Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

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Abstract

To know if seaweed products on the Swedish markets could contribute to a better ω3 polyunsaturated fatty acid (PUFA) nutritional status a selection of eight dried red and brown macroalgae were chosen for this study. The fat content was determined in all the algae and the fatty acid composition was examined by gas chromatography. Overall the seaweeds displayed a low fat content with 0.24 and 3.28g/100g dry weight (DW). The red algae *Porphyra yezoensis* had the highest content of PUFAs with 58.7% and also the highest content of eicosapentaenoic acid (EPA) with 49.8%, while *Undaria pinnatifida* displayed the second highest levels with 44.4% PUFA and 13.3% EPA. Remaining seaweeds had a PUFA content ranging between 0.98 and 30.1% and an EPA content between 0.00 and 4.10%. Expressed in mg/g DW the EPA content for *Porphyra yezoensis* was 6.15 mg/g DW and *Undaria pinnatifida* 1.34 mg/g DW. Majority of the species studied had a beneficial ω6/ω3 ratio with 0.17 and 4.88. Docosahexaenoic acid (DHA) was not detected in any of the samples

The richest source for EPA was *Porphyra yezoensis*. It could be a valuable source for ω3 PUFAs for to improve the ω3 status in humans.

Keywords: macroalgae, rhodophyta, phaeophyta, ω3 fatty acids, polyunsaturated fatty acids

Sammanfattning

Långkedjade ω3 fettsyror behövs för att upprätthålla viktiga fysiologiska funktioner i cellmembranen. Det är vanligt med ett för lågt intag av dessa fettsyror vilket kan leda till diverse bristsymptom. Ett större intag skulle därför vara fördelaktigt för folkhälsan.

Efterfrågan på fiskolja som är en källa till ω3 fettsyror har ökat och för att kunna möta ett eventuellt större behov behövs alternativ. Eftersom fettsyrasammansättningen i tång är fördelaktig med avseende på hög procenthalt fleromättade fettsyror av totalt fettinnehåll är de av intresse. Två av de kvantitativt viktigaste fleromättade fettsyrorna är eikosapentaensyra (EPA) och dokosahexaensyra (DHA).

För att undersöka om tångprodukter på den svenska marknaden kan bidra till en bättre ω3 status gjordes ett urval av åtta torkade röda och bruna makroalger. Fettmängden bestämdes och fettsyrasammansättningen undersöktes i en gaskromatograf. Tången uppvisade låga fetthalter, mellan 0,24 och 3,28g/100g torrvikt. Rödalgen *Porphyra yezoensis* hade högst procenthalt fleromättade fettsyror (58,7%) samt högst innehåll av EPA (49,8%). *Undaria pinnatifida* hade näst högst nivåer med 44,4% fleromättade fettsyror samt 13,3% EPA. Övriga sjögräs hade nivåer mellan 0,98 och 30,1% fleromättade fettsyror samt 0,00 och 4,10% EPA. Uttryckt i mg/g torrvikt var innehållet EPA i *Porphyra yezoensis* 6,15 mg/g och *Undaria Pinnatifida* hade 1,34 mg/g. De flesta sjögräsen hade en fördelaktig ω6/ω3 ratio mellan 0,17och 4,88.

Dessa resultat visar att Porphyra yezoensis är en möjlig källa för EPA och skulle kunna nyttjas för en bättre ω3 status.

Nyckelord: macroalger, rhodophya, phaeophyta, ω3 fettsyror, fleromättade fettsyror

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Abbreviations

PUFA	Polyunsaturated fatty acid
MUFA	Monounsaturated fatty acid
SFA	Saturated fatty acid
LCP	Long chain polyunsaturated fatty acid
EPA	Eicosapentaenoic acid
DPA	Docosapentaenoic acid
DHA	Docosahexaenoic acid
RNI	Recommended nutritional intake
DW	Dry weight
FAME	Fatty acid methyl ester
AA	Arachidonic acid
ALA	Alpha-linolenic acid
LA	Linoleic acid

1 Introduction

Although the levels ingested of ω3 long chain polyunsaturated fatty acids (LCP) in humans are generally low, an adequate amount is needed since they act as a structural component of the cell membrane and are important for various physiological functions. Pregnant and lactating women are especially dependent on a sufficient intake for the child's well being. Large quantities accumulate in the brain the first two years of living, hence it is important for social development, communication and IQ (Hibbeln et al. 2007). Also adverse effects on factors causing cardiovascular disease and type-2 diabetes make these fatty acids an important tool in prevention of disease (Kromhout et al. 1985; Hendrich 2010)

To meet an increasing demand of fish oil additional sources for ω3 LCPs are needed. Partly to protect the diminishing fish stock but also to find more energy efficient alternatives. There are also problematic aspects of using fish oil as supplement because of off-taste and toxic substances (Certik & Shimizu 1999). As the fatty acid composition of seaweeds is advantageous regarding the high levels of PUFAs they are certainly an area of interest. Seaweeds can be grown practically everywhere in the world and can be cultured in large volumes. There are plenty of varieties and many of them are used in the cuisine in the Far East. The nutritional composition is interesting with high levels of protein and fibres, and although a low content of fat the proportions of PUFAs are often over 50%. Of certain interest are EPA and DHA since they are two of the quantitatively most important PUFAs.

1.1 Objectives

Seaweeds could be a potential source of the ω3 fatty acids EPA and DHA accounting for two of the quantitatively most important LCPs. The objective is to investigate the fat content and composition of a selection of red and brown seaweeds on the Swedish market and to investigate if they have adequate amounts to contribute to a better ω3 status in humans.

1.2 Interests

To meet an increasing demand for fish oil it is necessary to find alternative sources. Other sources are needed to protect the diminishing fish stocks to have a sustainable production of fish oil. Seaweeds are known for having a high percentage of LCPs of total fat content, some of which are difficult to get from terrestrial sources. Of particular interest are EPA and DHA that mainly come from marine sources and are needed for important physiological functions in the body. The ω3 PUFA status in humans is generally low and seaweeds could act as an additional source. Seaweeds are interesting because they can be consumed as a part of a meal instead of a supplement contributing to flavour and a sensation of fullness.

2 Background

2.1 Environmental aspects

The vast majority of ω3 oils come from fish from the oceans, a source that is facing overexploitation. FAOs report "Review of the state of the world marine fishery resources" (2011) divides the fish stock into three categories; not fully exploited fully exploited or overexploited. The largest category is fully exploited with 57.4% followed by overexploited with 29.9%. To meet a growing demand of fish oil there is no possibility of expansion. If not properly handled the situation might get worse, which was the case previous years. In 2008 the category fully exploited fish made up for 53.3% and 28% was estimated to be overexploited, 3% was depleted and 1% recovering from depletion. It has been an increase in overfishing since the 70´s with 10% overfished stocks in 1974 to 26% in 1989 (FAO 2010). Although large quantities of the world production of fish, an increasing number now over 40% are from aquaculture, the main part of the captured fish are used as feed in aquaculture (FAO Fisheries and Aquaculture Department 2013).

There are some problematic aspects of using fish oil such as off-taste and odour, potentially toxic substances, presence of fatty acids with unfavourable properties such as arachidonic acid (AA) and also variations in quality of the oil due to seasonable differences. If ω3 PUFAs were to be used world wide as a prophylactic drug it would be necessary with additional sources. The world production of fish oil would not be able to meet the demands and alternatives as algae could be a potential source (Certik & Shimizu 1999). The annual demand for EPA in Japan is 125 tonnes and the world wide demand is estimated to be much greater, about 300 tonnes. Also because of the fluctuant quality, price and availability of fish oil a more reliable source would be useful. Oil from microalgae has the ability to compete with fish oil in both price and quality (Belarbi et al. 2000; Sanchez Mirón et al. 1999).

2.2 EPA and DHA as essential fatty acids

EPA and DHA are two of the quantitatively most important fatty acids. They are ω3 PUFAs with one of the double bonds located at the third carbon from the methyl end. They are not essential *per se* since they can be synthetized from α-linolenic acid (ALA) in the human body, but are crucial for the function of membrane structural lipids.

Some fatty acids cannot be synthesized in the body and are therefore essential. Since essential fatty acids are impossible to acquire in other ways they have to be ingested as a part of the diet. Linoleic acid (LA) and ALA are the parent essential fatty acid that by chain elongation, chain shortening or desaturation can be transformed into LCPs with ≥ 20 carbon atoms and ≥ 3 double bonds. LA is the precursor of AA, an important ω6 fatty acid that also can be found in considerable amounts in meat. ALA is the precursor for the two major LCP ω3 fatty acids, EPA and DHA which can be found in high concentrations in fish (Muskiet et al. 2004).

EPA and DHA are known for having numerous health benefits; adverse effects on risk factors for cardiovascular disease, coronary heart disease and type 2 diabetes and plays an important role as a structural component of the cell membranes. It is especially important for the developing brain where it accumulates in large amounts for the first two years bot pre- and postnatal, why it is also important for pregnant and lactating women to get sufficient amounts.

