THE PHYSICOCHEMICAL, SENSORY CHARACTERISTICS AND STORAGE STUDIES OF SEAWEED (*Kappaphycus alvarezii*) SORBET

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UNIVERSITI MALAYSIA SABAH
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THESIS SUBMITTED IN PARTIAL FULFILLMENT FOR THE DEGREE OF MASTER OF SCIENCE

FACULTY OF FOOD SCIENCE AND NUTRITION
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2016

DECLARATION

I hereby declare that the material in this thesis is my own except for quotations, excepts, equations, summaries and references, which have been duly acknowledged.

14 November 2016

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ABSTRACT

Seaweed (*Kappaphycus alvarezii*), which can be locally found in Sabah, East Malaysia, was used to develop seaweed sorbet. Seaweed powder was incorporated into sorbet mixes at different percentages (0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0%). The seaweed sorbet contents were found to be low in fat (0.12 to 0.16%), protein (0.07% to 0.26%), crude fiber (0.81% to 2.61%), ash (0.11% to 0.62%) and high moisture value (83.87% to 79.55%). %). Mineral content detected in the control sorbet was sodium (0.005), potassium (23.735), calcium (3.434), magnesium (1.144), copper (0.008) and zink (0.001) mg in 100g sample. Sorbet with seaweed powder was found to have sodium (14.680 to 38.052), potassium (96.382 to 237.726), calcium (19.954 to 49.417), magnesium (11.421 to 34.515), copper (0.013 to 0.202) and zink (0.023 to 2.275) mg in 100g sample. The BIB ranking test showed that sorbet with 0.5%, 1.0% and 1.5% seaweed powder was the most favorable sorbet. Hedonic test on sorbet attribute such as colour, aroma, body, texture, seaweed taste, flavor, after taste, melting, balanced taste and overall acceptance found that there was no significant difference at $p<0.05$ between sorbet containing 0.5%, 1% and 1.5% seaweed powder. The increase of seaweed powder into sorbet resulted in a significant increment ($p<0.05$) of viscosity and hardness; on the contrary it decreased the overrun value. The first dripping time and complete melting time prolonged as the percentage of seaweed powder increased. For the total phenolic content on the seaweed sorbet, the values ranged from 0.05 to 0.14 mg PGE/g dry extract (water extraction) and 0.41 to 1.33 mg PGE/g dry extract and the control sorbet was 0.03 mg PGE/g dry extract (water extraction) and 0.17 mg PGE/g dry extract (ethanol extraction). FRAP value for water extraction of seaweed sorbet and control sorbet ranged from 5.67 to 7.89 mM ferric reducing to ferrous in 1 mg of dry extract which was higher compared to the control sorbet at 3.85 mM ferric reducing to ferrous in 1 mg of dry extract respectively. Meanwhile, for ethanolic extract, seaweed sorbet and control sorbet were found to have 9.06 to 7.89 mM ferric reducing to ferrous in 1 mg of dry extract and 3.85 mM ferric reducing to ferrous in 1 mg of dry extract, respectively. Control sorbet, sorbet with 0.5% and 4% seaweed powder recorded an IC50 value of 127.27 mg/ml, 84.38 mg/ml and 48.39 mg/ml respectively. The pH, yeast and mold test for the control and seaweed sorbet during storage showed no significant deterioration within the storage period; however, the total plate count showed a significant decreasing count from day 0 to month 6. Paired comparison test showed the sorbet with 1.5% seaweed powder scored better than the sorbet with 1% seaweed powder in terms of colour, aroma, texture, seaweed taste, sweetness and balanced taste to its reference sample. The majority which was 82.41% from 199 consumers were wiling to purchase this product while 7.59% were not willing to purchase and 10.05% were not sure on purchasing the product. In conclusion, the seaweed powder from *Kappaphycus alvarezii* had improved the sorbet on nutritional and textural physical values as compared to the control sorbet and therefore could be potentially commercialized in the market.

