The Fucoxanthin Content and Antioxidant Properties of *Undaria pinnatifida* from Marlborough Sound, New Zealand

Adah Yee Chuen Fung

A thesis submitted to

Auckland University of Technology University

in partial fulfilment of the requirements for the degree of

Master of Applied Science (MAppSc)

2012

School of Applied Sciences

Primary Supervisor: Dr Jun Lu

Secondary Supervisor: Dr Nazimah Hamid

Table of contents

Contents

List of Figures

List of Tables

Statement of Originality

"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 nor material which to a substantial extent has been accepted for the qualification of any other degree or diploma of a university or other institutions of higher learning, except where due acknowledgement is made in the acknowledgement".

Signed ____ _____

Date 27/08/2012

Acknowledgement

I would like to show my gratitude to my primary supervisor Dr Jun Lu for giving me advice, knowledge and endless support for materials and equipment.

I am deeply indebted to my secondary supervisor Dr Nazimah Hamid, whose advice, guidance and offered invaluable assistance throughout my research with patience. I would not be able to complete my thesis and research on time without her help and sacrifices.

I would also like to express my sincere thanks to Dr John Robertson and Dr Chris Pook for their assistance with my HPLC analysis. Many thanks to the Chemistry and Biology technicians and Applied Sciences staff especially Brid Lorigan for their help throughout the year.

Special thanks to my friends from the *U.pinnatifida* research project Jessica, Wilfred, Sayvisene, Kevin and April, and also Bruce and Clara for their help and unconditional support in assisting me to complete this thesis. It has been a pleasure to work with them. I had a lot of fun and wonderful memories that I would never forget.

Finally, I would like to express my love and gratitude to my beloved families and friends for their understanding and endless support throughout the duration of my studies.

Abstract

Undaria pinnatifida (*U.pinnatifida*) is a type of brown seaweed native to the temperate shores of Japan, Korea and China. It was introduced to New Zealand accidently through ships travelling from Asia in 1987. Since then, it has widely spread to areas frequented by vessels in New Zealand. This study was carried out to investigate the fucoxanthin content and antioxidant properties of *U.pinnatifida* collected from the Marlborough Sounds, New Zealand.

U.pinnatifida was collected from two locations - Port Underwood and Pelorus Sound of the Marlborough Sounds, from their appearance in late autumn (June) to the onset of senescence in early summer (November), 2011. Results showed that freeze dried *U.pinnatifida* harvested from Port Underwood had higher fucoxanthin content in the blade particularly in July, August and September compared to Pelorus Sound. The sporophyll which is usually discarded when processing *U.pinnatifida* as a dried food product was also found to contain a significant amount of fucoxanthin, but all lower than that in the blade, throughout the harvest season.

New Zealand *U.pinnatifida* was further processed in this study to produce cut wakame, which was the most popular form of commercially dried products available in New Zealand. The New Zealand processed *U.pinnatifida* was compared to four other commercial dried products from Korea (Ottogi and Chung Jung Won) and Japan (Wako Shokai and Riken) in terms of their fucoxanthin content and antioxidant activities. For antioxidant measurement, 1,1- diphenyl-2-picrylhydrazyl assay (DPPH) and cupric reducing antioxidant capacity (CUPRAC) were utilised. Processed *U.pinnatifida* had lower fucoxanthin content and antioxidant capacities compared to the freeze-dried *U.pinnatifida*. Fucoxanthin content and antioxidant activity of New Zealand processed *U.pinnatifida* was not significantly different from other commercial samples.

In conclusion, sporophyll is a useful resource for fucoxanthin extraction, and New Zealand *U.pinnatifida* is a valuable food or nutraceuticals resource, as it has similar fucoxanthin content and antioxidant activity compared with Japanese and Korean *U.pinnatifida* products.

Chapter 1

1 Introduction

In answer to society's demand for a better lifestyle and increased longevity, consumers have developed an increasing interest towards consuming nutraceuticals and functional foods that are rich in natural bioactive compounds. Among the well-known bioactive compounds, antioxidants are the most important compounds required by our human body in order to stay healthy. Antioxidants sacrifice themselves by inhibiting further oxidation reactions that produce free radicals, which cause cell damage or death and consequently induce different kinds of serious chronic diseases, including cancer and atherosclerosis. Seaweed or sea vegetables are rich in polysaccharides, vitamins, minerals, bioactive substances like polyphenols, proteins, lipids and carotenoid that possess antioxidant, antibacterial, antiviral and other beneficial functions. Marine products are currently of considerable interest in the food and pharmaceutical industries for the development of antioxidants (Ngo, Wijesekara, Vo, Van Ta, & Kim, 2010).

Seaweeds are harvested for different purposes and utilised differently around the globe. In the Pacific and Asian cultures, seaweed has long been incorporated into human diets such as "nori" (*Porphyra sp.)* as sushi wrappings; "hijiki" (*Hizikia fusiforme* (Harvey) *Okumaru*), "kombu" (*Laminaria sp.*), "wakame" (*U.pinnatifida* (Harvey) *Suringar*) that are consumed in soups, salads and vegetable dishes, and "Limu Palahalaha (*Ulva fasiata*) in Hawaiian snacks. Besides these traditionally uses, seaweeds are also incorporated in modern commercial food products in Asian markets, most commonly in confectionery products (Foodnavigator, 2006). In western countries, seaweeds are mainly developed as a hydrocolloid used in processed food in the form of additives (from E400 to E407), or for pharmaceutical industry purposes as a tablet encapsulation agent. However, with the increase of Asian cuisine consumption in western countries together with the known health benefits of seaweed consumption; other cultures have started to include seaweed into their diets.

Seaweeds are classified into three types of taxa depending on their pigmentation: red (*Phylum Rhodophyta*), brown (*Class Phaephyceae*) and green (*Phylum Chlorophta*)

(Tierney, Croft, & Hayes, 2010). Several studies on different seaweed species have shown that brown seaweeds have a higher antioxidant capacity than red or green seaweed (Jiménez-Escrig, Jiménez-Jiménez, Pulido, & Saura-Calixto, 2001; Matanjun, Mohamed, Mustapha, Muhammad, & Ming, 2008; Prabhasankar et al., 2009). Phlorotannins are the only group of tannins present in brown seaweed. These extracts have shown protective effects against hydrogen peroxide-induced cell damage by acting as free radical scavengers (Tierney et al., 2010), reducing agents and metal chelators (Ngo et al., 2010; Tierney et al., 2010). On the contrary, fucoxanthin is the dominant carotenoid in brown seaweeds. Although less attention has been paid to the physiological effects of carotenoid in seaweeds, fucoxanthin has recently attracted much attention due to its strong antioxidant properties that showed significant anti-cancer, anti-obesity and anti-inflammatory effects (Miyashita & Hosokawa, 2008).

The objective of this study was to examine the fucoxanthin content and antioxidant properties of *U.pinnatifida*, a type of brown seaweed obtained from the Marlborough Sounds, New Zealand. *U.pinnatifida* is an unwanted organism in New Zealand under Section 164c of the Biosecurity Act 1993. However in the late 2010, a new policy was implemented that allowed farming and harvesting of *U.pinnatifida* for commercial use. This research is the first study conducted to examine the antioxidant properties and fucoxanthin content of New Zealand *U.pinnatifida* across its growing season, and in fresh, processed and commercial *U.pinnatifida.*

1.1 World production of aquaculture

The term “aquaculture” is generally defined as the cultivation of freshwater and saltwater organisms. In most Western countries, aquaculture is commonly described as the finfish and shellfish aquaculture of the country without reference to seaweed aquaculture. However in Asian countries that are the major producers of seaweed, seaweed aquaculture was considered to be equal or even superior to other aquaculture sectors. According to the Fishery and Aquaculture Statistics Yearbook , the production of seaweeds and other aquatic plants worldwide was 10.1 million tonnes in 2000 and this has increased to 15.8 million tonnes in 2008 bringing in a total value revenue of US$ 7.4 billion in 2008 compared to US$5.6 billion in 2000 (FAO, 2010; Werner, Clarke, & Kraan, 2006). The production of aquatic plants since 1970 had an

average annual growth rate of 7.7 percent, where 99.6 percent of production and 99.3 percent by value in 2008 were dominated by seaweeds (Garibaldi et al., 2010).

To date, countries in East and Southeast Asia still dominate the production of seaweed in the world (99.8 percent by quantity and 99.5 percent by value in 2008) (Garibaldi et al., 2010). China is currently the leading producer of seaweed in the world. China alone comprised 62.8 percent of the world's seaweeds production by quantity followed by Indonesia (13.7 percent), Philippines (10.6 percent), Republic of Korea (5.9 percent) and Democratic People's Republic of Korea (2.8 percent) (Garibaldi et al., 2010). Although Japan is one of the countries where seaweed is heavily consumed in the consumers' daily diet, Japan only produces 2.9 percent of seaweed in the world. Nevertheless in terms of value, Japan remained the second most important producer in the world due to its high-valued Nori production. Most seaweed species cultured in East Asia are fundamentally used for human consumption, except for certain Japanese kelp that is utilised as a raw material for iodine and algin extraction. Besides this, *Eucheuma* in Southeast Asia is cultivated mainly for carrageenan extraction (Garibaldi et al., 2010).

Chile was reported to be the most exported seaweed culturing country outside Asia. In 2008 Chile produced 21 700 tonnes of farmed seaweeds, followed by Africa with 14 700 tonnes (Garibaldi et al., 2010). United Republic of Tanzania and Madagascar mostly exported the *Eucheuma* farmed seaweed; whereas South Africa cultivated seaweeds as marine feed (Garibaldi et al., 2010).

1.1.1 Seaweed production and value worldwide

According to the Food and Agriculture Organization (2002), a review of the global production and value of brown, red and green seaweeds used in aquaculture from 1991-2000 showed brown seaweeds consistently remaining as the most voluminous and profitable seaweeds produced in aquaculture (Figure 1). Brown seaweeds comprised approximately double the tonnage and value of red seaweeds, while green seaweeds had the least production in comparison. The volume of brown seaweed production increased dramatically from 1991-1993, and remained relatively constant through to 1998, with a tonnage of around 5-5.5 million (wet tonnes) at the end of 2000. However, the commercial value of brown seaweed remained constant at around

US$1.8 billion. The volume and value of these seaweeds are largely constituted of *Laminaria japonica* followed by *U.pinnatifida* from China (FAO, 2002).

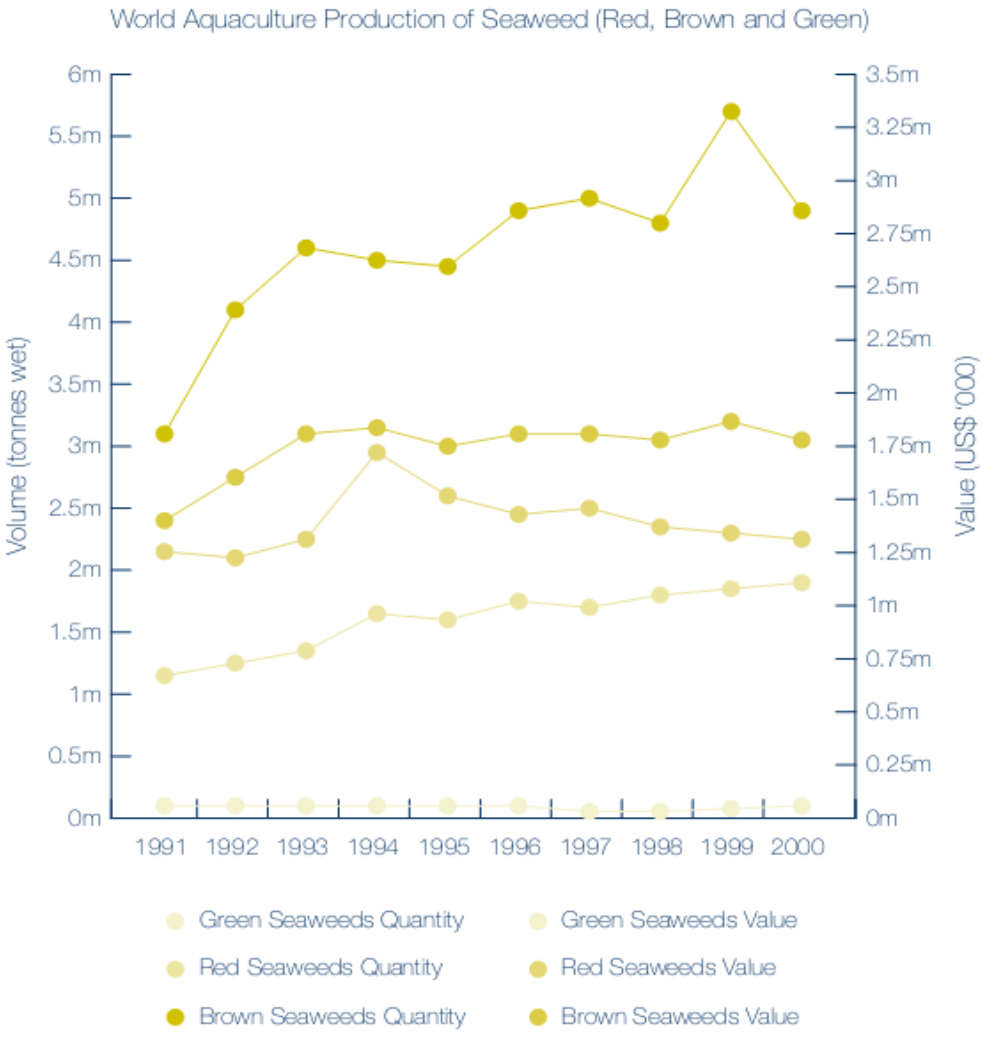

Figure 1 Graph depicting brown, red and green seaweed global production and values in aquaculture in 1991-2000 (adapted from FAO, 2002)

Garibaldi (2010) reported that in 2008, brown seaweed production remained the highest among cultured seaweeds; *Laminaria japonica* (4.8 million tonnes) followed by *U.pinnatifida* (1.8 million tonnes). The second highest production of cultured seaweeds are red seaweeds; *Eucheuma* seaweeds (*Kappaphycus alvarezii and Eucheuma spp*., 3.8 million tonnes), *Porphyra spp*., (1.4 million tonnes) and *Gracilaria spp*. (1.4 million tonnes). It is apparent that the demand for brown seaweeds remained high and profitable over the past 20 years (1991-2008) and would most likely continue to do so in the following years.

1.1.2 Aquaculture in New Zealand

Aquaculture in New Zealand has grown from a small business in the late 1950s to a significant economic activity from 1980 onwards (Figure 2). It has an average annual growth rate of 13 percent by total sales earning over the years until 2010 (Fisheries, 2010). The industry utilised over 7,700 hectares of sea space, and exports alone generated sales about $306 million during the year ending September 2011 (FAO, 2012a; Fisheries, 2010). Aquaculture in New Zealand contributed to around 20 percent of the value of the country's total seafood, and approximately 66 percent of all aquaculture production exported (FAO, 2012a; Fisheries, 2010). This makes aquaculture a vital part of New Zealand's future in the export industry.

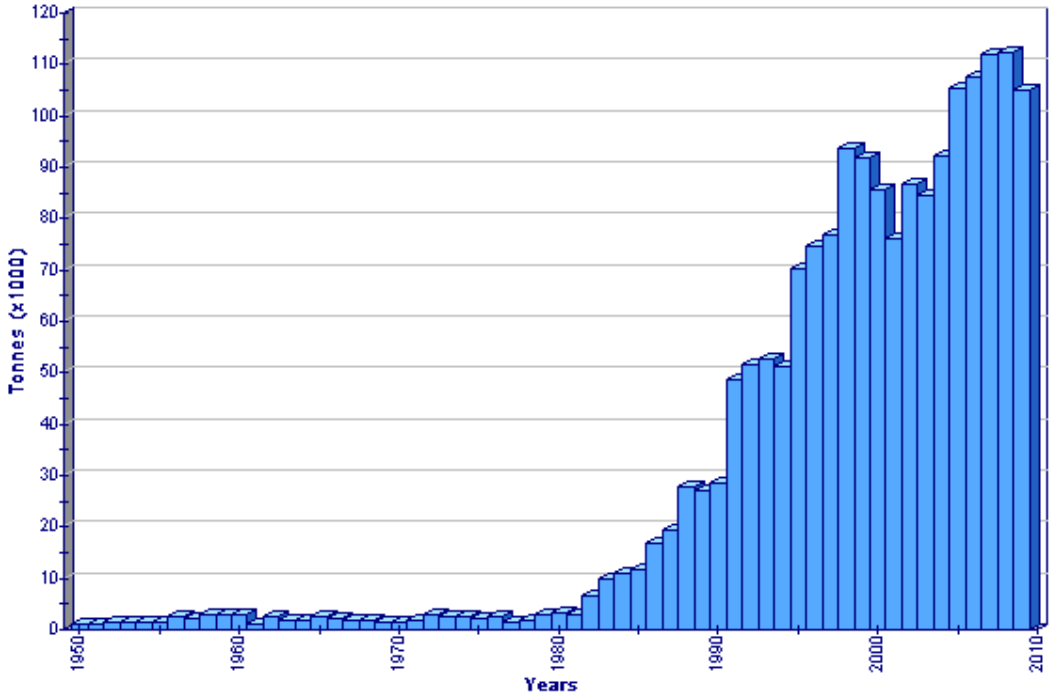

Figure 2 Graph shows the total reported aquaculture production in New Zealand (1950- 2010) according to the FAO statistics chart (adapted from FAO, 2012a).

New Zealand has three main aquaculture species that together contribute to more than 90 percent in terms of value and volume of total aquaculture production. Greenshell mussels have been a New Zealand seafood delicacy for over a century and are the leading aquaculture export product in the country, worth over $181 million in 2006 (Fisheries, 2008). New Zealanders have been farming these mussels for the past 30 years and since then, domestic and international demands have grown dramatically.

King salmon is the second largest aquaculture species exported from New Zealand. It was introduced to the country with a number of other salmon species as a sport fish in the early 1900s, but only King salmon adapted to the environment. In 2006, a further NZD$42 million worth of King Salmon was exported mainly to Japan followed by Australia and USA (Fisheries, 2008). Pacific oysters was the third largest exported aquaculture species from New Zealand and was worth NZD$18 million in 2006 (Fisheries, 2008).

The New Zealand government has recognized aquaculture as a key area for the development of its economy in a rapid pace but yet in an environmentally sustainable manner (Aquaculture, 2012b). This industry does not only bring export dollars into New Zealand, but will also create more opportunities for employment and service industries into the country. Therefore in 2006, the aquaculture industry in New Zealand developed a strategy that aimed to achieve sales of $1billion per annum by 2025, with sales target that was equivalent to New Zealand's wool and wine industries (Aquaculture, 2012b; Burrel, Meehan, & Munro, 2006; Fisheries, 2008). In order to meet the $1 billion target, innovations in existing and new space, species, products and markets are required.

In May 2010, the New Zealand government permitted the harvest of *U.pinnatifida* for the first time for commercial use, which was a million dollar business in Asia. The revised rules allowed farming in selected heavily infested area; harvest when grown on artificial surfaces or cast ashore in selected areas, and from natural surfaces except part of a specific programme to control *U.pinnatifida* (Aquaculture, 2012a; Forestry, 2010). The Ministry of Agriculture and Forestry (MAF) announced three geographical areas; Wellington, Marlborough sounds and Banks Peninsula, where farming and harvesting of *U.pinnatifida* was allowed subject to MAF approval (Aquaculture, 2012a). This edible seaweed has great potential to expand the aquaculture sector of New Zealand locally and internationally. It can be harvested for human consumption, health and pharmaceutical products and also as fertiliser and fish feed. In 2004, Aquaculture New Zealand (AQNZ) estimated that *U.pinnatifida* could return between $500/tonne for bulk seaweed used in agricultural products to more than $1000/tonne for higher grade *U.pinnatifida* for human consumption (Carter, 2004). However with the revised rule in 2010, the increased flexibility for harvesting

and farming of *U.pinnatifida* would encourage more economic potential. Based on overseas values, the return value of *U.pinnatifida* may exceed the estimated value made by AQNZ in 2004.

1.2 History of *U.pinnatifida*

U.pinnatifida (Harvey) Suringar 1873 is a Japanese kelp native to the temperate shores of north-western Pacific along most of the coasts of Japan (Wallentinus, 2007); southern Korea and Chenshan Island of the Zhoushan Archipelago near Shanghai in China (Hay & Villouta, 1993). *U.pinnatifida*, also known as wakame in Japan are edible brown seaweed (*Phaeophyceae*) in the order *Laminariales*, that play a crucial role in marine environments both as food and marine habitats. Historically, *U.pinnatifida* was traditionally regarded as a luxury food in both Korea and Japan (Nisizawa, Noda, Kikuchi, & Watanabe, 1987). However with increased *U.pinnatifida* faming and harvesting in recent years, overproduction of *U.pinnatifida* has resulted in dramatic price reduction. Although the market for *U.pinnatifida* in Japan is large, *U.pinnatifida* is more in demand in the Republic of Korea than in Japan.

