraction (n=4 for *A. esculenta* and *P. palmata*, n=3 for *S. latissima*).

	Neutral lipids		
FAME	*A. esculenta*	*S. latissima*	*P. palmata*
C12:0	0.03	0.03	n.d.
C13:0	n.d.	0.03	n.d.
C14:0	7.58	11.72	7.70
C14:1n-5c	n.d.	0.13	n.d.
C15:0	0.25	0.49	0.21
C16:0	24.76	13.94	46.26
C16:1n-7c	7.75	3.86	3.15
C16:1n-5c [a)]	0.09	0.15	0.50
C17:0	0.14	0.18	n.d.
C16:2n-6c [a)]	0.04	0.11	n.d.
C16:2n-4c [a)]	0.12	0.18	n.d.
C17:1n-7c [a)]	0.06	0.09	n.d.
C18:0	2.24	0.78	1.80
C18:1n-9c	19.07	9.46	6.98
C18:1n-8c [a)]	0.15	0.12	n.d.
C18:1n-7c [a)]	n.d.	n.d.	1.02
C18:2n-6c	7.95	8.87	0.47
C18:3n-6c	0.79	0.67	n.d.
C20:0	0.60	0.44	n.d.
C18:3n-3c	4.23	5.15	0.13
C20:1n-9c	0.07	n.d.	0.27
C18:4n-3c [a)]	7.20	11.83	0.30
C22:0	n.d.	n.d.	0.08
C20:2n-6c	0.06	0.08	n.d.
C20:3n-6c	0.35	0.24	n.d.
C20:4n-6c	6.11	15.64	0.29
C22:1n-9c	0.03	n.d.	0.27
C20:4n-3c [a)]	0.41	0.59	n.d.
C20:5n-3c	9.68	15.12	29.37
C24:0	n.d.	n.d.	0.07
C21:5n-3c [a)]	0.08	n.d.	n.d.
C24:1n-9c	n.d.	n.d.	1.12
C22:6n-3c [a)]	0.15	0.09	n.d.

5.3 Free fatty acid fraction in *A. esculenta, S. latissima* and *P. palmata*

The analysis of the FFA fraction resulted in the detection and quantification of 25, 25 and 27 FAs for *A. esculenta, S. latissima* and *P. palmata,* respectively. In *S. latissima* and *P. palmata* the free fatty acids are the most substantial fraction, containing 59.3 and 42.3 % of the total fatty acid content, respectively, while for *A. esculenta* these constitute 17.6 %. The same 10 fatty acids that predominated in the NL fraction were also the most abundant in the FFA fraction for all species. While some of the same trends in the distribution of fatty acids can be observed, there is a substantially larger amount of SFAs in the FFA fraction in all three algae, as shown in **figure x**. The increase of SFAs results in the subsequent decrease of PUFAs, while the amount of MUFAs are relatively similar in both fractions. Of the three seaweeds, *S. latissima* had the largest variation in MUFAs, with elevated values of both C16:1n-7c and C18:1n-9c, 3,9 and 9.5 % in the NL fraction increased to 10.4 and 14.9 % in the FFA fraction. The most sizable variation is observed in *P. palmata*, containing far less EPA, 3.2 % in the FFA fraction compared to the 29.4 % in the NL fraction, as such many of the PUFAs found only in small amounts in the NL fraction are found in slightly higher concentrations in the FFA fraction, albeit still < 2 % of total fatty acid content. The 10 predominating FAs constitute more than 90 % of total fatty acids in the FFA fraction for all species.

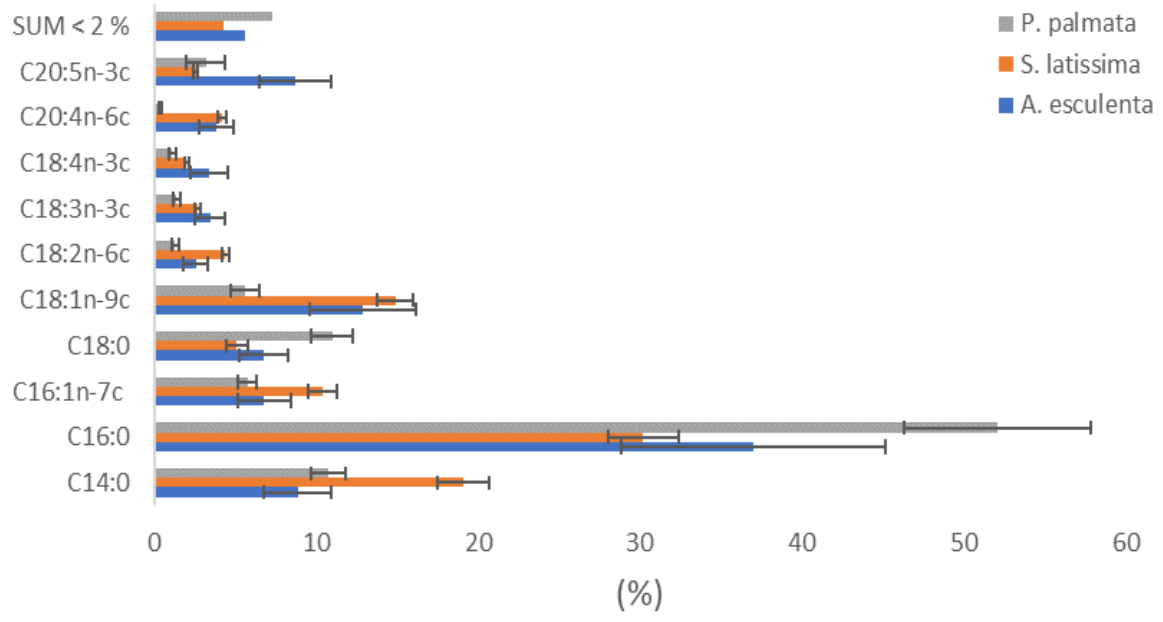


Figure 9: Fatty acid profile for fatty acids in the FFA fraction that constitutes of more than 2 % of total fatty acid content, in at least one of the fractions. SUM < 2% is the summarized contribution of the remaining fatty acids (n=4 for *A. esculenta* and *P. palmata*, n=3 for *S. latissima*, error bars = ±SD).

Comparatively to the NL fraction the highest amount of SFAs in the FFA fraction was observed in *P. palmata* with 75.9 % of total fatty acid content, while *A. esculenta* and *S. latissima*, contained 54.5 and 56.7 %, respectively. The MUFAs and PUFAs constituted of 21.3 and 24.16 %, 26.3 and 17.0 %, 16.1 and 8.0 % of total fatty acids in *A. esculenta* and *S. latissima* and *P. palmata*, respectively.

Of the EFAs in the FFA fraction, *A. esculenta* contained the highest amounts of ALA (3.5 %) while *S. latissima* contained the largest quantities of LA (4.4 %). The highest values for n-3 PUFAs are found in *A. esculenta* whereas *S. latissima* express the largest sum of n-6 PUFAs. The distribution of SFAs, MUFAs, PUFAs in the FFA fraction is displayed in **figure x**, along with values for n-3 and n-6 PUFAs. Presented in **table x** is the total fatty acid profile for *A. esculenta, S. latissima* and *P. palmata* in the FFA fraction.

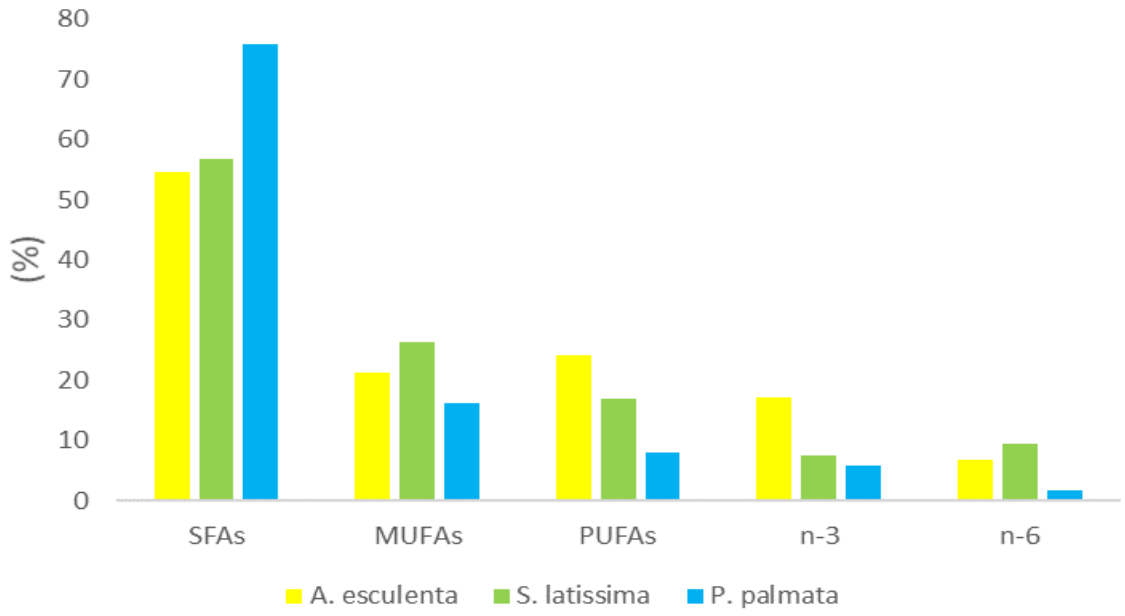


Figure 10: A graphical representation of SFA, MUFA, PUFA, n-3 and n-6 distribution relative to total FA content in the FFA fraction for each respective species (n=4 for *A. esculenta* and *P. palmata*, n=3 for *S. latissima*).

Table 5: Fatty acid content (%) for each respective FA in the FFA fraction (n=4 for *A. esculenta* and *P. palmata*, n=3 for *S. latissima*).

