Changes in lipid, fatty acid, α-tocopherol and phytosterol content of New Zealand *Undaria pinnatifida* with the time and location

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Attestation of Authorship

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person (except where explicitly defined in the acknowledgements), nor material which to a substantial extent has been submitted for the award of any other degree or diploma of a university or other institution of higher learning.

Sayvisene Boulom

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Abbreviations

AA: Arachidonic acid

DHA: docosahexaenoic acid

DW: dried weight

EPA: eicosapentaenoic acid

FA: fatty acid

FAMEs: fatty acid methyl esters

GC-FID: gas chromatography flame ionised detector

GC-MS: gas chromatography mass spectrometry

IS: Internal standard

LC-PUFAs: long chain polyunsaturated fatty acids

n-3 : omega-3

n-6: omega-6

n-9: omega-9

MUFA: monounsaturated fatty acids

PUFA: polyunsaturated fatty acids

SFA: saturated fatty acids

Abstract

Seasonal changes in the contents of lipids, fatty acids, phytosterol and α-tocopherol in the brown macroalgae *Undaria pinnatifida* were investigated in this research. The *Undaria* samples were collected monthly from June to December 2011 from mussel lines in the Marlborough Sounds. Lipids were measured on a dry weight (DW) basis. *U. pinnatifida* had a low lipid content which ranged from 17.07 to 45 mg/g dried weight (DW) in blade and between 20.70 and 63 mg/g in the sporophyll. In New Zealand, *U. Pinnatifida* is always present and growing in summer although its degradation was reported in summer in Japan due to high sea temperatures. The sea temperatures in the Marlborough Sounds (New Zealand) are cool in summer and are conducive for *Undaria* growth.

The saponifiable lipid fraction was derivatised into fatty acid methyl esters (FAMEs) and analysed by gas chromatography flame ionised detector GC-FID to identify and quantify the individual fatty acids in *U. pinnatifida.* It was found that the level of SFAs increased from winter to summer. In December the most abundant SFA was palmitic acid (C16:0) which was up to 6.49 mg/g in the blade and the predominant MUFA was oleic acid (C18:1n-9c) with a highest level of, 3.40 mg/g, also in the blade.

In December, *U. pinnatifida* was also found to be a richer source of PUFAs than the SFAs and MUFAs. These PUFAs consisted of both omega-6 (n-6 or ω-6) and omega-3 (n-3 or ω-3) PUFAs. The abundant n-6 PUFAs are C18:2n-6, C18:3n-6 and C20:4n-6 also reached a maximum in December. By contrast some of the PUFAs decreased in summer. C18:3n-3, C18:4n-3 and C20:5n-3 fatty acids were higher in winter. The New Zealand *U. pinnatifida* showed high concentrations of n-3 fatty acids and demonstrated a nutritionally balanced of omega-6 (n-6): omega-3 (n-3) ratio.

The unsaponifiable lipid fractions examined contained two principal phytosterols; fucosterol and 24 methylenecholesterol, and the fat-soluble vitamin α-tocopherol. These non-saponifiable compounds were identified by high pressure liquid chromatography with UV/Vis detection (HLPC-UV) and gas chromatography mass spectrometry (GC-MS), followed by GC-FID analysis for quantitation. The unsaponifiable lipid content represented less than 1% of total lipids in *U. pinnatifida*.

α-Tocopherol was the only fat-soluble vitamin identified in New Zealand *Undaria*. Fucosterol and 24 methylenecholesterol were found higher concentrations in the winter. The content of fucosterol ranged from 146 to 338 µg/g and 24 methylenecholesterol was between 8.4 and 48 µg/g. However, the content of α-tocopherol was relatively high in winter and spring (13 and 14 µg/g) and then slightly decreased in summer (9.6 µg/g). This study showed that *U. pinnatifida* from New Zealand can be a balanced source of fatty acids and additionally contained the useful antioxidant α-tocopherol.

Chapter 1 General introduction

1.1 Outline of research

Wakame or *Undaria* species are of Asian origin and were originally found in Japan, Korea and China (Hay & Villoula, 1993). These seaweeds are produced and commercially sold in the Asian markets where they are largely used in agriculture and industry (Sánchez-Machado, López-Cervantes, López-Hernández, & Paseiro-Losada, 2004a). Hay and Villoula (1993) noted that *Undaria* species were introduced to the Wellington harbour, in around 1987 by ships from Asia. Since then they have populated the New Zealand coastal environment (Hay & Luckens, 1987). However, the harvesting these seaweeds was not permitted until in May 2010, when the government gave the go-ahead for commercial use of this seaweed (MAF, 2010).

Seaweeds are marine algae and their growing conditions depend on geography and temperature. Hence its chemical and nutritional composition may vary with location and seasonal changes (Nelson, Phleger, & Nichols, 2002). For example, Nelson et al. (2002) demonstrated that lipid contents of seaweeds (Phaeophyta, Rhodophyta and Chlorophyta) increased in winter and decreased in summer.

Generally, all algae have a low fat content compared to oil seeds. However, they are rich in polyunsaturated fatty acids (PUPAs) that can be beneficial to health (Dawczynski, Schubert, & Jahreis, 2007; Norziah & Ching, 2000). Many macroalgae contain important fatty acids (C12-C22) (Kumari, Kumar, Gupta, Reddy, & Jha, 2010). Brown algae namely *Laminaria* spp, *Undaria* sp and *Hizikia* sp have high levels of oleic acid (C18:1, n-9), alpha-linolenic acid (C18:3, n-3) and eicosapentaenoic acid (EPA) C20:5n-3. Long chain n-3 polyunsaturated fatty acids (LC-PUFAs) are found largely in marine fish or fish oil. However, as humans are not able to synthesize them; they have to obtain them through diet. For this reason, brown macro-algae like *Undaria pinnatifida* could be a new source of n-3 PUFAs, and are commercially interesting because they are cultivated large scale in the sea (Kumari et al., 2010). Moreover, *Undaria* species also provide other nutritional, nutraceutical and perceived health benefits (Simopoulos, 2008).

There is very little knowledge on fatty acids and other lipid fractions present in New Zealand *Undaria pinnatifida*. Hence, this study will focus on the determination of

changes in the total lipid, fatty acids, phytosterols and α-tocopherol in *Undaria* from different months and locations. This research will generate new information regarding the lipid composition of New Zealand brown seaweed (*Undaria*), which may have potential commercial interests.

1.2 Background of seaweed

Undaria species are known as different names in many countries. They are commonly known as *Wakame* (Japanese), *miyeuk* (Korean), *haijiecai* and, *quandai-cai* (Chinese) as well as Japanese kelp, sea mustard and apron-ribbon (English). Their formal taxonomy is shown in Table 1 (Wallentinus, 2007):

Table 1 : Taxonomy of *Undaria* species

Class	Phaeophyceae
Order	Laminariales
Family	Alariaceae
Genus	*Undaria*
Species	*pinnatifida*

(Wallentinus, 2007)

The brown algal genus *Undaria*, a laminarian kelp, consists of three species *U. pinnatifida* (Harvey.) Suringar, *U. undarioides* (Yendo) Okamura, and *U. peterseniana* Okamura (Parsons, 1994). These species are used as fresh and dried food in Asia. Moreover, *Undaria pinnatifida* is commercialised and is extensively cultivated in Japan, China and Korea.

1.3 Physiology of *Undaria pinnatifida*

In the mature state, *Undaria pinnatifida* presents a distinctive form, namely the leaf and reproductive parts. The "leaf" is the common part consumed as food and is also called "blade". The skeleton of the leaf is known as the midrib, and is also consumed in China. *Undaria pinnatifida* also develops an enormous reproductive organ called the "sporophyll". It can produce millions of zoospores that produce female and male gametophytes. Additionally, the sporophyll is also called "Mekabu" which is eaten in Korea, China and Japan. Japanese researchers believe that the sporophyll is rich in compounds with functional and nutraceutical properties, namely the antioxidants that

are anticancer agents (Lee, Hayashi, Hashimoto, Nakano, & Hayashi, 2004; Gudiel-Urbano & Goni, 2002).

1.3.1 **Biology and life cycle of *Undaria* species**

Undaria species have a similar life annual cycle to other kelp like Laminariales, which are heteromorphic and diplohaplontic (Figure 1). They have a large sporophyte with the separation the microscopic female and male gametophytes (Wallentinus, 2007). The length of sporophytes varies according to geography. The total length could reach from 1 m in nature to 3 m in the culturing farms (Herbreteau, Coiffard, Derrien, & De Roeck-Holtzhauer, 1997). However it is approximately 1 m in length in Mediterranean countries and New Zealand due to high turbidity (Silva, Woodfield, Cohen, Harris, & Goddard, 2002). Hay and Luckens (1987) reported that in August 1987, *U. pinnatifida* have found in 7 m depth and were measured 1.3 m in length with fully developed sporophylls. *Undaria* species are known to have high reproductive capacity as its reproductive organ or sporophyll is large and can produce zoospores throughout the year that contribute to its high potential colonization (Wallentinus, 2007). The zoospores are motile or biflagellate spores which can mobilize in the sea after liberation (Hay & Gibbs, 1996).

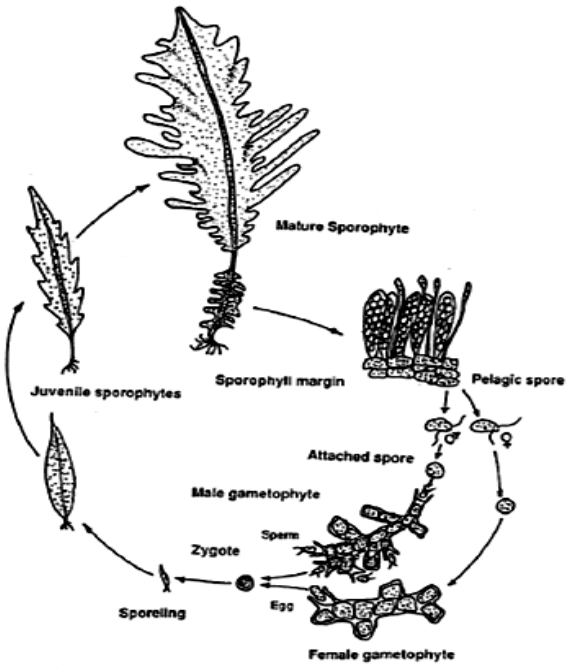

Figure 1 : Life cycle of *Undaria pinnatifida* (Hunt, Chadderton, Stuart, Cooper, & Carruthers, 2009)

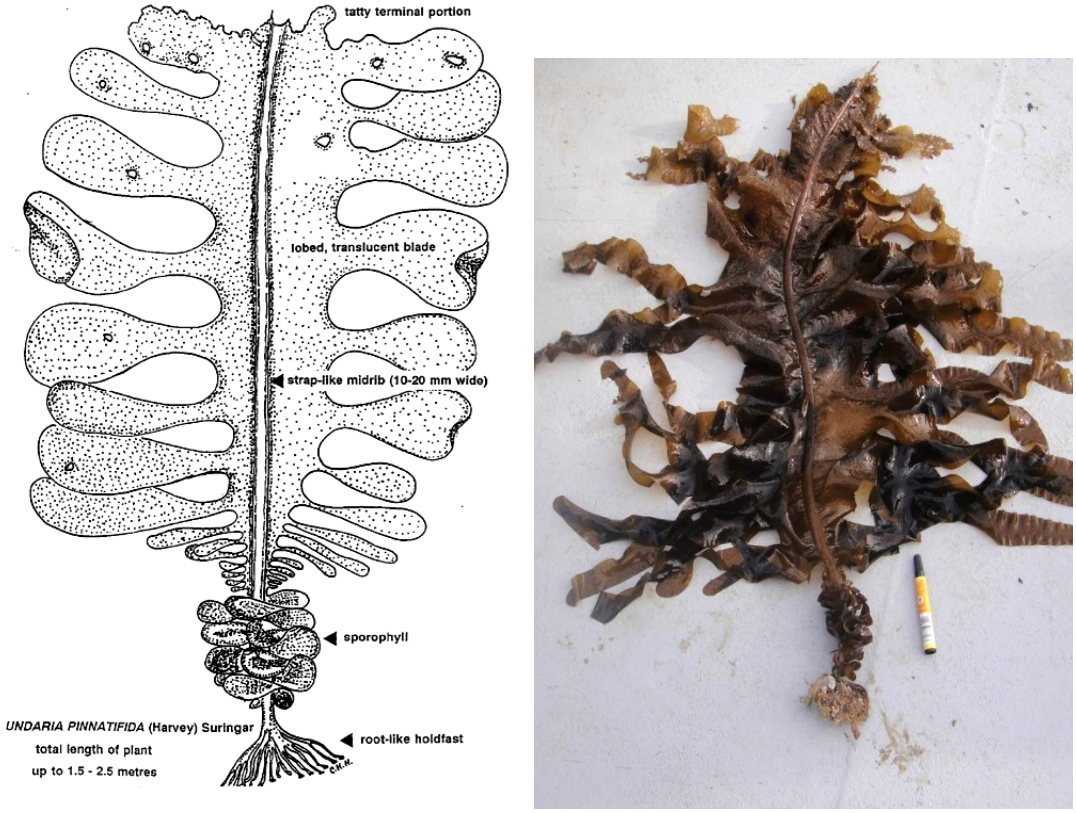

Figure 2 : Mature plant of *Undaria pinnatifida* 1.6 meters

1.3.2 **Geographic and environmental conditions of *Undaria's* growth**

The *Undaria* species have the ability to adapt and develop in different geographical environments. In nature, the Japanese kelp attaches and groups on rocks and reefs from 1 to 15 m of depth. In Japanese farms, *Undaria* can grow at 0.5-5 m depth depending on the clarity of the seawater. However, *Undaria pinnatifida* can be found in different areas around New Zealand coasts. In particular, it has colonised the mussel farms in the Marlborough Sounds where they attach to mussel lines and clusters of mussels.

Undaria pinnatifida occurs along the Japanese coasts and different locations in different continents. Their zoospores and sporophytes grow and mature in wide range of temperatures. Sporophytes usually develop in late winter and will die in the summer. It grows best between 5 and 15ºC (FAO, 2011). The optimal temperature for growing young sporophytes is between 14-17°C, while the older kelp will develop better at lower temperature. For example, the growth of young sporophytes in North East Honshu, Japan was between 4 and 25°C (Wallentinus, 2007). Moreover, the liberation of zoospores occurs during the summer when the surface temperature ranges 17-22ºC after old sporophytes die (Gibbs, Hay, & Dodgshun, 1998; Yamanaka & Akiyama, 1993).

In New Zealand *Undaria* sporophytes differ from their Japanese counterparts. Hay and Luckens (1987) reported that in Japan in mid-winter (November-December), with sporophytes 20-25 cm length whereas the NZ sporophytes ranged from 70 cm to 1.3 m at the same season in Wellington (July-August). *U. pinnatifida* are always present in Wellington waters due to the relatively small annual variation of sea temperature where surface water ranged from 9-19ºC (Hay & Luckens, 1987).

1.4 Global spread of *Undaria pinnatifida*

Undaria pinnatifida is considered as the third most invasive seaweed in the world. *Undaria* spreads widely due to its great capacity to colonize. It sometimes creates fouling problems for native marine species. However, there are also intentional introductions of this seaweed to some areas for farming because *Undaria* (Wakame) has economic value as a human food source (Wallentinus, 2007).

Different pathways have introduced *Undaria*. Generally, commercial vessels and aqua-culture activities accidentally introduced *U. pinnatifida*. In Europe, *Undaria pinnatifida* colonized the northern Mediterranean and north-eastern Atlantic by two ways, namely by boat and introduction of oysters (Herbreteau et al., 1997). In the northern Mediterranean, it was found in France in 1971 and in Italy in 1992. In the North Atlantic Sea, *Undaria* was introduced to Spain and the Netherlands by transplantation of Japanese oysters and by ships in 1990 and 1999 respectively. The introduction of *Undaria* in the south of United Kingdom was by recreational boats in 1994. In the Southwest Atlantic and East Pacific Ocean, *Undaria* has spread to Argentina and California by shipping (ballast or hull) in 1992 and 2000 respectively (Silva et al., 2002).

For commercial interests, *Undaria pinnatifida* was intentionally farmed in certain aqua-cultural areas. Experimental farming has been carried out in Brittany, Northwest France since 1983 (Herbreteau et al., 1997; Perez, Kaas, & Barbarroux, 1984). As a result, it spread later around European countries. *Undaria* cultivation has been common in Japan, Korea and China since the 1930s. *Undaria pinnatifida* is not only used for human consumption but is also utilised for animal feed. It has also been cultivated for feeding abalone (Marinho-Soriano, Fonseca, Carneiro, & Moreira, 2006).

1.5 Eradication and management of *Undaria pinnatifida*

Although *Undaria pinnatifida* is widely utilised as fodder and human food, especially in Asia, it is also considered an invasive plant or vegetative pest, which has colonized native marine alga in many countries. For this reason, eradication and management of *Undaria* species have been put in place in many countries. The aims are the control of its population or to limit its colonization (Wallentinus, 2007). Removing non-native *Undaria* from colonized areas has been attempted to clean the affected areas. In the Mediterranean, eradication of mature *Undaria* has taken place in Venice during the reproductive period. The population of *Undaria* decreased but re-colonization of this species was complete after two years.

Mechanical eradication and removal of *Undaria* must be done before its zoospore period because mature sporophytes and their gametophytes will release viable zoospores and rapidly recolonise the cleaned area (Wallentinus, 2007). In the UK, when *Undaria* was first discovered in the English south coast, all *Undaria* was removed. However, it appeared again due to the already released spores. In the Netherlands, efforts have been made to eradicate these species because it hinders mussel harvests. All in all, these attempts have failed due to its microscopic spores and gametophytes, which can grow under suitable environmental conditions (Wallentinus, 2007).

1.6 History of New Zealand *Undaria pinnatifida*

Undaria species were accidentally introduced to New Zealand by ships and ballast seawater. As noted earlier, *Undaria* was discovered on the Wellington coast in 1987 and in Timaru in 1988. Then it later spread to many New Zealand harbours such as the Marlborough Sounds, Picton, Lyttelton, Oamaru, Otago and Port Chalmers (Figure 3) (Hay & Villoula, 1993). New Zealand has suitable conditions for *Undaria* to thrive because of the relatively low sea temperatures throughout the year. Furthermore, its zoospores have good tolerance and persistence to seasonal condition. An experiment at the Cawthron Institute showed that *Undaria* zoospores were able to survive for 50-100 days in darkness at 26°C in the laboratory in Pelorus Sounds, Marlborough. Juveniles and mature sporophytes could also be present throughout the year in the region with low temperature ranges. A maximal summer temperature in New Zealand is between 15-19ºC (Stuart, Hurd, & Brown, 1999)

In 2000, the Ministry of Agriculture and Fisheries (MAF) put in place the management and control of *Undaria pinnatifida* by educating fishermen and eradicating it when found (Cassidy, 2009). The Bio-security Act obligated owners to clean the sporophytes from boat hulls and free areas from this plant for three years (Hunt et al., 2009). However, protection of local biodiversity was not successful. While harvesting *Undaria* species was prohibited before 2010, MAF authorised its harvesting for future commercial interests of marine macro-algae (MAF, 2010).

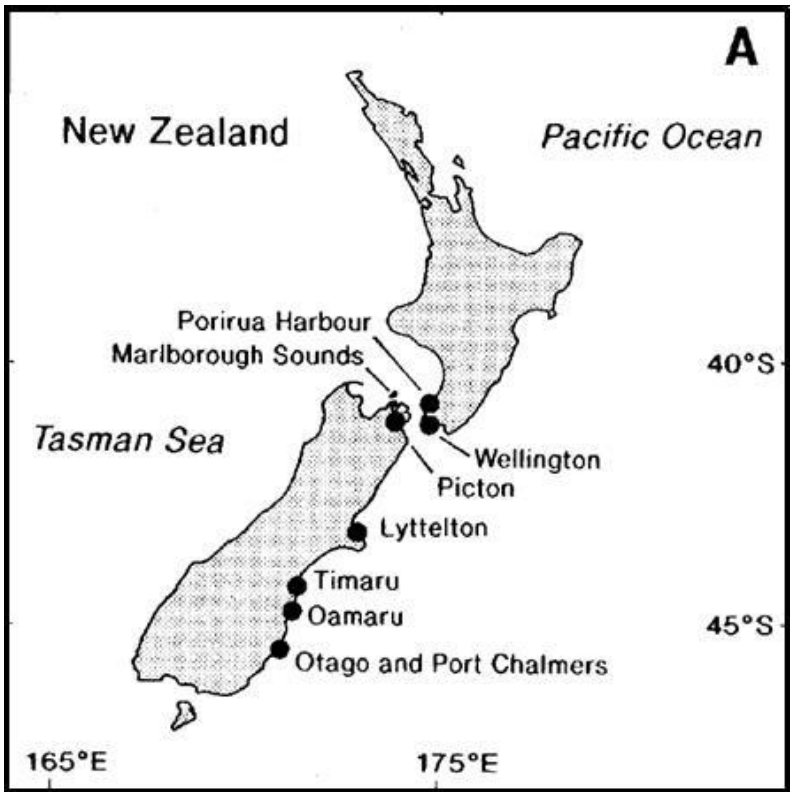

Figure 3 : Localities in New Zealand where *Undaria* was discovered in the early 1990's (Hay & Villoula, 1993)

1.7 Aquaculture and industrial utilization of *Undaria* species

1.7.1 Cultivation and global production of *Undaria pinnatifida*

In Asia, *Undaria* cultivation has been practiced since the 1930s especially in China, Korea and Japan (Hay & Gibbs, 1996). At the beginning, *Undaria* was harvested from the wild. When consumption of wakame became popular, the farming of *Undaria* responded to the market requirements. France was the first European country where experimental *Undaria* cultivation was done (Perez et al., 1984).

The principal steps of culturing *Undaria* are: collection of gametophytes (sporeling), seeding and insertion of plantlets on culture grounds (Gibbs, Brown, Forrest, & Dodgshun, 2000; Gibbs et al., 1998). Juveniles or gametophyte were traditionally collected from the sea. However, the Chinese developed sporeling methods that could be done in nursery farms. Zoospores were harvested by seeding onto collector frames (Weddy Gibbs et al., 1998; Tseng, 2001). Then these frames were removed to nursery tanks with enriched with seawater maintained at 10-17ºC and an illumination of about 10 μmol photons m^{-2} s^{-1}. If the temperature exceeded 25ºC, gametophytes died. The young sporophytes were removed from the seeding strings when plantlets reached 1-2 cm length they were immediately inserted into main culture ropes and the ropes placed in *Undaria* farms. *Undaria* would reach its maturity in 4-6 months after culturing and become 2 m in length in good conditions (Gibbs et al., 2000; Yamanaka & Akiyama, 1993).

Vegetative and reproductive growth are two morphological developments of *Undaria* (Gibbs et al., 1998). It increases its length and width during vegetative growth while increasing the thickness of blade and stipe when the sporophyte development begins (Choi, Kim, Lee, & Nam, 2007).

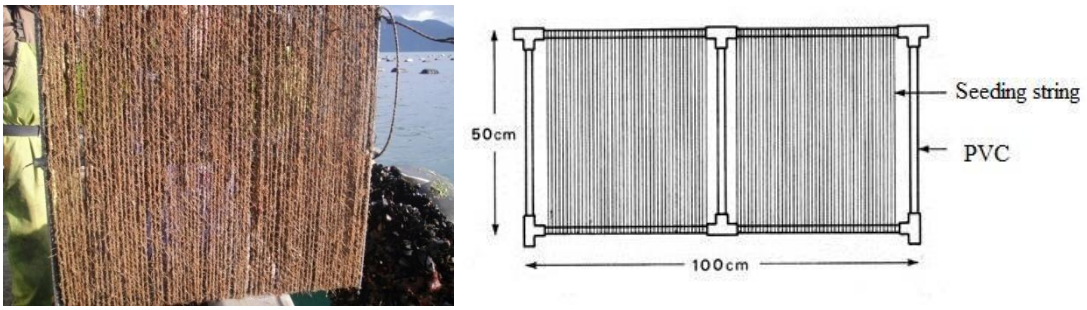

Figure 4 : Collector frame including seeding strings

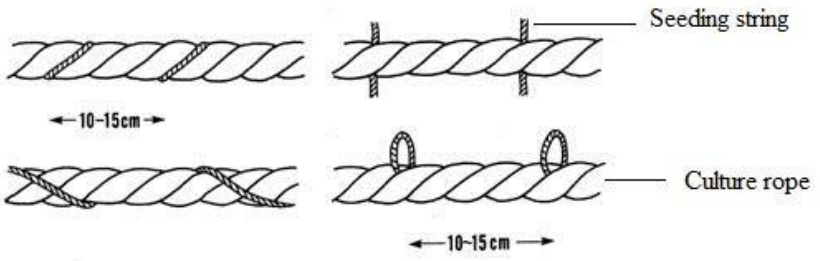

Figure 5 : Culture rope with seeding strings

1.7.2 **Global production and markets of *Undaria pinnatifida***

Aquatic production plays an important role in providing human and animal food sources. Seaweeds are important aquatic plants (FAO, 2008). In 2008, the total amount of aquatic production including animals and plants was globally 68.3 million tonnes which was worth an estimated US$106 billion. About 15.8 million tonnes of aquatic plants were produced by aquaculture and were valued at US$ 7.4 billion. Production of aquatic plants was dominated by seaweeds (99.6% of the total production of aquatic plants) (FAO, 2008). The main seaweed production is centralized in East and Southeast Asia (Table 2). Most aquatic production was used for human consumption. However, some algae are raw materials for iodine and alginate production. For example, *Eucheuma*, a major seaweed species is produced in Southeast Asia and used for carrageenan extraction. Moreover, Chile is another main seaweed producing country outside of Asia with 21 700 tonnes of production in 2008.

Table 2 : Global production of aquatic plants (FAO 2008)

Countries	**Quantity (%)**
China	*62.8*
Indonesia	13.7
Philippines	10.6
Republic of Korea	5.6
Japan	2.9
Democratic Peoples' Republic of Korea	2.8

Only a few seaweed species are cultivated globally in high quantity. These seaweeds are Japanese kelp (*Laminaria japonoca*), Eucheuma seaweed sp. (*Kappaphycus alvarezii* and *Eucheuma* spp.), Wakame (*Undaria pinnatifida*) and nori (*Porphyra* spp.). Their production was estimated to be approximately 4.8, 3.8, 1.8 and 1.4 million tonnes respectively (FAO 2008). In 2008, Japan became the main market of wakame. Japanese domestic demand of this product was at approximately 350,000 – 400,000 tonnes (dry weight) per annum. However, there was a shortage of *Undaria* production in China and Korea (Onodera, Yoshie-Stark, & Suzuki, 2008). On the other hand, while *Undaria pinnatifida* is considered as an invasive plant in New Zealand it grows very well in New Zealand mussel farms. The quantity of fresh *Undaria* in mussel farms was estimated in 2011 at approximately 4,000 tonnes which could turn it to a lucrative potential export (Aquaculture, 2011).

1.7.3 **Processing wakame for the food industry**

Undaria pinnatifida is processed into different products in Japan and various *Undaria* products are present in Japanese markets (Yamanaka & Akiyama, 1993). For example, Suboshi wakame, Haiboshi wakame, salted wakame, boiled and salted wakame and finally dried cut wakame. The wakame or commercial dry *Undaria* is processed differently according to Japanese tradition and location (Nisizawa, Noda, Kikuchi, & Watanabe, 1987). The traditional processing methods are sun drying and ash-drying. Suboshi wakame is an easy processing method. *Undaria* is washed and dehydrated by sunshine. The second traditional Japanese processing is the haiboshi method (Watanabe & Nısizawa, 1984). This process is different to the first method as the *Undaria* is mixed with ash. Then it is subjected to sun-drying and washed. After washing, wakame is dried again by sunshine.

Since the 1960s, blanch-salted and dried-cut wakame have been popular ways of processing wakame in Japan. Firstly, Fresh *Undaria* is washed with fresh water to remove undesirable matter. It is then blanched at 80ºC for about one minute then cooled quickly with water. After that, a 30% salt solution is added into the blanched seaweed and the mixture put in preserving tanks for 24 hours. The excess water and midrib are removed from the salted seaweed. This salted wakame is packed and freshly sold. Dried cut wakame is another popular commercial wakame in Japan. On an industrial scale, blanched salted *Undaria* is washed with fresh water to remove excess salt, and then centrifuged to remove excess water from the product. It is cut into suitable pieces and dried with a rotary-type flow-through dryer. Finally, it is classified the size by sieves and foreign mater is removed before packing for sale (Nisizawa et al., 1987).

The processes above lead to different forms of wakame. The principal criteria of quality are taste, colour, foreign mater and shelf life. Colour and shelf life mainly depend on the thermal treatment during the manufacturing process. The desirable colour of processed wakame is fresh green (Yamanaka & Akiyama, 1993). The change of fresh green chlorophyll depends on the temperature and time of blanching. The best colour of wakame is obtained when *Undaria* is heated between 80 and 95ºC for about 30-60 seconds. Too high a temperature would degrade chlorophyll to phaeophytin which results in a yellowish or brown wakame. Brown wakame during storage is an indicator of blanching failure. This failure leads to soft tissues during long storage due to autolysis of blade cells (Nisizawa, 1987). Quick cooling after blanching avoids

overheating and preserves the fresh green colour. Proper blanching of wakame contributes to longer storage life with a fresh green color that can last 6-7 months.

Foreign matter is also important criteria. In the modern industry, metal detectors are utilized to remove some foreign matter. However, a visual check on conveyor belts is still required before packing. A manufacturer could expect 1,000 pieces of foreign matter / ton of dried wakame. The highest quality of wakame would contain no more than 50 foreign bodies per tonne (Yamanaka & Akiyama, 1993).

1.8 Application of seaweeds

1.8.1 **Industrial uses**

Seaweeds are important marine sources of raw materials for industry. Industrially, seaweed species have been utilised to produce hydrocolloids like agar, carrageenan, and alginate. For instance, brown seaweed, *Sargassum confusum* is extracted for alginate. This hydrocolloid has been used as a raw material in industry especially in stabilizing ice cream, making dental and other impression materials, and sizing textiles (Tseng, 2001).

Cultivation of the *Laminaria* species was successful in China but recently this species has been replaced with *Sargassum confusum* for alginate production. Interestingly, iodine and mannitol are by-products of this production. In 2001, China was the biggest alginate producer with an annual maximal capacity of 13,000 tonnes for pharmaceutical and cosmetic uses.

In general, seaweeds can provide important supplements required by humans. Iodine is important in human diets and algae contain important quantities of iodine, especially in *Laminaria* species (Madhusudan, Manaj, Rahul, & Rishi, 2011). In China, only 40% of the population had iodine deficiency. *Laminaria* thallus contained 5% of iodine. In 2000, Chinese iodine deficiency was solved by using alginate by-products (Tseng, 2001).

Brown algae have a possible therapeutic role due to their sulfated polyanions (Fitton, 2003). *Undaria* can be extracted to produce fucoxanthin and fucoidan which have antioxidant and anti-cancer properties (Terasaki et al., 2009). Brown seaweed has fucose-containing sulfated polysaccharides, which is an effective drug against uraemia.

Propylene glycol alginate sulphate extracted from alga was found effective in treating heart and brain diseases (Tseng, 2001).

Macro-algae have also been used in cosmetic treatments. Seaweeds are added as raw materials in a wide range of therapies and products, such as massage paste, marine mud and algae treatments, which inhibit skin damage (Fitton & Irhimeh, 2008). It is also believed that alga message creams could relieve rheumatic pain and remove cellulite (Fitton, 2003). Hydrocolloids in seaweeds could restore elasticity and suppleness of the skin, alginate and carrageenan can provide moisture retention properties to the skin (Bang et al., 2011). Additionally, *U. pinnatifida* apparently has anti obesity properties (Choi, Kim, Kim, Kim, & Kim, 1999) and decreases potential carcinogens (Gudiel-Urbano & Goni, 2002). Brown algae is also a rich source for PUFA with an omega-6: omega-3 ratio of about 1 and have high potential to prevent inflammatory, cardiovascular and nervous system disorders (van Ginneken, Helsper, de Visser, van Keulen, & Branderburg, 2011).

1.8.2 **Agricultural uses**

Seaweeds are good biomass for agriculture. They have been used as green fertilizer, animal fodders and soil conditioning agents (Marinho-Soriano et al., 2006). The cultivation of alga species such as *Laminaria*, *Undaria* and *Porphyra* has been developed. Seaweed fertilizers have been used in agriculture in many countries such as France, Canada, China, Japan, United States, England and South India because of its rich nitrogen and potassium chloride (KCl) content (Bang et al., 2011; Madhusudan et al., 2011; Tseng, 2001). They could also be used as green manure and soil conditioner. In India, the use of seaweeds in coconut farms either as fresh manure or the seaweed compost resulted in a superior yields (Madhusudan et al., 2011).