International shipping in the 20th century was responsible for the global spread of *U.pinnatifida*. It was accidently introduced with oysters that were exported from Japan to the French Mediterranean Coast (Wallentinus, 2007). *U.pinnatifida* was then intentionally transplanted from the Mediterranean Sea to Brittany, north western France in 1983 for farming purposes; and was later seen dispersed across the north-eastern Atlantic through recreational boats or shipping (Wallentinus, 2007). In the late 1980s, it was reported in both New Zealand and Australia and was accidently introduced by shipping from Asia (Forrest, Brown, Taylor, Hurd, & Hay, 2000). It also spread to Argentina in the early 1990s (Wallentinus, 2007). Since the early 2000s, *U.pinnatifida* was reported on all continents except Africa and Antarctica (Wallentinus, 2007). *U.pinnatifida* was discovered in Los Angeles Harbour, Southern California in March 2000 (Silva, Woodfield, Cohen, Harris, & Goddard, 2002).

1.2.1 Description

U.pinnatifida is an annual, heteromorphic life-cycle plant that is yellowish-brown to brown in colour. A mature *U.pinnatifida* sporophyte (spore-producing phase) can be divided into the blade (lamina), sporophyll and root-like formations, haptera (Figure 3). It can reach a total length of 1-2 metres in its native habitat; 3 metre cultivation has

also been recorded in Japan (Silva et al., 2002). The blade consists of a midrib that runs in the central of the seaweed with a large, translucent blade on both sides that can be up to 50-80cm wide. The sporophyll of the sporophyte grows at the base of the blade. The sporophyll has a characteristic thickened sinuate structure that develops along its two flattened edges and is a more yellowish colour than the blade. The mature sporophyll can grow up to 20cm in length and 4cm in width. Juvenille *U.pinnatifida* had a holdfast, stipe and undivided blade (Figure 4a). It was often not easily distinguished from a juvenile New Zealand kelp *Ecklonia radiata,* until the development of midrib or sporophyll becomes visible (Figure 4b).

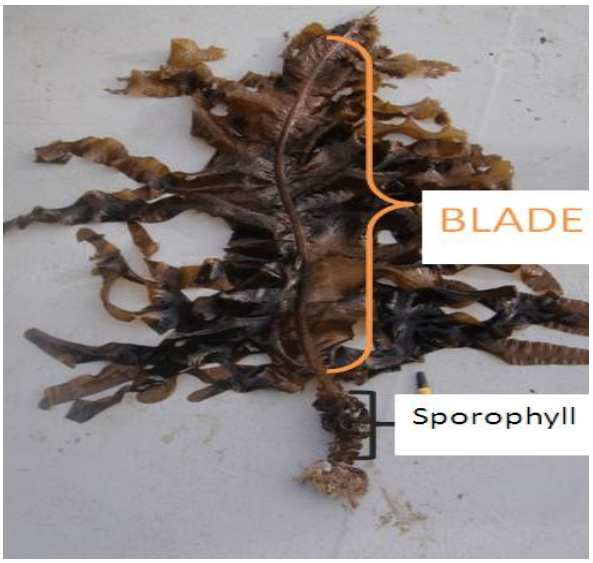

Figure 3 A mature *U.pinnatifida* collected from Marlborough Sounds, New Zealand (Photo credits: Sayvisene Boulum)

(a) (b)

Figure 4 (a) Young *U.pinnatifida* (b) comparison between New Zealand kelp *Ecklonia radiata* (left) *and U.pinnatifida* (right) (adapted from MAF, 2012).

1.2.1 Biology

U.pinnatifida has a heteromorphic, diplohaplontic life cycle that alternates between separate microscopic female and male gametophytes, and with a macroscopic sporophyte (Figure 5). *U.pinnatifida* is a cold season plant exhibiting maximum photosynthetic rates during winter and it deteriorates in the late summer and autumn; growing best in sea temperature of less than 17^{o}C (Gibbs, Hay, & Dodgshun, 1998). During summer as the sea surface temperature warms, the sporophyll of the sporophyte releases millions of minute (8-9 x 4-5 µm), motile, biflagellate asexual spores. The optimum liberation of spores was in the range between 17-22^{o}C (Hay & Gibbs, 1996). The spores then attached to the substratum and rapidly developed into microscopic filamentous male or female gametophytes. This usually occurs 1 to 6 hour after liberation, but they can remain swimming and viable in the water for several days. The female gametophytes consists of one to a few cells, bearing the oogonia but is relatively large in diameter (Hay & Gibbs, 1996; Wallentinus, 2007). The male gametophyte bearing the antheridia is multicellular and filamentous. The gametophytes have been reported to have a dormancy period for at least three years (Fisheries, 2001) that could act as a seed bank especially at low light environment (Wallentinus, 2007). As the sea temperature increases to above 24-25^{o}C (Hay & Gibbs, 1996), they develop into thick walled, spherical cells filled with chromatophores and enter resting stages, which allow them to survive adverse conditions and even temperatures up to 30^{o}C (Hay & Gibbs, 1996).

When the sea surface temperatures drop with the onset of winter, sex organs are formed in gametophytes. The adherence and germination of zoospores are optimum when the sea temperature was below 20^{o}C and gametophytes attained sexual maturity within 20 days. When the temperature fell below 20^{o}C, zoospores released from the antheridia fertilised the non-motile oospheres retained in the female gametophytes, and the resultant diploid cell develops into a new young sporophyte plant (Hay & Gibbs, 1996). The young sporophyte become apparent in mid-late autumn and grows rapidly throughout winter to spring (May to November in New Zealand). FAO (2012) reported that *U.pinnatifida* has a growth rate of 1cm per day. Mature sporophytes are by then ready to be harvested for consumption or commercial purposes. The sporophyte then releases its spores in the late spring or summer before entering senescence phase in late summer and autumn.

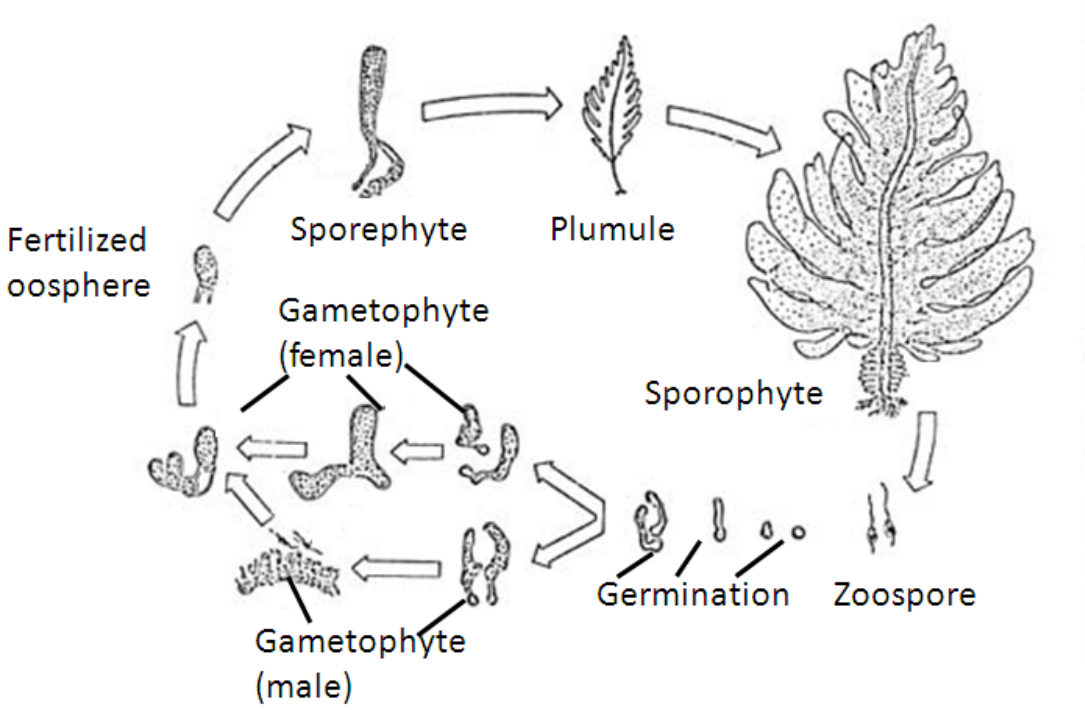

Figure 5 Life cycle of *U.pinnatifida* alternating of generations (redrawn from Tae, 2012).

1.2.1 Global production for *U.pinnatifida*

U.pinnatifida has a potential economic value both as a food source and to the pharmaceutical industry. According to Fishery and Aquaculture Organization of the United Nations (2012), a review of the total world *U.pinnatifida* production showed a tremendous increase from 300,000 tonnes in 2002 to 2.4 million in 2006 and remained constant at approximately 1.8 million tonnes since 2008 (Figure 6). China, Korea and Japan are currently the three leading countries producing *U.pinnatifida* in the world. *U.pinnatifida* harvested in South Korea dominated the overall production, constituting 42% of the total wet weight, followed by *Porphyra,* 28.4% and *Laminaria*, 26.4% in 2006 (Yoon, 2008). Since the mid 1970's, domestic cultivation of *U.pinnatifida* in Japan slowly declined. Gradually, increasing demand for *U.pinnatifida* and *U.pinnatifida* products from locals could no longer be compensated from Japan production alone. Hence *U.pinnatifida* was mainly imported from Korea since the early 90's (Hay & Gibbs, 1996). However, the product from Korea was considered poor by all Japanese processing companies and an alternative country was sought for high quality of *U.pinnatifida* to Japan (Hay & Gibbs, 1996). China has recently become the main producer of *U.pinnatifida* due to the earthquake in early March 2011 in Japan, which

has devastated the main production base in Fukushima. *U.pinnatifida* exports from China increased by nearly 30% to $5.5 million in March 2011 (Yan, 2011).

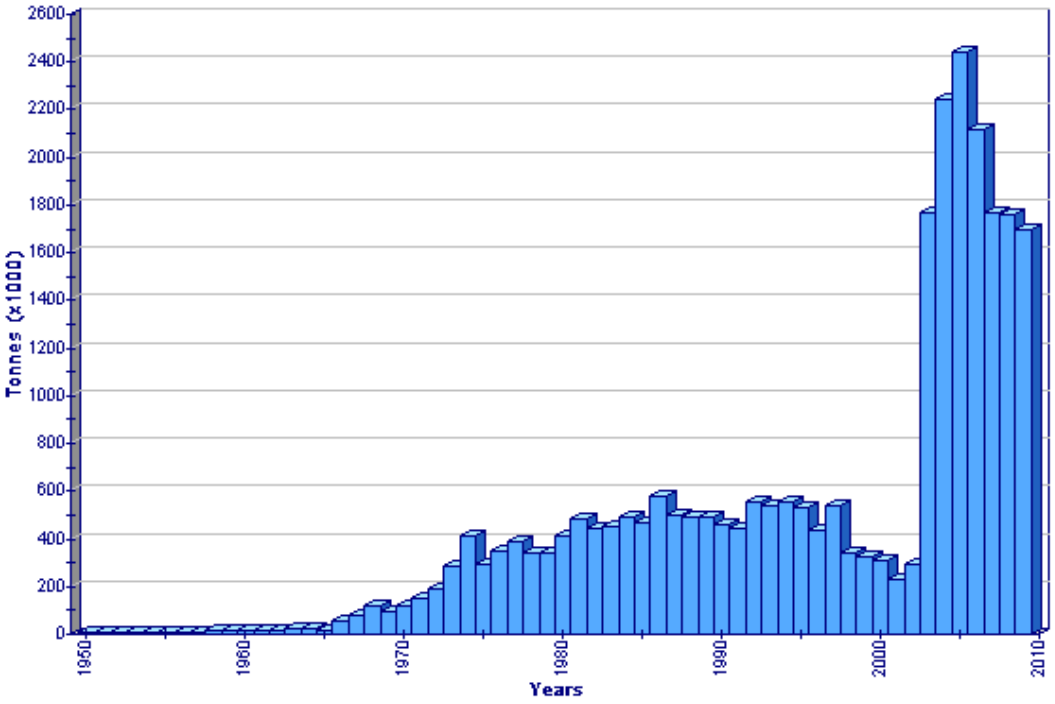

Figure 6 Global production for *U. pinnatifida* from 1950-2010 (adapted from FAO, 2012b).

1.2.1 *U.pinnatifida* (wakame) as food

U.pinnatifida is essentially a staple of the Japanese and Korean diets. It has been harvested from nature over several centuries and preserved in various ways. It is most commonly found in miso soup and salad in Japan. In the Korean culture, wakame soup are served to the pregnant and nursing women as it was known by the locals as an effective way of stimulating healthy breast milk production and recovery from labour. Wakame was not only delicious but was high in fibre and low in joule content that increased its popularity to be consumed as a daily part of the diet (Nisizawa et al., 1987). Although *U.pinnatifida* is brown in colour when harvested, preservation of *U.pinnatifida* turns them green. Wakame is produced various form of processing methods (Nisizawa et al., 1987; Watanabe & Nisizawa, 1984).

1.2.1.1 Suboshi and haiboshi wakame

Suboshi wakame was one of the oldest methods to preserve *U.pinnatifida* (Nisizawa et al., 1987; Watanabe & Nisizawa, 1984). After harvesting, *U.pinnatifida* is

washed repeatedly with seawater and then freshwater. The central midrib is removed leaving the blades and they are then dried in the sun or a hot air dryer. Although simple, this method often produces products that fade and soften during storage due to the remaining activities of various enzymes such as chlorophyllase and alginate. To overcome this, *haiboshi wakame* is developed. This process is similar to *suboshi wakame* but ash from wood or straw are mixed into fresh seaweed, dried in the sun for two to three days, then place into a plastic bag and kept in the dark. The alkalinity of the ash inactivated the enzymes. The seaweed is then washed and midrib removed before being re-dried again. *Haiboshi wakame* has a deeper colour and can be kept for a longer period.

1.2.1.2 Ita wakame

This product is a stretched wakame that undergoes a similar processing procedure as *suboshi wakame (Nisizawa et al., 1987)*. *U.pinnatifida* is spread on a hurdle of reed to dry in the sun that results in a thin board product. They are then shaped into a uniform size before packaging.

1.2.1.3 Blanched and salted wakame

Blanched and salted wakame is reported to be the major wakame product (Nisizawa et al., 1987; Watanabe & Nisizawa, 1984). Fresh wakame was firstly blanched into water 80^{o}C for 1 minute and cooled quickly in cold water for another minute. Then 30% of salt is added to its actual wet weight, and mixed thoroughly. The seaweed is then left to cure for 24 hours to dehydrate the wakame. The excess liquid is drained and the seaweed is stored at -10^{o}C. Midribs are then removed from the blade prior for sale. This product is manufactured on a large scale, has a fresh green colour and can be stored for longer periods at low temperature.

1.2.1.4 Cut wakame

Cut wakame is one of the most popular dried wakame products due to its convenient form. It is regularly found in various instant foods such as noodles and soups. Like blanched and salted wakame, wakame is boiled and salted to remove excess water. However instead of storing the product at lower temperature, cut wakame is dried in a flow through dryer. Therefore, this product can be stored at room temperature. It has a long storage life and fresh green colour when rehydrated.

1.2.1.5 Other wakame products

The wakame sporophyll also known as mekabu in Japanese is considered to have a lower utility value as a food product. However in recent years, some Japanese started consuming mekabu. Mekabu has been reported to be richer in minerals and phytonutrients than its blade and midrib (Choice, 2012). It is also very low in calorie and fat. Sporophylls are harvested as a by-product to extract bioactive compounds such as fucoxanthin and fucoidon for nutraceutical applications (Ngo et al., 2010).

1.3 Distribution of *U.pinnatifida* in New Zealand

The first discovery of *U.pinnatifida* in New Zealand was in the Wellington Harbour in 1987 (Stuart, 2004). The kelp was unintentionally introduced into the country from ballast water discharged via shipping from Asia. Since its first discovery in Wellington harbour, *U.pinnatifida* widely spread to many East coast ports and harbours between Gisborne and Stewart Island (Figure 7). Translocation of *U.pinnatifida* within New Zealand occurred via vessel fouling due to the close association of many populated areas frequented by vessels. This included ports and Harbours at Gisborne, Wellington, Porirua, Malborough Sounds, Nelson, Lyttelton, Akaroa, Timaru, Oamaru Bluff and Halfmoon bay (Stuart, 2004). Researchers suggested that drifting mooring buoys and towed navigational buoys were responsible for the dispersal (Wallentinus, 2007). More recently, marine farming activities caused the translocations of *U.pinnatifida* in Big Glory Bay, Goldern Bay, Wainui Bay and the Firth of Thames (Stuart, 2004).

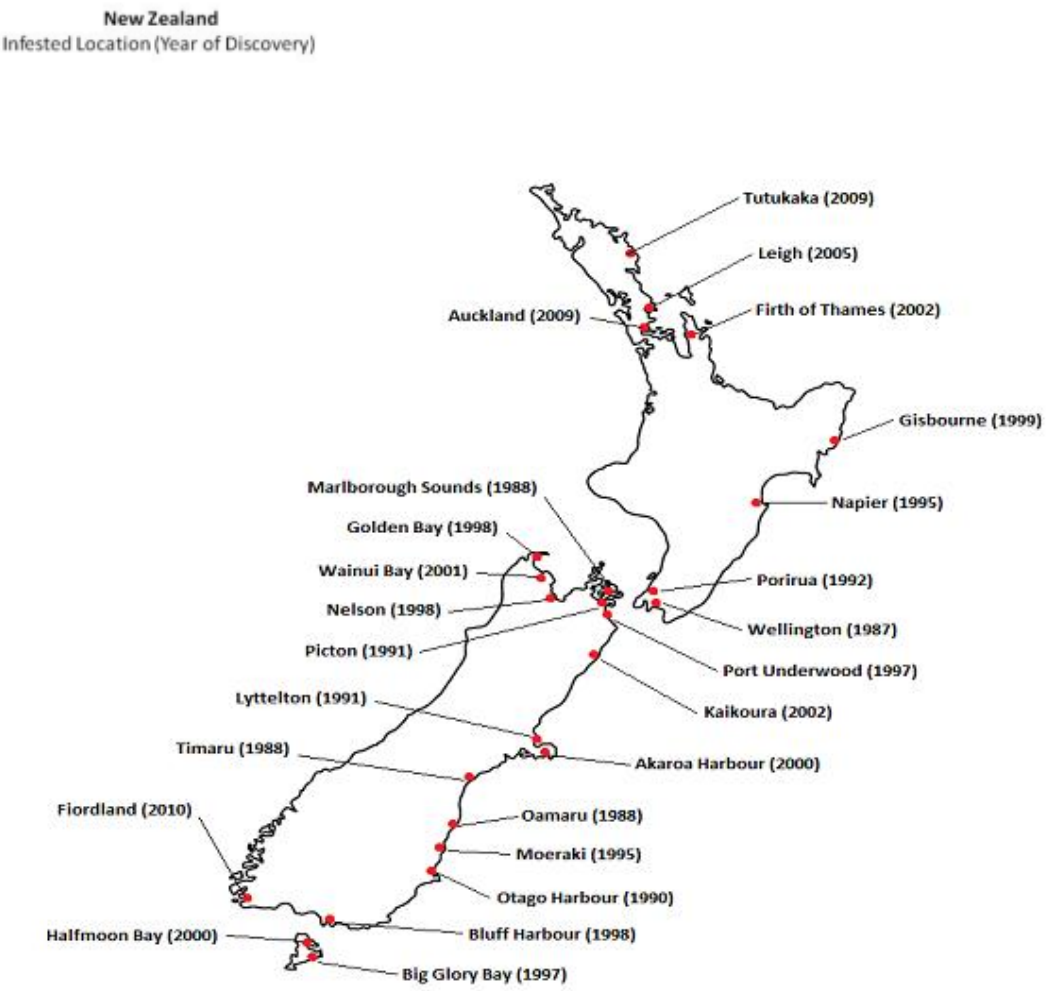

Figure 7 Known dispersal of *U.pinnatifida* about New Zealand with its year of discovery (redrawn from Carter, 2004 and Stuart, 2004)

U.pinnatifida did not appear to be an aggressive species but was described to be the third most invasive seaweed in Europe (Dean & Hurd, 2007; Wallentinus, 2007) and ranked top 100 invasive species in the world. They often contribute to a fouling problem which affects ships, boats and also structures used in aquaculture and molluscs growing on the seabed(Wallentinus, 2007), due to their ability to colonize artificial substrates and disturbed areas rapidly. They are often found on rock and immersed artificial substrates, such as hulls of boats and mooring ropes. It also has a high tolerance for extreme conditions and its gametophytes are able to survive being out of water for more than a month, which explains its introduction to distant areas. They are also capable of competing with other seaweeds for resources and often seen growing from the low water neap tide mark down to 15-18m depth. In New Zealand,

the Ministry of Fisheries (2001) described *U.pinnatifida* as having potential impacts to decrease the recruitment of paua by displacement of native coralline algae; displacement of native macroalgal communities; and decreased encrusting and sub-canopy sessile diversity.

