PUMS 99:1

UNIVERSITI MALAYSIA SABAH

BORANG PENGESAHAN STATUS TESIS

JUDUL: DETERMINATION OF VITAMIN A AND B IN THREE SPECIES OF SABAH EDIBLE SEAWEEDS

IJAZAH: SARJANA MUDA SAINS MAKANAN DAN PEMAKANAN

SESI PENGAJIAN: 2006-2010

Saya MATTHEW TAN CHEE SIANG

(HURUF BESAR)

mengaku membenarkan tesis (LPS/ Sarjana/ Doktor Falsafah) ini di simpan di Perpustakaan Universiti Malaysia Sabah dengan syarat-syarat kegunaan seperti berikut:

1. Tesis adalah hakmilik Universiti Malaysia Sabah.
2. Perpustakaan Universiti Malaysia Sabah dibenarkan membuat salinan untuk tujuan pengajian sahaja.
3. Perpustakaan dibenarkan membuat salinan tesis ini sebagai bahan pertukaran antara institusi pengajian tinggi.
4. ** Sila tandakan (/)

[ ] SULIT (Mengandungi maklumat yang berdarjah keselamatan atau kepentingan Malaysia seperti yang termaktub di dalam AKTA RAHSIA RASMI 1972)

[ ] TERHAD (Mengandungi maklumat TERHAD yang telah ditentukakan oleh organisasi/badan di mana penyelidikan dijalankan)

[✓] TIDAK TERHAD

Disahkan oleh:

(TANDATANGAN PENULIS)

(TANDATANGAN PUSTAKAWAN)

Alamat Tetap: 173, LORONG BERLIAN 5/1, TAMAN BERLIAN, 09000 KULIM, KEDAH

DR. PATRICIA MATANJUN
Nama Penyelia

Tarikh: 20/5/2010

Tarikh: 20/5/2010

CATATAN: * Potong yang tidak berkenaan.
* Jika tesis ini SULIT atau TERHAD, sila lampiran surat daripada pihak berkuasa/organsasi berkenaan dengan menyatakan sekali sebab dan tempoh tesis ini perlu dikelaskan sebagai SULIT dan TERHAD.
* Tesis dimaksudkan sebagai tesis bagi Ijazah Doktor Falsafah dan Sarjana secara penyelidikan, atau disertasi bagi pengajian secara kerja kursus dan penyelidikan, atau Laporan Projek Sarjana Muda (LPSM).

DETERMINATION OF VITAMIN A AND B IN THREE SPECIES OF SABAH EDIBLE SEAWEEDS

MATTHEW TAN CHEE SIANG

THIS DISSERTATION IS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE DEGREE OF FOOD SCIENCE WITH HONORS IN FOOD SCIENCE AND NUTRITION

SCHOOL OF FOOD SCIENCE AND NUTRITION
UNIVERSITI MALAYSIA SABAH

2010

DECLARATION

I hereby declare that the work in this thesis is my own except for quotations and summaries which have been duly acknowledge.

16 April 2010

MATTHEW TAN CHEE SIANG
HN2006 - 3820

CERTIFICATION

NAME : **MATTHEW TAN CHEE SIANG**

MATRIC NO. : **HN2006-3820**

TITLE : **DETERMINATION OF VITAMIN A AND B IN THREE SPECIES OF SABAH EDIBLE SEAWEEDS**

DEGREE : **BACHELOR OF FOOD SCIENCE WITH HONORS**

VIVA DATE : **12 MAY 2010**

DECLARED BY

1. **SUPERVISOR**
   DR. PATRICIA MATANJUN

2. **EXAMINER 1**
   ASSOC. PROF. DR. MOHD ISMAIL ABDULLAH

3. **EXAMINER 2**
   PN. NOR QHAIRUL IZZREEN MOHD. NOOR

4. **DEAN**
   ASSOC. PROF. DR. MOHD ISMAIL ABDULLAH

ACKNOWLEDGEMENT

My greatest gratitude goes to the supports and guidance from people who encouraged and inspired me along the journey of completing my thesis. First of all, I would like to express my highest gratitude to God for giving me strength and wisdom to plan my work until the day I completed my thesis.

I owe my deepest gratitude to my supervisor, Dr. Patricia Matanjun for her understanding, encouragements and personal guidance that have made this thesis possible. Her wide knowledge and her logical way of thinking have been of great value for me along the path to the completion of my thesis.

I would like to show my gratitude to Pn. Nor Qhairul Izzreen Mohd Noor for providing her HPLC column to me for my analysis on HPLC instrument.

I would like to express my warm and sincere thanks to SSMP seniors: Ms. Wong Jin Yi, Mr. Chia Kah Fei, Mr. Tin Hoe Seng, Ms. Ng Xue Ni, Mr. Birdie and Ms. Pei Teng for providing assistances, valuable information and teaching me to operate the instruments for my analysis.

