UNIVERSITI PUTRA MALAYSIA

NUTRITIONAL COMPOSITION AND HEAVY METAL CONTENT OF FARMED AND WILD SEAWEED (GRACILARIA CHANGII)

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NUTRITIONAL COMPOSITION AND HEAVY METAL CONTENT OF FARMED AND WILD SEAWEED (*GRACILARIA CHANGII*)

By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the degree of Master of Science

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NUTRITIONAL COMPOSITION AND HEAVY METAL CONTENT OF FARMED AND WILD SEAWEED (*GRACILARIA CHANGII*)

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Chair: Professor Amin bin Ismail, PhD
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Gracilaria changii is edible seaweed that is cultivated commercially or found in its natural habitat. However, there is limited information regarding its nutritional composition and heavy metal content. In addition, no comparison between farmed and wild *G. changii* in terms of nutritional composition and heavy metal was found. Furthermore, there is insufficient previous information on mineral and heavy metal content of surface water for farmed (culture pond) and wild *G. changii* (mangrove forest). Proximate composition, mineral, water soluble vitamin, fat soluble vitamin, sugar, fatty acid, amino acid and heavy metal of farmed and wild *G. changii* were determined. Moisture content was found to be 74.47% in farmed *G. changii* and wild *G. changii* (71.12%). Protein, carbohydrate, total dietary fiber and ash were detected in farmed (17.11, 44.88, 32.43 and 32.60 g/ 100 g) and wild *G. changii* (12.30, 42.77, 40.53 and 41.27 g/ 100 g). Atomic absorption spectroscopy of the ashes showed that farmed and wild *G. changii* contained higher amount of potassium (2688.603-5504.927 mg/ 100 g) and sodium (887.168-2680.427 mg/ 100 g) than calcium, zinc, iron and copper. Positive correlation between *G. changii* and its surface water was found for zinc, copper and potassium (0.888, 0.972 and 1.000). For water soluble vitamins, vitamin C (0.076-0.337 mg/ 100 g), niacin (0.019-0.078 mg/ 100 g) and pyridoxine (0.004-0.006 mg/ 100 g) were detected. Palmitic acid (0.624-0.687 g/ 100 g) was the highest fatty acid detected and make up more than 80% of the total fatty acid detected. In terms of amino acids, valine (7.33-7.89%) and leucine (7.30-7.84%) were the highest essential amino acid found. Cadmium, lead, mercury and arsenic were found in farmed and wild *G. changii* although only mercury was within the permitted level according to Malaysian Food Regulations 1985. Negligible amount of cadmium, lead, mercury and arsenic were detected in culture pond and mangrove forest surface water. A positive correlation was found for cadmium and lead for wild *G. changii* and its surface waters. There is a positive correlation for arsenic content for farmed *G. changii* and its surface water. Seaweeds are used as ingredient to form gelling agent so the heavy metal content will be diluted during the manufacturing process. The heavy metal content does not reflect the safety consumption of *G. changii*. In summary, *G. changii* can be beneficial to the food industry as a supplement or halal ingredient due to its attractive nutritional properties such as dietary fiber, minerals and amino acids.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Sarjana Sains