Although Biosecurity New Zealand lifted the restriction on *U.pinnatifida* harvest, *U.pinnatifida* remains an unwanted organism in New Zealand under section 164c of the Biosecurity Act 1993. The main emphasis was trying to remain *U.pinnatifida* free in some valued areas, and curb the spread of *U.pinnatifida* to the Sub-Antartic and the Chatham Islands (Wallentinus, 2007).

1.3.1 The advantage of harvesting *U.pinnatifida* in New Zealand

Since its discovery in New Zealand, several food processing companies, exporters, mussel farmers and horticulturists have become interested in the possibility of commercially cultivating *U.pinnatifida* for export to Japan (Hay & Gibbs, 1996). New Zealand is famous for its high standards of coastal water quality. For example, shellfish harvested in New Zealand are one of the few in the world that do not require depuration before processing due to the pathogen free aquatic environment and relative absence of inorganic toxins (Fisheries, 2008). This will give New Zealand a greater competitive edge to produce high standard *U.pinnatifida* in global markets. Besides that, Asian consumers traditionally prefer to buy fresh *U.pinnatifida* but it was impossible for Asian immigrants in New Zealand as *U.pinnatifida* here was imported frozen or dry-packed. Thus, harvesting *U.pinnatifida* has potential market growth in both New Zealand and Asia.

In commercial mussel farms, *U.pinnatifida* is found growing along with the mussels on the longlines supported by floats (Figure 8). This often causes problems in harvesting the mussels. As *U.pinnatifida* was previously not allowed to be harvested, mussel farmers discarded these valuable resources back to the sea. Thousands of tonnes of *U.pinnatifida* that grew on these mussel lines around the country went to waste every year. Moreover, attempts to remove this fouling pest over the years have been futile. Thus, harvesting *U.pinnatifida* can benefit both the economy of the country, turning this fouling pest from a disadvantage to an advantage.

Figure 8 *U.pinnatifida* growing along with mussel in Marlborough Sounds (Picture credits: Sayvisene Boulum)

The concentrations of mass seaweed production in Asia result from cultural and historical traditions as well as social-economic aspects. In recent years, other countries such as North America and Europe have attempted efforts to establish seaweed aquaculture. However there are only a few places in the world where *U.pinnatifida* is able to grow. China, Japan and Korea are currently the largest producers exporting *U.pinnatifida*. Outside Asia, France was reported to cultivate *U.pinnatifida* for local demands in a small scale (5-8 tonnes wet weight per year) (Werner et al., 2006). It is sold as dried wakame on the French food market but the production is less stable due to the limited demand for *U.pinnatifida* in the French market. Due to the increased Asian immigrants and overseas students in New Zealand and Australia; Oceania is predicted to have a greater demand for *U.pinnatifida*.

1.4 Antioxidants in general

The formations of reactive oxygen species (ROS) such as superoxide anion, hydroxyl radical and hydrogen peroxide are natural byproducts of the normal metabolism of oxygen that have crucial roles in homeostasis and cell signaling in human body. Under normal circumstances, cells are able to defend themselves against ROS damage with enzymatic antioxidants such as superoxide dismutase, catalase and glutathione peroxidase (Tierney et al., 2010), and non-enzymatic antioxidants; Vitamin E, C and glutathione. However, during times of environmental stress (eg, heat

exposure or UV), ROS levels increase gradually and the production of antioxidants in the human body doesn't compensate for the increase. Accumulation of ROS in the body can result in oxidative damage to cellular components leading to cell death and tissue injury. This is associated with the onset of a variety of chronic disease states in human including certain cancers (Matanjun et al., 2008) and inflammatory diseases (Tierney et al., 2010). Hence, consumption of antioxidants has been advocated to be useful for health as they are found to help neutralise these excess free radicals produced in the body (Matanjun et al., 2008).

Antioxidants are described as a "substance that when present in low concentrations relative to the oxidisable substrate significantly delayed or reduced oxidation of the substrate" (Halliwell, Zhao, & Whiteman, 2000). They protect the body by reacting with the ROS to halt the process of oxidation with cellular. One antioxidant molecule can only react with a single free radical. Therefore, there is a constant need to replenish antioxidant resources endogenously or through supplementation. Many natural and synthetic compounds have been investigated over the decades for their efficacy to protect against oxidative stress (Heo & Jeon, 2009). Antioxidants from natural sources are preferred by consumers due to the concerns about the toxic and carcinogenic effects of synthetic antioxidants.

Plants, including fruits and vegetables are already well known sources that contained a wide range of antioxidants. Recently, there has been increased interest in the antioxidant capacity of algae due to epidemiological evidence linking the habitual consumption of seaweed to reduced risk of particular chronic diseases in the Japanese and Chinese (Yuan, 2007). Seaweed was reported to contain a range of antioxidants that are highly beneficial such as fucoxanthin (Yuan, 2007) and fucoidan that are not found in fruits and vegetables.

1.5 Antioxidant in seaweed

The strong antioxidant activities in seaweeds are present as chemical protection mechanisms. Microalgae are frequently exposed to a combination of strong light and high oxygen concentrations that lead to the formation of ROS and other strong oxidising agents but damage in the structural components (polyunsaturated fatty acids) or any serious photodynamic damage are seldom found. In order to survive these

harsh marine environments, seaweeds have developed strong protective antioxidative defense systems that consist of an array of antioxidative compounds that may or may not work synergistically to limit oxidation.

Seaweeds contain antioxidant substances of very different nature that may either be water soluble or lipid soluble compounds. Water soluble antioxidants such as polyphenols, phycobiliproteins and vitamins (vitamin C) react with oxidants in the cell cytosol and blood plasma as an excellent free radical scavenger (de Quiros, Frecha-Ferreiro, Vidal-Perez, & Lopez-Hernandez, 2010; Sies, 1997). On the contrary, lipid soluble compounds such as carotenoids and tocopherols can act as free radical scavenger and singlet oxygen quenchers (Airanthi, Hosokawa, & Miyashita, 2011; Sachindra et al., 2007) that protect cell membranes from lipid peroxidation. Brown seaweed species examined in several experiments has been reported to have a higher antioxidant capacity than red or green seaweed (Jiménez-Escrig et al., 2001; Matanjun et al., 2008; Prabhasankar et al., 2009). The following sections will cover some of the active antioxidant compounds that are rich in brown seaweeds.

1.5.1 Polyphenol

Polyphenols in general are categorized into distinct groups according to their structures by the presence of several hydroxyl groups on aromatic rings, such as the flavonoids, phenolic acids, stilbenes and lignans (Tierney et al., 2010). These compounds can be biosynthesised through either the polyketide acetate/malonate pathway (phlorotannins) or shikimate/ phenylpropanoid pathway (flavonoids) or both (Tierney et al., 2010; Yuan, 2007). They are widely found in the plant kingdom as secondary metabolites responsible for pigmentation, reproduction, growth and has mechanism defense against pathogens (Yuan, 2007; Yvonne, 2007). Polyphenols have demonstrated multifunctional antioxidant activity, due to their phenol rings acting as electron traps to scavenge peroxy, superoxide anions and hydroxyl radicals.

Phlorotannins are the only group of tannins present in brown seaweed. They comprised polymers of phloroglucinols (1,3,5- trihydroxybenze) that are biosynthesised through the acetate/malonate pathway, constituting up to 1 to 15% of the dry weight of brown algae (Burtin, 2003; Yuan, 2007). The brown to black coloration of *Phaephyceae* results from phlorotannins and their oxidation products. They are reported to be highly hydrophilic (Yvonne, 2007) and are also suggested to be

responsible for the higher antioxidant capacities of brown seaweeds (Airanthi et al., 2011). These extracts have shown protective effects against hydrogen peroxide-induced cell damage by acting as free radical scavengers (Tierney et al., 2010), reducing agents and metal chelators (Ngo et al., 2010; Tierney et al., 2010).

Phlorotannins have a potential application in functional food ingredients (Shibata, Ishimaru, Kawaguchi, Yoshikawa, & Hama, 2008). Shibata et al. (2008) investigated the antioxidant activities of phlorotannins isolated from the Japanese Laminarian brown seaweeds, *Eisenia bicycles, Ecklonia cava* and *Ecklonia kurome*. When a complex of crude phlorotannins and soybean protein was prepared, the pentamer and hexamers of phoroglucinol showed pronounced affinity for the soybean protein. The complex had almost four times stronger DPPH radical scavenging activity than that of the lyophilized soybean protein extract alone (Shibata et al., 2008). Phlorotannins extracted from these Japanese laminarian also showed significant radical scavenging activities against the superoxide anion and 1,1-diphenyl-2-picrylhydrazyl (DPPH) that were twice as effective as catechin, ascorbic acid and α–tocopherol.

1.5.2 Lipophilic antioxidants

Lipophillic antioxidants are important to the oxidative status of marine algae to protect themself from cell damage against not only dessication during tidal fluctuations but also photooxidative stress from UV radiation (Yuan, 2007). Seaweeds are rich in fatty acids especially in the lipophilic extracts (Huang & Wang, 2004). Huang & Wang (2004) reported that lipophilic constituents from seaweeds have an increased antioxidative property with increasing content of unsaturated fatty acid and low polarity of the chemical components allowing it to be readily dissolved in the lipid fraction of food. These components are useful as natural antioxidants in the food industry and extend food shelf life by retarding lipid oxidation and as a potential dietary supplement to provide health benefits.

1.5.2.1 Carotenoids

The recognition of important bioactive molecules of pigments in seaweed lipids has long been acknowledged (Huang & Wang, 2004; Yuan, 2007). Chlorophylls are the major photosynthetic pigment whereas carotenoids are the secondary photosynthetic pigment. Although both classes of pigments have antioxidant activity, it was the compounds in carotenoids which have strong biological effects to prevent disease

(Sachindra et al., 2007). Most carotenoids are polyunsaturated hydrocarbons containing 40 carbon atoms and two terminal ring systems. Those carotenoids which are composed entirely of carbon and hydrogen are known as carotenes, whereas those that also contain oxygen are termed xanthophyll (Roh, Uddin, & Chun, 2008). carotenoids are polyunsaturated hydrocarbons containing 40 carbon atoms and two terminal ring systems. Those carotenoids which are composed entirely of carbon and hydrogen are known as carotenes, whereas those that also contain oxygen are termed xanthophyll (Sachindra et al., 2007).

The carotenoid extracted from brown seaweeds is predominately rich in fucoxanthin followed by violoxanthin as the second major xantophyll and β-carotene, probably the single carotene (Burtin, 2003; Haugan & Liaaen-Jensen, 1994). β-carotene are well known as electron donors to free radicals and particularly as oxygen quenchers in vitro and vivo (Yuan, 2007). The strong antioxidative properties of β-carotene in fruits and vegetables have been well established. However relatively less attention had been paid to the physiological effects of carotenoid in seaweeds. Fucoxanthin on the other hand had recently attracted much attention due to its strong antioxidant properties that show significant anti-cancer, anti-obesity and anti-inflammation effects (Miyashita & Hosokawa, 2008). Fucoxanthin will be discussed in section 1.9.

1.5.2.2 Tocopherol

Tocopherol is another lipophilic compound that had strong antioxidant activity. It has been extracted from several brown seaweeds (*Fucus vesiculosus, Fucus serratus, Hijikia fusiformis* and *Laminaria digitata*), with δ-tocopherol making up the majority; with γ-tocopherol and traces of α-tocopherol also found (Le Tutour et al., 1998).

1.6 Mechanism of Antioxidants

Different responses of radical chain reactions are anticipated with regard to the type of antioxidants involved. Therefore for convenience purposes, antioxidants are traditionally classified into two classes; primary or chain breaking antioxidants and secondary or preventative antioxidants.

1.6.1 Primary (Chain Breaking) Antioxidants

When a free radical loses or gains an electron, a second radical is formed through the process. The second radical then undergoes the same reaction and continue to generate more unstable products until termination occurs. Primary antioxidants act by stopping these free radicals from participating in further radical chain reactions, either through delaying or inhibiting the initiation of reaction by scavenging or by inactivating free radicals as shown in the equation below.

$$L\bullet + AH \rightarrow LH + A\bullet$$

$$LO\bullet + AH \rightarrow LOH + A\bullet$$

$$LOO\bullet + AH \rightarrow LOOH + A\bullet$$

Thus, primary antioxidants: AH scavenge the free radicals of lipid radical: L• halting radical initiation or alkoxyl: LO•, peroxyl: LOO• interrupting the propagation step and forming a low reactivity antioxidant radical A• that prevents further reaction from occurring.

Polyphenol (PPH) for example is a strong chain-breaking antioxidant. It inhibits lipid peroxidation by rapidly donating a hydrogen atom to the peroxyl radical (ROO•) to form alkyl hydroperoxide (ROOH).

$$ROO\bullet + PPH \rightarrow ROOH + PP\bullet$$

The polyphenol phenoxyl radical (PP•) produced is then stabilised by further donation of a hydrogen atom and formation of quinines, or by reacting with another radical, including another phenoxyl radical, thereby interrupting the initiation of a new chain reaction (Prior, Wu, & Schaich, 2005).

1.6.1 Secondary (Preventive) Antioxidants

Secondary antioxidants retard the rate of chain initiation by scavenging initiating radicals before new radical chain reactions occur. For example, metal chelators are preventive antioxidants that chelate metal ions such as Fe^{2+} and Cu^{2+}, thereby halting metal-catalyzed initiation reaction and decomposition of lipid hydroperoxides. Iron was suggested to be the main responsible metal ion responsible for the formation of hydroxyl radicals in vivo (Huang, Ou, & Prior, 2005). Fe^{2+} was of concern because it was

readily oxidized by hydrogen peroxide (H_2O_2) and generates hydroxyl radicals. This is known as the Fenton reaction.

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^{\bullet} + OH^{-}$$

Researchers have often designed antioxidant assays by generating hydroxyl radicals without interference from other ROS using Fenton reactions like in the hydroxyl radical scavenging assay.

Antioxidant capacity assays have been broadly divided into two groups; Hydrogen atom transfer reactions (HAT) and electron transfer reactions (ET). However, only ET will be focused because both antioxidant capacity assays used in this study are ET based assays.

1.7 Electron transfer assays

ET based assays act by measuring the reduction of any compounds like radicals, carbonyls and metals (M) (Prior et al., 2005) in a sample by transferring an electron from potential antioxidants (AH).

$$M(III) + AH \rightarrow AH^{\bullet} + M(II)$$

The relative reactivity is determined primarily by the ionisation potential of the reactive functional group and deprotonation causing this reaction to be pH dependent. The reaction is also described to be slower and may take up minutes to hours to complete. The antioxidant capacity is calculated by the percent decrease in the product rather than in terms of kinetics like in the HAT assays (Prior et al., 2005).

In most ET assay, antioxidants in the sample reduce the coloured probe (oxidising agent) and cause colour changes that can be measured using a spectrophotometer. The colour changes may cause an increase or decrease in absorbance depending on the type of probe used and degree of colour changes is correlated to the concentration of antioxidants in the sample.

1.7.1 DPPH radical scavenging assay

DPPH (2,2-diphenyl-1-picrylhydrazyl or 1,1-diphenyl-2-picrylhydrazyl) radical is one of the few stable organic nitrogen centered free radical, which is effectively scavenged by antioxidants. It has a deep purple colour that is promptly decolourised

by antioxidants. The scavenging ability of the test compound can be determined at 515nm using a spectrophotometer after 30 minutes of incubation. It is simple, yet rapid and also inexpensive as the assay only requires a DPPH radical reagent, which is commercially available. This explains its widespread use for antioxidant screening in macroalgae as seen in Table 1.

DPPH assay was initially classified as an ET assay but subsequent studies using DPPH assay have reported that DPPH radicals may also act through the HAT mechanism. This phenomenon is also evident in two other ET assays; Folin-Ciocalteu Reagent (FCR) and Trolox equivalent antioxidant capacity (TEAC) (Karadag, Ozcelik, & Saner, 2009). Huang et al., (2005) suggested that as hydrogen atom abstraction in the DPPH assay was a marginal reaction that occurred very slowly depending on the solvent like methanol, ethanol and acetone, it should be mainly considered as an ET reaction. However, Prior et al., (2005) considered the DPPH assay as having both ET and HAT mechanisms because of the difficulty that exists in interpreting inhibition mechanisms of the DPPH radical without understanding the composition and structures of antioxidant tested.

Table 1 Reported antioxidant activities in extracts of the *Phaeophyceae* class using the DPPH radical scavenging assay

Phaeophycean species	Extraction solvent	Antioxidant capacity (mean values	References
Angophora crassifolia	Methanol	9.43 µg mg^{-1}	Airanthi et al., 2011
Ascophyllum nodusum	70% Acetone	EC_{50} 18.5 µg ml^{-1}	Wang, Jónsdóttir, & Ólafsdóttir, 2009
Cystoseira hakodatensis	Methanol	65.32 µg mg^{-1}	Airanthi et al., 2011
Delma australis	Methanol	IC_{50} 1.60 mg ml^{-1}	Vinayak, Sabu, & Chatterji, 2011
Dictyopteris delicatula	Methanol	IC_{50} 0.66 mg ml^{-1}	Vinayak et al., 2011

Ecklonia kurome	Aqueous	22 µmol CatEq ml^{-1}	Kuda & Ikemori, 2009
Ecklonia stolonifera	Aqueous	32 µmol CatEq ml^{-1}	Kuda & Ikemori, 2009
Eisenia bicyclis	Methanol	58.63 µg mg^{-1}	Airanthi et al., 2011
Fucus serratus	70% Acetone	EC_{50} 11 µg ml^{-1}	Wang et al., 2009
Fucus vesiculosus	70% Acetone	EC_{50} 10.7 µg ml^{-1}	Wang et al., 2009
	30-35% Ethanol	IC_{50} 11.9 µg ml^{-1}	Zaragozaì et al., 2008
	50-70% Ethanol	IC_{50} 26 µg ml^{-1}	Zaragozaì et al., 2008
	Methanol/Water	EC_{50} 3.07 g DMg $^{-1}$	Jiménez-Escrig et al., 2001
Hizikia fusiformis	80% MeOH	IC_{50} 1.41 mg ml^{-1}	Karawita et al., 2005
	Diethyl ether	23.62%	Siriwardhana, Lee, Kim, Ha, & Jeon, 2003
Kjellmaniella crassifolia	Methanol	33.46 µg mg^{-1}	Airanthi et al., 2011
Laminaria hyberborea	70% Acetone	EC_{50} 25.8 µg ml^{-1}	Wang et al., 2009
Laminaria ochroleuca	Methanol/Water	EC_{50} 29.12 g DMg $^{-1}$	Jiménez-Escrig et al., 2001
Padina antillarum	50% Methanol	IC_{50} 0.337 mg ml^{-1}	Chew, Lim, Omar, & Khoo, 2008
Padina Tetrastomatica	Methanol	14.78%	Chandini, Ganesan, & Bhaskar, 2008
	Methanol	IC_{50} 0.61 mg ml^{-1}	Vinayak et al., 2011

Sargassum horneri	Methanol/Chloroform	43.82%	Hong-Yu, Bin Wang, Chun-Guang Yu, Qu, & Su, 2010
	Methanol	28.50 µg mg^{-1}	Airanthi et al., 2011
Sargassum fusiforme	Methanol/Chloroform	24.20%	Hong-Yu et al., 2010
Sargassum kjellmanianum	Methanol/Chloroform	58.25%	Hong-Yu et al., 2010
Sargassum macrocarpum	Aqueous	8 µmol CatEq ml^{-1}	Kuda & Ikemori, 2009
Sargassum marginatum	MeOH	11%	Chandini et al., 2008
	MeOH	IC_{50} 2.87 mg ml^{-1}	Vinayak et al., 2011
Sargassum myriocystum	MeOH MeOH/Chl Ethyl acetate n-butanol n-hexane/chl water extract	15% 18% -5% 25% 12% 10%	Badrinathan S. et al., 2011
Sargassum. Pallidum	Methanol/Chloroform	29.42%	Hong-Yu et al., 2010
Sargassum ringgoldianum	Aqueous	17 µmol CatEq ml^{-1}	Kuda & Ikemori, 2009
Sargassum silliquastrum	Aqueous	5 µmol CatEq ml^{-1}	Kuda & Ikemori, 2009
Sargassum sp.	Methanol	54.9%	Patra, Rath, Jena, Rathod, & Thatoi, 2008
	Aqueous	IC_{50} 1.18 mg ml^{-1}	Yangthong, Hutadilok-Towatana, & Phromkunthong, 2009

Sargassum thunbergii	Methanol/Chloroform	38.55%	Hong-Yu et al., 2010
Spatoglossum aspermum	Methanol	IC_{50} 0.98 mg ml^{-1}	Vinayak et al., 2011
Spatoglossum variable	Methanol	IC_{50} 1.01 mg ml^{-1}	Vinayak et al., 2011
Stoechospermum marginatum	Methanol	IC_{50} 0.56 mg ml^{-1}	Vinayak et al., 2011
Turbinaria conoides	Methanol	17.23%	Chandini et al., 2008
U.pinnatifida	Methanol/water	EC_{50} 45.86 g DMg^{-1}	Jiménez-Escrig et al., 2001

1.7.1 Cupric ion reducing antioxidant capacity (CUPRAC)

The CUPRAC assay, introduced by Apak, Guclu, Ozyurek, & Karademir (2004) is a variant of the ferric reducing antioxidant power (FRAP) assay but utilises Cu^{2+} instead of Fe^{3+}. Since its introduction, it has been widely used to measure the antioxidant capacity in fruits (Guclu, Altun, Ozyurek, Karademir, & Apak, 2006), vegetables (Koksal & Gulcin, 2008) and plants (Apak et al., 2007; Celik et al., 2008). The current study carried out is the first to be performed on seaweed.