1.8.3 **Human consumption of brown seaweed (*Undaria pinnatifida*)**

Seaweed consumption is well known in Asian countries especially in China, Japan and Korea due to its beneficial nutrients (FAO, 2011). Since ancient time, seaweeds have been considered as a sea vegetable for human food in these countries (Honya, Kinoshita, Ishikawa, Mori, & Nisizawa, 1994). For example, more than 1.6 kg of dry

weight per capita has been consumed annually in Japan (Dawczynski et al., 2007; Murata & Nakazoe, 2001). Different parts of seaweeds can also be eaten. Fresh or dry fronds (blades) are commonly used for various types of meals (Yamanaka & Akiyama, 1993). For example, *Undaria pinnatifida* is frequently used in seaweed salad and miso, where the dried cut seaweed is added directly after rehydrating with water (Onodera et al., 2008). Cut dry wakame is also used in instant noodles (Nisizawa et al., 1987)

In China, the midrib of *Undaria* separated from blade, is processed into fresh green seaweed salad or cut dried brown *wakame*. The sporophyll or the reproductive part of *Undaria* is known as "Mekabu" which can be served as a salad or as a vegetable. It is commercially sold in the dry form or packed in liquid, sometimes mixed with ginger. Currently, fresh and dry cut seaweed, and seaweed products are increasingly popular in the European market due to perceived nutritional and health benefits (Dawczynski et al., 2007). Moreover, European consumers are looking for high quality products with good colour, consistency and nutritional values (Dawczynski et al., 2007). In some areas, consumption of *Undaria pinnatifida* is also recognised because this marine plant contains high amount of sulphated polysaccharides namely sulphated fucans and fucoidans which have potential antiviral property (Plaza, Cifuentes, & Ibáñez, 2008).

Nowadays, consumption of seaweeds is a new trend in Europe. However, few types of alga are authorized for human consumption due to safety issues. In France, the only 5 brown seaweed species allowed for consumption are *Ascophyllum nodosum, Fucus serratus, Fucus vesiculosus, Himanthalia elonga*te and *Undaria pinnatifida* (Holdt & Kraan, 2011).

Chapter 2 Saponifiable fractions of lipid in *Undaria pinnatifida*

2.1 Introduction

Various types of lipids are found in plant membranes and are important components of the adipose tissue together with proteins and carbohydrate (Nawar, 1996). Glycol esters of fatty acids account for 99% of the lipids in plants and animals. This chapter will focus on the total lipid and fatty acid methyl esters content of *Undaria pinnatifida*. These different elements, that affect the constitution of lipids and fatty acids, including biological and environmental influences, are compared.

There are many definitions of lipids. Christie (1990) defined lipids as "fatty acids and their derivatives, and substances related biosynthetically or functionally to these compounds". Lipids contain different compounds, which are soluble in low polarity organic solvents such as chloroform, diethyl ether, hexane and benzene. Lipids such as triglycerides, phospholipids, glycolipids, sphingolipids and waxes that can be hydrolysed in basic or acidic conditions to give alcohols and acidic species are called saponifiable lipids (O'Keefe, 2008). Murata and Nakazoe (2001) reported that the main lipids in marine algae were phospholipids.

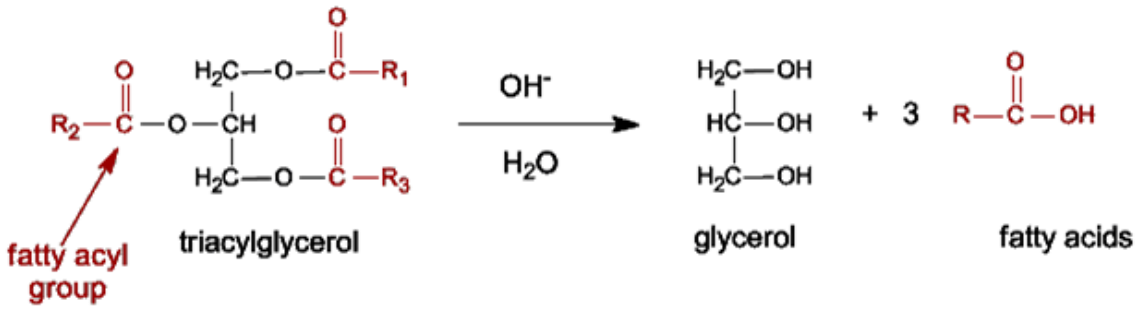

Figure 6 : Saponifiable lipids

(Jakubowski, 2011)

Non-saponifiable lipids include sterols, fatty alcohols, fat-soluble vitamins (namely carotenoids, vitamin A, D, E and K), phospholipids and other substances (Holdt & Kraan, 2011).

Algae contain relatively low amounts of lipids compared to other plant seeds such as soy and sunflower. However, these lipids consist of essential fatty acids and functional

lipid fractions, namely omega-3 fatty acids, phytosterol and fat soluble-vitamins. In the brown seaweed, the range of total lipid content was reported to be between 1 and 4.5 g/100g dry weight (Dawczynski et al., 2007). There have been conflicting ideas about the lipid content in seaweeds (Bhaskar, Hosokawa, & Miyashita, 2004; Khotimchenko, 2005; Murata & Nakazoe, 2001). Murata and Nakazoe (2001) claimed that phospholipids were the main source of lipids in marine algae. However, Bhaskar et al. (2004) and Khotimchenko (2005) argued that the glycolipids are the major lipid class in all seaweed, followed by neutral and phospholipids (Holdt & Kraan, 2011).

2.2 Fatty acids in *Undaria pinnatifida*

Fatty acids are classified according to their chain length, number, configuration and position of double bonds and additional other groups. They are mainly separated depending on their degree of saturation existing as saturated fatty acids and unsaturated fatty acids. The structure of fatty acids consists of a chain and one carboxyl groups.

Mono unsaturated fatty acids consist of single double bonds while poly-unsaturated fatty acids have one or more double bonds. Fatty acid compositions depend on the plant species. Vegetable oils would contain mainly saturated and unsaturated 16 and 18-carbon fatty acids (C16-C18), while marine lipids, especially algal species are made up of C14-C22 fatty acids (Holdt & Kraan, 2011).

2.2.1 **Fatty acid classifications**

Marine algae can be important sources of essential fatty acids as long chain unsaturated fatty acids are often found in marine algae. Fatty acids of seaweeds are generally linear chained, even though there are some long chain ones with one or more double bonds (Figure 7) (Lobb & Chow, 2008)

2.2.1.1 Saturated fatty acids

The term saturated refers to a hydrocarbon chain with only single carbon-to-carbon bonds, which are chemically less reactive. Generally, the melting points increase with the length of carbon chain. In *Undaria pinnatifida*, these fatty acids are lauric (C12), myristic (C14), pentadecylic (C15), palmitic (C16), margaric (C17), stearic (C18), and arachidic (C20) acids, Palmitic acid is the predominant fatty acid (Moreau, Lampi, & Hicks, 2009; Murata & Nakazoe, 2001).

2.2.1.2 Unsaturated fatty acids

Unsaturated fatty acids are more reactive than saturated fatty acids due to their double bonds. Herbreteau et al. (1997) reported that C18 and C20 saturated fatty acids are commonly found in *U. pinnatifida*. The most common MUFA in *Undaria pinnatifida* are C12:1 (lauroleic acid), C14:1 (myristoleic acid), C16:1 (palmitoleic acid), C17:1 (cis-10-heptadecenoic acid) and C18:1 (oleic acid) (Nisizawa et al., 1987). Oleic acid is the main MUFA in seaweed and accounted for 6.79 and 22.64 % of the total fatty acid in canned *Himanthalia elongate* and dried *Undaria pinnatifida* respectively (Sánchez-Machado et al., 2004a; Khotimchenko, 2003).

Long chain polyunsaturated fatty acids (LC-PUFAs) are not only present in marine fishes but are also found in macroalgae (Fleurence, Gutbier, Mabeau, & Leray, 1994). These LC-PUFAs are an important source of essential fatty acids which are made up of omega-6 (n-6 or ω-6) and omega-3 (n-3 or ω-3). The main PUFA in *Undaria pinnatifida* are C18:2n-6 (linoleic acid), C18:3n-6 (γ-linolenic acid), C18:3n-3 (α-linolenic acid), C18:4n-3 (stearidonic acid), C20:4n-6 (arachidonic acid) and C20:3n-5 (eicosapentaenoic acid) fatty acids (Figure 7). These compounds have more than two double bonds which not only play a critical role in nutritional properties but also contribute anti-inflammatory, anti-cancer and obesity effects and cardiovascular disease (Plaza et al., 2008). For instance, essential fatty acids are important for children's growth and development (Newton, 1996). The long chain omega-3 PUFA arachidonic acid (AA) and eicosapentaenoic acid (EPA) have been extensively investigated (Khan et al., 2007). Their functional roles will discuss more in section 2.6.

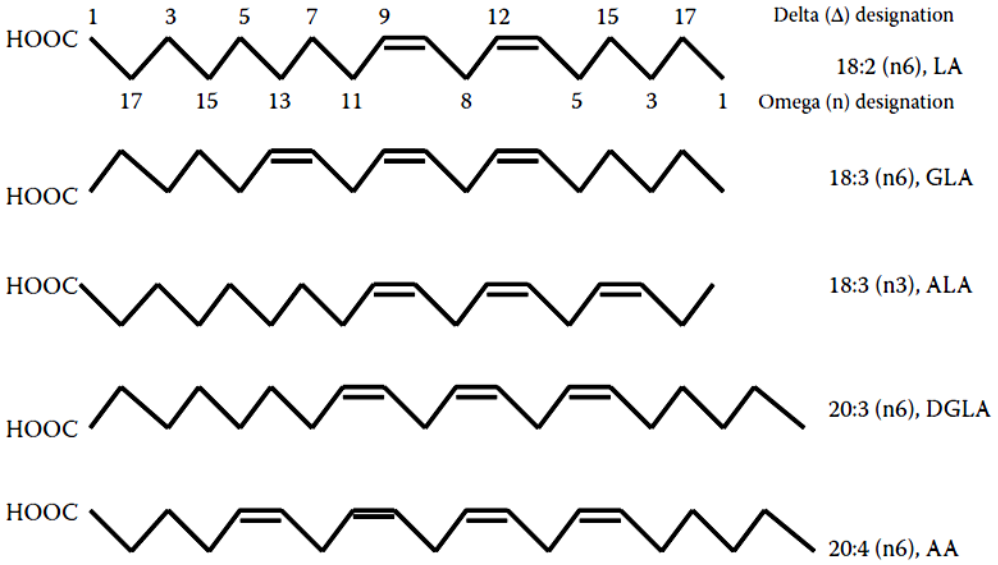

Figure 7 : Chemical structure of some LC-PUFA: LA, GLA, ALA, DGLA and AA (Wanasundara & Wanasundara, 2006)

2.2.1.3 Desaturation and elongation of fatty acids and precursors of eicosanoids

Both omega-6 and omega-3 PUFAs play an important role in biological functions. Omega-6 and omega-3 fatty acids that are considered as hormone-like compounds and include eicosanoids (prostaglandins, leukotrienes, lipoxins, etc.) (Lands, 1992). These compounds are involved in many biological activities in the human body and are precursors of other important compounds (Newton, 1996).

Linoleic and α-linolenic acids serve as hormonal processors in human metabolisms and are parents of other long chain PUFA. Figure 8 shows the pathways of DHA (docosahexaenoic acid) and decosapentaenoic acids formation from linoleic and linolenic acids. The principle pathway involves desaturation and elongation. The process of elongation added more carbon atoms to fatty acid, while desaturation led to the addition of double bonds to the carboxyl end of fatty acid molecules (Newton, 1996). These processes use some specific enzymes for synthesizing LC-PUFA, such as Δ^6, Δ^5 and Δ^4-desaturase and elongase. Δ^6 and Δ^4–desaturase that can generate both omega-6 and omega-3 fatty acids. However, a high intake of LA can interfere with the process of desaturation and elongation (Simopoulos, 2008).

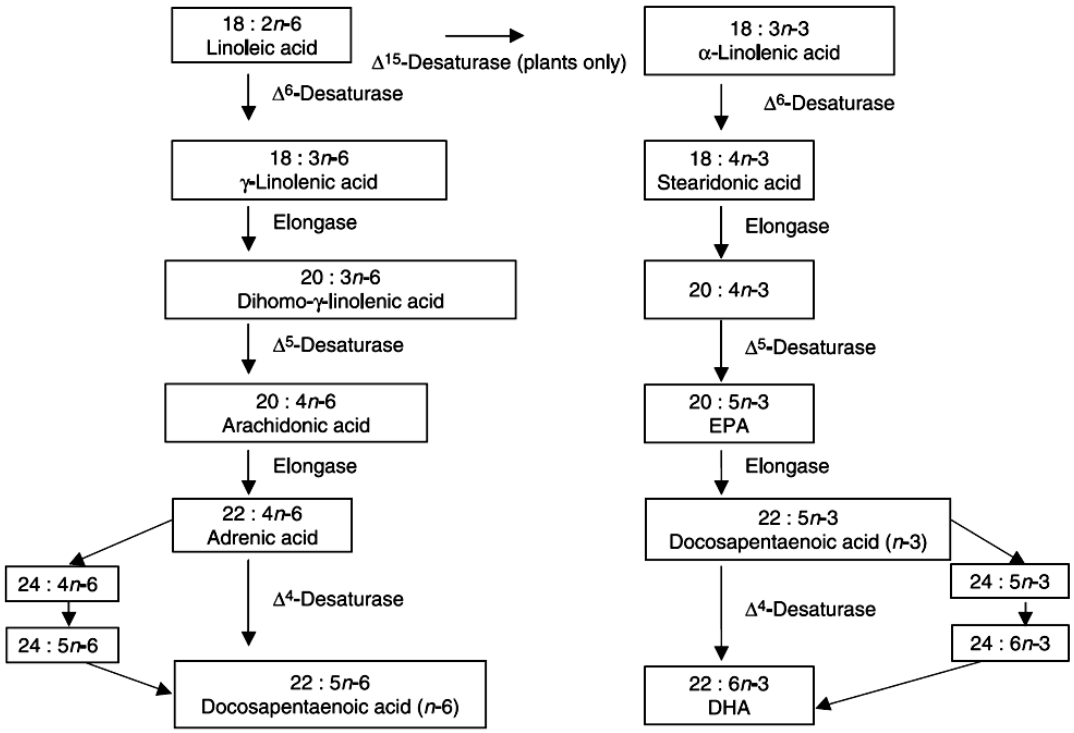

Figure 8 : Desaturation and elongation of n-6 and n-3 long-chain PUFA (Appleton, Rogers, & Ness, 2008).

It is interesting to note that dietary LC-PUFA can contribute to many biological processes. Linoleic and linolenic acids act like precursors or "parent" compounds of omega-6 and omega-3 LC-PUFA (Guil-Guerrero, 2007). Humans can metabolize these compounds into 20 and 22 carbon atoms by the process of elongation and desaturation (Figures 8). On one hand, linoleic acid can be conjugated to other important polyunsaturated fatty acid, such as gamma-linolenic acid (GLA), dihomo-gamma linolenic acid (DGLA) and arachidonic acid (AA) (Newton, 1996). On the other hand, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are derived from alpha-linolenic acid. EPA is dominant in brown seaweed including *Undaria pinnatifida*, while DHA is rich in both freshwater and marine fish oil (Dawczynski et al., 2007).

Enzymatic activities are not only involved in desaturation and elongation processes but can also influence several biological functions (Figure 9). 5 and 15-lipoxygenase and cyclooxygenase play an important role in producing 1and 2 series prostaglandins, 15-HETrE and 4-series leukotrienes (immune cells) which leads to diverse biological effects (Simopoulos, 2008; Wanasundara & Wanasundara, 2006).

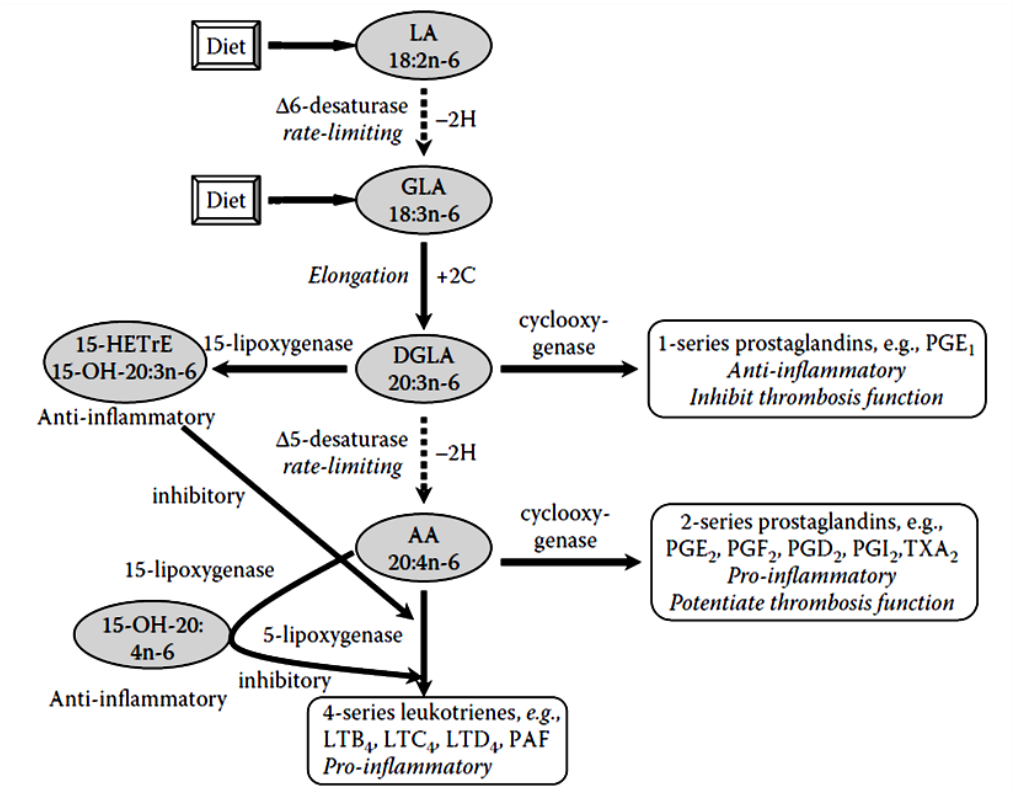

Figure 9 : Illustrating elongation and desaturation of dietary linoleic and γ-linolenic acids (Wanasundara & Wanasundara, 2006)

2.3 Other classes of lipids

2.3.1 **Phospholipids**

Phospholipids or phosphoglycerides are important lipids of animal and vegetable membranes (Holdt & Kraan, 2011). Phospholipids act as fat-soluble and water-soluble compounds, which have hydrophilic and hydrophobic characteristics. Phospholipids in plant cells play a crucial role in transporting materials and maintaining the structure of plants (Erickson, 2008).

Phospholipids in seaweed vary between 10% and 20% of the total lipids. Phospholipids in marine oils are more resistant to oxidation (rancidity) and have a high amount of fatty acids like EPA and DHA. These characteristics give better bioavailability and better spectrum of health benefits for humans and animals (Holdt & Kraan, 2011).

2.3.2 **Glycolipids**

Glycolipids are carbohydrate-attached lipids, which are associated with cell membranes. They play important roles in providing energy and serve as markers for cellular

recognition (Holdt & Kraan, 2011). Glycolipids consist of monoglycosyldiacylglycerol (MGDG), diglycosyldiacylglycerol (DGDG), trimethyl-betaalaninediacylglycerol and sulphaquinovosyldiacyl-glycerol. The components of glycolipids vary depending on algal types. In brown seaweed, MGDG content varies from 26% to 47%, DGDG content from 20% to 44% and sulphaquinovosylglycerol content from 18% to 52%, of total glycolipids (Dembitsky et al. 1990). Khotimchenko (2003) reported that *Undaria* contained the highest quantity of saturated and mono-unsaturated fatty acids in sulphaquinovosyldiacyl-glycerol.

2.4 Changes in fatty acid profile with environmental and seasonal conditions

The environment and seasonal conditions have an effect on seaweed growth and lipid content. For *Undaria*, the season and water temperature has a direct effect on growth rate. Hay and Villouta (1993) showed in their study in Wellington that *Undaria* started growing at the end of winter (June and July in New Zealand) and reached the maximal size of up to 4 m in spring (August and October) with average water temperatures of between 10.2 and 11.4 ºC (Hay & Villoula, 1993). *Undaria* degenerated in the summer due to high sea temperatures. The temperature and season affected not only growth rate but also changed the lipid composition of seaweeds. Nelson et al. (2002) found that total macro-algal lipid content increased during winter and spring for all the algae species and declined in summer (Nelson et al., 2002).

In the brown seaweed (*Egregia menziesii*), total fatty acid of was highest in spring (13.3 mg/g of dry weight) and lowest at 6.3 mg/g in summer (Nelson et al., 2002). Temperature also had a major effect on the individual fatty acids in seaweed cell membranes. Phleger (1991) explained that low temperature would increase the level of unsaturated fatty acid levels in polar lipids that would lower melting points and maintain lipids in a liquid state for normal protoplasmic viscosity. He further explained that arachidonic acid levels in marine fish were higher in warmer water. In macroalgae, the saturation level of fatty acid compositions was also controlled by temperature. red seaweed, *Palmaria palmate* had higher levels of EPA at 11°C and arachidonic acids at 15°C. In the brown seaweed, *Laminaria japonica*, (n-6) PUFA content reached the highest level during the warm months. In contrast, (n-3) PUFA level was the highest during the cold months (Nelson et al., 2002).

2.5 Nutritional and functional lipids

2.5.1 **Essential fatty acids**

Fatty acids play an important role in human diets, especially what are called the essential fatty acids (Plaza et al., 2008). The essential fatty acids are defined as substances, which are essential for growth and maintenances of biological processes (Webb, 2006). Additionally, these substances cannot be synthesized by human or animal and can only be obtained by dietary intake. The important essential fatty acids are omega fatty acids such as omega-3 and omega-6. Only two omega-6 and omega-3 fatty acids are essential for humans. These are alpha-linolenic acid (C18:3n-6) and linoleic acid (C18:2n-3). Other fatty acids are considered as "conditionally essential", for example gamma-linolenic acid,

2.5.2 **The importance of lipid in foods and human health**

The essential fatty acids linoleic and linolenic acid provide eicosanoids which produce hormone-like activity and regulate diverse body functions (Meschino, 2007; Zhou & Nilsson, 2001). Fatty acids, especially n-3 PUFA, have several biological effects in human and animal. Marine oil has been subjected to many studies and specific PUFA have interesting medical application against diseases (Fleurence et al., 1994).

Long chain unsaturated and essential fatty acids are claimed to prevent heart diseases, and have anticancer, anti-obesity and antioxidant properties (Li & Watkins, 2006). Generally, the marine n-3 polyunsaturated fatty acids exert anti-arteriosclerosis, anti-hypertension, anti-inflammation, immune-regulation effects, etc. (Plaza et al. 2008; Khan et al. 2007; Maeda et al. 2005).

2.5.3 **Diet and lipid consumption**

Many diseases are related to dietary intake. However, all diets containing these fatty acids are not considered healthy. For example, Western diets contain more omega-6 than omega-3. On the other hand, Mediterranean diets that are considered healthy have a balanced of n-6 and n-3 ratio. Ideally, the ratio of n-6/n-3 essential fatty should be

around 1. The ratio of n-6/n-3 was approximately 1:1 in prehistoric human diet (Simopoulos, 2002).

The Western diet's ratio is between 15:1 and 17:1 due to excessive amount of omega-6. Hence, Western diets are deficient in omega3 (Simopoulos, 2002). American intakes of omega-6 (DHA) and 3 (EPA) were reported to be 50 mg per capita/day and 80 mg per capita/day respectively. These sources were mainly from fish and poultry. As a result, the ratio of n-6/n-3 was about 15-20:1, which was considered as "being deficient" in omega-3. This excess of n-6 would lead to much pathology including cardiovascular diseases, cancer, inflammatory and autoimmune diseases (Simopoulos, 2008) .This ratio can also vary. On one hand, the European Nutritional Societies reported that human diet with 5:1 ratio n-6/n-3 ratio will have health benefits (Simopoulos, 2008) whereas the World Health Organization (WHO), recommends that the ratio of n-6/n-3 in human food should not exceed 10 (Sánchez-Machado et al., 2004a).

2.6 Previous research of lipids and fatty acids

2.6.1 **Analysis of saponifiable lipids**

2.6.1.1 Total lipid extraction methods

Lipids are organic soluble and insoluble in water. Selecting the suitable organic solvents plays an important role in lipid extraction because selective solvents and the solubility of lipids are main criteria for extracting lipids from the cell membranes of plants. There are two types of lipids. Triglycerides are non polar, while phospholipids and glycolipids are polar lipid. Several procedures have been utilized to extract lipids (Wrolstad et al., 2005). The basic method of fat extraction from foods was the Soxhlet procedure. A sample was refluxed with solvent (hexane, petroleum ether or diethyl ether) for a few hours and then the total lipid was obtained by evaporating the non-polar solvent. However, non-polar lipid are removed from the sample (Wrolstad et al., 2005).

Christie (1990) reported the use of chloroform and methanol to extract lipids from samples. This type of extraction can extract both polar and non-polar lipids. Another popular solvent extraction method was the Folch method developed by Lees and Sloane-Stanley (1957). Lipid was extracted by methanol/chloroform (1:2 v/v) or methanol/chloroform/water (1:2:1 v/v/v) (Folch, Lees, & Sloane-Stanley, 1957). Finally, Bligh and Dyer (1959) used a modified Folch's procedure that involved two

steps of solvent extraction. First, a mixture of methanol/chloroform/water (2:1:1 v/v/v) was used and then the sample was re-extracted with a second mixture of methanol/chloroform/water (1:1:1 v/v/v) (Bligh & Dyer, 1959). The extract was separated clearly into two layers. Lipids were dissolved in the organic phase (chloroform) and precipitated in the bottom, while the aqueous phase (methanol/water) was on the top layer. This aqueous phase would wash out most water soluble compounds.

The Folch and Bligh's methods have been applied widely for analysing total lipid of seaweeds (Dawczynski et al., 2007; Sánchez-Machado et al., 2004a). In *Undaria pinnatifida*, total lipid content was found to vary from 1 to 6.4 g/100g (Dawczynski et al., 2007; Fleurence et al., 1994).

2.6.1.2 Fatty acid analysis methods

Fatty acid profiles of seaweeds have been intensively studied. Generally, these studies have used commercial dried wakame available in the markets. Thus, the profiles would depend on plant origin (Dawczynski et al., 2007; Fleurence et al., 1994; Ortega-Calvo, Mazuelos, Hermosin, & Saiz-Jimenez, 1993) (see Appendix1).

Fatty acid analysis of food lipids are commonly done by gas chromatography. To analyse fatty acids in animals and plants, the complex structures of lipid have to be hydrolysed into individual FAs prior gas chromatographic analysis. This hydrolysis is called esterification or transesterification, which transfer lipid into fatty acid methyl ester (FAME). Preparation of FAMEs can be done by several methods that use either acidic or alkaline catalysts (Meier, Mjøs, Joensen, & Grahl-Nielsen, 2006). The fatty acids can result from the esterification or transesterification of the triacylglycerol. Then FAMEs can then be identified by GC and GC-MS analysis. Esterification could use basic, acidic and enzymatic catalysts. The use of boron trifluoride, sulphuric acid, anhydrous hydrogen chloride in methanol, and lipase are well documented (Scrimgeour, 2005). The common acidic catalysts are 14% (w/v) BF_3 in methanol (Wrolstad et al., 2005), 10% (w/v) BCl_3 in methanol, 5% anhydrous HCl in methanol (Sánchez-Machado et al., 2004a) and 1% to 2% concentrate sulphuric acid in methanol. On the other hand, basic hydrolysis utilized for transesterification include 0.5 N sodium methoxide and 1:4 (v/v) tetrathylguanidine (TMG) in methanol (Christie, 1990).

These methods have both advantages and disadvantages. On one hand, the BF_3 method can methylate free fatty acids quickly. Christie (1990) claimed that fatty acids can be methylated quickly with BCl_3. However, this reagent has not been since because BCl_3 was too concentrated and resulted in artefacts of FAME and loss of PUFA. Moreover, shelf life of the chemical reagent could create the problem for efficiency of transesterification (de La Cruz, Lopez Hernandez, & Simal Lozano, 2000), methanolic HCl method was stable for 1 or two days. Thus, it should be prepared freshly before using. Wrolstad et al. (2005) explained that acid catalyst including BF_3 could change configuration of double bonds which lead to misinterpretation of conjugated fatty acid such as conjugated linoleic acid (CLA).

On the other hand, basic catalysts could perform better than acidic counterpart on lipid rich in conjugated fatty acids. Sodium methoxide or TMG would avoid problem of isomerisation and artefact of FAMEs. In contrast, these methods were unable to methalate free fatty acids and N-link (amid bond) fatty acids which could be found in sphingolipids (Christie, 1990). After transesterification, FAMEs are analysed by gas chromatography (GC) or occasionally by high performance liquid chromatography (HPLC).

2.7 Materials and methods

2.7.1 **Materials**

2.7.1.1 Sampling and drying methods

Undaria were mainly collected fresh from different mussel farms in the Marlborough regions (Figure 10). Port Underwood and Pelorus Sound are two locations with the former being an exposed site and the latter a sheltered site. Of a total of nine farms, three farms were located in the exposed area and six farms in the sheltered area. Twenty plants from each farm were harvested each month. The blades of the seaweeds were separated from the sporophyll on the boat, and each sample was placed in numbered and labelled bags. These samples were then frozen overnight prior to being air freighted to Vitaco Limited, a freeze-drying plant in Auckland, to be lyophilised in bulk within 48 hours of frozen storage. Freeze drying samples aimed to prevent lysis of cell membrane and loose of water-soluble compounds. After drying, the blade and sporophyll samples were ground and passed through a sieve (600 μm). They were stored in plastic containers in a dark cupboard to avoid oxidation when in contact with light.

In addition, *Undaria* was also collected from Shelly Bay and Worser Bay, Wellington (Figure 10) and Great Barrier Island, Auckland (See at Appendix I, Figure I. 1). These samples were dried in an oven at 60°C for 3 days.

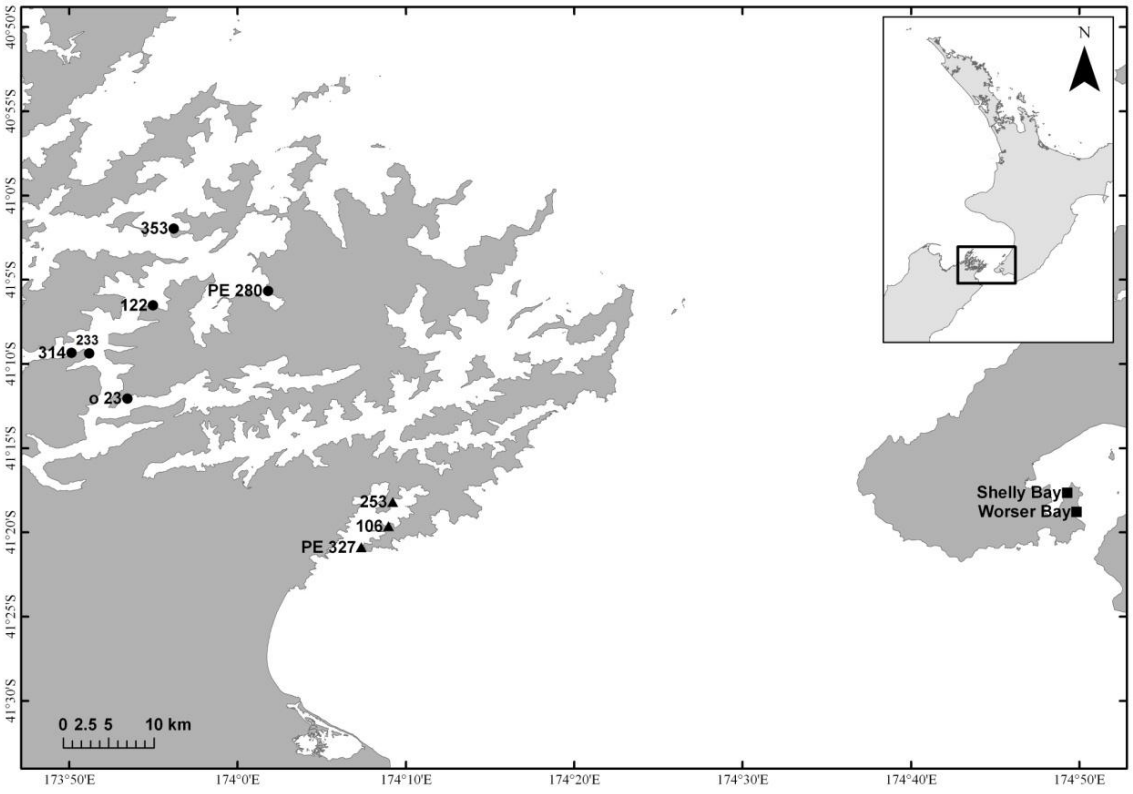

Figure 10 : Locations of *Undaria* collection.

New Zealand *Undaria* was not only compared in terms of lipid content between locations and months but also compared to vegetable oils and lipids found in marine animals. Olive, linseed, canola, wheat germ and rice bran oils were selected vegetable oils while scallop and commercial fish oils were representatives of marine animal oils. This selection was based on the characteristics of these oils. On one hand, vegetable oils would confirm some short and medium chains of fatty acids while marine animal oil and tissue would provide long chain polyunsaturated fatty acids.

