THE EFFECT OF SOAKING DURATION TO *KAPPAPHYCUS ALVAREZII SP*. SEAWEED POWDER

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Submitted in accordance with the requirement of the Universiti Teknikal Malaysia Melaka (UTeM) for Bachelor Degree of Manufacturing Engineering (Hons.)

by

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2021

DECLARATION

I hereby, declared this report entitled "The Effect of Soaking Duration to *Kappaphycus Alvarezii Sp.* Seaweed Powder" is the result of my own research except as cited in references.

Signature : ……………………………………

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Date : 15th SEPTEMBER 2021

APPROVAL

This report is submitted to the Faculty of Manufacturing Engineering of Universiti Teknikal Malaysia Melaka as a partial fulfilment of the requirement for Degree of Manufacturing Engineering (Hons). The member of the supervisory committee is as follow:

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ABSTRAK

Baru baru ini, kesedaran mengenai hubungan antara diet dan kesihatan yang menyebabkan pengambilan rumpai laut semakin mendapat perhatian. Oleh itu, perkembangan serbuk rumpai laut perlu ditingkatkan di atas permintaan yang tinggi dan untuk pemasaran serbuk rumpai laut yang lebih maju. Tujuan kajian ini adalah untuk menyiasat kesan jangka masa rendaman kepada *Kappaphycus Alvarezii sp.* dalam penghasilan serbuk rumpai laut melalui kaedah rawatan rendaman. Dalam penghasilan serbuk rumpai laut, signifikasi parameter jangka masa rendaman telah dijalankan pada rumpai laut. Berdasarkan kajian terdahulu, terdapat hanya sedikit kajian berkenaan kesan jangka masa rendaman kepada penghasilan serbuk rumpai laut. Oleh itu, kesan jangka masa rendaman dikaji untuk mendapatkan jangka masa rendaman yang optimum dan menganalisis kandungan logam berat di dalam rumpai laut serta jumlah kandungan kelembapan rumpai laut yang boleh menjamin kualiti rumpai laut yang tinggi seterusnya meningkatkan jangka hayat serbuk rumpai laut. Tempoh rendaman didapati optimum pada 2 jam rendaman kerana rumpai laut menunjukkan pengembangan dan tekstur yang diinginkan. Kandungan logam berat Arsenik (As), Kadmium (Cd) dan Plumbum (Pb) dipostulat dalam pengurangan kandungan logam berat pada jangka masa rendaman yang optimum iaitu selama 2 jam berdasarkan trend grafik melalui tinjauan kritikal. Pada jangka masa rendaman yang dioptimumkan selama 2 jam juga memperoleh kandungan kelembapan yang memenuhi kehendak iaitu pada 14.67%. Imbasan Elektron Mikroskopi (SEM) analisis dijalankan untuk mengkaji struktur morfologi kesan jangka masa rendaman pada masa yang berbeza terhadap rumpai laut. Pada rendaman optimum 2 jam, struktur morfologi menunjukkan tekstur lebih halus dan berkembang sejajar dengan peningkatan kandungan lembapan serta permukaan rumpai laut yang lebih bersih kerana benda asing telah disingkirkan Oleh itu, ketersediaan rumpai laut boleh menjadi sumber bahan mentah dalam pengeluaran serbuk rumpai laut yang menyumbang kepada sektor industri, terutama dalam makanan dan produk farmaseutikal dengan cara yang lestari.

ABSTRACT

Recently, people have become more conscious of the relation between diet and health, leading to increasing attention on the consumption of seaweeds. Therefore, the development of seaweed powder needs to be enhanced due to high demand and for advanced marketing of seaweed powder. The purpose of this study is to investigate the effect of soaking duration on *Kappaphycus Alvarezii sp.* seaweed powder during soaking treatment. In order to produce seaweed powder, significant soaking durations have been carried out on the seaweed. Based on the previous study, there has only little study on how soaking duration affects the production of seaweed powder. Hence, the effect of soaking duration was studied to analyse the toxicity of heavy metal content inside seaweed and the amount of moisture content of seaweed to guarantee a high seaweed quality and increase the shelf life of seaweed powder. The optimum soaking duration was observed to be 2 hours of soaking, at which seaweed exhibits desirable expansion and texture. The heavy metal contents of Arsenic (As), Cadmium (Cd) and Lead (Pb) were postulated to be reduced at the optimized soaking duration of 2 hours, based on critical review of the graph trend. The optimized soaking duration of 2 hours also showed an acceptable moisture content of 14.67%. Next, surface morphology structure was observed by carrying out Scanning Electron Microscopy (SEM) analysis to study the effect of various soaking duration towards seaweed. At optimum soaking of 2 hours, the surface morphology indicated smooth texture and obtained desired expansion corresponding to the increased moisture content and the surface was cleaner after soaking treatment as all the impurities had been removed thoroughly. Thus, the abundantly available seaweed can provide as a source of raw materials in the production of seaweed powder which will contribute to the industrial sector, especially in food and pharmaceutical productions that favour sustainability.

DEDICATION

Only

my appreciated mother, Romsiah Binti Abdullah

my adored sister and brothers, Amirul, Syafina and Nazrul

To my kindhearted supervisor, Ts. Dr. Rose Farahiyan binti Munawar

To my co-supervisor, Ts. Dr. Jeefferie bin Abd Razak

for giving me moral support, money, cooperation, encouragement and also understandings

Thank You So Much & Love You All Forever

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LIST OF ABBREVIATIONS

As	-	Arsenic
Cd	-	Cadmium
Pb	-	Lead
H_20_2	-	Hydrogen peroxide
HNO_3	-	Nitric acid
ICP-MS	-	Inductively coupled plasma mass spectrometry
JECFA	-	Joint FAO/WHO Expert Committee on Food Additive
LOD	-	Loss on drying
SEM	-	Scanning Electron Microscopy

LIST OF SYMBOLS

kV	-	Kilovolt
%	-	Percent
℃	-	Degree celcius
g	-	Gram
h	-	Hour
nm	-	Nanometre
$mgkg^{-1}$ -	-	Milligrams Per Kilogram
ml	-	Millilitre
µm	-	Micrometre

CHAPTER 1

INTRODUCTION

This chapter explains the background of the study, problem statement, objectives, research scopes, and the significance of this research. As requested by the respective industry (Saanen Sdn. Bhd.), the *Kappaphycus Alvarezii sp.* seaweed name applied throughout the study representing the investigated seaweed species.

1.1 Research Background

Seaweeds or macroalgae are an economically viable and environmentally important part of marine habitats. Seaweed is the largest producer, a shelter and even necessities for aquatic ecosystems. They create an underwater forest as the significant food source for marine herbivores, offer protection for fish and preserve marine endophytes. Seaweed horizontal distribution is influenced by substratum, tide action, and wave amplitude and their common habitats are sandy and rocky (Zainee *et al*., 2019). Seaweed populations where most common habitats are sandy and rocky. The distribution of seaweed was studied in an area of 500 m^2 from lower intertidal levels to high intertidal regions (Babitha and Subramaniam, 2016). Moreover, macroalgae are generally classified and come in a multitude of varieties. Those three main divisions are Chlorophyta, Phaeophyta, and Rhodophyta, based on their pigmentation (Syeda Fatima Manzelat *et al*., 2018).

Over the past few years ago, tropical algae are currently attracting attention in biodiversity studies. Presently, seaweeds have drawn critical worldwide attention because of their possible use include anti-biofilm activity, biofuels, bioremediation, fertilizer, as fish feed, as food or food ingredients, in pharmacology in cosmeceutical formulation, and in such other applications as filters or for obtaining minerals (Gomez Zavaglia *et al*., 2019).

Furthermore, aquaculture cultivation with the balance produced through natural harvesting provides 96% of world seaweed production for human use and various applications. On a fresh weight, its annual basis is ca.24.9 million tons (Radulovich *et al*., 2015).

Kappaphycus Alvarezii sp. are among the largest tropical red algae, with a high growth rate (double in biomass in 15 to 30 days) (Bhuyar *et al*., 2020). Cultivation of *Kappaphycus Alvarezii sp*. has recently been found in Semporna, Sabah (Ilias *et a*l., 2017). *Kappaphycus Alvarezii sp.* are stiff, fleshy, solid, and coarse thalli with 1–2 cm diameter axes and branches that grow up to 2 m tall. It may be discovered as a flat reef 1 to 17 m deep underwater or close or unattached to fractured coral (Bhuyar *et al*., 2020). *Kappaphycus Alvarezii sp.* has the highest growth rate among other *Kappaphycus* seaweeds made it favorable for food and pharmaceutical applications (Kumar *et al*., 2015). *Kappaphycus Alvarezii sp.* is a kappa carrageenan source, a phyllocoloid generally used to stabilize and thicken in food, cosmetics, and pharmaceutical industries (Mohammad *et al*. 2019). Seaweed is stuffed with vitamins and protein and also various nutritious content. The characteristics of seaweed dietary fiber are related to its physicochemical properties made it essential to improve functional properties in food and beverages. However, this seaweed is being revalued in recent years and gradually introduced into the diet, especially for vegans and vegetarians (Penalver *et al*., 2020).

Soaking is the treatment when solid food is softened by submerging it for hours in liquid, usually water. Soaking is suitable for improving the nutritional content of the soaked food and its taste. Nonetheless, foods are often soaked to transform them into a mild shape that helps to mix seamlessly. The shortage of quantity of soaking water can contribute to a rough texture and stiff structure. However, this method is held responsible for removing a certain percentage of vital nutrients into the water. Soaking should be in proper condition because leaching of material depends on the soaking conditions, particularly on time of soaking (Siah *et al*., 2014).

Hence, this current work aims to prepare the Kappaphycus Alvarezii sp. seaweed powder by utilizing the soaking process on the raw material. The proper soaking process conditions are subjected to soaking durations with varied parameters. This is due to limited previous studies on how the soaking process would affect seaweed powder characteristics.

The effect of the soaking process is further analyzed on the seaweed in terms of heavy metal content and moisture content. Scanning Electron Microscopy (SEM) is used to characterize the morphological and structural of seaweed. Therefore, proper soaking conditions can be determined as to produce a good seaweed powder by evaluating the characteristics and structures obtained through different soaking conditions.

1.2 Problem Statement

This study points up the preparation and characterization of seaweed powder, significantly, *Kappaphycus Alverazii sp* seaweed via the soaking process. In order to convert the seaweed into powder, the seaweed must undergo several processes. Several procedures need to be conducted and start with soaking, cutting, drying and grinding. However, the soaking process is highlighted in this study. The soaking treatment is one of the methods used to improve the nutrition value of raw materials in the manufacturing of food products besides being an additional advantage towards saving energy cost and cost-effectiveness in industries. This study can help expand some good advantages of macroalgae that focus on the health benefits of seaweed and its use in various end-use industries. Furthermore, the seaweed market in world production is getting high demand from 2011 to 2015 (Ferdouse *et al*., 2018).

However, mostly algae have absorption capability and become a great concern. They can accumulate heavy metals and are widely recognized in oil-related pollution caused by ship leakage and waste generated by local shore facilities (Tornero and Hanke, 2016). Furthermore, biosorption has developed as a viable alternative to traditional methods for removing heavy metal ions from effluents produced by various industries, which eventually reach and contaminate freshwater bodies (Kanamarlapudi *et al*., 2018). Heavy metals are pertaining to any metal that features a comparatively high density, toxic, poisonous at low concentrations. Besides, the moisture content of the food material is vital to consider the food is suitable before consumption. Moisture content relates to the freshness and stability for storing the food for a long period and determines the actual quality of the food before consumption.

Moreover, to date, the limitation of current studies has only been considered on the soaking concentration and soaking temperature, however, its characterization with the effect of soaking duration on the seaweed needs to be exploited. To provide a vast potential market of seaweed as the main source of raw materials globally and the industrial sector, it needs to upgrade seaweed powder production through soaking treatment on seaweed. Hence, to overcome the limitations, optimization of the seaweed powder processing will be applied throughout this study. However, due to the restriction of the COVID pandemic, the heavy metal analysis study conducted via postulating the critical review analysis while moisture content and SEM analysis by experimental study. Scanning Electron Microscopy (SEM) analysis enables the characterization of material structures of the seaweed due to different parameters during the soaking process. Figure 1.1 shows a summary of the problem statement and research gap of seaweed powder.

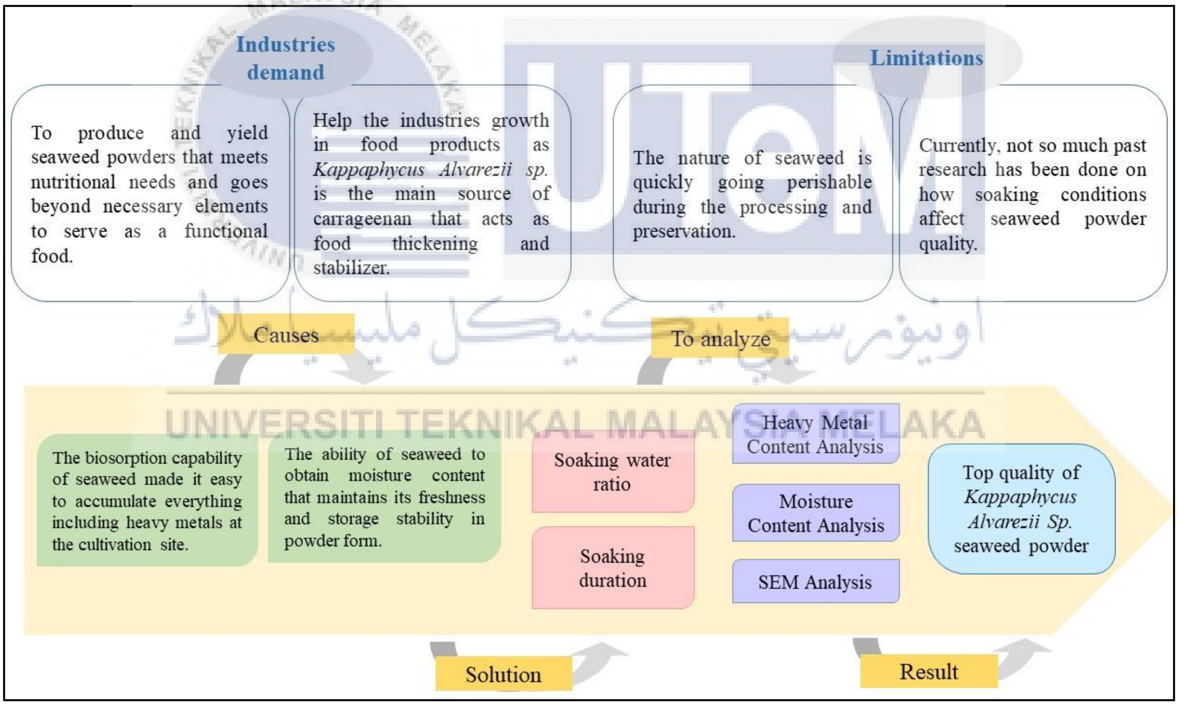


Figure 1.1: The overview of problem statement and research gap.

1.3 Objectives

The objectives are as follows:

i. To prepare *Kappaphycus Alvarezii sp.* seaweed powder with a proper soaking condition particularly soaking durations.

ii. To study the effect of soaking treatment towards heavy metal content and moisture content of *Kappaphycus Alvarezii sp.* seaweed.

iii. To characterize seaweed structure via Scanning Electron Microscopy (SEM) analysis.

1.4 Research Scopes

The study mainly focuses on soaking treatment on seaweed on how soaking duration effects the heavy metal content, moisture content and to investigate the characterization of the selected algae, *Kappaphycus Alverazii sp.* This study performs in accordance with several scopes. For the seaweed powder preparation, the soaking treatment was carried out on dried seaweed while at the same time it helps in removing impurities. The dried seaweed usually contains foreign matters and needs to sort out manually with naked eyes before the soaking process.

The focused objective of this study is to investigate the effect of soaking durations towards *Kappaphycus Alvarezii sp.* seaweed powder. The soaking process is chosen because it is environmentally friendly and does not require an organic solvent, which is very cost-effective. Before industrial processing proceeds, the seaweed must be softened by soaking due to its rough, rugged and dry texture. However, the predetermined soaking water ratio is 1 part of dried seaweed to 10 parts of distilled water (1:10). The optimized parameter in this study is soaking duration. Soaking with varied parameters of duration is used in this study.

Then, the heavy metals analysis and moisture content analysis are studied, as mention in the second objective. Heavy metals analysis has been postulated and supported via critical review analysis based on previous research. Heavy metals testing detects metals that are harmful in both small and large amounts, such as lead, mercury, arsenic, and cadmium inside seaweed. This is important towards presented *Kappaphycus Alvarezii* are safe as seaweed consumption is growing steadily in human dietary fiber intake. The purpose of analyzing the moisture content of seaweed is to measure the amount of water content of seaweed after going through a varied duration of soaking treatment. Moisture content is vital towards product shelf life, freshness, quality, and resistance to bacterial contamination. As to reach the third objective of this study, the characterization methods were using Scanning Electron Microscopy (SEM) of seaweed. Scanning Electron Microscopy (SEM) analysis was performed to study the surface morphology and structure of *Kappaphycus Alvarezii sp.* seaweed after the soaking process at different durations. The mapping matrix of the objectives and scopes describes as in Figure 1.2.