KOMPOSISI PEMAKANAN DAN KANDUNGAN LOGAM BERAT RUMPAI LAUT *GRACILARIA CHANGII* YANG DITERNAK DAN LIAR

Oleh

MOHD NAEEM BIN MOHD NAWI

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Gracilaria changii merupakan rumpai laut yang diternak secara komersial atau boleh dijumpai di tempat tinggal semulajadinya. Terdapat maklumat yang terhad berkenaan komposisi nutrisi dan kandungan logam berat di dalamnya. Tambahan lagi, tiada perbandingan pernah dibuat antara *G. changii* yang diternak dan liar. Selain itu, tiada maklumat berkenaan logam galian dan logam berat di dalam permukaan air untuk *G. changii* yang diternak (kolam) dan liar (paya bakau). Analisis komposisi proksimat, logam galian, vitamin larut air, vitamin larut lemak, gula, asid lemak, asid amino dan logam berat di dalam *G. changii* yang diternak dan liar telah dijalankan. Terdapat sebanyak 74.47% kandungan air di dalam *G. changii* yang diternak dan liar (71.12%). Protein, karbohidrat, jumlah serat pemakanan, dan abu telah dikesan di dalam *G. changii* yang diternak (17.11, 44.88, 32.43 dan 32.60 g/ 100 g) dan liar (12.30, 42.77, 40.53 dan 41.27 g/ 100 g). Spektroskopi penyerapan atom abu telah menunjukkan bahawa *G. changii* yang diternak dan liar mempunyai kandungan kalium (2688.603-5504.927 mg/ 100 g) dan natrium (887.168-2680.427 mg/ 100 g) yang lebih tinggi berbanding kalsium, zink, besi dan kuprum. Korelasi positif antara *G. changii* dan permukaan airnya telah didapati untuk logam galian zink, kuprum dan kalium (0.888, 0.972 and 1.000). Vitamin C (0.076-0.337 mg/ 100 g), niasin (0.019-0.078 mg/ 100 g) dan piridoksina (0.004-0.006 mg/ 100 g) merupakan vitamin larut air yang telah dijumpai. Asid palmitik (0.624-0.687 g/ 100 g) merupakan asid lemak tertinggi dan merupakan 80% dari kesemua asid lemak yang dikesan. Valin (7.33-7.89%) dan leusin (7.30-7.84%) merupakan asid amino perlu yang tertinggi yang dapat dikesan. Kadmium, plumbum, merkuri dan arsenic telah dijumpai di dalam *G. changii* yang diternak dan liar walaubagaimanapun hanya merkuri masih di bawah paras yang dibenarkan oleh Akta Peraturan Makanan 1985. Kandungan kadmium, plumbum, merkuri dan arsenik yang sedikit telah dikesan di dalam permukaan air kolam dan paya bakau. Terdapat korelasi positif bagi kandungan arsenik antara *G. changii* dan persekitaran airnya. Rumpai laut digunakan sebagai ramuan bagi membentuk agen penjelian jadi kandungan logam berat akan menjadi kurang semasa proses penghasilan. Kandungan logam berat tidak meggambarkan keselamatan penggunaan *G. changii*. *G. changii* boleh digunakan sebagai ramuan halal kerana sifat nutrisi yang menarik.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

AD	Anno Domini
BC	Before Christ
DOF	Department of Fisheries
DOFS	Department of Fisheries Sabah
DW	Dry Weight
EPP	Entry Point Project
GC-FID	Gas Chromatography-Flame Ionization Detector
HCMS	Human Cytomegalovirus
HDL	High density lipoprotein
HIV	Human Immunodeficiency Virus
HPLC	High Pressure Liquid Chromatography
HSV	Human Simplex Virus
ICP-MS	Inductively Coupled Plasma - Mass Spectrometer
LDL	Low density lipoprotein
NKEA	National Key Economic Areas
RSV	Respiratory Synctial Virus
UHPLC	Ultra High Pressure Liquid Chromatography
WAT	white adipose tissue

CHAPTER 1

INTRODUCTION

1.1 Background

Algae are classified into microalgae and macroalgae. Seaweeds are part of the macroalgae group. The classification is dictated by the different zones where the algae are found. Macroalgae occupy the littoral zone while microalgae habituate both benthic and littoral zones. Littoral zone is part of the ocean that is close to the shore. On the other hand, benthic zone is at the bottom of the ocean and includes the sediment surface (Peinado, Girón, Koutsidis and Ames, 2014). Seaweeds belong to the macroalgae group while phytoplankton is a type of microalgae.

Seaweeds are grouped to brown (Phaeophyta), green (Chlorophyta) and red (Rhodophyta). The variation in color is due to pigmentation during photosynthesis process. In addition, it can also be attributed to morphological and anatomical characters as reported by Manivannan, Thirumaran, Karthikai, Anantharaman and Balasubramanian (2009).

According to McLachlan and Bird (1986) *Gracilaria* are among the major edible seaweeds of Rhodophyta. *Kappaphycus* and *Gracilaria* both are part of Rhodophyta. Large scale farming for *Kappaphycus* is already established in Malaysia. On the other hand, farming for *Gracilaria* is still at an early stage and small scale in nature.