CUPRAC utilises neocuproine (2,9-dimethyl-1, 10- phenanthroline) as a chromogenic oxidising reagent which forms a colour compound, Cu(I)-chelate as a result of redox reaction with a reducing antioxidant in the following manner:

$nCu(Nc)_2^{2+}$ + n-electron reductant

-> $nCu(Nc)_2^{+}$ + n-electron oxidized product + nH^{+}

In this reaction, bis(neocuproine) copper(II) chelate oxidised the reactive reducing antioxidants to the corresponding oxidised product producing a highly coloured Cu(I)-Nc chelate, which shows maximum absorption at 450nm and can be detected using a spectrophotometer.

The FRAP assay has been extensively criticised for its in adequacies. Introduction of the CUPRAC assay has made it a more favorable assay and seen as a good replacement for FRAP (Apak et al., 2004; Apak et al., 2007; Guclu et al., 2006). The standard redox potential of Cu (II/I)-neucuproine is 0.6V, which is much lower than Fe^{3+}-TPTZ with a redox potential of <0.7V. Hence CUPRAC is able to measure a greater variety of antioxidant compounds such as glutathione type compounds, which is not detectable when using FRAP (Celik et al., 2008). The copper reaction kinetics are also observed to be faster than iron, which essentially complete within 30 min (Apak et al., 2004). CUPRAC also offers a more selective total antioxidant measurement. Prior et al., (2005) stated that reducing sugars and citric acid, which are not true antioxidants but oxidisable substrates in other similar assays are not oxidised with the CUPRAC reagent. Moreover, the redox reactions for CUPRAC is carried out close to physiological pH (pH 7) as opposed to the acidic pH (pH 3.6) in FRAP, which may suppress the reducing capacity due to protonation on antioxidant compounds. This method also allows both hydrophilic and lipophilic antioxidants to be measured simultaneously(Apak et al., 2004; Apak et al., 2007).

CUPRAC has been extensively used to analyse polyphenol (Apak et al., 2007; Celik et al., 2008; Guclu et al., 2006). In a study by Celik et al., (2008), the CUPRAC, and Folin-Ciocalteu Reagent assay, a colorimetric assay commonly used to assess polyphenolic antioxidants, had the highest correlation compared to other antioxidant assays (ABTS/persulfate and FRAP). This was because like the Folin Ciocalteu Reagent assay, CUPRAC was able to oxidise phenolic hydroxyl groups of antioxidants to their corresponding quinines. Therefore, CUPRAC is a suitable assay to assess the total antioxidant capacity in seaweed, as polyphenols are found in abundance in brown seaweed.

1.8 Fucoxanthin

Fucoxanthin is an abundant marine xanthophyll that contains an allelic bond and two epoxy groups (Figure 9). It is estimated to account for more than 10% of total carotenoid produced in nature (Miyashita & Hosokawa, 2008; Nakazawa, Sashima, Hosokawa, & Miyashita, 2009; Terasaki et al., 2009). This characteristic lipid component of brown seaweeds is bound to several proteins, together with chlorophyll a, to form fucox-Chl a-protein complexes in the thylakoid, where it acts as a light

harvesting and energy transferring pigment (Kim, Shang, & Um, 2011). Fucoxanthin in particular has been extensively investigated with respect to its strong antioxidant activity. Yan et al. (1999) demonstrated that the major active compound isolated from the carotenoid extract in *Hijikia fusiformis* was fucoxanthin that showed strong DPPH radical scavenging activity. The electron spin resonance method employed to investigate the quenching ability of fucoxanthin against the organic radicals DPPH, radical adduct of nitrobenzene with linoleic acids (NB-L) and 12-doxyl-steric acid (12-DS) indicated that in the presence of fucoxanthin, the ESR signals for these radicals are significantly decreased by 28%, 57%, and 66% respectively (Sachindra et al., 2007). From the structural point of view, it is suggested that the presence of the unique double allenic carbon (c-7', 201.84ppm) and two hydroxyl groups in fucoxanthin confer additional stability and resonance stabilisation within the conjugated double bond structure are responsible for the higher antioxidant activities (Sachindra et al., 2007; Yan, Chuda, Suzuki, & Nagata, 1999; Yuan, 2007). Although fucoxanthin is known for its strong antioxidant activities, investigations on its involvement in the antioxidant system are limited and vague (Airanthi et al., 2011).

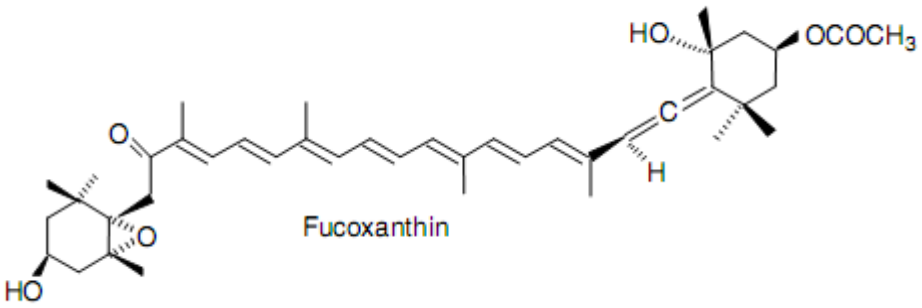

Figure 9 Structure of fucoxanthin.

In principle, the double bond in the polyene chain of a carotenoid enables it to exist in two configurations, *trans* and *cis*, that describe the disposition of substituent groups. All-*trans* fucoxanthin (~88%) was the major isomer of fucoxanthin found in fresh *U.pinnatifida* (Holdt & Kraan, 2011) and in most natural sources, followed by a mixture of 13-cis and 13' cis isomers (~9%) and 9'-cis isomer when stored in dark (Nakazawa et al., 2009). The *trans* form of fucoxanthin are generally more stable thermodynamically than its *cis* counterpart due to the dipoles of the substituent at

either side that reduces steric hindrance. Nakazawa et al. (2009) reported that the *trans* form of fucoxanthin had a faster uptake and incorporation into cellular lipids than its *cis* counterparts. However, the *cis* isomers were found to exert a higher inhibitory effect on human leukaemia (HL-60) cells compared to their *trans* counterparts. Fucoxanthin also exists in another form; fucoxanthinol, which is found in human intestinal cells and mice after consumption of fucoxanthin (Maeda, Hosokawa, Sashima, Funayama, & Miyashita, 2007; K. Miyashita et al., 2011). This suggests that fucoxanthional is the active form of fucoxanthin in biological systems.

Fucoxanthin in its pure form is vulnerable to oxidation. Nonetheless it is fairly stable in the presence of co-existing antioxidants such as polyphenol. Fucoxanthin identified in the dried form of algae stored at ambient temperature (Miyashita & Hosokawa, 2008) is present in lower amounts indicating that the process of drying could decomposed fucoxanthin. The content of fucoxanthin was also reported to vary significantly with season and the life cycle of the algae, peaking between the winter and spring (mature phase of sporophyte) and lowest during summer (senescence phase) (Terasaki et al., 2009). Studies involving the quantification of fucoxanthin both in wild and cultured algae are limited. Quantification of fucoxanthin content in several brown seaweeds based on the published literature is summarized in Table 2.

Table 2 Reported fucoxanthin contents of the Phaeophyceae class

Phaeophyceae species	Fucoxanthin content	References
U.pinnatifida		Mori et al., 2004
young thallus	0.32 mg g^{-1}	
commercial-dried	0.33 mg g^{-1}	
female gametophyte	1.64 mg g^{-1}	
male gametophyte	2.67 mg g^{-1}	
Scytosiphon lamentaria		
young thallus	0.24 mg g^{-1}	
germlings	0.56 mg g^{-1}	
Petalonia binghamiae		
young thallus	0.43 mg g^{-1}	
germlings	0.58 mg g^{-1}	
Laminaria religiosa		
young thallus	0.24 mg g^{-1}	

Ecklonia radiate	1.65 mg g^{-1}	Czeczuga & Taylor, 1987
Carphophyllum mashalocarpum	1.17 mg g^{-1}	
C. plumosum	1.44 mg g^{-1}	
Cystophora retroflexa	0.46 mg g^{-1}	
Sargassum sinclairii	0.54 mg g^{-1}	
Fucus serratus	0.56 mg g^{-1}	Haugan & Liaaen-Jensen, 1994
Cytoseira hakodatensis		Terasaki et al., 2009
Lateral branch	1.9 mg g^{-1}	
Fusiform branch	0.5 mg g^{-1}	
Main axis	0.5 mg g^{-1}	
Sargassum confusum		
Main branch (young)	1.7 mg g^{-1}	
Vesicle	2.9 mg g^{-1}	
Leaf	2.8 mg g^{-1}	
Lateral branch	2.1 mg g^{-1}	
Main branch	1.6 mg g^{-1}	
Main axis	0.7 mg g^{-1}	
Laminaria digitata	0.468 mg g^{-1}	Holdt & Kraan, 2011
Laminaria japonica	0.178-0.213 mg g^{-1}	
Ascophyllum nodosum	0.172-0.272 mg g^{-1}	
Fucus serratus	0.495-0.720 mg g^{-1}	
F. vesiculosus	0.340 mg g^{-1}	
Laminaria japonica (raw)	0.187 mg g^{-1}	Kanazawa et al., 2008
Undiara pinnatifida		
Raw	0.111 mg g^{-1}	
dry	0.084 mg g^{-1}	
Eisenia bicycles (raw)	0.077 mg g^{-1}	
Sargassum fulvellum (raw)	0.065 mg g^{-1}	
Hizikia fusiformis (raw)	0.022 mg g^{-1}	

1.8.1 Fucoxanthin as therapeutic agents

Due to its strong antioxidant properties, fucoxanthin showed remarkable therapeutic activities including anti-cancer (Nakazawa et al., 2009), antihypertensive (Tierney et al., 2010), anti-inflammatory (Heo et al., 2010; Shiratori et al., 2005) and anti-obesity effects (Maeda, Hosokawa, Sashima, Funayama, & Miyashita, 2005; Maeda et al., 2007). The mechanism underlying fucoxanthin-induced apoptosis in human leukemia cell HL-60 cells remains unclear. However, it was suggested that fucoxanthin suppressed the level of Bcl-2 protein, an apoptosis-suppressing protein, which down regulated the signal for apoptosis and hence reduced the viability of human colon cancer cell lines (Nakazawa et al., 2009). Fucoxanthin was also found to induce cell cycle arrest during G_0/G_1 phase mediated through the up-regulation of $P21^{WAF1/Cip1}$, which then inhibited the proliferation of colon cancer cells (Das et al., 2005; Okuzumi et al., 1993). Recently, fucoxanthin showed a protective effect against DNA damage and UV-B radiation when human fibroblasts were irradiated with UV-B radiation (Heo & Jeon, 2009). Cells pre-treated with fucoxanthin at 5, 50 and 100 µm/ml prior to UV-B radiation had a survival rate of 59.3%, 76.68% and 81.47%, respectively, whereas cells without fucoxanthin showed merely 43% survival rate (Heo & Jeon, 2009).

Inflammation is the fundamental process that the human body reacts to during infection, irritation or other injury in order to kill pathogen and initiate wound healing. When inflammation occurs, mediators of inflammation trigger inflammatory cells (neutrophyles, monocytes, macrophages and mast cells) to the inflamed area to kill pathogen and resulted in generation of superoxide anion and nitric oxide. However, prolonged or excessive inflammation responses induced excessive generation of ROS that are harmful to human body. Therefore, antioxidant and anti-inflammatory activities are closely related in the regulation of homeostasis in the human body. Fucoxanthin has an excellent anti-inflammatory effect and inhibits the production of nitric oxide by suppressing inducible nitric oxide synthase and cyclooxygenase 2 protein expressions (Heo et al., 2010). The releases of inflammatory cells are also significantly reduced with the addition of fuxcoxanthin in a dose-dependent manner (Heo, Park, Lee, & Jeon, 2005; Shiratori et al., 2005). Prolonged exposure to inflammatory cells and ROS causes cell destruction, therefore removal of excessive inflammatory cells are crucial to prevent chronic diseases.

As an anti-obesity agent, rats and mice fed with fucoxanthin were found to have significantly lower abdominal white adipose tissue (WAT) weights and body weights than their counterparts (Maeda et al., 2007). Western and Northern blot analyses showed obvious signals of uncoupling protein 1 (UCP 1) and its mRNA in mice fed with fucoxanthin than in control diets. This suggested the up-regulation of UCP1 expression in these mice that promote oxidation of fatty acids and heat production in WAT mitochondria leading to more efficient burning of fat; this result in loss in WAT and body weights. Moreover, intake of fucoxanthin accelerated the bioconversion of omega-3 and omega-6 polyunsaturated fatty acids to arachidonic acid and docosahexaenoic acid (Airanthi et al., 2011); important fatty acids that serve as key inflammatory intermediates and primary structural components of the brain and retina respectively.

1.8.2 Fucoxanthin as a dietary supplement and its application in food

Fucoxanthin is an effective and excellent natural food constituent that promotes health. Mice fed with a 0.27% fucoxanthin diet, equivalent to around 0.25mg.kg body weight/day for 4 weeks did not show any abnormalities or side effects (Maeda et al., 2005). Several companies in the USA have developed fucoxanthin as a dietary supplement, mostly as a weight loss supplement such as FucoPure, LipoxanThin and FucoThin (Sahelian, 2012). It was reported that fucoxanthin supplement worked by triggering UCP-1 to signal fatty acids to generate energy rather than remain stored as body fat.

Sensory evaluation has been conducted to investigate the consumer acceptability of fucoxanthin when incorporated into pasta (Prabhasankar et al., 2009). It was reported that consumers found the pasta acceptable with an incorporation of up to 20% fucoxanthin as an ingredient. Cooking and rigorousness of pasta making did not affect the quality of fucoxanthin. Oryza Oil & Fat Chemical CO., LTD, a Japanese company further incorporated fucoxanthin into other food including beverages, cakes and spreads (Oryza, 2011). The company also incorporated fucoxanthin into cosmetics products as fucoxanthin had activities that inhibited various enzymes involved in skin turnover and promoted collagen production.

1.9 Objectives

The aims of this study were as follows: (i) to determine the optimum month for fucoxanthin extraction from late autumn (June) to the onset of senescence in early summer (November) (ii) to determine fucoxanthin content of *U.pinnatifida* from two locations (Port Underwood and Pelorus Sound) in the Marlborough Sounds, New Zealand (iii) to investigate the fucoxanthin produced in different parts (blade and sporophyll) of *U.pinnatifida* (iv) to investigate the differences in fucoxanthin content between New Zealand fresh *U.pinnatifida,* New Zealand processed *U.pinnatifida*, and commercial dried *U.pinnatifida* (v) to examine the differences in the DPPH scavenging activity and reducing activity using the CUPRAC assay between New Zealand fresh *U.pinnatifida,* New Zealand processed *U.pinnatifida*, and commercial dried *U.pinnatifida*.

Chapter 2

2 Materials and Method

2.1 Materials

2.1.1 *U.pinnatifida* cultivation and sampling

U.pinnatifida sporophytes were harvested monthly from June 2011 to November 2011, from Port Underwood and Pelorus Sound of Marlborough Sound, New Zealand (Figure 10). *U.pinnatifida* grew on existing horizontal mussel ropes that spanned approximately 110m supported by buoys.

In this study, *U.pinnatifida* growing on two farms from Port Underwood (PE327 and 106) and two farms from Pelorus Sound (122 and 353) were selected for fucoxanthin analysis. Approximately 20 plants were collected from each farm and washed several times with seawater on the boat to remove foreign matter and sands. Each *U.pinnatifida* plant was then dried with paper towels. Blade and sporophyll were separated and kept in individual bags. These samples were then frozen and air-freighted to Vitaco Limited, a freeze-drying plant in Avondale, Auckland, to be lyophilised in bulk within 48 hours of frozen storage.

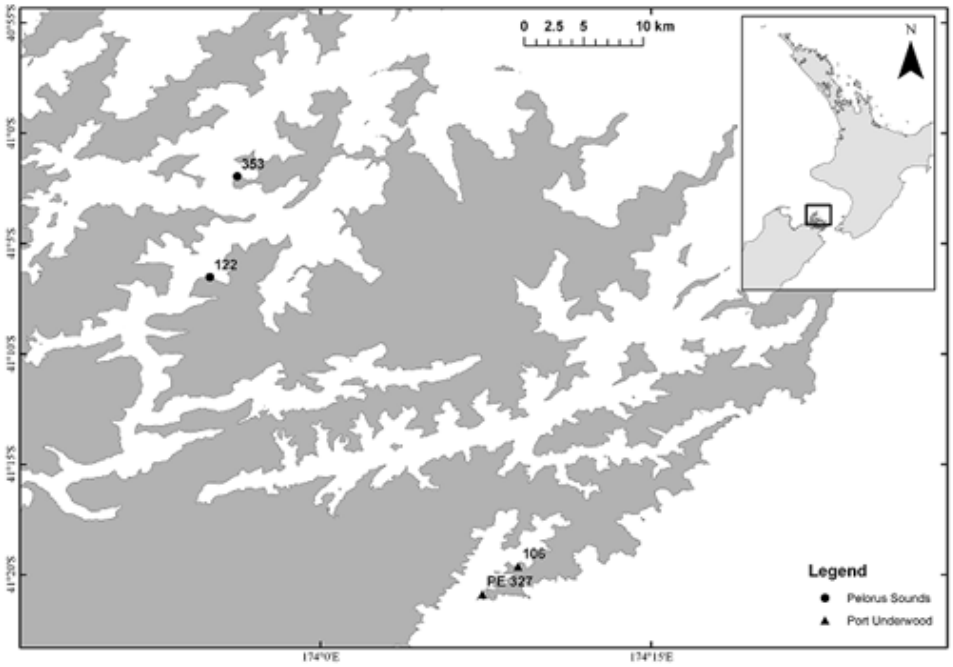

Figure 10 Map of the Marlborough Sounds showing mussel farm locations where *U.pinnatifida* was collected.

Table 3 The coordinates of mussel farms from which *U.pinnatifida* was collected

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"
Pelorus Sounds	122	41° 06' 30.89"	173° 54' 58.05"
	353	41° 01' 56.95"	173° 56' 12.55"

2.2 Chemicals and reagents

Methanol, hexane and chloroform were purchased from Thermofisher (Auckland, New Zealand) and were of HPLC grade. 2, 2- Diphenyl-1-picrylhydrazyl (DPPH), fucoxanthin and canthaxanthin standards were purchased from Sigma-Aldrich (St. Louis, MO, USA). Japanese and Korean wakame commercial products were purchased from a Korean Supermarket in Auckland. All the other chemicals and reagents used were obtained from the Auckland University of Technology (AUT) Applied Science laboratory.