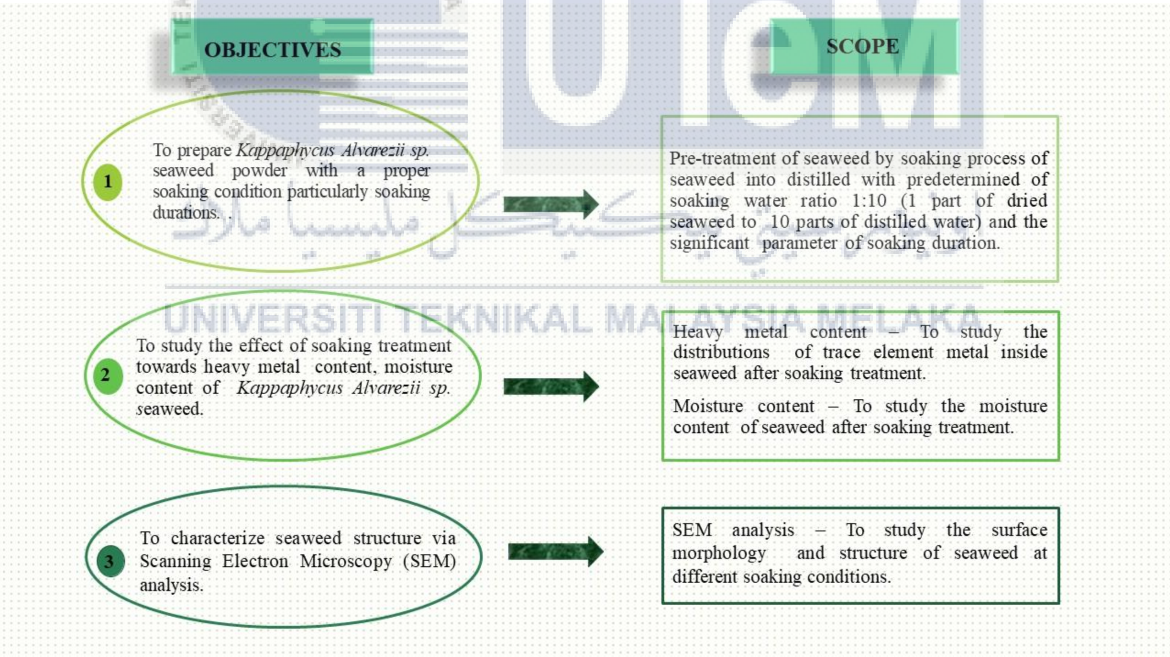


Figure 1.2: The mapping matrix of the objectives and scopes

1.5 Significant of Research

Marine life has independent of the mind of marine algae. Polysaccharides, proteins containing all necessary amino acids, polyunsaturated fatty acids, vitamins and minerals are rich in seaweeds (Wells *et al.*, 2017). Seaweed is a very multifunctional product generally used in the food indirect human consumption. In addition, the potential for seaweed development continues to grow for numerous applications. It is imperative to investigate the effective method for developing seaweed powder under proper soaking durations. In addition, a study on the processing of seaweed powder is conducted out and in order to optimized conditions to produce seaweed powder.

Thus far, a few detailed studies have been carried out on the effect of the seaweed soaking process as a raw material in the production of seaweed powder.*Kappaphycus Alvarezii*, the algae species, has various advantages and has been an attraction from the researcher. Aside from benefits to the food industry, nanocellulose extracted from seaweed is generally used as a reinforcing material for polymer composite due to good increasing abilities. Besides, a foliar spray made from seaweed extract is applied to green and fleshy vegetables, fruits, orchards, and horticultural plants to promote quicker development and production.

Today, besides the source of nutrients, seaweed is also present to treat several diseases related to bacterial infection, cancer, tumor, allergy, aging and inflammation. On the other hand, green material as the raw material is also a concentrate. Instead of other chemical sources, it provides an alternative raw material, which gives sustainable powder production. Thus, seaweed production uses mineral wealth as raw materials in the production of seaweed powder, which has a low impact on human well-being and the surroundings. It is more beneficial to optimize the analysis of natural materials for current and future applications.

CHAPTER 2

LITERATURE REVIEW

This chapter explains, in particular, the theory and study that described and carried out by various researchers years ago. Related material from previous research is extracted a source and explored in more detail.

2.1 Seaweed

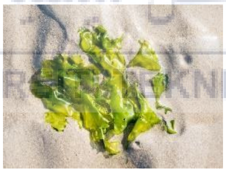
Seaweeds, otherwise referred to as marine algae belong to the most superficial plants. Algae are non-flowering photosynthetic macrophytes found in the ocean tidal zones (Rao *et al.*, 2018). Seaweed is plant-like organisms, diversity, and the seaweed that physique the benthos varied in size from microscopic single-celled forms to gigantic macrophytes and divided into main subgroups algae (Rindi, 2020). Seaweed is such an innovative product and extensively used for human consumption both fresh and dried and further processed applications (Stevant *et al.*, 2017).

Regarding Zugcic *et al.* (2018), the incorporation of seaweed in the food matrix has improved the physicochemical and nutritional properties and technological aspects of foods. It is a great source of nutrients to decrease the pollution globally (Gujar *et al.*, 2019).

2.1.1 Classification of seaweed

As per Leandro *et al.* (2020) seaweed is grouped taxonomically into three broad and distinct classes focused on the color of the chlorophyta (green seaweed) thallus, Rhodophyta (red seaweed) and Phaeophyceaeae (brown seaweed). Khan *et al.* (2018) stated that macroalgae (seaweed) are multicellular, large-size algae that can be seen with the naked eye, whereas microalgae are microscopic single cells that can be prokaryotic, like cyanobacteria (Chloroxybacteria) or eukaryotic, like green algae. Brown seaweed are typically wide and vary from an immense kelp, often 20 m long while red seaweed is typically smaller, usually from a few centimeters to a few meters in length. The red seaweed is not only red, and are often purple, or brownish red. Green seaweed is also small, similar in size to red seaweed. The taxonomic categorization of algae is still a source of continuous modifications and disputes (Rao *et al*., 2018). Table 2.1 shows the pigment colour varies in three common algae group.

Table 2.1: The pigment colour of three common algae group (Gomez Zavaglia *et al*., 2019)

Pigment Class	Green Seaweed	Brown Seaweed	Red Seaweed
Chlorophylls	Chlorophyll a and b, and derivatives	Chlorophylls b and c, and derivatives	Chlorophylls a and d, and derivatives
Carotenoids	ß-carotene, xanthophylls	Fucoxanthin and Xanthophyll,ß-carotene	Xanthophylls
Phycobiliproteins	-	-	Phycoerythrin and phycocyanin
Example	 *Ulva lactuca sp.*	 *Sargassum sp.*	 *Kappaphycus Alvarezii sp.*

2.1.2 Structure and composition of seaweed

Seaweed's chemical composition provides their chemical structure with improved nutritional benefits, leading to human protein-based nutrients, including essential amino acids, minerals, and vitamins. The chemical properties of seaweed differ with organisms, age, collection and ecological factors (habitat, temperature, and season) that may either promote or hinder nutrient biosynthesis (Aroyehun *et al*.,2019) and genetic differences among species (Maehre *et al*., 2014).

Prominently, marine seaweed contains large quantities of nitrogenous compounds. Some elements, such as free amino acids, chlorophyll, nitrate and nitrite nitrogen, ammonium ions, and nucleic acids, are derived from a tiny amount of non-protein nitrogen found in seaweed (Daniel Tomé *et al*. 2019). Eventually, the total protein value varies based on the depth of the sample taken (Kasimala *et al*., 2015). Protein quality depending on the bioavailability and protein amino acids (Elango *et al*., 2012).

Minerals and trace elements at some point make up roughly 4% of total human body mass, play a vital part in bone formation, control various bodily functions, and maintain the body's water balance when combined with water. Seaweeds have high vitamin and minerals content, which delivers an apparent health benefit (Abdul Khalil *et al*., 2017) in Table 2.2. Seaweeds are allowed to extract inorganic compounds from the environment as a powerful source of minerals. (Misurcova, 2011). The nature of different polysaccharides throughout the seaweed walls justified that different seaweed species have different mineral systemic absorption. High metal sorption of brown seaweed over the green and red seaweed efficiency may depend on alginic acid and alginate.

Table 2.2: Chemical composition of seaweed (Abdul Khalil *et al*., 2017)

Compounds	Composition percentage
Water	80–90 %
Carbohydrates	Dry weight basis containing of 50 %
Lipids	1–3 %
Minerals	7–38 %
Protein contents	Highly variable (10–47 %) with high proportions of essential amino acids

2.1.3 Applications of seaweed

Seaweeds, sometimes known as "sea vegetables," quickly gained popularity in Asian cultures, where they have been traditionally been regarded as kings and gods cuisine. Marine is the tremendous contributor of structurally distinct natural resources found primarily in biological entities. Seaweed produces many essential functional ingredients for health benefits and is incorporated into foods for daily consumption (Ranga Rao, 2018). Hydrocolloids in seaweed are widely used in many food formulations to improve quality attributes and shelf-life. Hydrocolloids, such as alginate, carrageenan and agar, act as thickening and gel formation. It is also review as a pinpoint for food formulations and product development (Shannon and Abu-Ghannam, 2019).

In this day and age, humans served food to satisfy starvation, fulfill dietary needs and in the purpose to reduce chronic risk. It becomes the most extraordinary global demand for seaweed and in Asian countries, seaweed is vastly consumed as a traditional product. Currently, seaweed also poses as a food ingredient in making ice-creams, yogurts, pasta, cheeses, bread, snacks, frankfurters, and beef patties (Mohammad *et al*., 2019). This also stated by Circuncisao *et al*. (2018) in which different amounts of *Laminaria spp.* have been added to boost the iodine content of a novel probiotic yoghurt. As seaweed is a unique source of hydrocolloid, they tend to provide numerous ingredients to the food, pharmaceutical, cosmetic, textiles, paper, and biotechnology industries as stabilizers and thickeners emulsifiers and fillers (Abdul Khalil *et al*., 2018). Carrageenan is a kind of linear sulfated polysaccharide isolated from edible red seaweeds, primarily from the *Kappapphycus Alvarezii* species. Carrageenan is a thickening ingredient that is commonly used in the preparation of pizzas, desserts, gels, canned meals (Nakhate and van der Meer, 2021) and chocolate milk, cottage cheese, whipped cream, instant products, yogurt, frozen desserts, sauces (Ruthiran *et al*., 2018). Figure 2.1 summarized the seaweed application in food product.

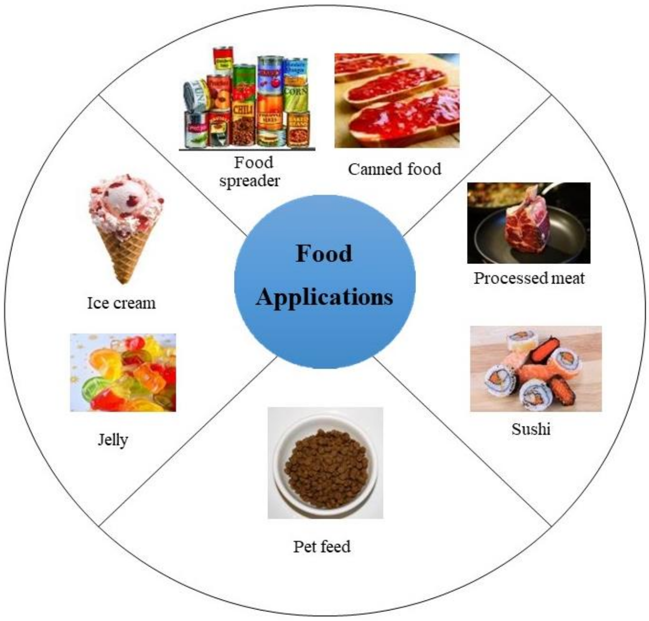


Figure 2.1: Seaweed application in food products (Abdul Khalili *et al*., 2018)

Apart from providing nutritional assistance, seaweed is also utilized to treat a variety of ailments as it acts as antimicrobial, antiviral, antifungal, anti-allergic, anticoagulant, anticancer, antifouling, and antioxidant properties are examples of biological disorders (Pati *et al.* 2016). Aside from their antibiotic properties, algal extracts have also been employed as vermifuges (destroying intestinal worms like Ascaris) and antiscorbutic, apart from cough, belly, chest, bladder, and renal problems (Rao *et al*., 2018). Seaweed extracts are also applied for face and skincare products, anti-aging cream, regenerative skin cream, emollient products, anti-irritant products, sun protection cream and hair care products (Veluchamy and Palaniswamy, 2020). In order to enhance their properties owing to their capacity to protect and maintain goods, bioactive compounds in seaweed are integrated especially into cosmetics (Pinela *et al*., 2018). Furthermore, due to the large possible biomass yields and growth methods that do not keep pace for agricultural land or freshwater, seaweed interest in using microalgae and macroalgae as biofuel sources In recent years, seaweed also was potentially used as an anti-fouling agent in the development of paints. Asparagopsis, Laurencia (red algae), and Sargassum (brown algae) act as vital sources of anti-fouling compounds (Veluchamy and Palaniswamy, 2020). Figure 2.2 explained the non-food applications of seaweed.

According to Morais *et al*. (2020), the usage of green seaweed, particularly Ulva sp., exhibited high protein content and high levels of mineral components present in oysters, while also providing a cost-effective approach to harness the positive effects of seaweeds in farm animals. Microalgae have been used to assess environmental toxicants as a biological technique. Heavy metals are found in wastewater are from metal smelters, paint manufacturers, inorganic fertilizers, agricultural activities, leather tanning, electroplating, alloy and battery manufacturing, and other industrial waste materials disposal (Arumugam *et al*., 2018). Heavy metal removal from wastewater is a serious environmental issue today, and it has gained recognition because heavy metals damage pose to the environment and social things, particularly when they exceed regulatory limits. As, Cd, Cr, Cu, Hg, Mn, Ni, Pb, and Zn are heavy metals that are of main concern in the environment due to their toxicity (Agoro *et al*., 2020)

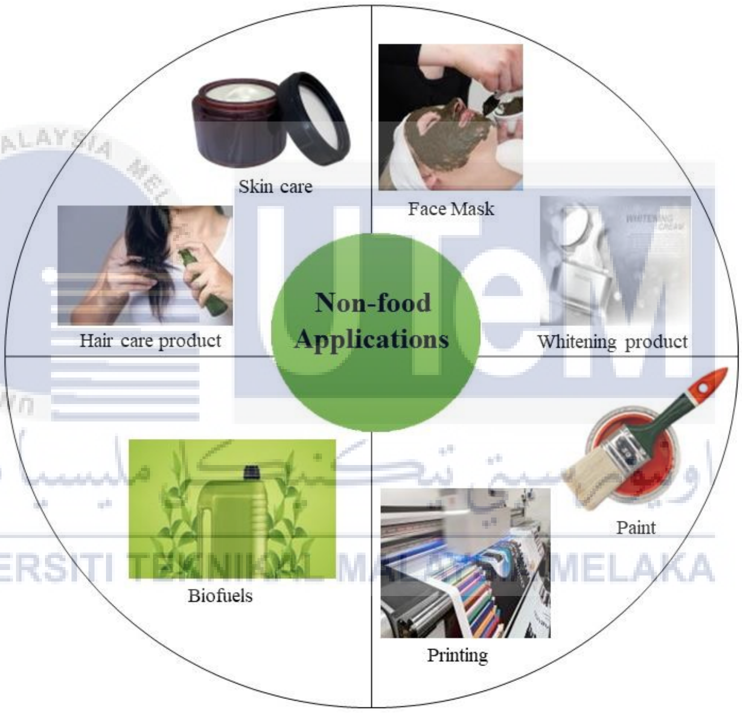


Figure 2.2: Seaweed in non-food application (Leandro *et al*., 2020)

Wastewater is a by-product of every phase of operation. Seaweed points contribute to various sectors and are commonly explored and utilized as an adsorbent in wastewater treatment to reduce usable activated carbon. The establishment of biosorption to remove heavy metals is a dependable technique (Ramírez Calderon *et al*., 2020). Biosorption is a type of sorption when the sorbent is a biologically derived substance. Biosorption is now widely regarded as a straightforward, cost-effective, and ecologically acceptable method of eliminating contaminants. The use of algae for heavy metal ion biosorption in wastewater can be environmentally friendly, inexpensive, and practical (Ortiz Calderon *et al*. 2017). The Table 2.3 shows the uses of red seaweed in removing heavy metals.