My sincere thanks conveyed to the lab assistants of SSMP: Pn. Zainab, Pn. Marni, Mr. Taipin and Ms Ireen for providing me chemicals and laboratory consumables and teaching me to operate the equipments throughout my laboratory sessions.

Last but not least, my deepest gratitude goes to my beloved parents and sibling for their endless support to fuel my journey until the day of the completion of this thesis.

Matthew Tan Chee Siang
16 April 2010

ABSTRACT

The objective of the study was to determine pro-vitamin A (β-carotene), vitamin B_1, vitamin B_2 and vitamin B_3 in three Sabah edible seaweeds namely red seaweed, *Kappaphycus alvarezii*, green seaweed, *Caulerpa lentillifera* and brown seaweed, *Sargassum polycystum*. Pro-vitamin A (β-carotene), vitamin B_1 and vitamin B_2 were identified and quantified using High Performance Liquid Chromatography with reversed phase C_{18} column. Vitamin B_3 was identified by using Liquid Chromatography – Mass Spectrometry for shorter analysis time and better accuracy. *K. alvarezii* was found to contain β-carotene (2.33 ± 0.25 mg/100g of dry weight), vitamin B_1 (1098.40 ± 46.79 mg/100 g of dry weight) and vitamin B_2 (67.63 ± 5.15 mg/100 g of dry weight) which were significantly higher ($p<0.05$) for vitamin B_1 and B_2 among the three seaweeds but significantly lower for β-carotene among the three seaweeds. Meanwhile, *C. lentillifera* contained β-carotene (112.36 ± 0.28 mg/100 g of dry weight) with higher significant difference ($p<0.05$) among the three seaweeds, vitamin B_1 (15.57 ± 0.25 mg/100 g of dry weight) which is significantly lower than the vitamin B_1 content in *K. alvarezii* and vitamin B_3 was detected. *S. polycystum* was analysed to contain β-carotene (49.29 ± 0.52 mg/100 g of dry weight) which is significantly higher than the β-carotene content in *K. alvarezii* and vitamin B_3 was detected as well. However, there were no significant differences ($p>0.05$) between *C. lentillifera* and *S. polycystum* in vitamin B_1 content. In conclusion, *K. alvarezii* contained the highest content of vitamin B_1 and B_2 while *C. lentillifera* contained the highest amount of β-carotene among the three seaweeds.

ABSTRAK

PENENTUAN VITAMIN A DAN B DALAM TIGA SPESIS RUMPAI LAUT SABAH YANG BOLEH DIMAKAN

Tujuan kajian ini adalah untuk menentukan Pro-vitamin A (β-karoten), vitamin B_1, vitamin B_2 and vitamin B_3 di dalam tiga rumpai laut Sabah yang boleh dimakan iaitu rumpai laut merah, Kappaphycus alvarezii, rumpai laut hijau, Caulerpa lentillifera and rumpai laut perang, Sargassum polycystum). Pro-vitamin A (β-kerotin), vitamin B_1 and vitamin B_2 telah dikenalpasti dan ditaksir dengan penggunaan Kromatografi Cecair Prestasi Tinggi dengan kolum C_{18} fasa berbalik. Vitamin B_3 pula dikenalpasti dengan Kromatografi Cecair – Spektrometri Jisim untuk pemendekkan masa analisa dan ketepatan. K. alvarezii didapati mengandungi β-karoten (2.33 ± 0.25 mg/100g berat kering), vitamin B_1 (1098.40 ± 46.79 mg/100 g berat kering) and vitamin B_2 (67.63 ± 5.15 mg/100 g berat kering) dengan perbezaan lebih tinggi yang signifikan ($p<0.05$) bagi vitamin B_1 dan B_2 tetapi perbezaan signifikan yang lebih rendah ($p<0.05$) bagi β-karoten antara ketiga-tiga rumpai laut. Sementara itu, C. lentillifera mengandungi β-karoten (112.36 ± 0.28 mg/100 g berat kering) dengan perbezaan signifikan yang tinggi ($p<0.05$) antara ketiga-tiga rumpai laut, vitamin B_1 (15.57 ± 0.25 mg/100 g berat kering) and vitamin B_3. S. polycystum telah dianalisa mengandungi β-karoten (49.29 ± 0.52 mg/100 g berat kering) dan vitamin B_3. Akan tetapi, didapati tiada perbezaan yang signifikan ($p>0.05$) antara C. lentillifera dan S. polycystum dalam kandungan vitamin B_1. Kesimpulannya, K. alvarezii mengandungi kandungan vitamin B_1 dan B_2 yang paling tinggi manakala C. lentillifera mengandungi kandungan β-karoten yang paling tinggi di antara ketiga-tiga rumpai laut.