	Free fatty acids		
FAME	*A. esculenta*	*S. latissima*	*P. palmata*
C10:0	0.10	n.d.	n.d.
C12:0	0.05	0.02	n.d.
C13:0	0.16	n.d.	0.57
C14:0	8.87	19.10	10.74
C14:1n-5c	n.d.	0.15	n.d.
C15:0 (14-methyl) [a)]	n.d.	n.d.	0.14
C15:0	0.74	1.68	0.72
C16:0	37.03	30.22	52.07
C16:1n-9c [a)]	n.d.	0.25	0.12
C16:1n-7c	6.80	10.42	5.77
C16:1n-5c [a)]	0.22	0.21	0.36
C17:0	n.d.	0.27	0.20
C16:2n-6c [a)]	n.d.	0.33	n.d.
C16:2n-4c [a)]	0.27	0.11	0.15
C17:1n-7c [a)]	0.07	0.19	n.d.
C18:0	6.78	5.13	11.02
C18:1n-9c	12.90	14.90	5.62
C18:1n-8 [a)]	0.90	n.d.	n.d.
C18:1n-7 [a)]	n.d.	n.d.	1.82
C18:1n-5 [a)]	n.d.	0.16	n.d.
C18:2 other [a)]	n.d.	n.d.	0.25
C18:2n-6c	2.57	4.43	1.31
C18:3n-6c	0.28	0.28	0.07
C20:0	0.66	0.32	0.21
C18:3n-3c	3.46	2.69	1.41
C20:1n-9c	0.28	0.04	0.30
C18:4n-3c [a)]	3.40	2.00	1.15
C20:2n-6c	n.d.	0.03	n.d.
C22:0	n.d.	n.d.	0.08
C20:3n-6c	0.08	0.14	n.d.
C20:4n-6c	3.83	4.21	0.39
C22:1n-9c	0.14	n.d.	0.32
C20:4n-3c [a)]	0.21	n.d.	0.06
C20:5n-3c	8.70	2.58	3.22

C24:0	0.11	n.d.	0.10
C24:1n-9c	n.d.	n.d.	1.76
C22:6n-3c [a)]	1.37	0.16	0.06

[a)] FA identified through NIST 2014 library searches and not confirmed by Restek Food Industry FAME Mix; n.d. = not detected.

5.4 Polar lipid fraction of *A. esculenta, S. latissima* and *P. palmata*

In the polar fraction 24, 22 and 21 FAs were identified and quantified for *A. esculenta, S. latissima* and *P. palmata,* respectively. Of all the fractions, the polar fraction expresses the least variation in total FA content, ranging from 32.3 % in *P. palmata* to 19.1 % in *S. latissima*, with *A. esculenta* containing 25.5 %. Consistent with the other fractions, the same fatty acids predominate in the polar fraction as well, however in none of the three species is C18:0 observed containing >2 % of total fatty acid content. The distribution of FAs in the polar fraction is equivalent to that of the neutral lipid fraction, with no massive variations. Most notably is the increase of C14:0 and the decrease of EPA in *S. latissima,* the reduced EPA contents in *P. palmata,* as well as the reduced amounts of OA and higher values of three PUFAs in *A. esculenta* with the largest variety seen in the SDA content. As seen in **Figure 11**, through all three species the 9 predominating FAs constitute more than 95 % of total fatty acids in the PL fraction.

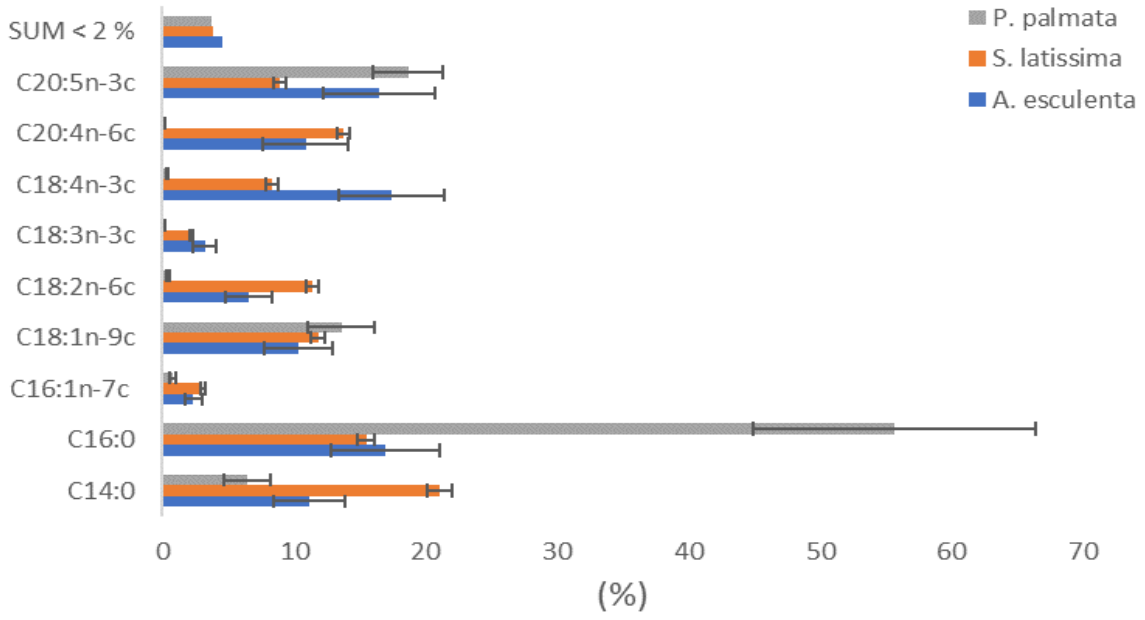


Figure 12: Fatty acid profile for fatty acids in the PL fraction that constitutes of more than 2 % of total fatty acid content, in at least one of the fractions. SUM < 2% is the summarized contribution of the remaining fatty acids (n=4 for *A. esculenta* and *P. palmata*, n=3 for *S. latissima*, error bars = ±SD).

Coherent with both the NL and the FFA fraction the highest amount of SFAs are seen in *P. palmata* with 63.7 % of total fatty acid content, while *A. esculenta* and *S. latissima,* contained 28.6 and 38.0 %, respectively. The MUFA content of all three macroalgae is relatively equal varying only a few percent, from 16.4 % in *P. palmata* to 13.1 % in *A. esculenta.* In the PL fraction both *A. esculenta* and *S. latissima* express high PUFA values, 58.3 and 46.6 %, respectively, with *P. palmata* containing significantly less (19.2 %).

Regarding content of EFAs in the PL fraction, *A. esculenta* was found to contain the highest proportion of ALA (3.2 %) while *S. latissima* contained the most LA (11.4 %). Just like the FFA fraction, the highest values for n-3 PUFAs are found in *A. esculenta* whereas *S. latissima* express the largest quantities of n-6 PUFAs, as highlighted in **Figure 13**. The total fatty acid profile in the polar lipid fraction for *A. esculenta, S. latissima* and *P. palmata* is presented in **Table 6**.

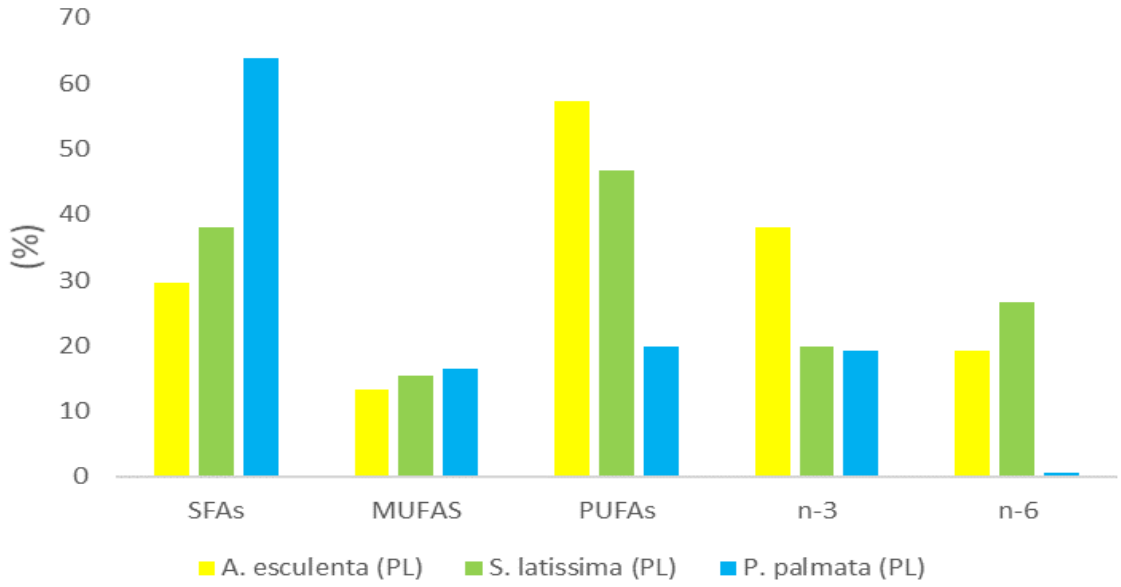


Figure 13: A graphical representation of SFA, MUFA, PUFA, n-3 and n-6 distribution relative to total FA content in the PL fraction for each respective species (n=4 for *A. esculenta* and *P. palmata*, n=3 for *S. latissima*).

Table 6: Fatty acid content (%) for each respective FA in the PL fraction (n=4 for *A. esculenta* and *P. palmata*, n=3 for *S. latissima*).

	Polar lipids		
FAME	*A. esculenta*	*S. latissima*	*P. palmata*
C14:0	11.14	21.09	6.41
C15:0	0.52	0.60	0.24
C16:0	16.93	15.51	55.66
C16:1n-7c	2.37	3.09	0.76
C16:1n-5c [a)]	0.10	0.23	0.29
C17:0	0.07	0.12	0.09
C16:2n-6c [a)]	0.07	0.12	n.d..
C16:2n-4c [a)]	0.16	0.13	n.d..
C17:1n-7c [a)]	0.05	0.05	n.d..
C18:0	0.52	0.56	0.35
C18:1n-9c	10.35	11.83	3.52
C18:1n-7c [a)]	0.28	n.d..	0.28
C18:1n-5c [a)]	n.d..	0.16	n.d..
C18:1n-1-yn [a)]	n.d..	n.d..	0.12
C18:2n-6c	6.58	11.38	0.12
C18:3n-6c	1.09	0.65	0.01
C20:0	0.39	0.16	0.02
C18:3n-3c	3.21	2.18	0.03
C20:1n-9c	0.05	n.d..	0.16
C18:4n-3c [a)]	17.46	8.37	0.39
C20:2n-6c	0.11	0.10	n.d..
C22:0	n.d..	n.d..	0.09
C20:3n-6c	0.42	0.58	n.d..
C20:4n-6c	10.91	13.76	0.12
C22:1n-9c	n.d..	n.d..	0.16
C20:4n-3c [a)]	0.34	0.34	n.d..
C20:5n-3c	16.51	8.92	18.71
C24:0	0.02	n.d..	0.03
C24:1n-9c	n.d..	n.d..	0.45
C22:6n-3c [a)]	0.41	0.09	n.d..