Table 2.3: Application of red seaweed used in removing heavy metals content in wastewater treatment (Arumugam *et al*., 2018)

Seaweed	Type of wastewater	Studied parameters	Treatment Conditions	Heavy Metal	Treatment performance
Gracilaria sp.	Landfill leachate	Gel/Adsorbent concentration = 10, 20, 50, and 100mg/L t = 10 d	pH = 8	Fe Cd As Ni	Fe = 100% (t = 1, Ion = 10 mg/L) Cd = 100% (t = 5 d, Ion = 100mg/L) As = 100% (t = 5 d, Ion = 50 mg/L) Ni = 98% (t = 10 d, Ion = 50 mg/L)
Osmundea Pinnatifid sp.	Simulated Wastewater	pH = 2–9 t = 0–3 h Biomass = 5–80 g/L Ion concentration = 50–400 mg	pH = 5 t = 60 min Biomass = 1 g Biomass size = 0.5 mm Ion concentration T = 25°C rpm = 500	Cu2+ Cd2	Cd2+= 57.29% (pH = 5, biomass = 20 g/L, ion concentration = 100 mg/L, t = 180min) Cu2+= 50.89% (pH = 5, biomass = 20 g/L, ion concentration = 100 mg/L, t = 180min) Cd2+= 62.9% (pH = 5, biomass = 20 g/L, ion concentration = 100 mg/L, t = 60 min) Cu2+= 69.15% (pH = 5, biomass = 20 g/L, ion concentration = 100 mg/L, t = 60 min) Cd2+= 75.36% (pH = 5, biomass = 20 g/L, ion concentration = 100 mg/L, t = 60 min) Cu2+= 70.22% (pH = 5, biomass = 20 g/L, ion concentration = 100 mg/L, t = 60 min) Cd2+= 75.84% (pH = 5, biomass = 20 g/L, ion concentration = 50 mg/L, t = 60 min) Cu2+=71.64% (pH = 5, biomass = 20 g/L, ion concentration = 50 mg/L, t = 60 min)
Chondracanthus Chamissoi sp.	Aqueous Solution	pH Pb(II) = 2-5.5 Cd(II) = 2-7	pH = 4 Adsorbent = 20 mg Ion concentration = 70 mg/L t = 48 h	Pb(II) Cd(II)	Pb(II) = 1.37 mmol/g Cd(II) = 0.76 mmol/g
Ceramium virgatum sp	Simulated wastewater	pH = 2–8 t = 0–120 min Biomass = 1–40 g/L T = 20, 30, 40, and 50°C	Biomass size = 0.5 mm Biomass = 10 g/L rpm = 100 pH = 5 t = 60 min T = 20°C	Cd2+	96% (pH = 5, Ion concentration = 10 mg/L, T = 20°C 97% (pH = 5, Ion concentration = 10 mg/L, T = 20°C biomass = 10 g/L, t = 60 min)

2.1.4 Summary of literature review of seaweed

From the study, it was discovered that seaweed is an abundant resources extensively benefits for human consumption. Seaweed derived nutrient and compounds has led it to biological activities. Seaweed nutritional value has high potential to be market value due to its various applications. The summary of finding on seaweed is presented in Table 2.4.

Table 2.4: A summary of literature review of seaweed

References	**Sub- topic**	**Main Findings**
Rao *et al.* (2018)	Seaweed	• Primitive non-flowering photosynthetic macrophytes and found in tidal areas of the oceans.
Rindi (2020)		• Plant-like organisms, diversity, its physique benthos varied in size from microscopic single-celled forms to gigantic macrophytes and divided into main subgroups algae.
Stevant *et al.* (2017)		• Such an innovative product, extensively used for human consumption both fresh and dried and further processed applications.
Žugcic *et al.* (2018)		• Incorporation into food matrix has improved the physicochemical and nutritional properties and technological aspects of foods.
Gujar *et al.*, 2019		• A great source of nutrients to decrease the pollution globally.
Leandro *et al.* (2019)	Classification of seaweed	• Taxonomically organized in three large distinct groups based on colour of the thallus: -Chlorophyta (green seaweed) -Rhodophyta (red seaweed) -Phaeophyceae (brown seaweed)
Muhammad Imran khan *et al.* (2018)		• Macroalgae - Multicellular, large-size algae, Microalgae - Microscopic single cells and may be prokaryotic, similar to cyanobacteria (Chloroxybacteria), or eukaryotic like green algae (Chlorophyta).
Rao *et al.*, 2018		• The taxonomic categorization of algae is still a source of continuous modifications and disputes.
Zavaglia *et al.*, 2019		• (Green Seaweed) Chlorophylls: Chlorophyll a & b and

		derivatives Carotenoids: ß-carotene, xanthophylls Example: *Ulva lactuca sp* • (Brown Seaweed) Chlorophylls: Chlorophylls b and c, and derivatives Carotenoids: Fucoxanthin and Xanthophyll, ß-carotene Example: *Sargassum sp.* • (Red Seaweed) Chlorophylls: Chlorophylls a and d, and derivatives Carotenoids: Xanthophylls Phycobiliproteins: Phycoerythrin and phycocyanin Example: *Kappaphycus Alvarezii sp.*
Aroyehun *et al*. (2019)		• Chemical composition: varies with species, maturity stage, sample collection, ecological factors (habitat, temperature, and season) that stimulate or inhibit the biosynthesis of its nutrient composition.
Maehre *et al*. (2014)	Structure and composition of seaweed	• Genetic are difference among species
Daniel Tomé *et al*. (2019)		• Contains small amount of non-protein nitrogen whereas source for some compounds such as free amino acids, chlorophyll, nitrate and nitrite nitrogen, ammonium ions, and nucleic acids.
Kasimala *et al*. (2015)		• Variations in total protein value depending on the depth of the sample collected.
Elango *et al*. (2010)		• Protein quality depending on the bioavailability and protein amino acids.
Abdul Khalil *et al*. (2017)		• Seaweeds have high vitamins and minerals content Chemical composition of seaweed: -Water: 80–90 % -Carbohydrates: Dry weight basis containing of 50 % -Lipids: 1–3 % -Minerals: 7–38 % -Protein contents: Highly variable (10–47 %) with high proportions of essential amino acids
Mišurcová (2011)		• Allowed to extract inorganic compounds from the environment as a powerful source of minerals.
Ranga Rao (2018)	Applications of seaweed	• Produces many essential functional ingredients for health benefits incorporated into foods for daily consumption.

Shannon and Abu-Ghannam (2019)		• Hydrocolloids review as a pinpoint for food formulations and product development.
Mohammad *et al*. (2019)		• As food ingredients in making ice-creams, yogurts, pasta, cheeses, bread, snacks, frankfurters and beef patties.
Circuncisao et al. (2018)		• Different amounts of *Laminaria spp*.seaweed have been added to boost the iodine content of a novel probiotic yoghurt.
Abdul Khalil *et al.* (2018)		• Hydrocolloid provide numerous ingredients to the food, pharmaceutical, cosmetic, textiles, paper, and biotechnology industries as stabilizers and thickeners emulsifiers and fillers.
Nakhate and van der Meer (2021)		• Carrageenan as thickening ingredient that is commonly used in the preparation of pizzas, desserts, gels and canned meals.
Ruthiran *ns.* (2018)		• Carrageenan is also used in chocolate milk, cottage cheese, whipped cream, instant products, yogurt, frozen desserts, sauces.
Pati *et al*. (2016)		• Treat a variety of ailments and acts as antimicrobial, antiviral, antifungal, anti-allergic, anticoagulant, anticancer, antifouling, and antioxidant properties.
Rao *et al*. (2018)		• Algal extracts employed as vermifuges (destroying intestinal worms like Ascaris) and antiscorbutic apart curing cough, belly, chest, bladder, and renal problems.
Veluchamy and Palaniswamy (2020)		• Seaweed extracts applied for face and skincare products, anti-aging cream, regenerative skin cream, emollient products, anti-irritant products, sun protection cream and hair care products.
Pinela *et al*. (2018)		• Bioactive compounds in seaweed are integrated especially into cosmetics.
Veluchamy and Palaniswamy (2020)		• Seaweed potentially as an anti-fouling agent in the development of paints. *Asparagopsis*, *Laurencia* (red algae), and *Sargassum* (brown algae) act as vital sources of anti-fouling compounds.
Morais *et al*. (2020)		• The usage of green seaweed Ulva sp. exhibit high protein content and high levels of mineral components present in oysters while providing cost-effective approach to harness the positive effects of seaweeds in farm animals.
Arumugam *et al*. (2018)		• Heavy metals are found in wastewater are from metal smelters, paint manufacturers, inorganic fertilizers, agricultural activities, leather tanning, electroplating, alloy and battery manufacturing, and other industrial waste materials disposal.
Agoro *et al*. (2020)		• As, Cd, Cr, Cu, Hg, Mn, Ni, Pb, and Zn are heavy metals that are of main concern in the environment due to their toxicity.
Calderon *et al*. (2020)	Seaweed application in wastewater treatment	• The establishment of biosorption to remove heavy metals is a dependable technique.
Ortiz Calderon *et al.* (2017)		• The use of algae for heavy metal ion biosorption in wastewater is environmentally friendly, inexpensive and practical.

2.2 Red Seaweed (Rhodophyta)

Rhodophyta, or red seaweed, is a large category of macroalgae that includes about 7000 species (Ismail *et al*., 2020). Food has historically been made from the whole algae of red seaweeds. Cian *et al.* (2015) stated that red algae are notable for having high carbohydrates, proteins, and minerals compared to other seaweeds. Red algae containing agar and carrageenan have been harvested for various uses including food, pharmaceuticals, and biotechnological applications (Torres *et al*., 2019). Unlike the other two types of seaweed, green and brown, red seaweed are a major source of biologically active materials. Polysaccharides such floridean starch and sulfated galactans like carrageenans and agars are found in red seaweed. They also comprise many kinds of minerals, unsaturated fatty acids, amino acids, vitamins, phycobiliproteins, various pigments, phycolectins, and mycosporine-like amino acids distinguishing them from any other terrestrial plant. (Cian *et al*. 2015). Numerous Malaysian islands provide a variety of environments for the growth of marine macroalgae (seaweeds) such as the species of *Kappaphycus Alvarezii*, *Eucheuma denticulatum*, and *Gracilaria manilaensis*, which are commercially grown (Phang *et al*., 2019).

2.2.1 *Kappaphycus Alvarezii sp*. Seaweed

Kappaphycus Alvarezii sp. is a red macroalga and is very well known for its industrial use, which is affable to cultivate. *Kappaphycus Alvarezii sp.* is a tough, fleshy, firm marine seaweed up to 6 feet in length and approximately 0.5 inches in diameter and it is growing to 2 m long (Deepika, 2018) and its scientific classification described in Table 2.5. *Kappaphycus Alvarezii sp.* is rising to heavy carbonaceous materials of detrital origins. *Kappaphycus Alvarezii sp*. or commercially known as *Eucheuma cottonii*, is an economically significant tropical Rhodophyta that highly demanded cell wall polysaccharide, carrageenan most industrially important carrageenophyte in the world while has demonstrated its aptitude as a bioethanol source, antioxidant, and heavy metal absorber (Ilias *et al*., 2017). It is rich in phytochemical constituents such as phenolic compounds and micro and macronutrients (Mohammad *et al*., 2017). The Philippines supplied 55% of the total output of k-carrageenan extracted from red seaweed, followed by Indonesia (38%) and

Malaysia (2.5%) in Southeast Asia (Bono *et al*. 2011). Implementation of *Kappaphycus Alvarezii sp.* into the food system could bring a tremendous opportunity for marketing in the dairy industry, high fiber functional foods, antibacterial agent properties and high overall phenolic content.

Table 2.5: Scientific classification of *Kappaphycus Alvarezii sp.* seaweed (Charu Deepika, 2018)

Domain	Eukaryota
Phylum	Rhodophyta
Class	Rhodophyta
Order	Gigartinales
Family	Solieriaceae
Genus	Kappaphycus

2.2.2 Properties and Composition of *Kappaphycus Alvarezii sp.* Seaweed

Seaweed is considered to be a balanced food of low-calorie count and strong fiber and mineral composition, with large quantities of protein, vitamins and trace minerals, and a broad variety of naturally occurring substances not present in other species. The potential triggers as a natural source of functional ingredients render this resource especially desirable. The protein level ranged from 10-50 % of dry weight in red seaweed and was higher than macroalgal classes and other foods (Vieira *et al*., 2018). Red and green algae contain a greatest number of phenolic compounds, such as flavonoid phenolic acids and bromophenols, which have been used for medicinal uses because of the response of those elements to proteins, e.g. enzymes or cellular receptors (Ismail *et al*. 2020). Table 2.6 explains the chemical composition of *Kappaphycus Alvarezii sp.*

Table 2.6: Chemical composition of *Kappaphycus Alvarezii sp.* seaweed (Abdul Khalil *et al*., 2018)

Chemical content	Composition (wt. %)
Moisture	0.89%
Carbohydrate	65.20%
Protein	3.40%
Fat	1.10%
Ash	11.57%

2.2.3 Morphology characteristics of *Kappaphycus Alvarezii sp.* seaweed

Morphological characteristics of *Kappaphycus Alvarezii sp.* included the measurements included thallus long (primary thallus, branch I, branch II, and branch III), internodes (primary and secondary), thallus width (main, second tier, and third), and branch numbers for *Kappaphycus Alvarezii sp* (I, II and III). The diameter of the biggest thallus is the main diameter, the first width of the branch is the secondary diameter, and the diameter of the branches II and III is the tertiary diameter as in Figure 2.3 (Fadilah *et al*., 2016). According to Cabrera *et al*. (2019), the main axis is relatively straight, absent of secondary branching towards the apical area, the branch is open and irregular, and the apices of the secondary branches in the apical section are somewhat flexible, thin, and small. The *Kappaphycus Alvarezii sp.* transverse and longitudinal cross section are shown as in Figure 2.4.

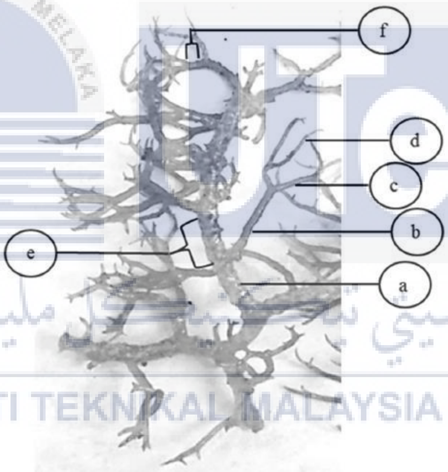


Figure 2.3: Morphology of *Kappaphycus Alvarezii sp.* seaweed: a) the main thallus, b) branch I, c) branch II, d) Branch III, e) primary internodes, f) secondary internodes (Fadilah *et al*., 2016).

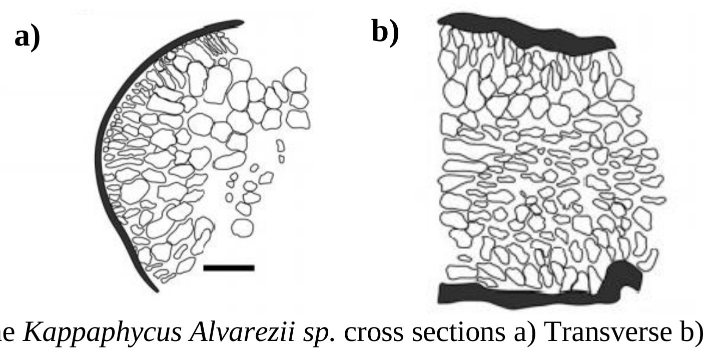


Figure 2.4: The *Kappaphycus Alvarezii sp.* cross sections a) Transverse b) Longitudinal (Cabrera *et al*. 2019)

2.2.4 Summarization of Literature Review of Red Seaweed (Rhodophyta)

In summary, red seaweed is the globally growing seaweed and *Kappaphycus Alvarezii sp.* is one of the species red seaweed. *Kappaphycus Alvarezii sp.* are one of the popular species that is easy to cultivate and well known for its industrial use. The naturally nutritious composition of *Kappaphycus Alvarezii sp.* made it compatible to be functional food within the sort of powder. The morphology characteristics of *Kappaphycus Alvarezii sp* are also summarised in Table 2.7.

Table 2.7: A summary of literature review of red seaweed (Rhodophyta)

References	Sub- topic	Main Findings
Ismail *et al*. (2020)	Red seaweed	• A large macroalgae group of roughly 7000 species.
Cian *et al*. (2015)		• Notable for having high carbohydrates, proteins and minerals compared to other seaweeds.
Torres *et al*. (2019)		• Containing agar and carrageenan have been harvested for various uses including food, pharmaceuticals and biotechnological applications
Cian *et al*. (2015)		• Comprises many kinds of minerals, unsaturated fatty acids, amino acids, vitamins, phycobiliproteins, various pigments, phycolectins, and mycosporine-like amino acids distinguish them from terrestrial plants.
Phang *et al*. (2019)		• Numerous Malaysian islands provide a variety of environments for the growth of marine macroalgae (seaweeds) such as the species of *Kappaphycus Alvarezii, Eucheuma denticulatum and Gracilaria manilaensis.*
Deepika (2018)	*Kappaphycus Alvarezii sp.* Seaweed	• A tough, fleshy, firm marine seaweed up to 6 feet in length and approximately 0.5 inches in diameter and it is growing to 2 m long. Scientific classification of *Kappaphycus Alvarezii sp.* : Domain: Eukaryota Phylum: Rhodophyta Class: Rhodophyta Order: Gigartinales Family: Solieriaceae Genus: Kappaphycus
Ilias *et al*. (2017)		• Has emanded cell wall polysaccharide, carrageenan most industrially important carrageenophyte in the world while has demonstrated its aptitude as a bioethanol source, antioxidant and heavy metal absorber.