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVATION

AACC	American Association of Cereal Chemists
ADP	Adenosine diphosphate
ATP	Adenosine triphosphate
API	Atmospheric Pressure Ionisation
BHT	Butylated hydroxytoulene
DCM	Dichloromethane
DNA	Deoxyribonucleic acid
ESI	Electrospray ionization
FAD	Flavin Adenine Dinucleotide
FMN	Flavin mononucleotide
HN-APCI	Heated nebulizer-atmospheric pressure chemical ionization
HPLC	High Performance Liquid Chromatography
KOH	Potassium hydroxide
LC-MS	Liquid Chromatography- Mass Spectrometry
MeOH	Methanol
NAD	Nicotinamide Adenine Dinucleotide
NADP	Nicotinamide Adenine Dinucleotide Phosphate
PDA	Photodiode array
PI	Positive ion
PTFE	Polytetrafluoroethylene
RNA	Ribonucleic acid
THF	Tetrahydrofuran
UPLC	Ultra Performance Liquid Chromatography
UV-Vis	Ultraviolet-visible spectrophotometry

LIST OF SYMBOLS

°C	Degree Celsius
<	Less than
±	plus or minus
mg	milligram
ml	millilitre
%	percentage
nm	nanometre
µg	microgram
α	alpha
β	beta
m/z	mass to charge ratio
M	molar
m	metre
v/v	volume to volume ratio
V	Volt
eV	electronvolt
N	normal
ppm	parts per million
ppb	parts per billion
RCF	relative centrifugal force
RE	retinol equivalent
psi	pounds per square inch

CHAPTER 1

Introduction

1.1 Research background

Seaweed has been a food source for populations in Asia since the beginning of time specifically in Japan and China. In China, seaweed was regarded as a luxurious delicacy for the king and the most honourable guest during 600 B.C. Meanwhile, in Japan, it has been used in cooking ever since the eighth century (Chapman and Chapman, 1980). Besides being a food source for humans, seaweeds were utilised in many other ways such as the source of agar, alginate, carrageenan, fertilizers, animal feed, as biomass for fuel, cosmetics, integrated aquaculture and waste water treatment. Today, the seaweed industry are producing a wide range of products with the estimated total value of US$ 5.5-6 billion whereby food products for human consumption contribute US$ 5 billion of the total estimated value (McHugh, 2003). A total of 7.5-8 billion tonnes of wet seaweed which are harvested from naturally growing and cultivated seaweed have been used in the industry annually (McHugh, 2003).

Malaysia's geographical settings provide habitats that encourage the proliferation of seaweed in the country. According to Phang (2006), Malaysian marine algae had reached the total of 375 specific and infraspecific taxa consisting Chlorophyta, Rhodophyta, Phaeophyta and Cyanophyta. Seaweed or benthic marine algae is mainly categorized into three major groups which are Phaeophyta (Brown), Rhodophyta (Red) and Chlorophyta (Green) according to its pigmentation. The main uses of brown, red and green seaweed are as food and the extraction of hydrocolloids.

The difference between the seaweed is the type of hydrocolloid extracted such as alginate, agar and carrageenan (McHugh, 2003).

Sabah has a wide range of edible seaweeds with substantial benefits to human. *Kappaphycus alvarezii* (Rhodophyta), *Caulerpa lentillifera* (Chlorophyta) and *Sargassum polycystum* (Phaeophyta) are edible seaweeds found in the waters of Sabah (Zemke-White and Ohno, 1999; Phang, 2006). Besides having the common nature as the source of food, *C. lentillifera* has medicinal value in lowering high blood pressure (Trono, 1999). According to Darcy-Vrillon (1993), seaweeds are rich sources of non-starch polysaccharides, minerals and vitamin. Seaweeds contain vitamin A, vitamins from group B including vitamin B_{12}, vitamin C and vitamin E (Chapman and Chapman, 1980; Burtin, 2003). Brown and green seaweed provide an average amount between 500 to 3000 mg/kg of dry matter for vitamin C which is equivalent to the level in parsley, blackcurrant, and peppers, whereas red seaweed contains 100 to 800 mg/kg. In addition, it was claimed that brown seaweeds contain higher levels of vitamin E than green and red seaweed (Burtin, 2003).