During pregnancy it expresses on a mental level with less symptoms of depression. A study conducted on pregnant women who were given either ω3 PUFAs or placebo showed that participant from the ω3 group had a lower prevalence and symptoms of depression (Su et al. 2007). The development of the brain is dependent on an adequate intake, whereas pregnant women in the U.S. are advised to limit their consumption of fish to 340 g per week, given with the reason that pregnant women should be less exposed to neurotoxins. Based on these recommendations an observational cohort study was conducted to see possible health benefits or drawbacks. Positive effects were seen for the child development rather then adverse when eating more than 340 g per week. When eating less the children had an increased risk of being in the lowest quartile when measuring IQ. Also other outcomes like suboptimal social development, communication, prosocial behaviour and fine motor ability were associated with a decreased intake of fish. For all of the outcomes measured the result showed that the consumption was correlated to the amount of fish consumed. The less ingested the higher probability of adverse effects (Hibbeln et al. 2007).

A decreased risk of cardiovascular disease (CVD) in epidemiological studies has been associated with intake of oily fish. The mortality from CVD has also been shown to have an inverse relation (Kromhout et al. 1985). DHA has been shown to decrease the hypertension and stroke related behaviour changes in a certain strain of rats more prone for these diseases. The rats life is thereby prolonged and also positive results for learning performance and lowered systolic blood pressure was a result of DHA consumption (Minami et al. 1997).

Omega 3 PUFAs have been shown to have positive effects also in other age related diseases. Type 2 diabetes, which is also a disease strongly correlated with obesity is influenced by what fatty acids consumed. Trans fatty acids are known for having a negative effect while it is still under investigation whether ω3 fatty acids have a positive effect (Hendrich 2010). The Nurses Health study which is a long running investigation of women's health with over 200.000 participants found that a higher intake was correlated to lower prevalence of type 2 diabetes, although also clinical trials have to be performed to confirm (Salmerón et al. 2001). Another age related disease is sarcopenia. Probable beneficial effect on the muscle protein synthesis when eating supplementation with ω3 fatty acids has been seen and also prevention from sarcopenia from emerging (Smith et al. 2011).

2.2.1 Nutritional recommendations for ω3 PUFAs

EFSA has set dietary reference values for fats including EPA and DHA. In general for fats the recommendations are in E% but for LCPs and cholesterol it is expressed in mg. E% stands for energy percentage and states how much of the total calorie count that comes from various macronutrients. The level for adequate intake (AI) for EPA plus DHA is 250 mg/day for adults. Pregnant and lactating women should have an intake of 100 to 200 mg higher than the basic recommendations. For infants from 6 months and children up to 24 months the AI is 100 mg for DHA with no recommendations set for EPA (EFSA Panel on Dietetic Products Nutrition and Allergies 2010).

Nordic Nutrition Recommendations (2012) writes that an intake up to 200 and 250 mg/day DHA + EPA has been associated with a lower risk of cardiovascular disease. ALA which is a precursor for DHA and EPA is recommended to make up for at least 0.5E% of the total energy intake. For pregnant and lactating women they advise 200 mg DHA per day. Young children between 6 and 23 months should have 1E% from ω3 fatty acids including DHA but it is not specified in mg. They also write that the ratio between ω6 and ω3 is not set since there is not enough evidence to prove a relation between the two (Nordic Nutrition Recommendations 2012).

The US-institute of medicine based the AI of ALA on the median intake of the US population. The recommendation was made claiming it is very rare with ω3 LCP deficiency in healthy individuals. They do not have specific recommendations for EPA and DHA instead the dietary reference intake (DRI) is set for ALA. For adults, children 1 to 3 years old and children 4 to 18 years old the RDI is between 0.6 and 1.2 % of the daily energy consumed (IoM 2006).

EFSA writes that many authorities have set separate recommendations for ALA, EPA and DHA because it is unsure how well the conversion from ALA works and that the fatty acids might have different functions in the body (EFSA 2010).

No recommendations have been set for the proportions of EPA and DHA. There is not enough research done to suggest that either of the fatty acids would be more beneficial than the other. In supplements the proportions normally range between 1:2 and 2:1. Supplements on the market in Kansas city summer of 2003 had a predominant ratio of 180 mg EPA and 120 mg DHA (Harris 2004).

Of importance is also the ratio of ω6/ω3 PUFAs. Based on the recommendations for adequate intake of ω6 and ω3 PUFAs Simopoulos (2002) suggests that a ratio of 1:1 or 2:1 should be considered as a dietary guideline. Although various studies have shown that a ratio between 1:1 and 4:1 had good results depending on what disease investigated. Humans historically evolved on a ratio of 1 while a western diet has a ratio of 15:1 – 16.7:1. The high ratio in the western diet is a result of the progressions in agriculture which rapidly changed the amount of ω6 fatty acids consumed.

2.2.2 A world wide deficiency

Data available for EPA and DHA intake is scarce. One study conducted by Elmadfa & Kornsteiner (2009) showed data for PUFA intake from 14 countries and intake fore EPA/DHA from twelve countries. It concluded that all countries reported higher intake for ω6 PUFAs than needed to prevent deficiency symptoms (>2.5E%), consisting mainly of LA. However the intake was smaller than the recommendations from WHO/FAO (5 to 8E%). The intake of ω3 is drastically lower, however, the recommendations set are also lower. An intake of >0.5 to 1E% of mainly ALA was seen in nine out of 14 countries. The remaining five countries had an intake below 0.5E%, an intake very close to the limit of what would be inadequate for staying healthy. For EPA and DHA there were big differences between the countries. The lowest intake was found in China with 0.3%E and the highest in Japan with 1.05E%.

Data is also available from the U.S. An observational study made with food surveys including 14 388 participants collected data for intake of fish and ω3 fatty acids. The intake varied depending on gender and age, a higher intake was found in older adults and men, still few consumed recommended levels. The average intake from food was for ALA 1.5g/day, EPA 23 mg/day and DHA 63 mg/day. Data was also registered for intake of food and additional supplements obtaining higher levels for ALA 1.6g/day, EPA 41g/day and DHA 72g/day. These intakes displays levels that does not live up to the recommendations, suggested is that food supplementation might be needed to meet the daily demands (Elmadfa & Kornsteiner 2009).

2.2.3 Limitations of EPA and DHA synthesis in humans

The same enzymes are used for converting ALA and LA to their respective long chain ω3 PUFA. Therefore it is a competition of which fatty acid the enzymes transform. When the amount of ALA consumed increases the conversion to EPA and DHA augments and the same with LA to AA. One of the most important enzyme is the Δ6-desaturase which catalyses the conversion of LA to GLA and ALA to stearidonic acid (Russo 2009). Since the affinity of Δ6-desaturase is higher for LA it takes 10 times more ALA to reduce the production of GLA from LA (Mohrhauer et al. 1967). For long it was unknown how the formation of DHA from EPA was carried out. A proposed enzyme called Δ4-desaturase was thought to be responsible; it was also thought to catalyse the formation of ω6 docosapentaenoic acid (DPA) from adrenic acid. The enzyme is not found in humans but the gene expressing it has now been found in the microalgae Isochrysis and works specifically on ω3 and ω6 C_{20} PUFAs (Pereira et al. 2004). The production of DHA in humans use another pathway first producing tetracosahexaenoic acid (C 24:6 ω3) by several elongation and desaturation steps. By β-oxidation of this compound DHA (C22:6 ω3) is formed. The enzymes required for this process comes from both the endoplasmatic reticulum (ER) and the peroxisomes. First synthesis of C_{24} ω3 and ω6 fatty acids take place in the ER after which they are transferred to the peroxisomes for β-oxidation. After completed formation the fatty acids move back into the ER for being utilized for membrane lipid biosynthesis (Baykousheva et al. 1995).

There are great limitations of the synthesis of EPA from plasma ALA, around 0.2% is transformed. Plasma EPA on the contrary has 63% available for production of DPA and DPA has 37% available for transformation to DHA. Therefore dietary EPA might be a suitable precursor for DHA since there is a greater transfer of mass than it is in the biosynthesis from ALA (Pawlosky et al. 2001).

2.2.4 Vegetable oil and fish oil as sources for EPA and DHA

Since DHA and EPA can be obtained from vegetable oil by bio-conversion and from fish oil directly it is of interest to se how well the body can assimilate the fats from these sources. In vegetable oils it is mainly ALA present whereas fish oil contains both EPA and DHA. Several studies examined the conversion of ALA to EPA and DHA with various results. The difference in concentration of DHA in body tissues between consuming rapeseed oil containing ALA or fish oil with very long chain fatty acids has been examined in an experiment with rats. The rats were first fed with a diet low in ALA. When given the same amounts of oil the group fed with fish oil had higher concentrations of DHA in all the tissues examined. About two times more vegetable oil was needed to get the same amount of DHA. A quicker recovery of DHA concentrations in the nervous tissue was also seen with fish oil (Bourre et al. 1997). One study found that the conversion is very limited in humans, especially to DHA. The conversion was investigated in a group of African-American people with chronic illness. In 12 weeks the plasma EPA levels rose with 60% and the DPA levels with 25%. The concentration of DHA in the blood plasma did not rise at all (Harper et al. 2006).