2.3 Methods

2.3.1 Sample preparation

Prior to extraction, all freeze-dried *U.pinnatifida* samples were ground using a Breville CG2B Coffee 'n' Spice Grinder and sieved through a 600µm sieve to obtain fine powder. Each sample was stored in individual 200mL PET bottles and kept in the dark at room temperature until use.

2.3.2 Fucoxanthin extraction

All fucoxanthin extraction was carried out away from direct sunlight to reduce the possibility of oxidation by sunlight. Freeze dried sample weighing 100 mg was mixed with 15 ml of methanol and stirred using a magnetic bar for an hour at room temperature for an hour. The sample was filtered using a Whatman No.1 filter paper (Thermofisher, New Zealand) to remove the solids. Hexane (15ml) and water (15 ml) were added to the methanol extract and vortexed for 1 minute to remove non-polar and water-soluble compounds, respectively. The mixture was then transferred to a separation funnel and left to partition into two distinct layers. The upper phase (hexane) was discarded. The lower phase (methanol:water) was collected into a

centrifuge tube and vortexed for 1 minute with 10 ml of chloroform to extract fucoxanthin. The mixture was inserted into a 50ml poylproplene Nalgene centrifuge tube and centrifuged in a Sorvall RC5C instruments using the Fiberlite F21-8x50y rotor, Thermofisher USA at 17,000 g for 15 minutes at 4^{o}C. The organic (lipid) phase, settled to the bottom while the aqueous phase remained on the top. The aqueous phase was removed using a pipette dropper leaving the organic layer undisturbed.

The organic phase was then dried completely using a rotary evaporator at 30^{o}C. Methanol (5 ml) was added to the dried extract and the resulting solution was transferred into a glass vial. The sample in the glass vial was then flushed with argon gas to prevent oxidation and stored at -80^{o}C until further use. Seven *U.pinnatifida* samples (blade and sporophyll) from each farm were selected for the analysis.

2.3.3 HPLC analysis

HPLC was used for the quantitative analysis of the sample collected. The HPLC system consisted of a LC-20AT pump system (Shimadzu), a UV-Vis SPD-20A (Shimadzu) absorbance detector and online analysis software (LC solution version 1.25). Fucoxanthin was separated on a Luna 5µm C18 (2) (4.6mm x 250mm, Phenomenex) column. The mobile phase used was 100% methanol with a flow rate of 1ml/min and the sample injection volume was 20µl. The detection wavelength was set at 450nm to detect fucoxanthin.

2.3.3.1 Stock standards

Fucoxanthin standards (0.0078µg/ml, 0.0156µg/ml, 0.0312 µg/ml, 0.0624 µg/ml and 0.125µg/ml) were prepared in methanol and stored at -80^{o}C when not in use. Canthaxanthin was used as the internal standard. Fresh canthaxanthin standard (0.03125µg/ml) was prepared in acetone before each HPLC analysis. Fucoxanthin standard or sample (0.5 ml) was mixed with 0.5ml of canthaxanthin standard and filtered through a 0.22µm membrane filter (Phenomenex) before HPLC analysis. Each fucoxanthin standard curve set was injected in duplicates before and after the injections of all the samples.

The calibration curve was plotted:

$$\frac{peak\ area\ of standard}{peak\ area\ of\ internal\ standard} vs \frac{concentration\ of\ standard}{concentration\ of\ internal\ standard}$$

A best-fit linear regression curve was constructed. The concentrations of fucoxanthin in each sample were determined using the calibration curve. The ratio of the peak area of the analyte and the internal standard was used in the determination of the fucoxanthin concentration.

2.3.4 HPLC quality control parameters

2.3.4.1 Accuracy and precision

Accuracy and precision of the assay were determined for both intra- and inter runs. For intra-run accuracy and precision, five replicates of fucoxanthin extract were extracted from the same *U.pinnatifida* sample according to the method in section 2.3.2. The concentration of fucoxanthin for each replicate was then determined according to methods in section 2.3.3 and 2.3.3.1. The mean and standard deviation of fucoxanthin concentration were determined to calculate the coefficient of variation of the samples.

Coefficient of variation was calculated as below:

$$Coefficient\ of\ variation = \frac{Standard\ deviation\ of\ five\ replicates}{Mean\ of\ five\ replicates}\ X\ 100$$

The coefficient of variation was expressed as a percentage. The coefficient of variation around the mean observed samples concentration was 10%, indicating precision and accuracy of the method. The experiment was then repeated for an additional four days using the same *U.pinnatifida* sample to obtain the coefficient of variation for the inter-run accuracy and precision (reproducibility). The coefficient of variation around the mean observed in the inter-run samples was 14%, indicating reproducibility of the method when measured on different occasions.

2.3.4.2 Recovery

A spike and recovery experiment was carried to measure the extraction efficiency of the fucoxanthin method used in this study. A 2mg/ml fucoxanthin standard prepared in 100% methanol was added to five replicates of fucoxanthin extracts of known concentrations. The standard (125µl) was added to 100 mg of the freeze dried sample and mixed with 15 ml of methanol before stirring the replicates using a magnetic bar for an hour at room temperature. Fucoxanthin was then extracted according to the method in section 2.3.2 and concentrations of fucoxanthin were determined according to methods in sections 2.3.3 and 2.3.3.1.

The recovery was expressed as a percentage and calculated as shown below:

$$Recovery = \frac{C_{spike} - C_{sample}}{C_{added}} \; x \; 100$$

C_{spike} was the concentration of the analyte determined from the spike and recovery experiment. C_{sample} was the concentration of the known analyte. C_{added} were the sum concentration of the standard added and the concentration of the known analyte.

The recovery of this method was 75%, indicating reproducible of the procedure. These reproducibility is comparable to those published in Kai, Quitan, Sasaki, & Goto, (2011)(75% of recovery rate).

2.3.4.3 Instrument error

The instrument error of the HPLC employed in this study was measured. Fucoxanthin standard solution (0.0078µg/ml) was measured five times using the HPLC. The coefficient of variation around the mean observed in the run was 5%, indicating accuracy and precision of the instrument.

2.3.5 Processing *U.pinnatifida*

Cut wakame is one of the most popular dried wakame products because it is ready to be consumed once rehydrated. It is used for various instant foods such as noodles and soups. Most commercial products found sold in the Asian Supermarket in Auckland are processed in this form. Therefore, to investigate the antioxidant potential of New Zealand *U.pinnatifida*, the *U.pinnatifida* harvested was processed similarly to the commercial products using the method of Kantono, (2011).

Fresh New Zealand *U.pinnatifida* harvested from Farm 327 Port Underwood in the Marlborough Sounds were rinsed and placed into plastic bags on the boat. The samples were then transported to the AUT laboratory via airfreight overnight to be further processed into wakame. The seaweed was washed repeatedly with tap water to remove sand and other foreign matter; defective leaves were also removed. Salt (300g) was mixed with 1 litre of tap water in a beaker and heated up to 80^{o}C using a hot plate. The *U.pinnatifida* was blanched at 80^{o}C for 1 minute and cooled quickly in a beaker filled with ice water for another minute. The *U.pinnatifida* was then cured (3:10 (w: w) ratio of salt to *U.pinnatifida*) for 48 hours to dehydrate the *U.pinnatifida*. Cured *U.pinnatifida* was then briefly rinsed under running tap water to remove the salt and

was placed into plastic bags that were pierced. Heavy weights were then placed on the bags for 48 hours to further remove water. After pressing, the *U.pinnatifida* was dried using an oven for 24 hours at 60^{o}C. Dried seaweed was placed into zip-lock bag and stored in the dark at room temperature until use.

2.3.5.1 Total antioxidant preparation

All extraction was carried out away from direct sunlight to reduce the possibility of oxidation. Methanol (15 ml) was added to 300 mg of sample and stirred with a magnetic stirring bar for an hour at room temperature. The sample was filtered using a Whatman No.1 filter paper to remove the solids. The methanol extract was then dried using a rotary evaporator at 30^{o}C. Twenty ml of methanol was added to the dried extract and the resulting solution was transferred into a glass vial. The sample in the glass vial was then flushed with argon gas to prevent oxidation of fucoxanthin and stored at -80^{o}C until further use. Commercial Japanese (Wako Shokai and Riken), and Korean wakame products (Ottogi and Chung Jung Won) were used for comparison with the wakame produced in the laboratory. Analysis of each sample was repeated five times. These Methanol extracts were used for antioxidant analysis (DPPH radical Scavenging activity assay and CUPRAC).

2.3.1 Analysis of antioxidant activity

2.3.1.1 DPPH Radical Scavenging Activity

DPPH radical scavenging activity was measured using the method of Duan, Zhang, & Wang, (2006). Two ml of sample was mixed with 2.0ml of 0.16mM DPPH on methanol and incubated at room temperature for 30min in the dark. After incubation, the absorbance was measured at 517nm. The reading on the spectrophotometer (Ultraspec 2100 pro UV/VIS spectrophotometer, Amersham Pharmacia Biotech) was zeroed using methanol as the blank.

The scavenging activity was calculated as follows:

$$\text{Scavenging (\%)} = \left[1 - \frac{(Abs_{sample} - Abs_{sample\ blank})}{Abs_{control}}\right] X\ 100$$

Where Abs_{sample} was the absorbance of the sample with treatment, $Abs_{sample\ blank}$ was the absorbance of sample with 2.0ml of methanol and $Abs_{control}$ was the absorbance of 2.0ml of methanol with the treatment.

2.3.1.2 CUPRAC

The CUPRAC reagent was prepared according to the method described by Apak et al. (2004). Copper (II) chloride solution (10-2M) was prepared by weighing 426.2mg of CuCl2•2H2O and dissolving it in 250ml distilled water. Ammonium acetate (NH4Ac) buffer at pH7.0 was prepared by dissolving 19.27g of NH4Ac into 250ml of distilled water. Neocuproine, (Nc) solution (7.5 X 10-3 M) was prepared by dissolving 3.9mg of Nc in 96% ethanol that was further diluted to 25ml with 96% ethanol.

One ml of Cu (II), Nc and NH4Ac buffer solutions were added into a test tube. Sample (1ml) and distilled water (0.1ml) were then added to the initial mixture to make up the final volume of 4.1ml. The test tubes were manually shaken for a few seconds and left in the dark for 30 minutes. After incubation, the absorbance was measured at 450nm against a reagent blank. The reading on the spectrophotometer (Ultraspec 2100 pro UV/VIS spectrophotometer, Amersham Pharmacia Biotech) was zeroed using distilled water as the blank. The absorbances of the samples were recorded.

2.3.1 Statistical analysis

Results of all tests were considered significant if $P \leq 0.05$. Minitab® (Version 16), one-way ANOVA was carried out to test for differences in the fucoxanthin content between months, farms, locations, processed and freeze dried U.pinnatifida and commercial products. Where significant differences occurred, Tukey's HSD was employed to examine where that effect occurred. One way ANOVA using Minitab® (Version 16) was also carried out to test for differences in the DPPH scavenging assay and CUPRAC assay between processed and freeze dried U.pinnatifida and commercial products. Where significant differences occurred, Tukey's HSD was employed to examine where that effect occurred. The association between fucoxanthin and antioxidant activities of New Zealand *U.pinnatifida* was evaluated using Pearsons correlation (Minitab® Version 16).

All data analyzed using ANOVAs met the assumptions of equal variance and homogeneity using Minitab® (Version 15).

Chapter 3

3 Results

3.1 Fucoxanthin content in *U.pinnatifida*

The HPLC chromatogram of the fucoxanthin extracted from blade and sporophyll of New Zealand *U.pinnatifida* is shown in Figure 11. Fucoxanthin was detected using the UV-VIS spectrum at 450 nm. The peak corresponding to all-trans-fucoxanthin was detected at the retention time of 3.6 min. The two peaks ascribed as the cis-isomer of fucoxanthin were detected at retention times of 4.3 min and 4.5 min. The peak detected at a retention time of 8.3 min was the internal standard, canthaxanthin. An unidentified peak was detected at a retention time of 6.2 min in both blade and sporophyll. However the unidentified peak was not further purified for identification as this was not an objective of the research carried out.

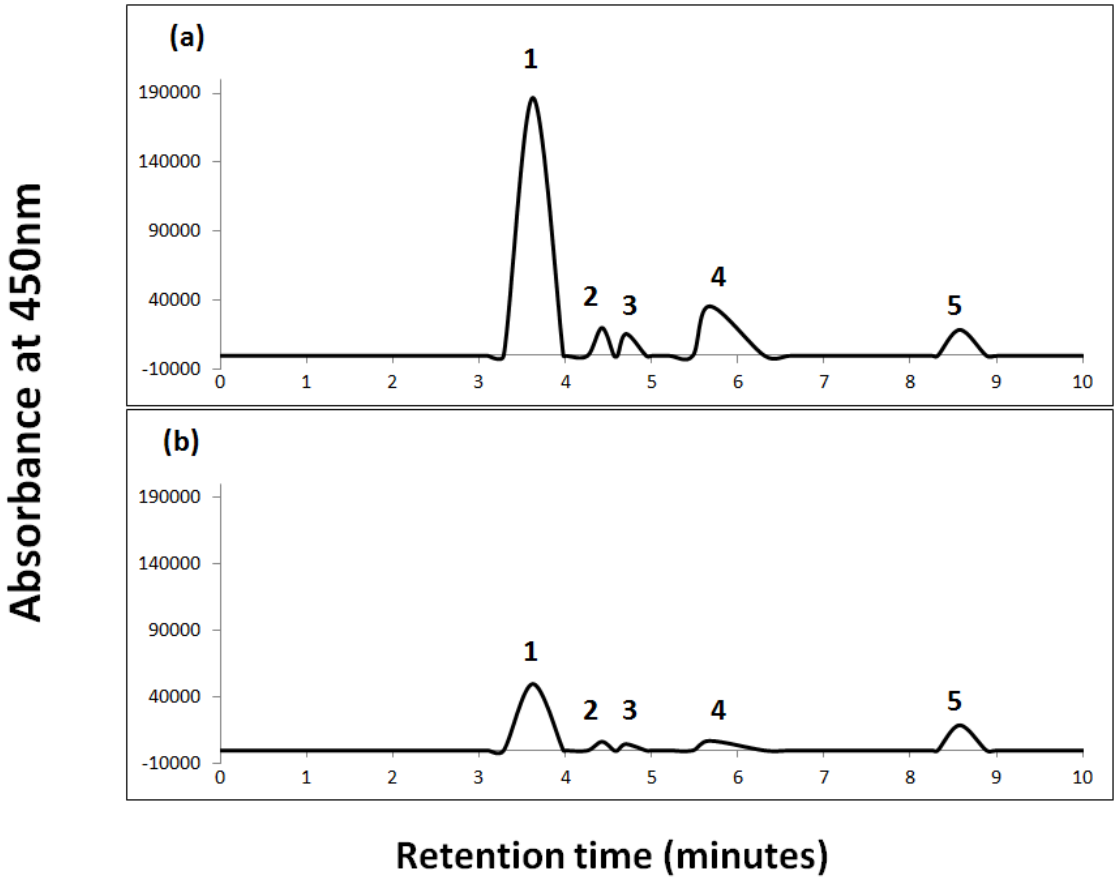

Figure 11 HPLC chromatograms of (a) blade and (b) sporophyll detected in New Zealand *U.pinnatifida*. Peaks: (1) all-trans-fucoxanthin (2&3) cis-isomer of fucoxanthin (4) unidentified peak (5) canthaxanthin, internal standard

3.1.1 Monthly variation of fucoxanthin content in blade and sporophyll

Figure 12 shows the variation in fucoxanthin content of blade and sporophyll of *U.pinnatifida* collected over the months of June to November 2011 from four different farms in the Marlborough Sounds. Months at which seaweeds were not collected are left blank. In all four farms the blade part of the seaweed generally had a higher content of fucoxanthin compared to the sporophyll.

Although sporophyll showed a similar trend, the changes with different months were not discernible. In addition, fucoxanthin content of both blade and sporophyll content from farm 353 were similar in August, October and November.

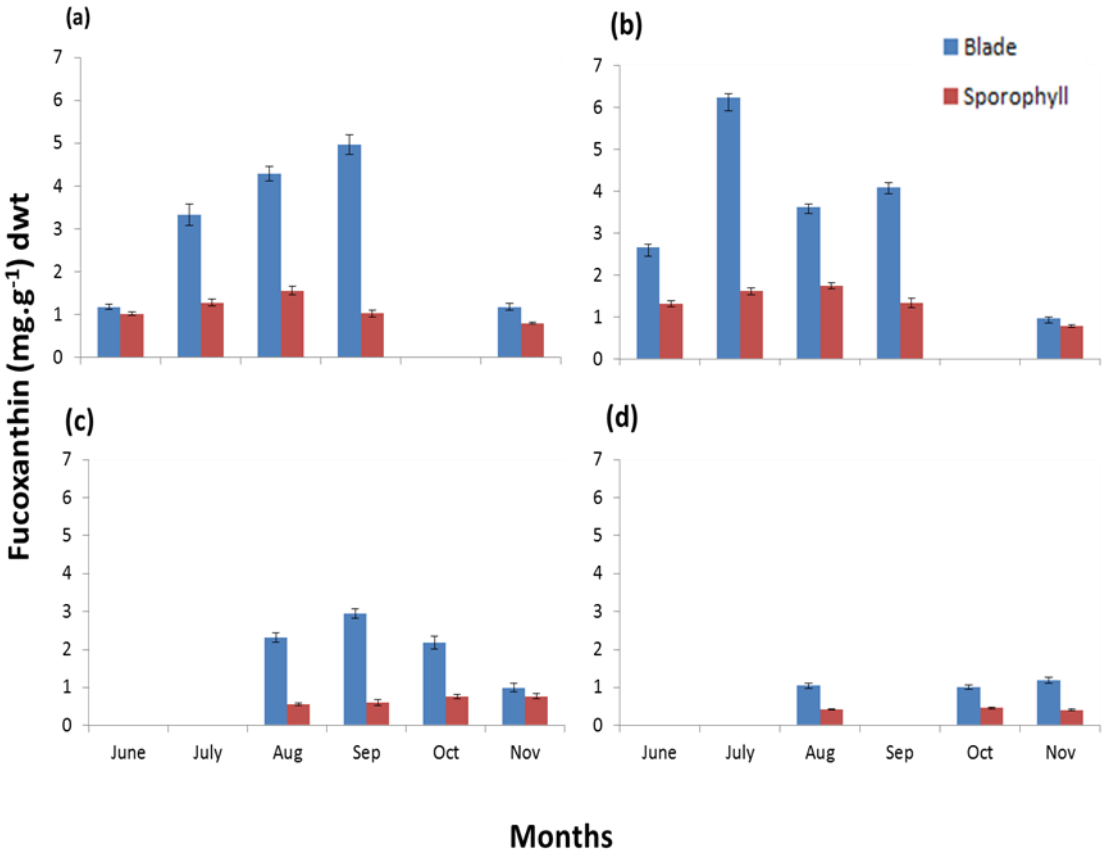

Figure 12 Monthly variations in the fucoxanthin content of blade (blue) and sporophyll (red) collected on June to November 2011 from Port Underwood (a) farm 327 (b) farm 106, and Pelorus Sound (c) farm 122 (d) farm 353.
All the values are mean ± SE of seven samples (period when seaweeds were not collected are left blank

3.1.1 Monthly variation in the fucoxanthin content from the blade and sporophyll - comparisons within farm (327, 106, 122 and 353)

3.1.1.1 Blade

There was a significant difference in the monthly changes of fucoxanthin content in the blade obtained from farms 327 and 106 in Port Underwood (Table 4). Fucoxanthin content of the blade from farm 327, was significantly high ($P < 0.001$) in July, August and September compared to June and November. Samples from July had the highest amount of fucoxanthin but were not significantly different to the August sample. As for the sample from farm 106, fucoxanthin content was significantly higher ($P < 0.001$) for July samples compared to the June, August September and November samples.

On the other hand, samples from Farm 122 in August, September and October were significantly higher ($P < 0.001$) than November in fucoxanthin content. However there was no significant difference between the monthly changes in fucoxanthin content from farm 353.

3.1.1.2 Sporophyll

In farm 327, the fucoxanthin content was significantly high ($p= 0.007$) in August compared to November. As for the sample from farm 106, fucoxanthin content was significantly high ($p = 0.002$) in July and August compared to November. These samples were however not significantly different to the June and September samples. There was no significant difference between the monthly changes in fucoxanthin content from farms 122 and 353.