Most *Gracilaria* species are reported to be from tropical waters such as Thailand, Malaysia and Indonesia. Sreenivasan, Ibrahim and Mohd Kassim (2010) reported that *Gracilaria* grows greatly in hot and humid countries. According to Brodie and Zuccarello (2007) *Gracilaria* is one of the most omnipresent genus of Rhodophyta. In Malaysia, 20 species of *Gracilaria* have been identified up to 2004 (Lim and Phang, 2004). *Gracilaria changii* (*G. changii*) is one the *Gracilaria* species that can be found in Malaysia.

Xia and Abbott (1987) were the first to have recorded the presence of *G. changii* in Malaysia. *G. changii* was extensively found in the mangrove forest along the west coast of Peninsular Malaysia (Lim and Phang, 2004; Phang, Shaharuddin, Noraishah and Sasekumar, 1996). One of the main usage of *Gracilaria* is it serves as a building block for the extraction of agar or carrageenan. In addition, *Gracilaria* is also important in the production of tissue culture media (Jahara and Phang, 1990; Glickman, 1987).

Gracilaria is also one of the more ubiquitous agarophytic seaweeds found in Malaysia. *Gracilaria* is now primarily cultured for agar production in the food industry (Phang et al., 1996). According to Andersen (2005) *Gracilaria* contribute approximately 53% of the total agar production. *G. changii* possess high yields of agar in the range of 12 to 25% dry weight (Phang et al., 1996). Furthermore, it also contains high yields of agarose in the range of 13 to 16% dry weight. Both agar and agarose also have high gel strength and can be used for improving binding characteristics, consistency and presentation in ground meat products (Chen, Chen, Zhou, Li, Ma, Nishiumi, and Suzuki, 2014).

The Malaysian government has identified the seaweed industry as one of the industries that have the potential to be a source of income for the country. Seaweeds have the probability to be utilized as a raw material or ingredient for the manufacturing of pharmaceutical and nutraceutical products. Malaysia is one the biggest contributors towards the production of red seaweed from the genus *Euchema* sp. and *Kappaphycus* sp. Rao, Mantri and Ganesan (2007) reported that seaweeds are rich source of minerals. It was also reported that nutrient composition of seaweeds is dependent on multiple factors such as species of the seaweeds and geographic area where the seaweeds were found. It was also found that season of the year during harvesting process and temperature of water could also affect nutritional composition of seaweeds (Tabarsa, Rezaei, Ramezanpour and Waaland, 2012). Seaweeds are a great source of compounds such as carotenoids, dietary fiber, protein and minerals as reported by Fleuerence, Gutbier, Mabeau and Leray (1994).

Seaweeds are widely consumed in east asian countries such as Japan and China. It can be said that approximately 25% of all foods in Japan consist of seaweeds. Seaweeds can be served in many forms in Japan and is considered to be the main source of income for Japan's fishermen (Norziah and Ching, 2000). It was also reported that the nutritional composition of seaweeds are usually dependent on the biochemical compositions. Biochemical compositions usually includes proteins, carbohydrates, vitamins, minerals, and amino acids.

G. changii in Malaysia could be found in the wild or in cultured pond. Farmed and wild *G. changii* are found in different kinds of water and environment. Farmed *G. changii* are grown in cultured ponds with other organisms such as fish and prawns. As of now, Department of Fisheries Malaysia (DOF) carried out pond cultivation for *G. changii* in Merbok, Kedah (Yow, Lim and Phang, 2011).

1.2 Problem statements

Farmers in Malaysia are having difficulty in culturing *G. changii* in culture ponds throughout the country which can be due to environmental factors or its nutrient content. There is also a need to find an alternative source of gelatin that is Halalan Toyyiban or made from halal sources according to Islamic law. Previous research on nutritional composition of *G. changii* was limited to proximate and few additional nutrients. In addition, no additional information on heavy metal composition of *G. changii* is available. Besides that, no comparison between nutritional composition of farmed and wild *G. changii* was done previously.