The conversion is higher for women than for men, which might be because of oestrogen that can regulate the conversion. When pregnant, the levels of oestrogen rise and might stimulate the conversion since the demand for DHA increase. However it might still be insufficient for pregnant and lactating women since the demand is even higher. If the demand is low for basic maintenance of membranes the conversion might be sufficient even though it is less (Burdge & Calder 2005). A study performed on young women found that the conversion was considerably higher for women than for men with a net fractional conversion of ALA resulting in 21% EPA and 9% DHA. These result indicate that the conversion when pregnant might be even higher making up for a larger part of the daily need because of the higher levels of oestrogen (Burdge & Wootton 2002).

The conversion of LA to AA is very efficient at the expense of ALA conversion. However there are studies that found that even when the conversion is low it can be sufficient. A study conducted on men found that the conversion to EPA was 6% and to DHA 3.8%. Based on that 2 g ALA would in an American diet be enough to provide for 75 to 85% of the daily requirement for some individuals (Emken et al. 1994). These studies suggest that the conversion to DHA is very limited whilst the conversion to EPA and is more efficient. What was not investigated is how well the conversion from EPA to DHA works, which might be more relevant when regarding seaweeds. ALA might still be an alternative for fish oil and is worth considering as an alternative. More research has to be done on how well the conversion works and under what circumstances.

2.2.5 Sources of long chain fatty acids

Animals and higher plants lack the requisite enzymes to synthetize PUFAs longer than C_{18} in any significant amounts. Therefore we rely on other organism able to perform the formation or by consuming animals that accumulate the fats in their tissue. Fish and fish oil contain high amounts of these fatty acids and are therefore good source of LCPs. The fatty acids in fish originate from microalgae, the primary producers of ω3 EPA, DHA and DPA. The health benefits prescribed to fish oil therefore also apply to oil from microalgae (Certik & Shimizu 1999).

Microalgae are a valuable source of long chain fatty acids since they can be grown on various substrates in many different environments. They are fast growing and can be grown throughout the year. It is also easier to control the quality of the oil compared to other sources and therefore get PUFAs that can be used for pharmaceutical purposes (Certik & Shimizu 1999).

Macroalgae are also a source of PUFAs. Even though they have a low fat content, the amount is equal to or higher than that of land growing vegetables. The content of important PUFAs indicates that macroalgae could be used to prepare low fat foods with a favourable composition of fat (Kumari et al. 2009). The composition of fat is interesting since it for many seaweeds consists of more than 50% unsaturated fatty acids (Herbreteau et al. 1997). Especially red algae have shown to have a content of PUFAs close to half of the fat content in many cases consisting of the quantitatively important EPA (Fleurence et al. 1994). Table 1 gives an overview of what amounts of EPA and DHA found in previous research in the seaweeds investigated in the present study.

Table 1. Percentage of total lipid content found in seaweeds in previous studies

Fatty acid	EPA 20:5 ω3	DHA 22:6 ω3	ΣPUFA
Seaweed	% total fatty acids	% total fatty acids	
Red algae			
Porphyra yezoensis	10.4[a] - 50.1[b]	-	33.9[a] – 63.9[b]
Palmaria palmata	46.6[c]	0.5[c]	53.2[c]
Chondrus crispus	18.7[c]	0.3[c]	45.8[c]

Brown algae			
Hizikia fusiforme	42.4[a]	-	57.0[a]
Laminaria japonica	16.2[a]	-	39.9[a]
Undaria pinnatifida	13.2[a]	-	73.7[a]

[a] (Dawczynski et al. 2007) [b](Noda 1993) [c] (Fleurence et al. 1994)
- not detectable or below 0.1% of total lipid content

2.3 Macroalgae

Macroalgae are also referred to as seaweeds and have a size that ranges from a few cm up to 20 m. It is the size that differs them from the microalgae that are smaller and often consists of only one cell. The pigmentation is the main characteristic for the classification and divides them into three subcategories; brown algae known as phaeophyta, red algae known as rhodophyta and green algae known as chlorophyta. Some seaweeds have a colour not matching with their classification. In those cases plant scientists decides based upon other characteristics in what category they belong (FAO 2003).

Most edible seaweeds grow in a marine environment whereas also freshwater species exists. Because of their habitat and versatility they grow all over the world and have been used by man for hundreds of years. In East Asia they have been both harvested from the sea and cultivated as use for food for humans and feed for animals. It is an industry on the rise, now also utilised in other areas such as for chemicals and pharmaceuticals. This fast growing industry is now one of the largest marine sources regarding the quantity of the biomass (Lüning & Pang 2003).

Recent years seaweeds cultured either in the ocean or in tanks made up for 33% of the biomass produced in the aquaculture sector, meaning 21 million tonnes worldwide. Nearly all of the biomass comes from culture-based systems with minor volumes harvested from the wild. The annual production growth for the last years has been between 5 to 10% on a year-to-year basis. Asia is by far the largest producer with an almost non-existent production in other parts of the world (FAO Fisheries and Aquaculture Department 2013).

2.3.1 Consumption of algae

Most of the algae on the market are used for human consumption. They are often consumed minimally processed but can also act as an ingredient in other products in the form of carrageenan, agar and agarose. The processing often involves cleaning the algae from other plants and animals and to preserve them in a way that keeps their natural characteristics, for example by drying. The most consumed algae is Nori from the species *Porphyra* which is a kind of red algae. Nori is known in the western world mainly because of its use in sushi dishes, which gained in popularity the last decades. In table 2 the major products derived from seaweeds are listed. Their main uses are stated and also the market value to get a comprehension of the proportions of their use (Radmer 1996).

Table 2. The major products derived from seaweed, their use and market value in in 1993

Product	Use	Market value (million $ US)
Nori	Food	1800
Wakame	Food	600
Kombu	Food	600
Alginates	Food products Paper products Biomedical applications	230
Carragenans	Food products Cosmetics Pharmaceutical products	100
Agars	Food produtcs Biomedical applications	160
Agarose	Biotechnology applications	>50
Manure (”Maerl”)	Agriculture	10
Seaweed meal	Animal Feed	5
Liquid fertilizer	Agriculture	5
Phycobiliproteins	Biomedical uses	2

(adapted from Radmer 1996)

When instead considering the biomass, Kombu (*Laminaria japonica*) has the highest production with 4.8 million tonnes, Eucheuma that is used mainly for carrageenan 3.8 million tonnes, Wakame (*Undaria pinnatifida*) 1.8 million tonnes and Nori (*Porphyra yezoensis*) 1.4 million tonnes (FAO 2010).

Japan has the highest consumption of seaweed and over 20 species are consumed. From 1949 to 1996 the Japanese population consumed an average of 5.5 g/day and the consumption has been stable over the years (Matsumura 2001). Based on commercial sales the consumption goes up to 10 g/day or 4 kg/year and person (Beverly & Sokimi 2000). Even though little variation between the years, the trend of what algae consumed has changed. In the 1950´s Japan started to transit into a more westernized diet (Drewnowski & Popkin 1997). It is believed that Kombu became less popular and Wakame increased in popularity (Katamine et al. 1986).

2.3.2 Nutritional aspects

The Japanese are one of the world's longest living people and some types of cancer are rare. One major difference is their high intake of seaweed. The high seaweed intake has shown to have health benefits not seen in other parts of the world. An elevated intake of iodine might be a contributing factor as the average Japanese consume more than in the western world (Zava & Zava 2011). Other benefits prescribed to seaweed consumption is the high levels of fibres. It is higher than in terrestrial vegetables and could provide 12.5% of the daily need in an 8g serving, a considerable amount that could be valuable to include in the diet. Seaweed contains many types of fibres including carrageenan, agar and alginates. These are soluble fibres that can protect against cardiovascular and gastrointestinal diseases. They can also increase the feeling of satiety, reduce the intestinal absorption and function as an bulking agent to speed up the passage through the intestines and favour the intestinal micro flora (Brownlee et al. 2005).

Another feature that distinguishes seaweeds from terrestrial vegetables is the high mineral content. They contain a large variety of macro minerals and trace elements. In red algae the content varies between 20.6 and 21.3% and in brown algae between 30.1 and 39.3%. Land growing vegetables contain between 5 and 10% with exceptions like spinach that comes close to that of seaweed with 20.4% (Rupérez 2002). Including seaweeds in the diet could help to fulfil the daily requirements for many important minerals. MacArtain et al. (2007) writes that an 8g serving of dry *Palmaria palmata* contains more iron than 100g of sirloin steak, as much as 6.4 mg compared to 1.6 mg. An 8g serving of *Ulva lactuca* provides 260 mg calcium, which is 37% of the recommended nutritional intake (RNI) for an adult male. The same amount of cheese would contribute with about 5% of the RNI. The bioavailability of calcium from seaweed is unknown, but it is a source worth consideration (Titchenal & Dobbs 2007).