Mohammad *et al*. (2017)		• Rich in phytochemical constituents such as phenolic compounds, micro and macronutrients.
Bono *et al*. (2014)		• The Southeast Asian countries have produced 96.5% of the total production of k-carrageenan extracted from red seaweed, 55% is contributed by the Philippines, followed by Indonesia (38%) and Malaysia (2.5%).
Vieira *et al*. (2018)		• The protein level ranged from 10-50 % of dry weight and higher than macroalgal classes and other foods.
Ismail *et al*. (2020)		• Contains a greatest number of phenolic compounds, such as flavonoid phenolic acids and bromophenols used for medicinal uses because of the response of those elements to proteins, e.g. enzymes or cellular receptors.
Abdul Khalil *et al*. (2018)		• Chemical composition of *Kappaphycus Alvarezii sp.* seaweed: -Moisture: 0.89% -Carbohydrate: 65.20% -Protein: 3.40% -Fat: 1.10% -Ash: 11.57%
Fadilah *et al*. (2016)	Morphology characteristics of *Kappaphycus Alvarezii sp.* seaweed	• The primary diameter is the diameter of the largest thallus, the secondary diameter is the first diameter of the branch, and the tertiary diameter is the diameter of the branches II and III.
Cabrera *et al*. (2019)		• Main axis is relatively straight, absent of secondary branching towards the apical area, the branch is open and irregular, and the apices of the secondary branches in the apical section are somewhat flexible, thin, and small.

2.3 Pretreatment method

Before analytical analysis by scientific equipment and instruments can be used to analyze samples, the untreated raw material must be appropriately handled and prepared. This first step is crucial to the entire analytical process since it helps avoid contamination, enhance accuracy, and reduce the possibility of result distortion. Pretreatment is the act of treating something ahead of time as a preliminary or preparatory treatment. Pretreatment removes foreign materials from fabrics and improves the uniformity, hydrophilic characteristics, and affinity for dyestuff. Fundamental pretreatment, pretreatment with chemicals, and pretreatment without chemicals are the three types of pretreatment (Xin Wang, 2021). The suitability of different types of pretreatment for processing seaweed is likely to vary with differences in seaweed chemical composition (Gallagher *et al*., 2018). The primary pretreatment methods include physical, chemical, and biological approaches (Kumar and Sharma, 2017).

The conventional pretreatment method that has been utilized in the sample preparation is the washing process. Pretreatment with washing is an efficient way to remove sand, grime, and high quantities of alkaline metals like potassium and chlorine. Furthermore, it has been found that washing is a standard initial pretreatment step and has been reported in a number of papers on biofuel production from macroalgae (Adams *et al*., 2014). The process of extraction for seaweed hydrocolloid commonly involving alkaline pretreatment, formaldehyde pretreatment and acid treatment. Table 2.8 shows the overview of pretreatment in the extraction of seaweed hydrocolloid.

Table 2.8: The overview on pretreatment in the extraction of seaweed hydrocolloid (Abdul Khalil *et al*., 2018)

Extraction process	Extraction process basis
Alkali pre-treatment	Improve hydrocolloid gelling characteristics by converting unstable sulphate molecules into 3, 6-anhydro-L-galactopyranose (3,6-AG)
Formaldehyde pre-treatment	Increase the alginate production in brown seaweed by removing colour pigments and cross-linking phenolic chemicals in seaweed tissue.
Acid pre-treatment	Clarified the phenolic chemicals and formaldehyde residue while encouraging the conversion of insoluble alginate salts (calcium, magnesium, etc.) to soluble salts.

Moreover, in this study, soaking treatments are done towards seaweed. However, there is limited and only a few detailed on how soaking pretreatment on seaweed. Therefore, this study focused on investigating the application of soaking pretreatments with the parameter of soaking durations towards the production of *Kappaphycus Alvarezii sp.* seaweed powder.

2.3.1 Soaking Method

Soaking is a method to soften the hard texture foods by immersing them in liquid, preferably water, for hours. Theoretically, soaking is intended to enhance the nutritious value and taste of the soaked food. However, sometimes food is merely soaked to turn it into a soft shape that helps to mix smoothly. Soaking of food is done in many cuisines to prepare varied recipes.

Soaking is mainly used to improve the body processing potential for nutrients from ingested food. The process of soaking was described as an efficient way to break down the gluten content of the raw food to make it simpler and fuller to absorb food. However soaking phase of soybean flour resulted in an increment of deterioration in protein level with soaking time (Agume *et al*., 2017). Meanwhile, in the soaking process for germination of seed, Bashabsheh *et al*. (2018) mentioned that optimization of soaking duration for seeds is of an essence as a too-long period of soaking causes bacterial infection of the embryo prior to the actual germination and unsuitable time causes inactivation of the enzyme assisting the germination. Soaking seeds in water softens the seed coat, removes germination inhibitors, and allows for adequate water uptake, all of which are necessary for seed germination, resulting in a higher germination percentage (Ikhramullah *et al*., 2017).

2.3.1.1 Soaking water ratio

Water level or other liquid is also playing a vital role in the soaking process. However, during soaking, the high water level made the soluble nutrient in the food easily dissolve in the solution. This is agreed by Nadeem *et al*. (2017), whereby exosmosis of enzymes and hormones was produced by increased water availability, which caused leaching of the necessary soluble food stores in the seeds. However, Siah *et al*. (2014) mentioned that was the most significant factor are soaking duration as compared to dry seaweed to soaking water ratio. Table 2.9 explains the effects of soaking water ratio and soaking duration towards seaweed paste gel strength, whiteness, moisture content and protein content of *Kappaphycus Alvarezii sp.*

Table 2.9: The effect of soaking water ratio and soaking duration *Kappaphycus Alvarezii sp.* seaweed paste (Siah *et al*., 2014).

Variables	Description
Measurement of gel strength	Due to the leaching of water-soluble protein responsible for gel formation, an increase in the dry seaweed to water ratio and soaking time resulted in a drop in the gel strength of seaweed paste.
Measurement of colour (whiteness)	A higher ratio of dried seaweed to soaking water and a longer soaking time resulted in a whiter-colored seaweed paste.
Measurement of moisture content	When dried seaweed was immersed for a longer period of time, it expanded more. The absorption of water during the soaking process promotes to the growth of the seaweed, and the moisture content was found to have risen as a result.
Measurement of protein content	When seaweed was soaked in higher soaking water ratios for a long period of time, it had a low protein content.

2.3.1.2 Soaking duration

Soaking duration in an experiment is one of the parameters commonly used to determine the effect towards the results by varying the soaking duration. Studies by Ikhramullah *et al*. (2017) indicate that in the germination and seedling growth of peach stones, the interaction between seed rated and soaking duration has significant effects on the seed germination growth, seedlings height and seedling diameter. Meanwhile, in the kidney bean soaking, the soaking process would be increasing the sodium content, potassium content, calcium content, phosphorus content and magnesium content of the kidney bean (Alu and Ahiwe, 2018). This finding is similar to Kpanja *et al*. (2016). It stated that soaking and fermentation on the proximate composition, levels of anti-nutritional factors, oil quality and mineral element composition of Castor noted an increase in most of the relative components due to soaking. According to Chigwedere *et al*. (2019) during soaking, pulses attain equilibrium moisture content and to reach a maximum size and weight, soften to a consistent value. While in natural fiber material, the strength and toughness of a composite are affected by its exposure to water absorption over a period of time (Fang *et al*. 2017). Treated kenaf fibers produce higher strength of composite and improved further with the increasing of soaking time (Abdul Muiz *et al*., 2018). Figure 2.5 shows beans that had been soaked entirely, as well as beans that were partially and totally impermeable (soakability approximately 40%).

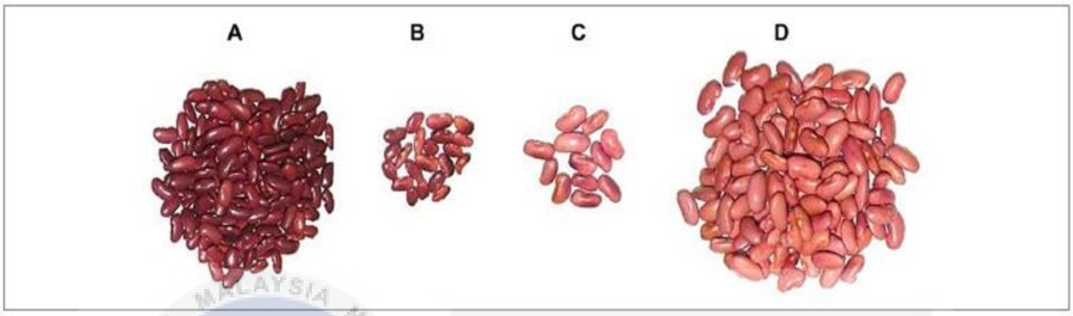


Figure 2.5: Non-homogeneity of soakability showing nonhydrated (A), slightly hydrated (B), almost fully hydrated (C), and fully hydrated (D) beans after soaking in demineralized water at 25 °C for 16 hr. (Chigwedere *et al*., 2019)

2.3.3 Summary of literature review of pretreatment method

In summation, pretreatment are done before analytical analysis on material. Soaking method are subjected soaking duration of experiment and a fixed soaking water ratio. The details of literature review of soaking method summarize in Table 2.10.

Table 2.10: A summary of literature review of pretreatment method

References	Sub- topic	Main Findings
Xin Wang (2021)		• Divided into fundamental pretreatment, pretreatment with chemicals, and pretreatment without chemicals.
Gallagher *et al*. (2018)	Pretreatment method	• The suitability of different types of pretreatment for processing vary with differences in seaweed chemical composition
Kumar and Sharma (2017)		• The primary pretreatment methods include physical, chemical and biological approaches.

Adams *et al*. (2014)		• Soaking seeds in water softens the seed coat, removes germination inhibitors, and allows for adequate water uptake, all of which are necessary for seed germination, resulting in a higher germination percentage,
Abdul Khalil *et al*. (2018)		• The overview on pretreatment in the extraction of seaweed hydrocolloid: Alkali pre-treatment: Improve hydrocolloid gelling characteristics by converting unstable sulphate molecules into 3, 6-anhydro-L-galactopyranose (3, 6-AG). Formaldehyde pre-treatment: Increase the alginate production in brown seaweed by removing colour pigments and cross-linking phenolic chemicals in seaweed tissue Acid pre-treatment: Clarified the phenolic chemicals and formaldehyde residue while encouraging the conversion of insoluble alginate salts (calcium, magnesium, etc.) to soluble salts.
Agume *et al*. (2017)		• Soaking phase of soybean flour resulted in an increment of deterioration in protein level with soaking time.
Naif Bashabsheh *et al*. (2018)	Soaking method	• Optimization of soaking duration for seeds is of an essence as a too-long period of soaking causes bacterial infection of the embryo prior to the actual germination and unsuitable time causes inactivation of the enzyme assisting the germination.
Ikhramullah *et al*. (2017)		• Soaking seeds in water softens the seed coat, removes germination inhibitors, and allows for adequate water uptake, all of which are necessary for seed germination, resulting in a higher germination percentage
Muhammad Kashif Nadeem *et al*. (2017)		• High-water availability caused leach down of the essential soluble food reserves in the seeds, exosmosis of enzymes and hormones reduced protein synthesis and respiration rate were reasons behind this mechanism.
Siah *et al.* (2014)		• The soaking duration was the most significant factor compared to dry seaweed to soaking water ratio. • Effect of soaking water ratio and soaking duration Kappa*phycus Alvarezii sp*. seaweed paste:

		-Measurement of gel strength: An increase in dry seaweed to water ratio and soaking duration resulted in a reduction of gel strength of seaweed paste due to the leaching of water-soluble protein.
		-Measurement of colour (whiteness): An increase in dry seaweed to soaking water ratio and soaking duration produced whiter- coloured seaweed paste. -Measurement of moisture content: The dry seaweed expanded more when soaked for a longer time. The absorption of water during soaking treatment contributes to the expansion of seaweed and it was observed that the moisture content had increased accordingly. -Measurement of protein content: Low protein content in the seaweed when the seaweed was soaked in higher soaking water ratios over an extended period.
Ikhramullah *et al*. (2017)	Soaking duration	• The germination and seedling growth of peach stones, the interaction between seed rated and soaking duration has significant effects on the seed germination growth, seedlings height and seedling diameter.
Alu and Ahiwe (2018)		• In the kidney bean soaking, the soaking process would be increasing the sodium content, potassium content, calcium content, phosphorus content and magnesium content of the kidney bean
Kpanja *et al*. (2016)		• Soaking and fermentation on the proximate composition, levels of anti-nutritional factors, oil quality and mineral element composition of Castor noted an increase in most of the relative components due to soaking.
Chigwedere *et al*. (2019)		• During soaking, pulses attain equilibrium moisture content and to reach a maximum size and weight, soften to a consistent value.
Fang *et al*. (2017)		• While in natural fiber material, the strength and toughness of a composite are affected by its exposure to water absorption over a period of time
Abdul Muiz *et al*. (2018)		• Treated kenaf fibers produce higher strength of composite and improved further with the increasing of soaking time.

2.4 Characterization of *Kappaphycus Alvarezii sp.* seaweed

Generally, the elemental compositions, structure and morphology of *Kappaphycus Alvarezii sp.* evaluated by using several microscopy and physical analysis. However, heavy metal analysis, moisture content analysis and Scanning Electron Microscopy (SEM) analysis were discussed were discussed in detail for the characterization of seaweed powder obtained in this study. Hence, due to the various factors such as cost and availability for characterization and analysis the Scanning Electron Microscopy (SEM) with Energy Dispersive X-Ray Analysis (EDX) was not used.

2.4.1 Heavy metals content

Industrial manufacturing is mainly responsible for the destruction of the environment. Ponds, ponds, and seas are overflowing with pathogens and trash. Trace elements were amongst harmful chemicals at dangerous levels. The global heavy metals that are worrying are chromium (Cr), nickel (Ni), copper (Cu), arsenic (As), cadmium (Cd), mercury (Hg), and lead (Pb) (Islam *et al*., 2015) while according to Cao *et al.* (2014), inorganic hazardous contaminants include heavy metal ions, arsenide, and fluorides. Chen *et al*. (2018) stated that seaweeds can rapidly accumulate elevated concentrations of metals, such as Cd and Cu. The macro, trace, and toxic elements varies widely in seaweed (Astorga-España *et al*., 2015). Biomass immersed in running water water has contributed to the elemination of some compounds (Michalak *et al*., 2018). According to studies, heavy metals can accumulate in red seaweed (Chen *et al*., 2018).

2.4.1.1 Heavy metals content analysis

Many advanced techniques may be used to investigate a systemic approach to seaweed biosorption, including FTIR (Fourier Transform Infrared Spectroscopy), ICP-OES (Inductively Coupled Plasma-Optical Emission Spectrometry) and SEM-EDX-EDX (Scanning Electron Microscopy with Energy Dispersive X-Ray Spectroscopy) (Izabela Michalak *et al*., 2018). According to Deniz *et al*. (2018), before and after the biosorption process, FTIR is used to assess shifts in the vibration frequency of functional classes of biosorbents, showing which groups are active in the biosorption process. ICP-OES is an effective and sensitive technique that helps the whole natural and enriched biomass to evaluate the mineral content after digestion into mineral acids. (Katarzyna Godlewska *et al*., 2018). Meanwhile, Deniz and Tezel (2018) mentioned that, SEM-EDX helps the assessment of the biomass surface elemental composition and the distribution of metal ions. These techniques can be described by biosorption metal ions of *Cladophora Glomerate sp.* seaweed in details in the Table 2.11.

Table 2.11: The biosorption analysis of *Cladophora Glomerate sp.* seaweed by using different techniques (Izabela Michalak *et al*., 2018)

FTIR	ICP-OES	SEM-EDX
-Samples of the natural *Cladophora Glomerate sp.* (C_O) and soaked *Cladophora Glomerate sp.* (SC_A) and enriched biomass of seaweed Enriched soaked *Cladophora Glomerate sp.* (ESC_A) and Enriched natural *Cladophora Glomerate sp.* (EC_O) dried for 24h at 80°C. -For FTIR analysis, Potassium bromide (KBr) disks containing 1.5 mg of the seaweed sample and 200 mg of KBr were prepared. The spectra were measured in the mid IR range (4000-400 cm-1) on a Bruker spectrophotometer (Bruker FT- IR IFS66/s, Billerica, Massachusetts, USA). Analysis of FTIR found that the carboxyl and hydroxyl groups were successful for Cr (III), Mg (II) and Mn (II) ion capture.	-The natural *Cladophora Glomerate sp.* (C_O) and natural *Cladophora Glomerate sp.* (C_A), soaked *Cladophora Glomerate sp.* (SC_A) as well as enriched with metal ions biomass while Enriched natural *Cladophora Glomerate sp*. (EC_O) and Enriched soaked *Cladophora Glomerate sp.* (ESC_A) was mineralized with nitric acid– 0.5 g of biomass and 5.0 mL of 69%Nitric Acid (HNO3) in a microwave oven.	-The natural *Cladophora Glomerate sp.* (C_O), soaked *Cladophora Glomerate sp.* (SC_A) as well as enriched with metal ions biomass Enriched natural *Cladophora Glomerate sp*. (EC_O) and Enriched soaked *Cladophora Glomerate sp.*(ESC_A) - 2.5 % of glutaraldehyde (Sigma) was fixed and ethanol dehydrated in the tests (from 30 % till 100 % concentration). - Samples coated with gold using a sputter coater and observed at 15 kV in scanning electron microscope. BRUCKER Energy Dispersive X-ray method, research was investigated. The increased quantity of Mg, Cr, and Mn during the biosorption process was discovered by SEM- EDX elemental review.