[a)] FA identified through NIST 2014 library searches and not confirmed by Restek Food Industry FAME Mix; n.d. = not detected.

5.5 Total fatty acid content in *A. esculenta, S. latissima* and *P. palmata*

Calculations made by plotting average area for each respective fatty acid in every fraction against the total response of all fractions within all individual species, provides a picture of the distribution of FAs in the algae as a whole. Displayed in **Figure 14** is a graphical representation of the total content of selected FA classes in *A. esculenta, S. latissima* and *P. palmata*. Shown in **Table 7** are values of selected individual important dietary FAs found in the three macroalgae.

It has been established that seaweeds growing in artic and temperate waters have the tendency to amass PUFAs (greave *et al.* 2002). In this study three species typically growing along the Norwegian coast were selected in order to elucidate their respective lipid contents, their distribution of neutral lipids, polar lipids and free fatty acids and the amount of each FA in the fractions. Through comparison of total response in all fractions between the species it is seen that *A. esculenta* and *S. latissima* contain 2.4 and 3.3, respectively, times higher amounts of FAs than *P. palmata.* This may be caused by the fact that the blades of *P. palmata* are layered, resulting in unsatisfactory disruption in the cryopulverization. *P. palmata* was also difficult to mill into fine powder, as such the liquid-liquid extraction may have yielded lower amounts of fatty acids. This may also be caused by seasonal, geographical or environmental factors.

The macroalgae with the absolute highest SFA values of total FA content is *P. palmata,* with an amount of 66.9 %, while *A. esculenta* express the lowest proportion of the three at 37.4 %. A study conducted by Kang *et al.* (2005) concluded that a PUFA/SFA ratio of 1-1.5 is favorable for reduced risk of cardiovascular disease. *A. esculenta, S. latissima* and *P. palmata* express PUFA contents of 40.0, 31.6 and 17.6 %, respectively, giving them PUFA/SFA ratios of 1.1, 0.7 and 0.3. Both *A. esculenta* and *S. latissima* exhibit ratios that could be potentially beneficial for human health, although *S. latissima* is somewhat lacking.

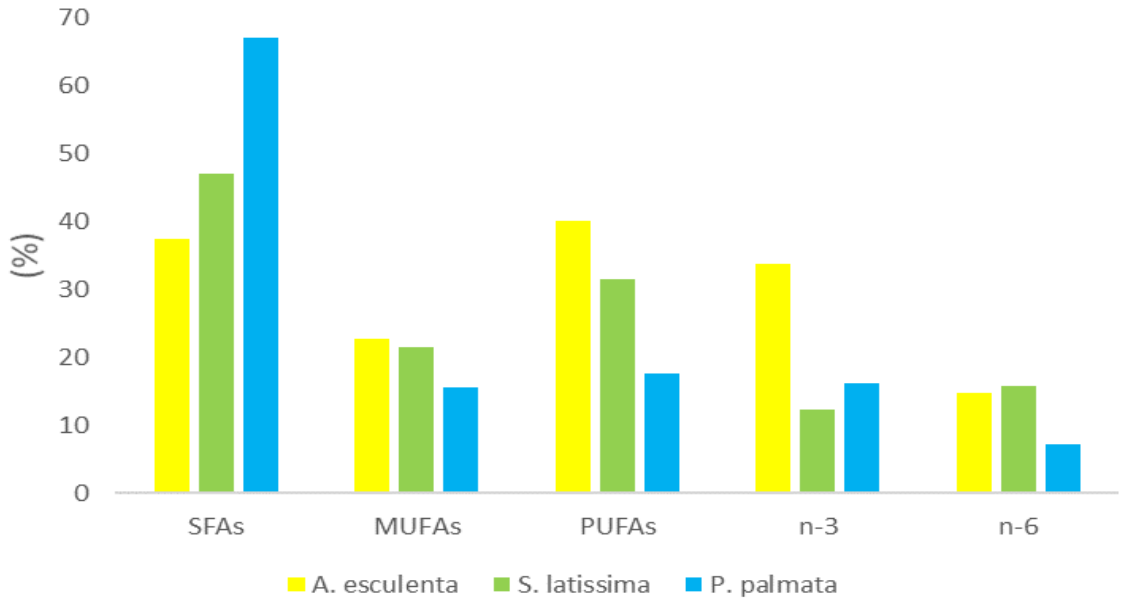


Figure 14: A graphical representation of SFA, MUFA, PUFA, n-3 and n-6 distribution relative to total FA content in each respective species (n=4 for *A. esculenta* and *P. palmata*, n=3 for *S. latissima*).

The importance of a balanced n-6/n-3 ratio has been established by several authors (Saini and Keum 2018, Simopoulos 2002). Where ratios of 1-2:1 have been reported to have suppressive effects on cardiovascular, inflammatory and autoimmune diseases (Russo 2009, Simopoulos 2002). However, this subject remains heavily debated and the official stance of FAO (2010) is that there is insufficient evidence to make such claims, and that further research is needed to determine the impact this have on human health. The calculated ratios for *A. esculenta*, *S. latissima* and *P. palmata* are 0.44, 1.29 and 0.44, respectively, suggesting that all three species could potentially impact human health positively if consumed.

The studied macroalgae contain many important dietary FAs in significant amounts, highlighted in **Table 7**, especially *A. esculenta* and *S. latissima.* These two seaweeds consist largely of FAs with established beneficial health effects, 54.0 and 43.5 %, respectively. The MUFA OA is present in relatively large quantities in all species, a FA reported to have several positive health effects, among which is slight anti-inflammatory effects, decrease of LDL cholesterol and aiding diabetes II patients with improved glucose control and insulin sensitivity (Calder 2015). Both essential fatty acids (LA and ALA) are present in similar proportions in *A. esculenta* and *S. latissima*, while lacking in *P. palmata.* The presence of AA and EPA, two well established FAs with crucial roles in the development and preservation of the brain and nervous system (Harauma *et al.* 2017) and the prevention of CVD (Siwardhana *et al.* 2012), as well as the FAs SDA and DHA, albeit the latter in miniscule amounts, further

substantiates the claim that the FA composition of these macroalgae may positively effect overall human health if incorporated in a balanced diet.

Table 7: Fatty acid content (%) of selected important dietary FAs prevalent in macroalgae relative to total fatty acid content (n=4 for *A. esculenta* and *P. palmata*, n=3 for *S. latissima*).

FA	*A. esculenta* [%]	*S. latissima* [%]	*P. palmata* [%]
OA	*15.77*	*13.15*	*8.56*
LA	6.65	6.72	0.82
ALA	3.83	3.12	0.67
SDA	9.13	5.34	0.69
AA	6.92	8.51	0.28
EPA	11.24	6.50	14.86
DHA	0.43	0.13	0.03
Σ	53.98	43.46	25.90

OA: oleic acid; LA: linoleic acid; ALA: alpha linolenic acid; SDA: stearidonic acid; AA: arachidonic acid; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid; Σ: summarized amount of selected FAs.

6 Conclusions

The in-house developed and validated method for extraction of lipids by LLE, utilization of off-line SPE for lipid fractioning, and derivatization of FAs into FAMEs with subsequent analysis by GS-MS, yielded satisfactory identification of fatty acids. A total of 32, 27 and 28 FAs were identified in *A. esculenta, S. latissima* and *P. palmata*, respectively, some of which were only found in a certain lipid fraction. Both *A. esculenta* and *S. latissima* were found to contain relatively large quantities of PUFAs, 40.0 and 31.6 %, respectively, while P. *palmata* expressed values far less (17.6 %), as such *A. esculenta* displays the most favorable PUFA/SFA ratio of 1.1 followed by *S. latissima* and *P. palmata* who expressed ratios of 0.7 and 0.3, respectively. All species contained relatively low amounts of MUFAs (~20 %). Among the predominating FAs are LA, ALA, SDA, AA, and EPA, which are established as important dietary fatty acids, and are especially predominating in *A. esculenta* and *S. latissima*, compared to *P. palmata.* The n-6/n-3 ratio displayed in all three species could be considered favorable for human health (< 2:1), with *A. esculenta* and *P. palmata* both exhibiting values of 0.44. The effects of consumption of n-3 FAs on human health and the ratio between consumed n-6 and n-3 fatty acids remain a heavily debated subject, thus, forming the basis of many clinical trials. Assessing *A. esculenta, S. latissima* and *P. palmata* from a purely FA composition point of view, gives rise to the belief that all three species display nutritionally beneficial profiles, especially *A. esculenta* and *S. latissima*. These contain large amounts of PUFAs linked to improved human health, through prevention and decreased risk of chronic diseases, as well as maintenance of crucial bodily and cognitive functions. Preliminary results suggest that consumption of these macroalgae may positively effect human health, however, further research is needed to conclusively determine all three as beneficial for overall human health. To best understand the total impact on human health other factors should also be considered, such as compounds like polysaccharides, proteins, mineral content, polyphenols and heavy metals potentially present. The quantitation of fatty acids present in the species could have been elucidated by the use of internal standards and previously determined RRF-values, however, due to un-homogenized internal standards, accurate calculations were impossible. Thus, additional sample preparations must be done and analyzed to acquire representative values for the FAs contained in the neutral lipid, free fatty acid and polar lipid fraction of *A. esculenta, S. latissima* and *P. palmata*.

7 References

Adkins, Y., & Kelley, D. S. (2010). Mechanisms underlying the cardioprotective effects of omega-3 polyunsaturated fatty acids. *The Journal of Nutritional Biochemistry, 21*(9), 781-792.