Some algae such as *Porphyra tenera* and *Palmaria palmata* have a high protein content, as much as up to 35% and 47% respectively (Fleurence 1999). Some seaweeds could therefore be a potential source of proteins, although the bioavailability is under investigation. For *Palmaria palmata* the presence of fibre and the cell wall encapsulating the proteins make the digestibility low. It is suggested that the bioavailability can be increased by fermentation and different forms of processing (Marrion et al. 2003).

The fat content of seaweed is generally low, between 1 and 6 g per 100g DW (Herbreteau et al. 1997; Dawczynski et al. 2007). The composition of fat is interesting because of the high proportions of PUFAs, a substantial amount made up of ω3 fatty acids. Compared to land growing plants they are richer in ω3 and ω6 PUFAs since they come from a marine environment. Red algae have concentrations ranging between 19.0–50.0% of the total fatty acid methyl ester (FAME) content (Fleurence et al. 1994). Some varieties of *Porphyra* sp. and *Hizikia fusiforme* have PUFA concentrations above 50% of the fat content (Dawczynski et al. 2007). Brown algae seem to have lower concentrations of EPA than red algae, between 13 and 40%. However the total amount of PUFAs can reach to over 70% (Dawczynski et al. 2007).

2.3.3 Bioavailability of lipids

Absorption and bioavailability is to take into consideration when regarding nutritional aspects. The bioavailability of lipids from seaweeds has been poorly investigated, even less so in humans. Brown seaweeds seem to have gained more interest than red and the subject of research has often been the fibre content investigated as a potential source of dietary fibre, with lipid bioavailability as a side-track. The results are inconsistent displaying fat digestibility both lower and higher compared to a control diet. Lower digestibility of both fat and protein has been seen in a seaweed containing diet compared to a control diet in a study made on rats (Gudiel Urbano & Goñi 2002). A study executed in the same manner also performed on rats gave higher digestibility for fat but lower for protein (Suzuki et al. 1993).

Brown and red algae contain fucoxanthin, a carotenoid that has been seen to have biological properties beneficial for human health. Moreover it seems to have an impact on fat absorption from seaweeds affecting the levels of highly unsaturated fatty acids absorbed by the body. An increase of AA and DHA has been seen in rat liver when eating a diet containing certain types of algae. It is believed that fucoxhantin up regulates the enzyme activity of Δ6-desaturase, an important enzyme in the synthesis of DHA and AA (Airanthi et al. 2011).

2.3.4 Unwanted substances in algae food products

Some substances found in seaweed can be problematic at to high concentrations. The surrounding environment during growth affects the content and consequently the product to be consumed. Mineral content of seaweed are generally high and therefore also unwanted heavy metal assimilation can be a problem. High levels of cadmium and inorganic arsenic have been found in seaweed products in Spain. Many products exceeded the levels set for inorganic arsenic set by French legislation making them unsuitable for human consumption (Almela et al. 2002; Besada et al. 2009). Based on the Japanese algae consumption in addition to arsenic the levels for iodine, mercury and cadmium are above the dietary intake values for many seaweed products. Mercury has been found in products from Japan, Canada and Norway with higher levels in seaweeds from Japan. Some levels high enough to be a source for unwanted exposure. Also traces of radioactive compounds have been found in some seaweeds, however the low levels might be naturally occurring uranium decay (Van Netten et al. 2000). Elevated levels of iodine can be a problem if the consumption of seaweed is too high. High levels of iodine could especially be a problem for pregnant and lactating women (Livsmedelsverket 2015). The levels ingested by Japanese are much higher than recommended by the Swedish food agency, 6 to 20 times more. It is uncertain how a high intake expresses with both positive and negative outcomes reported (Zava & Zava 2011).

2.3.5 A need for vegetarian sources?

The amount of vegetarians in the world have recently been estimated by Elimear et al. (2010) at the Economical and social research institute (ESRI) of Dublin. It was concluded that approximately 22% of the world population is vegetarian, meaning one and a half a billion people. As expected vegetarians were more prevalent in low-income countries but also great variations between low-income countries were found. Probable reasons are the availability, the price and also cultural differences. In high-income households there were smaller differences between countries. A slightly higher prevalence of vegetarians was seen with increased income. The income is connected with whether vegetarian by choice or necessity a distribution of 5 and 95%. In the United Kingdom a new trend has been seen from 1960´s. Then about 0.5% of the population was vegetarian a number that has increased to over 2%. This trend is probably true for more developed countries.

Vegetarians and vegans generally eat less fat than omnivores; the fat also comes from others sources resulting in lower EPA and DHA status. Studies have shown that the there is almost no DHA in vegetarian and vegan diets. If eggs are consumed the levels can be somewhat higher, providing 20 mg/egg (Sanders 2009). One study found that the intake of both LA and ALA appears to be higher in vegetarian and vegan diets although the overall intake of ω3 LCPs seems to be lower. The timespan of consuming a diet excluding or partially excluding animal products did not seem to matter for the levels of EPA and DHA in blood plasma since it did not differ between long and short-term vegetarians and vegans. This study also found that when excluding animal products from the diet the body produces endogenous EPA and DHA resulting in low but stable levels (Rosell et al. 2005).

The conversion from ALA has been specifically investigated in vegetarians since it is the predominant source in a plant based diet. The findings from a study made with vegetarian males suggest that the levels of total PUFAs increased in platelet phospholipids and plasma phospholipids eating a diet moderate or high in ALA. The levels of EPA also increased, but not the levels of DHA (Li et al. 1999). Although the intake of both ALA and LA seems to be higher in vegetarians the ratio seems to be at disadvantage. The higher intake of LA makes the intake of ALA low in comparison resulting in unfavourable conditions for conversion. According to Sanders (1999) the suggested ratio of LA:ALA for vegetarians should be between 4:1 and 10:1 for the conversion to work optimal but the current status is greater with a ratio of 15:1 – 20:1. Therefore a lowered intake of LA could be recommended to reach this status. It has also been claimed that the ratio is not of importance for the conversion, it is rather the total intake of ALA and LA. EPA formation can benefit from lowering the intake of LA, but the formation of DHA can increase just by having a higher intake of ALA (Goyens et al. 2006). That would be beneficial for vegetarians since they have an overall higher intake of ALA.

Plant sources for ω3 long chain fatty acids are only derived from the sea from seaweeds and microalgae. The fat content of these plants are generally low with the exception of some microalgae. Therefore it can be challenging to get adequate amounts from these sources. Consequently vegetarians and vegans mainly have to rely on the conversion from ALA to meet the daily requirement. Davis and Kris-Etherton therefore encourage non-meat eaters to make dietary changes to optimize the conversion into these long chain ω3 fatty acids (Davis & Kris-Etherton 2003).

1 Material and method

1.1 Methodology

To evaluate if seaweed products on the Swedish markets can be a source for ω3 LCPs a selection of brown and red seaweeds were chosen to represent the algae available. The selection was made upon researching the Internet for the range of products available. With not many species to choose from it resulted in six species, one in duplicate because it was available from Japan and Iceland. Excluded from the study was products containing oil, like seaweed crisps and seaweed salads. Including them would affect the total fat content and it would be difficult to calculate the amount of the fatty acids of interest.

The fat content and composition were determined in order to approximate how much ω3 LCP the seaweeds can contribute with. The experiment was carried out in a laboratory with techniques described further in the following section. In short, extraction of fat was performed to determine the total fat content needed to calculate the amount of different lipids. Qualitative and quantitative analyses were performed in a gas chromatograph to determine the fatty acid composition and the percentage of total fat for the individual fatty acids.

In addition a qualitative literature study was carried out in order to present previous research on the topic. A literature study can serve several purposes; presenting results from other studies so they can be compared with any new findings, exploring if there are gaps in previous research that is needed to answer the research question and puts the present study in a larger context (Creswell 2003). Finally the results were compared to previous findings and assumptions were made to evaluate seaweeds possible utilisation as a source for ω3 LCPs.

1.2 Sample collection

Macroalgae from rhodophyta and phaeophyta were chosen to represent the two most common divisions of seaweeds on the market. The algae were purchased in Sweden 2015 and all the algae were dried. Table 3 presents the scientific names and the commercial names.

Table 3. Algae from two different classes used for lipid analysis

Classification	Name	Commercial name
Red algae/Rhodophyta	Porphyra yezoensis	Nori
	Palmaria palmata	Dulse
	Chondrus crispus	Carrageen moss/ Irish moss
Brown algae/Paeophyta	Hiziki fusiforme	Hijiki
	Laminara Japonica	Kombu
	Undaria pinnatfida	Wakame
	Undaria pinnatfida (sprout)	Mekabu

1.3 Determination of dry weight

For comparison of fat content, the algae were dried additional to the drying done before purchase. The algae were cut to smaller pieces of approximately 1×1 cm. Roughly 2 g of each alga were weighed in duplicates in crucibles. The crucibles were put into an oven with the constant temperature of 85°C and the samples were dried until no further changes in weight. The samples were weighed after 21, 44 and 68 hours to control when all moisture had evaporated. The dried weights were noted.