The nutritional composition of seaweeds had been the interest of many researchers recently. Based on the research done by McDermid and Stuercke (2003) on 22 species of edible Hawaiian macroalgae comprising of 6 Chlorophyta, 4 Phaeophyta and 12 Rhodophyta, provitamin A (β-carotene) was the only constantly detectable vitamin measured by spectrophotometer, followed by vitamin C which displayed detectable level through high performance liquid chromatography analysis, while vitamin B_1, vitamin B_2 and vitamin B_3 were rarely detectable under the same method of analysis. *C. lentillifera* was found to contain high amount of vitamin E with moderate quantity of provitamin A (β-carotene), vitamin B_1, vitamin B_2 and vitamin B_3 (Ratana-arporn and Chirapart, 2006). According to a more recent research by Matanjun *et al.* (2008) on three tropical edible seaweeds found in the waters of Sabah, there were no significant differences of vitamin C level among *Eucheuma cottonii* (*K.*

alvarezii), *C. lentillifera* and *S. polycystum* . It was also found that *S. polycystum* has the highest level of vitamin E (α-tocopherol) compared to *C. lentillifera* and *E. cottonii*.

Seaweeds provide a wide range of vitamins which includes provitamin A (β-carotene), vitamin B_1, vitamin B_2, vitamin B_3, vitamin C and vitamin E (Chapman and Chapman, 1980; McDermid and Stuercke, 2003; Burtin, 2003). For provitamin A (β-carotene), it is beneficial to the humans' vision (Combs, Jr., 2008). Meanwhile, vitamins of group B generally support and increase the metabolism rate, maintaining skin health, strengthen the immune and nervous system, preventing anemia by promoting cell growth and division and reducing the risk of pancreatic cancer (Grosvenor and Smolin, 2002). As for vitamin C, it has benefits such as strengthening the immune system, trapping free radicals, regenerates Vitamin E, encourage the absorption of iron in the intestine, forming conjunctive tissue and protidic matrix of bone tissue (Burtin, 2003). According to Burtin (2003), vitamin E has a role in preventing cardiovascular disease by inhibiting the oxidation of low density lipoprotein (LDL). Besides that, it prevents the formation of prostaglandins and thromboxan in arachidonic acid chain.

Generally, seaweeds contain various types of vitamins that have potentials in food and pharmaceutical industries. Vitamins are required in the body for various physiological functions that are important to life such as enzyme cofactors, biological antioxidant, cofactors for metabolic oxidation-reduction reactions, hormones and photoreceptive cofactor in vision (Combs, Jr., 2008). At the present time, consumers and the food industry has develop a huge interest on products which are commonly known as functional foods that can promote health and well-being (Sloan, 1999). Therefore, seaweeds have good potential as natural sources of functional ingredients due to the content of many bioactive compounds that may have antioxidant for example vitamin C and vitamin E, antibacterial, antiviral and anticarcinogenic properties (Plaza *et al.*, 2008).

Sabah is one of the largest producers of seaweed in Malaysia especially red seaweed (Biusing and Hashim, 2009) with high income profit to Malaysia if these resources were utilised especially in food and pharmaceutical industries. Analysis of seaweeds' nutrient contents especially in vitamin contents had been well documented in a number of regions in the world but unfortunately, there are no comprehensive vitamin analysis report been done on local edible seaweed found in the waters of Sabah.

The stability of vitamins is the major concern in the usage of vitamin in food, diet supplements and as pharmaceuticals. In most cases, fat soluble vitamins, vitamin C, vitamin B_2, and biotin have weak stability against oxidation while some vitamins are prone to degradation by reaction with different factors during storage and preparation of sample (Combs, Jr., 2008). Thus, identification and quantification of vitamin of seaweed through analysis will encourage proper handling during storage and processing in food and pharmaceutical industries in order to preserve different vitamins in the products.

1.2 Objectives

The objective of this study was to identify, quantify and compare provitamin A (β-carotene), vitamin B_1 and B_2 in red seaweed, *Kappaphycus alvarezii*, green seaweed, *Caulerpa lentillifera*, and brown seaweed, *Sargassum polycystum*, from Sabah waters by using High Performance Liquid Chromatography. Another objective was to identify vitamin B_3 in the three edible Sabah seaweeds by using Liquid Chromatography – Mass Spectrometry.

CHAPTER 2

LITERATURE REVIEW

2.1 Seaweeds in the world and Malaysia

Seaweeds are distributed widely in all oceans of the world, but utilised in limited areas as food and as soil conditioners for centuries. With the inventions from time to time, the extractions of extracts from seaweed have been made possible in the industry (McLachlan, 1985). Seaweeds for the purpose of food are derived mainly from China, Japan and Korea, with roughly 94% attained by cultivation. Meanwhile, alginophytes are harvested in 15 countries but six of these countries account for more than 80% of the total harvest; all are from natural stocks except for a great quantity of *Laminaria* cultivated in China. Natural carrageenophytes, from 12 countries, now account for only 20% of the total harvest; the remainder is cultivated *Eucheuma* species, 99% of which is produced in only two countries, the Philippines and Indonesia. Of the four categories of commercial resources of seaweeds considered, agarophytes are spread more evenly over a greater number of countries; they come from 20 countries (McHugh, 1991).