Albracht-Schulte, K., Kalupahana, N. S., Ramalingam, L., Wang, S., Rahman, S. M., Robert-McComb, J., & Moustaid-Moussa, N. (2018). Omega-3 fatty acids in obesity and metabolic syndrome: a mechanistic update. *The Journal of Nutritional Biochemistry, 58*, 1-16.

Allsopp, P., Crowe, W., Bahar, B., Harnedy, P. A., Brown, E. S., Taylor, S. S., . . . McSorley, E. M. (2016). The effect of consuming Palmaria palmata-enriched bread on inflammatory markers, antioxidant status, lipid profile and thyroid function in a randomised placebo-controlled intervention trial in healthy adults. *European Journal of Nutrition, 55*(5), 1951-1962

Andrade-Eiroa, A., Canle, M., Leroy-Cancellieri, V., & Cerdà, V. (2016). Solid-phase extraction of organic compounds: A critical review (Part I). *TrAC Trends in Analytical Chemistry, 80*, 641-654.

Anyanwu, R. C., Rodriguez, C., Durrant, A., & Olabi, A. G. (2018). Micro-Macroalgae Properties and Applications. In *Reference Module in Materials Science and Materials Engineering*: Elsevier.

Barbosa, M., Fernandes, F., Pereira, D. M., Azevedo, I. C., Sousa-Pinto, I., Andrade, P. B., & Valentão, P. (2017). Fatty acid patterns of the kelps Saccharina latissima, Saccorhiza polyschides and Laminaria ochroleuca: Influence of changing environmental conditions. *Arabian Journal of Chemistry*.

Barbosa, M., Valentão, P., & Andrade, P. B. (2016). Biologically Active Oxylipins from Enzymatic and Nonenzymatic Routes in Macroalgae. *Marine Drugs, 14*(1), 23.

Barceló-Coblijn, G., & Murphy, E. J. (2009). Alpha-linolenic acid and its conversion to longer chain n−3 fatty acids: Benefits for human health and a role in maintaining tissue n−3 fatty acid levels. *Progress in Lipid Research, 48*(6), 355-374.

Bhaskar, N., Kinami, T., Miyashita, K., Park, S.-B., Endo, Y., & Fujimoto, K. (2004). Occurrence of Conjugated Polyenoic Fatty Acids in Seaweeds from the Indian Ocean. In *Zeitschrift für Naturforschung C* (Vol. 59, pp. 310).

Biancarosa, I., Belghit, I., Bruckner, C. G., Liland, N. S., Waagbø, R., Amlund, H., . . . Lock, E.-J. (2018). Chemical characterization of 21 species of marine macroalgae common in Norwegian waters: benefits of and limitations to their potential use in food and feed. *Journal of the Science of Food and Agriculture, 98*(5), 2035-2042.

Bligh, E. G., & Dyer, W. J. (1959). A RAPID METHOD OF TOTAL LIPID EXTRACTION AND PURIFICATION. *Canadian Journal of Biochemistry and Physiology, 37*(8), 911-917.

Blikra, M. J., Løvdal, T., Vaka, M. R., Roiha, I. S., Lunestad, B. T., Lindseth, C., & Skipnes, D. (2019). Assessment of food quality and microbial safety of brown macroalgae (Alaria esculenta and Saccharina latissima). *Journal of the Science of Food and Agriculture, 99*(3), 1198-1206.

Burden, D. W. (2012). Guide to the disruption of biological samples-2012. Random Primers, 12(1), 1-25.

Burlingame, B., Nishida, C., Uauy, R., & Weisell, R. (2009). Fats and Fatty Acids in Human Nutrition: Introduction. *Annals of Nutrition and Metabolism, 55*(1-3), 5-7.

Calder, P. C. (2015). Functional Roles of Fatty Acids and Their Effects on Human Health. *Journal of Parenteral and Enteral Nutrition, 39*(1S), 18S-32S.

Chandini, S. Kumar and Ganesan, P. and Suresh, P. V. and Bhaskar, N. (2008) Seaweeds as a source of nutritionally beneficial compounds - A review. Journal of Food Science and Technology, 45 (1). pp. 1-13

Christie, W. W., & Han, X. (2012). Chapter 2 - Chromatographic analysis of lipids: general principles. In W. W. Christie & X. Han (Eds.), *Lipid Analysis (Fourth Edition)* (pp. 21-54): Woodhead Publishing.

Christie, W. W., & Han, X. (2012). Chapter 3 - Lipid extraction, storage and sample handling. In W. W. Christie & X. Han (Eds.), *Lipid Analysis (Fourth Edition)* (pp. 55-66): Woodhead Publishing.

Christie, W. W., & Han, X. (2012). Chapter 4 - Analysis of simple lipid classes. In W. W. Christie & X. Han (Eds.), *Lipid Analysis (Fourth Edition)* (pp. 69-90): Woodhead Publishing.

Christie, W. W., & Han, X. (2012). Chapter 5 - Chromatographic analysis of phospholipids and glycosyldiacylglycerols. In W. W. Christie & X. Han (Eds.), *Lipid Analysis (Fourth Edition)* (pp. 91-124): Woodhead Publishing.

Christie, W. W., & Han, X. (2012). Chapter 7 - Preparation of derivatives of fatty acids. In W. W. Christie & X. Han (Eds.), *Lipid Analysis (Fourth Edition)* (pp. 145-158): Woodhead Publishing.

Christie, W. W., & Han, X. (2012). Chapter 8 - Gas chromatographic analysis of fatty acid derivatives. In W. W. Christie & X. Han (Eds.), *Lipid Analysis (Fourth Edition)* (pp. 159-180): Woodhead Publishing.

Christie, W. W., & Han, X. (2012). Chapter 9 - Isolation of fatty acids and identification by spectroscopic and related techniques. In W. W. Christie & X. Han (Eds.), *Lipid Analysis (Fourth Edition)* (pp. 181-211): Woodhead Publishing.

Colombo, M. L., Risè, P., Giavarini, F., De Angelis, L., Galli, C., & Bolis, C. L. (2006). Marine macroalgae as sources of polyunsaturated fatty acids. *Plant Foods for Human Nutrition, 61*(2), 67-72.

Connor, W. E. (2000). Importance of n−3 fatty acids in health and disease. *The American Journal of Clinical Nutrition, 71*(1), 171S-175S.

Devle, H., Rukke, E.-O., Naess-Andresen, C. F., & Ekeberg, D. (2009). A GC – magnetic sector MS method for identification and quantification of fatty acids in ewe milk by different acquisition modes. *Journal of Separation Science, 32*(21), 3738-3745.

Devle, H., Ulleberg, E. K., Naess-Andresen, C. F., Rukke, E.-O., Vegarud, G., & Ekeberg, D. (2014). Reciprocal interacting effects of proteins and lipids during ex vivo digestion of bovine milk. *International Dairy Journal, 36*(1), 6-13.

Dewick, P. M. (2009). Medicinal natural products: *a biosynthetic approach*. John Wiley & Sons. 3rd ed.

Eder, K. (1995). Gas chromatographic analysis of fatty acid methyl esters. *Journal of Chromatography B: Biomedical Sciences and Applications, 671*(1), 113-131.

El-Shoubaky, G. A., Moustafa, A. M. Y., & Salem, E. A. E. (2008). Comparative phytochemical investigation of beneficial essential fatty acids on a variety of marine seaweeds algae. *Res. J. Phytochem., 2*(1), 18-26.

FAO. (2010). Fats and fatty acids in human nutrition. Report of an expert consultation. FAO Food and nutrition paper, 91: 166.

FAO. (2018). The State of World Fisheries and Aquaculture 2018 - *Meeting the sustainable development goals*.

Fernandes, H., Salgado, J. M., Martins, N., Peres, H., Oliva-Teles, A., & Belo, I. (2019). Sequential bioprocessing of Ulva rigida to produce lignocellulolytic enzymes and to improve its nutritional value as aquaculture feed. *Bioresource Technology, 281*, 277-285.

Fleurence, J., Gutbier, G., Mabeau, S., & Leray, C. (1994). Fatty acids from 11 marine macroalgae of the French Brittany coast. *Journal of Applied Phycology, 6*(5), 527-532.

Folch, J., Lees, M., & Stanley, G. H. S. (1957). A SIMPLE METHOD FOR THE ISOLATION AND PURIFICATION OF TOTAL LIPIDES FROM ANIMAL TISSUES. *Journal of Biological Chemistry, 226*(1), 497-509

Foseid, L., Devle, H., Stenstrom, Y., Naess-Andresen, C. F., & Ekeberg, D. (2017). Fatty Acid Profiles of Stipe and Blade from the Norwegian Brown Macroalgae Laminaria hyperborea with Special Reference to Acyl Glycerides, Polar Lipids, and Free Fatty Acids. *J Lipids, 2017*, 1029702.

Gaillard, C., Bhatti, H. S., Novoa-Garrido, M., Lind, V., Roleda, M. Y., & Weisbjerg, M. R. (2018). Amino acid profiles of nine seaweed species and their *in-situ* degradability in dairy cows. *Animal Feed Science and Technology, 241*, 210-222.

Garcia-Vaquero, M., & Hayes, M. (2016). Red and green macroalgae for fish and animal feed and human functional food development. *Food Reviews International, 32*(1), 15-45.

Gebauer, S. K., Psota, T. L., Harris, W. S., & Kris-Etherton, P. M. (2006). n−3 Fatty acid dietary recommendations and food sources to achieve essentiality and cardiovascular benefits. *The American Journal of Clinical Nutrition, 83*(6), 1526S-1535S.

Graeve, M., Kattner, G., Wiencke, C., & Karsten, U. (2002). Fatty acid composition of Arctic and Antarctic macroalgae: Indicator of phylogenetic and trophic relationships. *Marine Ecology Progress Series, 231*, 67-74.

Guiry, W. (2019). AlgaeBase. Retrieved from http://www.algaebase.org/search/species/detail/?species_id=1

Guiry, W. (2019, March 27). The Seaweed Site: information on marine algae. Retrieved from http://www.seaweed.ie/index.php

Harauma, A., Yasuda, H., Hatanaka, E., Nakamura, M. T., Salem, N., & Moriguchi, T. (2017). The essentiality of arachidonic acid in addition to docosahexaenoic acid for brain growth and function. *Prostaglandins, Leukotrienes and Essential Fatty Acids, 116*, 9-18.