In addition, others techniques of Inductively Coupled Plasma Mass Spectrometry (ICP-MS) are advantageous of its great sensitivity and multi-element on-line detection capacity set it apart from other species detectors. (Xiaoping Yu *et al*., 2019). The use of MS allows researchers to not only gain information on the material qualitative and quantitative contents, but also to identify the analytes the structure and molar masses. For the speciation analysis of trace As, Hg, Se, and Sb, combining various separation methods with ICP-MS has become normal practice. (Marcinkowska and Barałkiewicz, 2016). Although on this contemporary MS detection techniques can contribute in a better comprehension of experimental data and species identification, many analytical laboratories are unable to support such equipment due to their high cost and high maintenance costs.

2.4.2 Moisture Content

Moisture determination is one of the most important and most widely used measurements in the processing and testing of foods. Of even greater significance, however, effect of moisture on the stability and quality of foods. According to Muzaffar and Kumar (2016), the physical, chemical and microbiological stabilities of fruit powders are strongly influenced by their moisture sorption characteristics. Dry foods usually produce 25 % or less of moisture, with a water behavior level of 0 to 0.60, while moisture in staple foods varies from 15 % to 50 %, with a water content of 0.60 to 0.85 (Mishra *et al*., 2017).

Furthermore, Mitra *et al*. (2017) stated that the food powders moisture content could influence the powders bulk properties. The bulk properties of food powder including the bulk density and the flowability of the product and matched to the efficacy of the method of food grinding. As the moisture content of the product reduces, the grinding phase will improve as the material with less moisture is more delicate (Hwabin Jung *et al*., 2018). Most free-flowing dry powders usually accumulate moisture during transportation and storage in conditions with strong relative humidity (Juarez Enriquez *et al*., 2017).

2.4.2 Moisture Content Analysis

Moisture content analysis is a measurement of the total water content of a foodstuff, usually measured as a percentage by weight on a wet basis, is the moisture or water content. Also common are dry food goods to prevent food spoilage. Drying food lowers the amount of humidity required to support microbial growth, thus increasing the shelf life of the product, which is the best option when food storage is not possible. The Figure 2.6 illustrate the common moisture measure methods of food.

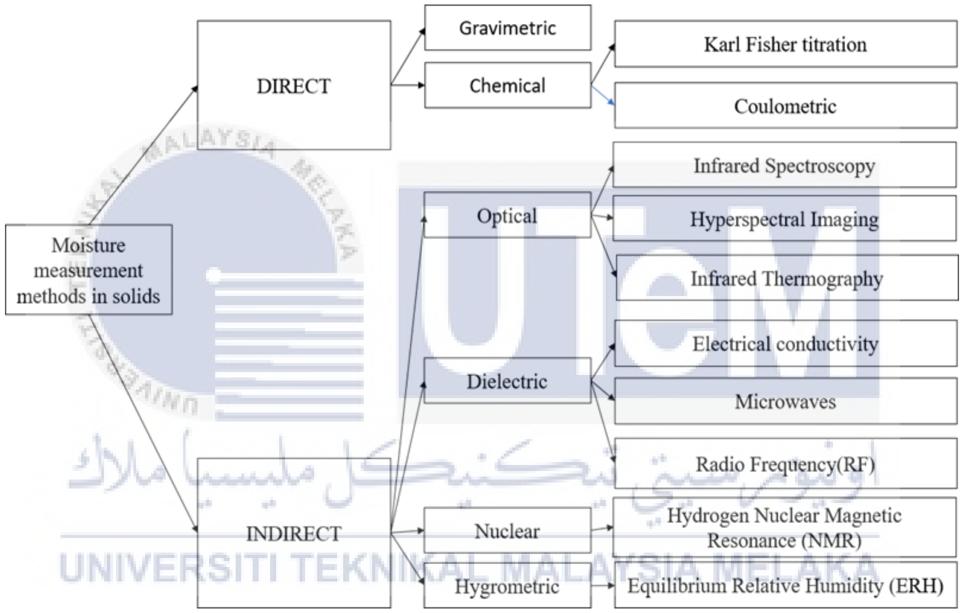


Figure 2.6: Moisture content measurement methods for food product (Zambrano *et al*., 2019)

Direct methods are usually conducted in a laboratory setting where complex environmental conditions and specialized equipment are necessarily required. In the gravimetric system and in the chemical method, a direct approach is established. Methods of chemistry, namely Karl Fisher and Coulometric titration. The gravimetric technique is a basic laboratory method generally employed in a number of industries (Wernecke & Wernecke, 2014). The moisture content is measured on the basis of the original and final masses of the sample, taking into consideration that the removal of water is responsible for all weight loss. A popular laboratory system used to measure the moisture content of liquids and solids is the Karl Fischer titration technique (Zambrano *et al*., 2019). A reference curve corresponding to the amount of the reagent used to titrate the sample's water is used to determine the moisture content. The method to colorimetric titration leverages cobalt chloride color differences to measure the moisture content of food items after drying.

The optical, dielectric, nuclear and hygrometric methods provide indirect approaches. With the exception of hygrometric techniques, all indirect methods utilize electromagnetic radiation. The choice of the spectrum range depends on the absorption, reflectance and propagation of the targeted material, the estimated moisture content of the sample, the form of water bond and the comparison between the targeted material and water in terms of optical properties (Erba *et al*. 2016). Meanwhile, for the calculation of samples with a homogeneous surface, optical measurement techniques are more effective. One of the most common methods for remote sensing of soil moisture content is infrared (IR) spectroscopy. The measuring concept is based on the ability of molecules and atoms to be stimulated by different wavelengths of light absorption (Fraden, 2016).

Thermography detectors in the infrared (IR) range can be used to create thermographic pictures or as photon-sensitive sensors (photodiodes) in near-infrared (NIR) systems (Maroy *et al*., 2016). Hyperspectral imaging has arisen as a valuable tool for non-destructive calculation and visualization of food substance moisture content. Using the principles of capacitance and resistance, when a mild electrical current is transferred using a sample touch procedure, electrical conductivity is usually used in which the functional moisture meters run. The microwave method is commonly used in the farming, clothing, construction, and food industries to assess moisture content, among others (Kurik *et al*., 2016). On the basis of transmitted, absorbed, or mirrored microwaves, or through resonance methods, the moisture content may be calculated.

Radiofrequency (RF) used to calculate moisture content of products with a homogeneous structure, rendering them ideal for gas moisture measurements. The method of nuclear magnetic resonance hydrogen (NMR) is a flexible basic laboratory method commonly employed in the dairy, medicinal and cosmetic industries. The material moisture content is proportional to the observed signal's strength (Proietti *et al*., 2015). Instead of moisture content, hygrometric methods depend on relative humidity equilibrium (ERH).

Relative humidity (RH) sensors are reasonably easy to use as a means of calculating food products water behavior which is suitable for most application.

2.4.3 Scanning Electron Microscopy (SEM) Analysis

The electron scanning microscope (SEM) is the most powerful and commonly utilized electron beam machine globally. The need gradually drives SEM usage for material characterization to gain images and comprehensive details up to three dimensional on the microstructure, the composition, and the crystallography of the material of interest. The SEM can produce analytical information via the interaction between the electron beam and the sample surface to yield high spatial resolution, elemental chemistry data that can be quantified to determine material composition (Dirk, 2017).

Carrageenan are present in the red seaweed *Kappaphycus Alvarezii.* This k-carrageenan inside *Kappaphycus Alvarezii* act as suspending and emulsifying stablizer, thickener, binder and gelling agent. K-carrageenan is a linear anionic sulfate polysaccharide which is reticulated, blocky morphology structure as described by Boston *et al* (2014) in Figure 2.7. In addition, Chan *et al* (2013) state that k-carrageenan gel stored at 18℃ appeared porous, while k-carrageenan gel stored at 25℃ and 4℃ appeared smooth. Meanwhile, Wasoh *et a*l (2014) did mentioned that the raw *Kappaphycus Alvarezii sp.* exhibited a smooth surface with some impurities that appears to be salt crystalloid deposition covering the area. The bulge fibrils the surface since the *Kappaphycus Alvarezii* is a typical type of seaweed with huge amount of hydrocolloid substance compounds.

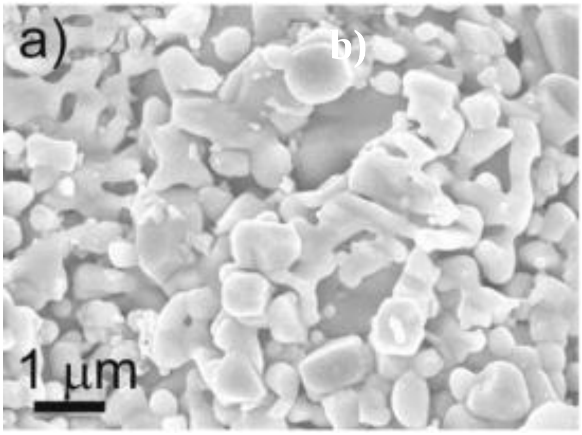


Figure 2.7: SEM micrographs of k-carrageenan of *Kapaphycus Alvarezii sp*. (Boston *et al*., 2014)

The results of SEM in Figure 2.8 shows drying techniques in the manufacture of semi refined carrageenan did not affect the morphological form, they did affect the diameter size. The images revealed a homogeneous dispersion and an amorphous, uneven shape. (Eko Nurcahya Dewi *et al*., 2015).

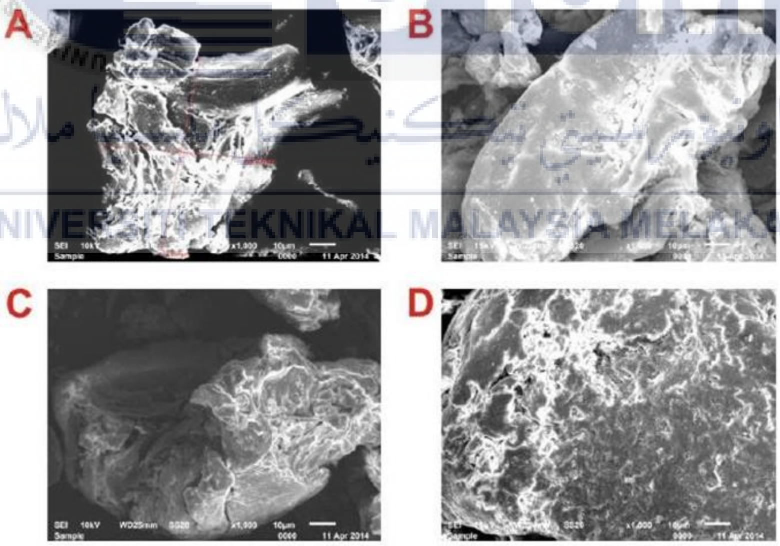


Figure 2.8: The SEM images of Semi Refined Carrageenan powder (1000X): (A) Sun Drying, (B) Solar Tunnel Drying, (C) Oven and (D) Commercial (Eko Nurcahya Dewi *et al*., 2015)

2.5.2 Summary of Literature Review on Material Characterization and Analysis

From the comprehensive literature review on past journals, the characterization and analysis of seaweed observation can be sum up as follows:

a) Heavy metals content analysis of seaweed is to study the elemental composition inside seaweed after soaking treatment. A systemic approach to investigate seaweed via biosorption of heavy metals can be measured by FTIR (Fourier Transform Infrared Spectroscopy), ICP-OES (Inductively Coupled Plasma-Optical Emission Spectrometry), SEM-EDX-EDX (Scanning Electron Microscopy with Energy Dispersive X-Ray Spectroscopy) or ICP-MS (Inductively Coupled Plasma –Mass Spectrometry).

b) Moisture content analysis in this study analyzes the amount of water or moisture at different soaking parameters. Determining moisture content is critical for guaranteeing food product quality, product processibility, shelf life, usefulness and product freshness. Improper level of water content in seaweed powder is prone to microbial growth and spoilage.

c) Scanning Electron Microscopy (SEM) analysis is used to study the surface morphology and structure of seaweed NC before and after soaking treatments are conducted. Moreover, SEM is also important to study that impurities such as salt deposition have been completely removed during the treatment.

CHAPTER 3

METHODOLOGY

Chapter three thoroughly describes the step-by-step methodologies and approaches employed to meet the objectives of this study. The discussion consists of the flowchart of experimental work concerned during this study, experimental materials and experimental method by referring attentively to the specification and particular of the previous study in Chapter 2. This chapter proposed the best method, technique, equipment and apparatus in reaching the study objective.

3.1 Introduction

This chapter divided into two parts which are experimental materials and experimental procedure, to investigate the effect of soaking duration towards the production of *Kappaphycus Alvarezii sp.* powder. Concurrently, the preparation of seaweed powder must meet the study objective and scope as discussed in Chapter 1. The Figure 3.1 depicts a flowchart of the experimental studies and procedures and Figure 3.2 shows the details of the experimental procedures in Sections A and B.

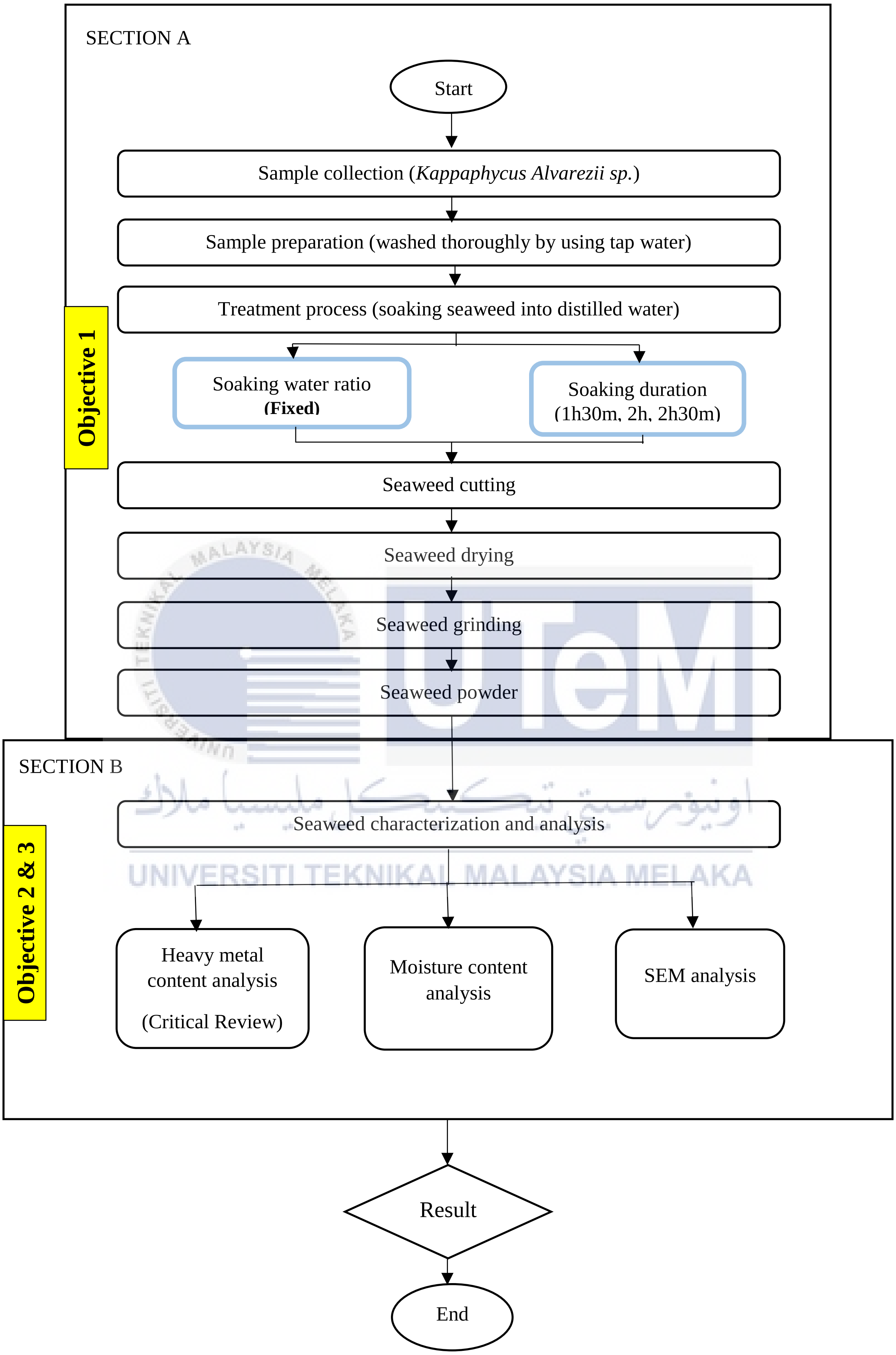


Figure 3.1: The flowchart of the experimental studies and procedures.