Statistics of the resources of seaweeds worldwide are just an approximate because accurate surveys are difficult, time-consuming, and costly and can usually be justified only when industrial exploitation is being considered. However, these informations have been useful in guiding industrial attentions to expected locations for harvesting (McHugh, 1991). At the present time, there are approximately 221 species of seaweed which comprises of 32 chlorophytes, 125 rhodophytes and 64 phaeophytes being utilised worldwide. An estimated number of 145 species (66%) are use for food which includes 79 rhodophytes, 28 chlorophytes and 38 phaeophytes. More than half of the rhodophytes and phaeophytes are use for phycocolloid production; 41 species for alginates, 33 for agar and 27 for carrageenan. 24 species are use in traditional medicines. About 25 species are use in agriculture, including animal feed and fertiliser, while at least 2 species are use in the production of paper in Italy (Zemke-White and Ohno, 1999).

The extensive ranges of products manufactured by the seaweed industry have an approximated annual value of US$ 6 billion. Besides 83% of the total revenue contributed by food products, the remaining sum was made up by seaweed hydrocolloids, fertilizers, animal feed addictives and others. The industry utilises approximately 7.5 – 8 million tonnes of wet seaweed annually that were harvested from wild and cultivated seaweed. Commercial harvesting takes place in roughly 35 countries between the Northern and Southern Hemispheres, from cold, through temperate, to tropical waters (McHugh, 2003).

Malaysia has a widespread coastline of the total 3432 km, with 418000 km^2 of continental shelf bordered by many islands, providing various habitats for the growth of seaweeds. These islands form groups along the coastlines of Peninsular Malaysia, and east Malaysia which includes Sabah and Sarawak (Phang, 2006). Rocky shores and sandy bays with mudflats and mangroves alternately, islands with coral reefs offer habitats for the wide range of seaweed species found in Malaysian waters (Phang, 1998). Equatorial climate controlled by monsoon wind systems, with the Northeast Monsoon blowing between the period of November and March, while the Southwest Monsoon brings rain from May to September affects the tropical seaweed in Malaysian waters (Phang, 1998). The saline level of Malaysian waters range between 28 and 34 ppt, while surface water temperature range between 27°C and 29°C. Semi-diurnal tides occur on the west coast Peninsular Malaysia, while the east coast experiencing a mixed tidal system. Mixed tidal systems take place in Sabah and Sarawak (Phang, 2006).

Malaysian marine algae had reached the total of 375 specific and infraspecific taxa consisting of 102 taxa of Chlorophyta, 182 Rhodophyta, 72 Phaeophyta and the remaining taxa of Cyanophyta (Phang, 2006). Basically, seaweeds in Malaysia were used for the purpose of food, animal feed, fertiliser and traditional medicine (Zaneveld, 1959; Hooper, 1960; Burkill, 1966; Phang, 1984). Seaweeds were harvested and consumed either as raw or added into salads include the Rhodophytes, *Gracilaria changii, Gracilaria tenuispitata, Eucheuma* (*Kappaphycus*) species and the Chlorophytes, *C. lentillifera* and *Caulerpa racemosa. Corallina* (Amphiroa) is mashed and fed to children to expel worms in the intestines meanwhile broth made up of *Sargassum* and *Turbinaria* is a source of iodine and 'cools' the body system, traditionally used by the Chinese (Phang, 2006). Most of the seaweeds here were not cultivated except *Eucheuma* species in Semporna, Sabah for the extraction of semi-refined carrageenan with the monthly production of 60 to 100 tonnes dry weight and the harvested seaweeds were sun dried on the platforms of houses constructed on the reefs and sold at RM1.10 (US$1 = RM3.80) per kg dry weight with the moisture content of 32 to 35% (Phang, 2006).

2.2 Classification of seaweeds

Seaweeds are known as attached, macroscopic marine algae that are classified according to its pigmentations of green (Chlorophyta), brown (Phaeophyta) and red (Rhodophyta) (Sumich and Morrissey, 2004). Brown seaweeds are generally large in size with different varieties whereby giant kelps are usually 20 m long, thick, leather like species ranging from 2 – 4 m in length and smaller species with the length of 30 – 60 cm. As for the red seaweeds that occasionally exist in purple or brownish red instead of red most of the time are normally smaller size with the range of few centimetres to approximately a metre in length. Meanwhile, the green seaweeds are particularly small in size with a similar range of sizes to the red ones (McHugh, 2003).