2.7.1.2 Processing of New Zealand seaweed

In New Zealand, a range of imported seaweed products are available. There is little knowledge on the processing wakame. Hence, experiments on New Zealand *Undaria* will provide a basic understanding about the chemical and nutritional changes from

fresh seaweeds to the processed products that were commercial wakames from Japan and Korea.

Fresh seaweed was delivered from mussel farms in Marlborough Sounds and the blade and sporophyll were separated. The samples were cleaned and washed with tap water to eliminate adherent material. Cleaned seaweed was blanched at 80ºC for about 1 minute and the salt was added to blanched seaweed in a 3 : 10 salt : seaweed ratio and cured for 48 hours. During the curing, excess of liquid was removed by pressing with a heavy metal block for 48 hours. After curing, the midrib was removed from blade and all parts of seaweed were dried in oven dryer at 60°C for 48 hours. Finally, dried New Zealand *Undaria* was stored in zip lock bag for further analysis.

2.7.2 **Chemical reagents**

Tridecanoic acid (C13) was the internal standard and a commercial 37 fatty acid methyl ester standard mix were used as reference for unknown fatty acids of our samples. These standards were purchased from Supelco-Sigma Aldrich. Hydrochloric acid was also from this company. Acetonitrile, chloroform, methanol, ethanol and toluene were HPLC-grade. These reagents were from Thermo Fisher New Zealand. Potassium carbonate and hexane were analytical grade from Thermo Fisher. In addition, 5α-cholestan was used as internal standard and to quantify phytosterol concentration. It was also purchased from Sigma Aldrich.

2.8 Methods

2.8.1 **Total lipid extraction**

Total fat was extracted by different methods. Soxhlet lipid extraction and two solvent extraction methods were used for optimisation in this study.

2.8.1.1 Soxhlet lipid extraction

Undaria powder (2g) was Soxhlet extracted for 3 hours with hexane. The extract was evaporated in the rotary evaporator at 40ºC under reduced pressure (approximately 20 mmHg). The total lipid content was determined by weight difference.

2.8.1.2 Solvent extraction of lipid content

Solvent extraction methods were based on methanol and chloroform extraction methods. The first method was utilized methanol/chloroform/water extraction which was modified by Bligh and Dyer (1959). 0.5g of dried seaweed was hydrated by 0.7 mL of Milipor Q water then 3 mL of methanol/chloroform (2:1 V/V) was added. The mixture of seaweed and solvents sat in an ultrasonic bath for 10 min and then 1 mL of chloroform and 1 mL of water was added. The mixture was shaken by a vortex stirrer. After shaking, it was centrifuged for 5 min at 6000 rpm and separated into two layers. The lower (chloroform) layer was collected and dried under a nitrogen stream at 60ºC in water bath.

Second method was based on partition between chloroform and methanol known as the Folch method (1957). Samples were extracted with 2:1 chloroform/methanol. First, 2 g of freeze dry sample placed in glass bottle with 14 mL of 2:1 chloroform/methanol. The bottle was closed under nitrogen and shaken with vortex stirrer for 2 minutes. The mixture was filtered through Whatman paper No41.

The residue of the first filtration was re-extracted with 5 mL of the solvent mixture and shaken in vortex mixer, filtered through the same Whatman paper No 41 and mixed with the first filtrate. The combined extracts were dried under a stream of nitrogen and dried weight of resulting residue considered as the total lipid content of *Undaria* sample (Sánchez-Machado et al., 2004a).

2.8.2 **Preparing fatty acid methyl esters (FAMEs) and derivatised fats**

Fatty acid methyl ester was prepared using BF_3/methanol. Total lipid extracted in section 2.8.1.2 was added a 15 mL glass vial with a screw-cap. 0.4 mL 0.5 N NaOH in methanol was added and saponification done by heating in water bath at 100°C for 5 min. The tube was cooled with tap water and 0.4 mL 14% BF_3 was added. Then the tube was placed in water bath at 100°C for 5 min to esterify the fatty acids. After cooling with tap water, 0.8 mL of water was added and the tube was placed in centrifuge for 5 min at 1000 g. The organic phase (top layer) was transferred to 2 mL vials and analysed by GC.

Fatty acid methyl esters were prepared by the transesterification method of de La Cruz et al. (2000). Dried powder of samples (0.75g) were weighed and placed into 50 mL a screw-topped tube (de La Cruz et al., 2000). Then 2 mL of toluene and freshly prepared

5% methanolic HCl (3 mL) were added to the sample and the tube mixed thoroughly in a vortex mixer. The tube was closed under nitrogen to avoiding lipid oxidation and heated at 70ºC in water bath for 2 hours. After cooling by tap water, 4 mL of 6% aqueous K_2CO_3 and 2 mL of toluene were added and the mixture was shaken in a vortex mixer. The mixture was then centrifuged for 5 minutes at 373 g and the organic phase in the top layer carefully into the beaker and dried with anhydrous Na_2SO_4 (Sánchez-Machado et al., 2004a).

2.8.3 **Identification of fatty acids by GC**

The FAME mixtures were analysed by the gas chromatograph (Shimadzu GC-2010) with a split-splitless injector and flame ionisation detector (FID). The individual FAMEs were separated and identified by Zebron ZB-Wax capillary column (30 m x 0.25 mm x 0.25 µm) from Phenomenex. The oven program was 140ºC to 245ºC at 5ºC/min then hold for 15 minutes. The gas carrier was nitrogen with a linear velocity of 20 cm/sec with a 20:1 split. The temperature of the detector was 250ºC. Injection volume was 1 µL.

2.8.4 **Quantification of fatty acid methyl esters**

Quantification of fatty acids was calculated by comparing a known amount of internal standard peak area of the C-13 internal standard and peak area of the analyte. The calculation method was as follows:

Calculation of individual fatty acid

$$\mathbf{Ci} = \frac{\mathbf{Pi} \times \mathbf{Cis} \times \mathbf{L} \times \mathbf{100}}{\mathbf{Pis} \times \mathbf{W}}$$

Ci : concentration of individual analyte (g/100g DW)

Cis : Concentration of internal standard (mg/mL)

L : total reagents (mL)

Pis : peak area of internal standard

Pi : peak area of analyte

W: sample weight (g)

2.9 Statistical analysis

Results are expressed as means ± SD (n=3). Significance between season and location at $P < 0.05$, were analysed by one-way analysis of variance (ANOVA) followed by post hoc Tukey testing if a significant different was found. This was carried using the Minitab software (version 16). Multivariate analysis was used to evaluate different variables between location and months. Principle Components Analysis (PCA) was performed by Xlstat 2011.

2.10 Results and discussions

2.10.1 **Geographical conditions**

2.10.1.1 Temperature and rainfall

Geographical conditions would have generally an influence on development and chemical changes of plants. In this study, temperature and rainfall were taken into consideration. These conditions provided by National Institute of Water & Atmospheric Research (NIWA) (NIWA, 2011) and the Weather Online (Weatheronline, 2011). Nelson was the referenced station while Table 4 shows changes of rainfall and temperature in 2011 respectively. According to this report, there was not much variation of temperature in this region. The average of temperature was between minimum 11.7ºC and maximum 13.8ºC.

Table 3 : Monthly temperature and rainfall for Nelson 2011

Months	**Max T (°C)**	**Min T (°C)**	**Mean T (°C)**	**Rain (mm)**
January	23.5	12	17.75	75
February	23	12	17.5	72
March	22.5	10.3	16.4	75
April	18.2	8	13.1	80
May	15.5	4	9.75	83
June	13.5	3.2	8.35	90
July	12.2	2	7.1	87
August	13.2	3.4	8.3	82
September	17.2	5	11.1	85
October	17.3	6	11.65	89
November	21	8	14.5	77
December	21.9	11	16.45	79
Total				**979**

(Weatheronline, 2011)

Monthly temperature and rainfall were recorded by Weather Online (UK). The air temperature in Nelson varied about 7ºC between winter and summer. The hottest month was in January and maximum temperature was 23.5ºC. The lowest temperature was in July and about 2ºC. On the other hand, the weather-and-climate site gives as average of ~80 mm per month (nearly 1000 mm / year)

2.11 Total lipid contents

2.11.1 **Comparison of total lipids from the three methods**

In this study, the samples collected in September 2010 and were homogenised and oven dried. Then 5 replicates were subjected to each method. These methods were described in section 2.8.1 and the results were showed in Table 5.

Table 4 **: Comparison of three extraction methods**

(Mean ± SE mg/g, n = 5, no significant different, P > 0.05, T-test)

Methods	**Total lipid content**
Soxhlet	31.6 ± 0.32[a]
Folch	31.9 ± 0.58[a]
Bligh	42.4 ± 0.97[a]

a : No significant difference

Table 5 shows that there was no statistically significant difference between each method. The Bligh method showed a higher standard error between replicates while the Soxhlet method utilized more solvent and was expensive. The Soxhlet is the standard method for foods. Because the Folch method was most suitable for the small samples and cheap, it was chosen for this study.

2.11.2 **Comparison of total lipid between freeze dried and oven dried samples**

Because lipids could be subject to thermal degradation if seaweed is dried at 60ºC, a comparison was made between freeze drying and oven drying at 60ºC for 3 days. The result of the comparison are shown in Table 6.

Table 5 **: Comparison of freeze dried and oven dried samples**

a: level of significant difference, values are expressed as mean and standard error (mg/g, n = 3, no significant different, P > 0.05, T-test)

Type of samples	Total lipid content
Freeze dry	28.4 ± 0.01 [a]
Oven dry	26.7 ± 0.12 [a]

The result showed that, at least gravimetrically, there is no significant difference between freeze dried and oven dried samples by using one-way T-test ($P > 0.05$).

2.11.3 Comparison of total lipid between commercial wakames and processed New Zealand *Undaria pinnatifida*

It is assumed that some of the chemical compounds in seaweed would be partially degraded after processing. For this reason, analysing total fat content of commercial *Undaria* (dried wakame) and NZ processed *Undaria* could provide a measure of the quality of products. Four commercial dried cut wamakes viz. Japanese Riken wakame Chan, Japanese Woko Shakai, Korean Ito wakame and Korea Chung Jung Won seaweed were compared to New Zealand processed *Undaria*. Additionally, commercial wakame is produced from blade. New Zealand *Undaria* was processed according to the method in section 2.7.1.2 and midrib was removed from frond. The results are shown in Table 7.

Table 6 : Total lipid contents of processed Wakame

Type of samples	Total lipid content
Riken wakame Chan	38.2 ± 0.10 [b]
Woko Shakai wakame	34.2 ± 0.06 [c]
Ito wakame	34.7 ± 0.12 [b, c]
Chung Jung Won seaweed	43.6 ± 0.14 [a]
Blade	21.7 ± 0.07 [d]
Midrib	9.6 ± 0.10 [e]
Sporophyll	34.6 ± 0.08 [c]

a: level of significant difference, values are expressed as mean and standard error (mg/g, n = 3, P < 0.05, ANOVA, Tukey post-hoc test)

There were significant differences between commercial wakames and New Zealand processed blade and midrib of *Undaria* ($P < 0.05$) (Table 7). The range of total lipids varied from 9.6 to 43.6 mg/g. The Japanese and Korean wakame had significantly higher lipid content than the New Zealand blade and midrib ($P < 0.05$). However, New Zealand processed sporophyll had similar amount of lipid as Japanese Woko Shakai which was 34.6 and 34.2 mg/g respectively. The New Zealand *Undaria* samples used in this study were not mature yet. Thus, processing step resulted in decreasing its total lipid content.

2.11.4 **Comparison of total lipid from blade, midrib and sporophyll**

Lipids are a source of energy for plant and are stored in various parts of plant. Lipids are stored in the seed of soy bean for reproduction of future young plant. For this reason, investigating the lipid content in blade, midrib and sporophyll would provide interesting information because these parts of *Undaria* were consumed and commercialized in China and Japan.

Table 7 : Total lipid in different parts of *Undaria* sporophyte

Type of samples	**Total lipid content**
Blade	22.5 ± 0.07^{a}
Midrib	17.7 ± 0.17^{a}
Sporophyll	58.2 ± 0.38^{b}

All values are expressed as mean and standard error (mg/g dry weight, n=5). All samples came from farm 327. Different superscript letters (a, b, c) indicate significant difference between different types of samples using the Tukey post-hoc tests ($P < 0.05$)

The *Undaria* sporophyll contained more total fat than blade and the sporophyll while there was no significant difference between blade and midrib (Table 8) ($P < 0.05$).

2.11.5 **Comparison of total lipid from different seasons**

Environmental conditions have a direct effect on biochemical formation of terrestrial and marine plants. The results of seasonal and environmental changes in *Undaria* were showed in Figure 11.

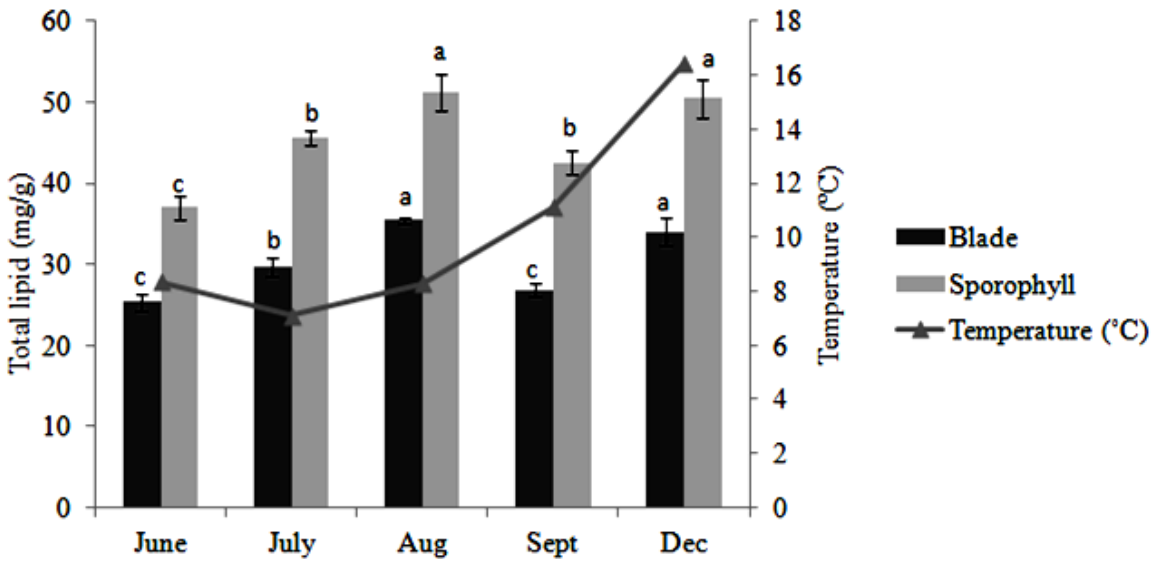

Figure 11: Seasonal changes of total lipid contents

All values are expressed as mean and standard error bar (mg/g dry weight, n=3). All samples came from farm 327. Different superscript letters within the total lipid contents indicate statistical difference between months using Tukey post-hoc tests ($P < 0.05$).

Figure 11 shows that the season and the environment had an influence on lipid formation in *Undaria pinnatifida*. Total lipids in the blade and the sporophyll increased during the winter then decreased during the spring, increased at the beginning of summer. The peak lipid content was found in the end of winter (August). Where total lipids of blade and the sporophyll were 35.5 and 51.3 mg/g respectively. Another peak was also found in beginning of summer (December). The total lipid in the blade was 34 mg/g and 50.4 mg/g for the sporophyll. Lowest amount of lipids were in June when the algae started growing again. The lipid contents of the blade and the sporophyll were 25.3 and 37 mg/g respectively. According to the records, the Port Underwood summer in 2011 was cold with an average temperature of approximately 16ºC. This temperature appeared to be suitable for the development of *Undaria*.

2.11.6 **Comparison of total lipid from different locations**

The geography of farms would have an impact on biochemical growth of *Undaria pinnatifida*. The lipid content of *Undaria* from different locations was compared. In August 2011, *Undaria* was collected from Port Underwood (PUW), Worser Bay and Shelly Bay (Wellington) and Great Barrier Island (Auckland). The blade and sporophyll were separately subjected to lipid analysis. Three plants from each location were used. Port Underwood and Worser Bay were considered as exposed sites.

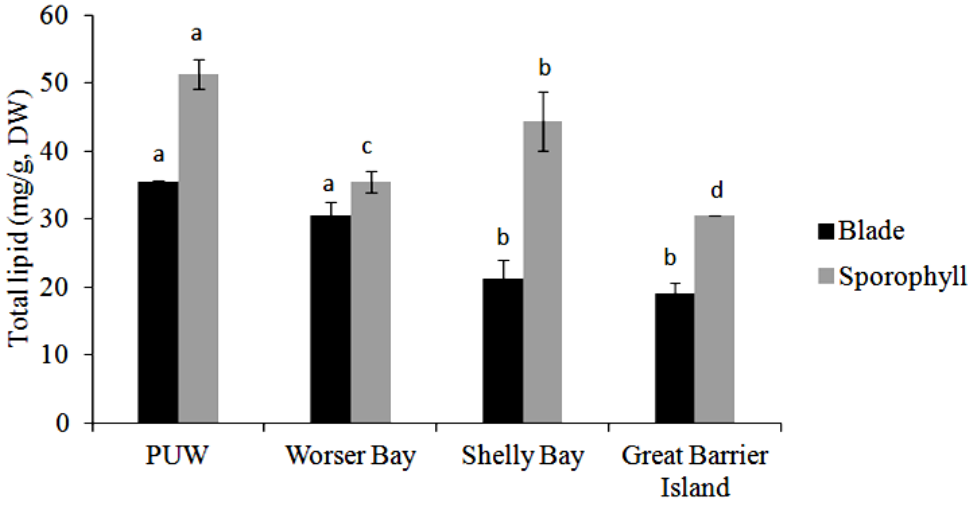

Figure 12: Total lipid of *Undaria* from different locations

All values are expressed as mean and standard error bar (mg/g dry weight, n=3). All samples came from farm 327. Different superscript letters within the total lipid contents indicate statistical difference between locations using Tukey post-hoc tests ($P < 0.05$).

Results are shown in Figure 12 and appendix V (Table V.3). The geographical locations had direct influence on lipid formation. There was not only significant difference ($P < 0.05$) of lipid content between blade and sporophyll but also between sheltered and exposed sites. On one hand, the lipid content of blade from exposed sites was higher than from sheltered sites (Shelly bay) and Great Barrier Island. *Undaria* from PUW and Worser Bay consisted of 35.46 and 30.45 mg/g respectively while total lipid of counterpart was 21.2 and 18.96 mg/g respectively.

On the other hand, sporophylls from Port Underwood contained the highest lipid content while the lowest lipid content was found in one of Great Barrier Island. It is interesting to notice that double amount of lipid was found in the sporophyll from Shelly Bay compared to its blade. This information explained that *Undaria* from Great Barrier Island was smaller than other locations and there was high variation of lipid content in sheltered sites between the blade and the sporophyll.

2.12 Size of *Undaria pinnatifida* and lipid content

To investigate any connection between maturity and lipid content, 25 plants of different sizes were analysed. The samples were collected from two exposed and sheltered sites

in October 2011. Then total lipid, length and weight were measured. These data was plotted as linear regression to show the relation between size of plant and its chemical compound (see in Figure 13, equation1 and appendix II).

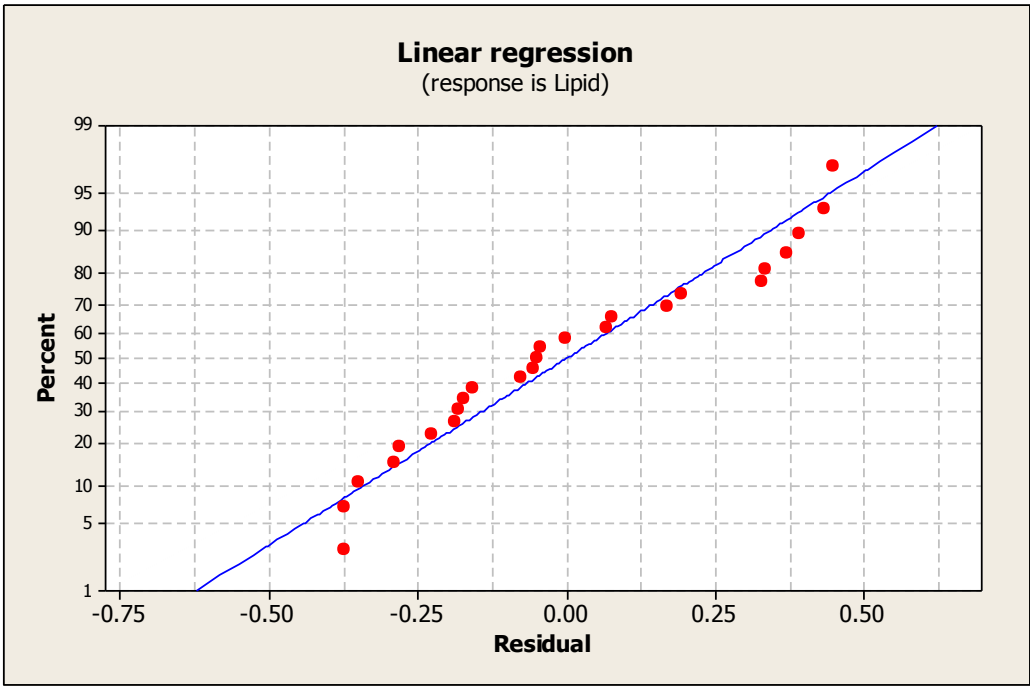

Figure 13 : Linear regression between total lipid contents and size of plants

Equation 1: The linear regression equation of lipid

$$\textbf{Lipid} = 0.705 + 0.0308\ \textbf{Length} + 0.0124\ \textbf{weight}\ (R^2 = 88.7\%)$$

Equation1 showed that there was a significant correlation between size of plants and its total lipid. It increased with both length and weight. This information confirmed that mature algae would produce more lipids than younger ones. However, this study was only used 25 plants and was considered as a preliminary study only.

2.13 Pilot study of fatty acid methyl esters (FAMEs)

2.13.1 **Identification of FAMEs by GC-FID**

Identification of unknown fatty acids was done by comparison with a fatty acid methyl ester (FAME) mixture (Supelco No. 47885-U). This standard mixture and *Undaria* sample were analysed by GC-FID. Unknown fatty acids were identified by comparing

the retention time of standard mixture and one of unknown samples. The identification of NZ *Undaria* fatty acids was showed in Figure 14 and 15.

The Figure 14 demonstrated overlapped chromatograms between mix standards and an *Undaria* samples. This technique could identify 17 individual fatty acids from dried *Undaria*. However, this standard mixture could not identify all unknown FAMEs. Unknown fatty acid was compared with retention time from the result of Sanchez Machado et al. (2004) because this FAME was prepared by their method.

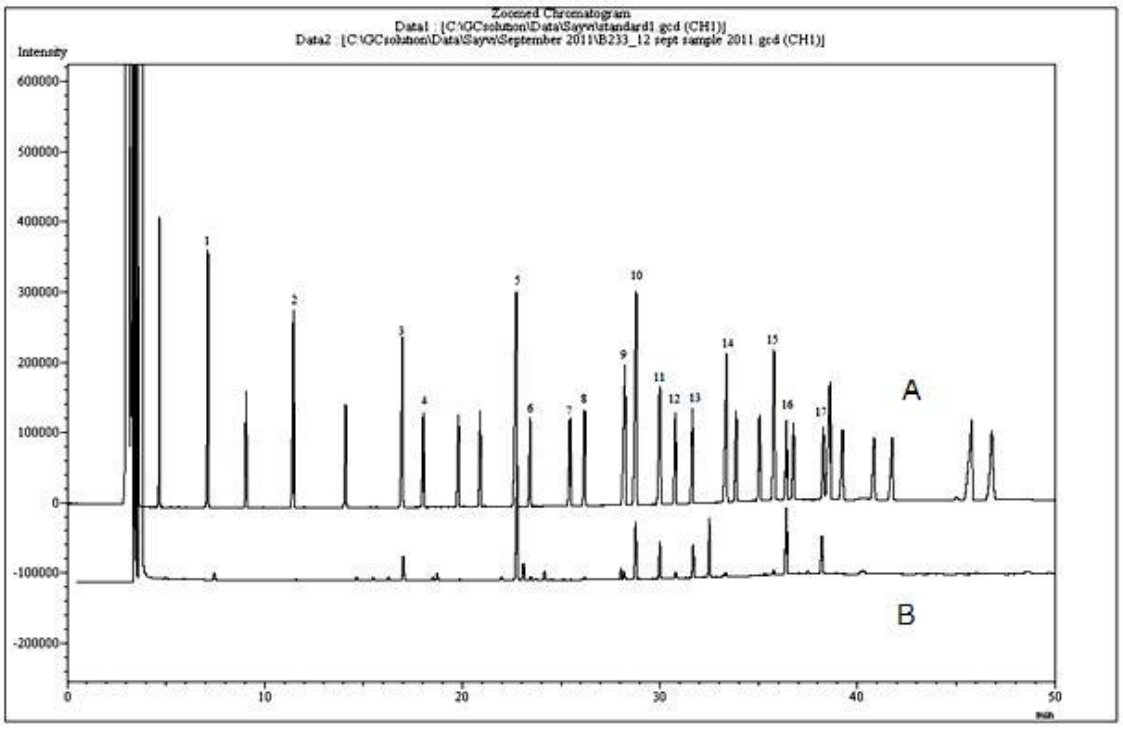

Figure 14: Comparison of fatty acid profile of NZ *Undaria* (B) and mix standards (A) : 1:C10, 2: C12, 3: C14, 4: C14:1, 5: C16, 6: C16:1, 7: C17, 8: C17:1, 9: C18, 10: C18:1n-9c, 11: C18:2n-6c, 12: C18:3n-6c, 13: C18:3n-3, 14: C20, 15: C20:3n-6, 16: C20:4n-6 and 17: C20:5n-3.

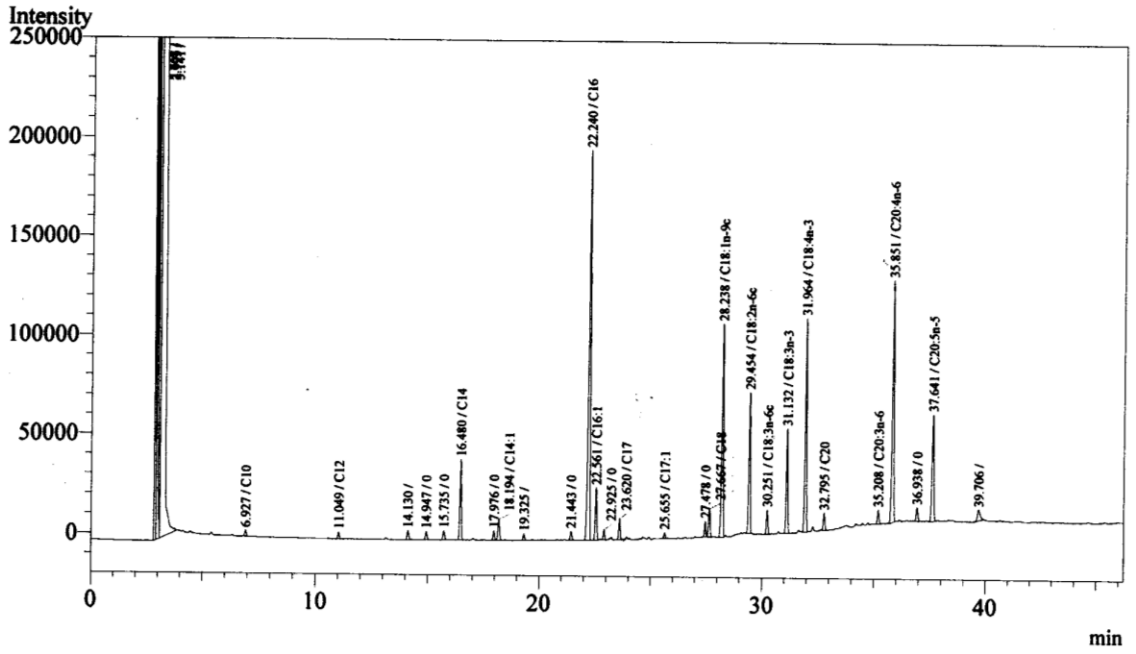

Figure 15: Chromatogram of New Zealand fatty acid profile from GC-FID with retention time

Additionally, Figure 15 showed a chromatogram of 18 identified fatty acid methyl esters by GC-FID. This figure shows additional fatty acids which did not match the standard mixture. For examples, the retention time indicated 32.334 min was C18:4n-3 by comparison with other studies (see appendix IV, Table IV.2). However, many small peaks can be unable to identify (see Figure 19).

2.13.2 **A comparison of two FAME esterification methods.**

In this section, FAMEs were prepared by the methanolic BF_3 and methanolic HCl methods of transesterification. After subjecting to acidic catalysis, FAMEs were identified by chromatographic analysis and fatty acid profiles of NZ *Undaria* were shown in Table 9.

The comparison between these methods is to determine recoveries of fatty acids. Table 9 shows that the recoveries of fatty acid methyl esters by these methods were significantly different ($P < 0.05$). The BF_3 methanol method was able to recover more saturated (SFA) and monounsaturated fatty acids (MUFA) while the methanolic HCl method could derivatise more saturated and polyunsaturated fatty acids (PUFA). For example, SFA was found 51.38% of total fatty acids by methanolic BF3 method but it

was only 28.58% of total fatty acid from the methanolic HCl method. In contrast, the methanolic HCl method could recover 58.93% of total fatty acids while it was found only 19.9% of total fatty acids (also see Apprendix III). Moreover, the reagents of methanolic HCl method were more stable and less expensive. Thus, this method was chosen for transesterification of FAMEs in this study.

Table 8 : Fatty acid profiles of New Zealand *Undaria pinnatifida* by acidic catalysis

	Methanolic BF_3	**Methanolic HCl**
C14	5.10 ± 0.48	3.97 ± 1.27
C16	39.91 ± 0.49	19.44 ± 5.82
C17	0.49 ± 0.09	2.76 ± 0.28
C18	4.87 ± 0.42	1.23 ± 0.17
C20	0.99 ± 0.13	1.19 ± 0.52
∑SFA	**51.38 ± 1.63[a]**	**28.58 ± 8.02[b]**
C14:1	0.39 ± 0.04	1.56 ± 0.29
C16:1	0.74 ± 0.048	1.84 ± 0.20
C17:1	0.38 ± 0.07	1.24 ± 0.31
C18:1n9c	27.82 ± 0.26	7.84 ± 2.09
∑MUFA	**29.34 ± 0.85[a]**	**12.48 ± 2.89[b]**
C18:2n6c	4.76 ± 0.18	7.62 ± 3.02
C18:3n6	1.02 ± 0.07	1.80 ± 1.10
C18:3n3	1.66 ± 0.08	8.62 ± 0.51
C18:4n3	2.60 ± 0.14	16.94 ± 2.76
C20:3n6	0.65 ± 0.27	0.75 ± 0.16
C20:4n6	6.74 ± 1.33	13.85 ± 1.28
C20:5n3	2.48 ± 0.12	9.37 ± 1.81
∑PUFA	**19.90 ± 2.19[a]**	**58.93 ± 10.64[b]**
n6	**13.17**	**24.01**
n3	**6.74**	**34.92**
n6/n3	**1.96**	**0.69**

Results are mean ± standard error (% of total fatty acids, n = 3). Different superscript letters in row are significantly different ($P < 0.05$, by one-way ANOVA and Tukey test). ∑SFA: sum of saturated fatty acid, ∑MUFA: sum of monounsaturated fatty acid, and ∑PUFA: sum of polyunsaturated fatty acids.

2.13.3 **Repeatability and feasibility of Methanolic HCl method**

In this section, the Methanolic HCl method was used to analyse different oils and compared the results to other studies and New Zealand *Undaria* was also compared to these oils. Using marine animals and terrestrial vegetable oils could provide the feasibility and repeatability of fatty acid methylation method. Methanolic HCl method (tested in section 2.14.2) was utilised to analyse both marine and terrestrial oils then their results were compared with different research results of similar products. Fish oil

and scallop muscle were represented long chain polyunsaturated fatty acids (Ackman, 2006; Indarti, Majid, Hashim, & Chong, 2005). Vegetable oils would refer to short and medium chain of fatty acids (Cheruvanky & Thummala, 1991; Dunforda & Zhang, 2003). Especially, C18:1 n-9c was biochemically characteristic to edible vegetable oils such as canola, olive and sunflower oils (Moser, 2008; Mourente, Good, & Bell, 2005).

Table 10 showed comparison between New Zealand *Undaria pinnatifida* and other oils. Commercially available fish oil and scallop tissue were used as references for Methanolic HCl method. The results showed that HCl method was able to recover main fatty acid compositions as similar as other studies. For instance, in fish oil and scallop tissue, C14, C16 and C18 were the main constituents of saturated fatty acids in marine animal oils. The various significant fatty acids recovered and identified C18:1n-9 and C24:1n-9 were represented MUFA while long chain PUFA C18:2n-6, C18:3n-3, C18:4n-3, C20:5n-6, C20:5 n-3 and C22:6n-3 were gave similar pattern to published results (Ackman, 2006; Cheruvanky & Thummala, 1991; Indarti et al., 2005).