1.3 Significance of the study

The understanding of nutritional composition of farmed and wild *G. changii* can be utilized to help aquaculture producers in improving its production. Comparison between nutritional composition of farmed and wild *G. changii* can be used to identify the differences in growing conditions in culture ponds. All of this data can be used to help farmers in improving their cultivation and production of *G. changii* at other culture ponds in Malaysia. The improvement will lead to higher production of *G. changii* and place Malaysia in a good position to be a leader in the agar and carrageenan industry. Besides that, it will also benefit farmers by creating wealth and job opportunities as well. In addition, seaweed farming is an Entry Point Project (EPP) under the National Key Economic Areas (NKEA) Agriculture. NKEA Agriculture is projected to create approximately RM28.9 billion in cumulative gross national income (GNI) and 109335 new jobs by 2020. The EPP aims to increase yields and commercialization of seaweed farming industry.

G. changii also have good gel forming abilities that make it suitable to be used as an alternative source of gelatin made from halal sources for the benefits of Muslim consumers.

1.4 Objectives

1.4.1 General objectives

The general objective was to investigate nutritional composition and heavy metal content of farmed and wild *G. changii*.

1.4.2 Specific objectives

- To determine and compare nutritional composition of farmed and wild *G. changii* which includes proximate composition, minerals, water soluble vitamin, fat soluble vitamin, sugar, fatty acid and amino acid.
- To determine and compare heavy metal content of farmed and wild *G. changii*.
- To determine and compare heavy metals and minerals composition of surface waters for farmed and wild *G. changii*.
- To determine correlation between heavy metals and minerals composition of *G. changii* and its surface waters.

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APPENDIX

Table 1.1: Conversion Factors (F) for Specified Food

Food commodity	Type	N factor
Cereal product	Wheat, hard, medium or soft	5.83
	Wholemeal or flour or bulgur	5.83
	Flour, medium or low extraction	5.70
	Macaroni. Spaghetti, wheat pastes	5.70
	Bran	6.31
Rice		5.95
Rye, barley, oats		5.83
Pulses, nuts and seeds	Groundnuts	5.46
Treenuts	Almond	5.18
	Brazil nuts	5.71
	Coconuts, chestnuts, treenuts	5.30
Seeds	Sesame, safflower, sunflower	5.30
Milk and milk products		6.38
Edible fats and Edible Oil		6.38
	Margarine, Butter	6.38
Other foods		6.25

Source: Food Act (1983); Food Regulations (1985)

Table 1.2 Retention Time for Water Soluble Vitamins

Nutrient	Retention Time (minutes)
Thiamin	1.863
Pyridoxine	2.490
Vitamin C	4.840
Niacin	5.273
Cobalamin	6.587
Riboflavin	7.533
Folic Acid	9.263

Table 1.3 Retention Time for Fat Soluble Vitamin

Nutrient	Retention Time (minutes)
Retinol	6.327
Ergocalciferol	10.097
Cholecalciferol	10.489
Menaquinone	11.541
Tocopherol	13.152
Phylloquinone	17.028

Table 1.4 Retention Time for Sugar

Nutrient	Retention Time (minutes)
Fructose	8.096
Glucose	9.547
Sucrose	13.412
Maltose	11.541

Table 1.5 Retention Time for Detected Fatty Acids

Nutrient	Retention Time (minutes)
Palmitic Acid (C16:0)	17.16
Heptadecanoic Acid (C17:0)	17.71
Oleic Acid (C18:1)	18.17
Trans Fatty Acid	18.20

Table 1.6 Retention Time for Amino Acids

Amino Acids	Retention Time (Minutes)
Histidine	1.950
Serine	2.775
Arginine	3.080
Glycine	3.199
Aspartic Acid	3.732
Glutamic Acid	4.590
Threonine	5.057
Alanine	5.463
Proline	6.143
Cystine	7.039
Lysine	7.096
Tyrosine	7.169
Methionine	7.307
Valine	7.439
Isoleucine	8.166
Leucine	8.245
Phenylalanine	8.347