2.2.1 Chlorophyta

Chlorophyta is one of the main groups of algae and comprising several selected marine orders and ganera from the tropical regions (Amsler, 2009). Chlorophyta consists of the second highest number of taxa in Malaysian waters with 13 species and seven varieties of *Caulerpa* have been documented, primarily in coral reefs. Current collections signify that eight of these that are still generally found are *C. lentillifera, C. peltata, C. racemosa, C. scalpelliformis, C. serrulata, C. sertulariodes, C. taxifolia and C. verticillata*. The coral reefs are also dominated by species of *Halimeda* (*H. discoidea, H. opuntia, H. tuna*), the erect coralline algae which contribute towards reef building with the calcium carbonate retained in their cell walls. Other green seaweeds inhabiting coral areas include *Anadyomene plicata, Boodlea montagnei, Cladophoropsis* species, *Dictyosphaeria cavernosa, Valonia aegagropila, Cladophora fascicularis, Bryopsis pennata, Codium* species, *Udotea javensis, Udotea flabellum, Tydemmania expeditionis, Bornetella* species and *Neomeris* species. Several species of *Enteromorpha* and *Ulva* are found in the nutrient rich shores and mudflats. *Enteromorpha intestinalis, E. chlathrata, Ulva lactuca* and *U. fasciata* are commonly seen covering small rocks, stones, driftwood and sandy patches along beaches which are frequently visited by people (Phang, 2006).

The basic characteristics of Chlorophyta are: the natural habitat is mainly fresh water but it can also be found in salt water and humid land areas, the flagellate cells are isokont in which the flagella are similar in structure even though they have different length, the chloroplast is enclosed by the double membrane of the chloroplast envelope without additional envelope of the endoplasmic reticulum, the thylakoids within the chloroplast are grouped to form lamellae, the chloroplasts are green in colour due to the uncovered chlorophyll by the accessory pigments, only chlorophyll a and b are present in Chlorophyta, they have a characteristic set of accessory pigments such as xanthophylls lutein, zeaxanthin, violaxanthin, antheraxanthin and neoxanthin, the storage polysaccharide is starch that exists as grains, the circular molecules of chloroplast DNA are concentrated in numerous small nucleoids that are widely distributed in the chloroplast. All the characteristics above are also shared with higher plants or at least the higher plants with flagellates stages in their life cycles (Hoek *et al.*, 1995).

Caulerpa lentillifera **(Chlorophyta)**

Classification:

Empire	Eukaryota
Kingdom	Plantae
Subkingdom	Viridaeplantae
Phylum	Chlorophyta
Class	Bryopsidophyceae
Order	Bryopssidales
Family	Caulerpaceae
Genus	Caulerpa
Species	lentillifera

Source: Guiry and Guiry, 2009

Caulerpa lentillifera (Figure 2.1) is an edible green algae utilised widely in fresh salad and it is also known as sea grapes or green caviar. *Caulerpa lentillifera* has delicate taste and soft, succulent texture (McHugh, 1991). It naturally grows on sandy or muddy bottom in shallow protected waters (Barsanti and Gualtieri, 2006).

It is small in size, exists in loose mats on coral rubbles and rocks, to 30 mm tall; the terete stolon widespread, to 2 mm in diameter, bearing long, descending branches, to 20 mm long, with laxly branched, filiform rhizoids at the ends; the few,

References:

Amsler, C. D. 2009. *Algal Chemical Ecology.* Springer, Birmingham, Alabama.

Ardrey, R. E. 2003. *Liquid Chromatography - Mass Spectrometry: An Introduction.* John Wiley & Sons. Huddersfield, United Kingdom.

Barsanti, L., Gualtieri, P. 2006. *Algae: anatomy, biochemistry, and biotechnology,* CRC Press, Florida.

Beck, W. S. 1991. *Hematology* (5 ed.). MIT Press. Massachusetts.

Biusing, R., Hashim, M. 2009. Management and Development of the Malaysian Seaweed Industry: the way forward. *Paper presented at the Malaysian Seaweed and Carrageenan R&D Forum, Kota Kinabalu.*

Burkill, I. H., 1966. A Dictionary of the Economic Products of the Malay Peninsula. 2 vols. Ministry of Agriculture and Cooperatives, Kuala Lumpur, Malaysia.

Burtin, P. 2003. Nutritional Value of Seaweeds. *Electronic Journal of Environment, Agricultural, and Food Chemistry.* **4:** 498-503.

Careri, M., Bianchi, F., Corradini, C. 2002. Recent advances in the application of mass spectrometry in food-related analysis. *Journal of Chromatography A.* **970**: 3-64.

Chapman, V. J., Chapman, D. J. 1980. Seaweeds & their uses. Chapman & Hall, New York. P. 62-97.