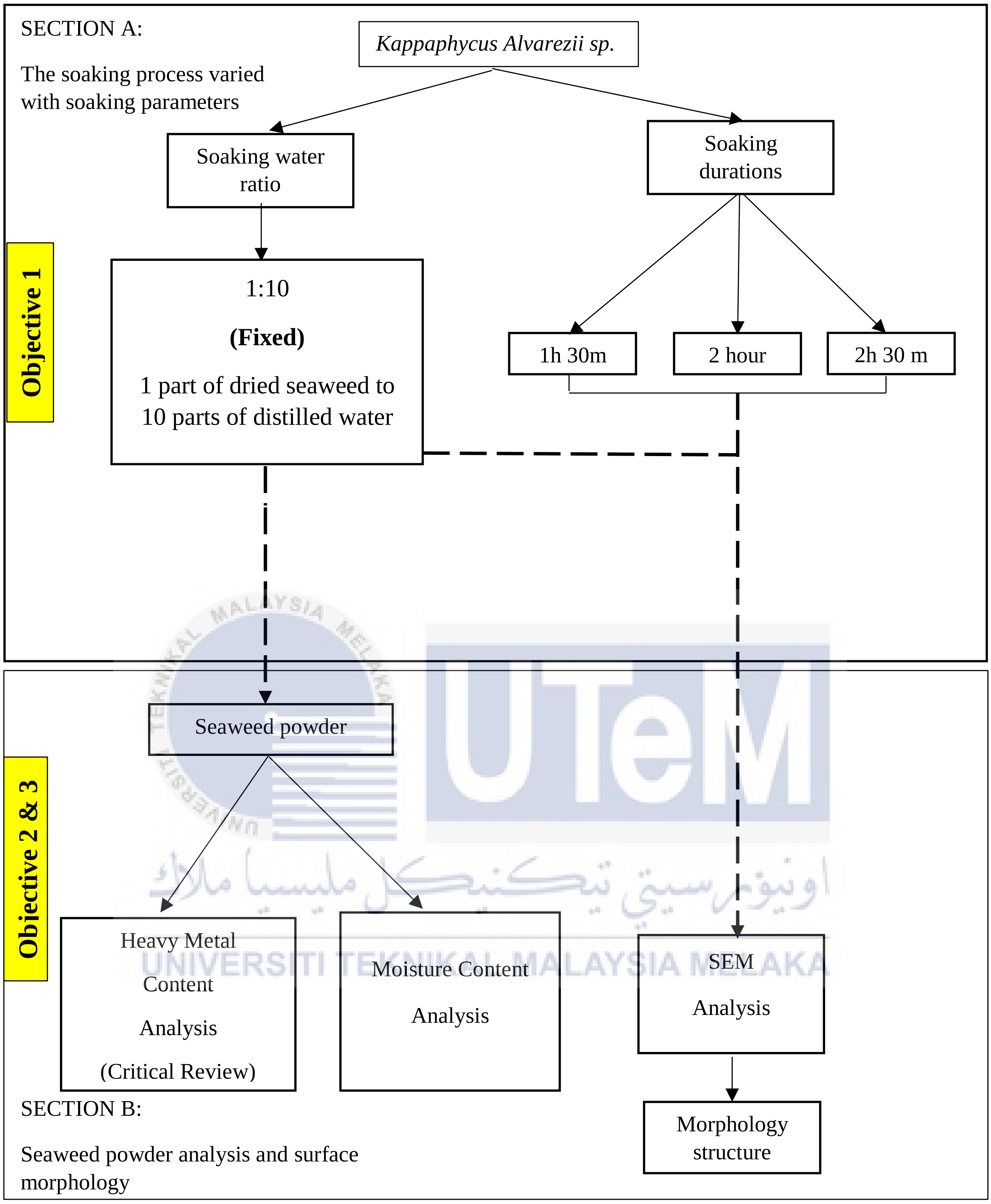


Figure 3.2: Details on experimental works in Section A and B

3.2 Experimental Work based on Objective 1

This subsection explains the experimental work involved in Objective 1 from the beginning of raw resources preparation to the production of seaweed powder.

3.2.1 Experiment Materials

The following element reiterates the experimental materials utilized in Objective 1, including raw resources, liquid, equipment, machinery, gloves, laboratory glassware and other functional products as the main experimental materials used in this research.

3.2.2 Raw Materials

Seaweed possessed as a raw material for the soaking process in this study. *Kappaphycus Alvarezii sp.* as shown in Figure 3.3 was chosen due to its current industrial needs, rich in nutrition, abundant resources, and sustainability. In this analysis, the seaweed was collected from a local company called Saanen Sdn Bhd, based in Melaka, Malaysia, which is also part of this research study. For the beginning of sample preparation, the manual sorting process of seaweed removes foreign matter such as sand, dried sea animals, and nylon strings used in the cultivation process. Seaweed is repeatedly washed to remove impurities and other adhered substances that are visible to the naked eye.

Figure 3.3: The *Kappaphycus Alvarezii sp.* seaweed

3.2.3 Chemical and other substances

In this experiment, no chemical substances are involved in soaking process of transforming the seaweed into powder form. The only pure substance that is involved in this study is distilled water. The seaweed soaked into the distilled water during the soaking process to homogenized and grind into powder.

3.2.4 Experimental Equipment

All the equipment subjected to Objective 1 are beaker, stirring hot plate, magnetic stirring bar, glassware, ruler, scissor, stainless steel stirrer, screw cap bottle, aluminum foil, mini pulverize machine seaweed powder grinder and drying oven.

3.2.5 Experimental Methods

Experimental methods were conducted to produce seaweed in powder form for the *Kappaphycus Alvarezii sp*. seaweed consists of sample preparation, soaking, cutting, oven-drying, and grinding. The methods were performed in order to effectuate the first objective of study analysis.

3.2.6 Sample Preparation

The first step in this process was to prepare the raw material of red seaweed, *Kappaphycus Alvarezii sp*. For each sample, an estimated 90g of red seaweed was prepared and the total samples of this experiment are three samples. Initially, wash the red seaweed repeatedly to remove any contaminants under running water. Preparation of seaweed samples was carried out with the primary purpose of washing to reduce toxins in the raw materials used. The seaweed contained impurities and foreign matter which had to be adequately filtered out well before soaking.

3.2.7 Design of Parameter

The soaking duration act as the study parameters in this study. Soaking water ratio is determined as the fixed-parameter in this study. The predetermined water ratio is one part of dried seaweed to ten parts of the distilled water (1:10) while soaking duration is the significant parameters for this study. Thus, three samples with different soaking duration were designed and depicted in the constructed design in the Table 3.1.

Table 3.1: Design parameters of the experiment

<table>
<tr><th>Number of samples</th><th>Soaking duration</th><th>Soaking water ratio</th></tr>
<tr><td>Sample 1</td><td>1 hour 30 minutes</td><td rowspan="3">(1:10)

fixed

1 part of dried seaweed to 10 parts of distilled water.</td></tr>
<tr><td>Sample 2</td><td>2 hours</td></tr>
<tr><td>Sample 3</td><td>2 hour 30 minutes</td></tr>
</table>

Previous studies demonstrated that the soaking process is suitable pretreatment for transforming *Kappaphycus Alvarezii sp.* seaweed into powder. The chosen method is due to the studies by past researchers due to its method reliability and validity. Soaking is one of the simplest pretreatment processes by only using distilled water instead of other chemical substances. The soaking duration are designed with varying parameters and the soaking water ratio is the fixed parameter. Meanwhile, the drying duration and temperature are set to 10 hours and 60°C accordingly. Figure 3.4 portrayed the whole process in this experiment.

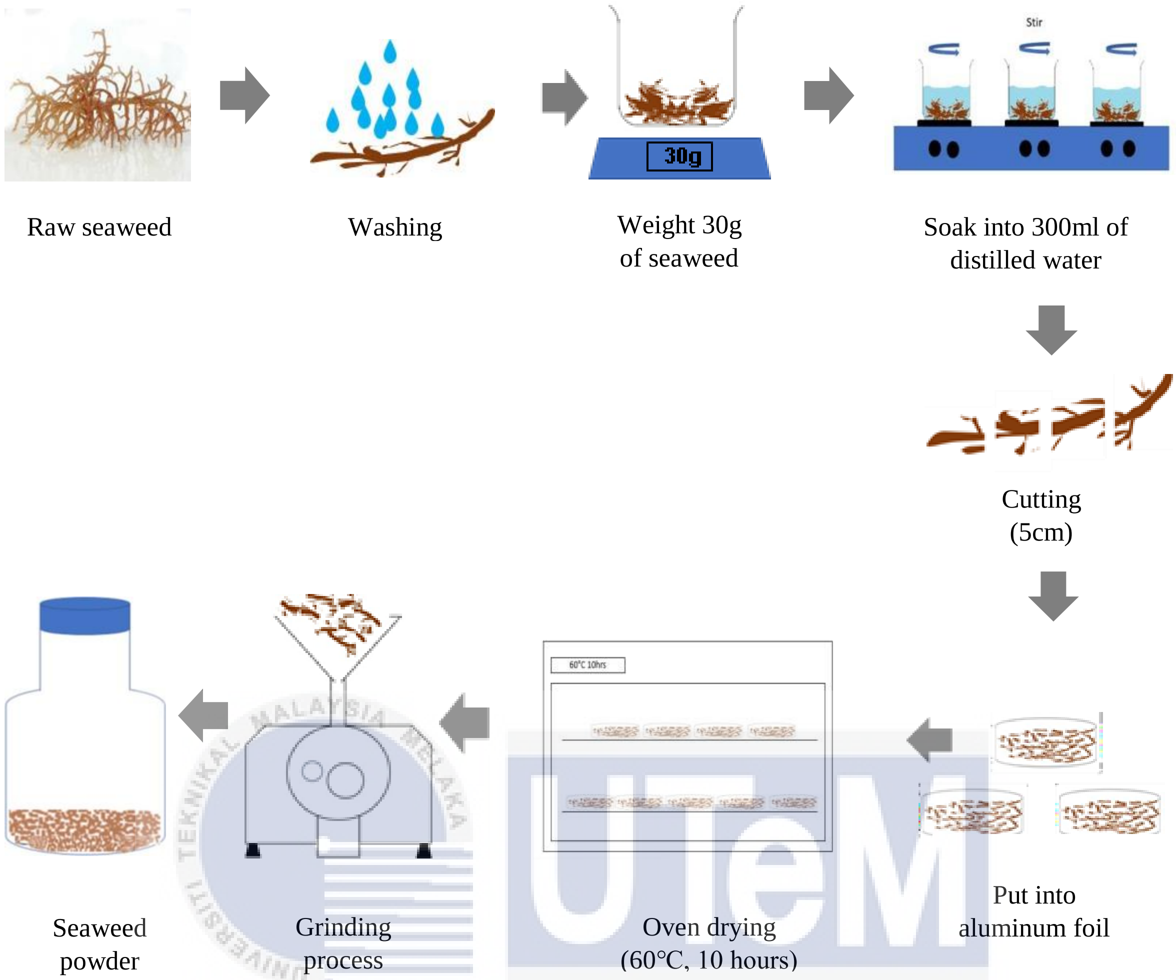


Figure 3.4: Illustration of preparation of seaweed powder

3.2.8 Soaking pretreatment process

In the soaking process, one sample was prepared with 90g in seaweed weight but replicated three times by means 30g at a time. The soaking process is replicated three times to make sure all the seaweed is soaking and keep the stirring process from being disrupted as the seaweed expands and becomes denser as the soaking duration goes by. The soaking process takes place in a fixed ratio (1:10), containing 30g of red dried seaweed soak into 300ml of distilled water. During soaking, without adding temperature, place the beaker containing soaked seaweed on the hot stirred plate to keep the soaked seaweed with distilled water submerged wholly and keeps circulating as in Figure 3.5. The soaking takes places were left soaked according to designed of significant parameter which is 1 hour 30 minutes, 2 hours and 2 hours 30 minutes.

Figure 3.5: The soaking treatment on seaweed

3.2.9 Cutting Process

After soaking, the size reduction of seaweed is done by cutting process the seaweed into 5cm each in length. Size reduction is made for the easy in grinding process as in Figure 3.6.

Figure 3.6: The cutting process on soaked seaweed

3.2.10 Drying Process

Place the seaweed sample into aluminum foil and dried it in the drying oven at optimized temperature and duration of 60°C for 10 hours as shown in Figure 3.7.

Figure 3.7: The drying process on seaweed (10h, 60℃)

3.2.11 Grinding Process

Then, the resulting smaller pieces of dried seaweed were ground down by seaweed powder grinder as shown in Figure 3.8. The seaweed is ground efficiently soft to medium-hard depending on the brittleness of the samples rugged texture. The seaweed needs to be ground repeatedly until obtained the desirable flowy properties of powder food product.

Figure 3.8: The grinding process

3.3 Experimental Work Based on Objective 2

This section continually explained the experimental analysis involved in Objective 2, which is the heavy metal content analysis and moisture content analysis.

3.3.1 Experimental Equipment

The equipment used in this analysis are MB25 moisture analyzer, spatula, lab glassware and disposable aluminum sample pan.

3.3.2 Experimental Methods

Experimental methods to be performed are determining the heavy metal content of the sample and moisture content of the seaweed powder. The methods used are to cover the second objective of this research analysis.

3.3.3 Determination of Heavy Metal Content

The determination heavy metal of dried seaweed samples were digested using the hot-block digestion procedure for metals concentration. Sample weighting 1g were ground using digestion tube, 10ml concentrated HNO_3 (Merack Germany) was added to the digestion tube and covered with watch glass in the mouth of it. The mixture was left overnight and digestion tube was placed into a block digester (ATM600 BLOCK, Australia). Heated it at 95 °C for 1.5 hours then allowed to cool before added 7 ml H_2O_2 and continue to digest the sample for another 2 hours until the digest sample was clear. Additional HNO_3 not exceeding 5 ml was added to maintain a wet digest. The diluted equaregia (mixture of nitric acid and hydrochloric acid with a volume ratio of 1:3) was added to the dissolved digest residue and the solution was transferred into a 50 ml volumetric flask. Deionized water was added to bring the volume of the solution up to 50 ml and the solution was filtered using 0.45 μm filter paper (Whatman, U.S.) to remove any particulates. Lastly, the solution was analyzed using the inductively coupled plasma mass spectrometry ICP-MS as shown in Figure 3.9. Based on the previous study, it is expected that the heavy metal content of the *Kappaphycus Alvarezii sp*. is low. The growth dynamic of the seaweed in the subtidal zone on the reef and sandy-coral regions indicated that *Kappaphycus Alvarezii sp.* is low heavy metal and in permittable amount for human consumption. However, due to restriction of the COVID-19 across the world, the heavy metal content analysis were conducted by postulating the data through critical review analysis.

Figure 3.9: Inductively Coupled Plasma Mass Spectrometry (ICP-MS) machine

3.3.4 Moisture Content Analysis

The moisture content of the *Kappaphycus Alvarezii sp*. is an analysis to determine the amount of moisture content of the powder. Seaweed powder analyzed by the 'Loss on Drying (LOD) method. Moisture content analysis is done by constant weight recording while the sample is heated and dried through a halogen lamp or other infrared radiator. If the sample does not lose weight, the difference between the two weights is measured to calculate the moisture content. Press the temperature or time keypad to decide the drying parameters, add the seaweed powder sample, and start the test process. The Figure 3.10 is the Ohaus MB25 moisture content analyzer that is suitable for repetitive activities conducted by users at all ability levels. Based on previous research, high amounts of moisture can reduce the value of the products and longevity due to a higher risk for microbial growth. The food product must have low moisture content without spoiling the quality, nutritional value, and better shelf life. It is expected that powder moisture content of powder is below 15%.

Figure 3.10: MB25 Moisture Analyzer

3.4 Experimental Work Based on Objective 3

This section explained the experimental characterization in Objective 3 by utilizing the Scanning Electron Microscopy (SEM) Analysis.

3.4.1 Scanning Electron Microscopy (SEM) Analysis

A scanning electron microscope (SEM) was commonly used to analyze and determine the surface morphology of seaweed at different soaking duration. In this analysis, SEM machine as in Figure 3.11 was used to ascertain the surface morphology of red seaweed before and after the soaking process to investigate the composition of the seaweed morphology surface at different soaking duration parameters. Before the study, a thin layer of samples was positioned on the aluminum studs and coated with gold using a mini sputter coater. The pictures were taken with an acceleration voltage of 2 kV with a resolution of up to 10 nm and an improvement from 5 to 1 000 000X. Based on the previous research, it is expected that the surface structure of the untreated sample of seaweed exhibited a smooth *Kappaphycus Alvarezii sp.* surface with some impurities and the seaweed structure becomes smoother after soaking.

Figure 3.11: Scanning Electron Microscope (SEM)

CHAPTER 4

RESULTS AND DISCUSSIONS

This chapter provides observations and results from data analysis of the experimental works on preparing *Kappaphychus Alvarezii sp.*powder. All results obtained are discussed in this chapter along with the study outcomes. The experiments were carried out according to the flow chart presented in Figures 3.1. Due to the restriction of the COVID pandemic, heavy metal analysis were done via the critical review analysis. In this chapter, those objectives are accomplished and the findings are presented. Scanning Electron Microscopy (SEM) is used to study the surface morphology of seaweed before and after the soaking process of seaweed at different soaking parameters. On top of that, this chapter would comprise a discussion on the study for the production of *Kappaphycus Alvarezii sp.* powder.