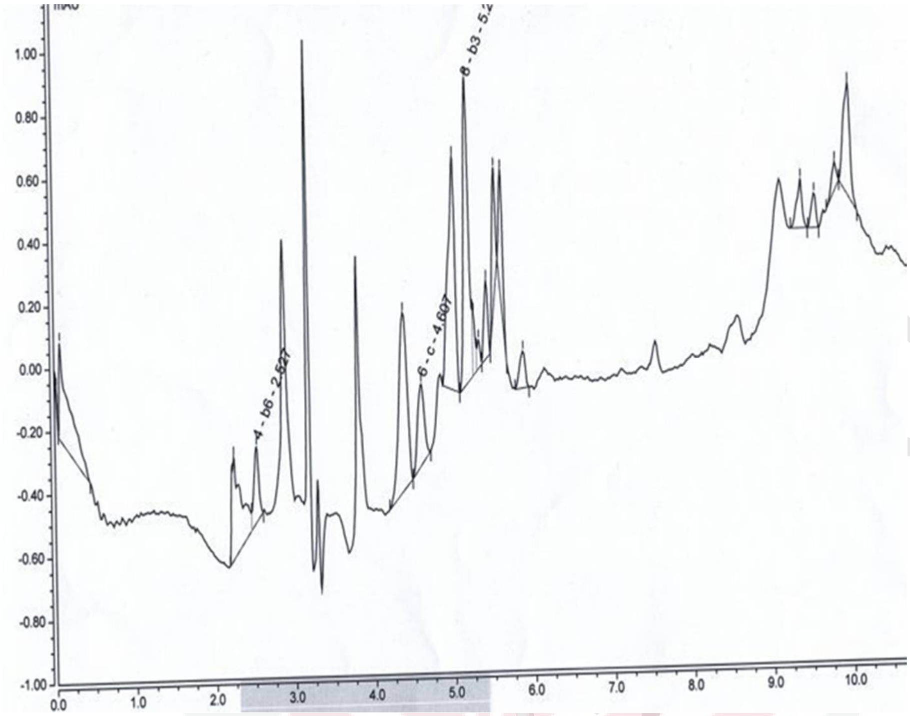

Figure 1.1 Wild *Gracilaria changii* Water Soluble Vitamins Chromatogram

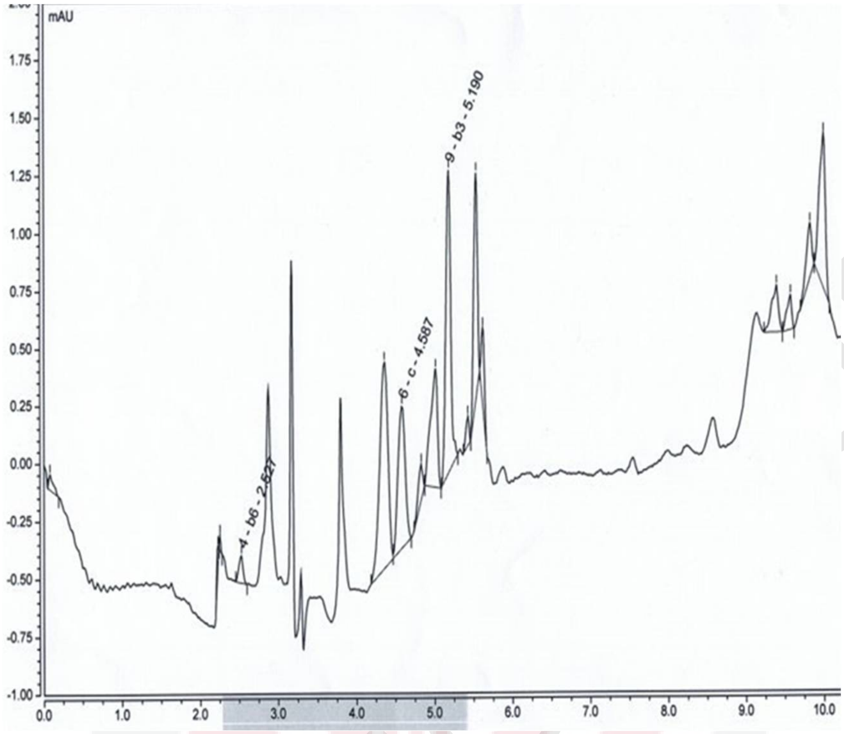

Figure 1.2 Farmed *Gracilaria changii* Water Soluble Vitamins Chromatogram

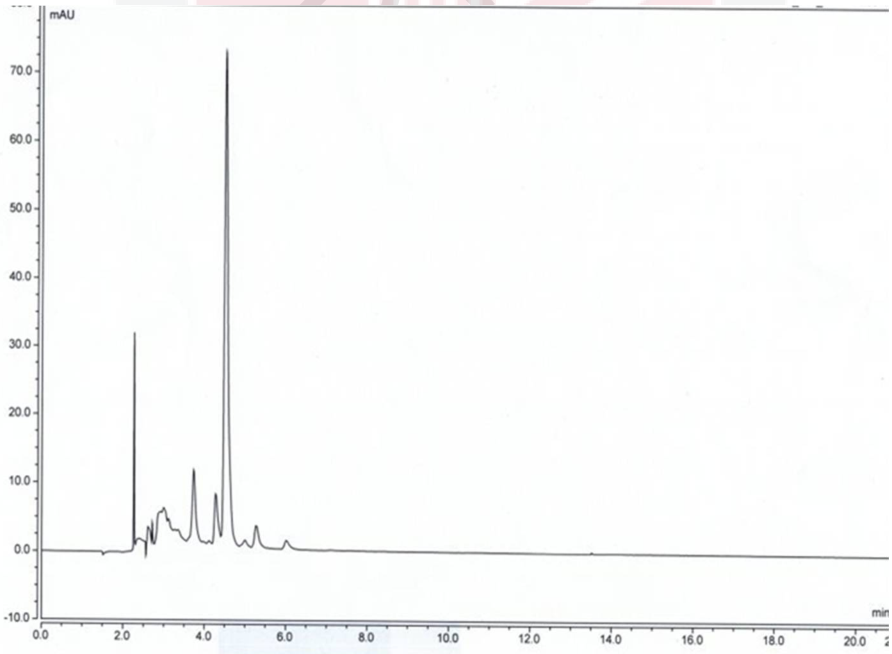

Figure 1.3 Wild *Gracilaria changii* Fat Soluble Vitamins Chromatogram