The samples used for the lipid extraction and lipid analysis were not dried before analysis and therefore the DW of the samples used was calculated.

1.4 Lipid extraction

The seaweeds were cut into pieces 0.5×0.5 cm and grinded in a mortar before prepared in duplicates with 0.5g for each sample. Water was added (2ml) and the algae were left to swell until all water was absorbed. The lipids were extracted with the hexane isopropanol method described by Hara & Radin (1978). The samples were homogenized with 25 ml of HIP (hexane:isopropanol 3:2) for 5 min using an Ultra-Turrax. Approximately 5 ml of the total HIP volume was used to rinse the homogenizer between each sample. Between the different species the homogenizer was thoroughly cleaned. Na_2SO_4 (6.67%, 9ml) was added and the samples were shaken before left for the hexane phase to separate. The hexane phase was transferred to evaporation flasks and the hexane was let to evaporate with a flow of N_2. When evaporated 0.5 to 1 ml hexane was added and the mixture was transferred to small test tubes.

1.5 Lipid analysis

1.5.1 Methylation

Methylation of fatty acids were performed according to the method described by Appelquist (1968). Dry methanol (2 ml) was added to the test tubes with hexane and lipids and the samples were heated at 60°C for 10 min. The samples were taken out of the heater, 3 ml BF_3 was added and they were put back in the heater at 60°C for 10 more min. The samples were cooled under running water before adding 2 ml 20% NaCl and 2 ml hexane. With a vortex the samples were shaken vigorously before left for the hexane phase to separate. If not separated properly the test tubes were centrifuged at 3000 rpm for 5 min. The upper phase was collected and transferred to smaller pre weighed test tubes. The samples were flushed with N_2 until dry and weighed for determination of FAME content. The FAME were dissolved in 0.2 ml hexane and put in the freezer at -18°C until further trials.

1.5.2 Thin Layer Chromatography (TLC)

Thin layer chromatography was performed to control that the fatty acids were methylated. A solvent of hexane, ether and acetic acid (85:15:1) was made to act as a mobile phase. The stationary phase consisted of a 20×20 silica plate. The plate was prepared by drawing a line 2 cm from the bottom edge and making 8 dots along the line writing under each dot what sample to apply and 2 additional dots for the references. Before put into the mobile phase 5μL of the methylated samples and 10μL of the standards were put on the silica plate. After 1 hour the mobile phase almost reached the top of the silica plate and the plate was taken out to dry for 10 min before put into a chamber with iodine. After approximately 20 to 30 min when the samples were clearly visible the plate was taken out and the samples were compared to the TLC standards.

1.5.3 Gas Chromatography (GC)

The fatty acids were analysed using a gas chromatograph (Varian, CP3800, Stockholm, Sweden) equipped with flame ionisation detector (FID) and split injector and fitted with a 50m length × 0.22mm i.d. × 0.25µm film thickness BPX 70 fused silica capillary column (SGE, Austin, TX, USA). The samples were injected by a CP8400 auto sampler (Varian AB, Stockholm, Sweden) and the temperature of the column was programmed to 158°C for 5 min and then to increase with 2°C/min until reaching a temperature of 220°C which was remained for 8 min. The injector temperature was 230°C and the detector temperature was 250°C. The fatty acids were identified by comparison to retention times of the standard mixture GLC-68A (Nu-Check Prep, Elysian, MN, USA). Peak areas were integrated using Varian Galaxy chromatograph workstation software (Varian AB, Stockholm, Sweden). The carrier gas used was helium at 22cm/s and a flow rate of 0.8ml/min. The make up gas was nitrogen.

2 Results

2.1 Dry weight and lipid content

The results from drying shows that the algae were semi-dried before packed for distribution. *Palmaria palmata* and *Chondrus crispus* had higher water content than the other products. The lipid content varied between the species, with a lowest content of 0.24g/100g for *Chondrus crispus* and a highest content of 3.28g/100g for *Undaria pinnatifida* (mekabu). All of the algae had the nutritional value printed on the package. For five of them the fat content stated was lower, for two of them it was higher, one of the packages had the nutritional value printed but did not state the fat content. Table 4 shows the dry matter, total lipid content and the fat content printed on the package.

Table 4. DW of seaweeds expressed in g/100g semi-DW, total lipids expressed in g/100g DW, fat content determined by producer

Algae	DM ± SD	Total lipids ± SD	Fat content on package
Red algae/Rhodophyta			
Porphyra yezoensis	93.1±0.0	1.23±0.13	0.1
Palmaria palmata	83.5±0.2	0.30±0.02	0
Chondrus crispus	81.8±1.5	0.24±0.20	1.3
Brown algae/Paeophyta			
Hizikia fusiforme	88.0±0.0	1.34±0.02	0
Laminaria japonica	93.5±0.1	2.07±0.05	Not stated
Undaria pinnatifida[a]	90.7±0.4	1.01±0.02	0
Undaria pinnatifida[b]	90.9±0.1	0.75±0.09	1.4
Undaria pinnatifida (mekabu)	92.9±0.1	3.28±0.44	0

[ab]Undaria pinnatifidia from two different producers

2.2 Lipid composition

2.2.1 Polyunsaturated fatty acids

The amount of fatty acids from the different fatty acid groups are shown in tables 5 and 6. The fatty acid compositions are stated in table 7 and 8. PUFAs were found in varying concentrations. For the species *Porphyra yezoensis, Palmaria palmata, Undaria pinnatifida*[a] and *Undaria pinnatifida*[b] the concentration of ω3 PUFAs were higher than for ω6 PUFAs. Highest levels of PUFAs was found in *Porphyra yezoensis* (58.7%) as well as the highest levels of ω3 PUFAs (50.0%).

DHA (22:6ω3) was not found in any of the samples (data not shown). DPA (22:5ω3) was only found in *Palmaria palmata* in the low concentration 0.45% of total FAME content. EPA (20:5ω3) was found in all seaweeds except *Palmaria palmata* and *Chondrus crispus* in various concentrations. *Porphyra yezoensis* had the highest levels with 49.8% of total FAME content, the two brands of *Undaria pinnatifida* had the second highest levels 9.47 and 13.3%. When investigating the concentration of EPA expressed in mg/g of DW it displays a bit differently. *Porphyra yezoensis* again had the highest levels with 6.15 mg/g, *Undaria pinnatifida*[b] had the second highest with 1.34 mg/g and *Undaria pinnatifida* (mekabu) had 0.84 mg/g.

The most common ω6 PUFA was AA most abundant in *Undaria pinnatifida*[b] with 14.5%, the second highest levels were found in *Hizikia fusiforme* with 12.1%. When looking at mg/g of DW the highest levels were found in *Undaria pinnatifida* (mekabu) with 2.20 mg/g DW and the second highest were found in *Laminara japonica* with 1.78 mg/g DW because of their higher fat content.

2.2.2 Monounsaturated fatty acids

All the seaweeds had a fairly high percentage of monounsaturated fatty acids (MUFAs), between 10.7 and 32.9% as shown in table 7 and 8. Both *Undaria pinnatifida* seaweeds had concentrations in the top range. Overall the brown seaweeds had a higher content compared to the red. The two most abundant fatty acids in this class were oleic acid 18:1ω9 and gondoic acid 20:1ω9. Oleic acid was most abundant in *Laminaria Japonica* with 30.7% and gondoic acid was found in highest amount in *Undaria pinnatifida*[a] 23.7%.

2.2.3 Saturated fatty acids

The highest concentration of SFAs was found in *Chondrus crispus* at a level of 77.9%, this is displayed in table 7 and 8. High levels were also found in *Palmaria* *palmata* at 67.7%. For the remaining seaweeds levels between 19.7 and 50.8% was found.

Palmitic acid was the predominant saturated fatty acid (SFA) and was found in highest levels in *Chondrus crispus.*

Table 5. Fatty acid groups of seaweeds given in means (% of FAME content) and the ω6/ω3 ratio

Seaweed	*Porphyra yezoensis*	*Palmaria palmata*	*Chondrus crispus*	*Hizikia fusiforme*
Fatty acid group	mean ± SD	mean ± SD	mean ± SD	mean ± SD
SFA	21.8 ± 0.02	67.7 ± 2.01	77.9 ± 3.16	41.7 ± 1.74
MUFA	10.7 ±1.41	18.5 ± 1.02	14.0 ±0.98	19.0 ± 0.06
PUFA	58.7 ± 1.68	0.98 ± 0.25	1.29 ± 0.56	30.1 ± 0.31
PUFA ω3	50.0 ± 1.44	0.45 ± 0.01		13.1 ± 0.14
PUFA ω6	8.64 ± 0.24	0.53 ± 0.24	1.29 ± 0.56	17.0 ± 0.17
ω6/ω3	0.17 ± 0.00	1.17 ± 0.51		1.30 ± 0.00

Table 6. Fatty acid groups of seaweeds given in means (% of FAME content) and the ω6/ω3 ratio

Seaweed	*Laminaria japonica*	*Undaria Pinnatifida*[a]	*Undaria Pinnatifida*[b]	*Undaria pinnatifida (mekabu)*
Fatty acid group	mean ± SD	mean ± SD	mean ± SD	mean ±SD
SFA	42.8 ± 0.82	19.7 ± 0.88	19.9 ± 1.08	50.8 ± 3.48
MUFA	32.8 ± 0.07	32.9 ± 6.45	29.1 ± 0.33	29.6 ± 2.12
PUFA	22.3 ± 1.22	33.6 ± 2.40	44.4 ± 0.60	18.1 ± 1.32
PUFA ω3	3.79 ± 0.19	19.6 ± 3.16	23.1 ± 0.56	3.59 ± 0.25
PUFA ω6	18.5 ± 1.03	13.9 ± 0.75	21.3 ± 0.03	14.5 ± 1.07
ω6/ω3	4.88 ± 0.03	0.71 ± 0.15	0.92 ± 0.02	4.05 ± 0.02

[ab] Two different brands of the same species *Undaria pinnatifidia* was analysed.