Ching, C. Y., Norziah, M. H. 2000. Nutritional Composition of Edible Seaweed *Gracilaria changgi. Food Chemistry.* **68**: 69 - 76.

Cole, K. M., Sheath, R. G. 1990. *Biology of the red algae.* Cambridge University Press. New York.

Combs, G. F., Jr. 2008. The Vitamins: Fundamental Aspects in Nutrition and Health. New York, Elsevier Academic Press.

Darcy-Vrillion, B. 1993. Nutrional Aspects of the Developing Use of Marine Mircoalgae for the Human Food Industry. *International Journal of Food Science and Nutrition.* **44**: 23-35.

Edwards, G. I. 2000. *Biology the easy way* (3 ed.). Barron's Educational Series. New York.

Eitenmiller, R. R., Lin, Y., Landen Jr, W. O. 2008. *Vitamin Analysis for The Health and Food Sciences* (2 ed.). CRC Press. Florida.

Grosvenor. L. A., Smolin, M. B. 2002. Nutrition from Science to Life. Harcourt College Publishers, Florida.

Guiry, M. D. & Guiry, G. M. 2009. Algaebase version 4.2, world-wide electronic publication, National University of Ireland, Galway. http://www.algaebase.org/search/species/ Retrieved 15th October 2009.

Hart, D. J., Scott, K. J. 1995. Development and evaluation of an HPLC method for the analysis of carotenoids in foods, and the measurement of the carotenoid content of the vegetables and fruits commonly consumed in the UK. *Journal of Food Chemistry.* **54**: 101 - 111.

Hegazi, M. M., Pe´rez-Ruzafaa, A., Almelab, L., Candelac, M. E. 1998. Separation and identification of chlorophylls and carotenoids from *Caulerpa prolifera, Jania rubens* and *Padina pavonica* by reversed phase high-performance liquid chromatography. *Journal of Chromatography A.***829**. *153-159.*

Hoek, C., Mann, D. G., Jahns, H. M. 1995. *Algae: an introduction to phycology.* Cambridge University Press. New York.

Hooper, D., 1960. On Chinese Medicine: Drugs of Chinese Pharmacies in Malaya. The Garden's Bulletin Straits Settlements VII(1–15), 1–57.

Kamman, J. F., Labuza, T. P., Warthesen, J. J. 1980. Thiamin and Riboflavin Analysis by High Performance Liquid Chromatography. *Journal of Food Science*, **45:** 1497 - 1499.

Kolb, N., Vallorani, L., Milanovi, N., Stocchi, V. 2004. Evaluation of Marine Algae Wakame (*Undaria pinnatifida*) and Kombu (*Laminaria digitata japonica*) as Food Supplements. *Food Technology and Biotechnology*. **42(1):** 57-61.

LaCroix, D. E., Wolf, W. R., Kwansa, A. L. 2005. Rapid Trichloroacetic Acid Extraction and Liquid Chromatography Methodfor Determination of Nicotinamide in Commercial Cereals. *Cereal Chemistry*. **82(3):** 277-281.

Lako, Jimaima., Trenerry, V. C., Wahlqvist, M., Wattanapenpaiboon, N., Sotheeswaran, S. & Premier, R. 2007. Phytochemical flavonols, carotenoids and the antioxidant properties of a wide selection of Fijian fruit, vegetables and other readily available foods. *Food Chemistry*. **101(4):** 1727 – 1741.

Leporati, A., Catellani, D., Suman, M., Andreoli, R., Manini, P., Niessen, W. M. A. 2005. Application of a liquid chromatography tandem mass spectrometry method to the analysis of water soluble vitamins in Italian pasta. *Analytica Chimica Acta*. **531:** 87-95.

Lee, W. T., Yeow, C. W. 1984. *Seaweeds of Singapore*. NUS Press. Singapore.

Lim, P. E., Phang, S. M., 2004. Gracilaria species (Gracilariales, Rhodophyta) of Malaysia including two new records. Malaysian J. Science. **23(2)**: 71–80.

Lonsdale, D. 2006. A Review of the Biochemistry, Metabolism and Clinical Benefits of Thiamin(e) and Its Derivatives. *eCAM*. **3(1)**: 49-59.

Luo, X., Chen, B., Ding, L., Tang, F., Yao, S. 2006. HPLC-ESI-MS Analysis of Vitamin B_{12} in Food Products and in multivitamins-multimineral tablets. *Analytica Chimica Acta*. **562:** 185-189.

Matanjun, P., Mohamed, S. Mustapha, N. M., Muhammad, K. 2008. Nutrient content of tropical edible seaweeds, *Eucheuma cottonii, Caulerpa lentillifera and Sargassum polycystum. Journal of Applied Phycology, DOI 10.1007/s10811-008-9326-4.*

Mauseth, J. D. 2003. *Botany: an introduction to plant biology* (3 ed.). Jones & Bartlett Publishers, Massachusetts.