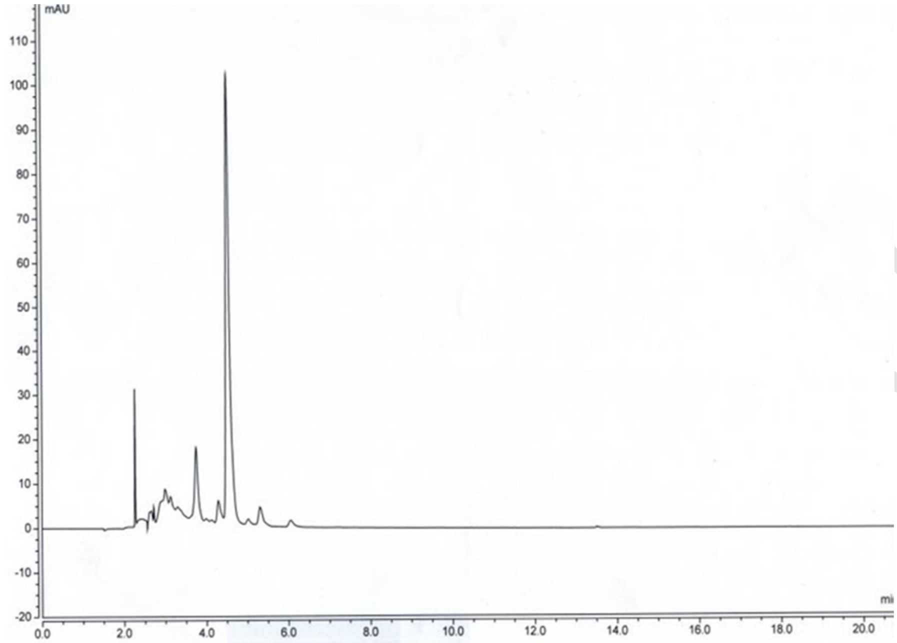

Figure 1.4 Farmed *Gracilaria changii* Fat Soluble Vitamins Chromatogram

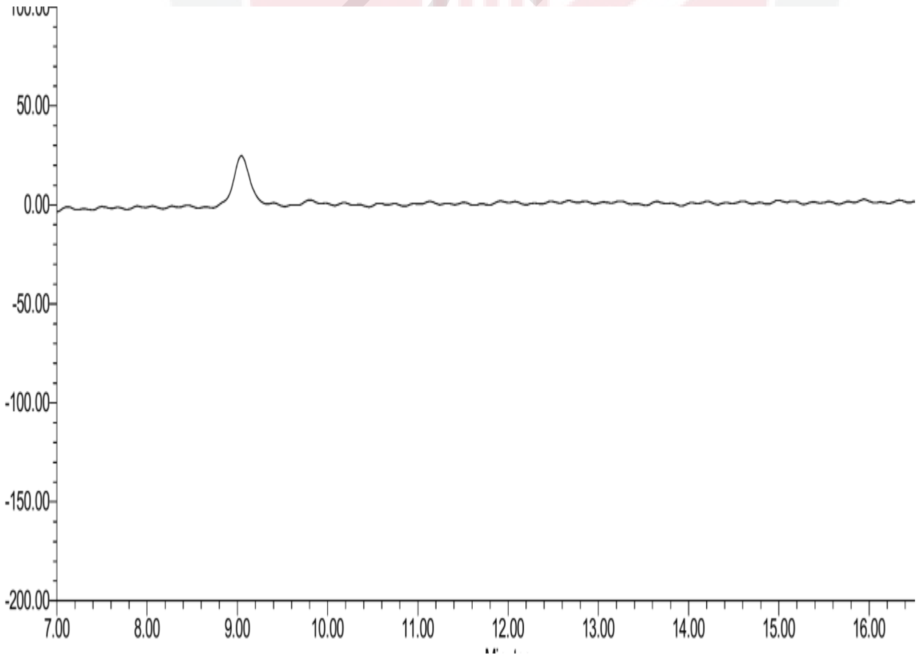

Figure 1.5 Wild *Gracilaria changii* Sugar Chromatogram

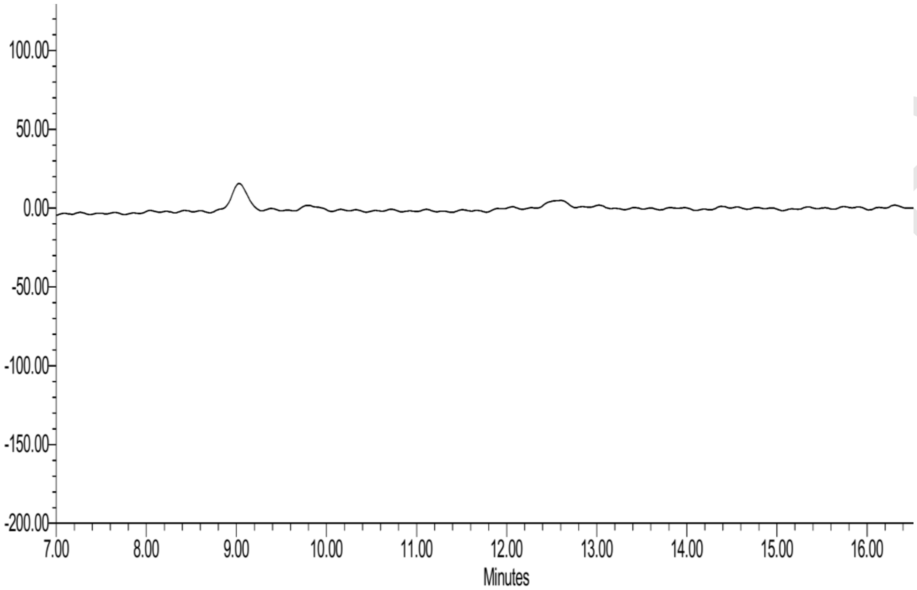

Figure 1.4 Farmed *Gracilaria changii* Sugar Chromatogram

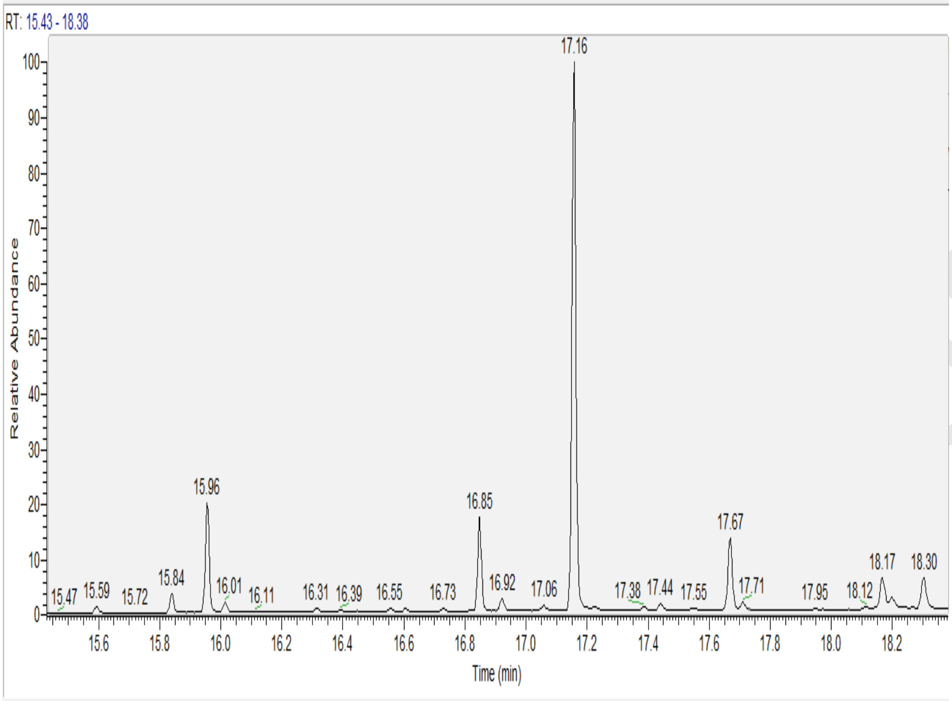