3.1.2 Monthly variation in the fucoxanthin content from the blade and sporophyll – comparisons between farms in Port Underwood (farm 327 and 106) and Pelorus Sound (farm 122 and 353)

3.1.2.1 Blade

Farm 106 had a significantly higher fucoxanthin content ($p = 0.004$) compared to farm 327 in June. However, there was no significant difference in fucoxanthin content between farms 327 and 106 in July, August, September and November.

Fucoxanthin content from farm 122 was significantly higher compared to farm 353 in August ($p = 0.001$) and September ($p = 0.007$). However, there was no significant different in fucoxanthin content between farms 122 and 353 in November.

3.1.2.2 Sporophyll

There was no significant difference in the fucoxanthin content between farms 327 and 106 in June, July August, September and November.

There was also no significant difference in fucoxanthin content between farms 122 and 353 in August and October. However, fucoxanthin content from farm 122 was significantly high ($p = 0.016$) compared to farm 353 in November.

Table 4 **Fucoxanthin content (mg.g^{-1}) of blade and sporophyll of *U.pinnatifida* collected on June to November 2011 from Port Underwood.**

Location	Port Underwood				Pelorus Sound			
Body Part	Blade		Sporophyll		Blade		Sporophyll	
Farm	327	106	327	106	122	353	122	353
Month								
June	1.17±0.11^{Ac}	2.67±0.42^{Bb}	1.01±0.09^{Aab}	1.33±0.14^{Aab}	NA	NA	NA	NA
July	4.96±0.45^{Aa}	6.24±0.64^{Aa}	1.27±0.15^{Aab}	1.62±0.17^{Aa}	NA	NA	NA	NA
August	4.28±0.33^{Aab}	3.62±0.28^{Ab}	1.55±0.20^{Aa}	1.75±0.15^{Aa}	2.31±0.25^{Aa}	1.05±0.13^{Ba}	0.56±0.06^{Aa}	0.42±0.03^{Aa}
September	3.32±0.51^{Ab}	4.10±0.31^{Ab}	1.02±0.16^{Aab}	1.34±0.21^{Aab}	2.95±0.25^{Aa}	1.00±0.11^{Ba}	0.60±0.16^{Aa}	0.46±0.03^{Aa}
October	NA	NA	NA	NA	2.18±0.34^{a}	NA	1.04±0.27^{a}	NA
November	1.17±0.15^{Ac}	0.98±0.23^{Ac}	0.79±0.04^{Ab}	0.79±0.07^{Ab}	0.99±0.23^{Ab}	1.19±0.15^{Aa}	0.76±0.13^{Aa}	0.40±0.02^{Ba}

All the values are mean ± standard error of seven samples (period when seaweeds were not collected are indicated with NA, not available). Significant differences ($p < 0.05$) with months for blade or sporophyll from a farm are indicated by different superscript lower letters (column). Significant differences ($p < 0.05$) with months between farms within a location; Port Underwood (farm 327 and farm 106) and Pelorus Sound (farm 122 and farm 353) for each body part are indicated by different superscript capital letters.

3.1.1 Environmental factors that might influence the monthly variations in fucoxanthin content from blade and sporophyll from farms 327, 106, 122 and 353 in the Marlborough Sound

The monthly variations in temperature and sunshine of Port Underwood and Pelorus Sound were not available. Therefore, the monthly total sunshine exposure and the mean of daily highest temperature recorded from a nearby station, Reeftom Ews at Blenhiem (42° 117", 171° 860") was selected to investigate the possible effects of environmental factors on the monthly changes in fucoxanthin content. Data on sunshine and temperature were obtained from NIWA, the National Institute of Water and Atmospheric Research (NIWA, 2012).

3.1.1.1 Sunshine and Temperature

U.pinnatifida has been reported to have a maximum amount of pigments at lower light levels of winter (Campbell, Bite, & Burridge, 1999; Dean & Hurd, 2007; Terasaki et al., 2009). Results from this study showed an increase in fucoxanthin content in the blade with a decrease in temperature and total sunshine exposure (Figure 13 a). The highest fucoxanthin content was found in blade when the temperature was below 12^{O}C, and total sunshine exposure was below approximately 118.2 hours.

The fucoxanthin content in sporophyll however showed no changes with temperature and total sunshine exposure as seen in Figure 13 (b).

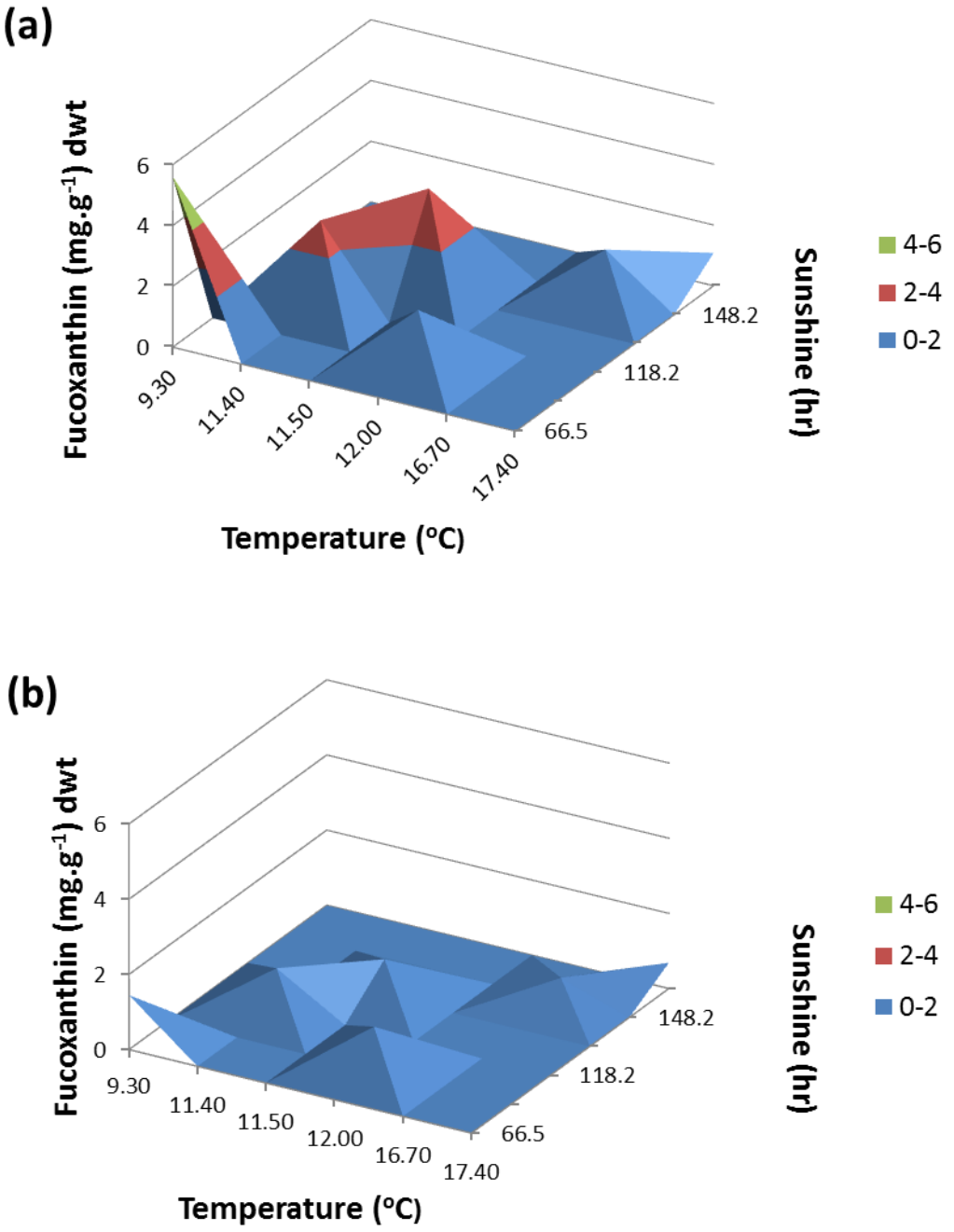

Figure 13 Fucoxanthin content in relation to total sunshine exposure (hour) and temperature (°C) (a) blade (b) sporophyll (*n* = 3).
The total sunshine exposure and temperature data was obtained from National Institute of Water and Atmospheric Research (NIWA, 2012).

3.1.1 Monthly variation in the fucoxanthin content of blade and sporophyll – comparison between two locations (Port Underwood and Pelorus Sound) in Marlborough Sound

Fucoxanthin content in the blade and sporophyll of *U.pinnatifida* obtained from two locations (Port Underwood and Pelorus Sound) were also determined. Only *U.pinnatifida* collected from August and November were considered because these

were the only two months where *U.pinnatifida* was collected in all four farms (327,106,122 and 353).

3.1.1.1 Blade

Port Underwood blade samples had significantly high ($p < 0.001$) fucoxanthin content in August compared to November (Table 5). Similarly, fucoxanthin content of the blade from Pelorus Sound was also significantly high ($p = 0.030$) in August compared to November.

Fucoxanthin content of the blade from Port Underwood was significantly higher ($p < 0.001$) compared to Pelorus Sound in August. However, fucoxanthin content from Port Underwood and Pelorus Sound was not significantly different in November.

3.1.1.2 Sporophyll

Fucoxanthin content of the sporophyll from Port Underwood was significantly high ($p < 0.001$) in August compared to November. However, fucoxanthin content in Pelorus Sound was not significantly different between August and November.

Fucoxanthin content from Port Underwood was significantly higher compared to Pelorus Sound in August ($p < 0.001$) and November ($p = 0.020$).

Table 5 Mean of fucoxanthin content ($mg.g^{-1}$) in blade and sporophyll of *U.pinnatifida* collected from Port Underwood and Pelorus Sound in August and November 2011.

Body Part	Blade		Sporophyll	
Location	Port Underwood	Pelorus Sound	Port Underwood	Pelorus Sound
Month				
August	3.95 ± 0.31^{Aa}	1.68 ± 0.19^{Ba}	1.65 ± 0.18^{Aa}	0.49 ± 0.05^{Ba}
November	1.07 ± 0.19^{Ab}	1.09 ± 0.19^{Ab}	0.79 ± 0.04^{Ab}	0.59 ± 0.08^{Ba}

All the values are mean ± standard error of fourteen samples.
Significant differences ($p < 0.05$) with months for blade or sporophyll from a location; Port Underwood and Pelorus Sound are indicated by different superscript lower letters.
Significant differences ($p < 0.05$) with months between location; Port Underwood and Pelorus Sound for each body part are indicated by different superscript capital letters.

3.1.1 Monthly variation in the fucoxanthin content of New Zealand processed and freeze dried *U.pinnatifida*

In order to study the effect of processing on fucoxanthin content, *U.pinnatifida* samples were collected from farm 327 in July, August and September to be processed into wakame.

There were no significant differences in the fucoxanthin content of processed and fresh *U.pinnatifida* with monthly changes (Table 6). However, fucoxanthin content in fresh *U.pinnatifida* was significantly high compared to processed *U.pinnatifida* in July ($p < 0.001$), August ($p < 0.001$) and September ($p < 0.001$).

Table 6 Fucoxanthin content (mg.g^{-1}) of fresh and processed *U.pinnatifida*. All the values are mean ± standard error of seven samples.

	Fucoxanthin content (mg.g^{-1})	
Type	Processed	Fresh
Month		
July	2.08±0.04^{Aa}	4.96±0.45^{Ba}
August	1.77±0.02^{Aa}	4.28±0.33^{Bab}
September	2.04±0.21^{Aa}	3.32±0.51^{Bb}

Significant differences ($p < 0.05$) with months for processed or fresh *U.pinnatfida* ndaria are indicated by different superscript lower letters.
Significant differences ($p < 0.05$) between processed and fresh *U.pinnatfida* with months are indicated by different superscript capital letters.

3.1.1 Comparisons of fucoxanthin content between New Zealand processed and Commercial *U.pinnatifida*

Fucoxanthin content between New Zealand (NZ) processed *U.pinnatifida* harvested in July and commercial dried *U.pinnatifida* products from Japan and Korea were compared. Fucoxanthin content in Japanese products (Wako Shokai and Riken) were significantly higher ($p < 0.001$) compared to NZ and Korean products (Ottogi and Chung Jung Won) (Table 7).

Table 7 Variation in the fucoxanthin content (mg.g^{-1}) in New Zealand (NZ) processed and commercial *U.pinnatifida* from Japan and Korea.

Products	Fucoxanthin content (mg.g^{-1})
NZ	2.08±0.04^{a}
Ottogi	2.00±0.07^{a}
Chung Jung Won	1.91±0.05^{a}
Wako Shokai	2.89±0.17^{b}
Riken	2.81±0.02^{b}

All the values are mean ± standard error of seven samples for fucoxanthin content.
Significant differences ($p < 0.05$) between months for each column are indicated by different superscript lower letters.

3.2 Antioxidant activity

3.2.1 DPPH scavenging activity and CUPRAC assay between – Monthly changes and comparison between New Zealand processed and freeze dried *U.pinnatifida*

DPPH scavenging activity did not show significant difference with months for both the processed and fresh *U.pinnatifida* (Table 8). However, DPPH scavenging activity in fresh *U.pinnatifida* was significantly higher compared to processed *U.pinnatifida* in July ($p < 0.001$), August ($p < 0.001$) and September ($p < 0.001$).

The CUPRAC assay also showed significant difference between the processed and fresh *U.pinnatifida* harvested in July, August and September (Table 8). Freeze dried *U.pinnatifida* had significantly higher absorbance readings than processed *U.pinnatifida* in July ($p = 0.008$), August ($p < 0.001$) and September ($p = 0.047$). Processed *U.pinnatifida* had a lower reducing activity than freeze dried *U.pinnatifida* as the decrease in absorbance in the CUPRAC assay was linked to a decrease in reducing activity (Apak et al., 2007).

Table 8 Comparison of DPPH (2,2-diphenyl-1-picrylhydrazyl free radical) radical scavenging assay (%) and CUPRAC assay (absorbance) for fresh and processed *U.pinnatifida* harvested in different months.

Assay	DPPH radical scavenging activity		CUPRAC assay	
Type	Processed	Fresh	Processed	Fresh
Month				
July	32.09 ± 3.03^{Aa}	87.18 ± 4.04^{Ba}	0.93 ± 0.07^{Ba}	1.41 ± 0.11^{Aa}
August	35.34 ± 3.51^{Aa}	91.29 ± 3.37^{Ba}	0.78 ± 0.02^{Ba}	1.30 ± 0.08^{Aa}
September	39.64 ± 2.50^{Aa}	85.01 ± 4.65^{Ba}	0.81 ± 0.02^{Ba}	1.20 ± 0.16^{Ab}

All the values are mean ± standard error of five samples.
Significant differences ($p < 0.05$) with months for processed or fresh *U.pinnatfida* are indicated by different superscript lower letters.
Significant differences ($p < 0.05$) between processed and fresh *U.pinnatfida* with months for each assay; DPPH radical scavenging activity and CUPRAC assay are indicated by different superscript capital letters.

3.2.2 DPPH scavenging activity and CUPRAC assay – Comparison between New Zealand processed and Commercial *U.pinnatfida*

U.pinnatifida processed in July was processed to investigate the differences in DPPH scavenging activity and CUPRAC of New Zealand (NZ) processed *U.pinnatifida* with commercial *U.pinnatifida* products (Table 9).

DPPH scavenging activities for NZ, Ottogi and Chung Jung Won were significantly higher ($p < 0.001$) compared to Wako Shokai and Riken.

The CUPRAC assay investigated the reducing ability of the sample, with higher absorbance readings related to stronger reducing activity. The reducing activity in Ottogi, Chung Jung Won, and Wako Shokai commercial samples were significantly higher ($p = 0.005$) compared to NZ processed *U.pinnatifida* and the commercial Riken sample.

Table 9 DPPH (2,2-diphenyl-1-picrylhydrazyl free radical) radical scavenging assay (%) and CUPRAC assay (absorbance) results for New Zealand (NZ) processed and commercial dried *U.pinnatfida*.

Products	DPPH (%)	CUPRAC assay
NZ	32.09±3.03[a]	0.92±0.07[b]
Ottogi	31.35±1.88[a]	1.20±0.05[a]
Chung Jung Won	31.49±2.51[a]	1.23±0.02[a]
Wako Shokai	26.02±3.93[b]	1.19±0.08[a]
Riken	29.53±3.48[b]	1.09±0.02[b]

All the values are mean ± standard error of five samples.
Significant differences ($p < 0.05$) between months for each column are indicated by different superscript lower letters.

Chapter 4

4 Discussion

4.1 Fucoxanthin content

4.1.1 Monthly variations of fucoxanthin content in the blade

A significant increase in fucoxanthin content was observed in the blade of seaweeds obtained from farms 327 and 106 (Port Underwood) from June and reached a maximum in July ($p < 0.001$). This was followed by a decrease in fucoxanthin content in August that remained constant in September before a rapid decrease in November. Collection of *U.pinnatifida* from the Pelorus Sound (farms 122 and 353), was only carried out in August, September, October and November as the seaweeds only started growing from August. Fucoxanthin content in the blade from farm 122 was significantly higher compared with farm 353 in August ($p = 0.001$) and September ($p = 0.007$). Samples from Farm 122 in August, September and October had significantly higher ($p < 0.001$) fucoxanthin content than in November. However, there was no significant difference between the monthly changes in fucoxanthin content from farm 353. Differences observed in the fucoxanthin content between farms from the same location could be due to the proximity of the farms from each other. Farms 327 and 106 were located approximately 2 kilometres apart from each other, whereas farms 122 and 353 were located approximately 10 kilometres away (Figure 10). The closeness of farms 327 and 106 in Port Underwood (only 2 kilometres apart) made it likely that these farms were exposed to similar environmental factors such as nutrients, sunlight, and temperature that have been reported to have an effect on the growth of seaweeds and their pigment contents (Campbell et al., 1999; Dean & Hurd, 2007; Terasaki et al., 2009)

Fucoxanthin content in the blade of seaweeds obtained from Port Underwood was similar to a study by Dean and Hurd (2007) who collected *U.pinnatifida* from Carey's Bay at the Otago Harbour, New Zealand from May to November 1996. The authors reported that the total pigment content, including fucoxanthin, increased steadily from May, reached a maximum in July, decreased in August, and remained constant until a rapid decrease in November (Dean & Hurd, 2007). Hence,

U.pinnatifida collected from the Marlborough Sounds had a similar growth pattern to the *U.pinnatifida* in the Otago Harbour.

Fucoxanthin content in the blade and sporophyll of *U.pinnatifida* collected from August and November from two locations (Port Underwood and Pelorus Sound) were compared because these were the only months where samples were collected in all four farms (Figures 12). Port Underwood was found to be a better location to harvest *U.pinnatifida* that had high fucoxanthin content in both blade and sporophyll especially in August. The differences in fucoxanthin content between seaweeds harvested from Port Underwood and Pelorus Sound could be due to differences in the growing environment. It has been reported that environmental factors were crucial in determining the growth and pigment content of seaweed (Barr & Rees, 2003; Campbell et al., 1999; Dean & Hurd, 2007). In this study, *U.pinnatifida* in Pelorus Sound grew later than those in Port Underwood. Hence, this limited the study to compare samples obtained from Port Underwood and Pelorus Sound in the months of August and November only. As Pelorus Sound was mainly surrounded by land and was located further away from the open sea compared with Port Underwood (Figure 10), the later growth of *U.pinnatifida* in the Pelorus Sound may be due to a higher freshwater imput from surrounding land washed into the sea. Freshwater was found to slow down the growth of seaweed and could even kill seaweed (Anderson, 2007; Trioba, 2007). In addition, the use of freshwater pumped from the Torrens River into saline "West Lakes" in South Australia was reported to have successfully controlled and eradicated *Caulerpa taxifolia* (*Chlorophyta*), a type of seaweed (Anderson, 2007).

4.1.2 Environmental factors

Fucoxanthin in all four farms investigated in this study showed to have variations in months except for farm 353 (Table 4). An important aspect of a successful invasion of a species is the ecophysiological characteristics to match the environment (Campbell et al., 1999; Dean & Hurd, 2007). The variations of fucoxanthin in months observed in *U.pinnatifida* might due to its highly plastic physiology and morphology to allow optimal resource acquisition and allocation in a wide range of environments. The variations in sunshine and temperature with fucoxanthin were considered in this study.