The commercial vegetable oils utilised in this comparison were wheat germ, rice bran, linseed, canola and olive oils. The main characteristic of these oils was consisted short and medium chains of fatty acids. For example, C16 and C18 were main SFA of many vegetable oils while canola oil contained additionally C20. It was interesting to notice that the common characteristic of vegetable oils was found important amount of C18:1 n-9. Thirdly, NZ *Undaria* consisted of SFA, MUFA and PUFA which were similar profiles to the marine and terrestrial oils. The HCl/methanol method was used to derivatise the lipids. Additionally, recovery of SFA, MUFA and PUFA for New Zealand *Undaria* confirmed repeatability of this method. Many authors agreed that the PUFA contents of *Undaria pinnatifida* are higher than one of SFA and MUFA (see Appendix IV, Table IV.2) (Dawczynski et al., 2007; Fleurence et al., 1994; Khotimchenko, 1998).

Table 9 : Fatty acid profiles of plant oils and marine animal and edible algae (% of total fatty acids)

Fatty acid	**Fish oil**	**Fish oil1**	**Scallop**	**Scallop1**	***Undaria***	**Wheat**	**Wheat 1**	**Rice**	**Rice1**	**Linseed**	**Linseed1**	**Canola**	**Canola1**	**Olive**	**0live1**
C10	1.2		1.39		**0.16**									0.21	
C12	0.86		0.46		**0.13**					0.09				0.18	
C14	7.41	6.3	2.05	2.1	**3.39**	0.25		0.67	0.4	0.29	3	0.3		0.3	4.5
C16	18.83	17.4	24.5	19.8	**18.81**	17.59	16.4	21.47	21.5	7.1	10.5	5.64	4.6	13.26	13.1
C17	1.12		1.05		**1.33**										
C18	3.53	2.9	8.17	4.2	**1.52**	0.97	0.7	2.45	2.9	3.9	3.1	1.82	2.1	2.88	2.7
C20	1.36		0.47		**0.7**	1.09	0.2	0.82		0.18		2.68	0.7	0.2	
C23	0.85		1.75												
ΣSFA	**35.16**	**26.6**	**39.85**	**26.1**	**26.02**	**19.9**	**17.3**	**25.41**	**24.8**	**11.57**	**16.6**	**10.43**	**7.4**	**17.04**	**20.3**
C14:1			0.64		**0.74**					0.09				0.16	
C16:1	9.46	8.1	0	2.6	**1.96**			0.24			2.7	0.24	0.2	0.79	3.1
C17:1	1.3		1.71		**0.65**			0.2						0.11	
C18:1n-9c	9.25	18.5	3.25	4.7	**8.9**	12.23	14.6	38.87	38.4	18.71	15.6	56.99	64.3	71.7	37.2
C18:1n-9t	3.09		2.36			1.19		0.76		0.59		3.06		8.16	
C22:1n-9c	1.01		2.43								2.1				
C24:1n-9c	1.57		0.95				0.2								
ΣMUFA	**25.68**	**26.6**	**11.34**	**7.3**	**12.25**	**13.41**	**14.8**	**40.07**	**38.4**	**19.4**	**20.4**	**60.28**	**64.5**	**80.92**	**40.3**
C18:2n-6c	1.49	5.8	1.47	0.5	**9.05**	54.15	56.8	31.39	34.4	16.64	12.3	19.79	20.2	0.53	9
C18:3n-6			0.43		**1.74**					0.09				0.19	
C18:3n-3	1.02	1.4	1.43	0.3	**6.49**	11.65	6.2	1.42	2.2	51.43	25.9	8.02	7.6	0.46	1.2
C18:4n-3	3.87	1.8	3.81		**14.98**			0.27		0.1	1	0.72		0.31	0.9
C20:3n-6			3.16		**0.86**	0.36		0.42		0.16		0.32		0.32	
C20:4n-6	1.38	0.5	0.48	1.2	**17.27**	0.27		0.24		0.36	0.4	0		0.13	0.4
C20:5n-3	18.77	7.8	14.15	18.5	**9.8**	0.25		0.23		0.12	5.2	0.32		0.1	4.7
C22:6n-3	9.08	8.1	23.87	22.9							4.4				4.3
ΣPUFA	**35.61**	**25.4**	**48.81**	**43.4**	**60.2**	**66.68**	**63**	**33.98**	**36.6**	**68.9**	**49.2**	**29.16**	**27.8**	**2.05**	**20.5**
Ratio n-6/n-3	**0.088**	**0.33**	**0.128**	**0.041**	**0.924**	**4.602**	**9.16**	**16.657**	**15.64**	**0.334**	**0.348**	**2.225**	**2.66**	**1.345**	**0.847**

Fish oil 1: Indarti et al. (2005), Scallop 1 = Ackman (2006), Rice 1: Rice bran oil (Cheruvanky & Thummala, 1991), Wheat 1 : Wheat germ oil (Dunforda & Zhang, 2003), Canola 1: Canola oil (Moser, 2008), Linseed 1: Linseed oil (Mourente et al., 2005), Olive 1: Olive oil (Mourente et al., 2005).

2.14 Identification of a fatty acid profile in New Zealand edible *Undaria*

These results are for fatty acid methyl esters (FAMEs) methanolic HCl preparation. Individual FAMEs was identified by comparing to retention time of 37 FAMEs mixed standard. Table 11 shows the 18 identified fatty acids from New Zealand *Undaria pinnatifida.*

Table 10 : Identification of fatty acid in edible NZ *Undaria* according to retention time

Peak	Common name	Systematic name*	Fatty acid	RT
1	Capric acid	Decanoic acid	C10	6.980
2	Lauric acid	Dodecanoic acid	C12	11.123
3	Myristic acid	Tetradecanoic acid	C14	16.566
4	Myristoleic acid	Cis-9-tetradecenoic acid	C14:1	18.258
5	Palmitic acid	Hexadecanoic acid	C16	22.299
6	Palmitoleic acid	Cis-9-hexadecenoic acid	C16:1	22.628
7	Margaric acid	Heptadecanoic acid	C17	23.717
8		Cis-10-heptadecenoic acid	C17:1	25.759
9	Stearic acid	Octadecanoic acid	C18	27.600
10	Oleic acid	Cis-9-octadecanoic acid	C18:1n-9c	28.319
11	Linoleic acid	9,12 octadecadienoic acid	C18:2n-6c	29.549
12	γ-linolenic acid	6,9,12-Octadecatrienoic	C18:3n-6	30.357
13	α-linolenic acid	9,12,15-octadecatrienoic acid	C18:3n-3	31.229
14	Stearidonic acid	Octadeca-6,9,12,15-tetraenoic acid	C18:4n-3	32.145
15	Arachidic acid	Eicosanoic acid	C20	32.930
16	Dihomo-g-linolenic	Cis-8, 11, 14-eicosatrienoic acid	C20:3n-6	35.313
17	Arachidonic acid	5,8,11,14- Eicosatetraenoic acid	C20:4n-6	36.129
18	Eicosapentaenoic acid	Cis-5,8,11,14,17-Eicosapentaenic acid	C20:5n-3	37.858

RT: Retention time, *:(O'Keefe, 2008)

This table shows common and systematic name, formula and retention time of individual fatty acid. New Zealand *Undaria* are rich in saturated, monounsaturated and long chain polyunsaturated fatty acids.

2.14.1 **Comparison of fatty acid profiles between freeze dried and oven dried samples**

Ideally, different samples had to treat the same way which would produce comparative results. However, the Marlborough Sound's samples were freeze dried while Wellington and Auckland samples were oven dried. To be able to compare these samples, test of difference between these dried systems would be necessary.

Table 11 : FAMEs of freeze dried and oven dried samples of September 2011

Fatty acid	**Oven dry**	**Freeze dry**
C10	0.10 ± 0.02	0.08 ± 0.01
C12	0.43 ± 0.03	0.41 ± 0.02
C14	0.78 ± 0.05	0.91 ± 0.10
C16	4.35 ± 0.08	4.68 ± 0.14
C17	0.42 ± 0.03	0.37 ± 0.03
C18	0.30 ± 0.00	0.40 ± 0.06
C20	0.22 ± 0.01	0.27 ± 0.02
∑SFA	**6.59 ± 0.24[a]**	**7.11 ± 0.39[a]**
C14:1	0.25 ± 0.01	0.26 ± 0.03
C16:1	0.52 ± 0.03	0.60 ± 0.07
C17:1	0.40 ± 0.14	0.25 ± 0.00
C18:1n9c	1.77 ± 0.09	1.91 ± 0.48
∑MUFA	**2.94 ± 0.27[a]**	**3.02 ± 0.59[a]**
C18:2n-6c	1.83 ± 0.11	1.80 ± 0.23
C18:3n-6	0.34 ± 0.01	0.33 ± 0.01
C18:3n-3	2.28 ± 0.11	2.01 ± 0.46
C18:4n-3	4.05 ± 0.28	3.84 ± 0.37
C20:3n-6	0.19 ± 0.03	0.21 ± 0.03
C20:4n-6	3.80 ± 0.10	3.70 ±0.31
C20:5n-3	2.41 ± 0.25	2.15 ±0.27
∑PUFA	**14.91 ± 0.88[a]**	**14.06 ± 1.68[a]**
Total fatty acid	**24.44 ± 1.39[a]**	**24.19 ± 2.66[a]**
n-6	**6.2**	**6.1**
n-3	**8.7**	**8.0**
Ratio n-6/n-3	**0.70**	**0.76**

Results are mean ± standard error (mg/g dry weight, n=3). Different superscript letters in row are significantly different ($P < 0.05$, by one-way ANOVA and Tukey test). ∑SFA: sum of saturated fatty acid, ∑MUFA: sum of monounsaturated fatty acid, and ∑PUFA: sum of polyunsaturated fatty acids.

The same samples were collected from Marlborough Sounds in September 2011. Then, one part was subjected to freeze drier and another part was dried by oven in AUT laboratory. Table 12 showed the results of fatty acid profiles between freeze dried and

oven dried methods. Overall results demonstrated that there was no significant difference within these methods ($P < 0.05$). For instance, two methods produced similar amounts of SFA, MUFA and PUFA that were 6.59, 2.94 and 14.91 mg/g. It was interesting to notice that thermal treatments were not significant effect on fatty acids profiles.

2.14.2 **Comparison of fatty acids between blade, midrib and sporophyll**

Every part of *Undaria pinnatifida* can be consumed. The blade is usually produced as dried cut wakame, the midrib is consumed like a fresh vegetable and the dried cut sporophyll iss commercially known as Mekabu. Hence, it was interesting to investigate fatty acid profile of these parts of *Undaria*.

Table 12 : Fatty acid profile of different morphological *Undaria* (September samples)

Fatty acid	**Blade**	**Midrib**	**Sporophyll**
C10	0.12 ± 0.01^{a}	0.11 ± 0.01^{a}	0.12 ± 0.00^{a}
C12	0.32 ± 0.14^{a}	0.12 ± 0.02^{a}	0.12 ± 0.01^{a}
C14	0.99 ± 0.16^{ab}	0.55 ± 0.01^{b}	1.49 ± 0.18^{a}
C16	5.68 ± 1.34^{b}	2.82 ± 0.08^{b}	12.02 ± 1.47^{a}
C17	0.35 ± 0.10^{a}	0.11 ± 0.01^{ab}	0.09 ± 0.01^{b}
C18	0.43 ± 0.14^{b}	0.31 ± 0.04^{b}	1.35 ± 0.20^{a}
C20	0.24 ± 0.03^{a}	0.14 ± 0.01^{b}	0.22 ± 0.02^{a}
ΣSFA	**8.13 ± 1.88^{b}**	**4.17 ± 0.18^{c}**	**15.41 ± 1.89^{a}**
C14:1	0.24 ± 0.02^{a}	0.09 ± 0.01^{c}	0.16 ± 0.01^{b}
C16:1	0.46 ± 0.03^{a}	0.18 ± 0.02^{b}	0.18 ± 0.02^{b}
C17:1	0.23 ± 0.02^{a}	0.06 ± 0.00^{b}	0.09 ± 0.01^{b}
C18:1n-9c	2.85 ± 1.01^{b}	1.41 ± 0.03^{b}	7.49 ± 0.93^{a}
ΣMUFA	**3.79 ± 1.08^{b}**	**1.75 ± 0.06^{c}**	**7.92 ± 0.97^{a}**
C18:2n-6c	1.75 ± 0.03^{b}	1.08 ± 0.04^{c}	2.31 ± 0.09^{a}
C18:3n-6	0.35 ± 0.00^{b}	0.21 ± 0.01^{c}	0.50 ± 0.03^{a}
C18:3n-3	1.84 ± 0.55^{a}	0.66 ± 0.05^{b}	0.80 ± 0.04^{ab}
C18:4n-3	3.31 ± 1.03^{a}	1.15 ± 0.11^{b}	0.88 ± 0.09^{b}
C20:3n-6	0.20 ± 0.02^{a}	0.13 ± 0.01^{a}	0.21 ± 0.04^{a}
C20:4n-6	3.70 ± 0.02^{b}	2.56 ± 0.05^{c}	5.19 ± 0.33^{a}
C20:5n-3	2.16 ± 0.51^{a}	0.87 ± 0.03^{b}	1.86 ± 0.24^{ab}
ΣPUFA	**13.31 ± 2.15^{a}**	**6.65 ± 0.29^{c}**	**11.74 ± 0.87^{b}**
Total fatty acid	**25.22 ± 5.11^{b}**	**12.56 ± 0.54^{c}**	**35.07 ± 3.73^{a}**
ratio n-6/n-3	**0.82**	**1.49**	**2.32**

Results are mean ± standard error (mg/g dry weight, n=3). Different superscribed letters in row are significantly different using one-way ANOVA and Tukey test ($P < 0.05$).

∑SFA: sum of saturated fatty acid, ∑MUFA: sum of monounsaturated fatty acid, and ∑PUFA: sum of polyunsaturated fatty acids. FA: fatty acids. n-3: omega-3. n-6: omega-

The results in Table 13 showed that there was significant difference between SFA, MUFA and PUFA in the different morphologies ($P < 0.05$). Higher SFA and MUFA were found in sporophyll than in blade. For instance, total of SFA and MUFA were 15.41 and 7.92 mg/g *Undaria* in sporophyll respectively while they were found 8.13 and 3.79 mg/g respectively.

The main components of SFA in the sporophyll were C16 and C18 which consisted of 12.02 and 1.35 mg/g. C18:1n-9 was the most dominant MUFA in sporophyll and it contained 7.49 mg/g. However, blade of *Undaria* contained more PUFA than sporophyll. There was 13.31 mg/g in blade while 11.74 mg/g was found in sporophyll. The main PUFA in blade were C18:3n-3, C20:3n-6, C20:4n-6 and C20:5n-3 while their concentration was 1.84, 3.31, 0.20, 3.70 and 2.16 mg/g.

2.14.3 **Fatty acid profile of processed New Zealand *Undaria***

Most wakame is a commercially sold in the dried cut *Undaria*, which was subjected to thermal processing. Analysing processed *Undaria* would provide new idea of changes in fatty acid profile.

Table 14 shows that fatty acid profile of processed *Undaria* was similar as dried *Undaria*. However, salting and pressing the seaweed during processing resulted in a decrease of fatty acid concentration. Total fatty acid content in sporophyll was still higher than in the blade and midrib. They were 29.34, 17.07 and 8.58 mg/g of dry weight. To compare unprocessed *Undaria* (table 13) and processed algae (Table 14), total fatty acids in blade were decreased from 25.22 mg/g to 17.07 mg/g. This process caused lost about 32%. This loss was less excessive in sporophyll. Total fatty acids in the sporophyll were diminished by about 16%.

Table 13 : Comparison of FAME from New Zealand processed *Undaria pinnatifida*

Fatty acid	Blade	Midrib	Sporophyll
C10	0.42 ± 0.02^{a}	0.15 ± 0.13^{b}	0.45 ± 0.02^{a}
C12	0.28 ± 0.20^{a}	0.12 ± 0.06^{a}	0.11 ± 0.02^{a}
C14	0.56 ± 0.01^{b}	0.35 ± 0.01^{c}	1.41 ± 0.07^{a}
C16	3.11 ± 0.08^{b}	1.76 ± 0.04^{c}	9.84 ± 0.33^{a}
C17	0.19 ± 0.01^{a}	0.04 ± 0.01^{c}	0.11 ± 0.03^{b}

C18	0.33 ± 0.01[b]	0.20 ± 0.03[b]	1.09 ± 0.04[a]
C20	0.09 ± 0.00[a]	0.09 ± 0.01[a]	0.19 ± 0.07[a]
ΣSFA	**4.98 ± 0.32[b]**	**2.71 ± 0.028[c]**	**13.20 ±0.58[a]**
C14:1	0.17 ± 0.09[a]	0.05 ± 0.01[a]	0.06 ± 0.01[a]
C16:1	0.40 ± 0.03[a]	0.31± 0.23[a]	0.13 ± 0.01[b]
C17:1	0.35 ± 0.03[a]	0.31 ± 0.24[a]	0.08 ± 0.01[b]
C18:1n-9c	1.79 ± 0.06[b]	0.95± 0.06[c]	7.42 ± 0.37[a]
ΣMUFA	**2.71 ± 0.32[b]**	**1.62 ± 0.54[c]**	**7.69 ± 0.40[a]**
C18:2n-6c	1.07 ± 0.05[b]	0.77 ± 0.02[b]	2.42 ± 0.39[a]
C18:3n-6	0.24 ± 0.01[b]	0.13 ± 0.01[c]	0.44 ± 0.03[a]
C18:3n-3	1.21 ± 0.07[a]	0.32 ± 0.04[b]	0.41 ± 0.03[b]
C18:4n-3	2.74 ± 0.18[a]	0.63 ± 0.10[b]	0.52 ± 0.06[b]
C20:3n-6	0.12 ± 0.01[b]	0.12 ± 0.00[b]	0.24 ± 0.01[a]
C20:4n-6	2.33 ± 0.13[b]	1.81 ± 0.12[b]	3.15 ± 0.20[a]
C20:5n-3	1.67 ± 0.11[a]	0.47 ± 0.05[c]	1.27 ± 0.09[b]
ΣPUFA	**9.39 ± 0.57[a]**	**4.24 ± 0.35[c]**	**8.45 ± 0.80[b]**
Total fatty acids	**17.07 ± 0.57[b]**	**8.58 ± 0.98[c]**	**29.34 ± 0.79[a]**
Ratio n-6/n-3	**0.67**	**2**	**2.84**

Results are mean ± standard error (mg/g dry weight, n=3). Different superscript letters in row are significantly different using one-way ANOVA and Tukey test ($P < 0.05$). ∑SFA: sum of saturated fatty acid, ∑MUFA: sum of monounsaturated fatty acid, ∑PUFA: sum of polyunsaturated fatty acids. n-3: omega-3. n-6: omega-6

Moreover, the sporophyll contained more SFA and MUFA while the blade consisted of higher PUFA than sporophyll and midrib. Total SFA and MUFA in the sporophyll were 13.20 and 7.69 mg/g respectively while they were 4.98 and 2.98 mg/g in blade respectively. Additionally, C14, C16 and C18 were dominant for SFA in the sporophyll and C18:1n-9c was the highest amount for MUFA in the sporophyll. Furthermore, total PUFA contained in blade was 9.39 mg/g. The important PUFA in blade was C18:3n-3, C18:4n-3, C20:4n-6 and C20:5n-3 which were essential fatty acids in *Undaria pinnatifida*. Processed *Undaria* of our experiment was still balancing between omega-6 and omega-3. This was good indicator for health promotion.

2.14.4 Comparison FAMEs between New Zealand processed *Undaria* and commercial wakame

Wakame is a popular processed *Undaria* product in Japan, Korea and China. Most of raw material was harvested from *Undaria* farms. However, our New Zealand *Undaria* was collected from mussel farms. Thus, comparison of fatty acid profile between our processed *Undaria* and commercial wakame would indicate quality of New Zealand edible brown algae. In this study, two commercial Japanese wakame (J1 and J2) and

Korean wakame (K1 and K2) were utilised to compare with New Zealand *Undaria*. The results showed in Table 15.

Table 14 : Fatty acid profiles of NZ processed *Undaria* and commercial wakame

Fatty acid	J1	J2	K1	K2	New Zealand
C10	0.07 ± 0.01^{a}	0.07 ± 0.00^{a}	0.08 ± 0.01^{a}	0.06 ± 0.00^{a}	0.20 ± 0.01^{a}
C12	0.08 ± 0.00^{b}	0.06 ± 0.01^{b}	0.09 ± 0.00^{ab}	0.12 ± 0.01^{a}	0.06 ± 0.01^{b}
C14	0.56 ± 0.04^{c}	0.72 ± 0.07^{bc}	0.73 ± 0.03^{b}	0.98 ± 0.03^{a}	0.58 ± 0.08^{c}
C16	3.42 ± 0.20^{bc}	4.46 ± 0.71^{ab}	4.01 ± 0.40^{abc}	4.92 ± 0.40^{a}	3.05 ± 0.44^{c}
C17	0.42 ± 0.05^{b}	0.45 ± 0.01^{b}	0.48 ± 0.01^{b}	0.59 ± 0.01^{a}	0.21 ± 0.04^{c}
C18	0.15 ± 0.02^{a}	0.25 ± 0.08^{a}	0.25 ± 0.00^{a}	0.28 ± 0.00^{a}	0.26 ± 0.04^{a}
C20	0.07 ± 0.01^{b}	0.12 ± 0.03^{ab}	0.16 ± 0.04^{a}	0.15 ± 0.04^{a}	0.11 ± 0.02^{ab}
∑SFA	**4.77 ± 0.32^{c}**	**6.14 ± 0.91^{b}**	**5.81 ± 0.29^{b}**	**7.10 ± 0.49^{a}**	**4.47 ± 0.64^{c}**
C14:1	0.20 ± 0.01^{b}	0.31 ± 0.06^{ab}	0.24 ± 0.01^{b}	0.43 ± 0.04^{a}	0.18 ± 0.02^{b}
C16:1	0.34 ± 0.01^{c}	0.53 ± 0.09^{ab}	0.45 ± 0.00^{bc}	0.66 ± 0.07^{a}	0.45 ± 0.05^{bc}
C17:1	0.58 ± 0.05^{a}	0.62 ± 0.01^{a}	0.45 ± 0.03^{b}	0.61 ± 0.07^{a}	0.21 ± 0.00^{c}
C18:1n-9c	1.43 ± 0.05^{a}	1.94 ± 0.45^{a}	1.95 ± 0.33^{a}	2.02 ± 0.15^{a}	1.38 ± 0.04^{a}
∑MUFA	**$2.54 \pm 0.12b^{c}$**	**3.41 ± 0.61^{a}**	**3.09 ± 0.37^{b}**	**3.72 ± 0.34^{a}**	**2.22 ± 0.12^{c}**
C18:2n-6c	1.24 ± 0.05^{b}	1.81 ± 0.38^{ab}	1.74 ± 0.24^{ab}	2.55 ± 0.03^{a}	1.37 ± 0.19^{b}
C18:3n-6	0.17 ± 0.01^{c}	0.27 ± 0.05^{b}	0.34 ± 0.00^{b}	0.55 ± 0.01^{a}	0.29 ± 0.04^{b}
C18:3n-3	2.44 ± 0.05^{bc}	3.53 ± 0.79^{ab}	2.68 ± 0.54^{bc}	4.19 ± 0.28^{a}	1.55 ± 0.21^{c}
C18:4n-3	6.36 ± 0.10^{b}	7.16 ± 0.44^{b}	6.27 ± 0.18^{b}	9.36 ± 0.71^{a}	3.53 ± 0.47^{c}
C20:3n-6	0.14 ± 0.02^{c}	0.15 ± 0.01^{c}	0.23 ± 0.00^{a}	0.19 ± 0.05^{b}	0.13 ± 0.01^{c}
C20:4n-6	3.37 ± 0.05^{cd}	3.77 ± 0.22^{c}	4.47 ± 0.09^{b}	5.44 ± 0.40^{a}	2.83 ± 0.01^{d}
C20:5n-3	4.04 ± 0.15^{a}	4.19 ± 0.06^{a}	4.47 ± 0.12^{a}	5.34 ± 0.22^{a}	1.46 ± 0.04^{b}
∑PUFA	**17.76 ± 0.43^{c}**	**20.88 ± 1.95^{b}**	**20.20 ± 1.17^{bc}**	**27.63 ± 0.17^{a}**	**11.17 ± 1.06^{d}**
Σ fatty acids	**25.27 ± 0.86^{d}**	**30.43 ± 3.47^{b}**	**29.10 ± 1.18^{c}**	**38.45 ± 2.53^{a}**	**17.85 ± 1.82^{e}**
Ratio n-6/n-3	**0.38**	**0.40**	**0.51**	**0.46**	**0.71**

Results are mean ± standard error (mg/g dry weight, n=3). Different superscript letters in row are significantly different using one-way ANOVA and Tukey test ($P < 0.05$). ∑SFA: sum of saturated fatty acid, ∑MUFA: sum of monounsaturated fatty acid, and ∑PUFA: sum of polyunsaturated fatty acids. n-3: omega-3. n-6: omega-6

Overall results showed that there was significant difference of total fatty acid between products and NZ processed *Undaria* ($P < 0.05$). Total fatty acid was varied from 17.85 to 38.45 mg/g. The highest concentration of fatty acid was found in K2 (38.45 mg/g) while NZ processed *Undaria* contained low amount of fatty acid (17.87 mg/g). Even though, our processed wakame presented low amount of total fatty acid, its SFA, MUFA and some individual PUFA were similar as Japanese commercial wakame (J1). Concentration of total SFA in NZ Undaria and J1 were 4.47 and 4.77 mg/g. Moreover,

there was no significantly difference for C14, C16 and C18 between New Zealand *Undaria* and J1. In addition, amount of total MUFA presented in New Zealand processed *Undaria* and J1 was 2.22 and 2.54 mg/g.

It was interesting to notice that C18:2n-6, C18:3n-3, C20:3n-6 and C20:4n-6 presented similar amounts in New Zealand processed products and J1. They were 1.37, 1.55, 0.13 and 2.83 mg/g respectively. Finally, we assumed that low amount of fatty acids in New Zealand processed *Undaria* was due to small plants compared to commercial wakame from Japan and Korea. Commercial products would utilize mature plants when processing.

2.14.5 **Comparison of fatty acid profiles between sheltered and exposed sites**

In this study, the two different sites in the Marlborough Sounds compared were Port Underwood, an exposed site and Pelorus Sounds, a sheltered site. Two farms from each site were selected. Farms PE327 and 106 were from Port Underwood and Farms 122 and 233 were from the Pelorus Sounds. The fatty acid distributions are summarized in Table 16.

Table 15 : Comparison of fatty acid methyl esters from *Undaria pinnatifida* blade from obtained from sheltered and exposed sites

Locations	**Exposed sites**		**Sheltered sites**	
Fatty acid	**B327**	**B106**	**B233**	**B122**
C10	0.10 ± 0.01^{ab}	0.07 ± 0.01^{b}	0.27 ± 0.10^{a}	0.18 ± 0.05^{ab}
C12	0.04 ± 0.02^{a}	0.07 ± 0.01^{a}	0.08 ± 0.03^{a}	0.06 ± 0.01^{a}
C14	1.00 ± 0.17^{a}	0.95 ± 0.14^{a}	0.72 ± 0.07^{a}	0.76 ± 0.05^{a}
C16	6.15 ± 1.15^{a}	5.90 ± 1.06^{a}	4.32 ± 0.04^{b}	3.63 ± 0.22^{b}
C17	0.20 ± 0.06^{ab}	0.10 ± 0.01^{b}	0.30 ± 0.04^{a}	0.29 ± 0.01^{a}
C18	0.53 ± 0.09^{b}	0.81 ± 0.08^{a}	0.54 ± 0.02^{b}	0.47 ± 0.07^{b}
C20	0.25 ± 0.01^{a}	0.26 ± 0.02^{ab}	0.21 ± 0.00^{bc}	0.16 ± 0.02^{c}
∑SFA	$\mathbf{8.26 \pm 1.52^{a}}$	$\mathbf{8.17 \pm 1.32^{a}}$	$\mathbf{6.44 \pm 0.30^{b}}$	$\mathbf{5.56 \pm 0.44^{b}}$
C14:1	0.26 ± 0.03^{a}	0.15 ± 0.01^{b}	$0.18 \pm 0.02a^{b}$	0.19 ± 0.02^{ab}
C16:1	0.58 ± 0.09^{a}	0.34 ± 0.06^{b}	0.39 ± 0.05^{ab}	0.45 ± 0.06^{ab}
C17:1	0.19 ± 0.03^{a}	0.06 ± 0.02^{b}	0.07 ± 0.01^{b}	0.09 ± 0.01^{b}
C18:1n-9c	3.15 ± 0.91^{a}	3.35 ± 0.75^{a}	1.78 ± 0.05^{b}	1.56 ± 0.07^{b}
∑MUFA	$\mathbf{4.17 \pm 1.07^{a}}$	$\mathbf{3.89 \pm 0.83^{a}}$	$\mathbf{2.41 \pm 0.12^{b}}$	$\mathbf{2.29 \pm 0.16^{b}}$
C18:2n-6c	1.65 ± 0.10^{ab}	1.90 ± 0.22^{a}	1.37 ± 0.09^{b}	1.37 ± 0.10^{b}

C18:3n-6	0.31 ± 0.03^{a}	0.27 ± 0.05^{ab}	0.19 ± 0.01^{b}	0.22 ± 0.03^{ab}
C18:3n-3	1.22 ± 0.26^{a}	0.74 ± 0.06^{b}	0.97 ± 0.09^{b}	1.36 ± 0.14^{a}
C18:4n-3	2.11 ± 0.43^{a}	0.91 ± 0.12^{c}	1.69 ± 0.22^{b}	2.46 ± 0.49^{a}
C20:3n-6	0.18 ± 0.00^{a}	0.17 ± 0.02^{a}	0.15 ± 0.01^{a}	0.16 ± 0.02^{a}
C20:4n-6	3.49 ± 0.10^{a}	2.78 ± 0.29^{b}	2.85 ± 0.05^{b}	3.15 ± 0.33^{a}
C20:5n-3	1.47 ± 0.16^{ab}	0.83 ± 0.10^{b}	1.39 ± 0.25^{ab}	1.48 ± 0.25^{a}
∑PUFA	**10.44 ± 1.08^{a}**	**7.60 ± 0.86^{b}**	**8.62 ± 0.71^{b}**	**10.20 ± 1.35^{a}**
Total fatty acid	**22.88 ± 3.66^{a}**	**19.66 ± 3.01^{a}**	**17.46 ± 1.13^{b}**	**18.05 ± 1.95^{b}**
Ratio n-6/n-3	**1.17**	**2.06**	**1.13**	**0.93**

Results are expressed as mean ± standard error (mg/g dry weight, n=3). Different superscript letters within a row are significantly different ($P < 0.05$, by one-way ANOVA and Tukey post hoc testing). ∑SFA: sum of saturated fatty acid, ∑MUFA: sum of monounsaturated fatty acid, and ∑PUFA: sum of polyunsaturated fatty acids.

Blade showed significant differences in the total sum of MUFA, PUFA and SFAs contents between exposed sites and sheltered sites ($P < 0.05$). The major fatty acids for SFA, MUFA and PUFA were the C16, C18:1n-9c and. C20:4n-6 respectively. In this study, principal component analysis (PCA) was carried out on the individual fatty acids from *Undaria* blade obtained from the sheltered and exposed farms. The samples were collected from exposed and sheltered sites in August and September 2011. The September samples were B327 and B233, whereas B327 and B280 were samples obtained in August. The PCA biplot in Figure 16 represented a total of 67.79% variance between factor 1 (F1) and factor 2 (F2), where 39.82% and 27.96% explained the variances for F1 and F2 respectively. B327A samples were from exposed sites were separated from the sheltered samples along factor 1 with positive scores. This was correlated to MUFA and PUFA fatty acids that included C14:1, C17:1, C18:2n-6, C18:3n-6, C18:3n-3, C18:4n-3, C20:4n-6 and C20:5n-3 (depicted in as square points). All these fatty acids were significantly higher ($P < 0.05$) as shown in Table 16 and in *Undaria* from exposed farms. Samples from B280A, and B233S obtained from the sheltered farms on the other had high negative scores. This was correlated to the content of C10, C12, C18 and C16:1 fatty acids. However, concentration of these fatty acids from the sheltered farms was not significantly different from exposed farms.