Figure 1.5 Wild *Gracilaria changii* Fatty Acid Chromatogram

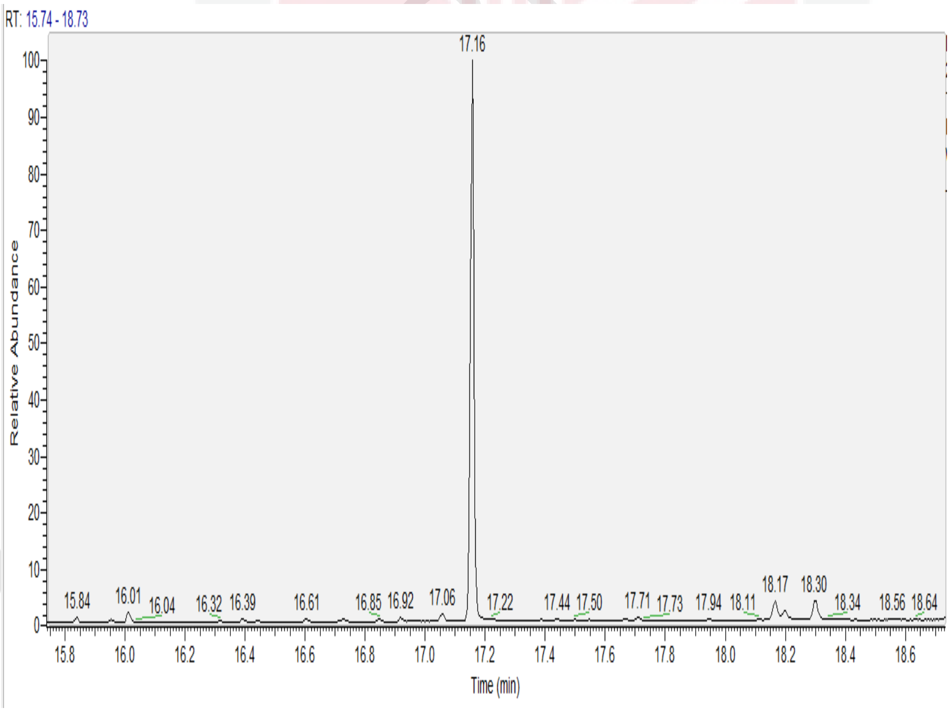

Figure 1.6 Farmed *Gracilaria changii* Fatty Acid Chromatogram

BIOADATA OF STUDENT

My name is Mohd Naeem bin Mohd Nawi. I was born in Kuala Lumpur on 31st October 1987. I have 7 siblings and I am the fourth among them. I graduated from Universiti Teknologi MARA (UiTM) in 2010 with Bachelor of Science (Hons) in Food Science and Technology. I have been working as a research officer in Institute for Medical Research (IMR) since 2010 until now. The degree that is being sought is Master of Science (Halal Product Science).

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