Hennion, M.-C. (1999). Solid-phase extraction: method development, sorbents, and coupling with liquid chromatography. *Journal of Chromatography A, 856*(1), 3-54.

Holdt, S. L., & Kraan, S. (2011). Bioactive compounds in seaweed: functional food applications and legislation. *Journal of Applied Phycology, 23*(3), 543-597.

Ichihara, K. i., Kohsaka, C., Tomari, N., Kiyono, T., Wada, J., Hirooka, K., & Yamamoto, Y. (2016). Fatty acid analysis of triacylglycerols: Preparation of fatty acid methyl esters for gas chromatography. *Analytical Biochemistry, 495*, 6-8.

Innis, S. M. (2008). Dietary omega 3 fatty acids and the developing brain. *Brain Research, 1237*, 35-43.

Jensen, R. G., & Clark, R. M. (1984). Methods of Lipid Analysis. *Journal of Pediatric Gastroenterology and Nutrition, 3*(2), 296-299.

Kang, M. J., Shin, M. S., Park, J. N., & Lee, S. S. (2005). The effects of polyunsaturated:saturated fatty acids ratios and peroxidisability index values of dietary fats on serum lipid profiles and hepatic enzyme activities in rats. *Br J Nutr, 94*(4), 526-532.

Kelly Fretwell. (2016). Biodiversity of the central coast. accessed Retrieved from https://www.centralcoastbiodiversity.org/sugar-kelp-bull-saccharina-latissima.html).

Kumari, P., Kumar, M., Gupta, V., Reddy, C. R. K., & Jha, B. (2010). Tropical marine macroalgae as potential sources of nutritionally important PUFAs. *Food Chemistry, 120*(3), 749-757.

Kumari, P., Reddy, C. R. K., & Jha, B. (2011). Comparative evaluation and selection of a method for lipid and fatty acid extraction from macroalgae. *Analytical Biochemistry, 415*(2), 134-144.

Lands, B., Bibus, D., & Stark, K. D. (2018). Dynamic interactions of n-3 and n-6 fatty acid nutrients. *Prostaglandins, Leukotrienes and Essential Fatty Acids, 136*, 15-21.

Liu, H.-Q., Qiu, Y., Mu, Y., Zhang, X.-J., Liu, L., Hou, X.-H., . . . Wang, F. (2013). A high ratio of dietary n-3/n-6 polyunsaturated fatty acids improves obesity-linked inflammation and insulin resistance through suppressing activation of TLR4 in SD rats. *Nutrition Research, 33*(10), 849-858.

Liu, K.-S. (1994). Preparation of fatty acid methyl esters for gas-chromatographic analysis of lipids in biological materials. *Journal of the American Oil Chemists' Society, 71*(11), 1179-1187.

Mæhre, H. K., Malde, M. K., Eilertsen, K.-E., & Elvevoll, E. O. (2014). Characterization of protein, lipid and mineral contents in common Norwegian seaweeds and evaluation of their potential as food and feed. *Journal of the Science of Food and Agriculture, 94*(15), 3281-3290.

MACOI - Portuguese Seaweeds Website. (2019). Alaria esculenta (Linnaeus) Greville. Retrieved from http://macoi.ci.uc.pt/spec_list_detail.php?spec_id=241

Malins, D. C., & Mangold, H. K. (1960). Analysis of complex lipid mixtures by thin-layer chromatography and complementary methods. *Journal of the American Oil Chemists' Society, 37*(11), 576-578.

Marinho, G. S., Holdt, S. L., Jacobsen, C., & Angelidaki, I. (2015). Lipids and Composition of Fatty Acids of Saccharina latissima Cultivated Year-Round in Integrated Multi-Trophic Aquaculture. *Marine Drugs, 13*(7), 4357-4374.

Marinho, G. S., Sørensen, A.-D. M., Safafar, H., Pedersen, A. H., & Holdt, S. L. (2019). Antioxidant content and activity of the seaweed Saccharina latissima: a seasonal perspective. *Journal of Applied Phycology, 31*(2), 1343-1354.

Matanjun, P., Mohamed, S., Mustapha, N. M., & Muhammad, K. (2009). Nutrient content of tropical edible seaweeds, Eucheuma cottonii, Caulerpa lentillifera and Sargassum polycystum. *Journal of Applied Phycology, 21*(1), 75-80.

McHugh, D. J. (2003). A guide to the seaweed industry FAO Fisheries Technical Paper 441. Food and Agriculture Organization of the United Nations, Rome.

Millar. A. (2011, July 30). Macroalgae: The facts. Retrieved from https://thefishsite.com/articles/macroalgae-the-facts

Morgan, K. C., Wright, J. L. C., & Simpson, F. J. (1980). Review of chemical constituents of the red algaPalmaria palmata (dulse). *Economic Botany, 34*(1), 27-50.

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

Murata, M., & Nakazoe, J.-i. (2001). Production and Use of Marine AIgae in Japan. *Japan Agricultural Research Quarterly: JARQ, 35*(4), 281-290. doi:10.6090/jarq.35.281

Neto, R. T., Marçal, C., Queirós, A. S., Abreu, H., Silva, A. M. S., & Cardoso, S. M. (2018). Screening of Ulva rigida, Gracilaria sp., Fucus vesiculosus and Saccharina latissima as Functional Ingredients. *International Journal of Molecular Sciences, 19*(10), 2987..

Palatnik, R. R., & Zilberman, D. (2017). *Economics of Natural Resource Utilization - the Case of Macroalgae*, Cham.

Patel, N. K., & Shah, S. N. (2015). 11 - Biodiesel from Plant Oils. In S. Ahuja (Ed.), *Food, Energy, and Water* (pp. 277-307). Boston: Elsevier.

Poole, C. F. (2003). New trends in solid-phase extraction. *TrAC Trends in Analytical Chemistry, 22*(6), 362-373.

Ramsden, C. E., Faurot, K. R., Zamora, D., Suchindran, C. M., MacIntosh, B. A., Gaylord, S., . . . Mann, J. D. (2013). Targeted alteration of dietary n-3 and n-6 fatty acids for the treatment of chronic headaches: A randomized trial. *PAIN®, 154*(11), 2441-2451.

Robertson, R. C., Guihéneuf, F., Bahar, B., Schmid, M., Stengel, D. B., Fitzgerald, G. F., . . . Stanton, C. (2015). The Anti-Inflammatory Effect of Algae-Derived Lipid Extracts on Lipopolysaccharide (LPS)-Stimulated Human THP-1 Macrophages. *Marine Drugs, 13*(8), 5402-5424.

Russo, G. L. (2009). Dietary n−6 and n−3 polyunsaturated fatty acids: From biochemistry to clinical implications in cardiovascular prevention. *Biochemical Pharmacology, 77*(6), 937-946.

Saini, R. K., & Keum, Y.-S. (2018). Omega-3 and omega-6 polyunsaturated fatty acids: Dietary sources, metabolism, and significance — A review. *Life Sciences, 203*, 255-267.

Salimon, J., Omar, T. A., & Salih, N. (2017). An accurate and reliable method for identification and quantification of fatty acids and trans fatty acids in food fats samples using gas chromatography. *Arabian Journal of Chemistry, 10*, S1875-S1882.

Sánchez-Machado, D. I., López-Cervantes, J., López-Hernández, J., & Paseiro-Losada, P. (2004). Fatty acids, total lipid, protein and ash contents of processed edible seaweeds. *Food Chemistry, 85*(3), 439-444.

Sappati, P. K., Nayak, B., VanWalsum, G. P., & Mulrey, O. T. (2019). Combined effects of seasonal variation and drying methods on the physicochemical properties and

antioxidant activity of sugar kelp (Saccharina latissima). *Journal of Applied Phycology, 31*(2), 1311-1332.

Schiener, P., Black, K. D., Stanley, M. S., & Green, D. H. (2015). The seasonal variation in the chemical composition of the kelp species Laminaria digitata, Laminaria hyperborea, Saccharina latissima and Alaria esculenta. *Journal of Applied Phycology, 27*(1), 363-373.

Schiener, P., Zhao, S., Theodoridou, K., Carey, M., Mooney-McAuley, K., & Greenwell, C. (2017). The nutritional aspects of biorefined Saccharina latissima, Ascophyllum nodosum and Palmaria palmata. *Biomass Conversion and Biorefinery, 7*(2), 221-235.

Seppänen-Laakso, T., Laakso, I., & Hiltunen, R. (2002). Analysis of fatty acids by gas chromatography, and its relevance to research on health and nutrition. *Analytica Chimica Acta, 465*(1), 39-62.

Simopoulos, A. P. (1999). Essential fatty acids in health and chronic disease. *The American Journal of Clinical Nutrition, 70*(3), 560s-569s.

Simopoulos, A. P. (2002). The importance of the ratio of omega-6/omega-3 essential fatty acids. *Biomedicine & Pharmacotherapy, 56*(8), 365-379.

Simpson, N. J. (2000). Solid-phase extraction: principles, techniques, and applications. CRC press.

Siriwardhana, N., Kalupahana, N. S., & Moustaid-Moussa, N. (2012). Chapter 13 - Health Benefits of n-3 Polyunsaturated Fatty Acids: Eicosapentaenoic Acid and Docosahexaenoic Acid. In S.-K. Kim (Ed.), *Advances in Food and Nutrition Research* (Vol. 65, pp. 211-222): Academic Press.

Smucker, R. A., & Pfister, R. M. (1975). Liquid Nitrogen Cryo-Impacting: a New Concept for Cell Disruption. *Applied Microbiology, 30*(3), 445-449.

Stévant, P., Indergård, E., Ólafsdóttir, A., Marfaing, H., Larssen, W. E., Fleurence, J., . . . Nordtvedt, T. S. (2018). Effects of drying on the nutrient content and physico-chemical and sensory characteristics of the edible kelp Saccharina latissima. *Journal of Applied Phycology, 30*(4), 2587-2599.