ABSTRAK

PENCIRIAN FIZIKOKIMIA, CIRI-CIRI SENSORI DAN KAJIAN PENYIMPANAN SORBET RUMPAI LAUT (Kappaphycus alvarezii)

Rumpai laut, Kappaphycus alvarezii yang boleh ditemui di Sabah, Malaysia timur telah di gunakan untuk membangunkan sorbet rumpai laut. Peratusan serbuk rumpai laut yang berbeza dicampur ke dalam campuran sorbet (0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, nd 4.0%). Sorbet rumpai laut mengandungi kandungan lemak yang rendah (0.124-0.156%), protein (0.070%-0.260%), serabut kasar (0.810%-2.610%), abu (0.105%-0.621%) dan kelembapan yang tinggi (83.87%-79.550%). Kandungan mineral dalam sorbet kawalan adalah natrium (0.005), kalium (23.735), kalsium (3.434), magnesium (1.144), kuprum (0.008) dan zink (0.001) mg dalam 100g sampel. Sorbet rumpai laut didapati mempunyai natrium (14.680-38.052), kalium (96.382-237.726), kalsium (19.954-49.417), magnesium (11.421-34.515), kuprum (0.013-0.202) dan zink (0.023-2.275) mg dalam 100g sampel. Ujian pemeringkatan BIB menunjukkan sorbet dengan 0.5%, 1.0% dan 1.5% serbuk rumpai laut adalah formulasi yang paling digemari. Atribut seperti warna, aroma, badan, tekstur, rasa rumpai laut, perisa, aftertaste, kadar pencairan, keseimbangan rasa dan penerimaan keseluruhan dalam ujian hedonic mendapati tiada signifikan ($p>0.05$) bagi formulasi sorbet yang mengandungi 0.5%, 1.0%. dan 1.5% serbuk rumpai laut. Peningkatan signifikan pada kelikatan dan kekerasan sorbet dan kesan sebaliknya terhadap nilai 'overrun' dengan penambahan serbuk rumpai laut. Masa jatuhan pertama dan selesai melebur sorbet semakin bertambah dengan penambahan serbuk rumpai laut. Kandungan fenolik untuk sorbet rumpai laut adalah 0.05-0.14 mg PGE/g ekstrak kering (ekstrak air) dan 0.41-1.33 mg PGE/g ekstrak kering manakala sorbet kawalan, 0.03 mg PGE/g ekstrak kering (ekstrak air) dan 0.17 mg PGE/g ekstrak kering (ekstrak ethanol). Nilai FRAP untuk ekstrak air sorbet rumpai laut adalah 5.67-7.89 mM penurunan ferik dalam 1 mg ekstrak berbanding sorbet kawalan, 3.85 mM penurunan ferik dalam 1 mg ekstrak manakala untuk ekstrak ethanol, sorbet rumpai laut dan sorbet kawalan, nilai FRAP adalah 9.06-7.89 mM dan 3.85 mM penurunan ferik in 1 mg ekstrak kering. Nilai IC50 untuk sorbet kawalan adalah 127.27 mg/ml. Sorbet yang mengandungi 0.5% serbuk rumpai laut mempunyai nilai IC50, 84.38 mg/ml manakala sorbet dengan 4% rumpai laut, nilai IC50 adalah 48.39 mg/ml. Nilai pH dan jumlah yis dan kulat untuk sorbet kawalan dan sorbet rumpai laut menunjukkan tiada kemerosotan kualiti sorbet manakala jumlah kiraan plat menunjukkan penurunan kiraan koloni yang signifikan pada hari 0 hingga bulan ke 6. Ujian perbandingan berganda menunjukkan sorbet dengan 1.5% serbuk rumpai laut adalah lebih baik daripada 1% serbuk rumpai laut dalam sorbet untuk atribut warna, aroma, rasa rumpai laut, kemanisan dan keseimbangan rasa berbanding sampel rujukan. Majoriti 82.41% daripada 199 pengguna ingin membeli produk ini, 7.59% tidak ingin membeli dan 10.05% tidak pasti akan membeli atau tidak. Kesimpulannya, serbuk rumpai laut daripada Kappaphycus alvarezii menambah baik nutrisi dan sifat fizikal sorbet berbanding sorbet kawalan seterusnya mempunyai potensi untuk di komersialkan di pasaran.