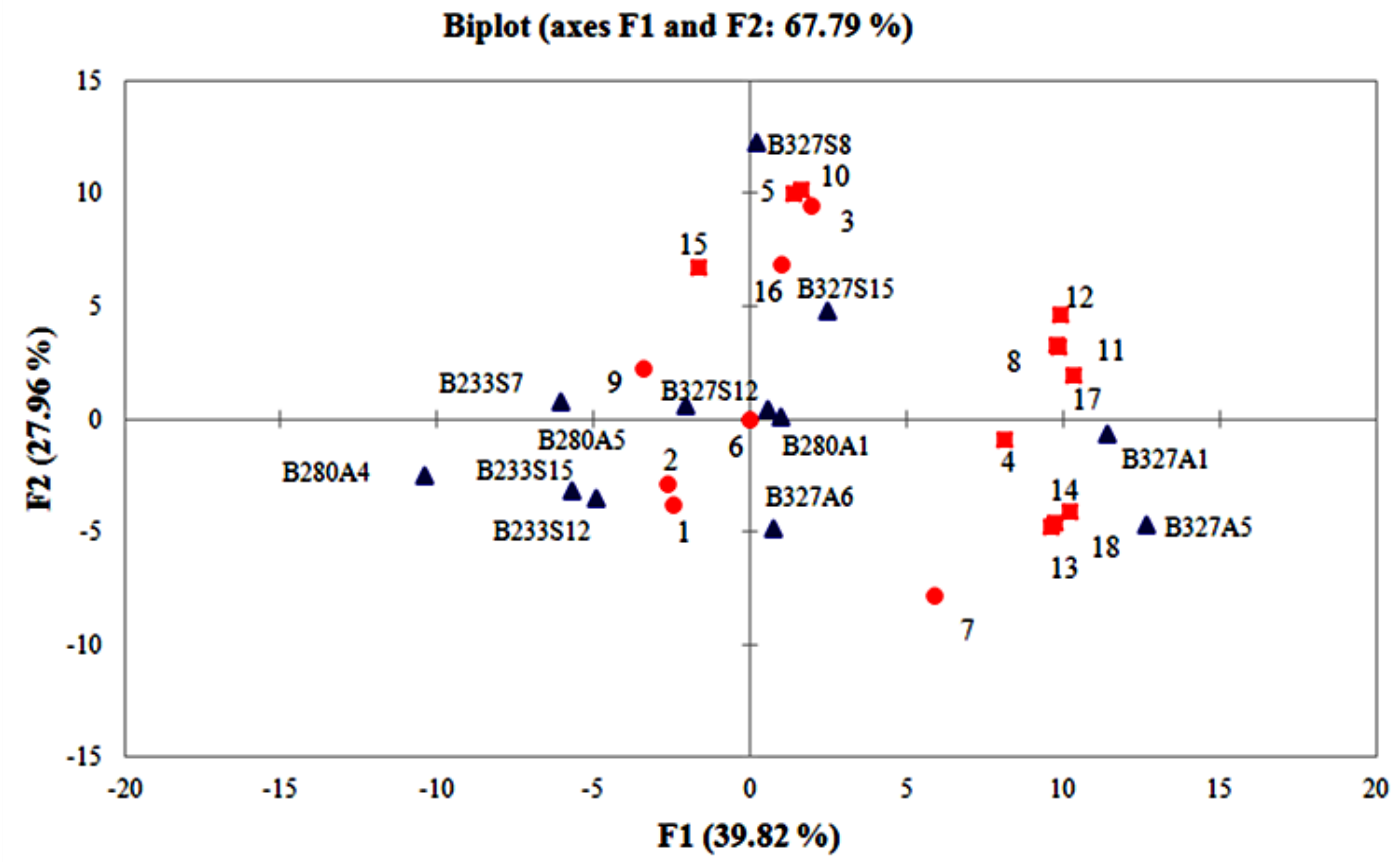

Figure 16: Bi-plots of F1 and F2 for fatty acids of *Undaria* blade between sheltered and exposed sites

B327 is blade samples from exposed farm, B233 and B280 are blade from sheltered farms. S: September and A: August.▲: different farms.●: no significant difference, ■: significant difference using Tukey post hoc test ($P < 0.05$) 1:C10, 2: C12, 3: C14, 4: C14:1, 5: C16, 6: C16:1, 7: C17, 8: C17:1, 9: C18, 10: C18:1n-9c, 11: C18:2n-6c, 12: C18:3n-6c, 13: C18:3n-3, 14: C18:4n-3, 15: C20, 16:C20:3n-6, 17: C20:4n-6 and 18: C20:5n-3.

Table 16 : Comparison of fatty acid methyl esters from *Undaria* sporophyll obtained from sheltered and exposed sites

Location	**Exposed sites**		**Sheltered sites**	
Fatty acid	**S327**	**S106**	**S233**	**S122**
C10	0.06 ± 0.01^{b}	0.05 ± 0.01^{b}	0.07 ± 0.01^{ab}	0.10 ± 0.003^{a}
C12	0.03 ± 0.002^{a}	0.04 ± 0.01^{a}	0.08 ± 0.02^{a}	0.08 ± 0.03^{a}
C14	$1.41 \pm 0.06b^{c}$	1.80 ± 0.07^{a}	1.29 ± 0.07^{c}	1.62 ± 0.07^{ab}
C16	9.27 ± 0.50^{c}	11.92 ± 0.63^{b}	7.17 ± 0.63^{d}	14.00 ± 0.54^{a}
C17	0.15 ± 0.02^{ab}	0.16 ± 0.01^{ab}	0.10 ± 0.02^{b}	0.19 ± 0.01^{a}
C18	1.28 ± 0.03^{a}	1.29 ± 0.11^{a}	0.58 ± 0.13^{b}	1.26 ± 0.04^{a}
C20	0.34 ± 0.06^{a}	0.31 ± 0.02^{a}	0.18 ± 0.09^{b}	0.34 ± 0.03^{a}
∑SFA	**12.54 ± 0.68^{c}**	**15.57 ± 0.86^{b}**	**9.47 ± 0.97^{d}**	**17.58 ± 0.72^{a}**
C14:1	0.12 ± 0.03^{b}	0.16 ± 0.01^{ab}	0.19 ± 0.02^{a}	0.17 ± 0.005^{ab}
C16:1	0.20 ± 0.02^{a}	0.22 ± 0.02^{a}	0.20 ± 0.02^{a}	0.22 ± 0.01^{a}
C17:1	0.08 ± 0.01^{a}	0.14 ± 0.08^{a}	0.10 ± 0.01^{a}	0.21 ± 0.10^{a}
C18:1n-9c	6.07 ± 0.23^{b}	8.19 ± 0.51^{a}	4.55 ± 0.54^{c}	9.18 ± 0.36^{a}
∑MUFA	**6.47 ± 0.29^{c}**	**8.70 ± 0.86^{b}**	**5.04 ± 0.59^{d}**	**9.78 ± 0.48^{a}**
C18:2n-6c	1.58 ± 0.03^{b}	1.95 ± 0.10^{a}	1.40 ± 0.05^{b}	1.87 ± 0.08^{a}
C18:3n-6	0.41 ± 0.04^{ab}	0.49 ± 0.03^{a}	0.22 ± 0.02^{c}	0.33 ± 0.03^{b}
C18:3n-3	0.36 ± 0.01^{a}	0.45 ± 0.07^{a}	0.34 ± 0.08^{a}	0.48 ± 0.05^{a}
C18:4n-3	0.50 ± 0.04^{a}	0.46 ± 0.07^{a}	0.34 ± 0.07^{a}	0.40 ± 0.02^{a}
C20:3n-6	0.28 ± 0.06^{a}	0.25 ± 0.01^{a}	0.19 ± 0.00^{a}	0.21 ± 0.01^{a}
C20:4n-6	3.84 ± 0.33^{a}	3.65 ± 0.29^{a}	2.50 ± 0.20^{b}	3.40 ± 0.19^{a}
C20:5n-3	0.96 ± 0.05^{a}	1.23 ± 0.14^{ab}	0.79 ± 0.08^{b}	1.30 ± 0.12^{a}
∑PUFA	**7.92 ± 0.55^{a}**	**8.48 ± 0.71^{a}**	**5.78 ± 0.51^{b}**	**7.98 ± 0.50^{a}**
Total FAs	**26.92 ± 1.52^{b}**	**32.75 ± 2.19^{a}**	**20.29 ± 2.07^{c}**	**35.34 ± 1.70^{a}**
Ratio n-6/n-3	**3.35**	**2.96**	**2.94**	**2.67**

Results are mean ± standard error (mg/g dry weight, n=3). Different superscript letters in row are significantly different ($P < 0.05$, by one-way ANOVA and Tukey test). ∑SFA: sum of saturated fatty acid, ∑MUFA: sum of monounsaturated fatty acid, and ∑PUFA: sum of polyunsaturated fatty acids.

The sporophyll also showed significant differences in the total sum of MUFA, PUFA and SFAs contents between exposed sites and sheltered sites ($P < 0.05$). The major fatty acids for SFA, MUFA and PUFA were the C16, C18:1n-9c and. C20:4n-6 like that found in blade.

There is significant difference in total fatty acid between farms in the sporophyll (Table 17, $P < 0.05$). Farm S327 and S106 have significantly higher amount of total lipid than S233 (sheltered farm). However, the total lipid contents of farm 122 is not significant different to S106. In contrast, the sporophyll concentrated more SFA and MUFA than

PUFA. In addition, sheltered farms contained significant higher amount of SFA and MUFA than exposed farms ($P < 0.05$).

The major SFA and MUFA in the sporophyll were similar as one of blade but these amounts were higher. For instance, C16 content of the sporophyll was at 14 mg/g in S122 (Table 17) while it was merely at 3.63 mg/g of dried weight in B122 (Table 16).

The sporophyll contained significantly higher amount of C18:1n-9 in the sporophyll than blade ($P < 0.05$). Its concentration in the sporophyll was at 9.18 mg/g of dried weight in the sporophyll (S122) but there was only 1.56 mg/g in blade (B122). It was interesting to notice that ratio between n-6 and n-3 was relatively higher in the sporophyll than in the blade. For example, this ratio was 1.17 in the blade from B327. But it was found 3.35 in the sporophyll from the same farm (Tables 16 and 17).

In this study, principal component analysis (PCA) was further carried out on the individual fatty acids from the *Undaria* sporophyll obtained from the sheltered and exposed farms. The September samples were S327S and S233S, whereas S327A and S233A were samples obtained in August. The PCA biplot in Figure 17 represented a total of 66.38% variance between factor 1 (F1) and factor 2 (F2), where 37.54% and 28.84% explained the variances for F1 and F2 respectively. S327A and S327S samples were from exposed sites were also separated from the sheltered samples along factor 1 with positive scores. This was correlated to SFA, MUFA and PUFA fatty acids that included C16, C17, C18, C20 , C18:1n-9, C18:3n-6, C20:4n-6 and C20:5n-3 (depicted in as square points). All these fatty acids were significantly higher ($P < 0.05$) as shown in Table 17 in *Undaria* from exposed farms. Samples from B233S obtained from the sheltered farms on the other had high negative scores. This was correlated to the content of C12, C14:1, C16:1 and C17:1 fatty acids. However, concentration of these fatty acids from the sheltered farms was not significantly different from exposed farms.

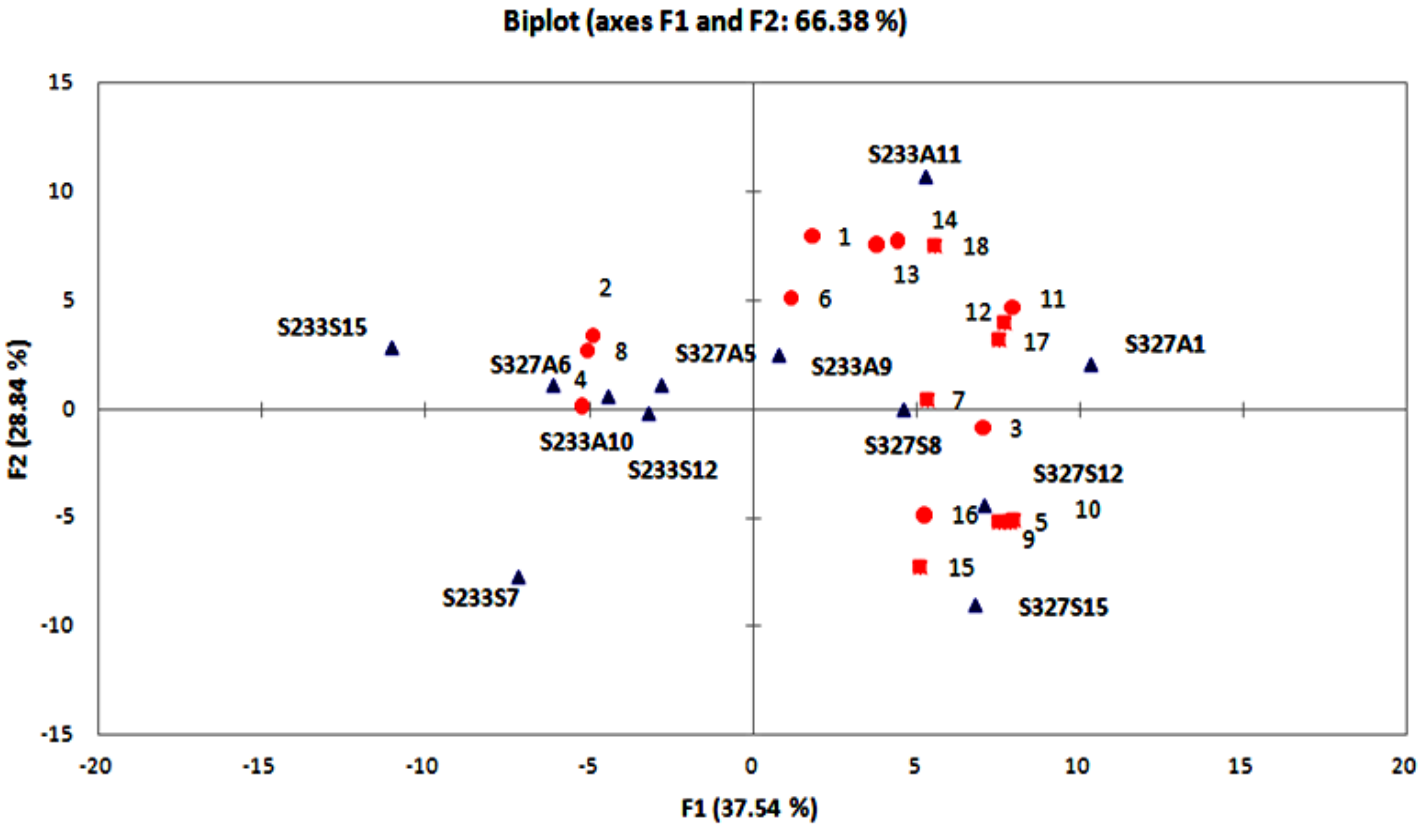

Figure 17: Bi-plots of F1 and F2 for fatty acids of *Undaria* sporophyll between sheltered and exposed sites

S327 is the sporophyll samples from exposed farm, S233 are the sporophyll from sheltered farms. S: September and A: August. ▲: different farms. ●: no significant difference, ■: significant difference using Tukey post hoc test ($P < 0.05$) 1:C10, 2: C12, 3: C14, 4: C14:1, 5: C16, 6: C16:1, 7: C17, 8: C17:1, 9: C18, 10: C18:1n-9c, 11: C18:2n-6c, 12: C18:3n-6c, 13: C18:3n-3, 14: C18:4n-3, 15: C20, 16:C20:3n-6, 17: C20:4n-6 and 18: C20:5n-3.

2.14.6 **Comparison of fatty acid distributions from different locations**

Locations from which *Undaria* was obtained showed significant differences in the total sum of SFA and MUFA between the exposed sites (Port Underwood and Worser Bay) and sheltered sites (Shelly Bay and Great Barrier Reef) ($P < 0.05$). The major fatty acids for SFA and MUFA were the C16, C18:1n-9c, C18:4n-3 and C20:4n-6 (Table 18).

Principal component analysis (PCA) was further carried out on the individual fatty acids from *Undaria* blade obtained from four different locations with sheltered and exposed farms. The samples were collected from the different locations were obtained in August and September 2011. The PCA biplot in Figure 18 represented a total of 61% variance between factor 1 (F1) and factor 2 (F2), where 39.82% and 27.96% explained the variances for F1 and F2 respectively. Farms from sheltered sites in Shelly Bay and the Great Barrier Island were separated from exposed farms in Worser Bay and Port Underwood along factor 1 with positive scores. Port Underwood and Worser Bay *Undaria* were correlated to MUFA and PUFA fatty acids that included C14:1, C16:1, C17:1, C18:2n-6c, C18:3n-3, C18:4n-3, C20:4n-6 and C20:5n-3 (depicted as square points). All these fatty acids were significantly higher ($P < 0.05$) as shown in Table 18 and in *Undaria* from exposed farms. Samples from Shelly Bay and the Great Barrier Reef, which were sheltered farms on the other hand, had negative scores. This was correlated to the content of C14, C16, C18 and C18:1n-9c fatty acids. However, only the concentration of the C18:1n-9c fatty acid from the exposed farms was significantly higher than the sheltered farms.

Table 17 : Comparison of fatty acid methyl ester from *Undaria* blade obtained from different locations

Fatty acid	Port Underwood	Shelly Bay	Worser Bay	Great Barrier Island
C10	0.13 ± 0.01^{a}	0.14 ± 0.05^{a}	0.19 ± 0.06^{a}	0.17 ± 0.02^{a}
C12	0.14 ± 0.07^{a}	0.08 ± 0.01^{a}	0.07 ± 0.01^{a}	0.08 ± 0.02^{a}
C14	0.80 ± 0.04^{ab}	0.56 ± 0.04^{b}	0.85 ± 0.13^{a}	0.74 ± 0.02^{ab}
C16	4.58 ± 0.02^{ab}	2.90 ± 0.25^{b}	5.65 ± 1.36^{a}	3.73 ± 0.11^{ab}
C17	0.47 ± 0.07^{a}	0.34 ± 0.04^{a}	0.30 ± 0.08^{a}	0.27 ± 0.05^{a}
C18	0.43 ± 0.14^{a}	0.25 ± 0.02^{b}	0.44 ± 0.14^{a}	0.52 ± 0.08^{a}
C20	0.16 ± 0.01^{a}	0.13 ± 0.02^{a}	0.12 ± 0.02^{a}	0.15 ± 0.01^{a}
∑SFA	**6.70 ± 0.36^{a}**	**4.40 ± 0.44^{c}**	**7.61 ± 1.80^{a}**	**5.67 ± 0.31^{b}**
C14:1	0.29 ± 0.02^{a}	0.19 ± 0.02^{b}	0.19 ± 0.01^{b}	0.18 ± 0.01^{b}
C16:1	0.57 ± 0.01^{a}	0.40 ± 0.05^{b}	0.40 ± 0.10^{b}	0.44 ± 0.02^{ab}
C17:1	0.27 ± 0.02^{b}	0.18 ± 0.03^{d}	0.36 ± 0.11^{a}	0.25 ± 0.08^{c}
C18:1n9c	2.04 ± 0.17^{b}	1.32 ± 0.13^{c}	2.74 ± 0.72^{a}	1.88 ± 0.22^{b}
∑MUFA	**3.17 ± 0.22^{a}**	**2.10 ± 0.24^{b}**	**3.69 ± 0.95^{a}**	**2.75 ± 0.34^{b}**
C18:2n6c	2.02 ± 0.03^{a}	1.14 ± 0.11^{c}	1.39 ± 0.06^{b}	1.41 ± 0.09^{b}
C18:3n6	0.33 ± 0.04^{ab}	0.23 ± 0.02^{b}	0.33 ± 0.02^{ab}	0.35 ± 0.03^{a}
C18:3n3	2.70 ± 0.07^{a}	1.73 ± 0.13^{b}	2.10 ± 0.53^{b}	1.27 ± 0.07^{c}
C18:4n3	4.73 ± 0.36^{a}	2.99 ± 0.20^{b}	4.14 ± 1.08^{a}	2.29 ± 0.17^{b}
C20:3n6	0.14 ± 0.02^{b}	0.12 ± 0.01^{b}	0.19 ± 0.02^{a}	0.16 ± 0.01^{ab}
C20:4n6	4.39 ± 0.16^{a}	2.57 ± 0.19^{b}	3.64 ± 0.28^{a}	2.97 ± 0.16^{b}
C20:5n3	2.93 ± 0.07^{a}	1.55 ± 0.11^{b}	2.34 ± 0.43^{a}	1.33 ± 0.11^{b}
∑PUFA	**17.24 ± 0.76^{a}**	**10.33 ± 0.77^{c}**	**14.14 ± 2.41^{b}**	**9.78 ± 0.64^{c}**
Total FA	**27.12 ± 1.34^{a}**	**16.82 ± 1.44^{c}**	**25.44 ± 5.16^{ab}**	**18.19 ± 1.29^{bc}**
n6/n3	**0.66**	**0.65**	**0.65**	**1.00**

Results are mean ± standard error (n = 5). Different superscript letters in row are significantly different ($P < 0.05$, by one-way anova and Tukey test). ∑SFA: sum of saturated fatty acid, ∑MUFA: sum of monounsaturated fatty acid, and ∑PUFA: sum of polyunsaturated fatty acids. Port Underwood and Worser Bay were located in exposed sites and its total fatty acid was higher than sheltered site (Shelly Bay) and Great Barrier Island (GBI).

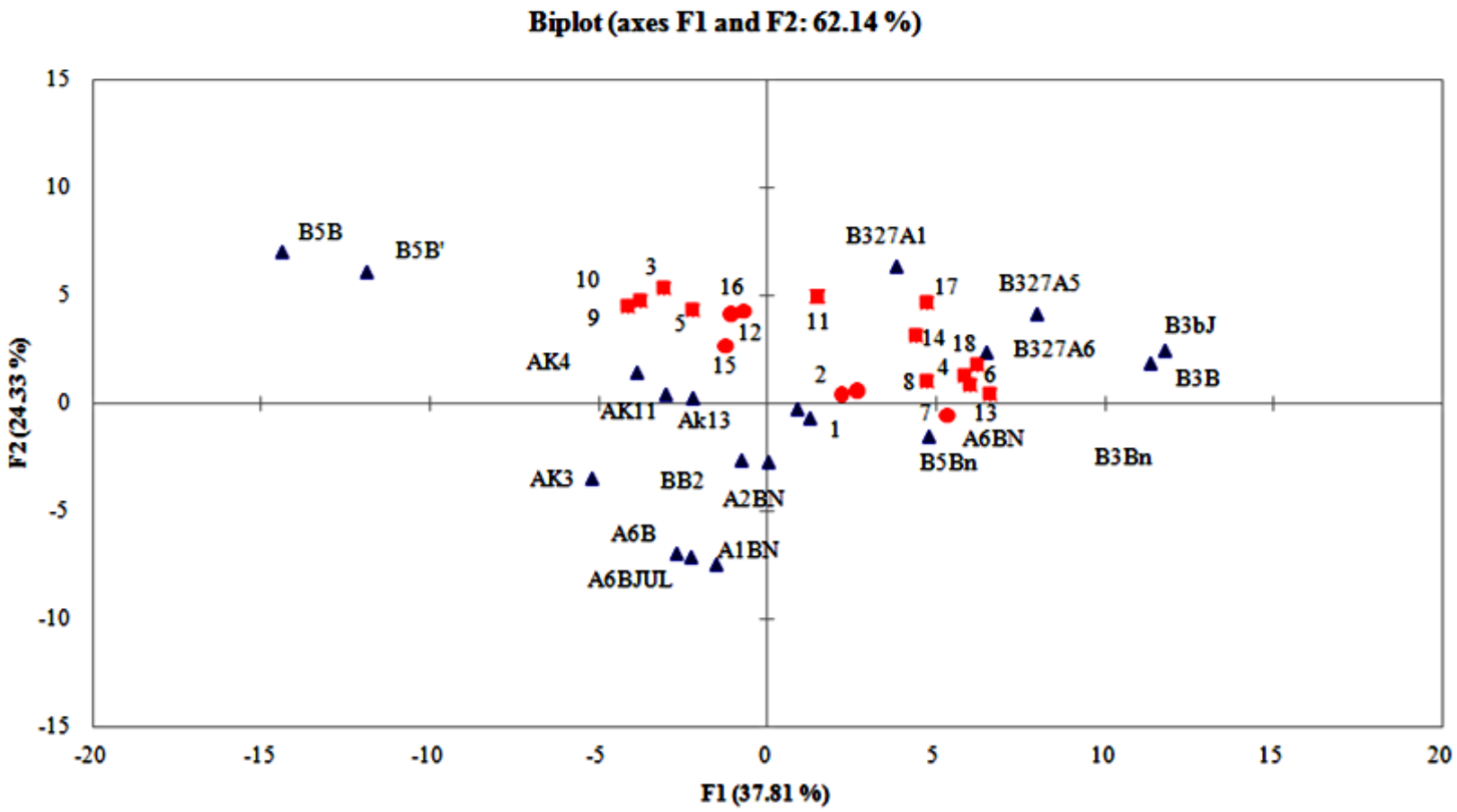

Figure 18: Bi-plots of F1 and F2 for FAMEs of *Undaria* from different locations

B327A is blade samples from 327 from harvested in August..●: no significant difference, ■: significant difference using Tukey post hoc test ($P < 0.05$) 1:C10, 2: C12, 3: C14, 4: C14:1, 5: C16, 6: C16:1, 7: C17, 8: C17:1, 9: C18, 10: C18:1n-9c, 11: C18:2n-6c, 12: C18:3n-6c, 13: C18:3n-3, 14: C18:4n-3, 15: C20, 16:C20:3n-6, 17: C20:4n-6 and 18: C20:5n-3. ▲: different locations, BB: Worser Bay, AB: Shelly Bay (Wellington) and AK: Great Barrier Island (Auckland)

Table 18 : Comparison of fatty acid methyl esters from *Undaria* sporophyll obtained from different locations (sporophyll)

Fatty acid	Port Underwood	Shelly Bay	Worser Bay	Great Barrier Island
C10	0.05 ± 0.01^{b}	0.15 ± 0.05^{a}	0.16 ± 0.06^{a}	0.09 ± 0.01^{a}
C12	0.29 ± 0.03^{a}	0.10 ± 0.04^{b}	0.10 ± 0.01^{b}	0.07 ± 0.02^{b}
C14	1.46 ± 0.22^{a}	1.36 ± 0.16^{ab}	0.93 ± 0.09^{b}	1.27 ± 0.11^{b}
C16	11.22 ± 0.24^{a}	9.66 ± 1.43^{ab}	4.78 ± 1.28^{b}	9.66 ± 0.76^{ab}
C17	0.13 ± 0.01^{a}	0.18 ± 0.05^{a}	0.12 ± 0.04^{a}	0.11 ± 0.01^{a}
C18	1.16 ± 0.08^{a}	1.76 ± 0.9^{a}	0.37 ± 0.11^{c}	0.95 ± 0.06^{b}
C20	0.25 ± 0.01^{a}	0.25 ± 0.05^{a}	0.13 ± 0.02^{b}	0.24 ± 0.02^{ab}
∑SFA	**14.56 ± 0.61^{a}**	**13.45 ± 2.75^{b}**	**6.58 ± 1.61^{d}**	**12.39 ± 0.99^{c}**
C14:1	0.17 ± 0.04^{a}	0.06 ± 0.01^{b}	0.11 ± 0.03^{ab}	0.11 ± 0.02^{ab}
C16:1	0.21 ± 0.01^{ab}	0.15 ± 0.02^{b}	0.38 ± 0.16^{a}	0.15 ± 0.02^{b}
C17:1	0.18 ± 0.05^{a}	0.17 ± 0.04^{a}	0.13 ± 0.05^{b}	0.17 ± 0.11^{a}
C18:1n9c	7.53 ± 0.18^{a}	4.95 ± 1.31^{c}	5.09 ± 0.92^{c}	6.22 ± 0.26^{b}
∑MUFA	**8.09 ± 0.27^{a}**	**5.33 ± 1.39^{c}**	**5.71 ± 1.16^{bc}**	**6.64 ± 0.40^{b}**
C18:2n6c	1.88 ± 0.03^{a}	1.77 ± 0.47^{a}	1.13 ± 0.15^{a}	1.65 ± 0.30^{a}
C18:3n6	0.48 ± 0.04^{b}	0.60 ± 0.08^{a}	0.26 ± 0.07^{c}	0.41 ± 0.03^{bc}
C18:3n3	0.49 ± 0.10^{b}	0.68 ± 0.13^{ab}	1.00 ± 0.37^{a}	0.48 ± 0.10^{b}
C18:4n3	0.47 ± 0.08^{b}	0.79 ± 0.25^{ab}	1.91 ± 0.81^{a}	0.40 ± 0.07^{b}
C20:3n6	0.30 ± 0.04^{a}	0.25 ± 0.02^{a}	0.13 ± 0.02^{b}	0.20 ± 0.01^{ab}
C20:4n6	3.91 ± 0.44^{ab}	4.37 ± 0.34^{a}	2.67 ± 0.35^{b}	3.25 ± 0.37^{c}
C20:5n3	1.12 ± 0.02^{b}	1.86 ± 0.38^{ab}	1.30 ± 0.17^{a}	1.09 ± 0.12^{b}
∑PUFA	**8.65 ± 0.74^{b}**	**10.32 ± 1.67^{a}**	**8.40 ± 1.94^{b}**	**7.48 ± 1.00^{c}**
Total FA	**31.30 ± 1.62^{a}**	**29.10 ± 5.81^{a}**	**20.70 ± 4.70^{c}**	**26.51 ± 2.40^{b}**
Ratio n-6/n-3	**3.15**	**2.09**	**0.99**	**2.78**

Results are mean ± standard error (n = 5). Different superscript letters in row are significantly different ($P < 0.05$, by one-way Anova and Tukey test). ∑SFA: sum of saturated fatty acid, ∑MUFA: sum of monounsaturated fatty acid, and ∑PUFA: sum of polyunsaturated fatty acids. FA: fatty acids

Sporophyll also showed significant differences in the total fatty acid, SFA and MUFAs contents between exposed Port Underwood and others sites (Worser Bay and Great Barrier Island) ($P < 0.05$). The major fatty acids for SFA, MUFA and PUFA were the C16, C18:1n-9c and. C20:4n-6 like that found in blade (Table 19).

However, the total lipid contents of farm Port Underwood is not significant different to Shelly Bay. The sporophyll contained significantly higher amount of C18:1n-9 in the sporophyll than blade ($P < 0.05$). In this study, principal component analysis (PCA) was further carried out on the individual fatty acids from *Undaria* sporophyll obtained from different locations. The samples were collected from exposed and sheltered sites in

August and September 2011. The PCA biplot in Figure 19 represented a total of 64.46 variance between factor 1 (F1) and factor 2 (F2), where 38.46% and 26% explained the variances for F1 and F2 respectively. Port Underwood (S327A and S106) and Shelly Bay sporophyll samples were from samples Worser Bay and Great Barrier Island along factor 1 with positive scores. Port Underwood and Selly Bay Undaria correlated to SFA, MUFA and PUFA fatty acids included C12, C14, C16, C18, C18:1n-9,C20:3n-6 and C20:4n-6 (depicted as square points). All these fatty acids were significantly higher ($P < 0.05$) as shown in Table 19.

Samples from Worser Bay and Great Barrier Reef on the other had high negative scores. This was correlated to the content of C10, C16:1 and C17:1, C18:3n-3 and C18:4n-3 fatty acids. However, concentration of these fatty acids was not significantly different from other locations.