Table 7. Fatty acid composition of three red seaweeds and one brown seaweed given in % of FAME content and mg/g ± SD of DW

Seaweed	Porphyra yezoensis		Palmaria palmata		Chondrus crispus		Hizikia fusiforme	
Fatty Acid	%	mg/g ± SD	%	mg/g ± SD	%	mg/g ± SD	%	mg/g ± SD
12:0	-		6.19	0.19 ± 0.03	-		-	
13:0	-		2.55	0.08 ± 0.01	-		-	
14:0	0.64	0.07 ± 0.06	14.2	0.43 ± 0.10	9.04	0.22 ± 0.19	5.79	0.78 ± 0.06
15:0	0.14	0.02 ± 0.00	-		1.33	0.03 ±0.02	0.43	0.06 ± 0.01
16:0	20.0	2.46 ± 0.40	38.4	1.15 ± 0.06	61.1	1.49 ± 1.33	32.3	4.33 ± 0.23
17:0	0.10	0.01 ± 0.01	0.26	0.01 ± 0.00	0.67	0.01 ± 0.01	0.14	0.02 ± 0.00
18:0	1.12	0.14 ± 0.07	5.34	0.15 ± 0.13	5.79	0.12 ± 0.07	1.00	0.13 ± 0.01
20:0	-		-		-		0.56	0.07 ± 0.01
22:0	-		0.29	0.01 ± 0.00	-		1.11	0.15 ± 0.00
24:0	-		0.39	0.01 ± 0.00	-		0.39	0.05 ± 0.00
14:1ω5	-		0.56	0.02 ± 0.01	-		-	
16:1ω9	1.79	0.22 ± 0.04	0.93	0.03 ± 0.00	1.69	0.03 ± 0.00	1.34	0.18 ± 0.01
16:1ω7	0.34	0.04 ± 0.04	1.85	0.05 ± 0.00	2.63	0.07 ± 0.06	3.03	0.41 ± 0.02
18:1ω9	3.46	0.42 ± 0.00	6.48	0.19 ± 0.02	8.10	0.19 ± 0.15	8.25	1.11 ± 0.03
18:1ω7	0.58	0.07 ± 0.02	4.39	0.13 ± 0.01	1.62	0.04 ± 0.04	0.22	0.03 ± 0.00
20:1ω9	3.28	0.40 ± 0.05	0.61	0.02 ± 0.01	-		-	
20:1ω7	-		-		-		1.34	0.18 ± 0.00
22:1ω11	-		-		-		4.23	0.57 ± 0.02
22:1ω9	1.26	0.15 ± 0.00	0.64	0.02 ± 0.01	-		0.63	0.08 ± 0.00
24:1ω9	-		3.06	0.09 ± 0.02	-		-	
18:2ω6	2.04	0.25 ± 0.05	0.53	0.02 ± 0.01	1.29	0.02 ± 0.01	4.00	0.54 ± 0.01
20:2ω6	0.82	0.10 ± 0.02	-		-		0.21	0.03 ± 0.00
18:3ω6	0.29	0.04 ± 0.01	-		-		0.17	0.02 ± 0.00
18:3ω3	0.21	0.03 ± 0.00	-		-		8.99	1.21 ± 0.02
20:3ω6	2.19	0.27 ± 0.05	-		-		0.47	0.06 ± 0.00
20:4ω6	3.30	0.41 ± 0.07	-		-		12.11	1.62 ± 0.00
20:5ω3	49.8	6.15 ± 1.09	-		-		4.10	0.55 ± 0.01
22:5ω3	-		0.45	0.01 ± 0.00	-		-	
Total identified	91.4		87.2		93.2		90.8	

Table 8. Fatty acid composition of 4 brown seaweeds given in % of FAME content and mg/g ± SD of DW

Seaweed	*Laminaria japonica*		*Undaria pinnatifida*[a]		*Undaria pinnatifida*[b]		*Undaria pinnatifida* (mekabu)	
Fatty Acid	%	mg/g ± SD	%	mg/g ± SD	%	mg/g ± SD	%	mg/g ± SD
12:0	-		-		-		-	
13:0	-		-		-		-	
14:0	6.34	1.31 ± 0.11	2.97	0.14 ± 0.17	5.17	0.52 ± 0.17	5.29	1.72 ± 0.08
15:0	0.19	0.04 ± 0.00	0.31	0.01 ± 0.02	0.12	0.01 ± 0.02	0.16	0.05 ± 0.01
16:0	30.0	6.20 ± 0.00	13.4	0.62 ± 0.75	12.0	1.21 ± 0.75	40.1	13.2 ± 2.86
17:0	0.27	0.06 ± 0.01	0.21	0.01 ± 0.01	0.14	0.01 ± 0.01	-	
18:0	5.08	1.05 ± 0.05	2.33	0.10 ± 0.12	2.30	0.23 ± 0.12	4.29	1.42 ± 0.35
20:0	0.95	0.20 ± 0.00	0.35	0.02 ± 0.02	0.21	0.02 ± 0.02	1.02	0.34 ± 0.10
22:0	-		-		-		-	
24:0	-		-		-		-	
14:1ω5	-		-		-		-	
16:1ω9	-		2.34	0.11 ± 0.14	1.03	0.10 ± 0.14	-	
16:1ω7	0.71	0.15 ± 0.00	0.60	0.03 ± 0.04	0.31	0.03 ± 0.04	0.53	0.17 ± 0.00
18:1ω9	30.7	6.35 ± 0.17	5.65	0.30 ± 0.39	7.88	0.80 ± 0.39	28.2	9.20 ± 0.61
18:1ω7	-		-		-		-	
20:1ω9	1.25	0.26 ± 0.01	23.7	1.15 ± 1.42	19.4	1.96 ± 1.42	0.90	0.29 ± 0.01
20:1ω7	-		-		-		-	
22:1ω11	0.15	0.03 ± 0.00	-		-		-	
22:1ω9	-		0.62	0.03 ± 0.04	0.57	0.06 ± 0.04	-	
24:1ω9	-		-		-		-	
18:2ω6	7.06	1.46 ± 0.12	4.00	0.18 ± 0.21	5.20	0.53 ± 0.21	6.15	2.00 ± 0.11
20:2ω6	-		-		-		-	
18:3ω6	1.99	0.41 ± 0.03	0.95	0.04 ± 0.05	1.05	0.11 ± 0.05	1.12	0.36 ± 0.02
18:3ω3	1.11	0.23 ± 0.02	10.2	0.49 ± 0.60	9.83	0.99 ± 0.60	1.02	0.33 ± 0.02
20:3ω6	0.86	0.18 ± 0.01	1.08	0.02 ± 0.01	0.59	0.06 ± 0.01	0.52	0.17 ± 0.02
20:4ω6	8.59	1.78 ± 0.14	7.91	0.36 ± 0.43	14.5	1.47 ± 0.43	6.75	2.20 ± 0.15
20:5ω3	2.68	0.56 ± 0.04	9.47	0.46 ± 0.58	13.3	1.34 ± 0.58	2.57	0.84 ± 0.06
22:5ω3	-		-		-		-	
Total identified	97.9		86.2		93.5		98.6	

[ab] Two different brands of the same species *Undaria pinnatifidia* was analysed.

2.3 Meeting the nutritional recommendations

There are no nutritional recommendations set for EPA and DHA separately. Therefore the estimated amount of seaweed needed for consumption is based on the amount of EPA and DHA recommended by the Nordic Nutrition Recommendations (2012) and the ratio of 2:1 (EPA:DHA) suggested by Harris (2004) in Cleveland Clinic Journal of medicine. The ratio of 2:1 (EPA:DHA) was also the most prevalent ratio for supplements in Kansas summer 2003.