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LIST OF ABBREVIATION

ABI	-	Asia Biotechnology Institute
ADA	-	The American Dietetic Association
AOAC	-	Association of Official Agricultural Chemists
Cfu	-	Colony forming unit
cP	-	centiPoise
DPPH	-	2,2 Diphenyl-1-picrylhydrazyl
EPP	-	Entry Point Project
EGCG	-	(+)-epigallocatechin gallate
FAO	-	Food and Agricultural Organization of the United Nations
FC	-	Folin-Ciocalteu
FRAP	-	Ferric Reducing Antioxidant Power
GAE	-	Gallic Acid Equivalent
GRAS	-	General Recognize As Safe
ICP-MS	-	Inductively Coupled Plasma - Mass Spectrophotometry
LAB	-	Lactic Acid Bacteria
MARDI	-	Malaysian Agricultural Research and Development Institute
mM	-	miroMol
NAP	-	National Agro-Food Policy
NCD	-	Non communicable Disease
NKEAs	-	National key economic areas
n-3	-	Omega 3 fatty acid
n-6	-	Omega 6 fatty acid
OA	-	Overall Acceptance
PGE	-	Phloroglucinol Equivalent
TPC	-	Total Phenol Content
WHO	-	World Health Organization
α-La	-	α- lactalbumin
β-Lg	-	β-lactoglobulin

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CHAPTER 1

INTRODUCTION

1.1 Study Background

Seaweed is a marine plant product containing compounds that are beneficial to human's wellbeing. It can be consumed as part of a balanced eating regimen as it can give fiber, protein, minerals, vitamins and low-fat carbohydrate source (Yuan and Walsh, 2006). Algal polysaccharides particularly assume a key role in reducing cholesterol levels because of their dietary fiber that has gel-firming properties and a bulking effect (Theuwissen and Mensink, 2008). Other than possessing nutraceutical components, seaweed also contains antioxidant and has antimutagenic, anticoagulant, anticancerous and antibacterial activities (Abirami and Kolsalya, 2011). Rhodophyta seaweed is particularly richer than other macroalgae in metabolites abundance and very diversified (Maschek and Baker, 2008).

Seaweed has been widely explored for food as it is known to have a rich source of nutrient (Rajapakse and Kim, 2011). Since frozen desserts are well accepted worldwide, particularly in a tropical country like Malaysia, the development of sorbet incorporated with seaweed contributes for a better health. The ingredients from natural resources such as sago (Patel, Jana, Aparnathi and Pinto, 2010), Ragi (finger millet) (Patel, Dharaiya and Pinto, 2014) and amla (Indian gooseberry) (Goraya and Bajwa, 2015) have been used in developing frozen desserts. Incorporation of a functional ingredient into products such as sorbet is a way of introducing a functional component in a person's diet. However, a moderate intake of food with different varieties is the key to a healthy diet plan (The American Dietetic Association (ADA), 2004).

Seaweed has been an important source of food production and its cultivation in Sabah started since 1978 (Adibi, Lim, Tan and Phang., 2012). Seaweed produced phycocolloid, a starch-like chemical such as algin, agar and carrageenan. A good

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source of kappa carrageenan is *Kappaphycus alvarezii (K. alvarezii)*, which is mainly cultivated in Malaysia, the Philippines and Indonesia (Awang, Ong and Anisuzzaman, 2012). In dairy application, high gelling properties originating from kappa carrageenan is highly valued (Distantina, Wiratni, Fahrurrozi and Rochmadi, 2011). Kappa carrageenan acts as a secondary hydrocolloid that can prevent the mix from wheying off during ice cream making (Goff and Hartel, 2013). Thus, incorporation of seaweed powder from *K. alvarezii* into sorbet might influence the physical characteristics of sorbet.

K. alvarezii is a type of red seaweed that can be found locally in Semporna, Sabah that is commonly known as *"agar-agar"*. This type of seaweed is prepared as savoury food, eaten raw and dipped in shrimp paste mix or incorporated with another type of vegetable as a salad. The flavour profile of seaweed that originates from sea water could contribute to the unpleasant taste such as fishy and salty. Sensory properties in new products need to be considered to limit the algae incorporation into products (Domínguez, 2013). Hence, sensory evaluation on sorbet added with seaweed determines the amount of seaweed that can be accepted in a seaweed sorbet.