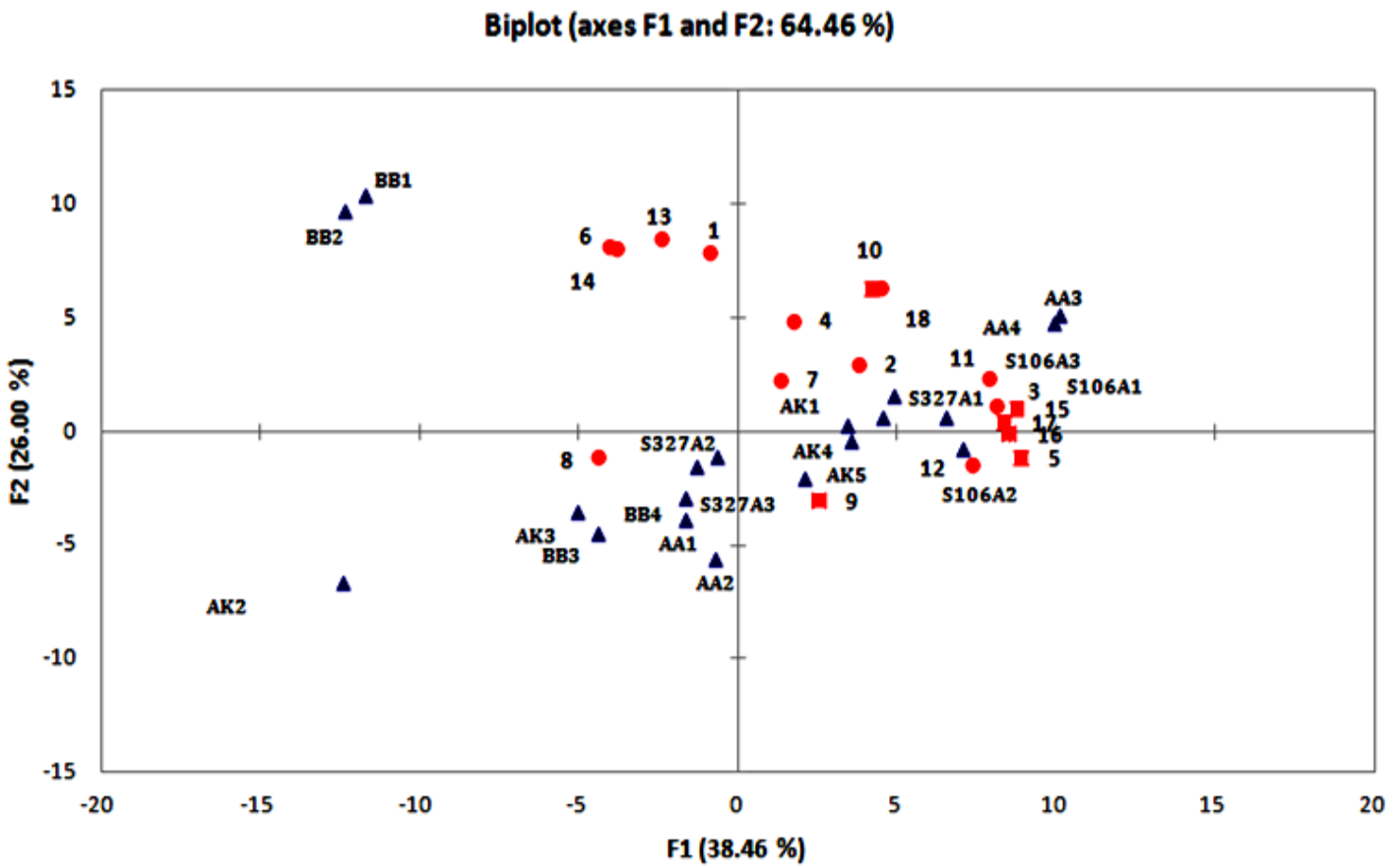

Figure 19: Bi-plots of F1 and F2 for FAMEs of *Undaria* from different locations

S327 and S106 are the sporophyll samples from farm 327 and 106 from harvested in August. ▲: different locations.●: no significant difference, ■: significant difference using Tukey post hoc test ($P < 0.05$) 1:C10, 2: C12, 3: C14, 4: C14:1, 5: C16, 6: C16:1, 7: C17, 8: C17:1, 9: C18, 10: C18:1n-9c, 11: C18:2n-6c, 12: C18:3n-6c, 13: C18:3n-3, 14: C18:4n-3, 15: C20, 16:C20:3n-6, 17: C20:4n-6 and 18: C20:5n-3. BB: Worser Bay, AB: Shelly Bay (Wellington) and AK: Great Barrier Island (Auckland)

2.14.7 **Comparison of fatty acid compound by seasons**

Biochemical formation of marine and terrestrial plants has seasonal effects (Nelson et al., 2002). In this section, the variation of fatty acid distribution by seasons was investigated. Since the underwater environment is not greatly affected by weather above the surface, only temperature was utilised as the climatic factor. Three seasons were taken into consideration in this study such as winter (June, July and August), spring (September) and summer (December). Samples were harvested monthly from the mussel lines on farm PE327. Results were showed in the Figure 20.

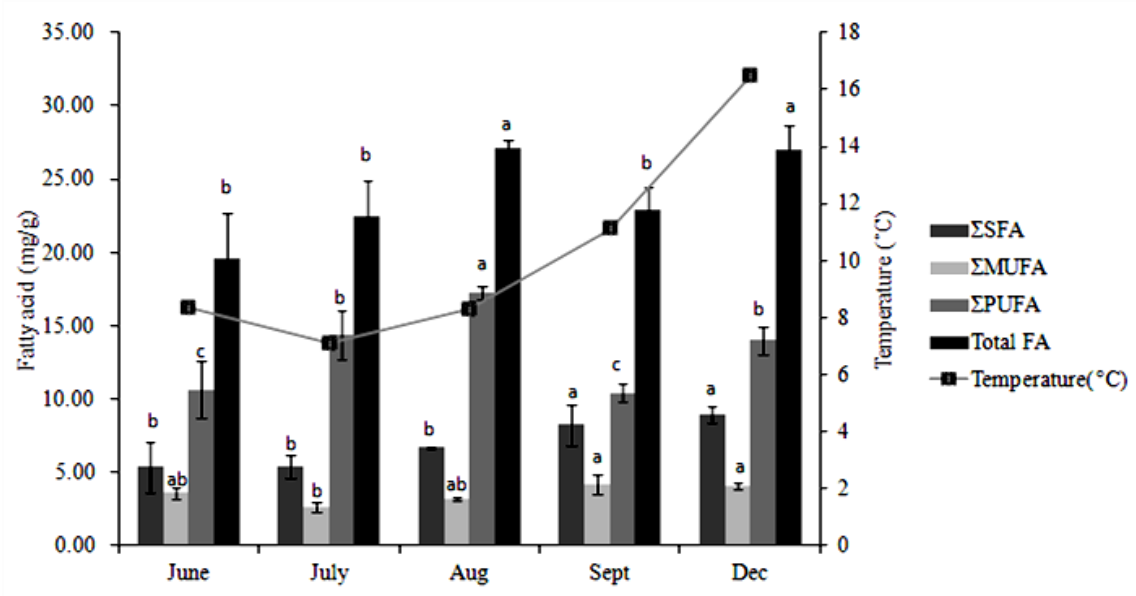

Figure 20: Monthly variation of fatty acids from farm PE327 of blade

Values are mean with standard error bar (n=3). Different superscript letters are significantly different ($P < 0.05$, by one-way ANOVA and Tukey test). ∑SFA: sum of saturated fatty acid, ∑MUFA: sum of monounsaturated fatty acid, and ∑PUFA: sum of polyunsaturated fatty acids

Results show that total fatty acids were significantly high during the winter with low temperatures and decreased slowly in spring with slow increase of temperature ($P < 0.05$). However, it was increased once again in summer. Saturated fatty acids were affected by the season (Guil-Guerrero, 2007; Renaud & Luong-Van, 2006). The highest concentration of SFA was reached in summer at 16ºC water temperature. In contrast, the lowest concentration was found in beginning of winter in June and July where the temperature was low (7-8ºC on average). In additional, New Zealand *Undaria*

accumulate the highest amount of C16 in both the blade and the sporophyll in summer (Appendix V, Figure V.4 and 5).

There was a significant increase of MUFA from winter to spring ($P < 0.05$). These compounds were reached a plateau on spring and summer. The highest concentration of MUFA was found in September and December with 4.17 mg/g. C18:1n-9 which is characteristic of *Undaria* MUFAs was found in higher concentration in the sporophyll in summer. For example, C18:1n-9 content is 9 mg/g in December (Appendix V, Figure V. 5).

There was significant difference in seasonal PUFA between winter, spring and summer ($P < 0.05$). The trend of PUFA was augmented during the winter then was dropped in spring. This concentration was increased again in summer. The highest amount of PUFA found 7.24 mg/g in August. Only SFA was increased with high temperature while PUFA was decreased with increase of temperature (Guil-Guerrero, 2007). *Undaria* produces more n-3 PUFAs in winter while n-6 PUFAs are found in higher concentrations in summer. The major n-3 PUFAs are C18:3n-3, C18:4n-3 and C20:5n-3. In contrast, the abundant n-6 PUFAs are C18:2n-6, C18:3n-6, C20:3n-6 and C20:4n-6 in December (Appendix V and Figure V.4).

In this study, PCA was also carried out to evaluate the seasonal variation of fatty acids. The PCA Bi-plots shown in Figure 21 explained 63.23% variance between factor 1 and factor 3 where 33.01% and 30.22% explained the variance for F1 and F2 respectively. *Undaria* samples harvested in winter from exposed farms (B106J, B327J, B327JL and B327A) have positive score along factor 1. These *Undaria* samples were correlated to MUFA and PUFA fatty acids included C14:1, C16:1, C17:1, C18:3n-6c, C18:3n-3, C18:4n-3, C20:4n-6 and C20:5n-3 (depicted as square points). These fatty acids are significantly different in winter ($P < 0.05$). Additionally, New Zealand *Undaria* accumulated more n-3 PUFAs in winter such as C18:3n-6c, C18:3n-3, C18:4n-3 and C20:5n-3. Others reported that MUFA and LC-PUFAs were accumulated in alga during cooler months especially in winter (Gerasimenko, Busarova, & Moiseenko, 2010; Honya et al., 1994). Kim et al. (1996) also reported that unsaturated fatty acids were higher in winter due to cold seasons.

B106S, B327S and B233O have high negative score and represented spring samples. In spring, *Undaria* contained significant amounts of C16, C18:1n-9c, C18:2n-6 and

C20:3n-6c ($P < 0.05$). In addition, these samples constituted n-6 and n-9 fatty acids like C18:1n-9c, C18:2n-6 and C20:3n-6c in spring. Honya et al. (1994) reported that brown alga produced maximal amount of n-6 PUFA during warm months and n-3 PUFA in cold months. However, there was interaction between winter and spring namely farm B122S, B233S, B106A and B280A. Furthermore, C16 was the most abundant SFA and was found on both sheltered and exposed sites in September and October. Additionally, C18:1n-9 was the abundant MUFA and was also found more in spring.

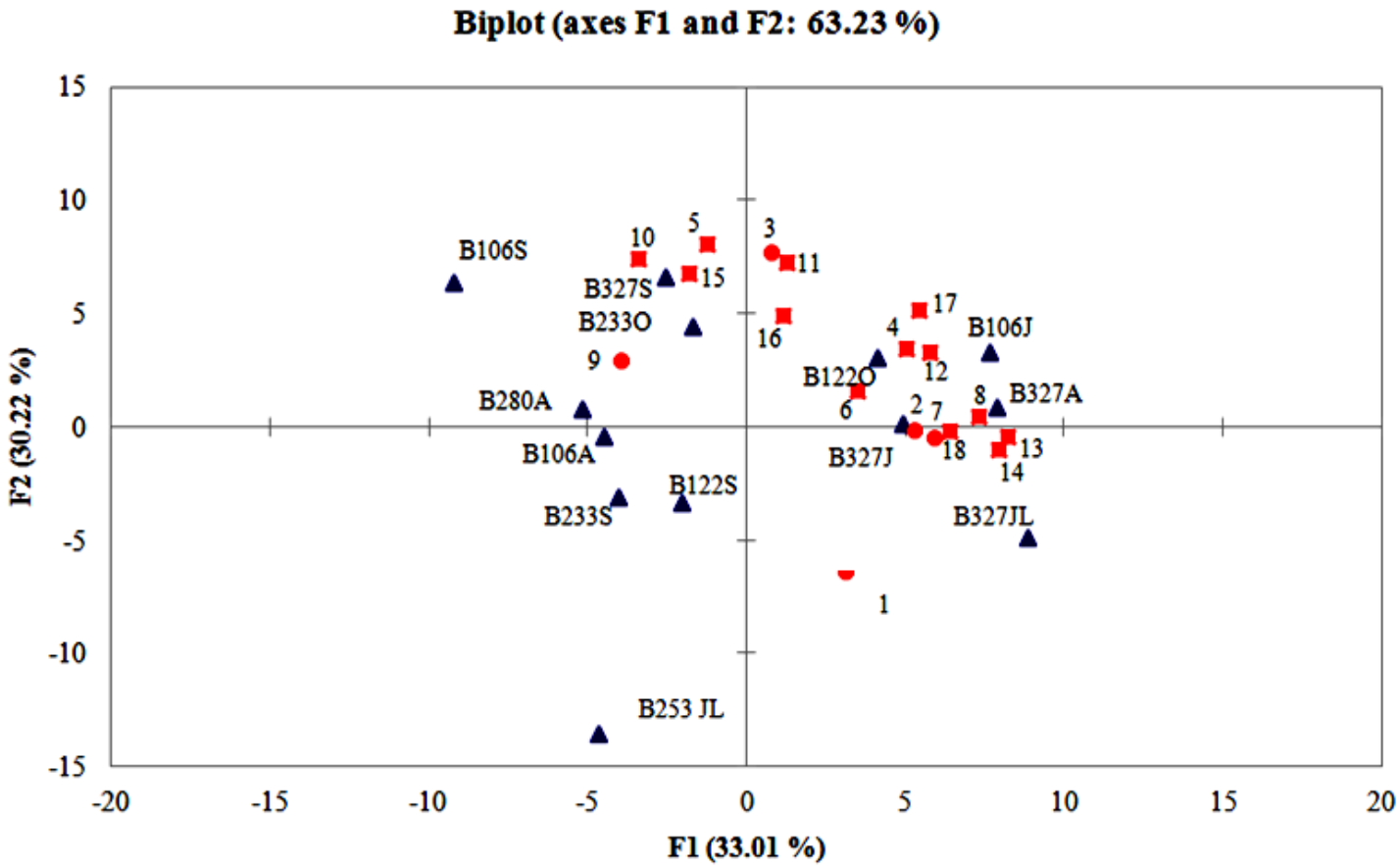

Figure 21 : Bi-plots of F1 and F2 for FAMEs of *Undaria* by months

B327 and B106 are blade samples from exposed farm, B233, B280 and B122 are blade from sheltered farms, S: September and A: August. ▲: locations. ●: no significant difference, ■: significant difference using Tukey post hoc test ($P < 0.05$) 1:C10, 2: C12, 3: C14, 4: C14:1, 5: C16, 6: C16:1, 7: C17, 8: C17:1, 9: C18, 10: C18:1n-9c, 11: C18:2n-6c, 12: C18:3n-6c, 13: C18:3n-3, 14: C18:4n-3, 15: C20, 16:C20:3n-6, 17: C20:4n-6 and 18: C20:5n-3. J: June, JL : July, A: August, S: September and O: October

2.14.8 **Comparison of fatty acid compositions between big and small *Undaria***

This section is an extended study that used December 2011 samples because *Undaria* sample form Port Underwood were bigger size. Big plants were randomly collected from different farms in Port Underwood such as PE327, 106 and 253 while the *Undaria* sample considered small were from mussel lines in farm 327. This differentiation would show that the size of *Undaria* has an effect on the fatty acid composition. The length and weight were measured as well as the total lipid and FAMEs. The results are shown in Table 20.

Table 19 : Comparison the FAMEs of New Zealand big and small plants (blade)

Fatty acid	**Big**	**%**	**Small**	**%**
C10	0.05 ± 0.02^{b}	0.13	0.12 ± 0.02^{a}	0.46
C12	0.04 ± 0.02^{b}	0.10	0.06 ± 0.00^{a}	0.24
C14	1.97 ± 0.24^{a}	5.29	1.21 ± 008^{b}	4.50
C16	13.91 ± 2.98^{a}	37.38	6.49 ± 0.36^{b}	24.05
C17	0.37 ± 0.16^{a}	1.01	0.22 ± 0.02^{b}	0.80
C18	1.48 ± 0.36^{a}	3.99	0.53 ± 0.04^{b}	1.95
C20	0.35 ± 0.14^{a}	0.94	0.28 ± 0.02^{b}	1.05
∑SFA	**18.17 ± 3.93^{a}**	**48.84**	**8.92 ± 0.54^{b}**	**33.05**
C14:1	0.18 ± 0.08^{a}	0.48	0.13 ± 0.01^{b}	0.48
C16:1	0.57 ± 0.26^{a}	1.52	0.37 ± 0.03^{b}	1.35
C17:1	0.08 ± 0.02^{b}	0.22	0.19 ± 0.06^{a}	0.70
C18:1n9c	8.76 ± 2.49^{a}	23.55	3.40 ± 0.13^{b}	12.59
∑MUFA	**9.59 ± 2.85^{a}**	**25.77**	**4.08 ± 0.23^{b}**	**15.12**
C18:2n6	2.12 ± 0.12^{a}	5.70	2.26 ± 0.16^{a}	8.38
C18:3n6	0.52 ± 0.06^{a}	1.39	0.43 ± 0.02^{a}	1.58
C18:3n3	0.91 ± 0.31^{b}	2.44	1.53 ± 0.08^{a}	5.67
C18:4n3	0.84 ± 0.52^{b}	2.26	2.85 ± 0.01^{a}	10.57
C20:3n6	0.22 ± 0.02^{b}	0.60	0.47 ± 0.18^{a}	1.73
C20:4n6	3.50 ± 0.32^{b}	9.41	4.60 ± 0.40^{a}	17.04
C20:5n3	1.33 ± 0.12^{b}	3.59	1.86 ± 0.07^{a}	6.88
∑PUFA (mg/g)	**9.44 ± 0.47^{b}**	**25.39**	**13.99 ± 0.92^{a}**	**51.84**
Total FAs (mg/g)	**37.20 ± 8.26^{a}**	**100**	**26.99 ± 1.70^{b}**	**100**
Total lipid (mg/g)	**45.0 ± 5.20^{a}**		**32.0 ± 7.50^{b}**	
Ratio n6/n3	**2.07**		**1.24**	
Plant length (Cm)	**96.25**		**51**	
Weight dry weight (g)	**122.34**		**43.56**	

Results are mean ± standard error (n = 3). Different superscript letters in are significantly different ($P < 0.05$, by one-way ANOVA and Tukey test). ∑SFA: sum of saturated fatty acid, ∑MUFA: sum of monounsaturated fatty acid, and ∑PUFA: sum of polyunsaturated fatty acids. %: percentage of total fatty acids

Overall, concentrations of total lipids, total fatty acids, SFA and MUFA were significantly different in big *Undaria* ($P < 0.05$). However, level of PUFA was significantly higher in small *Undaria.* For instance, sum of PUFA was 13.99 mg/g small ones but it was merely 9.44 mg/g.

The major SFAs was found to be significant different in C14, C16 and C18 between big and small *Undaria* ($P < 0.05$). Big *Undaria* contained double amount of C16 compared to small plants. It represented similar pattern for MUFA. The abundant MUFA was C18:1n-9 which counted 8.76 mg/g of dried weight in big plants and 3.40 mg/g of dried weight in small plants.

However, other individual long chain polyunsaturated fatty acids (LC-PUFAs) were found higher in small plants than in big plants. The significant difference of LC-PUFAs was C18:3n-3, C18:4n-3, C20:3n-6, C20:4n-6 and C20:5n-3 with concentration of 1.53, 2.85, 0.47, 4.60 and 1.89 mg/g respectively. The abundant PUFA was C20:4n-6 with 4.60 mg/g.

Table 21 shows that there was no significant difference of total lipid and fatty acid in the sporophyll between big and small *Undaria*. The amounts of total lipid and fatty acid were 63.25 and 40.96 mg/g of in big plants. However, only difference was sum of SFA which was 20.93 mg/g in big *Undaria* and 19.29 mg/g in small *Undaria*. Finally, the abundant FAs in the sporophyll were C16, C18:1n-9 and C20:4n-6 with amount of 16.03, 11.35 and 3.52 mg/g in big plant sporophyll.

Table 20 : Comparison of sporophyll FAMEs between big and small plants

fatty acid	Big	%	Small	%
C10	0.05 ± 0.01^{b}	0.13	0.08 ± 0.03^{a}	0.21
C12	0.02 ± 0.01^{a}	0.06	0.03 ± 0.00^{a}	0.08
C14	2.24 ± 0.16^{a}	5.46	2.12 ± 0.16^{a}	5.53
C16	16.03 ± 2.01^{a}	39.14	14.75 ± 1.71^{b}	38.53
C17	0.21 ± 0.06^{a}	0.50	0.12 ± 0.02^{b}	0.31
C18	1.86 ± 0.20^{a}	4.55	1.80 ± 0.11^{a}	4.69
C20	0.51 ± 0.01^{a}	1.25	0.39 ± 0.03^{a}	1.03
∑SFA	**20.93 ± 2.46^{a}**	**51.09**	**19.29 ± 2.06^{a}**	**50.38**
C14:1	0.10 ± 0.01^{a}	0.23	0.07 ± 0.00^{a}	0.19
C16:1	0.26 ± 0.04^{a}	0.63	0.20 ± 0.01^{a}	0.53
C17:1	0.06 ± 0.01^{b}	0.16	0.12 ± 0.06^{a}	0.30
C18:1n9c	11.35 ± 1.34^{a}	27.70	9.56 ± 1.03^{b}	24.98
∑MUFA	**11.76 ± 1.38^{a}**	**28.72**	**9.96 ± 1.10^{b}**	**26.00**
C18:2n6	2.03 ± 0.03^{a}	4.95	2.16 ± 0.12^{a}	5.65
C18:3n6	0.63 ± 0.05^{a}	1.54	0.65 ± 0.02^{a}	1.70
C18:3n3	0.37 ± 0.04^{a}	0.91	0.39 ± 0.05^{a}	1.01
C18:4n3	0.33 ± 0.02^{b}	0.81	0.44 ± 0.05^{a}	1.14
C20:3n6	0.32 ± 0.06^{a}	0.79	0.35 ± 0.04^{a}	0.90
C20:4n6	3.52 ± 0.38^{a}	8.58	4.06 ± 0.11^{a}	10.61
C20:5n3	1.07 ± 0.04^{a}	2.61	1.00 ± 0.08^{a}	2.60
∑PUFA	**8.27 ± 0.61^{a}**	**20.18**	**9.04 ± 0.46^{a}**	**23.62**
∑fatty acid	**40.96 ± 4.45^{a}**	**100**	**38.29 ± 3.61^{a}**	**100**
Total lipid	**63.25 ± 8.96^{a}**		**57.93 ± 9.96^{b}**	

Results are mean ± standard error (n = 3). Different superscript letters in are significantly different ($P < 0.05$, by one-way ANOVA and Tukey test). ∑SFA: sum of saturated fatty acid, ∑MUFA: sum of monounsaturated fatty acid, and ∑PUFA: sum of polyunsaturated fatty acids. %: percentage of total fatty acids

2.15 Discussion

2.15.1 **Mussel farms and *Undaria pinnatifida***

Undaria pinnatifida is actually considered as pest for the mussel farms in New Zealand. It hinders mussel harvesting. The quantity of this seaweed was approximately 4,000 tonnes and they grown on mussel lines around New Zealand (Aquaculture, 2011).

Figure 22 : *Undaria pinnatifida* on mussel lines from farm 327 in Port Underwood (July 2011).

According to our field observation, temperature and fresh water not only had an effect on mussels but on *Undaria's* growth. Even though, the temperature in Nelson-Marlborough region was suitable for *Undaria*, more fresh water from rainfall could hinder the growth of *Undaria*. Mussels and seaweeds in Pelorus Sounds grow slowly compared to the counterpart from Port Underwood farms (PUW) (Figures 22 and 23). Additionally, Pelorus Sounds is located near forestry sites. Rainfall not only brings the more fresh water into the sea but also sediment. These are the main reason why seaweeds in Pelorus Sounds were smaller.

Figure 23 : Mussel lines from farm 233 in July 2011 (Pelorus Sounds)

The saponifiable lipids of *Undaria pinnatifida* were classified into total lipids and fatty acid methyl esters. Both total lipids and FAMEs had seasonal and geographical variations.

2.15.2 **Total lipid contents**

Lipid contents in *Undaria* not only depended on biological difference but also environmental effects. In this study, analysing total lipid of blade, midrib and sporophyll demonstrated that the sporophyll contained higher concentration of total lipid than blade and midrib. The average of total lipid in the sporophyll is about 55 mg/g DW while ones of blade and midrib were 25 and 17 respectively. Previous studies have showed that total lipids in *U. pinnatifida* is ranged between 10.5 (Sánchez-Machado et al., 2004a) and 45 mg/g dry weight of blade (Dawczynski et al., 2007). Thus, NZ *Undaria* contained similar amount of total as counterparts.

In comparison between NZ processed *Undaria* and wakames from Japan and Korea, NZ *Undaria* consisted lower amount of total lipid than Japanese and Korean wakames. The total lipid of NZ *Undaria* was 21 mg/g DW while those from Japan and Korea were 43.6 and 3.82 mg/g DW respectively. The NZ *Undaria* samples were not mature yet and processing step resulted in decreasing total lipid contents.

Lipid formation has been directly affected by seasonal changes and increased in winter (Nelson et al., 2002; Renaud & Luong-Van, 2006). Generally, algae accumulate lipids during the cooler months (Gerasimenko et al., 2010). In this study, the highest lipid contents were found in winter (August). The concentration of lipid demonstrated 34 and 53 mg/g DW in blade and the sporophyll respectively. However, there was no significant difference of total lipid between winter and summer because water temperature in Port Underwood and Pelorus Sounds is always cold in summer (average 15-17ºC) (Hay & Villoula, 1993). The total lipids represented 51 mg/g DW in December. The sporophyte of *Undaria* is always present in mussel farms due low water temperature in the Marlborough Sounds. Additionally, we can observe that *Undaria* samples from Pelorus Sounds are always smaller than Port Underwood samples. The mussel farms in this area are located near timber exploitation so rainfall is brought fresh water and mud to mussel lines which are hindered *Undaria* and mussels in grown.

Furthermore, geographical conditions have also influenced on total lipid contents. Both blade and the sporophyll of *Undaria* from PUW had higher concentration of lipid than Wellington and Great Barrier Island samples. The average of lipid in blade was 35 mg/g DW from PUW. In contrast, it was found 23 and 20 mg/g DW from Shelly bay and Great Barrier Island respectively. This result showed that exposed site accumulated more lipid than sheltered site. In summary, *Undaria pinnatifida* consisted of low lipid

contents and was not a conventional source of energy (Sánchez-Machado et al., 2004a; Wong & Cheung, 2000). Moreover, Herbrteau et al. (1997) reported that low total lipid contents are normally found in all plants which adapted to a salty environment. In Asia, seaweeds including *Undaria* species have been degenerated in hot summer (Marinho-Soriano et al., 2006; Parsons, 1994). This study showed that cooler NZ summer is suitable to *Undaria* grown in the New Zealand and total lipid contents is not significant difference between winter and summer.

2.15.3 Fatty acid profiles

Fatty acid profiles have been a subject of interest for chemical analysis because FAs are an indicator of nutritional and functional properties in animal and human diets (Nisizawa, 1987). Even though, macroalgae contained low amount of lipid, brown algae are generally rich in C18 and C20 LC-PUFAs with isomer of n-3 and n-6 which are essential fatty acids for human diets (Watkins & Li, 2008). These following sections will focus on different aspects of fatty acid profiles.

2.15.3.1 Saturated fatty acids

In all the seaweeds, the most abundant saturated fatty acid is C16:0 (palmitic acid) (Terasaki et al., 2009). In *Undaria pinnatifida*, the C16:0 content was reported between 13.5 and 49.6 % of total fatty acids (see Appendix IV, Table IV.2) (Kim, Dubacq, Thomas, & Giraud, 1996). In this study, the palmitic acid was accounted for 4.58 mg/g DW (16.85 % of total fatty acids) in August samples. This amount was similar as Sánchez-Machado et al. report (2004).

Additionally, morphological parts of *Undaria* accumulated differently C16:0. The concentration of C16:0 was twice as high in the sporophyll compared to the blade. The sporophyll was 12.02 mg/g DW of C16:0 (34.3% of total fatty acids) while there was only 5.68 mg/g DW in blade (22.5% of total fatty acids) (see Section 2.15.2). Compared to the report of Nisizawa et al. (1987), it is lower than their result which was 64% 0f total lipid in the sporophyll. However, Ortega-Calvo et al. (1993) showed similar amount of C16 in *Undaria* blade as our NZ samples.

Furthermore, the concentration of C16 also varied by locations and seasons. This concentration is found higher in exposed sites where the size of plants was bigger than one in sheltered sites. The average amount of C16 was 5.11 mg/g DW in exposed sites while it was 3.31 in sheltered sites (see Section 2.15.6). Surprisingly, the C16:0 content

was increased from winter to summer. For example, this increase was from 4.58 mg/g DW (16.85 % of total of FAMEs) in the winter (August) to 6.49 mg/g DW (23.93% of total FAMEs) in summer (December). Additionally, Kim et al. (1996) reported that synthesis of triacylglycerols is still activated during the summer in brow alga in France. Moreover, the concentration of C16 in NZ summer was similar in Russian *Undaria* (26.8% of total FAMEs) (Khotimchenko, 1998). This information showed that short chain fatty acid, C16:0 was formed in warmer month in New Zealand summer.

2.15.3.2 Monounsaturated fatty acids (MUFA)

The amount of MUFA is the lowest compared to sum of SFA and PUFA. The distributions of MUFA are C14:1, C16:1, C17:1 and C18:1n-9c. The oleic acid (C18:1n-9c) is predominant MUFA in NZ *Undaria* and its content is 2.85 mg/g DW (7.52% of total fatty acids) in August samples. Sanchez-Machado et al. (2004a) reported this concentration was found in dried *Undaria* (6.79 %). NZ *Undaria* contained more oleic acid than previous report. Moreover, C18:1 n-9 is found high in vegetable oils which represented 38.4 % and 64.3% of total fatty acids in canola and rice bran oils respectively (Cheruvanky & Thummala, 1991; Moser, 2008). This fatty acid is characteristic to vegetable oils.

The concentration of C18:1n-9 is found higher in the sporophyll than in blade. The sporophyll contained about 7.49 mg/g DW (31.32% of total fatty acids) while it was 2.85 mg/g DW (11.30 % of total fatty acids). Nisizawa et al. (1987) reported that C18:1n-9 content was 25.2% of total fatty acid in *Undaria* sporophyll. Moreover, oleic acid concentration is also changed by location and seasons. This concentration was found higher in exposed sites than one in sheltered sites. In blade, oleic acid content of exposed *Undaria* is accounted for 3.25 mg/g DW (14.2% of total FAMEs) but one of sheltered *Undaria* is 1.17 mg/g DW (6.7% of total FAMEs) (see section 2.15.5). Dawczynsky et al. (2007) reported that the concentration of oleic acid in *Undaria pinnatifida* from Japan is accounted for 2.6% of total FAMEs which is comparable with one of NZ *Undaria*.

2.15.3.3 Polyunsaturated fatty acids (PUFA)

Brown algae contain long chain polyunsaturated fatty acids especially C18 and C20 PUFAs (Fleurence et al., 1994). Ortiz et al. (2006) demonstrated that blade of algae contained more MUFAs and PUFAs than the midrib. These authors agreed that brown

algae showed a greater content of C18 PUFAs than C20 PUFAs (Kumari et al., 2010; Ortiz et al., 2006). In this study, PUFAs are showed greater concentration than SFAs and MUFAs with isomer n-6 and n-3. The isomer n-6 PUFAs found in NZ *U. pinnatifida* are C18:2n-6, C18:3n-6, C20:3n-6 and C20:4n-6. Then its omega-3 PUFAs present C18:3n-3, C18:4n-3 and C20:5n-3. In comparison between blade, midrib and the sporophyll, major PUFAs of blade and midrib are C18:4n-3, C20:4n-6 and C20:5n-3 whereas ones of the sporophyll are C18:2n-6 and C20:4n-6. In blade, these concentrations are accounted for 1.84, 3.31, 3.70 and 2.16 mg/g DW. The PUFAs concentrations of NZ *Undaria* agreed with those of other authors (Fleurence et al., 1994; Guil-Guerrero, 2007). However, C20:5n-3 content in NZ *Undaria* is higher than one of French *Undaria* (0.53 mg/g DW) (Herbreteau et al., 1997). On the other hand, the C18:2n-6 and C20:4n-6 are characteristic of PUFAs in the sporophyll and are higher than ones in blade and midrib. These are accounted for 2.31 and 5.19 mg/g DW. Surprisingly, Nisizawa et al. (1987) reported that concentration of C18:2 n-6 and C20:4n-6 were found lower in Japanese wakame sporophylls..

Season and location have direct effects on biochemical formation in macroalgae (Hay & Villoula, 1993; Renaud & Luong-Van, 2006). The macroalgae collected in winter have higher energy value than one of summer (Renaud & Luong-Van, 2006). Temperature has influenced on fatty acid composition of cell membranes in algae. Low temperature affected on increased level of unsaturated fatty acid in polar lipid. This augmentation of unsaturation results in lower melting points and maintain lipid in liquid stat for normal protoplasmic viscosity (Nelson et al., 2002). Moreover, a red alga, *Palmaria palmata* present the highest amount of EPA at 11ºC and AA at 15ºC (Mishra, Temelli, Ooraikul, Shacklock, & Craigie, 1993). Honya et al. (1994) reported that n-6 PUFA concentrations of brown algae were maximal during the warm months whereas their n-3 PUFAs contents reached a maximum during cold months. In this study, PUFA contents are found the high level in winter. However, these concentrations are not significantly different in summer because New Zealand summer is cold with average 11ºC in December. For example, the total of PUFAs consisted of 63.5% of total fatty acids in winter and 51.8% in summer. This study showed similar results as other researches that LC-PUFAs are increased in cold month especially n-3 PUFAs namely C18:3n-3, C18:4 and C20:5n-3 found the great amount in August. For instance, these are 9.9, 17.4 and 10.8% of total FAs respectively. However, n-6 PUFAs are augmented in warmer summer. C18:2n-6, C18:3n-6, C20:3n-6 and C20:4n-6 are present higher level in

December with concentration of 8.4, 1.6, 1.7 and 17.0% of total FAs respectively (see Appendix V and Figure 4). Moreover, PCA analysis is also confirmed that LC-PUFAs are produced by New Zealand *Undaria* in cold month.