Stévant, P., Marfaing, H., Duinker, A., Fleurence, J., Rustad, T., Sandbakken, I., & Chapman, A. (2018). Biomass soaking treatments to reduce potentially undesirable compounds in the edible seaweeds sugar kelp (Saccharina latissima) and winged kelp (Alaria esculenta) and health risk estimation for human consumption. *Journal of Applied Phycology, 30*(3), 2047-2060.

Stévant, P., Marfaing, H., Rustad, T., Sandbakken, I., Fleurence, J., & Chapman, A. (2017). Nutritional value of the kelps Alaria esculenta and Saccharina latissima and effects of short-term storage on biomass quality. *Journal of Applied Phycology, 29*(5), 2417-2426.

Susanto, E., Fahmi, A. S., Abe, M., Hosokawa, M., & Miyashita, K. (2016). Lipids, Fatty

Acids, and Fucoxanthin Content from Temperate and Tropical Brown Seaweeds. *Aquatic Procedia, 7*, 66-75.

Tong, L., Zhang, L., Yu, S., Chen, X., & Bi, K. Analysis of the fatty acids from Periploca sepium by GC-MS and GC-FID.

Wan, A. H. L., Soler-Vila, A., O'Keeffe, D., Casburn, P., Fitzgerald, R., & Johnson, M. P. (2016). The inclusion of Palmaria palmata macroalgae in Atlantic salmon (Salmo salar) diets: effects on growth, haematology, immunity and liver function. *Journal of Applied Phycology*, *28*(5), 3091-3100.

Yamashita, M., & Fenn, J. B. (1984). Electrospray ion source. Another variation on the free-jet theme. *The Journal of Physical Chemistry*, *88*(20), 4451-4459.

Yuan, Y. V., Carrington, M. F., & Walsh, N. A. (2005). Extracts from dulse (Palmaria palmata) are effective antioxidants and inhibitors of cell proliferation in vitro. *Food and Chemical Toxicology*, *43*(7), 1073-1081.

8 Appendices

Appendix I: Reference standards

Table A.1: FAMEs present in the Restek Food Industry FAME Mix used as reference standards. Listed in order of elution with weight% contribution of each respective FAME in the mix.

FAME	Systematic name	Weight%
C4:0	Butanoic acid methyl ester	4.0
C6:0	Hexanoic acid methyl ester	4.0
C8:0	Octanoic acid methyl ester	4.0
C10:0	Decanoic acid methyl ester	4.0
C11:0	Undecanoic acid methyl ester	2.0
C12:0	Dodecanoic acid methyl ester	4.0
C13:0	Tridecanoic acid methyl ester	2.0
C14:0	Tetradecanoic acid methyl ester	4.0
C14:1n-5c	cis-9-Tetradecenoic acid methyl ester	2.0
C15:0	Pentadecanoic acid methyl ester	2.0
C15:1n-5c	cis-10-Pentadecenoic acid methyl ester	2.0
C16:0	Hexadecenoic acid methyl ester	6.0
C16:1n-7c	cis-9-Hexadecenoic acid methyl ester	2.0
C17:0	Heptadecanoic acid methyl ester	2.0
C17:1n-7c	cis-10-Heptadecenoic acid methyl ester	2.0
C18:0	Octadecanoic acid methyl ester	4.0
C18:1n-9c	cis-9-Octadecenoic acid methyl ester	4.0
C18:1n-9tr	trans-9-Octadecenoic acid methyl ester	2.0
C18:2n-6c	all-cis-9,12-Octadecadienoic acid methyl ester	2.0
C18:2n-6tr	all-trans-9,12-Octadecadienoic acid methyl ester	2.0
C18:3n-6c	all-cis-6,9,12-Octadecatrienoic acid methyl ester	2.0
C18:3n-3c	all-cis-9,12,15-Octadecatrienoic acid methyl ester	2.0
C20:0	Eicosanoic acid methyl ester	4.0

C20:1n-9c	cis-11-Eicosenoic acid methyl ester	2.0
C20:2n-6c	all-cis-11,14-Eicosadienoic acid methyl ester	2.0
C20:3n-6c	all-cis-8,11,14-Eicosatrienoic acid methyl ester	2.0
C21:0	Heneicosanoic acid methyl ester	2.0
C20:4n-6c	all-cis-5,8,11,14-Eicosatetraenoic acid methyl ester	2.0
C20:3n-3c	all-cis-11,14,17-Eicosatrienoic acid methyl ester	2.0
C20:5n-3c	all-cis-5,8,11,14,17-Eicosapentaenoic acid methyl ester	2.0
C22:0	Docosanoic acid methyl ester	4.0
C22:1n-9c	cis-13-Docosenoic acid methyl ester	2.0
C22:2n-6c	all-cis-13,16-Docosadienoic acid methyl ester	2.0
C23:0	Tricosanoic acid methyl ester	2.0
C24:0	Tetracosanoic acid methyl ester	4.0
C22:6n-3c	all-cis-4,7,10,13,16,19-Docosahexaenoic acid methyl ester	2.0
C24:1n-9c	cis-15-Tetracosenoic acid methyl ester	2.0

*Adapted from Devle et al (2009)

Appendix II: Internal standards

Table A.2: The internal standard utilized for the quantitation of FA profiles in the neutral lipid fraction.

Internal standard	Molecular weight [g/mole]	Concentration [mg/mL]	IS used [µL]	Amount IS [mg]	Moles IS
C19:0 TG	933.6	10	50	0.5	$5.356*10^{-7}$

Table A.3: The internal standard utilized for the quantitation of FA profiles in the free fatty acid fraction.

Internal standard	Molecular weight [g/mole]	Concentration [mg/mL]	IS used [µL]	Amount IS [mg]	Moles IS
C19:0 FFA	298.52	10	50	0.5	$1.675*10^{-6}$

Table A.4: The internal standard utilized for the quantitation of FA profiles in the polar lipid fraction.

Internal standard	Molecular weight [g/mole]	Concentration [mg/mL]	IS used [µL]	Amount IS [mg]	Moles IS
C19:0 PL	818.2	10	50	0.5	$6.111*10^{-7}$

Appendix III: RRF-values

Table A.5: RRF-values of FAMEs identified in *A. esculenta*, *S. latissima* and *P. palmata*. Listed in order of elution with molecular weight of each respective FAME.

FAME	RRF-value	Molecular weight [g/mole]
C10:0	0.85	172.27
C12:0	0.94	200.33
C13:0	1.11	214.35
C14:0	1.12	228.38
C14:1n-5c	1.24	226.38
C15:0	1.22	242.41
C15:0 (14-methyl)[a)]	1.22	256.43
C16:0	1.22	256.43
C16:1n-9c [a)]	1.18	254.43
C16:1n-7c	1.18	254.43
C16:1n-5c [a)]	1.18	254.43
C17:0	1.22	270.46
C16:2n-6c [a)]	1.18	252.43
C16:2n-4c [a)]	1.18	252.43
C17:1n-7c [a)]	1.22	268.46
C18:0	1.19	284.48
C18:1n-9c	1.16	282.48
C18:1n-8c [a)]	1.16	282.48

C18:1n-7c [a)]	1.16	282.48
C18:1n-5c [a)]	1.16	282.48
C18:1n-1-yn [a)]	1.16	280.48
C18:2n-6c other [a)]	1.01	280.48
C18:2n-6c	1.01	280.48
C18:3n-6c	0.99	278.48
C20:0	1.17	312.54
C18:3n-3c	0.98	278.48
C20:1n-9c	1.13	310.54
C18:4n-3c [a)]	0.98	276.48
C20:2n-6c	1.06	308.54
C20:3n-6c	1.18	306.53
C22:0	1.18	340.59
C20:4n-6c	0.96	304.52
C22:1n-9c	1.1	338.59
C20:4n-3c[a)]	0.96	304.52
C20:5n-3c	0.96	302.52
C24:0	1.19	368.65
C21:5n-3c[a)]	0.96	316.57
C24:1n-9c	1.01	366.65
C22:6n-3c [a)]	1.01	328.59

[a)] No previously calculated RRF-value

Appendix IV: Neutral lipid fraction *Alaria esculenta*

Table A.6: Summary table for the neutral lipid fraction in *Alaria esculenta.* Values for matchfactor, reverse matchfactor and probability were acquired through NIST 2014 library searches based on spectral information. Also included in the table are the average areas and retention times. Average amount represents total contribution of each respective fatty acid to total FA content in the fraction (µg/g DW). N=4.

FAME	Retention [%]	Matchfactor	Relative Matchfactor	Probability [%]	Avg. area	Avg. amount [µg]	SD
C12:0	10.83	821	896	59.9	170	2	0.25
C14:0	14.08	945	947	73.8	38722	368	63.34
C15:0	16.69	915	920	67.4	1285	11	1.96
C16:0	20.44	945	945	82.7	126485	1104	193.11
C16:1n-7c	22.84	948	948	42.2	39589	358	67.59
C16:1n-5c [a)]	23.45	853	905	22.8	459	4	0.81
C17:0	25.19	780	829	38	734	6	1.34
C16:2n-6c [a)]	26.06	833	851	38.7	224	2	0.49
C16:2n-4c [a)]	27.89	895	898	51.4	615	5	0.94
C17:1n-7c [a)]	28.26	781	801	18.3	301	2	1.24
C18:0	32.22	941	951	76.6	11466	102	20.35
C18:1n-9c	36.15	947	948	8.69	97383	891	178.70
C18:1n-8c [a)]	36.72	853	854	9.27	765	7	3.15
C18:2n-6c	42.94	954	955	36.7	40612	426	85.39
C18:3n-6c	46.678	933	935	63.7	4023	43	9.87
C20:0	48.79	919	952	52.4	3085	28	5.75
C18:3n-3c	49.15	950	951	68.9	21587	232	45.57
C20:1n-9c	51.12	823	860	25.6	365	3	0.60
C18:4n-3c [a)]	52.27	901	915	40.8	36775	386	125.22
C20:2n-6c	55.02	821	839	18.9	312	3	0.66
C20:3n-6c	57.37	903	940	52.4	1791	16	3.87
C20:4n-6c	59.04	956	956	75.9	31189	342	76.87
C22:1n-9c	60.1	754	774	32.6	133	1	0.28
C20:4n-3c [a)]	61.26	879	909	39.3	2100	23	3.93
C20:5n-3c	62.87	953	954	52.8	49433	545	107.38
C21:5n-3c [a)]	66.71	811	845	15.6	399	4	1.13
C22:6n-3c [a)]	71.15	883	906	28.1	779	8	1.40

[a)] Not represented by a reference standard in the Restek Food Industry FAME Mix.