Since only EPA was found in a considerable amount in the samples, only EPA is calculated for. This means that EPA would account for 133 to 166 mg/day. *Porphyra yezoensis* with the highest levels of EPA would in a 22g serving contribute with 133 mg EPA. *Undaria pinnatifida*[b] and *Undaria pinnatifida* (mekabu) have to be consumed in larger amounts, 99g and 158g respectively to reach the level of 133 mg. The remaining seaweed would have to be consumed in even larger amounts.

2.4 The ω6/ω3 ratio

The ratio of the ω6/ω3 PUFAs where found to be interesting (table 5 and 6). *Porphyra yezoensis* had the lowest ratio with 0.17, closely followed by *Undaria pinnatifida*[a] and *Undaria pinnatifida*[b] with 0.71 and 0.91. Overall the ratios where low with the highest ratio for *Laminara japonica* with 4.88. No ω3 lipids were found in *Chondrus crispus* therefore no ratio could be calculated.

3 Discussion

3.1 Lipid composition, fat content and factors influencing lipid composition

DHA was not detected in any of the samples. Previous research found low concentrations in *Chondrus crispus* and *Palmaria palmata* (Fleurence et al. 1994). The levels of EPA is of certain interest since it was found in high concentration in many of the algae, especially for the red algae *Porphyra yezoensis* with levels of 49.8% of FAME content and 6.15 mg/g DW. Other studies conducted on various *Porphyra* species found similar results with an EPA content between 35.5 and 50.1% of total lipids (Dawczynski et al. 2007; Noda 1993). The amount of lipids found is lower than in previous research with two to three times less (Noda 1993; Dawczynski et al. 2007; Blouin et al. 2006). Unexpectedly *Palmaria palmata* did not have any EPA. Earlier research displays levels comparable to that of *Porphyra* and also five times as much total lipids (Fleurence et al. 1994). The same results were found for *Chondrus crispus*. No EPA detected compared to levels around 20% found earlier, and almost three times less total lipids than expected (Fleurence et al. 1994). For *Chondrus crispus* it seemed to be difficult to get representative samples. Partly because the product consisted of different parts of the plant and also because it was not entirely free from contamination of other aquatic plants and animals.

The low fat content for *Palmaria palmata* and *Chondrus crispus* may be a coincidence since it correlates badly with previous findings. It might be this “brand” that has unusually low fat content or other extraction techniques were used. The low fat content might also affect the non-existent levels of EPA (table 7).

Among the brown algae the two brands of *Undaria pinnatifida* had lower fat content than in previous research, between two and five times less (Dawczynski et al. 2007; Herbreteau et al. 1997; table 8). Although one study showed similar results with 1.05g/100g DW, indicating that the amount of fat can differ considerably (Sánchez-Machado et al. 2004). The amount of EPA on the other hand (table 8) corresponded well with previous research with levels around 10% of total lipid content (Dawczynski et al. 2007).

AA was the predominant ω6 PUFA ranging between 6.75 and 14.5%, except for in *Palmaria palmata* and *Chondrus crispus* where it was not detected and in *Porphyra yezoensis* where the levels were lower with 3.30% (table 7 and 8). The proportion of AA is of interest since it affects the ω6/ω3 ratio. It is also of importance since it is an essential fatty acid. But because of the high amounts of LA in foodstuff leading to satisfying levels of AA it is of lesser importance to focus on when reaching the dietary reference values (EFSA 2010). Therefore a lower ratio is better for meeting the dietary reference values for ω3. Simopoulos (2002) writes that as humans developed on a ratio close to one excessive amounts of ω6 upsets the balance resulting in a pro-inflammatory state. The ratios for all the seaweeds were more profitable than an average western diet except for *Chondrus crispus* since no ω3 detected. Many of them had ratios close to one making them suitable for preparation of low fat foods with beneficial ω3 status.

To make a conclusion about whether red or brown seaweeds have the most beneficial composition is difficult because of the lack of ω3 PUFAs in two out of three of the red seaweeds. Earlier studies generalise and says that red seaweeds have higher levels of EPA (Fleurence et al. 1994; Dawczynski et al. 2007). Although it cannot be confirmed it cannot be ruled out. The low fat content might have contributed to various errors in the present study. What more could cause errors is that the percentage of identified lipids was between 86.2 and 98.5% (table 7 and 8). For the seaweeds with a low identification level this means that the ratio could turn out differently. Even so *Porphyra yezoensis* was found to have the best ω6/ω3 ratio (table 5) and also highest percentage of EPA (table 7).

The variations of fat content and composition can be caused by factors as area of cultivation, time of harvest, light, temperature, salinity etc. The temperature influences the concentration of long chain PUFAs meaning a low temperature gives a higher content and a high temperature the opposite (Bhaskar et al. 2004). Light and salinity both affect the fat content although it is not well known how. It seems to depend on what kind of seaweeds grown although some trends have been seen for a higher long chain PUFA content in high salinity (Floreto & Teshima 1998). Degradation by storage can also make a difference, especially for PUFAs that are highly sensitive. Extensive degradation during storage has been seen in previous studies affecting the fat composition (Mouritsen et al. 2013). Algae used in the other studies are mainly harvested fresh and dried not long before extraction of the lipids giving less time for storage degradation to occur. Taken these aspects in consideration it is not surprising that the fat content differs from previous research. Tough commercial algae in countries without indigenous traditions of consumption might face the reality of being stored for long periods of time.

Evaluating if seaweeds could be an adequate source of EPA and DHA these aspects are of importance. Majority of the seaweeds in western countries are sold dried and storage degradation may occur. The knowledge can also be used when cultivating to use favourable conditions for promoting a desirable fat content.

3.2 Evaluating seaweeds as a source of ω3 LCPs

The amount of EPA expressed in grams is quite interesting for some of the seaweeds. As stated in results a serving of 22 g would help reach the daily need of EPA. If the amount of total lipids would be even higher as in earlier studies these levels could be reached in smaller proportions, approximately 7 – 11g, since the percentage of EPA seems to be consistent throughout the studies with only the amount of fat fluctuating. This would also be the case for *Undaria pinnatifida*, whereas the serving size would still be quite large. The average intake of seaweeds for the Japanese population is estimated to be between 5.5 and 10g/day (Matsumura 2001; Gaudechoux 2000). Since seaweeds are not a part of the traditional western diet, the amount consumed by the Japanese is of importance to evaluate what is a realistic to eat on a daily basis. The Japanese seems to eat seaweeds mainly as a flavour enhancer meaning small amounts are used. Therefore it might be possible to consume at least equal amounts without making major changes in the diet. Nori (*Porphyra*) has the largest market value according to Radmer (1996) and also the majority is consumed unprocessed because of its popularity as a food algae. Because its popularity as a food algae it has properties liked by many consumers. It is also a seaweed many westerners are familiar with because of its use in sushi dishes. Considering these aspects Nori could be a useful source of EPA even in western countries.

To explore if any of the seaweeds are suitable the digestibility and bioavailability have to be further evaluated. Current research is ambiguous suggesting both better and poorer digestibility (Gudiel Urbano & Goñi 2002; Suzuki et al. 1993). To investigate the digestibility is certainly a field that has to be stressed since it lays the foundation not only for the absorption of fat but also for other nutrients. What is special for the bioavailability of fat is the presence of fucoxanthin up regulating Δ6-desaturase increasing the assimilation of LCPs (Airanthi et al. 2011). These effects are particularly interesting since they would make quite a difference when evaluating seaweeds as a source of EPA and DHA.

Since the conversion from ALA depends on various factors it is suggested that ω3 LCPs are ingested preformed (Davis & Kris-etherton 2003). That makes seaweeds a more appropriate source than terrestrial vegetables considering fat composition. Although the low fat content is at disadvantage; for example flaxseed oil has a high content of ALA and might work as a substitute for fish oil if the conversion is optimal. However, it is insecure to rely on the conversion since it is infeasible to keep track of the ω6/ω3 ratio, also since previous research shows that most people's ratio is at disadvantage (Simopoulos 2002). Meaning seaweeds are interesting because they are not dependent on conversion.

A drawback in seaweed consumption and therefore seaweeds as a source of ω3 is the unwanted substances cadmium, inorganic arsenic, mercury and iodine which have been found in elevated levels in seaweed products. It has been claimed that a an average Japanese consumer would exceeds the dietary intake levels recommended for these substances (Almela et al. 2002; Besada et al. 2009; Van Netten et al. 2000). Exceeding the levels is certainly problematic and it is unsure how widespread the substances are in commercial algae products. The area where cultured seems to be of great importance since the seaweeds assimilate substances from their close environment, this could be a tool to get the levels down. The current situation demands the product to be controlled before released on the market, a scenario that is not always the case because lack of legislation regarding heavy metals in seaweeds.