In addition, New Zealand *U. pinnatifida* has different LC-PUFA between locations. This study showed that MUFAs and PUFAs are abundantly found in Undaria from exposed sites whereas SFAs and other MUFAs are presented more in samples from sheltered sites. For example, samples from PE327 and Worser Bay were found more C14:1, C17:1, C18:2n-6, C18:3n-6, C18:3n-3, C18:4n-3, C20:4n-6 and C20:5n-3 (Figure 25). However, sheltered farms namely 233, 280 and Shelly Bay have more C10, C12, C18 and C16:1. We assumed that the exposed farms are faced to sea currant and temperature is lower that sheltered sites. Thus, PUFAs are formed better in these conditions. In summary, New Zealand *Undaria* consists of great amount of n-6 and n-3 PUFAs which will have good potential for perceived health benefits against diseases.

2.15.3.4 The omega-6/omega-3 ratio

Macroalgae represent an important source of n-6 an n-3 PUFAs that they contain in their cell membranes (Dawczynski et al., 2007). These PUFAs are precursors of eicosanoids which play an important role in influencing inflammatory processes and immune reactions (Calder, 2001). These classes of PUFAs have physiological functions and considered as essential FAs (Holdt & Kraan, 2011; Meschino, 2007) and their balance has an major influence on normal growth and development (Dawczynski et al., 2007). For these reasons, rich diet of n-6 and n-3 PUFAs are nutritional importance (Trautwein, 2001).

Many researchers reported that Western diets contain more n-6 PUFAs than n-3 PUFAs. Their ratio n-6 and n-3 are between 15:1 and 17:1 (Simopoulos, 2002). However, WHO recommended that this ratio has not assess 10 which will potentially provide health benefits (Sánchez-Machado et al., 2004a). In this paper, both blade and the sporophyll of New Zealand *Undaria* are considered as balance between n-6 and n-3. The range of this ratio in blade is between 0.93 and 2.5. However, it is higher in the sporophyll which ranges from 0.99 and 3.35. Even though, the ratio n-6/n-3 is slightly high in the sporophyll, it is considered as balance and is lower than WHO recommendation of diet in n-6 and n-3.

Chapter 3 Non-saponifiable fractions of lipid in *Undaria pinnatifida*

Undaria pinnatifida is not only rich in polyunsaturated fatty acids but also contains vitamins and phytosterols. These non-saponifiable lipids have nutritional and nutraceutical functions (Y. S. Lee, Shin, Kim, & Lee, 2004). In this section, the non-saponifiable lipid fractions of processed New Zealand *Undaria* are examined. Because geographical and environmental conditions have an important effect on chemical formation of alga (Popov, Marekov, Konaklieva, Panayotova, & Dimitrova-Konaklieva, 1987; Stuart et al., 1999), the results are compared to the size, season and locations

3.1 Phytosterol of macroalgae

3.1.1 **Definition of sterol (phytosterol) and derivative sterols**

Sterols are as hydroxylated steroid alcohols with a hydroxyl typically in the 3 position on the A ring. (Parish, Li, & Bell, 2008). Sterols are found in animals and plants, a well known animal example sterol is cholesterol (Figure 24). Sterols found in plants are usually called phytosterols (Piironen, Toivo, Puupponen-Pimia, & Lampi, 2003). Phytosterols are present in small amounts, and two common examples are stigmasterol and sitosterol (Abidi, 2001). The phytosterols are characterised by an additional alkyl group at C-24 on a cholesterol nucleus. Phytosterols with methylene or ethylidene substitutes such as 24-methylenecholesterol and fucosterol (Figures 25 and 26) are commonly found in plants (Parish et al., 2008)

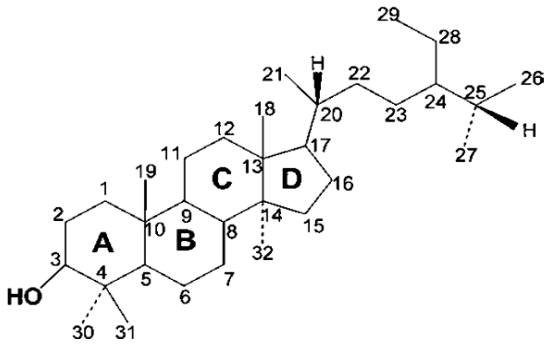

Figure 24: The structure of cholesterol.

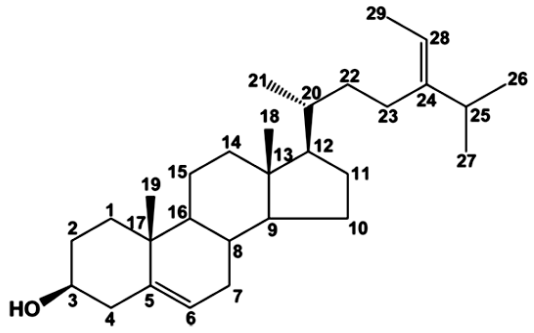

Figure 25 : The structure of fucosterol (found in *Undaria Pinnatifida*) (Bang et al., 2011)

Because fully systematic names are ring and complex, range of semi-systematic names are commonly used which can be confusing. Tables 22 and 23 illustrate possible synonyms and isomers of fucosterol and 24-methylene-cholesterol (Newburger, Uebel, Ikawa, Andersen, & Gagosian, 1979).

Table 21 : Synonyms and isomers of fucosterol:

Compounds	**Synonyms**
Fucosterol	Stigmasta-5,24(28)-dien-3-ol, (3β,24z)-
	Stigmasta-5,24(28)-dien-3-ol, (3β,24E)-;
	trans-24-Ethylidenecholesterol
	Fucosterin; 28-Isofucosterol 24(E)-Ethylidenecholest-5-en-3β-ol
	(24Z)-Stigmasta-5,24(28)-dien-3-ol

Table 22 : Synonyms and isomers of 24-methylenecholesterol

Compounds	**Synonyms**
24-methylenecholesterol	Chalinasterol(6CI)
	Ergosta-5,24(28)-dien-3b-ol (7CI,8CI)
	24-Methylcholesta-5,24(28)-dien-3b-ol
	24-Methylenecholest-5-en-3b-ol
	Cholesterol, 24-methylene-
	NSC 232664
	Ostreasterol

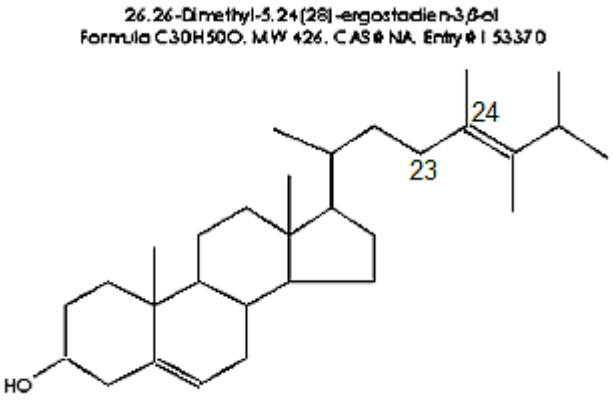

Figure 26: The structure of 24-methylenecholesterol

3.1.2 **Phytosterols in seaweed**

Phytosterols are bioactive compounds in all terrestrial and marine plants and algae. More than 200 types of phytosterols have been found (Lagarda, Garcia-Llatas, & Farre, 2006). Newburger et al. (1979) reported that seven phytosterol compounds had been identified in brown alga (*Agarum cribosum*). Fucosterol and 24-methylenecholesterol accounted for 88.7 and 10.8 % of the total unsaponifiable lipid fractions respectively. The main phytosterols in brown seaweeds are fucosterol and fucosterol derivatives (Sánchez-Machado, López-Cervantes, López-Hernández, & Paseiro-Losada, 2004b). Similarly, other authors reported that the amount of fucosterol varied between 83–97% of the total phytosterol content (662–2,320 μg/g dry weight) in *Undaria pinnatifida* and *Laminaria japonica* (Holdt & Kraan, 2011). Sánchez-Machado et al. (2004) further reported that 24-methylenecholesterol was found in *Undaria pinnatifida*. This phytosterol makes up 16.8 % of the total sterol content.

3.1.3 **Role of phytosterol in plants**

Sterols not only have a structure function in the membrane but also a metabolic role. In the membrane, their functions include regulation of the fluid and permeability that directly affect diffusion and active transport across membranes (Lagarda et al., 2006). Yankah (2006) agreed that the role of phytosterols was to stabilise and rigidify the plant membranes based on existing sterol/phospholipid ratio. Moreover, sterols associated with protein could further play important role as enzymes, receptors and signal transduction components (Piironen et al., 2003). Additionally, sterols are also precursors of other bioactive steroids such as biogenic precursors. These precursors

(brassinosteroids) are specially classified as growth substances and substrates for secondary plant metabolites (Piironen et al., 2003).

3.1.4 Biological properties of phytosterols and vitamin E for human health

Recently, there have been increasing interests in the study of functional and nutraceutical properties of phytosterol in human health (Yankah, 2006). Clinical research illustrated that consumption of plant sterol may help decrease blood cholesterol level (Lagarda et al., 2006). Other important properties of phytosterol include antifungal, antibacterial, anti-inflammatory, anti-tumour (Lichtenstein, 2000), anti-oxidant and anti-ulcerative properties (Sánchez-Machado et al., 2004b).

Brown seaweeds are rich in bioactive compounds and can play an important role in biological processes such as antioxidants, polysaccharides with antiviral action and n-3 fatty acid (Plaza et al., 2008). Compounds that are responsible for the antioxidant activities in brown macroalgae include carotenoids, vitamin E, chlorophyll (Rodriguez-Bernaldo de Quiros, Frecha-Ferreiro, Vidal-Perez, & Lopez-Hernandez, 2010) and fucosterol (Lee, Lee, Jung, Kang, & Shin, 2003).

3.1.4.1 Biological properties of fucosterol

The abundant phytosterol in brown algae is fucosterol, which has been shown to be active as an anti-diabetic, anti osteoporotic and antioxidant (Table 24). Moreover, Yankah (2006) reported that phytosterols can influence low density lipoprotein (LDP) cholesterol reduction in human. Antioxidant and inhibition of cholesterol absorption have been reported (Table 24).

Table 23 : Bioactive compounds and biological properties of brown algae

Brown seaweed	Bioactive compounds	Specific compounds	Possible health effect	References
Pelvetia siliquosa	*Phlorotannin*	*fucosterol*	*Anti-diabetic*	*Lee et al., 2004*
Undaria pinnatifida		fucosterol	Anti-osteoporotic	Bang et al. (2011)
Pelvetia siliquosa		fucosterol	Anti oxidant	Lee et al. (2003)

The antioxidant activities of fucosterol extracted from brown seaweed have been investigated by Lee et al. (2003). This study showed that fucosterol not only has antioxidant properties but also hepatoprotective activities. Lee et al. (2003) explained that fucosterol inhibited free radicals, which contribute to a large number of diseases

due to defect of immune system. Fucosterol increased antioxidative enzymes, including superoxide dismutase; catalase and glutathione peroxidise (protective enzymes). These are involved in oxygen detoxification and can reduce the risk of chronic diseases caused by free radicals (Lee et al., 2003).

Inhibition of cholesterol absorption is another characteristic of phytosterols, including fucosterol (Yankah, 2006). It was demonstrated that cholesterol with ethyl or methyl groups was hindered in intestinal absorption of phytosterols in humans and increased hydrophobicity. Increased hydrophobicity will create the mix between phytosterol and micelles which will block cholesterol absorption (Lagarda et al., 2006). For example, sitosterol and fucosterol displaced cholesterol from micelles solution and inhibited its absorption. Only 5% of phytosterols can be absorbed by the human intestine, while dietary cholesterol was absorbed between 30 and 60% by the intestine (Yankah, 2006). Daily intake of 2–3 g of phytosterols has been reported to reduce LDL-cholesterol levels by 9% to 20%, with considerable individual variability (Lichtenstein, 2000).

3.2 Vitamin E or α-tocopherol

Vitamins are organic compounds, which contribute to essential micronutrients. Vitamins are involved in many biological activities as coenzymes or precursors e.g. vitamins B6, B12 and folate, and the antioxidative defence system (ascorbic acid, carotenoid and vitamin E) (Gregory III, 1996).

Macroalgae have been reported to be rich in vitamins, and contain both water and fat-soluble vitamins (MacArtain, Gill, Brooks, Campbell, & Rowland, 2007). The common vitamins in algae are vitamins A, B, C and E (Lordan, Paul Ross, & Stanton, 2011). Vitamin E is the most abundant fat-soluble vitamin of non-saponifiable lipids in many algae. There are 4 different types of vitamin E including α-tocopherol (5,7,8-trimethyltocol), β-tocopherol (5,8-dimethyltocol), γ-tocopherol (7,8 dimethyltocol), and δ-tocopherol (8-methyltocol) (De Leenheer, De Bevere, Cruyl, & Claeys, 1978). Their different molecular structures were showed is summarized in Table 25 and shown in Figure 27.

Table 24 : Structures of Vitamin E in *Undaria pinnatifida*

(Miyashita & Takagi, 1987)

compounds	**R1**	**R2**	**R3**	**%**
Tocol	*H*	*H*	*H*	
α-tocopherol	CH3	CH3	CH3	99.4
β-tocopherol	Ch3	H	CH3	0.5
γ-tocopherol	H	CH3	CH3	0.1
δ-tocopherol	H	H	CH3	0

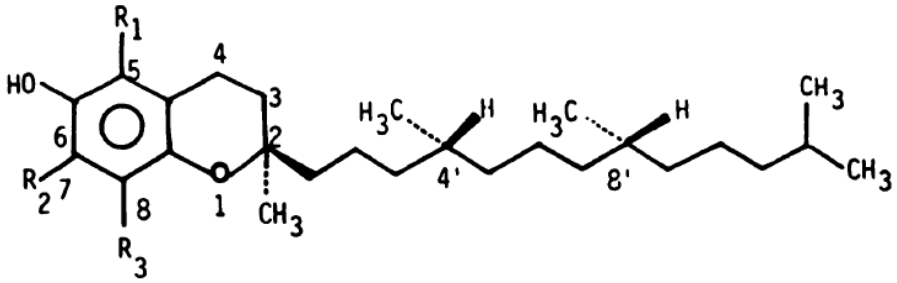

Figure 27: The basic structure of tocopherol

(De Leenheer et al., 1978)

The concentration of vitamins in algae depends on its exposure to sunlight (MacArtain et al., 2007). Lordan et al. (2011) reported that algae contained important sources of dietary antioxidants. The major antioxidant compounds in *Undaria* are vitamin C, E and polyphenols which constitute 1847, 145 and 6600 µg/g the dry weight respectively (Lordan et al., 2011). Additionally, Miyashita and Takagi (1987) reported that the vitamin E or α-tocopherol was the main fat-soluble vitamin in *Undaria,* and made up of more than 99% of the total vitamins (Table 25).

3.3 Extraction and analysis of non-saponifiable fractions in *Undaria*

3.3.1 **Analytical methods for non-saponifiable lipids**

The determination of phytosterols requires good methods for extraction, separation, purification, detection and quantification (Wrolstad et al., 2005). Generally, phytosterols have to be isolated from non-saponifiable fractions of lipids by solvent extraction. Various chromatographic clean-up procedures are used to purify

phytosterols, which are then subjected to chromatographic methods for identification (Abidi, 2001).

3.3.1.1 Isolation and extraction

Phytosterols in algal tissues are initially isolated by solvent extraction with chloroform-methanol, hexane, methylene chloride or acetone. This is followed by saponification and purification to enrich the sterol fraction. Fats and oils are saponified to form soluble fatty acid salts and glycerol whereas lipids that do not form soluble compounds are unaffected by saponification and can be extracted into non-polar solvents. As an example, for saponification of a lipid extract or vegetable oil, the oil samples can be mixed with 1M ethanolic potassium hydroxide and agitated over night. Then washing with distilled water several times can remove the saponified material and the solvent. Finally, unsaponifiable residues are subjected to chromatographic identification (Lagarda et al., 2006).

3.3.1.2 Purification techniques

Various purification techniques have been applied for cleaning sterols. Accessible and affordable purification techniques include column chromatography (CC) and thin layer chromatography (TLC). Abidi (2001) reported that column chromatography can purify large quantity sample of more than 200 mg. TLC is suitable technique for small amounts. Different fractions of small amount of lipid or non-saponifiable oil can be separated by silica gel TLC with suitable developing solvents.

Solid phase extraction (SPE), which is essentially small scale column/HPLC chromatography, provides rapid extraction and consumes less solvent. SPE has been employed in its reverse phase (RP) mode (octadecylsilica) form for isolating phytosterols from unsaponifiable extracts, while its normal phase (NP) mode is used to isolate trimethylsil (TMS) derivatives from unsaponifiable oils (Lagarda et al., 2006).

3.3.1.3 Chromatographic detections and quantitative analysis

Chromatographic techniques are commonly used to identify non-saponifiable fractions of lipids and its derivatives including GC, GC-MS and HPLC (Lagarda et al., 2006). Wrolstad (2005) claimed that capillary GC was the most convenient technique for quantitative measurement of non-saponifiable lipids. Non-polar stationary phases (100% polysiloxane phases) and slightly polar stationary phases (5% diphenyl-95%

dimethylpolysiloxane) are also used in this determination due to high thermal stability. However, at times trimethylsilyl (TMS) ether derivatization of phytosterol however was necessary prior to GC analysis for better resolution (Lagarda et al., 2006).

On the other hand, HPLC can be advantageous due to lower column temperatures and non-destructive detection. This technique is thus suitable for thermally unstable sterols. The common columns used are alkylsilica stationary phases and octadecylsilica columns, while organic solvents such as acetonitrile and methanol are utilised as mobile phases (Abidi, 2001). Various detectors have been employed with HPLC for sterol determination. These detectors include UV (200-210 nm), photodiode array detector (DAD), refractive index (RI), evaporative light scattering detector (ELSD) and mass spectrometry (MS) (Lagarda et al., 2006).

Both GC and HPLC have been utilised for quantitative measurement of phytosterols. However, GC-FID was the most common technique for sterol quantification due to a large linear range of response and reliable, low cost detector. As with almost all analytical techniques, the quantitation of sterols requires validation in terms of retention time, precision and absolute response factors (Abidi, 2001). Standard reference or internal standard (IS) methods have been used to compare retention times to unknown sterols (Lagarda et al., 2006).

3.4 Materials and methods

3.4.1 **Extraction of phytosterol from *Undaria***

Phytosterols were extracted by solvent saponification and extraction of the saponified mixture. Freeze dried *Undaria* was weighed carefully (1 g) and 27 mL of ethanolic KOH (1:8, v/v) added. The mixture was then heated in a water bath for 30 minutes at 80ºC. After cooling under tap water, the mixture was filtered with Whatman No1 paper. The Hexane (20 mL) and Milli Q water (20 mL) was added. The mixture was then transferred to a50 mL centrifuge tubes and spun at 357 G for 10 minutes. Non-saponifiable fractions of lipids (phytosterols) soluble in hexane were at the top layer. Finally, the fractions were stored in a GC vial at 5ºC until further analysis (Sánchez-Machado et al., 2004b).

3.4.2 **Qualification of non saponifiable fractions by HPLC and GC-MS**

Identification of unsaponifiable fraction of lipids was carried out using high pressure liquid chromatography (HPLC) followed by gas chromatography-mass spectrometry (GC-MS).

HPLC and GC-MS were used to qualify the different non-saponifiable fractions, while GC-FID was used to quantify the known fraction after HPLC and GC-MS. Firstly; the non-saponifiable extract from *Undaria* was dried completely by a stream of nitrogen and reconstituted with the mobile phase before injecting into a HPLC. The liquid chromatographic method was analysed by HPLC (Shimadzu LC-20AT) with a (SPD-20A UV/VIS detector. The detection wavelength was 205 nm. The non-saponifiable compounds were separated using a Nova-Pak C18 4µm column (Waters However, at times trimethylsilyl (TMS) ether derivatization of phytosterol however was necessary prior to GC analysis for better resolution (Lagarda et al., 2006).

15 cm x 3.9 mm i.d.,) with an isocratic 25:75 (v/v) methanol:acetonitrile mobile phase at 1.2 mL/min. The different fractions were collected and dried completely by a stream of nitrogen. The fractions were reconstituted with 30 µL of hexane before injecting it into a GC-MS.

The GC-MS was a Thermo Trace GC Ultra coupled to the mass selective detector Thermo TRACE DSQ quadripole. The instrument was controlled by the *Xcalibur* software. The mass range of GC-MS was set between 50 and 500. The GC was equipped with a Varian VF-5 ms with 30 m length, 0.25 mm diameter and 0.25µm film The inlet was set at 200^{o}C (because of a different way of measuring injector temperatures, on this instrument this is equivalent to approximately 250^{o}C or more) 60 kPa constant pressure with a 20:1 split. The temperature program was the same as that used for GC-FID analysis as described in section 3.4.3.

3.4.3 **Quantification of phytosterols by GC-FID**

The non-saponifiable fractions of lipid were determined using GC-FID. These fractions were analysed using a Shimadzu GC-2010 gas chromatograph with a split-splitless injector and flame ionisation detector (FID). The column was a 30 m Zebron ZB-5 0.25 mm inner diameter and 0.25 µm film thickness from Phemomenex. The temperature program was 180°C for 3 min then 6°C min^{-1} to 245°C followed by 3°C min^{-1} to 275°C and hold for 14 min. The carrier gas was nitrogen with a flow-rate of 50 mL/min. The

linear velocity was set up at 20 cm/sec while split rate was 20:1 with a head pressure of 138 kPa. The temperature of injector and detector were set at 290 and 300ºC respectively. The volume of injection was 1 µL.

Non-saponifiable lipids were quantified using a known internal standard, 5α-cholestane (C-8003) purchased from Sigma-Alrich as this phytosterol did not ocur in the nature.

Calculation of relative response factor (RRF)

Cholesterol and 5α-cholestane standards were used to estimate the RRF. These standards were mixed in ratio of 2:1, 1:1 and 2:1 and then subjected to GC analysis. Known weight of each standard and peak areas were used to calculate the RRF using the equation below (Hwang, Wang, & Choong, 2003).

$$\mathbf{RRF = (A_{cholesterol}) / (W_{cholesterol}) \div (A_{IS}) / (W_{IS})}$$

Here, $A_{cholesterol}$: peak area of cholesterol and A_{IS} : peak area of 5α-cholestane were obtained by GC. $W_{cholesterol}$ is weight (µg) of cholesterol used in analysis. W_{IS} is weight (µg) of 5α-cholestane used in analysis. IS is internal standard (5α-cholestane).

Quantification of phytosterols

The quantification of unsaponifiable lipids in seaweed was done according to the method of Hwang et al. (2003). The following equation was used to calculate the amount of cholesterol relative to the internal standard (IS).

$$\mathbf{Cholesterol\ (\mu g/g) = (A_{cholesterol} \div A_{IS}) * (W_{IS} \div RRF) * (1 \div W_{Sample})}$$

W_{Sample}: weight of sample

3.5 Results and discussion

3.5.1 Identification non-saponifiable fractions of lipids

As described above, tetermination of the unsaponifiable lipid fractions was carried out using HPLC to separate the lipid fractions followed by GC-MS analysis to identify the individual fractions. GC-FID was subsequently used to confirm (by retention times compared to standards) and quantify the GC-MS results.

3.5.1.1 HPLC separation of non-saponifable lipids

Measurement of non-saponifiable lipids was performed using HPLC as described in section 3.4.2. The chromatogram in Figure 28 showed four well separated peaks. Each fraction was collected and identified by GC-MS analysis.

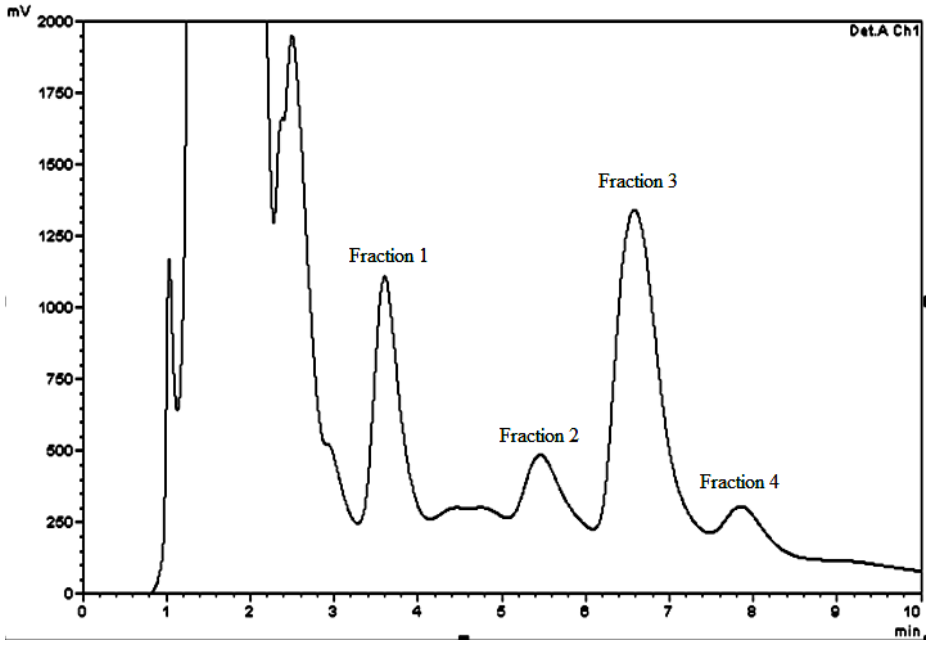

Figure 28: Chromatogram of non-saponifiable lipids from HPLC.

3.5.1.2 GC-MS identification

Two marine phytosterols were identified using GC-MS. 24-methylene cholesterol and fucosterol corresponded to fractions 2 and 3. However, fraction 1 and 4 were not the excellent fit. In addition, α-tocopherol (Vitamin E) was identified using a reference standard which was also confirmed by GC-MS because the content of α-tocopherol is low and cannot detect by the HPLC. Figure 32 shows the GC-MS chromatogram of unsaponifiable compounds from *Undaria* sample. Their retention times were compared to the different fractions obtained from HPLC. α-tocopherol, 24-methylenecholesterol and fucosterol appeared at retention times of 24.98, 28.81 and 33.51 min respectively. Their individual mass spectra, compared to the standard from the NIST library, are shown in Figures 29, 30 and 31. The major mass ions of fucosterol were 229, 281 and 314 m/z with a clear 412 molecular ion (Figure 29). For 24-methylenecholesterol 271, 314 and 315 m/z (Figure 30), fucosterol did not show a significant molecular ion but the pattern match is good and the retention time was an exact match for an authentic

standard. Finally, the major ions m/z of vitamin E was 165 and 430 was an excellent fit (Figure 31).

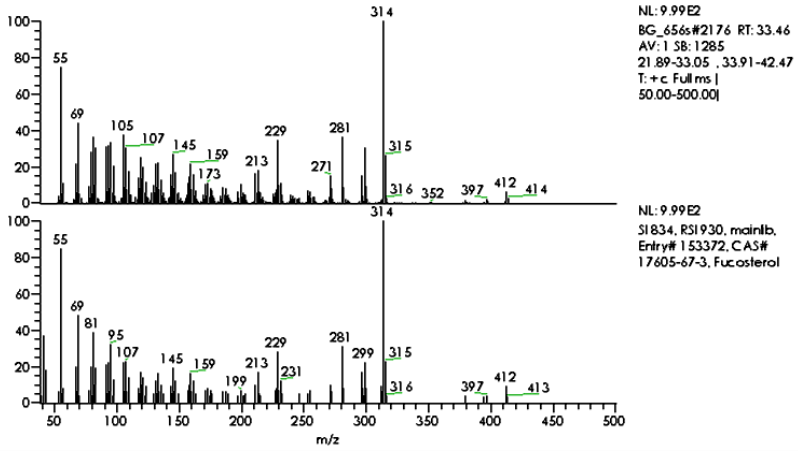

Figure 29: Mass spectrum of fucosterol

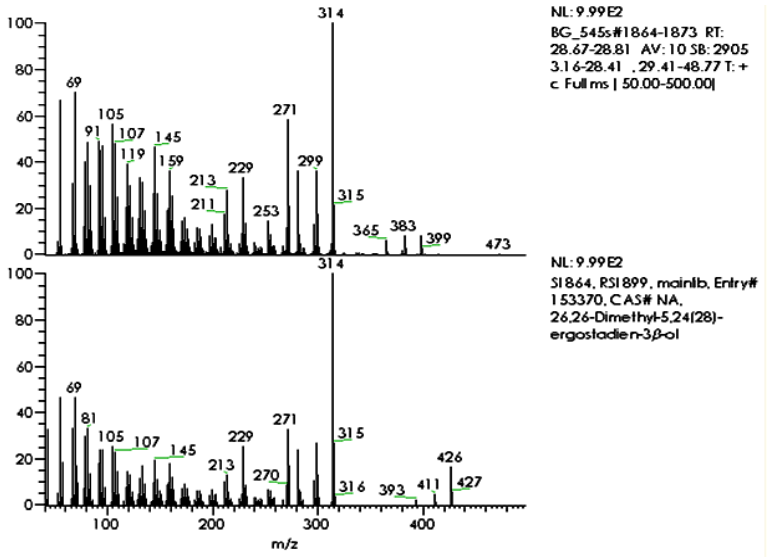

Figure 30: Mass spectrum of 24 methylenecholesterol

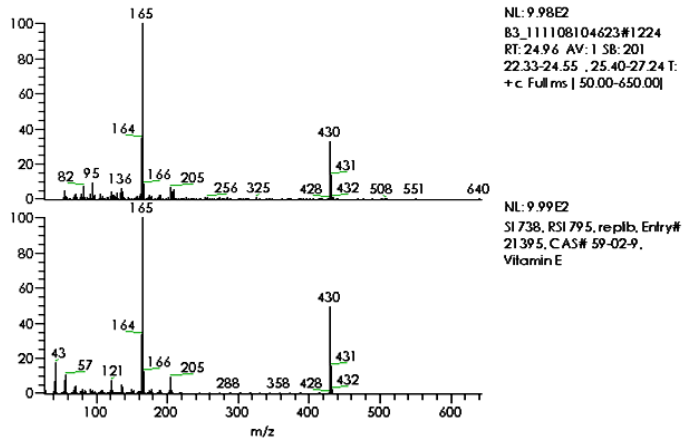

Figure 31: Mass spectrum of Vitamin E

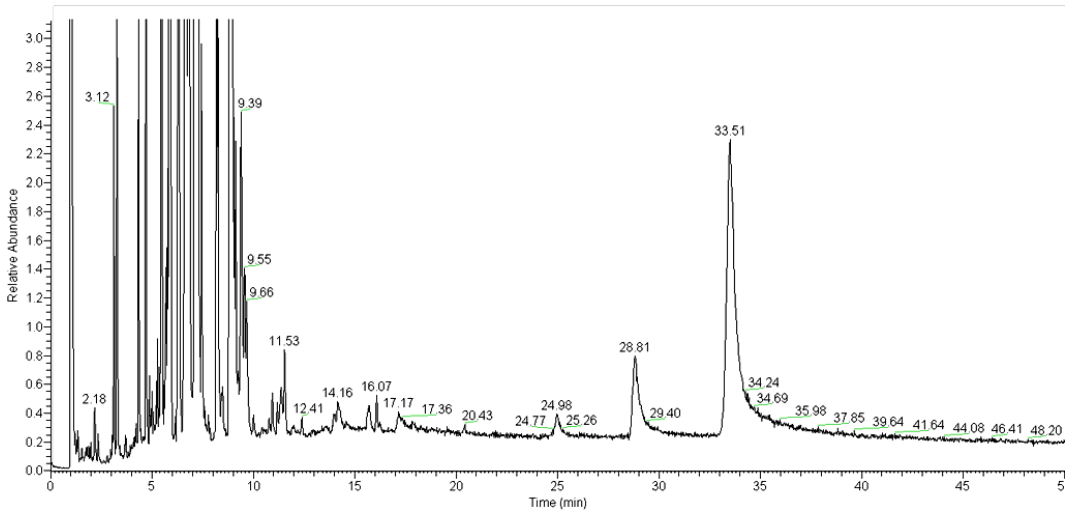

Figure 32 : Full mass range (50-500 m/z) Chromatogram from the GC-MS of the non-saponifiable fractions from *Undaria* sample

Vitamin E (24.98 min), 24 Mythylenecholesterol (28.81 min) and fucosterol (33.51 min)

3.5.1.3 Quantification of non-saponifiable fractions by GC-FID

Non-saponifiable fractions were quantitated by GC-FID. The elution times were similar to the GC-MS analysis. The chromatogram is shown in Figure 33 where the retention times of α-tocopherole, 24-methylenecholesterol and fucosterol were at 25.43, 27.04 and 30 min.