Appendix V: Neutral lipid fraction *Saccharina latissima*

Table A.7: Summary table for the neutral lipid fraction in *Saccharina latissima.* Values for matchfactor, reverse matchfactor and probability were acquired through NIST 2014 library searches based on spectral information. Also included in the table are the average areas and retention times. Average amount represents total contribution of each respective fatty acid to total FA content in the fraction (µg/g DW). N=3.

FAME	Retention [%]	Matchfactor	Relative Matchfactor	Probability [%]	Avg. area	Avg. amount [µg]	SD
C12:0	10.85	650	779	38.0	88	4.35	2.67
C13:0	12.23	754	828	51.6	79	2.27	1.72
C14:0	14.1	949	951	72.7	31778	903.46	1060.66
C14:1n-5c	15.84	874	884	46.8	364	9.36	11.14
C15:0	16.72	908	915	64.4	1321	34.48	41.45
C16:0	20.33	945	953	77.3	37777	985.97	1130.44
C16:1n-7c	22.81	923	924	30.5	10464	282.38	326.97
C16:1n-5c [a)]	23.47	866	888	19.2	404	10.89	12.60
C17:0	25.19	746	806	33.0	485	12.67	14.25
C16:2n-6c [a)]	26.06	870	879	47.4	293	7.91	10.07
C16:2n-4c [a)]	27.91	844	847	37.5	484	13.06	13.23
C17:1n-7c [a)]	28.29	773	793	22.2	243	6.35	5.08
C18:0	32.09	911	927	74.0	2101	56.23	58.16
C18:1n-9c	35.93	940	940	8.65	25650	704.10	830.92
C18:1n-5c [a)]	36.65	837	863	8.39	327	8.97	6.09
C18:2n-6c	42.90	943	954	35.6	24058	758.48	876.35
C18:3n-6c	46.7	879	883	35.9	1828	58.81	68.43
C20:0	48.78	912	917	67.7	1202	32.72	34.59
C18:3n-3c	49.16	940	941	66.8	13962	453.65	533.84
C18:4n-3c [a)]	52.3	892	902	36.1	32059	1041.66	1268.67
C20:2n-6c	55.02	816	838	37.5	216	6.48	5.42
C20:3n-6c	57.4	850	872	35.7	653	17.63	18.74
C20:4n-6c	59.07	956	956	76.4	42410	1406.69	1514.49
C20:4n-3c [a)]	61.27	876	905	48.6	1607	53.30	65.17
C20:5n-3c	62.88	952	952	56.5	40992	1359.66	1591.41
C22:6n-3c [a)]	71.18	829	837	19.4	242	7.62	7.56

[a)] Not represented by a reference standard in the Restek Food Industry FAME Mix.

Appendix VI: Neutral lipid fraction *Palmaria palmata*

Table A.8: Summary table for the neutral lipid fraction in *Palmaria palmata*. Values for matchfactor, reverse matchfactor and probability were acquired through NIST 2014 library searches based on spectral information. Also included in the table are the average areas and retention times. Average amount represents total contribution of each respective fatty acid to total FA content in the fraction (µg/g DW). N=4.

FAME	Retention [%]	Matchfactor	Relative Matchfactor	Probability [%]	Avg. area	Avg. amount [µg]	SD
C14:0	14.17	933	941	72.2	7443.75	26.94	9.08
C15:0	16.82	814	825	29.1	200	0.68	0.14
C16:0	20.5	923	939	75.4	44716.5	152.25	38.03
C16:1n-7c	22.93	892	892	19.7	3042.25	10.73	2.49
C16:1n-5c [a)]	24.42	864	882	37.7	481	1.60	0.88
C18:0	32.27	899	930	51.1	1739.75	6.11	1.30
C18:1n-9c	36.06	924	925	7.22	6747.25	24.10	5.16
C18:1n-7c [a)]	36.85	891	891	7.79	990	3.49	1.08
C18:2n-6c	43.01	873	874	12.3	458.5	1.87	0.45
C18:3n-3c	49.25	824	848	22.2	122.75	0.51	0.14
C20:1n-9c	51.23	846	846	27.3	258.25	0.92	0.34
C18:4n-3c [a)]	52.37	876	880	26.5	288	1.23	0.26
C22:0	58.92	780	803	61.4	82	0.28	0.09
C20:4n-6c	59.09	857	865	26.7	284	1.23	0.31
C22:1n-9c	60.55	827	827	36	265.5	1.17	0.92
C20:5n-3c	62.96	934	936	33.1	28385.25	123.48	23.56
C24:0	66.3	571	579	6.26	68.75	0.27	0.17
C24:1n-9c	67.68	899	900	53.7	1085	5.23	4.12

[a)] Not represented by a reference standard in the Restek Food Industry FAME Mix.

Appendix VII: Free fatty acid fraction *Alaria esculenta*

Table A.9: Summary table for the free fatty acid fraction in *Alaria esculenta.* Values for matchfactor, reverse matchfactor and probability were acquired through NIST 2014 library searches based on spectral information. Also included in the table are the average areas and retention times. Average amount represents total contribution of each respective fatty acid to total FA content in the fraction (µg/g DW). N=4.

FAME	Retention [%]	Matchfactor	Relative Matchfactor	Probability [%]	Avg. area	Avg. amount [µg]	SD
C10:0	8.24	877	878	70	162	0.8	0.44
C12:0	10.82	798	846	44.1	80	0.3	0.21
C13:0	12.19	888	932	56.4	254	0.9	0.52
C14:0	14.07	948	948	72.1	14044	47.6	27.80
C15:0	16.68	914	921	68.6	1179	3.6	2.26
C16:0	20.35	951	951	83.3	58615	185.3	105.55
C16:1n-7c	22.77	934	935	32.7	10770	34.8	20.56
C16:1n-5c [a)]	23.42	882	916	34.7	356	1.2	0.68
C16:2n-4c [a)]	27.9	848	903	20.4	425	1.4	0.97
C17:1n-7c [a)]	28.22	712	790	11.5	115	0.4	0.32
C18:0	32.08	944	955	74.4	10736	35.2	21.25
C18:1n-9c	35.86	948	949	10.2	20425	66.6	40.49
C18:1n-8c [a)]	36.59	902	904	6.75	1429	4.7	2.79
C18:2n-6c	42.78	940	957	31.9	4075	14.8	10.03
C18:3n-6c	46.65	860	865	38.9	436	1.6	1.18
C20:0	48.66	917	947	72.6	1044	3.3	2.19
C18:3n-3c	49.08	931	933	56.2	5473	21.0	13.14
C20:1n-9c	51.02	891	911	24.9	439	1.6	0.91
C18:4n-3c [a)]	52.21	913	932	43.8	5379	20.3	16.05
C20:3n-6c	57.32	833	859	26.4	124	0.4	0.24
C20:4n-6c	58.97	951	952	72	6066	23.7	15.31
C22:1n-9c	60.08	864	866	30.7	217	0.8	0.38
C20:4n-3c [a)]	61.23	874	900	41.9	339	1.4	0.77
C20:5n-3c	62.8	950	950	51.2	13771	54.3	33.96
C24:0	66.14	818	837	84.3	179	0.5	0.27
C22:6n-3c [a)]	71.15	938	939	42.2	2165	8.3	4.79

[a)] Not represented by a reference standard in the Restek Food Industry FAME Mix.

Appendix VIII: Free fatty acid fraction *Saccharina latissima*

Table A.10: Summary table for the free fatty acid fraction in *Saccharina latissima.* Values for matchfactor, reverse matchfactor and probability were acquired through NIST 2014 library searches based on spectral information. Also included in the table are the average areas and retention times. Average amount represents total contribution of each respective fatty acid to total FA content in the fraction (µg/g DW). N=3.

FAME	Retention [%]	Matchfactor	Relative Matchfactor	Probability [%]	Avg. area	Avg. amount [µg]	SD
C12:0	10.82	819	858	55	134	0.52	0.15
C14:0	14.14	905	909	76.2	142181	536.93	279.31
C14:1n-5c	15.82	905	911	55.7	1096	3.67	1.79
C15:0	16.7	945	945	68.8	12528	43.17	21.99
C16:0	20.54	942	943	84.6	224978	785.25	417.54
C16:1n-9c [a)]	22.36	778	797	20.8	1848	6.64	3.87
C16:1n-7c	22.89	928	929	40	77543	278.00	144.53
C16:1n-5c [a)]	23.48	907	910	29.1	1546	5.55	2.90
C17:0	25.24	918	922	61.3	1992	6.88	3.60
C16:2n-6c [a)]	26.08	873	882	30.3	2422	8.59	4.35
C16:2n-4c [a)]	27.91	885	885	41.4	849	3.03	1.57
C17:1n-7c [a)]	28.33	898	902	40.7	1447	4.96	2.53
C18:0	32.34	945	949	76.1	38162	133.51	67.73
C18:1n-9c	36.2	943	944	9.17	110932	407.48	217.78
C18:1n-5c [a)]	36.75	888	890	6.96	1182	4.65	2.86
C18:2n-6c	42.94	950	951	36	32971	141.03	78.96
C18:3n-6c	46.71	887	892	49.2	2070	9.02	5.00
C20:0	48.77	919	949	61.5	2362	8.64	4.70
C18:3n-3c	49.17	943	944	65.8	20006	86.95	46.63
C20:1n-9c	51.12	739	785	26.8	329	1.15	0.50
C18:4n-3c [a)]	52.27	902	914	42.9	14852	64.95	35.45
C20:2n-6c	55.03	691	696	24.6	254	1.03	0.63
C20:3n-6c	57.38	861	922	33.4	1038	3.87	2.35
C20:4n-6c	59.04	955	955	75.2	31359	139.89	76.00
C20:5n-3c	62.83	941	941	42.6	19175	85.97	47.54
C22:6n-3c [a)]	71.16	845	856	17.4	1188	5.02	2.66

[a)] Not represented by a reference standard in the Restek Food Industry FAME Mix.