4.1 Soaking treatment of *Kappaphycus Alvarezii Sp.* seaweed

The primary purpose of this study is to investigate the optimized soaking durations in producing seaweed powder products. The pretreatment was done by soaked the seaweed into the distilled water with the proper soaking duration. In this experiment, the soaking water ratio is fixed at one part of seaweed into ten parts of seaweed (1:10). In contrast, the soaking duration varies in parameters of 1 hour 30 minutes, 2 hours and 2 hours 30 minutes. Through the observation of the experimental result in Figure 4.1, soaking duration of 1 hour and 30 minutes resulted in partial rigidity and slightly chewier. At 2 hours, the seaweed mass increases approximately three times. The seaweed surface turns soft and expands to half solid texture. While at the soaking duration of 2 hours and 30 minutes, the seaweed turns softer and almost leaches. This is due to lower soaking duration lead to a decrease in water absorption by the seaweed. This is supported by Siah *et al.* (2014) mentioned that soaking at the shortest time will produce seaweed with a harder texture. Meanwhile, the soaking at longer time high water absorption by the seaweed will occurred and resulted in stickier and plasticizer texture. This is supported by Jumaidin *et al.* (2016) that water uptake of all materials increased with longer immersion time. The result shows that soaking at 2 hours would be the optimized soaking duration because after soaking treatment, the seaweed expands a great extent. This is further approved by Siah *et al.* (2014), soaking at desired extent is at the soaking duration of 120 min. Interestingly, Xiren and Aminah (2014) also reported *Sargassum muticum sp*. seaweed was soaked at optimum soaking duration of 2 hours in 100ml distilled water in the pretreatment to remove the seaweed odor. Moreover, over extended soaking duration of 2 hours and 30 minutes will caused the seaweed leached out, and this is corresponding Pasugdee *et al.* (2006) findings in which, 3 hours soaking period affected the nutrient leaching and feed stability.

Thus, it can be concluded that soaking treatment of seaweed are optimized at the duration of 2 hours and at the fixed soaking water ratio of 1:10. According to Reddy *et al.* (2018), soaking treatment of the dried sample in water was done to remove the salts and other impurities. Besides, it is important to remove this foreign matter before transform the seaweed into powder form and.

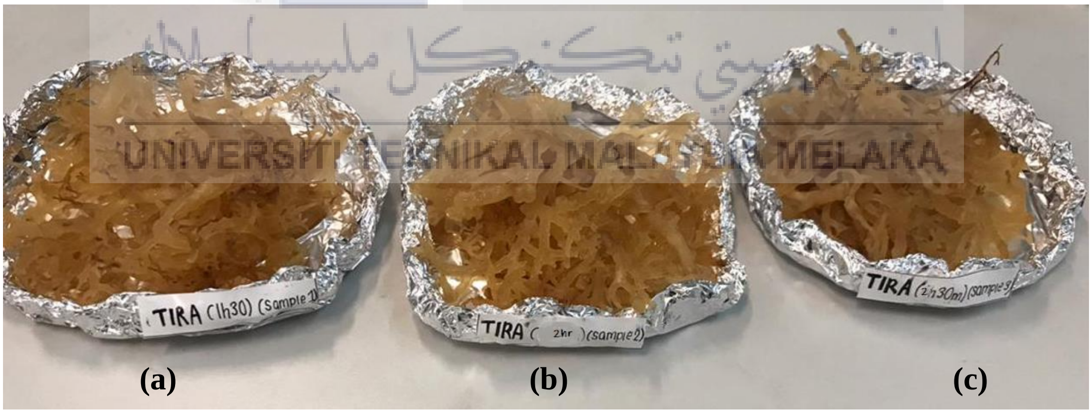


Figure 4.1: The *Kappaphycus Alvarezii sp.* seaweed after soaking treatment at a) 1 hours 30 minutes b) 2 hours c) 2 hours 30 minutes.

4.2 Heavy metal analysis

The heavy metal analysis for the *Kappaphycus Alvarezii sp.* seaweed powder is to analyze the metal contamination in the seaweed. This is the important analysis as to make sure this seaweed is safe before human consumption. But, only several studies have observed that the heavy metal content was lower after soaking treatment. Based on the graph in the Figure 4.1, without soaking treatment on seaweed, Mustafa *et al.* (2016) findings shows that the amount of metal content are high in which mercury (Hg), lead (Pb), Cadmium (Cd) and Arsenic (As) was 0.14 $mgkg^{-1}$, 0.26 $mgkg^{-1}$, 0.03 $mgkg^{-1}$ and 0.95 $mgkg^{-1}$ respectively. While with soaking pretreatment on seaweed, the heavy metal content are rather smaller as Xiren and Aminah (2017) observation. The amount had decrease to 0.46 $mgkg^{-1}$ for arsenic (As), 0.01 $mgkg^{-1}$ for cadmium (Cd) and 0.07 $mgkg^{-1}$ for lead (Pb). Meanwhile in Bui Huy Chich (2020) findings, with soaking treatment the mercury (Hg), lead (Pb), Cadmium (Cd) and Arsenic (As) content of seaweed also had smaller amount that is 0.01 $mgkg^{-1}$, 0.01 $mgkg^{-1}$, 0.05 $mgkg^{-1}$ and 0.01 $mgkg^{-1}$ accordingly. It has proven that with soaking treatment of seaweed, the heavy metals content can be reduced. This is stated by Zhang *et al.* (2020) stated that soaking pretreatment has proven that it can remove bioavailability of heavy metals.

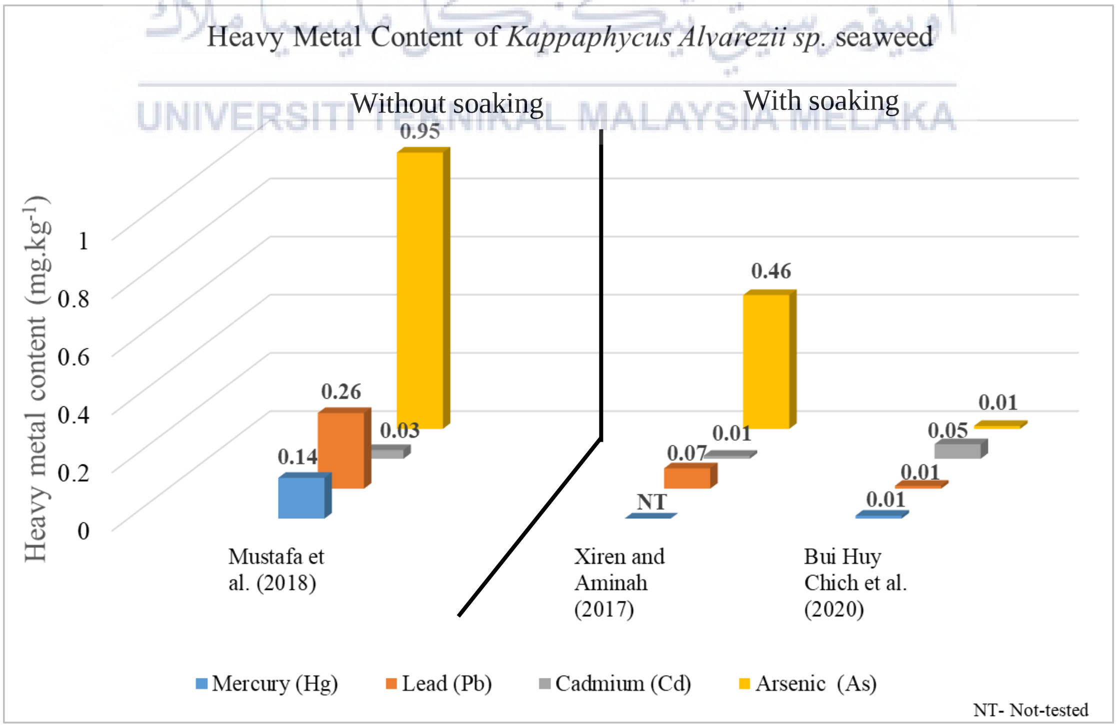


Figure 4.2: The overview of heavy metals content on seaweed with and without soaking treatment

Out of a number of the review journals that had been researched, only two journals had used the soaking duration in the soaking treatment that related to this study to be referred as a critical review. Due to this case, up to date, not many have researched on this seaweed material regarded soaking duration in order to produce seaweed powder. But, those two journals only stated the duration used during soaking and one of the journal are the same species of seaweed but different location. Despite the limited discussion from late research, the novelty on *Kappaphycus Alvarezii sp.* seaweed was still established.

The first journal was from Bui Huy Chich *et al.* (2020) that had used 80 minutes in the soaking treatment of seaweed. This researcher had used *Kappaphycus Alvarezii sp.* the same species but from difference location. The resulted shows from this study having the smaller amount of heavy metals content in the seaweed. Besides, the second journal which was from Xiren and Aminah (2017) had mentioned that the soaking treatment of the *Kappaphycus Alvarezii sp.* are by 117 minutes. Soaking duration that has been discussed previously optimized soaking duration of 2 hours. Hence, the hypothesized result of heavy metals content of Arsenic (As), Cadmium (Cd) and Lead (Pb) is postulated at 2 hours. All the data are arrange in the table and the data also had been plotted in the graph. The summarization of the soaking duration towards the heavy metals content of the seaweed review and postulated data can be referred to Table 4.1.

Table 4.1: The heavy metals content data

References	Soaking duration (min)	**Arsenic (As) content ($mgkg^{-1}$)**	**Cadmium (Cd) content ($mgkg^{-1}$)**	**Lead (Pb) Content ($mgkg^{-1}$)**
Bui Huy Chich *et al.* (2020)	80	0.01	0.05	0.01
Xiren and Aminah (2017)	117	0.46	0.01	0.07
Hypothesized Result	120	0.40	0.009	0.04

Therefore, the results were postulated on the graph. The red triangle in Figure 4.3, 4.4 and 4.5 indicated the hypothesized result. Based on the graph, the hyphothesized result of the heavy metals were postulated based on the Xiren and Aminah (2017). This is due to *Kappaphycus Alvarzii sp.* used are from the same species and from the same cultivation location in this study. The increase soaking duration on seaweed, the lower heavy metals content of seaweed. Based on previous study, the heavy metals of seaweed will be low at optimized hour of 2 hours of soaking that supported by Narsih *et al*. (2012) that mentioned the best treatment according to proximate analysis was a soaking time of 2 h. Meanwhile, as the results from the Bui Huy Chich *et al.* (2020) was far smaller because the metal contamination of seaweed are different growth dynamics and geochemical processes of seaweeds in a different stage of the life cycle. This is supported by Yong *et al*. (2017) that the genetic species, sea condition, and seasons and also the physiology and morphology of the seaweed will differ to each other. Moreover, the mercury (Hg) content was not found in Xiren and Aminah (2017) study as availability of heavy metals was varies consistently with their geographical origin and this is supported by Yong *et al*. (2017).

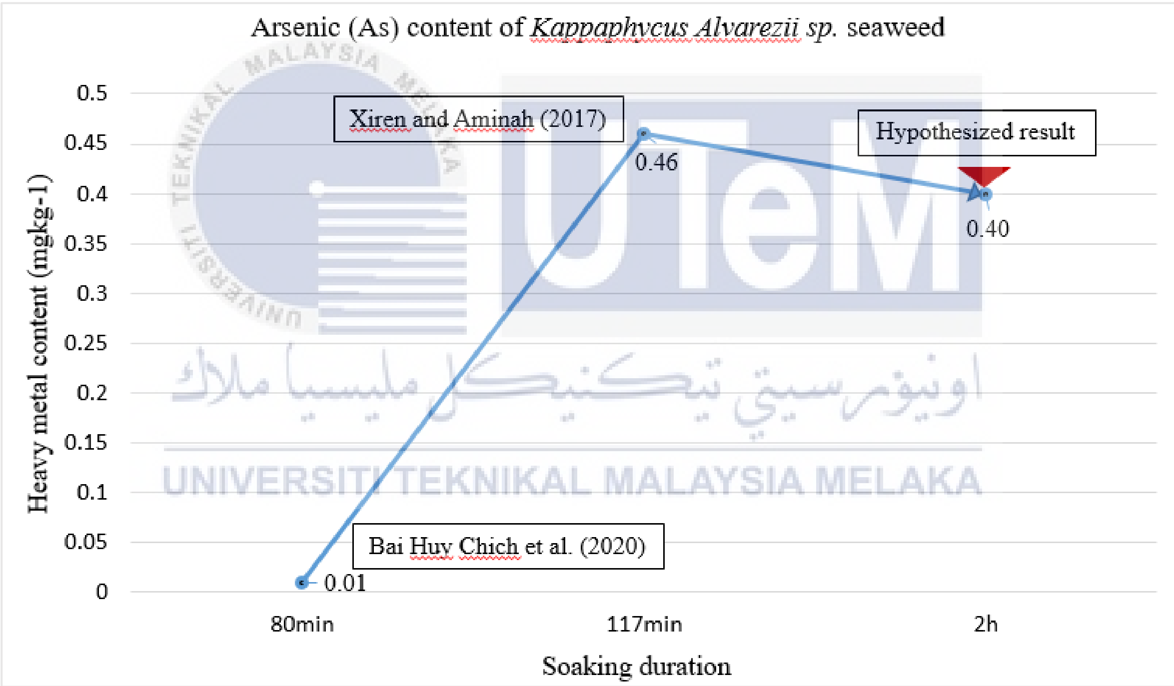


Figure 4.3: Arsenic (As) content of *Kappaphycus Alvarezii sp.* seaweed

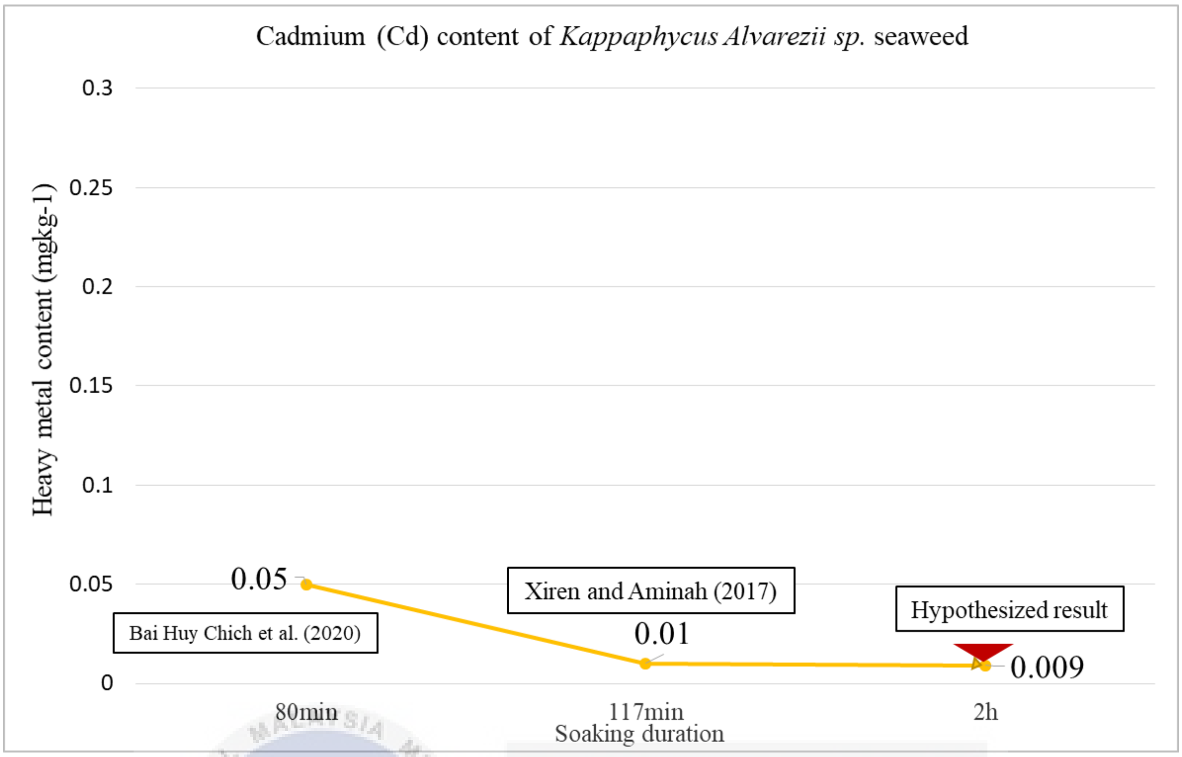


Figure 4.4: Cadmium (Cd) content of *Kappaphycus Alvarezii sp.* seaweed

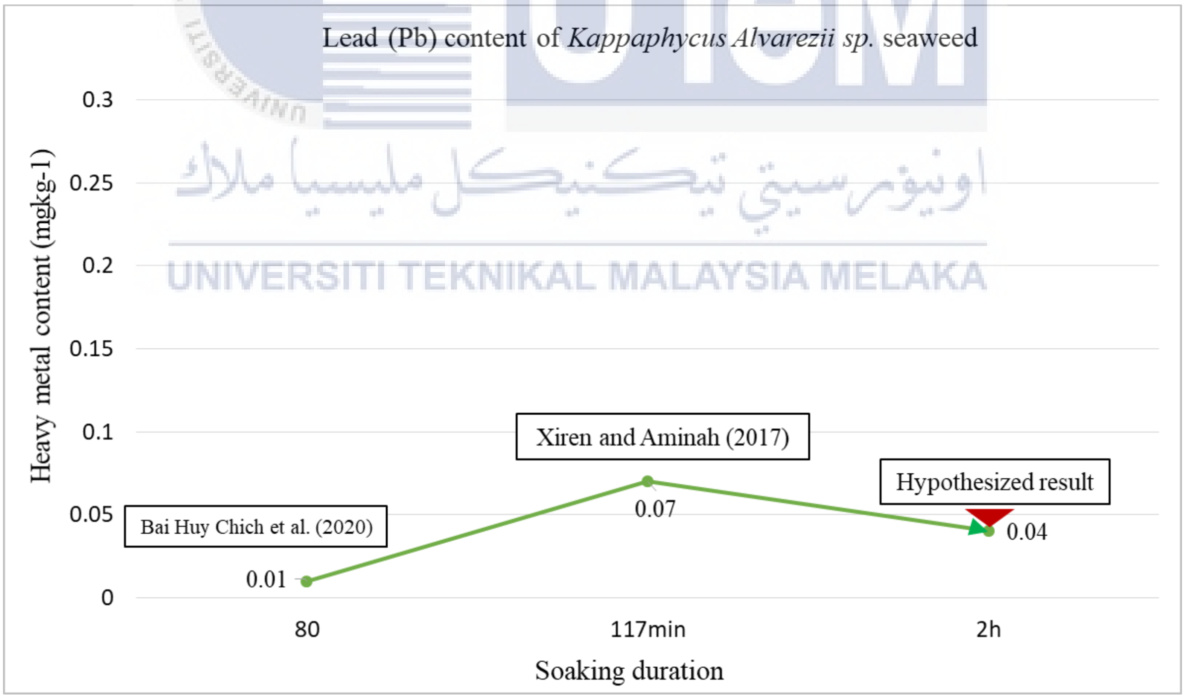


Figure 4.5: Lead (Pb) content of *Kappaphycus Alvarezii sp*. seaweed

The study findings show that based on the result on the graph, the heavy metals content in seaweed are at low content. This is supported by Tresnati *et al*. (2021). The bioaccumulation in *Kappaphycus Alvarezii sp.* was not permanent, so *Kappaphycus*

Alvarezii sp. is safe for human health because heavy metals can be released during post-harvest processing and carrageenan powder processing. Thus, it can be revealed that *Kappaphycus Alvarezii sp.* seaweed powder has met the specific standard of health requirement from Joint FAO/WHO Expert Committee on Food Additive (JECFA) and Health Council and National Medicine Academy of France in standard of < 1 $mgkg^{-1}$ for mercury (Hg), lead (Pb) < 2 $mgkg^{-1}$, Cadmium (Cd) <2 $mgkg^{-1}$ and Arsenic (As) < $mgkg^{-1}$.