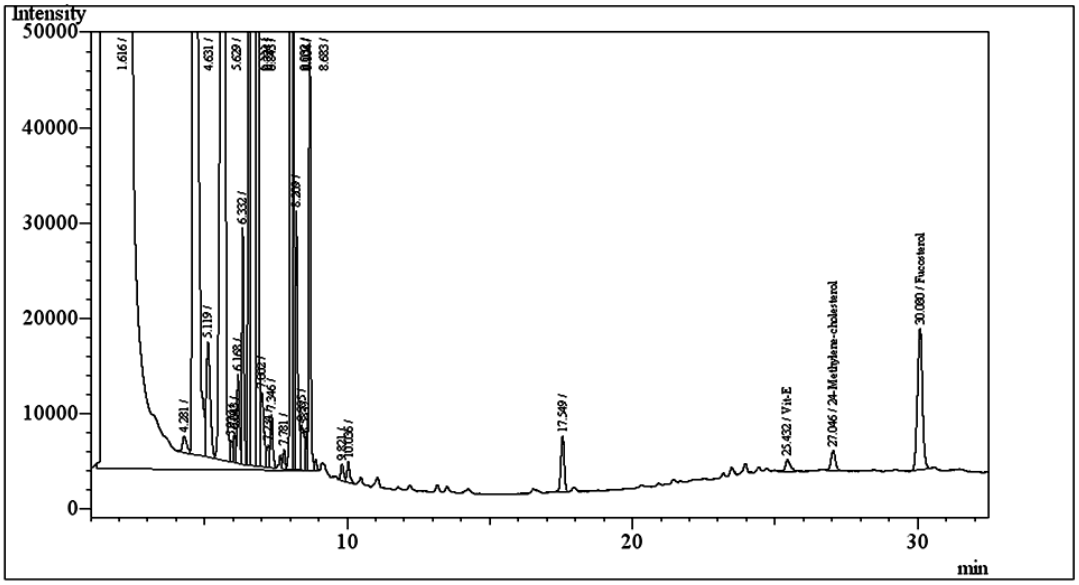

Figure 33 : Chromatogram of non-saponifiable fractions of lipids from GC-FID

3.6 Quantification of non-saponifible fractions in *Undaria*

3.6.1 Comparison of non-saponifiable lipids between big and small *Undaria*

The biochemical distributions of non-saponifiable lipid varied according to size of *Undaria*. In this study, different sizes of *Undaria* were collected from farm PE327 in Port Underwood in December 2012. The large plants measured 96.25 ± 5.34 cm in length and weighed 122.34 ± 7.65 g while the small plants were 51.4 ± 8.65 cm of length and 43.56 ± 9.13 g on average. Figure 34 shows that larger plants had significantly higher ($P < 0.05$) α-tocopherol, 24-methylenecholesterol and fucosterol than smaller ones. Fucosterol was the major phytosterol in *Undaria* samples and represented 338 µg/g dry weight. In contrast, the amount of fucosterol in the small plants was only 48 µg/g. of the other major phytosterol, 24-methylenecholesterol was 8 and 48 µg/g for small and big *Undaria* respectively (a reversal of that found for fucosterol). The concentration of α-tocopherol was also higher in larger *Undaria* samples, 32 µg/g in the large plants and 10 µg/g in the small ones. As shown in section 2.13, the total lipid level increased with plant size.

The levels of fucosterol and 24 methylenecholesterol of New Zealand *Undaria* were however lower than that reported by Holdt and Kraan. (2011). They reported 662 µg/g dry weight of fucosterol in *U. pinnatifida*. The low amount of non-saponifiable lipid fractions was attributed to immature *Undaria* samples used in our study. As noted before, Weddy Gibbs et al. (1998) reported that the level of total lipids was higher in mature marine and terrestrial plants.

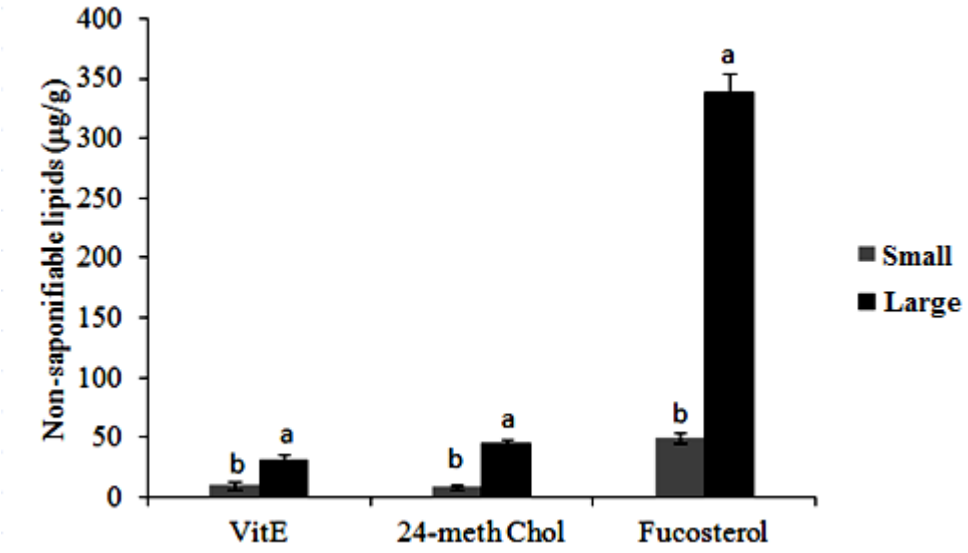

Figure 34 : Non-saponifiable lipids of small and large plants

All values are expressed as a mean with standard error bars (µg/g dry weight, n=3). All samples came from farm 327. Different superscript letters within the non-saponifiable lipid fractions indicate statistical difference between months using the Tukey post-hoc tests ($P < 0.05$). VitE: Vitamin E, 24-meth chol: 24-methylenecholesterol

3.6.2 **Seasonal variation of non-saponifiable lipids**

Popov et al. (1987) reported that season and water pollution influenced phytosterol composition in algae. Moreover, Honya et al. (1994) reported that the fucosterol of brown seaweed (*Laminaria japonica*) reached a maximum in winter and decreased rapidly in Autumn, when the highest amount of 24 methylenecholesterol was found. However, the amount of 24 methylenecholesterol is much less than fucosterol. Tocopherol content was also maximal in winter (Honya et al., 1994).

Tocopherol and phytosterol samples collected on a monthly basis from farm PE 327 (Port Underwood) were compared. Figure 35 shows that non-saponifiable lipid fractions namely fucosterol and 24-methylenecholesterol were significantly higher in winter ($P < 0.05$) (August) and showed a significant decrease in summer ($P < 0.05$) (December). Fucosterol concentration was highest in winter (146 µg/g) and the lowest in summer (49 µg/g). 24-methylenecholesterol showed a similar trend to fucosterol. The highest concentration was 28 µg/g in August while the lowest in December with 8.4 µg/g.

Interestingly, α-tocopherol levels showed a similar but smaller variation 14.1 µg/g in winter down to 9.6 in December. The content of α-tocopherol is low compared to another study (Lordan et al., 2011). They reported that the α-tocopherol contents is ranged between 145 and 174 µg/g dry weight (Lordan et al., 2011).

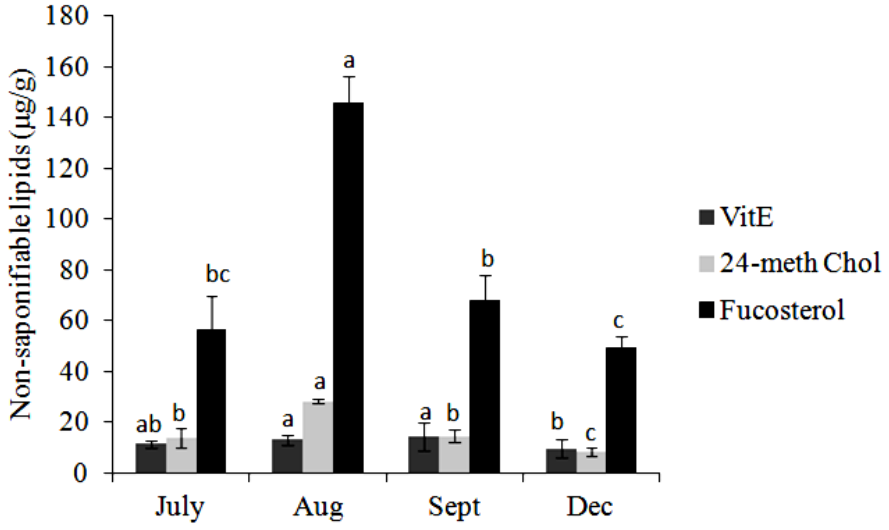

Figure 35 : Monthly changes of non-saponifiable lipid fractions

All values are expressed as mean and standard error bars (µg/g dry weight, n=3). All samples came from farm 327 (Port Underwood). Different superscript letters within the non-saponifiable lipid fractions indicated statistical difference between months using the Tukey post-hoc tests ($P < 0.05$). VitE: Vitamin E, 24-methchol: 24 methylenecholesterol

In conclusion, seasonal changes and maturity of *Undaria* had a significant effect on the levels and ratios the non-saponifiable lipids in *Undaria* from farm 327.

3.6.3 Comparison of non-saponifiable fractions between exposed and sheltered samples

Geographical conditions can play an important role in chemical formation in algae (Stuart et al., 1999). In this study, August samples were collected from farms: 327 and 122 were compared. 327 was an exposed site and farm 122 was a sheltered site. Figure 36 shows significant differences ($P < 0.05$) in fucosterol and 24 methylenecholesterol contents between the sheltered and exposed samples. The concentration of fucosterol was about two times higher in the exposed samples compared to the sheltered samples. 24-methylenecholesterol was also found to be significantly higher in exposed samples

($P < 0.05$). There was however no significant difference in α-tocopherol content between sheltered and exposed samples. The differences in fucosterol and 24 methylenecholesterol could be attributed to their growth rate. This is confirmed by the study in section 2.13. *Undaria* in sheltered sites grew more slowly because rainfall brings more fresh water and sediment to the mussel farms. The annual rainfall total was 979 mm in 2011 (see Section 2.11.1.1). These environmental effects will also hinder the growth of mussels, and by extension, *Undari* in Pelorus Sounds (Aquaculture, 2011).

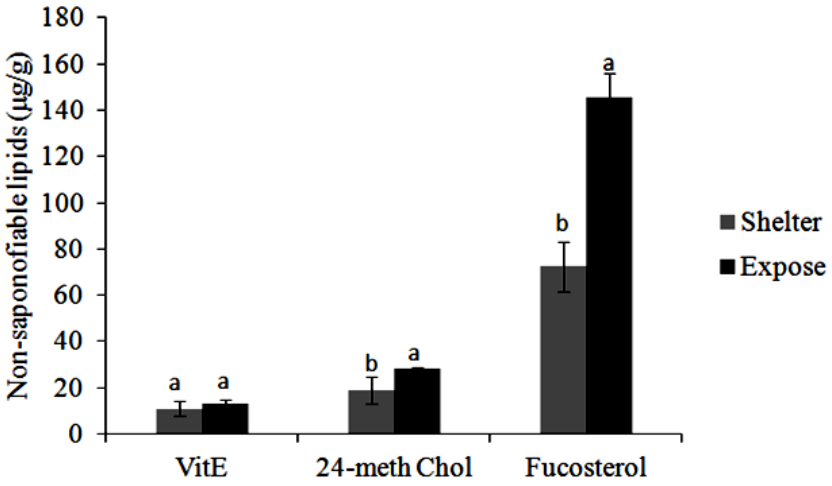

Figure 36 : Comparison of non-saponifiable lipids between sheltered and exposed sites

All values are expressed as mean and standard error bars (µg/g dry weight, n=3). Samples came from farm 327 (exposed site) and 122 (sheltered site). Different superscript letters within the non-saponifiable lipid fractions indicate statistical differences between months using the Tukey post-hoc tests ($P < 0.05$). VitE: Vitamin E, 24-meth chol: 24-methylenecholesterol

Chapter 4 Conclusion

The edible macroalga *Undaria pinnatifida* is now endemic in New Zealand and is being investigated at AUT for a range of nutritional components. This study is a part of the broader one and focussed the lipids in the plant. The total lipid content in different parts of the plant and the effects of different growing conditions both seasonal and location were investigated and for selected sites the fatty acid profiles and the levels of the major non-saponifiable lipids were examined in more detail.

Samples were principally sourced from Pelorus Sound and Port Underwood and at other times during the study, *Undaria* was also collected from Wellington harbour and Great Barrier Island for geographical comparison. Samples were collected on a monthly basis from mussel lines in the Marlborough Sounds from June until December 2011. In addition, the traditional salted and dried the wakame was prepared from New Zealand *Undaria* and the lipids compared with commercial wakame from Japan and Korea. It was found that the blade of *U. pinnatifida* had a relatively low lipid content of between 17.07 to 35.48 mg/g dry weight whereas the sporophyll was between 20.70 and 58.29 mg/g. These samples, collected from June to December, are considered as immature algae because Commercial wakame from Japan and Korea (prepared from the blade) had higher total lipids which varied between 38 and 43 mg/g and it is known that lipid levels in macro algae increase with the age of the plant.

U. pinnatifida was always present and grows throughout summer because of the relatively low water temperature (16ºC) whereas in Japan it degenerates and degrades in the high (25ºC) sea temperatures. Size also influenced lipid content. Larger *Undaria* samples (presumably older and more mature) were found to contain 45 to 63 mg/g lipid in the blade and the sporophyll respectively which is close to that obtained for the commercial Japanese product.

Samples from exposed sites also had a higher content of lipid compared to sheltered sites. Samples from Port Underwood and Worser Bay, both exposed sites, had higher lipid contents than samples from Pelorus Sounds and Shelly Bay. The sea currents and low temperatures in exposed sites encourage *Undaria* to produce more lipids.

Three classes of fatty acids were identified in New Zealand *U. pinnatifida* were saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs) and

polyunsaturated fatty acids (PUFAs). The levels of SFAs increased from winter to summer. The most abundant SFA was palmitic acid (C16:0) which increased from 4.2 mg/g in winter to 6.49 mg/g in the blade and similarly to 14.75 mg/g for the sporophyll in December. Oleic acid (C18:1n-9c) was the predominant MUFA and was at a maximum in summer. New Zealand *Undaria* were larger size in summer due to low temperature (16ºC) while Japanese *Undaria* died during summer when temperatures were in excess of 25ºC.

U. pinnatifida was also a rich source of polyunsaturated fatty acids. These FAs consist of both n-6 and n-3 PUFAs. The major n-6 PUFAs are linoleic acid (C18:2n-6), γ-linoleic acid (C18:3n-6) and arachidonic acid (C20:4n-6) which increased in spring and cold summer. The important PUFAs α-linoleic acid (C18:3n-3), stearidonic acid (C18:4n-3) and eicosapentaenoic acid (C20:5n-3) were higher in winter because Low temperature affected on increased level of unsaturated fatty acid in polar lipid. This augmentation of unsaturation results in lower melting points and maintain lipid in liquid stat for normal protoplasmic viscosity (Nelson et al., 2002). Exposed samples collected in winter had relatively higher levels of MUFAs and PUFAs namely, myristoleic acid (C14:1), cis-10-heptadecenoic acid (C17:1), linoleic acid (C18:2n-6), γ-linoleic acid (C18:3n-6), α-linoleic acid (C18:3n-3), stearidonic acid (C18:4n-3), arachidonic acid (C20:4n-6) and eicosapentaenoic acid (C20:5n-3). However, sheltered samples had more SFAs and MUFAs such as decanoic acid (C10), lauric acid (C12), stearic acid (C18) and palmitoleic acid (C16:1). Interestingly, the fatty acids of New Zealand *U. pinnatifida* showed high concentrations of n-3 and demonstrated a nutritional balance of n-6/n-3 ratio in both blade and the sporophyll. The n-6/n-3 fatty acid ratio varied between 0.9 and 2.5 in the blade and between 0.99 and 2.5 in the sporophyll. The World Health Organisation reported that this ratio should be lower than 10 in order to promote health benefits (Simopoulos, 2008). Hence New Zealand *Undaria* has a potential to provide health benefits to humans due to the high content of essential n-3 fatty acids.

Unsaponifiable fractions of lipids were less than 1 % of total lipids in *U. pinnatifida*. The three main components were the phytosterols predominantly fucosterol with small amounts of 24-methylenecholesterol. α-tocopherol, a fat-soluble, antioxidant, vitamin was also present in small amounts. The phytosterols and α-tocpoherol were directly influenced by monthly changes and were generally found in higher concentration in winter.

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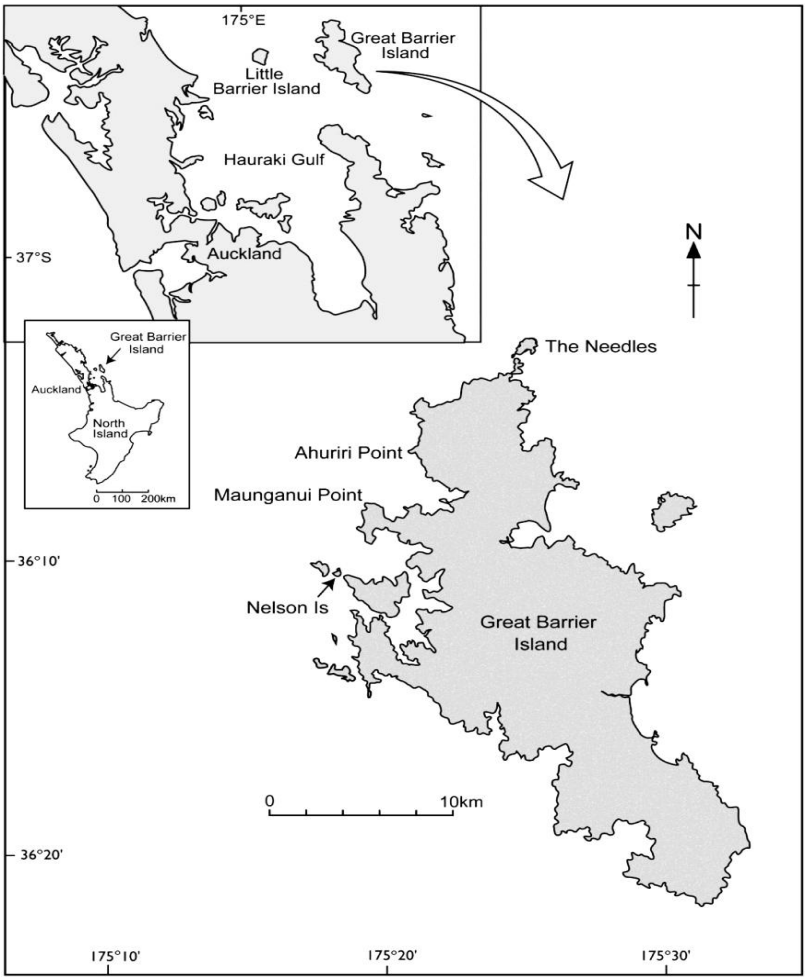
Appendices

Appendix I:

Table I. 1 : Co-ordination of seaweed collection

Location	Farms codes	Latitude	longitude
Port Underwood	PE 327	41° 20' 53.05"	174° 07' 20.96"
	106	41° 19' 37.74"	174° 08' 57.54"
	253	41° 18' 11.08"	174° 09' 13.43"
Pelorus Sounds	O23	41° 12' 003.01"	173° 53' 27.26"
	122	41° 06' 30.89"	173° 54' 58.05"
	233	41° 09' 21.64"	173° 51' 11.24"
	PE 280	41° 05' 39.22"	174° 01' 48.11"
	314	41° 09' 20.12"	173° 50' 07.64"
	353	41° 01' 56.95"	173° 56' 12.55"
Wellington	Shelly Bay	41° 17' 38.082"	174° 49' 16.110"
	Worser Bay	41° 18' 46.207"	174° 49' 49.678"

Figure I. 1 : Map of Great Barrier Island



(White, Coveny, Robertson, & Clements, 2010)

Appendix II : linear regression of lipid content

The regression equation is
Lipid = 0.705 + 0.0308 Length + 0.0124 weight

Predictor	Coef	SE Coef	T	P
Constant	0.7054	0.1341	5.26	0.000
Length	0.030793	0.006449	4.77	0.000
Weight	0.012362	0.004341	2.85	0.009

S = 0.279568 R-Sq = 88.7% R-Sq(adj) = 87.7%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	2	13.4845	6.7422	86.26	0.000
Residual Error	22	1.7195	0.0782		
Total	24	15.2040			

plant	Lipid (g/100g)	Length (Cm)	Weight (g)
1	2.5	35	30.9
2	2.3	45	47
3	1.6	27	9.2
4	1.3	27	11
5	1.7	15	6.9
6	1.2	18	7.8
7	2.5	35	23
8	3.72	59	65.3
9	1.2	19	7.3
10	2.123	25	56
11	1.2	19.2	6.2
12	2.9	55	45
13	3.1	49	56
14	2.78	55	49
15	2.7	55	47
16	2.9	43	65
17	2.67	45	75
18	1.2	17	21
19	1	10	5
20	1.5	11	7
21	3.1	45	55
22	1.2	19	7.9
23	2.2	35	20
24	1.2	10	9
25	1.5	23	7

Appendix III : Chromatogram of fatty acids by acidic analysis

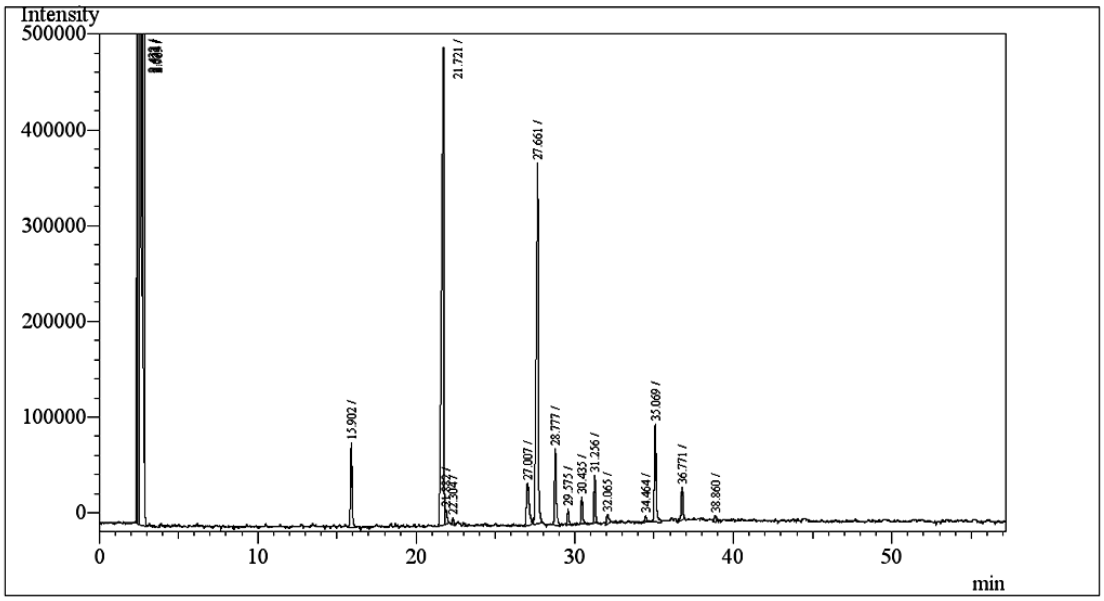


Figure III 2. : Chromatogram of FAME from methanolic BF_3 method

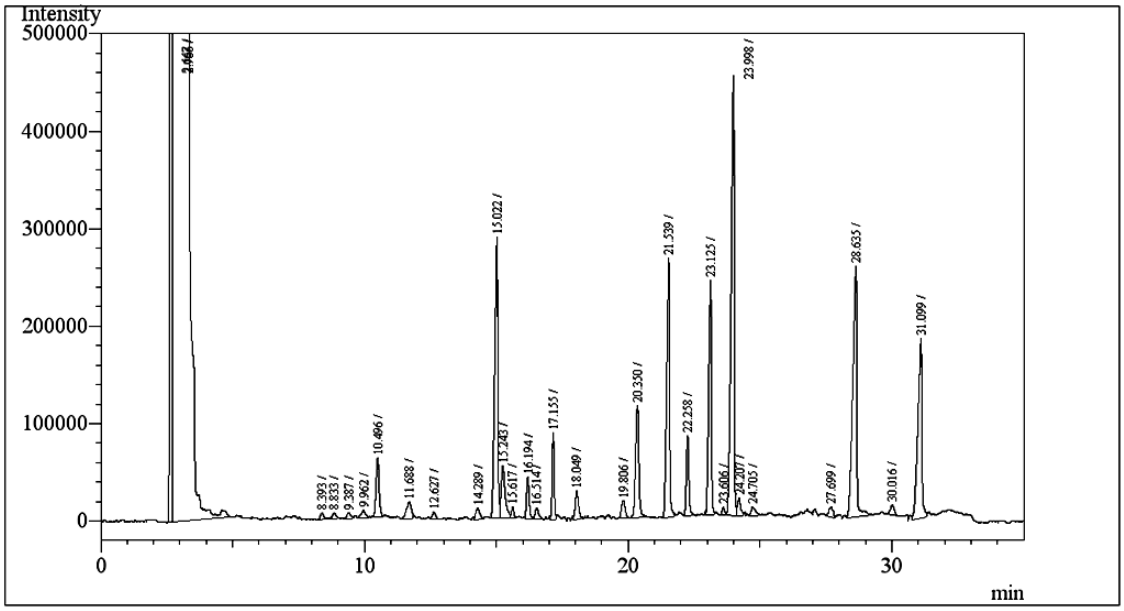


Figure III. 3 : Chromatogram of FAME from methanolic HCl method

Appendix IV:

Table IV. 2 : Fatty acid composition of *Undaria pinnatifida* (percentage of total fatty acids)

fatty acid	(Dawczynski et al., 2007)	(Sánchez-Machado et al., 2004a)	(Fleurence et al., 1994)	(Ortega-Calvo et al., 1993)	(Nisizawa et al., 1987)	(Khotimchenko, 2003)
C12				2.5		
C12:1					0.3	
C14	4.07	3.17	2.1	3.4	15.7	2.9
C15	0.21			0.4	0.6	
C16	13.5	16.51	15.9	25.5	49.6	33.1
C17	0.20					
C18	0.86	0.69	1.2	2.7	2.1	2.6
C20	0.39		0.4	0.8		
∑SFA						
C14:1					0.1	
C16:1	0.44	3.70	0.3	1	8.7	
C18:1n-9c	5.95*	6.70	10.2	22.7	10.7	15
C20:1					5.7	
∑MUFA						
C18:2n-6c	7.41	6.23	7.2	18.4	5.4	8.7
C18:3n-3	0.34	11.97	10.3		0.9	12.4
C18:3 n-6			1.2			
C18:4 n-3	25.80	22.60	21.1			6.2
C20:2			0.2	0.5		
C20:3n-6	0.57		1	18.4		
C20:3 n-3	0.14					
C20:4 n-6	13.30	15.87	17.5		2.3	11.3
C20:4 n-3		0.70	0.8			
C20:5 n-3	13.20	9.43	8.2			5.4
∑PUFA						
Total fatty acid						

*sum of C18:1

Appendix V

Table V. 3 : Total lipids from different locations

	PUW	Worser Bay	Shelly Bay	Great Barrier Island
Blade	35.46 ± 0.30^{a}	30.45 ± 1.96^{a}	21.20 ± 2.81^{b}	18.96 ± 1.70^{b}
Sporophyll	51.26 ± 2.21^{a}	35.48 ± 1.64^{bc}	44.39 ± 4.36^{ab}	30.50 ± 0.07^{c}

(Mean ± SE mg/g, n=3 DW). a, b, c significant difference level ($P < 0.05$, Tukey test)

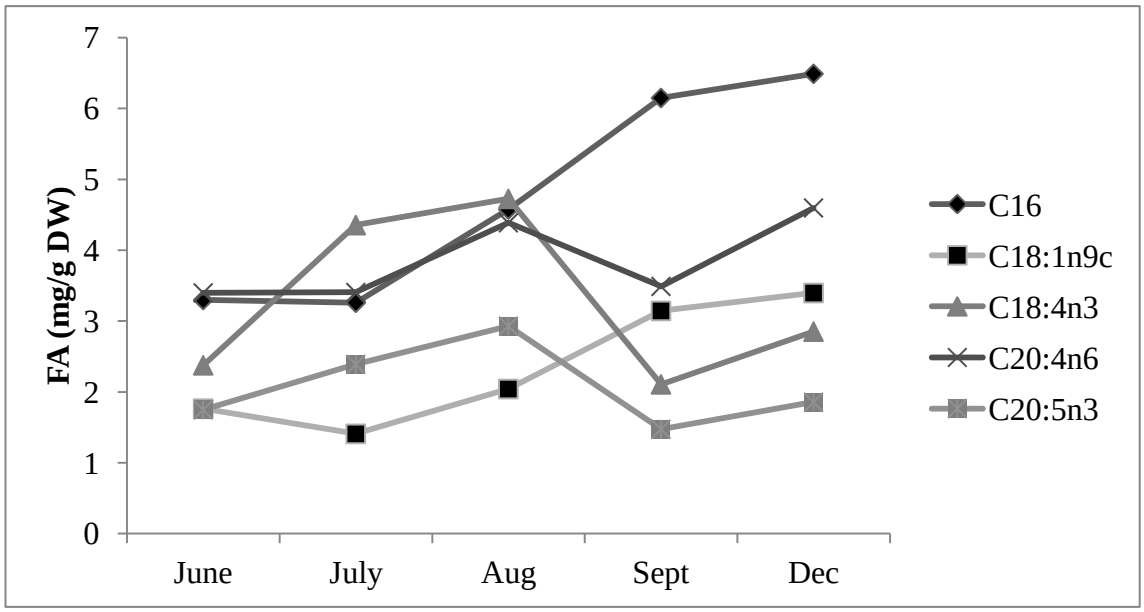


Figure V. 4 : Seasonal variation of fatty acid in blade

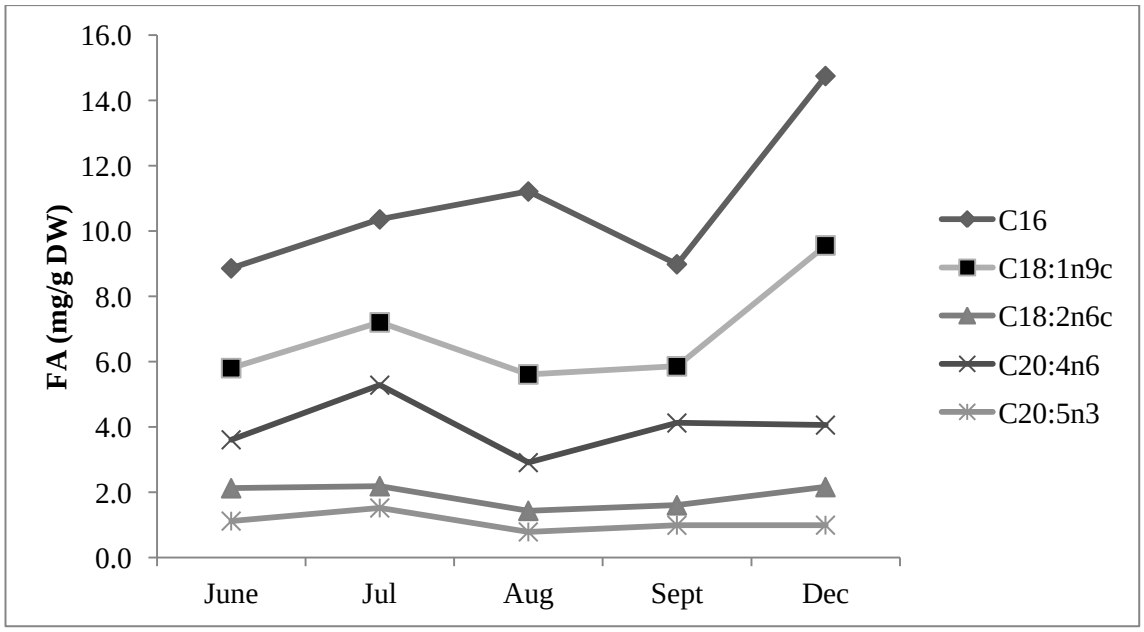


Figure V. 5 : Seasonal variation of fatty acids in the sporophyll

Appendix VI. Total lipid, fatty acids, phytosterol and fat soluble vitamin (Vit E)

Table VI. 4 : Different compositions of lipids

	Saponifiable lipid (µg/g)					Non saponifiable lipid(µg/g)			
	Total lipid	**ΣSFA**	**ΣMUFA**	**ΣPUFA**	**Total fatty acid**	**Vit-E**	**24-meth Chol**	**Fucosterol**	**T°C**
July	29666.7	5377.6	2645.6	14390.7	22413.9	11.4	13.6	56.7	7.1
Aug	35456.7	6704.3	3213.3	17239.3	27156.9	13.2	28.2	145.6	8.3
Sept	26883.3	8262.0	4174.9	10438.1	22875.0	14.1	14.5	68.1	11.1
Dec	34033.3	8921.5	4081.5	13990.9	26993.9	9.6	8.4	49.4	16.45

Vit-E: Vitamin E, 24-meth Chol: 24-methylene-cholesterol

Table VI. 5 : Percentage of individual lipid composition of total lipid

	Saponifiable lipid (%)			Non saponifiable lipid (%)			
	ΣSFA	**ΣMUFA**	**ΣPUFA**	**Total fatty acid**	**Vit-E**	**24-meth Chol**	**Fucosterol**
July	18.13	8.92	48.51	75.55	0.04	0.05	0.19
Aug	18.91	9.06	48.62	76.59	0.04	0.08	0.41
Sept	30.73	15.53	38.83	85.09	0.05	0.05	0.25
Dec	26.21	11.99	41.11	79.32	0.03	0.02	0.15