Mayne, S. T. 1996. Beta-carotene, carotenoids, and disease prevention in humans. *Journal of FASEB.* **10**: 690-701.

McDermid, K. J., Stuercke, B. 2003. Nutritional Composition of Edible Hawaiian Seaweeds. *Journal of Applied Phycology.* **15:** 513-524.

McDowell, L. R. 2000. *Vitamins in animal and human nutrition* (2 ed.). Wiley-Blackwell, Iowa.

McHugh, D. J. 1991. Worldwide distribution of commercial resources of seaweeds including Gelidium. *Hydrobiologia.* **221**: 19-29.

McHugh, D. J. 2003. "A Guide to Seaweed Industry." FAO Fisheries Techincal Paper. **441**: 105.

McLachlan, J. 1985. Macroalgae (seaweeds): industrial resources and their utilization. *Plant and Soil* **89**: 137-157.

Noiraksar, T., Ajisaka, T. 2008. Taxonomy and distribution of Sargassum (Phaeophyceae) in the Gulf of Thailand. *Journal of Applied Phycology.* **20**: 963-977.

Norziah, M. H., Ching, C. Y. 2000. Nutritional Composition of Edible Seaweed *Gracilaria changgi. Journal of Food Chemistry.* **68:** 69 - 76.

Phang, S. M., 1989. Notes on the Ecology of Some Marine Epiphytic Rhodophytes at Cape Rachado, Peninsular Malaysia. Malayan Nature J. **43:** 84–95.

Phang, S. M., 1998. The Seaweed Resources of Malaysia. In: A. T.Critchley, M. Ohno, Seaweed Resources of theWorld, pp. 79–91. Japan International Cooperation Agency, Yokosuka, Japan.

Phang, S. M., 1984. Seaweed Resources of Malaysia. Wallaceana **W38:** 3–8.

Phang, S. M., Maheswary, V., 1989. Phycocolloid Content of Some Malaysian Seaweeds. p 65–77. In: S. M. Phang, A. M. Sasekumar, S. Vikeneswary (Eds.), Proceedings Twelfth Annual Seminar Malaysian Society of Marine Sciences,.Kuala Lumpur, Malaysia.

Phang, S. M., Yoshida, T., 1997. Sargassum stolonifolium Phang et Yoshida sp. nov. from Penang Island, Peninsular Malaysia. In: I. A. Abbott (Ed.), Taxonomy of Economic Seaweeds: with reference to some Pacific and Caribbean species. Vol. VI, pp. 61–73. California Sea Grant College, California.

Phang, S. M. 2006. Seaweed resources in Malaysia: Current status and future prospects. *Aquatic Ecosystem Health & Management.* **9(2):** 185-202.

Plaza, M., Cifuentes, A., Ibanez, E. 2008. In The Search of New Functional Food Ingredients from Algae. *Trends in Food Science and Technology.* **19:** 31-39.

Ratana-arporn, P., Chirapart, A. 2006. Nutritional Evaluation of Tropical Green Seaweeds Calurpa Lentillifera and Ulva reticulata. Kasetsart. *J. Nat. Sci.,* **40:** 75-83.

Rosenthal, J. W., Carey, W. J. 2008. *Prevention of cancer: new research.* Nova Publishers, New York.

Smith, R. M. 2004. Column liquid chromatography. *Journal of Chromatography Library,* **69A:** 95-138.

Sloan, E. 1999. The New Market: foods for the not-so- healthy. *Journal of Food Technology.* **53**: 54-60.

Sumich, J. L., Morrissey, J. F. 2004. *Introduction to the biology of marine life* (8th ed.). Jones & Bartlett Publishers, Boston.

Tee, E. S., Lim, C. L. 1991. Carotenoid composition and content of Malaysian vegetables and fruits by AOAC and HPLC methods. *Food Chemistry*, **41:** 303 - 339.

Trono, G. C. 1999. Diversity of the seaweed flora of the Philippines and its utilization. *Hydrobiologia.* **398/399:** 1-6.

Vandamme, E. J. 1989. *Biotechnology of vitamins, pigments, and growth factors.* Springer, New York.

Williams, P. A., Phillips, G. O. 2006. *Gums and stabilisers for the food industry.* Royal Society of Chemistry, Cambridge.

Zaneveld, J. S., 1959. The Utilization of Marine Algae in Tropical South and East Asia. Economic Bot. **13:** 89–131.

Zemke-White, W. L., Ohno, M. . 1999. World seaweed utilisation: An end of century summary. *Journal of Applied Phycology.* **11:** 369-376.