4.3 Moisture content analysis

Moisture content analysis is to analyze the amount of water or moisture at different soaking parameters. Moisture has an impact on product processibility, shelf life, usefulness, and quality. As previous studies by researcher, the moisture content increase as the soaking time increase. The pretreatment on *Kappaphycus Alvarezii sp.* seaweed by soaking resulting amount moisture content as illustrated in Figure 4.6. Soaking the seaweed at 1 hour 30 minutes resulted a moisture content of 13.31%, 2 hours soaking resulting in 14.67%, and 2 hours 30 minutes having 9.20% of moisture content. It shows that soaking at 1 hour and 30 minutes and 2 hours has increase the moisture content accordingly. This is supported by Siah *et al* (2014) also that the absorption of water during soaking treatment contributes to the expansion of seaweed and the moisture content had increased. But, soaking at longer time at which at 2 hours and 30 minutes shows low amount of moisture content. This is due to high content of water inhibit the growth of bacteria and led to deterioration of seaweed moisture content. This is supported by Ojewumi *et al.* (2016) that mention the bacteria, yeast and molds responsible for deterioration need moisture for their metabolism. In addition, the moisture sorption isotherm integrates the hygroscopic properties of numerous constituents whose sorption properties may change duet physical/chemical interactions induced by heating and other pre/treatments (Sandulachi, 2012).

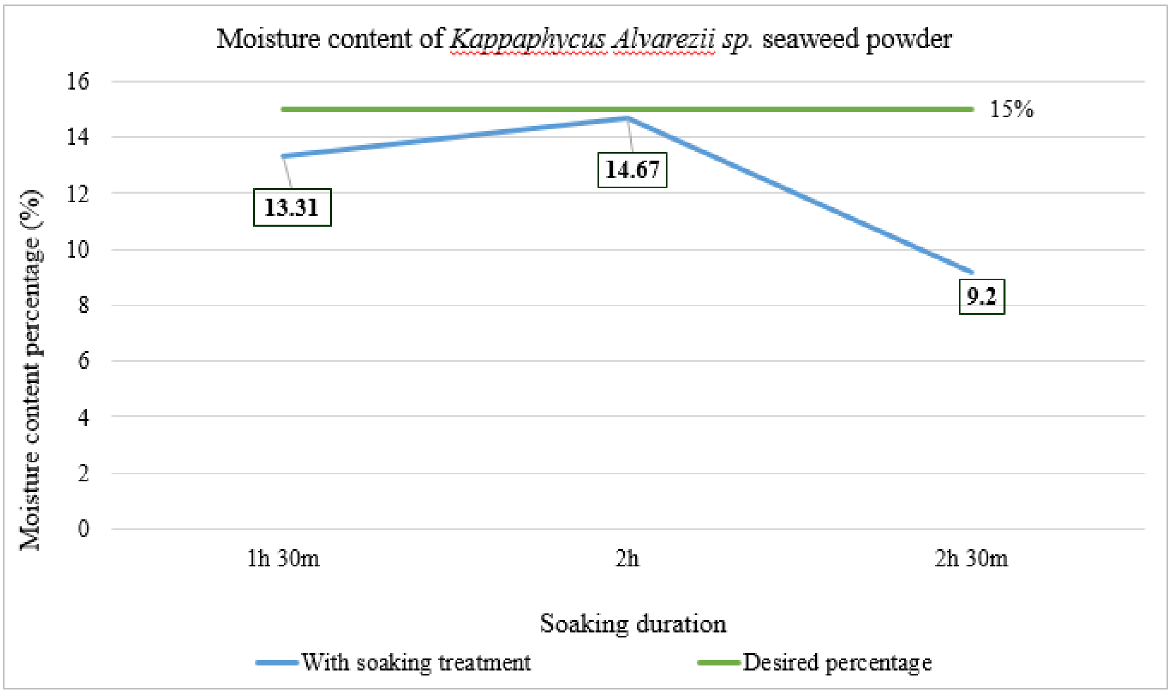


Figure 4.6: Moisture content of *Kappaphycus Alvarezii sp.* seaweed powder

At optimized hour of 2 hours, though, through the result soaking at 2 hours resulting the highest moisture content of the samples are still in an acceptable range, at which below 15% (Rosario *et al.*, 2019). This is also proven by Siah *et al.* (2014), whereas the optimum soaking duration for the moisture content paste are 2 hours. Hence, it can be concluded that, moisture content of *Kappaphycus Alvarezii sp.* at 2 hours exhibit moisture content at desirable amount. In addition, as seaweed will be grind into powder form with proper amount of moisture content will producing finer powder during grinding process (Jung *et al.*, 2018).

4.4 Scanning Electron Microscopy (SEM) Analysis

Scanning Electron Microscopy (SEM) analysis was used to obtain the surface morphology of the untreated seaweed and the seaweeds at three different soaking durations. The samples surface were examined with a variable pressure Scanning Electron Microscope (SEM), Carl Zeiss Model 1450 VP. The SEM micrograph resulted at the different parameters of the soaking process, there is an apparent alteration in seaweed morphology, a significant difference in terms of their level of smoothness and roughness texture of the seaweed. It is observed that Figure 4.6 (a) shown that the untreated surface of the biomass was found to be rough and porous. The seaweed exhibit a smooth surface with some impurities that appears to be crystalloid salt deposition covering the area (Wasoh *et al*., 2014). Also, the samples of raw seaweed resulted in a reticulated and blocky morphology. After soaking treatment in the Figure 4.6 (b), (c) and (d), it can be observed that soaking had caused seaweed surface to be smoothen. Also, as compared to untreated seaweed, the soaking treated seaweed has a cleaner surface and impurities such as salt has been removed thoroughly.

Besides, the morphology of seaweed still exhibits high rigidity at lower soaking durations. This is due to less water migration to seaweed during less time of soaking. It can be seen that the seaweed is still brittle and porous on the surface, as formed in Figure 4.6 (b). In contrast, at an optimized soaking duration that is 2 hours, the seaweed would expand and increase in weight as in Figure 4.6 (c). It is observed that seaweed expand at great extend and more smooth. Meanwhile, in the Figure 4.6 (d), the seaweed structure are smoother as compared to Figure 4.6 (b) and (c). Besides, the rigidity of Figure 4.6 (b) are due to correlation moisture content in the seaweed. As discussed previously, the high rigidity of Figure 4.6 (b) as compared to Figure 4.6 (c) are due to its moisture content is low at soaking 1 hour and 30 minutes (13.31%) while 2 hours is 14.67%. As seaweed moisture content increase, the seaweed expansion also increase. Unlike in Figure 4.6 (d), the seaweed surface having a shrinkage and this is due to its lower moisture content inside the seaweed. This is supported by Mahiuddin *et al*. (2018), the shrinkage in the food is directly proportional to the moisture loss. It is important to study surface morphology in characterizing the structural changes that occurred during the treatment.

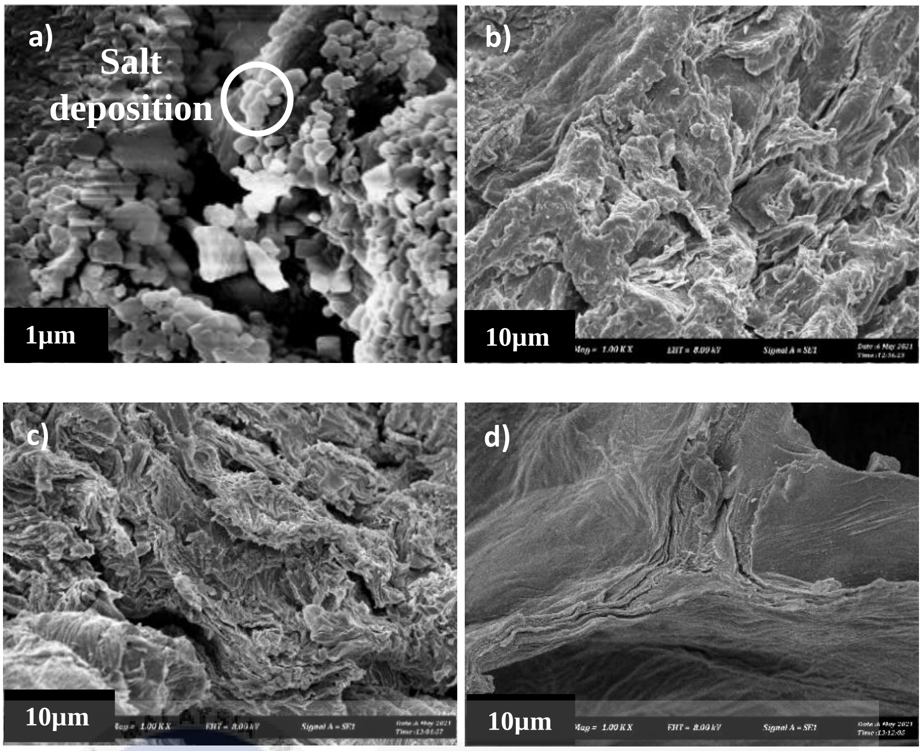


Figure 4.7: The SEM images of *Kappaphycus Alvarezii sp.* seaweed a) Untreated under magnification of 4000X (Wasoh *et al*., 2014) b) at soaking duration of 1 hour 30 minutes c) at soaking duration of 2 hours d) at soaking duration of 2 hour 30 minutes under magnification of 1000X

Thus, it can be concluded that, SEM images proved that soaking at 2 hours exhibit a great expansion of soaked seaweed. This has proven by Siah *et al.* (2014) mention that seaweed that absorbs water and expands to a certain extent is desired at soaking of 2 hours. Besides, through soaking treatment of seaweed, the surface of seaweed has become cleaner as impurities such as salt has been removed thoroughly, hence grinding of soft materials produce finer powders due to the softening effect of water (Jung *et al*., 2018).

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusion

In accordance with the first objectives, the seaweed powder of *Kappaphycus Alvarezii sp.* has been successfully produced by the soaking pretreatment method with optimized soaking duration and a fixed soaking water ratio of 1 part of dried seaweed to 10 parts of distilled water (1:10). The effects of different parameters of the soaking duration on seaweed were investigated. Before transforming the seaweed into powder form, soaking treatment of seaweed was conducted after the washing process to remove the impurities and proceed with the cutting, oven drying at constant temperature and time that is 60℃ and 10 hours. From the result obtained, it can be concluded that the optimum soaking duration for seaweed is 2 hours, as the seaweed resulted in great expansion and texture.

The second objective of this study was to analyze the heavy metals content of seaweed *Kappaphycus Alvarezii sp.* and to study the moisture content of seaweed at different soaking durations. For the heavy metals content, the result was postulated via critical review based on the graph trend. There were only two journals that had carried out soaking as a pretreatment as related to this study. This analysis was hypothesized critically by examining the graph trend based on the previous research data from Bui Huy Chich *et al*. (2020) and Xiren and Aminah (2017) at different soaking duration parameters. The heavy metals had been used studied are Arsenic (As), Cadmium (Cd) and Lead (Pb). As the optimized soaking hour is at 2 hours, the heavy metals postulated relatively lower than both journals. Heavy metals are very well-known for their toxicity to humans and the ecosystem, hence the low concentration of heavy metals in seaweed, the higher quality of the *Kappaphycus Alvarezii* sp. seaweed produced. Meanwhile, the moisture content of the seaweed powder has been successfully analyzed by a moisture content analyzer. The amount of moisture content substantially increases as soaking duration increases. At an optimized duration of 2 hours, the moisture content of the seaweed obtained is at an acceptable amount of 14.67%. The increase of soaking duration has proven to increase moisture and expansion. However, seaweed moisture deterioration occurred at 2 hours and 30 minutes of soaking duration as it is prone to microbial growth. This moisture content analysis is aligned to Scanning Electron Microscopy (SEM) analysis.

Besides, the third objective was to characterize the *Kappaphycus Alvarezii sp.* seaweed structure. The surface morphology of the seaweed had been analyzed by Scanning Electron Microscopy (SEM) analysis at different soaking durations. SEM analysis studied that the seaweed structure correlates to the moisture content of seaweed after soaking treatment. Lower soaking duration at 1 hour 3 minutes exhibits a rough and rigid structure, while at optimized soaking hours of 2 hours, the seaweed structure resulted in a smooth surface with great expansion. Meanwhile at 2 hours 30 minutes the shrinkage texture at low moisture content was caused by moisture deterioration due to the prolonged soaking duration. In addition, soaking treatment on seaweed revealed that impurities such as salt deposition were successfully removed thoroughly compared to untreated seaweed. Thus, these findings were aligned with the moisture content and the third objective of this study, which soaking duration had an effect on the moisture content and morphology of the seaweed after the soaking treatment.

5.2 Recommendations

This study has afforded an opportunity to exchange views towards production of *Kappaphycus Alvarezii sp*. seaweed powder. Further research needs to be done towards soaking treatment that can be improved. It is recommended to consider the combination of this soaking duration and soaking temperature such as varies between cold temperature and hot temperature. Furthermore, the future study should also considered the soaking time along with soaking temperature as this process also contribute to the production of the high quality of seaweed powder.

5.3 Sustainable Design and Development

The wise use of natural resources has as much beneficial influence on humans and the environment as it only has a minimum adverse impact. The implementation of seaweed has the advantage of promoting a healthier lifestyle sustainably. Seaweed is not only profoundly nutritious but also extremely versatile. Nowadays, vast application of seaweed has been incorporated into food, pharmaceutical, cosmetic industries and nutraceutical. The nutrient-dense nature of seaweeds makes them excellent candidates for the creation of novel functional food. Besides, biorefineries with seaweed as feedstock attract worldwide interest and include low-volume, high value-added products and vice versa. Biorefinery is a way to decrease the negative impact on the environment and create products with higher added value to get more economical and environmental benefits. They can be transformed into biofuels such as biogas, bioethanol, and biodiesel, replacing a part of fossil fuels. This is a great success that has introduced these biorefinery sources for future development in using green material as a raw material. However, it is valuable to investigate this newly developed biorefinery material further to produce highly sustainable, functional, and cost-effective products while advancing the bio-economy in the world. The concept of sustainable design was in purpose to reduce the detrimental effects on the environment issues.

5.4 Complexity

Throughout this study, there are difficulties and unexpected situations that come up at challenging and pressured periods. Increasingly case of the COVID-19 pandemic across the world had causes laboratory activities delayed and restricted laboratory use. This has made experimental works finished at a longer time. Moreover, the sudden Movement Control Order (MCO) announcement and only a few sectors are allowed at the MCO stage made the analysis that needs to be tested outside the lab are unavailable and need to be changed to critical review. The final year students should discuss with their supervisors the scope that aligned with the situation.

In order to overcome the problems, a critical review had been implemented into some part of the study analysis. The lack and limited study during soaking treatment in the production of seaweed powder has led to opportunities in novelty and originally of the study. The critical review has led to much complexity as knowledge on postulating results based on science and engineering elements and fundamentals. In this study, complex knowledge and solutions have been applied, such as optimum soaking duration to produce seaweed powder with the soaking pretreatment method. In the meantime, though it is hard, this study help in enhancing self-research-based knowledge and creativity in applied engineering principles.

5.5 Life Long Learning and Basic Entrepreneurship

The seaweed that has its potential application in different fields can be a global commercial market size due to booming biotechnology. This is because seaweed possesses unique compounds with several properties that are potential allies of human health, making them valuable compounds to be involved in biotechnological applications. Therefore, these results have studied the use of green material as the raw material without utilizing chemical substances to give a sustainable production and have been useful in entrepreneurship. The knowledge of lifelong learning is reviewed by suggestions for further advancement as stated in the recommendations part.

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