4.1.2.1 Sunlight and Temperature

U.pinnatifida was the only reported member of the *Laminariales* family that was a winter annual kelp with a maximal growth occurring at the start of winter to the end of spring when sunlight exposures are at the minimal (Dean & Hurd, 2007; Gibbs et al., 1998; Hay & Gibbs, 1996). Therefore in order to survive in such harsh environment, *U.pinnatifida* developed physiological characteristics to compete effectively with other fast growing macroalgae by increasing high metabolic rates during decreased daylength and increased canopy shading experienced during the growth phase of its life history (Campbell et al., 1999). Although fucoxanthin has strong antioxidant properties, its fundamental role in seaweeds served as a light harvesting and energy transferring pigment (Kim et al., 2011). As an accessory pigment, production of fucoxanthin was modulated by low light level during the winter period through the xanthophylls-cycle pathway (Terasaki et al., 2009). As seen in Figure 13, there was an increase in blade fucoxanthin with decrease in temperature and total sunshine exposure. It was reported that a higher pigment content was required at low light levels to harvest a similar number of photons compared to high light levels (Campbell et al., 1999). This strategy most possibly optimized pigment content in order to make best use of the light environment and conserve energy for other use such as growth (Campbell et al., 1999; Dean & Hurd, 2007).

The heat between air and the sea within a distance of the shore interchange frequently most noticeably between sea and land breezes through convection (Doshi, 2006). During daytime, warm air over land rises and cooler air over sea moves towards the land. Meanwhile at night, warm air over sea rises and cooler air over land moves towards sea. Therefore, air temperature is highly modified by sea surface temperature. In this study, the mean of daily highest temperature recorded from a nearby station, Reefton Ews at Blenhiem was selected because the sea surface temperature data was not available. Water temperature was found to be the most important factor influencing the life cycle and ecology of *U.pinnatifida* (Parsons, 1995). As mentioned in section 1.2.2, changes in water temperature largely govern the growth stage of *U.pinnatifida.* Like plants, *U.pinnatifida* seaweeds are photosynthetic and therefore produced pigments such as fucoxanthin for photosynthesis purposes (Dean & Hurd, 2007). It has been reported that *U.pinnatifida* exhibited maximum photosynthetic rates during the winter growth phase (when temperature is low) and lower

photosynthetic rates during summer senescence (when temperature is high) (Campbell et al., 1999). Therefore, the decrease in temperature and sunshine as seen in Figure 13 may have triggered a higher photosynthetic rate for growth, which in turn increased the fucoxanthin content in the seaweed.

4.1.3 Fucoxanthin content in sporophyll

There was no relationship between the fucoxanthin content in sporophyll with sunshine and temperature (Figure 13). Fucoxanthin content in the sporophyll was significantly less than blade for all months and all four farms investigated (Figure 12, $p < 0.001$). This may be because sporophylls are responsible for reproduction rather than to harvest light for growth. Hence, accessory pigments such as fucoxanthin are more abundant in blade in order to harvest light for growth. Nevertheless, sporophyll contained significant amount of fucoxanthin in all months and farms (~20-50% of fucoxanthin in the blade) that can be further extracted for commercial use. Sometimes, sporophyll is considered as a waste when *U.pinnatifida* is harvested as a food source. Extracting of fucoxanthin from sporophyll may reduce industrial waste and produce valuable health/nutraceutical products.

4.1.4 Differences in fucoxanthin content between New Zealand processed and freeze dried *U.pinnatifida*

In the present study, there was an average of 51.8% reduction in fucoxanthin content was in processed New Zealand samples compared to the freeze dried samples (Table 6). The processing of *U.pinnatifida* samples involved blanching the seaweed in 80^{o}C water for 1 minute, curing with salt for 48 hours to remove water, and finally oven drying for 24 hours at 60^{o}C (section 2.3.5). Thermal processing of the seaweed may be the main factor causing the reduction in fucoxanthin in the processed samples. Mise et al., (2011) reported that freeze dried *Cladosiohon okamuranus* (*Phaeophyta*) gave the highest fucoxanthin content compared to hot-wind dried and vacuum dried samples. Fucoxanthin content was reduced by 51 % in the samples dried with hot-wind at 60^{o}C compared to the freeze dried samples (Mise, 2011). The process of drying seaweed at high temperature decomposed fucoxanthin and even if fucoxanthin was able to withstand the drying process, it was be susceptible to oxidation when exposed to higher temperatures (Mise, 2011).

In this study, the *U.pinnatifida* was cured with salts for 48 hours to create a solute rich environment where osmotic pressure draws water out of seaweed and

microorganism to retard the growth of microorganism. Curing was reported to deactivate enzymes in seaweed to prolong its product shelf life (Nisizawa et al., 1987; Watanabe & Nisizawa, 1984). It could also be possible that prolonged curing drew water and other bioactive compounds out of the seaweed, which contributed to the loss of fucoxanthin in our samples.

4.1.5 Differences in fucoxanthin content between New Zealand processed and commercial *U.pinnatifida* products

New Zealand processed *U.pinnatifida* harvested in July from Port Underwood; farm 327 was selected to compare with Japanese and Korean products due to its higher content of fucoxanthin compared to August and September. Fucoxanthin content of New Zealand processed *U.pinnatifida* was not significantly different to the commercial dried Korean products (Ottogi and Chung Jung Won) but was significantly lower than the Japanese products (Wako Shokai and Riken) (Table 7). This could be due to variation in the fucoxanthin content of the fresh seaweed with different harvest locations. In this study, fucoxanthin content in New Zealand freeze dried samples varied with farms, locations and months. Furthermore, differences in processing methods may have caused the differences in fucoxanthin content of New Zealand *U.pinnatfida* compared to Japanese products (Wako Shokai and Riken). The method employed in the processing of *U.pinnatifida* in this study was a refined method of cut wakame that is commercially used (Kantono, 2011; Nisizawa et al., 1987; Watanabe & Nisizawa, 1984). The utilization of different processing parameters might account for differences in the fucoxanthin content between our samples and commercial wakame analysed. For instance, hot wind drying of seaweed was found to decrease more fucoxanthin compared to vacuum drying (Mise, 2011). Furthermore, increasing the temperature in hot wind and vacuum drying were shown to increase the decomposition of fucoxanthin in seaweed (Mise, 2011). In Japan, cut wakame was reported to be traditionally prepared by drying in a flow through dryer that pass through sieves to sort the different sized pieces (Nisizawa et al., 1987). It has not been reported how Korean cut wakame products are processed.

4.2 Antioxidant activity

Due to the presence of different components in the crude extracts of the biological tissue samples, it was relatively difficult to measure each antioxidant

component separately. Therefore, two assay methods were employed to evaluate the total antioxidant activity of *U.pinnatifida* samples in this study. The DPPH scavenging assay utilized a stable nitrogen centred free radical. DPPH are effectively scavenged by antioxidants through the donation of hydrogen, forming the reduced DPPH-H (Hong-Yu et al., 2010). Meanwhile, the CUPRAC assay utilized a chromogenic redox reagent, bis(neocuproine) copper (II) chelate that can be reduced to Cu(I)-chelate by reducing anitoxidants (Apak et al., 2007). Hence, the results of DPPH scavenging are indicative of the hydrogen donating ability of the seaweed extract to scavenge free radicals whereas CUPRAC measured the reducing activity of the seaweed extract.

4.2.1 Differences in the DPPH scavenging activity and CUPRAC reducing activity of New Zealand processed and fresh *U.pinnatifida*

In this study, both the DPPH radical scavenging assay and CUPRAC assay showed a significant reduction of the antioxidant activities in processed *U.pinnatifida* compared to fresh samples (Table 8). It has been reported that processed seaweeds generally contained less antioxidant activity than fresh seaweeds (Jiménez-Escrig et al., 2001; Yan et al., 1999).

It has been determined that the antioxidant effect of seaweed was mainly due to the antioxidant activity of phenolic compounds (Airanthi et al., 2011; Jiménez-Escrig et al., 2001). In this study, a relationship between the fucoxanthin content and antioxidant activities was evident, with DPPH showing a stronger correlation between fucoxanthin (r^2 = 0.879) than the CUPRAC (r^2= 0.752) assay. Hence, the reduction of antioxidant activity of processed seaweed compared to fresh samples in our study could be due to the loss of polyphenol and fucoxanthin during processing. The DPPH scavenging assay has been extensively employed to study the radical scavenging capacity of seaweed. The major active compounds in brown seaweed extracts detected with the DPPH scavenging assay have been reported to be polyphenol and fucoxanthin (Airanthi et al., 2011; Ganesan, Kumar, & Bhaskar, 2008). The radical scavenging capacity of seaweed was reported to be mostly related to their phenolic hydroxyl group (Jiménez-Escrig et al., 2001). Similarly, the correlation of Folin total phenolic contents of herbal teas with CUPRAC antioxidant capacities gave a linear correlation coefficient of 0.966 implying that the results of CUPRAC correlated well with the total phenolics content of herbal infusions (Celik et al., 2008). This is the first

reported study using the CUPRAC assay to evaluate antioxidant activity of seaweed. A reasonable correlation was found between fucoxanthin and the CUPRAC assay (r^2= 0.752). Further investigations are required to assess the involvement of other antioxidant activities measured using the CUPRAC assay apart from polyphenol and fucoxanthin in seaweed extracts.

Like fucoxanthin, drying also decreased the phenolic content by inactivating the enzymes, polyphenol oxdiases (Jiménez-Escrig et al., 2001; Lim & Murtijaya, 2007). Jimenez-Escrig et al., (2001) reported that the total phenolic content of processed *Fucus vesiculosus* (class *Phaephyceae*) decreased by 98% and DPPH scavenging activity decreased by 96% compared to the fresh material (Jiménez-Escrig et al., 2001). However, for New Zealand processed *U.pinnatifida* there was no such drastic decrease in the DPPH scavenging activity compared to fresh samples (an average of 52% of reduction). This could be due to *U.pinnatifida* being processed differently and/or contained a higher amount of other strong antioxidants that were not susceptible to loss during processing. Overall, the reduction of antioxidant activities in New Zealand processed *U.pinnatifida* compared to freeze dried samples as determined by the DPPH radical scavenging and CUPRAC assays could be due to the loss of polyphenol and fucoxanthin during processing.

4.2.2 Differences in DPPH scavenging activity and CUPRAC reducing activity between New Zealand processed and Commercial *U.pinnatifida* products

DPPH scavenging activities in NZ, Ottogi and Chung Jung Won were significantly higher compared to Wako Shokai and Riken (Table 9). However, the CUPRAC assay showed that the reducing activity in Ottogi, Chung Jung Won, Wako Shokai were significantly higher compared to NZ processed *U.pinnatifida* and Riken. Differences in the antioxidant capacity between these two assays may due to the selectivity of antioxidants that react differently in each assay.

Phlorotannins, the largest group of polyphenols have been reported to be the main antioxidant in brown seaweeds (Shibata et al., 2008; Yuan, 2007; Yuan & Walsh, 2006). They exist in many varieties of structures due to the different degree of polymerization (Ngo et al., 2010). In this study, differences in the DPPH radical scavenging activity and CUPRAC assays could be partly due to structural variations in

the phenolic compounds and the selectivity of antioxidant reacting to the assays. The CUPRAC reagent was found to be more selective (Apak et al., 2004). Simple sugars and citric acid, which are not true antioxidants are not oxidized with CUPRAC reagent and was capable of measuring thiol-type antioxidants such as gluthione. CUPRAC can also simultaneously measure hydrophilic and lipophillic antioxidants whereas the DPPH radical scavenging assay was solvent dependant (Huang et al., 2005; Prior et al., 2005).

The variation in antioxidant activities observed between New Zealand processed *U.pinnatifida* and commercial dried products in both assays may also be due to the involvement of antioxidants other than polyphenols. Fucoxanthin and tocopherol from brown seaweed have been demonstrated to have DPPH radical scavenging activity (Le Tutour et al., 1998; Sachindra et al., 2007; Yan et al., 1999). The differences in months and harvest locations of *U.pinnatifida* might further account for the differences in the antioxidant compounds of the dried products. Levels of the tocopherols in seaweed tissue were found to vary with season (Yuan, 2007).

Chapter 5

5 Conclusion

5.1 Site variation of fucoxanthin content in blade

5.1.1 Variation of fucoxanthin content

U.pinnatifida from Port Underwood has higher fucoxanthin content in the blade compared with that in *U.pinnatifida* blade harvested from Pelorus Sound. The growth of *U.pinnatifida* in Port Underwood occurred throughout the harvesting season, unlike in Pelorus Sound. Fucoxanthin content in blade from farms 327 and 106 collected from Port Underwood peaked in July, decreased in August and remained constant in September before a rapid drop in November. July was concluded to be the best month to extract fucoxanthin from *U.pinnatifida* harvested from farms in Port Underwood. However, *U.pinnatifida* harvested in August and September still contained significant amount of fucoxanthin.

5.1.2 Limitation of sampling

Due to budget, weather and time constraints, a complete collection from June to November from the four farms (327, 106, 122 and 353) was not accomplished. Farms collected from Pelorus Sound were surrounded by land and was further away in the ocean compared to Port Underwood. It was postulated that the high input of freshwater to the sea from the surrounding land in Pelorus Sound attributed to the later growth of *U.pinnatifida* in these areas. However, this cannot be verified in this study.

Among the major environmental factors that affected seaweeds are light, temperature, salinity, water motion, and nutrient availability (Campbell et al., 1999; Dean & Hurd, 2007; Stuart, 2004). However these environmental parameters for the sites investigated in this study were not actually measured. Thus, environmental parameters namely sunlight and air temperature were obtained from a nearby NIWA station in Blenheim (NIWA, 2012). The results may be biased towards farms 327 and 106 collected from Port Underwood, as Port Underwood was situated closer to Blenheim than the Pelorus Sound.

5.2 Fucoxanthin content in sporophyll

Fucoxanthin was also found in the sporophyll of *U.pinnatifida* throughout the harvesting season. Sporophyll of *U.pinnatifida*, also known as mekabu in Japanese, is considered to have a lower utility value as a food product compared to blade. Hence, it is usually dumped as fishery waste. Although, fucoxanthin content in sporophyll was significantly lower than that in blade, it remains a good source for fucoxanthin extraction. The mekabu extract has been reported to contain potential active substance that induce the non-oxidative apoptotic cell death and hence could possibly be a useful auxiliary drug to defeat diseases (Katsube, Yamasaki, Iwamoto, & Oka, 2003; Lee, Hayashi, Hashimoto, Nakano, & Hayashi, 2004; Nishibori, Itoh, Kashiwagi, Arimochi, & Morita, 2011).

5.3 Variation of fucoxanthin content and antioxidant activity in *U.pinnatifida*

New Zealand *U.pinnatifida* was successfully processed with the refined method of cut wakame. The processed *U.pinnatifida* in this study had lower fucoxanthin content and antioxidant capacity compared with the freeze dried sample, suggesting that processing could be responsible for the decrease.

Fucoxanthin content and DPPH scavenging activity of New Zealand processed *U.pinnatifida* was not significantly different to the commercial dried Korean products (Ottogi and Chung Jung Won). However, the fucoxanthin content of New Zealand processed *U.pinnatifida* was significantly lower than Japanese products (Wako Shokai and Riken) but the DPPH scavenging activity was significantly higher than the Japanese products. In contrast, the CUPRAC assay showed that the reducing capacity in Ottogi, Chung Jung Won, Wako Shokai were significantly higher compared to NZ processed *U.pinnatifida* and Riken. The discrepancy of fucoxanthin content and antioxidant activity between New Zealand processed *U.pinnatifida* and commercial products could be due to the variation in different harvesting locations and processing methods. On the contrary, the differences in the antioxidant capacity between DPPH scavenging assay and CUPRAC assay may due to the selectively of antioxidants that react differently in each assay.

5.4 Overall conclusion

In conclusion, *U.pinnatifida* harvested from Marlborough sound, New Zealand was found to have great potential for food and nutraceuticals developments. New Zealand processed *U.pinnatifida* has a significant amount of fucoxanthin, as well as free radical scavenging and reducing properties. These results were also compatible to the commercial *U.pinnatifida* products tested in this study. Hence, New Zealand *U.pinnatifida* has great potential to become a food resource. Furthermore, the pathogen free aquatic environment and relative absence of inorganic toxins of New Zealand water will give New Zealand *U.pinnatifida* a greater competitive edge to produce high standard seaweed-food in the global markets.

There has been increasing interest to develop fucoxanthin for nutraceutical applications due to its biological activities and potential health benefits to human. Fucoxanthin was found in the sporophyll of *U.pinnatifida* through the harvesting season, even though its level is significantly lower than that in the blade. Sporophyll has lower utility value as a food product compared to blade and is usually discarded as a by-product of *U.pinnatifida*. Hence, sporophyll can become a useful bioresource for fucoxanthin extractions which will reduce the waste production of *U.pinnatifida* harvest.

References

Airanthi, Hosokawa, M., & Miyashita, K. (2011). Comparative Antioxidant Activity of Edible Japanese Brown Seaweeds. *Journal of Food Science, 76*(1), C104-C111.

Anderson, L. W. J. (2007). Control of invasive seaweeds. *Botanica Marina, 50*, 418-437.

Apak, R., Guclu, K., Ozyurek, M., & Karademir, S. E. (2004). Novel total antioxidant capacity index for dietary polyphenols and vitamins C and E, using their cupric ion reducing capability in the presence of neocuproine: CUPRAC method. *Journal of Agricultural and Food Chemistry, 52*(26), 7970-7981.

Apak, R., Gueclue, K., Demirata, B., Oezyuerek, M., Celik, S. E., Bektasoglu, B., & Oezyurt, D. (2007). Comparative evaluation of various total antioxidant capacity assays applied to phenolic compounds with the CUPRAC assay. *Molecules, 12*(7), 1496-1547.

Aquaculture. (2012a). Areas designated for Undaria farming Retrieved 30-01-2012, from http://aquaculture.org.nz/2012/01/24/areas-designated-for-undaria-farming/

Aquaculture. (2012b). Industry Development Retrieved 30-01-2012, from http://www.aquaculture.govt.nz/industry_development.php

Badrinathan S., Suneeva S. C., Shiju T. M., P., G.K. C., & V., P. (2011). Exploration of a novel hydroxyl radical scavenger from *Sargassum myriocystum*. *Journal of Medicinal Plants Research, 5*(10), 1997-2005.

Barr, N. G., & Rees, T. A. V. (2003). Nitrogen status and metabolism in the green seaweed *Enteromorpha intestinalis*: an examination of three natural populations. *Ecology-Progress Series, 249*, 133-144.

Burrel, M., Meehan, L., & Munro, S. (2006). The New Zealand Aquaculture Strategy. Port Nelson, Nelson, New Zealand: Aquaculture Council Inc.

Burtin, P. (2003). Nutritonal value of seaweeds. *Electronic Journal of Environmental, Agricultural and Food Chemistry, 2*(4), 498-503.

Campbell, S. J., Bite, J. S., & Burridge, T. R. (1999). Seasonal patterns in the photosynthetic capacity, tissue pigment and nutrient content of different developmental stages of *Undaria pinnatifida* (*Phaeophyta : Laminariales*) in Port Phillip Bay south-eastern Australia. *Botanica Marina, 42*(3), 231-241.

Carter, H. D. (2004). Revised Policy Governing The Commercial Use of Undaria- An Exotic Asian Seaweed: Ministry of Agriculture and Forestry.

Celik, S. E., Oezyuerek, M., Altun, M., Bektasoglu, B., Gueclue, K., Berker, K. I., & Apak, R. (2008). Antioxidant Capacities of Herbal Plants Used in the Manufacture of Van Herby Cheese: 'Otlu Peynir'. *International Journal of Food Properties, 11*(4), 747-761.

Chandini, S. K., Ganesan, P., & Bhaskar, N. (2008). In vitro antioxidant activities of three selected brown seaweeds of India. *Food Chemistry, 107*(2), 707-713.

Chew, Y. L., Lim, Y. Y., Omar, M., & Khoo, K. S. (2008). Antioxidant activity of three edible seaweeds from two areas in South East Asia. *LWT - Food Science and Technology, 41*(6), 1067-1072.

Choice, N. (2012). Sea Vegetables - Mekabu Retrieved 02-02-2012, from http://www.nutritionistchoice.com.au/index.php?option=com_content&view=article&id=36&Itemid=93

Czeczuga, B., & Taylor, F. J. (1987). Carotenoid content in some species of the brown and red algae from the coastal area of New Zealand. *Biochemical Systematics and Ecology, 15*(1), 5-8.

Das, S. K., Hashimoto, T., Shimizu, K., Yoshida, T., Sakai, T., Sowa, Y., & Kanazawa, K. (2005). Fucoxanthin induces cell cycle arrest at G(0)/G(1) phase in human colon carcinoma cells through up-regulation of p21(WAF1/Cip1). *Biochimica Et Biophysica Acta-General Subjects, 1726*(3), 328-335.

de Quiros, A. R. B., Frecha-Ferreiro, S., Vidal-Perez, A. M., & Lopez-Hernandez, J. (2010). Antioxidant compounds in edible brown seaweeds. *European Food Research and Technology, 231*(3), 495-498.