3.3 Evaluation of method

This study displays lower amounts of total lipid content than other studies. One of the major differences is the method for extraction of lipids. Another solvent system consisting of chloroform and methanol was used, either in the proportions of 1:2 or 2:1. Mainly two methods were used, one of them was conducted by Bligh & Dyer (1959) and the other one by Folch et al. (1957). The two methods have been extensively used for many years and evaluated for how accurate they measure the lipid content. Both methods have accurate and repeatable results but the method from Bligh and Dyer underestimated the fat content when exceeding fat levels of 2% (Iverson et al. 2001). Since seaweeds have a lipid content in this range both would be appropriate methods. However the solvents used are of a toxic nature and replacement for them have been found. The method of Hara & Radin (1978) uses hexane and isopropanol which is less toxic. Their own evaluation is that is equally efficient with same amount of lipids extracted. Another study shows that the fat content extracted and determined gravimetrically was significantly lower than methods using chloroform (Gunnlaugsdottir & Ackman 1993). The results are from a study made on fishmeal with much higher amounts of fat than macroalgae. The difference would therefore be smaller in low fat products. It seems like the extraction method used in this study did not affect the amount of fat particularly.

Another major difference is the treatment of the seaweeds before the extraction. In previous studies the seaweeds were either pulverised or grounded (Fleurence et al. 1994; Dawczynski et al. 2007). To either pulverise or ground means very small parts were examined compared to grounding in a mortar and homogenised in an Ultra-Turrax as done in the present study. Because of the rough texture they were very hard to pulverise, ending up with both small and large fractions. Consequently the following extraction must have been less efficient.

3.4 Comparing fat content with list of nutrients

Comparing the values stated on the packages, the results from this and other studies conclude that the values in the list of nutrients are unreliable. Extraction methods used commercially in the industry are often mixed solvent extraction, continuous extraction, alkaline or acid hydrolysis. The most commonly used method is continuous extraction, sometimes with prior treatment of acid hydrolysis. This technique often uses petroleum spirits for extraction, needing a completely dry material and removal of mono and disaccharides before analysis. Major drawbacks of the technique is incomplete extraction leading to unreliable results (Greenfield & Southgate 1996). It can also be speculated that ignorance from producers concerning the importance of fat content results in poorly conducted analyses.

Accurate labelling is important for customers to have possibility for to make aware decision. If not stated accurately the purpose of labelling falls short. Moreover the labelling of “total fat” which is widely used, is unsatisfying in many ways. The classes of fat is equally important to the amount of fat and should therefore also be stated. Purchasing seaweeds for nutritional reason would mean that accurate labelling is needed.

3.5 Limiting factors and opportunities

Because of the large number of vegetarians in the world estimated by Elimear et al. (2010), there would be a demand for vegetarian sources. Maybe only vegetarians by choice would care for if the source is vegetarian or not, but since there is an increasing number of vegetarians it is of importance. Environmental aspects are also of importance where measures to protect the fish stocks have to be taken. To replace fish oil with oil from microalgae would be one measure but also to find other sources. Plant based sources would help to protect the fish stocks. It would also help to protect people living in coastal regions in developing countries since many of them are dependent on healthy oceans to make a living. It could also be said that the rapid increase of the world population implies that major challenges will be met in meeting the demand for ω3 oil.

To introduce seaweeds to peoples diets worldwide would be difficult since it is not a part of the traditional diet. Cultural obstacles when introducing unfamiliar food is a known phenomenon that is not easily overcome. To predict whether seaweed would face these challenges is almost impossible to predict. There have been some attempts to introduce seaweed products, but rather as an exclusive product than something to cook with everyday. Except Nori sheets for sushi and seaweed salad made from Wakame, seaweed crisps have been introduced as a "healthy" snack and in beauty products.

Besides eating them unprocessed as an addition to a meal they could also act as an ingredient. Microalgae have been included in various meat products such as meatballs and hot dogs as a texture agent, but does also contribute to lower meat content and important nutrients. Seaweeds have been used to make meat free burgers. An example is the Dutch weed burger that got a lot of attention in media. There are numerous alternatives if there would be an interest to develop them further.

3.6 Further research

Evaluating seaweeds as a source of long chain PUFAs research is possible in several areas. To start with there are great variations between species. To map out the species with highest levels of EPA and DHA would be a first step. Evaluating what growth conditions that are most favourable for promoting ω3 PUFAs is also of certain interest.

Methods of processing algae could affect both bioavailability and degradation in numerous ways. Most algae are evaluated fresh or dried but since often consumed in hot soups the effect of heating for heath sensitive lipids seems important. Also the effect of heating on polysaccharides is important since the bioavailability might change. Oxidation of lipids during storage both in fresh and dried algae is also an area of importance. How lipids are affected during processing and storage is a problem for many foodstuffs when not consumed fresh and would also be the case for seaweeds. Therefore it is necessary to examine the extent of the problem.

Digestibility and bioavailability needs to be thoroughly investigated to make sure that the body can assimilate nutrients including fat. In vivo studies on humans are needed to get a comprehensive picture of how well this process works.

4 Conclusion

Although the total lipid content was low the composition of fat is certainly interesting because of the high levels of PUFAs displayed for all species except *Palmaria palmata* and *Chondrus crispus*. Of particular interest is the high percentage of total lipid content of EPA found in *Porphyra yezonesis* (49.8%) and *Undaria pinnatifida* (13.3%). Correspondingly the amount of EPA expressed in mg/g DW was noticeable with 6.15 mg/g and 1.34 mg/g respectively. Furthermore it can be concluded that *Porphyra yezoensis* in a reasonably small amount could contribute to a better ω3 status in humans.

There are great variations in the total amount of fat in dried algae on the Swedish market. Evidently the fat content has to be further investigated to know to what extent seaweeds could contribute with ω3 LCPs. Arguing that EPA are needed in small amounts they could be a valuable source despite the low fat content. The ambiguous results for *Palmaria palmata* and *Chondrus crispus* compared with previous studies subsequently lays the ground for more research to unravel the reasons for the very low fat content found. Information about how the handling affects the fat content and composition is crucial to know if parts of the handling are to be done differently.

If handled in a correct way when cultured, processed and stored the amount and preservation of fat could be maximised. With that in mind some species of seaweeds are worth considering as a plant based alternative to fish oil.

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Appendix

Seaweeds, a healthy snack with healthy fats?

Seaweed grows practically everywhere in the world. In the coastal waters of India, the coast surrounding Ireland and the icy waters outside Alaska. They like many kind of habitats and thrive in in both sweet and salt water. There are mainly three subcategories known as red, brown and green seaweeds. Additionally there are also micro and marcroalgae. Macroalgae are what we refer to as seaweeds and microalgae are one cell organisms that have very different characteristics from their larger cousin and are not traditionally used as food. Currently they are used for making omega-3 oils for human consumption. But there are also great plans for these small bundles of energy when there is research about replacing fossil fuels with biofuel from microalgae. Seaweeds on the other hand have for centuries been used in the cuisine in the Far East. They are used in sushi dishes, soups, meat dishes, eaten with rice and also act as a flavour enhancer because of their umami taste.

Omega-3 oils are some interesting fats that are important for humans for to be healthy. They are building components of our cells and helps prevents different kinds of diseases such as cardiovascular disease (CVD) and type-2 diabetes. CVD is a class of diseases that involves heart and blood vessels and type-2 diabetes is an age related disease that causes high blood sugar. Omega-3 oils are also important for a healthy development of babies where they accumulate the fats in the brain the first two years of living starting in the womb.

Omega-3 oils mainly come from sources as fish or fish oil. There are some problematic aspects utilizing these sources. The main issue is the diminishing fish stocks due to overfishing of the seas. For us to have healthy oceans in the future, huge changes are needed in order to turn this trend. Therefore new sources have to be found. This is where algae come into the picture. Microalgae we know for sure can produce these oils but seaweeds need some looking at.

To find out if seaweed products on the Swedish market have potential to contribute with omega-3 oils a selection of eight dried red and brown seaweeds were chosen. The fat content was determined by extracting the fat from the algae with various solvents. Then the fat was weighed and the percentage of fat could be calculated. To get comparable results all of the algae were dried until no water was left affecting the weight of the algae. Not all of the fat in seaweeds are omega-3.

Therefore it had to be examined what fatty acids they have and in what proportions. The fatty acid composition was determined in a machine called a gas-chromatograph . A gas-chromatograph can separate fats depending on their structure. With the help of a sample with known fatty acids the unknown samples can be compared to figure out what fatty acids it contains.

The fat content was overall low in both the red and the brown seaweeds. They had a content between 0.2 to 3.3 %, about the same fat content as land growing vegetables. Omega-3 was found in all the seaweeds except one. One of the seaweeds called Nori had exceptionally high percentage of omega-3, over 50% of the total fat content. A 22g serving could contribute to a large part of the amount needed on a daily basis. This might not sound like a large amount of seaweeds, and it’s not. But if we compare it to what the Japanese consume (and they have the highest intake of seaweeds in the world) which is between 5 and 10 g/day, it seems a lot.

It can still be doable to consume these amounts. Since seaweeds are not the most popular dish in many parts of the world it needs to gain acceptance to be consumed in greater quantities, especially in the western world. What can be concluded is that seaweeds are an interesting source when it comes to omega-3 fatty acids but still more research has to be done in order to make any suggestion on whether seaweeds can contribute with these fatty acids or not.