Researches on antioxidants in *K. alvarezii* (Matanjun, Mohamed, Mustapha, Muhammad and Ming, 2008; Wai, Chun, Thau, Abas and Chin, 2015; Farah, Abdullah, Shahrul and Chan, 2015) are available; however there is a necessity to monitor the antioxidant activities and phenolic content from the previous method to determine the stability of seaweed sorbet with the suitable extraction technique. The results of antioxidant activities of plant materials and their extraction yields highly rely on extracting the solvent nature because antioxidant compounds consist of varied chemical characteristics and their ability to be soluble in the particular solvent (Sultana, Anwar and Asyraf, 2009). Furthermore, the researches on new bioactive compounds from marine sources are almost unlimited because of their great taxonomy diversity (Rasmussen and Morrissey, 2007; Plaza, Cifuentes and Ibanez, 2008).

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The campaign on healthy lifestyle among the Malaysian public can be seen on the changes in a better diet and active lifestyle. As the interest in healthy lifestyle expands, the chances of new products with beneficial ingredients are widely welcomed. The composition of saturated fats and cholesterol in milk fat in most ice creams has become a serious concern for health-conscious consumers and people suffering from coronary heart diseases and / or diabetes (Patel *et al.*, 2010). There are multifactorial factors that cause chronic diseases including unhealthy diets, demographic changes (migration, westernization and urbanization) and population risk behaviours, such as physical inactivity smoking and alcohol consumption (WHO, 2005).

Generally, plant-based food contains high dietary and lower saturated fatty acid compared to animal based food that changed some people to become vegetarian. This group of people would not be able to enjoy ice creams that contain dairy ingredients because it is against their religious belief, being a vegetarian or believing in other ideologies and may also avoid dairy due to lactose intolerance. Lactose intolerant patients suffer from indigestion of lactose in the small bowl which causes abdominal symptom such as bloating, nausea and abdominal pain. Thus, a non-dairy frozen dessert such as sorbet would be the best alternative for them.

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Sorbet is a frozen dessert that does not contain any dairy products as its ingredient. It is similar to ice cream without adding the milk, egg or fat and it is made with water, sugar and fresh fruits (Mashall, Goff and Hartel, 2003). It is a healthier alternative to ice cream since it is dairy-free and typically fat-free (Stogo, 1998). Those with high quality are expected to have a fluffy texture and are generally presumed to be at their best when consumed immediately after the freezing process. Some of the sorbet originating from France and Italy contain wines or liquors. Usually, sorbet is served fresh and sold directly to walk up customers at retail stands; though hardened sorbet can also be made available to the customer (Clark, Costello, Drake and Bodyfelt, 2009). In Malaysia, a frozen dessert such as sorbet and ice cream are enjoyed by people of all ages. Due to high demands, both local and international brand of frozen dessert can be found in the Malaysian market. Euromonitor

International reported that the retail volume of ice creams and frozen desserts in Malaysia was 40,400 tons that value RM656 million in 2015.

1.2 Problem statement

The taste of seaweed could possibly contribute to the problem in developing the extract as a functional ingredient. Sensory properties in new products need to be considered to limit the algae incorporation into products (Domínguez, 2013). Furthermore, seaweed hydrocolloids that possess gelling properties could set a drawback to the amount of seaweed incorporated into products. However, since seaweed is reported to have high nutritional and antioxidant properties, it is therefore of interest to investigate the product development potential of Sabah seaweeds, which are one of the under-utilised state resources.

1.3 Rationale for Research

Development of frozen dessert from seaweed such as *Kappaphycus alvarezii* is still scarce in the current market. To date, there is not a single study carried out on sorbet incorporated with *Kappaphycus alvarezii* and its nutritive value and storage studies. This study aimed to investigate the likelihood of enhancing the nutritional composition and improving the physical properties of sorbet utilizing *Kappaphycus alvarezii* with acceptable sensory properties. This study is of great importance to increase the utilization of a local produced raw material from Sabah's underutilized marine resource that has the potential to be developed into a local food industry