Dean, P. R., & Hurd, C. L. (2007). Seasonal growth, erosion rates, and nitrogen and photosynthetic ecophysiology of *Undaria pinnatifida* (*Heterokontophyta*) in southern New Zealand. *Journal of Phycology, 43*(6), 1138-1148.

Doshi, D. M. (2006). Transfer of Heat. In R. S. P. Ltd (Ed.), *New Living Science PHYSICS* (pp. 175-177): CBSE ADDA.

Duan, X. J., W.W. , Zhang, X. M. L., & Wang, B. G. (2006). Evaluation of antioxidant property of extract and fractions obtained from a red alga, Polysiphonia urceolata *Food Chem, 95*, 37-43.

FAO. (2002). The state of world fisheries and aquaculture, FAO, Sofia Retrieved 31-01-2012, from www.fao.org/sof/sofia/index_en.htm

FAO. (2010). Fishery and Aquaculture Statistics 2008 (pp. 1-100). Rome, Italy: Food and Agriculture Organization.

FAO. (2012a). National Aquaculture Sector Overview: New Zealand.

FAO. (2012b). Species Fact sheets: *Undaria pinnatifida* (Harvey) Suringar, 187 Retrieved 31-01-2012, from http://www.fao.org/fishery/species/2777/en

Fisheries. (2001). Action plan for unwanted species. 1-9 Retrieved 30-01-2012, from http://www.biodiversity.govt.nz/pdfs/seas/undaria_action_plan_dec01.pdf

Fisheries. (2008). Aquaculture in New Zealand Retrieved 31-01-2012, from www.aquaculture.govt.nz/files/pdfs/Aqua_NZ.pdf

Fisheries. (2010). New Zealand Fisheries at a Glance Retrieved 30-01-2012, from http://www.fish.govt.nz/en-nz/Fisheries+at+a+glance/default.htm

Foodnavigator. (2006). Seaweed tops list of innovative new ingredients Retrieved 31-01-2010, from http://www.foodnavigator.com/Science-Nutrition/Seaweed-tops-list-of-innovative-new-ingredients

Forestry, M. o. (2010). The commercial use of *Undaria pinnatifida*- an exotic Asian seaweed Retrieved 11-12-2011, from http://www.biosecurity.govt.nz/files/biosec/policy-laws/undaria-harvest-policy.pdf

Forrest, B. M., Brown, S. N., Taylor, M. D., Hurd, C. L., & Hay, C. H. (2000). The role of natural dispersal mechanisms in the spread of *Undaria pinnatifida* (*Laminariales, Phaeophyceae*). *Phycologia, 39*(6), 547-553.

Ganesan, P., Kumar, C. S., & Bhaskar, N. (2008). Antioxidant properties of methanol extract and its solvent fractions obtained from selected Indian red seaweeds. *Bioresource Technology, 99*(8), 2717-2723.

Garibaldi, L., Zhou, X., Vannuccini, S., Jara, F., Tsuji, S., Barros, P., & Ye, Y. (2010). World Review of Fisheries and Aquaculture. In R. Grainger (Ed.), *The State of World Fisheries and Aquaculture* (pp. 1-88). Rome, Italy.

Gibbs, W., Hay, C., & Dodgshun, T. (1998). A Manual for culturing Wakame (*Undiara pinnatifida*) 2. Plantlets (pp. 1-23). Cawthron: Cawthron Report No. 441.

Guclu, K., Altun, M., Ozyurek, M., Karademir, S. E., & Apak, R. (2006). Antioxidant capacity of fresh, sun- and sulphited-dried Malatya apricot (Prunus armeniaca)

assayed by CUPRAC, ABTS/TEAC and folin methods. *International Journal of Food Science and Technology, 41*, 76-85.

Halliwell, B., Zhao, K. C., & Whiteman, M. (2000). The gastrointestinal tract: A major site of antioxidant action? . *Free Radical Research, 33*(6), 819-830.

Haugan, J. A., & Liaaen-Jensen, S. v. (1994). Algal carotenoids 54. Carotenoids of brown algae (*Phaeophyceae*). *Biochemical Systematics and Ecology, 22*(1), 31-41.

Hay, C. H., & Gibbs, W. (1996). A Pratical Manual For culturing The Asian Sea Vegetable "wakame" (*Undaria Pinnatifida*): 1: Gametophytes. Nelson, New Zealand: Cawthron Institute.

Hay, C. H., & Villouta, E. (1993). Seasonality of the adventive asian kelp *Undaria pinnatifida* in New Zealand. *Seasonality of the adventive asian kelp Undaria pinnatifida in New Zealand, 36*(5), 461-476.

Heo, S. J., & Jeon, Y. J. (2009). Protective effect of fucoxanthin isolated from *Sargassum siliquastrum* on UV-B induced cell damage. *Journal of Photochemistry and Photobiology B-Biology, 95*(2), 101-107.

Heo, S. J., Park, E. J., Lee, K. W., & Jeon, Y. J. (2005). Antioxidant activities of enzymatic extracts from brown seaweeds. *Bioresource Technology, 96*(14), 1613-1623.

Heo, S. J., Yoon, W. J., Kim, K. N., Ahn, G. N., Kang, S. M., Kang, D. H., & Jeon, Y. J. (2010). Evaluation of anti-inflammatory effect of fucoxanthin isolated from brown algae in lipopolysaccharide-stimulated RAW 264.7 macrophages. *Food and Chemical Toxicology, 48*(8-9), 2045-2051.

Holdt, S., & Kraan, S. (2011). Bioactive compounds in seaweed: functional food applications and legislation. *Journal of Applied Phycology*, 1-55.

Hong-Yu, L., Bin Wang, Chun-Guang Yu, Qu, Y.-l., & Su, C.-l. (2010). Evaluation of antioxidant activities of five selected brown seaweeds from China. *Journal of Medicinal Plants Research, 4*(23), 2557–2565.

Huang, Ou, B. X., & Prior, R. L. (2005). The chemistry behind antioxidant capacity assays. *Journal of Agricultural and Food Chemistry, 53*(6), 1841-1856.

Huang, & Wang, B. G. (2004). Antioxidant capacity and lipophilic content of seaweeds collected from the Qingdao coastline. *Journal of Agricultural and Food Chemistry, 52*(16), 4993-4997.

Jiménez-Escrig, A., Jiménez-Jiménez, I., Pulido, R., & Saura-Calixto, F. (2001). Antioxidant activity of fresh and processed edible seaweeds. *Journal of the Science of Food and Agriculture, 81*(5), 530-534.

Kai, T., Quitan, A., Sasaki, M., & Goto, M. (2011). *Recovery of Valuable Components From Undaria Pinnatifida Using Supercritical CO2 and Microwave-Hydrothermal Extraction*. Kumamoto Univeristy. Minneapolis, MN.

Kanazawa, K., Ozaki, Y., Hashimoto, T., Das, S. K., Matsushita, S., Hirano, M., & Nakatsuka, M. (2008). Commercial-scale Preparation of Biofunctional Fucoxanthin from Waste Parts of Brown Sea Algae *Laminalia japonica*. *Food Science and Technology Research, 14*(6), 573-582.

Kantono, K. (2011). Consumer sensory testing of commercial and New Zealand Wakame. *Auckland University of Technology University, Auckland*.

Karadag, A., Ozcelik, B., & Saner, S. (2009). Review of Methods to Determine Antioxidant Capacities. *Food Analytical Methods, 2*(1), 41-60.

Karawita, R., Siriwardhana, N., L., K. W., , Heo, M. S., Yeo, I. K., Lee, Y. D., & Jeon, Y. J. (2005). Reactive oxygen species scavenging, metal chelation, reducing power and lipid peroxidation inhibition properties of different solvent fractions from *Hizikia fusiformis*. *European Food Research and Technology, 220*(3-4), 363-371.

Katsube, T., Yamasaki, Y., Iwamoto, M., & Oka, S. (2003). Hyaluronidase-Inhibiting Polysaccharide Isolated and Purified from Hot Water Extract of Sporophyll of *Undaria pinnatifida*. *Food Science and Technology Research, 9*(1), 25-29.

Kim, S. M., Shang, Y. F., & Um, B.-H. (2011). A preparative method for isolation of fucoxanthin from Eisenia bicyclis by centrifugal partition chromatography. *Phytochemical Analysis, 22*, 322–329.

Koksal, E., & Gulcin, I. (2008). Antioxidant activity of cauliflower (Brassica oleracea L.). *Turkish Journal of Agriculture and Forestry, 32*(1), 65-78.

Kuda, T., & Ikemori, T. (2009). Minerals, polysaccharides and antioxidant properties of aqueous solutions obtained from macroalgal beach-casts in the Noto Peninsula, Ishikawa, Japan. *Food Chemistry, 112*(3), 575-581.

Le Tutour, Benslimane, F., Gouleau, M., Gouygou, J., Saadan, B., & Quemeneur, F. (1998). Antioxidant and pro-oxidant activities of the brown algae, *Laminaria digitata, Himanthalia elongata, Fucus vesiculosus, Fucus serratus and Ascophyllum nodosum*. *Journal of Applied Phycology, 10*(2), 121-129.

Lee, J. B., Hayashi, K., Hashimoto, M., Nakano, T., & Hayashi, T. (2004). Novel antiviral fucoidan from sporophyll of Undaria pinnatifida (Mekabu). *Chemical & Pharmaceutical Bulletin, 52*(9), 1091-1094.

Lim, Y. Y., & Murtijaya, J. (2007). Antioxidant properties of Phyllanthus amarus extracts as affected by different drying methods. *Antioxidant properties of Phyllanthus amarus extracts as affected by different drying methods, 40*(9), 1664-1669.

Maeda, H., Hosokawa, M., Sashima, T., Funayama, K., & Miyashita, K. (2005). Fucoxanthin from edible seaweed, *Undaria pinnatifida*, shows antiobesity effect through UCP1 expression in white adipose tissues. *Biochemical and Biophysical Research Communications, 332*(2), 392-397.

Maeda, H., Hosokawa, M., Sashima, T., Funayama, K., & Miyashita, K. (2007). Effect of Medium-chain Triacylglycerols on Anti-obesity Effect of Fucoxanthin. *Journal of Oleo Science, 56*(12), 615-621.

MAF. (2012). *Undaria pinnatifida*: Marine Pest Guide Retrieved 02-02-2012, from www.biosecurity.govt.nz/files/pests/undaria/undaria-card.pdf

Matanjun, P., Mohamed, S., Mustapha, N. M., Muhammad, K., & Ming, C. H. (2008). Antioxidant activities and phenolics content of eight species of seaweeds from north Borneo. *Journal of Applied Phycology, 20*(4), 367-373.

Mise, T., Ueda, M., & Yasumoto, T. . (2011). Production of Fucoxanthin Rich Powder from *Cladosiphon okamuranus*. *Advance Journal of Food Science and Technology, 3*(1), 73-76.

Miyashita, & Hosokawa, M. (2008). Beneficial Health Effects of Seaweed Carotenoid, Fucoxanthin. In F. Shahid & C. Barrow (Eds.), *Marine Nutraceuticals and Functional Foods* (pp. 297-308): Taylor & Francis Group.

Miyashita, K., Nishikawa, S., Beppu, F., Tsukui, T., Abe, M., & Hosokawa, M. (2011). The allenic carotenoid fucoxanthin, a novel marine nutraceutical from brown seaweeds. *Journal of the Science of Food and Agriculture, 91*(7), 1166-1174.

Mori, K., Ooi, T., Hiraoka, M., Oka, N., Hamada, H., Tamura, M., & Kusumi, T. (2004). Fucoxanthin and Its Metabolites in Edible Brown Algae Cultivated in Deep Seawater. *Marine Drugs, 2*(2), 63-72.

Nakazawa, Y., Sashima, T., Hosokawa, M., & Miyashita, K. (2009). Comparative evaluation of growth inhibitory effect of stereoisomers of fucoxanthin in human cancer cell lines. *Journal of Functional Foods, 1*(1), 88-97.

Ngo, D.-H., Wijesekara, I., Vo, T.-S., Van Ta, Q., & Kim, S.-K. (2010). Marine food-derived functional ingredients as potential antioxidants in the food industry: An overview. *Food Research International, 44*(2), 523-529

Nishibori, N., Itoh, M., Kashiwagi, M., Arimochi, H., & Morita, K. (2011). In Vitro Cytotoxic Effect of Ethanol Extract Prepared From Sporophyll of *Undaria pinnatifida* on Human Colorectal Cancer Cells. *Phytotherapy Research, 26*, 191-196.

Nisizawa, K., Noda, H., Kikuchi, R., & Watanabe, T. (1987). The main seaweed foods in Japan. *Hydrobiologia, 151*, 5-29.

NIWA. (2012). Climate web access service Retrieved 24-02-2012, from http://www.niwa.co.nz/our-science/climate/our-services/data

Okuzumi, J., Takahashi, T., Yamane, T., Kitao, Y., Inagake, M., Ohya, K., & Tanaka, Y. (1993). Inhibitory effects of fucoxanthin, a natural carotenoid, on N-ethyl-N'-nitro-N-nitrosoguanidine-induced mouse duodenal carcinogenesis. *Cancer Letters, 68*(2-3), 159-168.

Oryza. (2011). Fucoxanthin; Dietary ingredient for prevention of Metabolic Syndrome and beauty enhancement. Tokyo, Japan: Oryza oil & Fat Chemical CO., LTD.

Parsons, M. J. (1995). Status of the introduced brown seaweed *Undaria* in New Zealand. *Conservation Advisory Science Notes*, 1-25.

Patra, J. K., Rath, S. K., Jena, K., Rathod, V. K., & Thatoi, H. (2008). Evaluation of antioxidant and antimicrobial activity of seaweed (*Sargassum sp*.) extract: A study on inhibition of glutathione-S-transferase activity. *Turkish Journal of Biology, 32*(2), 119-125.

Prabhasankar, P., Ganesan, P., Bhaskar, N., Hirose, A., Stephen, N., Gowda, L. R., & Miyashita, K. (2009). Edible Japanese seaweed, wakame (*Undaria pinnatifida*) as an ingredient in pasta: Chemical, functional and structural evaluation. *Food Chemistry, 115*(2), 501-508.

Prior, R. L., Wu, X. L., & Schaich, K. (2005). Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agricultural and Food Chemistry, 53*(10), 4290-4302.

Roh, M. K., Uddin, M. S., & Chun, B. S. (2008). Extraction of Fucoxanthin and Polyphenol from *Undaria pinnatifida* Using Supercritical Carbon dioxide with Co-solvent. *Biotechnology and Bioprocess Engineering, 13*(6), 724-729.

Sachindra, N. M., Sato, E., Maeda, H., Hosokawa, M., Niwano, Y., Kohno, M., & Miyashita, K. (2007). Radical scavenging and singlet oxygen quenching activity of marine carotenoid fucoxanthin and its metabolites. *Journal of Agricultural and Food Chemistry, 55*(21), 8516-8522.

Sahelian, R. M. D. (2012). Fucoxanthin supplement health benefit and adverse effects, dosage, uses - Does a fucoxanthin supplement use lead to weight loss? Does it have side effects? Is it a scam? Retrieved 13-02-2012, from http://www.raysahelian.com/fucoxanthin.html

Shibata, T., Ishimaru, K., Kawaguchi, S., Yoshikawa, H., & Hama, Y. (2008). Antioxidant activities of phlorotannins isolated from Japanese *Laminariaceae*. *Journal of Applied Phycology, 20*(5), 705-711.

Shiratori, K., Ohgami, K., Ilieva, I., Jin, X. H., Koyama, Y., Miyashita, K., & Ohno, S. (2005). Effects of fucoxanthin on lipopolysaccharide-induced inflammation in vitro and in vivo. *Experimental Eye Research, 81*(4), 422-428.

Sies, H. (1997). Oxidative stress: oxidants and antioxidants. *Experimental Physiology, 82*(2), 291-295.

Silva, P. C., Woodfield, R. A., Cohen, A. N., Harris, L. H., & Goddard, J. H. R. (2002). First Report of the Asian kelp *Undaria pinnatifida* in the Northeastern Pacific Ocean. *Kulwer Academic Publishers, 4*(333-338).

Siriwardhana, N., Lee, K. W., Kim, S. H., Ha, J. W., & Jeon, Y. J. (2003). Antioxidant activity of Hizikia fusiformis on reactive oxygen species scavenging and lipid peroxidation inhibition. *Food Science and Technology International, 9*(5), 339-346.

Stuart, M. D. (2004). Review of research on *Undaria pinnatifida* in New Zealand and its potential impacts on the eastern coast of the South Island. Wellington, New Zealand: Doc Science Internal Series 166.

Tae, J. J. (2012). Current Situation of seaweed aquaculture development in Rok Retrieved 22-03-2012, from http://www.fao.org/docrep/field/003/AB719E/AB719E06.htm

Terasaki, M., Hirose, A., Narayan, B., Baba, Y., Kawagoe, C., Yasui, H., & Miyashita, K. (2009). Evaluation of recoverable functional lipid components of several brown seaweeds (*Phaeophyta*) from Japan with special reference to fucoxanthin and fucosterol contents. *Journal of Phycology, 45*(4), 974-980.

Tierney, M. S., Croft, A. K., & Hayes, M. (2010). A review of antihypertensive and antioxidant activities in macroalgae. *Botanica Marina, 53*(5), 387-408.

Trioba, G. (2007). Seaweed Quality Manual: a practical guide for seaweed farmers, seaweed extension officers, buying agents, fisheries officers and exporters. Noumea: Secretariat of the Pacific Community.

Vinayak, R. C., Sabu, A. S., & Chatterji, A. (2011). Bio-prospecting of a few brown seaweeds for their cytotoxic and antioxidant activities *Evidence-based complementary and alternative medicine*: eCAM, 2011, 673083.

Wallentinus, I. (2007). Alien species alert: *Undaria pinnatifida* (wakame or Japanese kelp). Göteborg, Sweden: Göteborg University.

Wang, T., Jónsdóttir, R., & Ólafsdóttir, G. (2009). Total phenolic compounds, radical scavenging and metal chelation of extracts from Icelandic seaweeds. *Food Chemistry, 116*(1), 240-248.

Watanabe, T., & Nisizawa, K. (1984). The utilization of wakame (*Undaria pinnatifida*) in Japan and manufacture of haiboshi wakame and some of its biochemical and physical properties. *Hydrobiologia, 116*(9), 106-111.

Werner, A., Clarke, D., & Kraan, S. (2006). Strategic Review of the Feasibility of Seaweed Aquaculture in Ireland. In M. R. I. G. N. U. o. I. I. S. Centre (Ed.), (pp. 1-63): Parkmore Galway Ireland: Marine Institute.

Yan. (2011). Chinese marine product exports are on a roll China Daily European Weekly Retrieved 30-01-2012, from http://europe.chinadaily.com.cn/epaper/2011-05/06/content_12458093.htm

Yan, Chuda, Y., Suzuki, M., & Nagata, T. (1999). Fucoxanthin as the major antioxidant in Hijikia fusiformis, a common edible seaweed. *Bioscience Biotechnology and Biochemistry, 63*(3), 605-607.

Yangthong, M., Hutadilok-Towatana, N., & Phromkunthong, W. (2009). Antioxidant Activities of Four Edible Seaweeds from the Southern Coast of Thailand. *Plant Foods for Human Nutrition (Formerly Qualitas Plantarum), 64*(3), 218-223.

Yoon, G. H. (2008). Aquaculture in Korea. *Aquaculture, New 34, 16-17*.

Yuan. (2007). Antioxidants from Edible Seaweeds. In F. Shahidi & C. T. Ho (Eds.), *Antioxidant Measurement and Applications* (Vol. 956, pp. 268-301): American Chemical Society.

Yuan, & Walsh, N. A. (2006). Antioxidant and antiproliferative activities of extracts from a variety of edible seaweeds. *Food and Chemical Toxicology, 44*(7), 1144-1150.

Yvonne, Y. (2007). Marine Algal Constituents *Marine Nutraceuticals and Functional Foods* (pp. 259-296): CRC Press.

ZaragozaÌ, M. C., LoÌ•pez, D., P. SaÌ•iz, M., Poquet, M., PeÌ•rez, J., Puig-Parellada, P., & Mitjavila, M. T. (2008). Toxicity and Antioxidant Activity in Vitro and in Vivo of Two Fucus vesiculosus Extracts. *Journal of Agricultural and Food Chemistry, 56*(17), 7773-7780.