Appendix IX: Free fatty acid fraction *Palmaria palmata*

Table A.11: Summary table for the free fatty acid fraction in *Palmaria palmata.* Values for matchfactor, reverse matchfactor and probability were acquired through NIST 2014 library searches based on spectral information. Also included in the table are the average areas and retention times. Average amount represents total contribution of each respective fatty acid to total FA content in the fraction (µg/g DW). N=4.

FAME	Retention [%]	Matchfactor	Relative Matchfactor	Probability [%]	Avg. area	Avg. amount [µg]	SD
C13:0	12.27	909	912	61.3	913	1.10	0.06
C14:0	14.16	936	939	70.4	17314.75	20.65	1.34
C15:0 (14-methyl)[a)]	15.42	811	836	10.1	229	0.26	0.07
C15:0	16.8	910	916	63.7	1155.5	1.26	0.09
C16:0	20.52	930	944	75.5	83940.25	91.97	7.09
C16:1n-9c [a)]	22.46	838	838	29.7	199	0.23	0.06
C16:1n-7c	22.92	918	919	29.2	9293.75	10.52	0.74
C16:1n-5c [a)]	24.39	897	911	19.9	574.75	0.65	0.07
C17:0	25.34	841	849	63	325.5	0.36	0.03
C16:2n-4c [a)]	28.05	856	856	32.7	240.75	0.27	0.02
C18:0	32.35	936	952	73.3	17768	20.02	1.94
C18:1n-9c	36.05	932	932	7.11	9067.75	10.36	1.34
C18:1n-7c [a)]	36.84	916	917	7.92	2935.75	3.36	0.33
C18:2 other [a)]	39.81	802	826	10.4	404.75	0.56	0.19
C18:2n-6c	42.99	938	941	34.1	2108.25	2.76	0.36
C18:3n-6c	46.82	833	833	24.9	120.5	0.16	0.02
C20:0	48.9	862	875	44.9	344.5	0.39	0.05
C18:3n-3c	49.22	921	922	52.3	2272.75	3.08	0.34
C20:1n-9c	51.24	872	872	20.9	484.5	0.57	0.08
C18:4n-3c [a)]	52.35	909	919	50.4	1847.75	2.49	0.33
C22:0	58.89	846	848	72.6	129	0.14	0.03
C20:4n-6c	59.1	882	883	39.3	623.25	0.86	0.13
C22:1n-9c	60.54	882	889	38.4	519.75	0.62	0.11
C20:4n-3c [a)]	61.35	813	820	12.4	103.5	0.15	0.05
C20:5n-3c	62.91	929	930	31.7	5191.75	7.10	2.36
C24:0	66.27	789	793	61.5	162.5	0.19	0.08
C24:1n-9c	67.67	889	889	53.2	2838	3.71	0.56
C22:6n-3c [a)]	71.26	690	695	6.66	100.25	0.13	0.05

[a)] Not represented by a reference standard in the Restek Food Industry FAME Mix.

Appendix X: Polar lipid fraction *Alaria esculenta*

Table A.12: Summary table for the polar lipid fraction in *Alaria esculenta.* Values for matchfactor, reverse matchfactor and probability were acquired through NIST 2014 library searches based on spectral information. Also included in the table are the average areas and retention times. Average amount represents total contribution of each respective fatty acid to total FA content in the fraction (µg/g DW). N=4.

FAME	Retention [%]	Matchfactor	Relative Matchfactor	Probability [%]	Avg. area	Avg. amount [µg]	SD
C14:0	14.06	949	951	73.8	25329	1209.5	1671.3
C15:0	16.69	924	929	67.1	1190	48.9	66.8
C16:0	20.31	953	954	81.4	38493	1684.6	2324.7
C16:1n-7c	22.76	932	933	32.4	5390	232.8	318.8
C16:1n-5c [a)]	23.42	856	917	25.4	227	9.4	12.8
C17:0	25.18	805	832	34.4	166	6.7	9.2
C16:2n-6c [a)]	26.04	803	828	22.3	151	8.0	11.3
C16:2n-4c [a)]	27.82	881	881	37.2	356	13.3	18.0
C17:1n-7c [a)]	28.23	760	812	17.5	115	2.8	3.8
C18:0	32.03	892	915	72.4	1182	52.0	71.6
C18:1n-9c	35.88	936	937	7.84	23524	1076.1	1481.8
C18:1n-7c [a)]	36.59	898	899	8.08	634	29.2	40.3
C18:2n-6c	42.81	953	954	33.4	14967	780.0	1073.2
C18:3n-6c	46.65	926	928	64	2487	131.7	180.9
C20:0	48.7	919	944	55.8	893	40.0	54.9
C18:3n-3c	49.09	941	942	65.6	7305	398.7	549.5
C20:1n-9c	51.03	714	721	13.2	108	4.3	5.8
C18:4n-3c [a)]	52.27	910	922	49.8	39708	2178.3	3008.1
C20:2n-6c	54.99	841	844	29.2	261	12.8	17.6
C20:3n-6c	57.34	892	933	44.9	945	40.7	55.5
C20:4n-6c	59	952	953	74.3	24799	1376.8	1904.4
C20:4n-3c [a)]	61.23	864	894	32.9	780	41.6	57.3
C20:5n-3c	62.83	952	952	58.6	37546	2055.0	2832.2
C24:0	66.16	612	661	34.8	43	1.5	2.0
C22:6n-3c [a)]	71.14	866	868	29.5	921	40.5	55.4

[a)] Not represented by a reference standard in the Restek Food Industry FAME Mix.

Appendix XI: Polar lipid fraction *Saccharina latissima*

Table A.13: Summary table for the polar lipid fraction in *Saccharina latissima*. Values for matchfactor, reverse matchfactor and probability were acquired through NIST 2014 library searches based on spectral information. Also included in the table are the average areas and retention times. Average amount represents total contribution of each respective fatty acid to total FA content in the fraction (µg/g DW). N=3.

FAME	Retention [%]	Matchfactor	Relative Matchfactor	Probability [%]	Avg. area	Avg. amount [µg]	SD
C14:0	14.09	945	947	75.7	50474	33.76	2.29
C15:0	16.7	922	928	68.3	1432	0.88	0.07
C16:0	20.31	947	954	77.8	37127	22.85	2.23
C16:1n-7c	22.78	934	935	32.2	7393	4.69	0.31
C16:1n-5c [a)]	23.44	891	931	32.8	542	0.34	0.03
C17:0	25.18	830	853	42.1	283	0.18	0.05
C16:2n-6c [a)]	26.06	843	854	22.7	277	0.18	0.01
C16:2n-4c [a)]	27.88	846	847	35.5	312	0.20	0.01
C17:1n-7c [a)]	28.28	733	795	26.9	113	0.07	0.02
C18:0	32.05	891	923	66.8	1332	0.86	0.30
C18:1n-9c	35.91	940	940	9.51	28318	18.29	1.41
C18:1n-5c [a)]	36.62	818	819	6.87	386	0.25	0.07
C18:2n-6c	42.87	946	958	34	27229	20.19	1.33
C18:3n-6c	46.67	908	911	47.8	1547	1.17	0.06
C20:0	48.7	842	891	45.2	380	0.24	0.03
C18:3n-3c	49.1	931	933	58.3	5205	3.97	0.19
C18:4n-3c [a)]	52.25	913	929	46.3	20035	15.29	0.86
C20:2n-6c	55	833	838	21.3	244	0.17	0.01
C20:3n-6c	57.35	889	921	53.9	1389	0.88	0.04
C20:4n-6c	59.02	953	953	75.3	32925	25.66	1.01
C20:4n-3c [a)]	61.24	871	902	37.7	811	0.63	0.04
C20:5n-3c	62.82	949	949	51.2	21353	16.63	0.51
C22:6n-3c [a)]	71.16	873	876	22.9	217	0.16	0.01

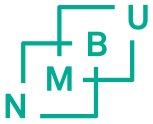
[a)] Not represented by a reference standard in the Restek Food Industry FAME Mix.

Appendix XII: Polar lipid fraction *Palmaria palmata*

Table A.14: Summary table for the polar lipid fraction in *Palmaria palmata*. Values for matchfactor, reverse matchfactor and probability were acquired through NIST 2014 library searches based on spectral information. Also included in the table are the average areas and retention times. Average amount represents total contribution of each respective fatty acid to total FA content in the fraction (µg/g DW). N=4.

FAME	Retention [%]	Matchfactor	Relative Matchfactor	Probability [%]	Avg. area	Avg. amount [µg]	SD
C14:0	14.15	929	937	71.6	7901	37.48	9.24
C15:0	16.79	873	875	53.6	290	1.29	0.152
C16:0	20.47	936	948	78.3	68629	305.85	40.48
C16:1n-7c	22.89	894	895	19	935	4.23	1.01
C16:1n-5c [a)]	24.38	851	872	28	354	1.53	0.69
C17:0	25.33	801	817	56	106	0.47	0.035
C18:0	32.22	888	924	51.8	1439	6.52	0.84
C18:1n-9c	36.06	942	942	11.9	16826	79.03	9.74
C18:1n-7 [a)]	36.79	901	903	7.34	1018	4.71	0.85
C18:1n-1-yn	39.43	723	6.98	182	150	0.58	0.48
C18:2n-6c	42.94	889	891	14.5	558	3.00	0.41
C18:3n-6c	46.78	765	765	12.7	77	0.45	0.14
C20:0	48.85	752	760	71	83	0.41	0.17
C18:3n-3c	49.21	833	846	25.4	147	0.82	0.09
C20:1n-9c	51.18	811	812	25.8	196	0.91	0.26
C18:4n-3c [a)]	52.32	883	891	29	480	2.66	0.23
C22:0	58.88	745	756	40.3	111	0.57	0.63
C20:4n-6c	59.05	738	739	20.7	143	0.86	0.34
C22:1n-9c	60.52	753	753	29.9	202	0.95	0.37
C20:5n-3c	62.91	943	945	38.6	23075	130.62	11.19
C24:0	66.28	548	548	16.1	35	0.17	0.08
C24:1n-9c	67.65	863	864	50.7	550	2.83	0.91

[a)] Not represented by a reference standard in the Restek Food Industry FAME Mix.



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