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1.4 Objectives

The objectives of this research are as outlined below: To study the physicochemical properties of sorb

a. To develop sorbet using *Kappaphycus alvarezii* seaweed
 incorporated with *Kappaphycus alvarezii* seaweed

b. et To study the storage quality of sorbet incorporated with *Kappaphycus alvarezii* seaweed

CHAPTER 2

LITERATURE REVIEW

2.1 Macro algal marine

Seaweed or macro-algae is a multicellular plant that lives either in seawater or freshwater environment. Photosynthetic pigment in seaweed with the aid of nutrient from water and also sunlight helps the photosynthesis process in seaweed that produces food (Joshi, Desai and Mulye, 2015). Distribution and variety of seaweed are determined by the types of environment that involve the pigments of plant, light, exposure, depth, temperature, tides and shore characteristics. The division of seaweed can be divided into Chlorophyta (green algae), Cyanophyceae (blue-green algae) Phaeophyta (brown algae) and Rhodophyta (red algae). Most of the red and brown algae are discovered in the marine environment and are industrially important. Normally, the blue-green algae can be found in the area such as freshwater and land.

In an Asian country, seaweed has been apart of the dietary intake whereas the Western countries are focusing mainly on the phycocolloid industry such as carrageenan, alginic acid and agar (Sen, 2005, Mabeau and Fleurence, 1993). The seaweed species namely *Hydroclathrus, Caulerpa, Euchema, Gracilaria* and *Acanthohora spp* are consumed as green salad vegetable while the coarse *Gracilaria* and *Euchema spp*. are pickled (Pawiro, 2008 and Sen, 2005). Dried *Laminaria spp*. known as kumbu in Japan is becoming a flavouring agent in food while *Phypora sp* is used in the production of nori seaweed wrap and usually used in sushi and kimbab. *Wakame* originating from *Undaria pinnatifida is* generally incorporated in bean curd soup or salad. In the food industry, carrageenan values depend on its yield and physical properties such as gel strength, gelling and melting temperature as well as the chemical properties (Distantina and Fahrurrozi, 2013).

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Seaweed hydrocolloids are located on the cell wall that contains polysaccharides (Venugopal, 2009). Polysaccharides from seaweed include agar, alginates, carrageenans and also minor compounds such as fucoidan and laminarin. Polysaccharide in a seaweed structure has high water holding capacity in compared to cellulosic fibers depending on the length and thickness of the fiber particles. Important functions of polysaccharide in technologies include being used as texturizers, stabilizers, emulsifiers, fat reducers, film formers, shelf life extenders and viscosity modifier. Seaweed can be widely utilized in developing various products and not only limited to developing food products. Products derived from seaweed are shown in Table 2.1.

Table 2.1: Seaweed derived products

Products/ Process/ Service	Source
Source of food / Nutraceutical	Alga-oligosaccharides (Agar, alginates, fucoidan)
Cosmetic	Emulsifier, anti-cellulite, anti-aging
Biopharmaceutical	Microbiocide
Industrial colloid	Agar, Agarose, Carrageenan
Horticulture	Fertilizer, soil conditioner, foliar spray (hormone growth)
Biofuel	Methane, methanol, ethanol
Weather Management	Reduction of carbon dioxide gas
Bioreactor	Vaccine

Source: Phang, 2010

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Commercial sources of Agar come from red seaweeds such *Gracilaria, Gelidium and Gelidiella* spp (Venugopal, 2009). Agar is made of two polysaccharides, agarose and agaropectin. It is a hydrophilic colloid, insoluble in cold water but soluble in boiling water. An essential characteristic of agar is its gelling property and the extensive variety of temperatures under which it holds this property. Agar usage is not limited in food application as a thickener, gelling agent and stabilizers but gel electrophoresis and chromatography.

Alginate originates from brown seaweed algae, namely *Laminaria hypernorea, Macrocystic pyrifera, Laminaria digitata, Ascophyllum nodosum, Laminaria japonica, Ecklonia maxima, Lessonia nigrescens* and *Durvillaea Antarctica* (Rehm, 2009). Alginic acid is composed of D-mannuronic acid (M), L-glucoronic (G) and alternates of M and G units. Alginate is utilized for thickening, suspending, balancing out, emulsifying, gel-framing, or film-shaping. Application of alginate is used for making ice cream and other dairy products, while the rest is used in other products, including shaving cream, rubber, or paint. In textiles, alginate is utilized to thicken fiber-reactive dye pastes, which facilitates the sharpness in printed lines and conserves dyes. Dental impression of teeth by dentists uses alginates (Nandini, Venkates and Nairi, 2008).

Fucoidan is a sulfated polysaccharide discovered from various species of brown seaweed. Sulfated fucan is the characteristic feature of the fucoidans. Past research has found that fucoidans contain antitumor, antimutagenic, anticomplementary, immunomodulating, hypoglycemic, antiviral, hypolipidemic and anti-inflammatory activities (Shanmugam and Mody, 2000). Anti-cogulative properties are also present in fucoidan ad the function of selections can be bound and blocked. The structure of fucoidans is in contrasts when they are extricated from diverse algal species; the structure of sulfated fucans of the marine invertebrate root is a less complex contrast with those algae (Venugopal, 2009).

Laminarin is another polysaccharide that can be found in brown seaweed, specifically *L. japonica*. Fundamentally, laminarin is made out of β-(1,3)- connected glucose containing a lot of sugars and a low part of uronic acids. Two sorts of polymeric chains are available in laminarin; G-chains with glucose toward the end and M-chains with mannitol as the terminal reducing end. A research conducted by Devillé, Gharbi, Dandrifosse and peulen (2007) found that laminarin is by all accounts a modulator of the intestinal digestion system by its impacts on mucus composition, intestinal pH and short chain unsaturated fat (SCFA) creation, particularly butyrate. The molecular structure, degree and length of branching and the monosaccharide constituents were found to be the connection to the antioxidant activity in laminarin (Choi, Kim and Lee, 2011).

Carrageenan is extracted from numerous types of red seaweeds including *Chondrus crispus, Gigartina stellate* and *Euchema spp.* The three major types of carrageenans are namely, kappa, iota and lambda. Carrageenan is a blend of galactans that convey fluctuating half-ester sulfate gatherings connected to one or a greater amount of the hydroxyl gatherings of the galactose units. Previous research has been done on the functionality of various carrageenan structures and sulfated galactants that are isolated from red seaweed (Lin, Tako and Hongo, 2000). Different types of carrageenan have a different chemical structure as shown in Figure 2.1. *Kappaphycus alvarezii* (*K. alvarezii*) is a good source of kappa carrageenan (Distantina, Wiratni, Moh and Rochmadi, 2011).

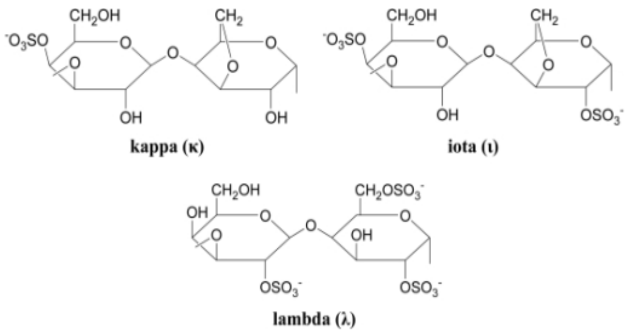

Figure 2.1: Chemical structure of different carrageenans.
Source : Falshaw, Bixler and Johndro (2001)

2.2 Seaweed in Malaysia

The agrobusiness segment assumes a vital part in Malaysia's financial growth that helps in giving provincial business, inspiring provincial salaries and guaranteeing national food security. Malaysia is located in South East Asia with a tropical climate. The coastline is 4,675 kilometers, specifically 2,068 kilometers in Peninsular Malaysia while East Malaysia has 2,607 kilometers of coastline. Henceforth, this provides an open door for the fishery industry to be created and this incorporates culturing of seaweeds.

In the Malaysian National Agro-Food Policy (2011-2020) (NAP4), kelp was recognized as one of the high-esteem products under the system of Entry Point Project 3 or EPP 3 (Venturing into Commercial Scale Seaweed Farming in Sabah). National key economic areas (NKEAs) within the entry point project (EPP) are focusing on transforming seaweed production in Sabah into high yielding commercial business. EPP's projects on seaweed are driven by the Department of Fisheries

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